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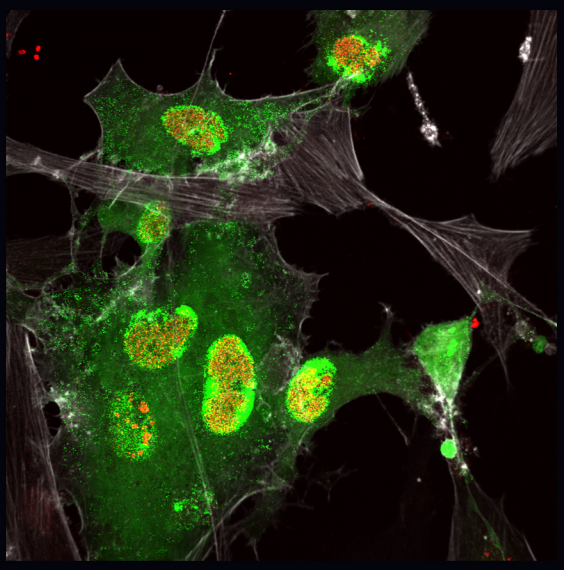
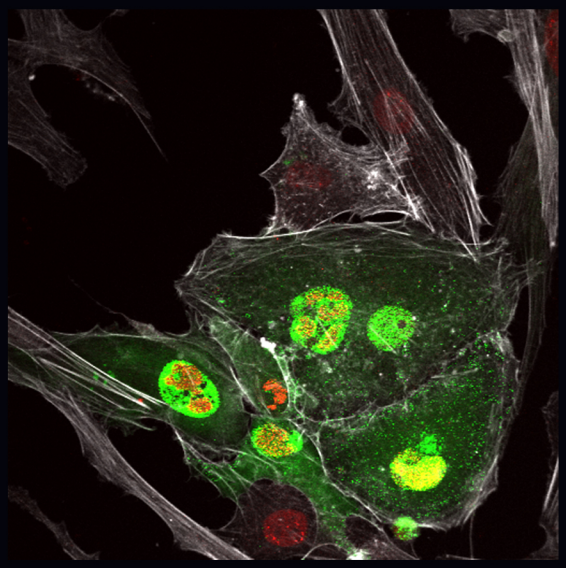


Nuclear capsid aggregates of Varicella-Zoster virus: assembly sites or dead-end depot?



Thesis submitted for the degree of
"Docteur en Sciences"

Marielle lebrun
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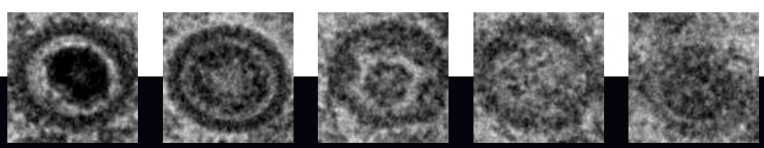

Et au bout du compte… J'y suis… Je crois que j'en ai rêvé cent fois, peut-être plus de ce jour où, seule devant mon clavier j'allais écrire ces quelques pages, l'épilogue de cette incroyable aventure… Quel chemin parcouru… Comment je me sens en cet instant précis ?… A peu près comme si j'étais Frodon, au bord de la crevasse du Destin, m'apprêtant à lancer l'anneau unique dans la lave s'écoulant à mes pieds, un mélange de soulagement et d'angoisse, de joie inexprimable et de profonde mélancolie. Il aura fallu 12 ans à JRR Tolkien pour écrire son livre, à peu près le temps qu'aura duré cette épopée sans nom. Que de gens à remercier, tous ces gens qui ont croisé ma route et à qui je dois cet happy end tant attendu…

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« Si tu aimes une fleur qui se trouve dans une étoile,

c'est doux, la nuit, de regarder le ciel. »

Le petit Prince, Antoine de Saint-Exupéry

A Isaline,

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GIGA-R,infection, Immunity and inflammation

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Nuclear capsid aggregates of Varicella-Zoster virus: assembly sites or dead-end depot?

Marielle LEBRUN

Thesis submitted for the degree of
"Docteur en Sciences"

Université de Liège

G I G A

Le virus de la Varicelle et du Zona (VZV) est un herpesvirus responsable de deux pathologies distinctes. La primo-infection a généralement lieu pendant la petite enfance et se manifeste, après une période d'incubation de 7 à 14 jours, par une éruption cutanée généralisée sous la forme de petites vésicules remplies de liquide contenant du virus infectieux. Lors de cette primo-infection, le virus va atteindre les ganglions nerveux périphériques de la chaine nerveuse dorsale où il établira un cycle latent. En cas de baisse de l'immunité, notamment lors du vieillissement, le virus peut se réactiver, atteindre une nouvelle fois la peau où il se manifeste par une éruption cutanée localisée et douloureuse qu'on appelle le Zona.

La particule virale infectieuse d'un herpesvirus est constituée de 3 parties distinctes. Premièrement : la nucléocaspide icosahédrale qui renferme le génome viral constitué d'ADN double brin ; deuxièmement : le tégument composé de protéines d'origine virale et cellulaire et troisièmement : l'enveloppe, une bicouche lipidique d'origine cellulaire dans laquelle sont enchâssées les glycoprotéines virales nécessaires à l'entrée du virus dans la cellule-hôte.

La première étape de l'assemblage des particules virales chez les herpesvirus se déroule dans le noyau. Cependant, le lieu exact où les procapsides sont assemblées, où le génome est empaqueté dans ces dernières pour former les nucléocapides matures et où le tégument interne est acquis est encore sujet à controverse. Afin de clarifier ces processus et leurs localisations, nous avons créé une souche recombinante du VZV exprimant la protéine de capside ORF23 (l'homologue de la protéine VP26 de HSV-1) fusionnée à l'eGFP. Dans un second temps, nous avons également généré des souches virales doublement fluorescentes où, en plus de la fusion ORF23-eGFP, une des protéines tégumentaires (ORF9, ORF21, ORF22, ORF38, ORF63/70, correspondant respectivement aux protéines d'HSV-1 pUL49, pUL37, pUL36, pUL21 and ICP22) est en plus fusionnée à un tag rouge.

Ces virus recombinants, nous ont permis d'identifier des structures fluorescentes denses dans les noyaux des cellules infectées, à la périphérie du compartiment de réplication viral. Nous avons démontré que ces structures apparaissent dans tous les types cellulaires testés et ce, à des temps aussi précoces que 8 à 12 heures post-infection. Elles contiennent des protéines constituant les procapsides et les capsides, ainsi que certaines des protéines tégumentaires précitées, à savoir ORF21, ORF22 et ORF38 alors qu'elles semblent dépourvues des protéines impliquées dans le

processus de réplication et de l'empaquetage de l'ADN. Par microscopie corrélative, nous avons démontré que ces structures renferment une grande quantité de capsides à différents stades de maturation, notamment de nombreuses procapsides ainsi que des capsides partielles, précurseurs de ces procapsides. Diverses techniques de microscopie de fluorescence sur cellules vivantes, nous ont permis de démontrer que ces structures sont dynamiques et qu'elles apparaissent avant l'accumulation massive de capsides dans le cytoplasme.

Nous avons également démontré que ces structures coïncident avec des corps nucléaires appelés PML bodies et dont les rôles dans la physiologie cellulaire sont extrêmement variés. Une de leur fonction présumée serait de pouvoir constituer une défense intrinsèque de la cellule contre les infections virales. De ce fait, beaucoup de virus ont développé des moyens pour contrecarrer cette défense, notamment le virus HSV-1, proche cousin du VZV. L'association des structures que nous avons identifiées avec les PML bodies pourrait soit être bénéfique au virus, soit représenter un moyen pour la cellule d'empêcher le passage des capsides nouvellement synthétisées vers le cytoplasme, limitant ainsi la croissance du virus.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

(H)CMV	(Human) CytoMegaloVirus
AIDS	Acquired immunodeficiency syndrom
ATCC	American Type Culture Collection
CDKI	Cyclin-dependent kinase I
CHPK	Conserved herpesvirus-encoded protein kinase
CKII	Casein kinase II
CMI	Cell-mediated immunity
CVSC	Capsid vertex specific component
Daxx	Death domain associated protein
DNA	Desoxyrobonucleic acid
DRG	Dorsal Root Ganglia
E	Early
EBV	Epstein-Barr Virus
EF-δ	Elongation factor-δ
eGFP	Enhanced green fluorescent protein
FLIP	Fluorescence loss in photobleaching
FRAP	Fluorescence recovery after photobleaching
HCF-1	Host-cell factor-1
HDAC-1/2	Histon deacetylase-1/2
hnRNP	Heterogenous nuclear ribonucleoprotein
HRP	Horseradish peroxydase
Hsc-70	Heat shock constitutive 70 kDa
HSF-1	Heat shock transcription factor 1
Hsp70/90	Heat shock protein 70kDa/90kDa
HSV	Herpes Simplex Virus
ICP0/4/8	Infected cell protein0/4/8
IDE	Insulin-degrading enzyme
IE	Immediate early
INM	Inner nuclear membrane
IRF3	Interferon regulatory factor 3
L	Late
LAT	Latency-associated transcript
LSCM	Laser scanning confocal microscopy
MAP	Microtubules associated protein

MCP	Major Capsid protein
MPRci	Cation indepededent mannose-6-phosphate receptor
MT	Microtubules
MTOC	Microtubules organizing center
NEC	Nuclear egress complex
NLS	Nuclear localization signal
OBP	Origin-binding protein
Oct-1	Octamer-binding transcription factor-1
ONM	Outer nuclear membrane
ORF	Open reading frame
PBMC	Peripherical Blood mononuclear cells
P-H2AX	Phospho-Histone H2 AX
PHN	Post-Herpetic neuralgia
PML	Promyelocytic leukemia
p-RPA	Phospho-Replication protein A
PRV	Pseudorabies virus
RC	Replication compartment
RER	Rough endoplasmic reticulum
RNP	Ribonucleoprotein
snRNP	Small nuclear ribonucleoprotein
Sp-1	Specificity factor-1
SP100	Speckled protein of 100kDa
SR protein	Serine (S)-Arginine (R)- rich protein
SUMO	Small ubiquitin-like modifier
Tag-RFP-T	Tag-Red fluorescent protein-T
TEM	Transmission electron microscopy
TG	Trigeminal ganglion
TGN	Trans-Golgi network
TK	Thymidine kinase
TNF	Tumor necrosis factor
VICE	Virus-induced chaperone-enriched
VZV	Varicella-Zoster-Virus

INTRODUCTION

1.Varicella-Zoster Virus

Varicella-zoster virus (VZV) is responsible for two distinct diseases: Varicella (also called Chickenpox), a mostly benign childhood disease and Zoster (also called Shingles), a more acute, localized and painful rash, highly frequent in the elderly.

1.1. History

(for review; (Weller, 1996), chapter I of (Arvin, 2000))

Varicella-zoster virus clinical manifestations have been reported already in the Ancient Greece but were originally thought to be a milder form of smallpox. The first attempt to prove tohat chickenpox was distinct from smallpox is attributable to William Heberden in 1767 but one had to wait the twentieth century and the work of Tyzzer to clearly assess this difference. At that time, chickenpox had already been proved to be an infectious disease by Steiner but the nature of the infectious agent was still unknown. In 1904, while Tyzzer was studying in the Philippines the susceptibility of monkeys to smallpox, an epidemic in the Bilibid prison retained its attention. While his colleagues were suspecting a mild form of smallpox, he noticed that most of the sick captives already had old scars typical of smallpox episode or vaccination. He then decided to inoculate vesicular fluid from their lesions in rabbit eyes and in monkeys and observed no sign of infection that normally appear with smallpox vesicular fluids. He also analyzed the lesions from the prisoners under a microscope and found gigantic multinuclear cells with nuclear inclusions that were not characteristic of smallpox lesions.

The relationship between the etiologic agents causing varicella and zoster was first suggested in 1892 by a paediatrician named Janos Von Bokay, who reported the high frequency of Varicella onset in children that had been in contact with persons suffering from Zoster. In 1921, Lipschutz characterized zoster lesions and noticed their strong similarities with the lesions described by Tyzzer. At the same time, Ernest Goodpasture was studying the enlarged cells characteristic of cytomegalic inclusion disease and observed also a remarkable resemblance with Tyzzer drawings (Goodpasture and Talbot, 1921). He then started a series of animal experiments to demonstrate that the nuclear inclusions were an hallmark of infection with herpes

virus (Goodpasture and Teague, 1923). By analogy, varicella was assumed to be caused by a virus.

In 1925, Kundratitz inoculated vesicular fluids from zoster lesions to healthy volunteers that developed varicella-like rashes. In 1943, Garland hypothesized that chickenpox and shingles, not only were caused by the same virus, but that, similarly to herpes simplex recurrent infections, Zoster was due to a reactivation of Varicella virus maintained latent in the host since the primo-infection (Garland, 1943). The same year, electron microscopy analysis of vesicular fluids from varicella and zoster lesions by Ruska supported the idea of a viral origin of these diseases. Several electron microscopy studies subsequently confirmed this result, brought other clues for a unique virus (Rake et al., 1948), and definitely proved the distinction between varicella and variola (Nagler and Rake, 1948).

In 1953, after many unfruitful attempts (Weller and Stoddard, 1952) that were later attributed to the highly cell-associated nature of VZV, Thomas Weller finally succeeded to establish a reproducible culturing method using intact infected cells as the inoculum (Weller, 1953). He spent the five following years to develop a neutralization test with human serum of varicella and zoster convalescent patients. This allowed him to finally prove the monistic theory supported by Garland and others and to give the virus its definite name: Varicella-Zoster Virus (Weller and Witton, 1958).

The sixtieth and the seventieth are characterized by the explosion of breakthroughs and new techniques development in the molecular branch of biology, among which, the discovery of type II restriction endonucleases and the birth of electrophoretic separation methods. This allowed Straus to analyze VZV genome and to demonstrate the variability between epidemiologically unrelated clinical isolates (Straus et al., 1983). He also brought another definite proof of the co-identity of Varicella and Zoster etiological agents, by analyzing the viral genome isolated from varicella and subsequent zoster lesions from the same patient (Straus et al., 1984). Two years later, Davison and Scott published the first complete sequence of a VZV Dumas strain (Davison and Scott, 1986).

1.2. Classification

The increasing numbers of identified viruses and the availability of their genome sequences have necessitated the development of classification methods. Several systems have coexisted over the years but the most broadly accepted is the one of the International Committee on Taxonomy of Viruses. This system divides the viral

world in orders, families, subfamilies, genera and species. Genome type, virion organization, capsid symmetry, viral life cycle and host types are the mains criteria defining the highest levels of classification. Since its first report in 1971, many changes have been brought and the actual 2012 release contains 7 orders, 96 families, 22 subfamilies and 420 genera. VZV, human herpesvirus 3, is an enveloped dsDNA virus with an icosahedral capsid belonging ergo to the herpesvirales order and herpesviridae family. Four characteristics are shared by all members of this family: (i) they express a large number of enzymes implicated in acid nucleic metabolism, DNA synthesis and processing of proteins; (2) genome synthesis and capsid assembly occur in the nucleus; (3) productive infection leads to host cell lysis and (4) they are able to establish and to maintain in the host a latent stage from which they can reactivate under specific conditions.

Subfamily	Reproductive cycle	Genome size	Host range	Genus	Species
alphaherpesvirinae	Short (~18h)	120-280 kbp	variable	*Simplexvirus*	HHV-1 (HSV-1), HHV-2 (HSV-2)
				Varicellovirus	HHV-3 (VZV), Suid herpesvirus-1 BoHV-1,-5, EHV-1
				Mardivirus	Marek-disease virus
betaherpesvirinae	Long	140-240 kbp	restricted	*Cytomegalovirus* *Muromegalovirus* *Roseolovirus*	HHV-5 (HCMV) Murine CMV HHV-6A, HHV-6B, HHV-7
gammaherpesvirinae		~180kbp	restricted	*Lymphocryptovirus* *rhadinovirus*	HHV-4 (EBV HHV-8 (KSHV)

Table 1. Representative members of the Herpesviridae family and characteristics of the subfamilies

This family contains three subfamilies, *alpha-*, *beta-*, *gamma-herpesvirinae* and a group of, so far, unclassified herpesvirinae (table 1 and 2).

While VZV genome sequence is closer to HSV-1, HSV-2 and PRV, it shares with MDV its very narrow host spectrum and its highly cell-associated nature. In cell culture, virtually no infectious particle are present in the supernatant, rendering synchronization experiments particularly tedious. Cell-free infection systems therefore rely on mechanical lysis of infected cells, followed by the removal of cellular debris and concentration of viral particles and still lead to quite low viral titre. Significantly higher yields can be reached if using freshly isolated human retinal pigment cells (Schmidt-Chanasit et al., 2008). A very recent publication has described

a similar approach but with a retina pigmented cells line available at the ATCC (Sloutskin et al., 2013).

Family	Virus	Productive (Lytic) Replication	Site of Latency
α	HSV-1	Epithelial and keratinocyte	Neuron
	HSV-2	Epithelial and keratinocyte	Neuron
	VZV	Epithelial, keratinocyte, T cell, sebocyte, monocyte, endothelial, Langerhans and PBMC	Neuron
β	HCMV	Macrophage, dendritic, endothelial, smooth muscle, epithelial and fibroblast	CD34+ HSC, monocyte
	HHV-6	T cell	BMP
	HHV-7	T cell	T cell
γ	EBV	B cell and epithelial	B cell
	KSHV	Lymphocyte	B cell

Common cell types in which the human herpesviruses, grouped by family, initiate productive lytic infection or establish latency are listed. PBMC, peripheral blood mononuclear cells. HSC, hematopoietic stem cell. BMP, bone marrow progenitor.

Table 2. Cells that support the different types of human herpesvirus infections (Penkert and Kalejta, 2011)

1.3. Pathology

1.3.1. Varicella

Varicella is a highly contagious, most of the time, benign disease, usually contracted during childhood and characterized by a generalized cutaneous rash accompanied by fever. The lesions resemble blisters and contain hundreds of infectious free particles that can be disseminated via airborne or direct contact to susceptible individuals (figure 1). It is important to note that, as described for cell culture systems, most of the ulterior viral dissemination steps occur via cell-contact dependant virus transfer.

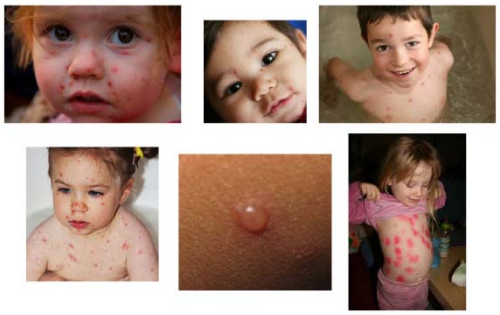


Figure 1. Clinical manifestation of Varicella

VZV enters the host via the upper respiratory tract mucosa where it replicates and reaches the associated lymphatic ganglia. This leads to a primary cell-associated viremia during which VZV will spread to several internal organs, such as liver and spleen. For a period of approximately 10 days, the virus proliferates in these organs, until the second viremia that will finally, via infected PBMC or T cells, convey the virus to the skin and result in the symptomatic phase of the disease. This "two

viremic steps" model has first been suggested by Fenner, revisited by Grose in 1981 (Grose, 1981) and has largely been accepted ever since. It provided a possible explanation for the relatively long incubation period (around 15 days) between the infection and the appearance of the cutaneous rash.

A more recent model has been proposed in which the T-Cells circulating in the tonsils or other lymphoïd tissues constituting the waldeyer's ring become infected and could directly transport VZV to the skin within a short time after entering the

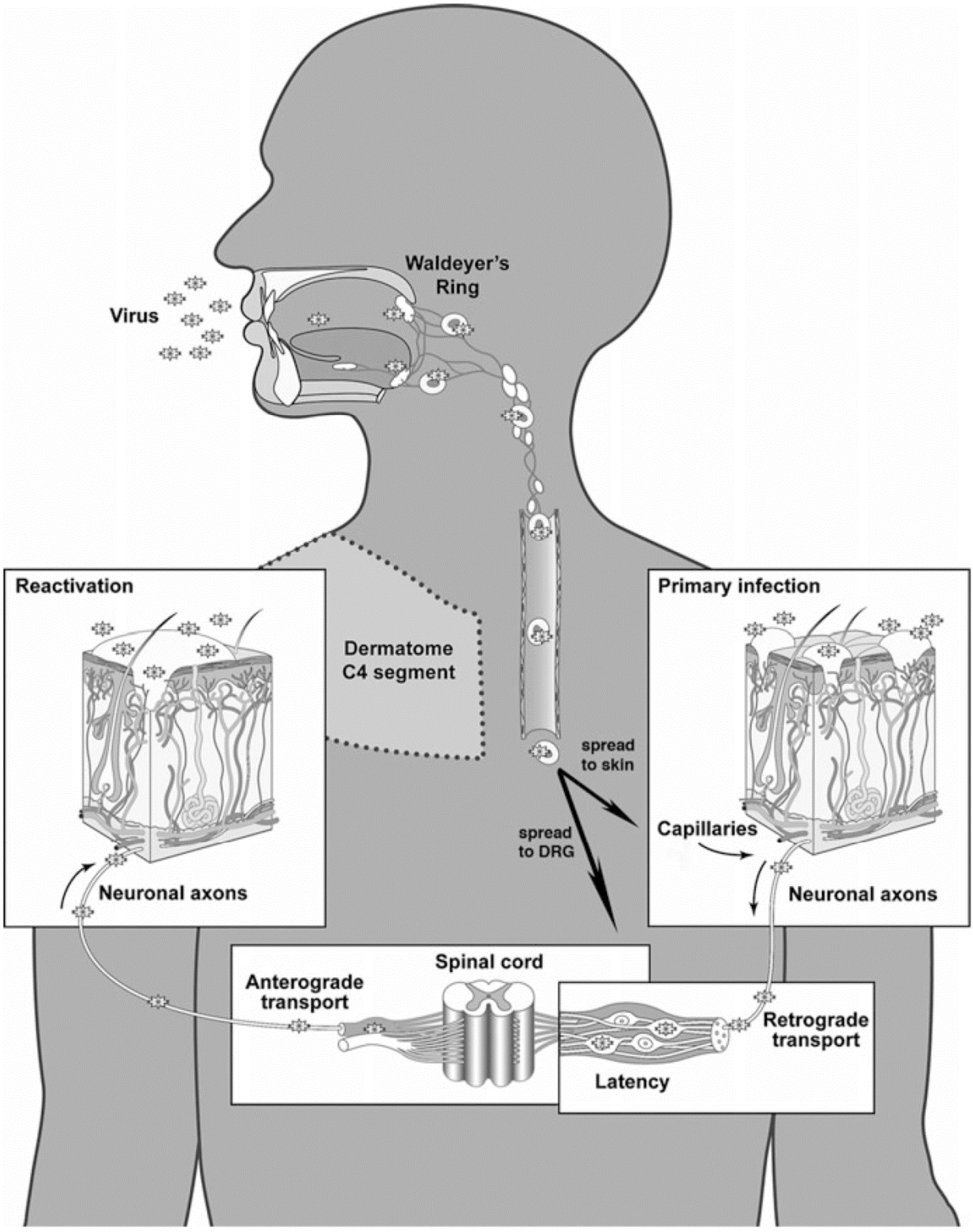


Figure 2. Model of primary and recurrent VZV infection (from Arvin et al., 2010)

circulation (figure 2) (Arvin et al., 2010). A potent innate immune response is then locally initiated and delays the cell-to-cell viral spread. The incubation period would then correspond to the time needed for the virus to overcome the immune barrier.

No matter the considered model, infected keratinocytes will fuse forming gigantic plurinuclear cells with the characteristic eosinophilic intranuclear inclusions, already described by Tyzzer in 1906. The evolution towards the vesicles is associated with a progressive "ballooning" degeneration of the cells (Arvin, 1996). It has been suggested that the efficient release of free viral particle specifically in the vesicular fluid is due to a downregulation in cation-independent mannose 6-phosphate receptors (MPR^{ci}s) characterizing the suprabasal layer of keratinocytes. This will be described further in the following chapters. Axons of sensory neurons present in this skin layer might provide the entrance door for the free viral particles present in the lesions. The virus, via an ascending route along the axons could then reach sensory ganglia in which it will establish the life-long latency. Another model has been proposed in which infected T-cells could transmit the virus directly in the neurons and satellite cells present in the ganglia. Recent data have demonstrated that symptomatic skin replication is not absolutely required for the virus to establish latency in the ganglia but favours it. This indicates that both models are not mutually exclusive. Dorsal root ganglia (DRG) and trigeminal ganglia (TG) are the main sites of latency although enteric ganglia can also be affected (Chen et al., 2011a; Gershon et al., 2012; Gilden et al., 2001; Mahalingam et al., 1990).

VZV is transmissible 24 to 48 hours before the exanthem begins. This probably occurs via respiratory droplets that may contains infectious particles released by the lesions frequently present in the oropharynx region. Of note, VZV has been shown to be present in the saliva of zoster patients, but also in asymptomatic VZV reactivation cases (Cohrs et al., 2008; Gershon, 2011; Leung et al., 2010; Mehta et al., 2008). If VZV is detectable in the saliva prior the onset of varicella rash remains to be determined. In an immuno-competent individual, the immune system clears the virus within a few days after the appearance of the cutaneous lesions. Complications are rare in healthy children but tend to be much more frequent in adults and in patients dealing with immunosuppressive diseases (like HIV or leukaemia) or specifically treated with immunosuppresors (transplanted patients for example). Among complications are hepatitis, pneumonias, encephalitis, meningitis and myelitis (Arvin, 1996; Echevarria et al., 1997; Fleisher et al., 1981; Guess et al., 1986; Heininger and Seward, 2006; Myers, 1979), resulting from uncontrolled localized viral replication, but the most

common are provoked by secondary bacterial infections, at the skin or the lung level (Guess et al., 1986; Heininger and Seward, 2006; Hill et al., 2005).

1.3.2. Zoster

Zoster, a clinical manifestation of VZV reactivation, is generally a localized, painfull rash, associated with elderly. VZV reactivation has been linked to a weakness of the immune system, the cell-associated immune response in particular. It is then not surprising that Zoster prevalence increases with age (for review, (Weinberg and Levin, 2010)) but also in patients suffering from diseases affecting their immune system (leukaemia, lymphoma and AIDS, for example) (Birlea et al., 2011; De Castro et al., 2011; Glesby et al., 1995) or treated with immunosuppressive drugs (grafted patients, autoimmune diseases) (Che et al., 2013a; Gourishankar et al., 2004). Reactivation occurs usually in a single ganglion in which viral replication is observed both in neuronal bodies and non-neuronal neighbouring cells. Infectious virions are able to reach back the skin of the corresponding dermatoma, via an anterograde route along the axons. Even though reactivation can take place in any latently-infected ganglion, the thoracic region is the most frequently affected area. This will result in pain, itching, parasthesias in the specific region, which is followed, a few days later, by a characteristic confined vesicular rash (figure 3). The pain is due to the localized inflammation within the ganglia and the destruction of some infected neurons. It usually disappears within 4 to 6 weeks but can frequently (up to 30% of the patients) persist for several months after the lesion disappearance, as a syndrome known as Post-Herpetic neuralgia (PHN). The etiology of PHN remains controversial. Two main theories, not mutually exclusive, are currently claimed: (i) excitability alteration of ganglionic (or even spinal cord) neurons and (ii) a low-level productive infection persistence in the ganglia. In some cases (around 10% of zoster cases) VZV reactivation takes place in the trigeminal ganglion, resulting in Herpes Zoster Ophtalamicus (HZO), that can permanently affect the patient vision. Other, less frequent, complications of Zoster includes encephalitis (0.2 to 0.5 % of zoster cases), myelitis, nerve palsies and motor paralysis (Weinberg, 2007). Immunosuppression augments the risk of morbidity and mortality associated with

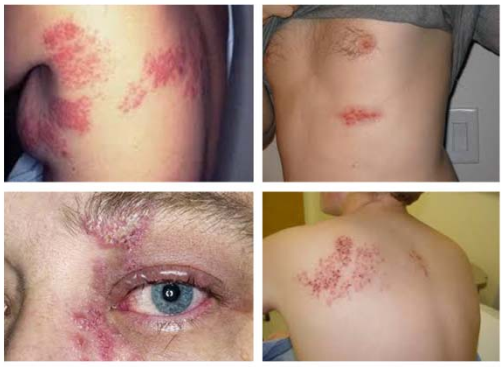


Figure 3. Clinical manifestation of Zoster

herpes Zoster. Pneumonias, cutanenous overall dissemination, visceral dissemination and neurological complications are quite frequent among these patients. It is now known that asymptomatic VZV reactivations frequently occur and probably favour the maintenance of cell-specific immunity against the virus (Birlea et al., 2011; Cohrs et al., 2008). "Zoster sine herpete" is an intermediate situation where the immune system is able to counteract the vesicular rash, but not the associated pain and/or other symptoms (Blumenthal et al., 2011; Gilden et al., 2010).

1.4. Epidemiology

Before the vaccination era, even though VZV was similarly present all over the world, the mean age at the primo-infection was highly dependent on the climate of the considered region. In temperate regions, chickenpox outbreaks were most frequently occurring in late winter and spring and over 90% of the population had contracted the illness naturally by the age of 9. In sub-tropical areas, epidemics were not seasonal and 50% of young adults (18-20 years) were still sero-negative (Lolekha et al., 2001). In 1974, a Japanese lab developed a live-attenuated vaccine (vOka) starting from the clinically isolated parental Oka strain (pOka) that was passaged on guinea pig cells, then serially passaged on Human cells (Takahashi et al., 1974). Shortly after, many small scale studies have been conducted to evaluate the efficacy of this vaccine and the maintenance of the protection after several years (Asano et al., 1982; Asano et al., 1975; Ozaki et al., 1984). The vaccine was first commercialized in Japan, in 1987 and, slightly later in Korea. In Japan, the vaccine is part of the voluntary vaccination program, with only 20-30% of coverage (Ozaki, 2013).

The Oka strain was later used in parallel by two biotech companies, Merck and GlaxoSmithKline to produce their own live attenuated vaccine, Varivax and Varilix, respectively. Systematic vaccination started in the USA in 1995, and is now also the applicable standard in Canada, and Taiwan. The single-dose vaccine has been proved to be efficient at 80%-85% and has greatly diminished the incidence of chickenpox. Mortality and morbidity associated with varicella has drastically fallen in the USA since its introduction (Nguyen et al., 2005).

In Europe, even though varilix is licensed in many countries, only a limited number of them have implemented a generalized vaccination program (Germany, Sicily). The reluctance to generalize the VZV vaccine in some countries partly finds its origin in conflicting models predicting the impact of VZV vaccination on Zoster occurrence in vaccinated and non vaccinated individuals. In fact, the twenty years of vaccination in the USA have brought a more global view and raised some concerns for the future

(Goldman and King, 2013a; Goldman and King, 2013b; Myers, 2013). As stated above, the vaccine, if administered in one single dose is efficient in around 80% of recipients. This means that 20% of vaccinated individuals will be susceptible to a wtVZV outbreak. Even if most of them will develop a relatively mild disease, the lesions carry infected particles that can be transmitted to naive individuals. During this "breakthrough varicella episode", the wild-type virus is able to reach the ganglia to establish a latency and to reactivate, causing Zoster. The VZV vaccine strain has also been shown to have the property to establish latency and to reactivate, although usually in mild forms. A two-dose schedule has recently been adopted in Canada and the USA and becomes the rule. This has been shown to allow >95% protection and should partially compensate for the strong drop in Cell-mediated Immunity specific to VZV that has been observed in the years following the vaccination and may account for the susceptibility of vaccinated person to contract breakthrough varicella (Kuter et al., 2004). Of course, only a limited number of data are currently available for the "two-doses" recipients. Note that a quite similar drop in the CMI against VZV is observed in the case of a true varicella episode in a naive patient (Weinberg and Levin, 2010).

The idea that frequent re-exposition to VZV during varicella epidemics are boosting our VZV-specific CMI was first claimed by Hope-Simpson (Hope-Simpson, 1965) and has been confirmed many times ever since (Brisson et al., 2002; Thomas et al., 2002). If true, it is conceivable that the introduction of the vaccine and the major drop in varicella outbreaks occurrence will manifest in a higher rate of zoster cases in the future, especially in people born prior the introduction of the vaccination campaign. Some conflicting reports have been published on zoster incidence comparing the pre- and the post-vaccination era. Even if all reports describe an increase in the number of Zoster cases, an ascending trend was already present before the introduction of the vaccine, complicating the interpretation of the results (Doerr, 2013; Goldman and King, 2013a; Goldman, 2005; Goldman and King, 2013b; Myers, 2013; Schmid and Jumaan, 2010; van Hoek et al., 2012).

1.5. Genome

VZV genome is a linear dsDNA molecule of ~125kbp (for review, (Cohen, 2010b)). It is divided in two successive unique regions flanked by inverted repeats (figure 4). The repeats surrounding the Unique Long region (U_L), the Terminal Repeat Long (TR_L) and the Internal Repeat Long (IR_L) are short DNA stretches of 88bp. The Unique Short region (U_S), much shorter that U_S region from HSV-1, is flanked by

longer repeats (IR_S, Internal Repeat Short and TR_S, Terminal Repeat Short) comprising the coding sequence of three genes, ORF62, ORF63 and ORF64 that, consequently, are present in two copies within the genome. ORF62 is identical to ORF71, ORF63 to ORF70 and ORF64 to ORF69 (Davison, 1991; Davison and Scott, 1986; Straus et al., 1981).

Two distinct ORFs (42 and 45) have been shown to constitute two exons of a long transcript which, after a splicing event, will give rise to a mature mRNA at the origin of a single protein (Visalli et al., 2007). Oppositely, the open reading frame 33 contains two overlapping in-frame genes that possess distinct transcription initiation sites but share the poly-A signal. The longer transcript, ORF33, is translated in a 605 amino acids protein whose C-terminal domain is identical to the protein of 302 amino acids produced from the shorter transcript, ORF33.5 (figure 4). These gene products are both essential for the capsid formation and will be described in details in the following paragraphs.

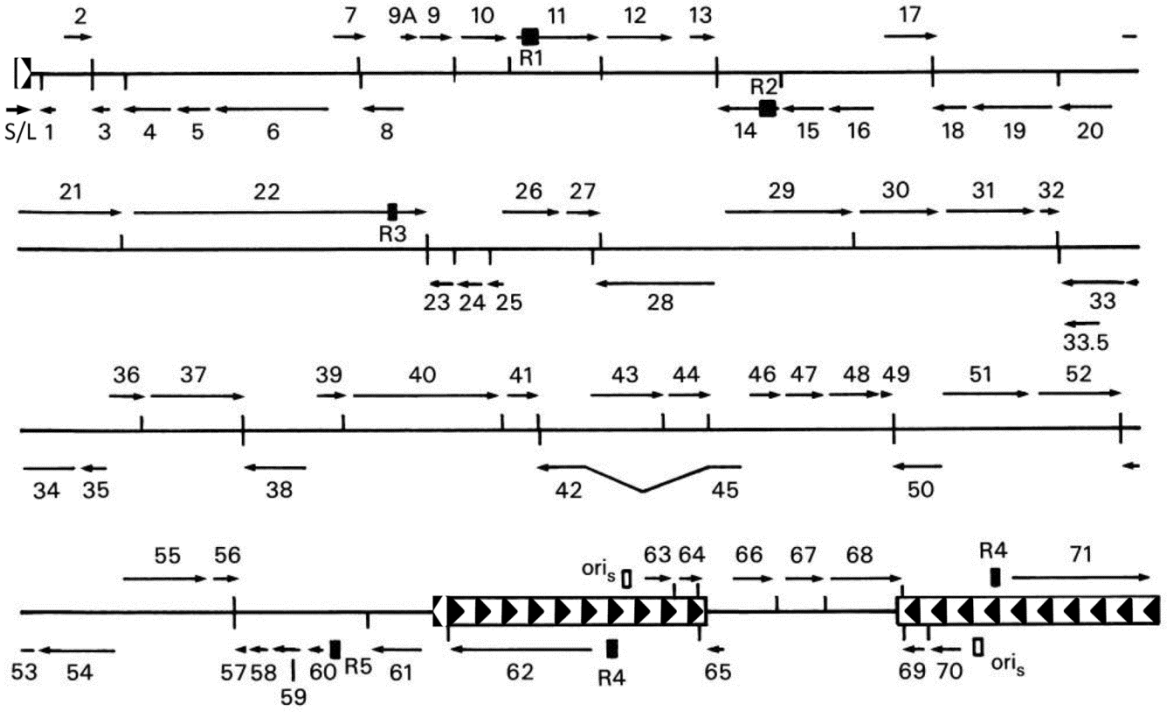


Figure 4. Schematic representation of the VZV genome. The digits indicate the ORFs and the arrows indicate their direction of transcription. The two replication origins are represented by open boxes (Ori_S) whereas the five repeated regions (R1 to R5) are represented by black boxes. The repeats flancking the unique regions are represented by boxes containing triangles (adapted from Davision, 1986).

The linear form of the genome has been shown to be the major form present within the virion (Davison, 1984; Kinchington et al., 1985). The presence of an unpaired C residue at the 3' end of TR_L and an unpaired G-residue at the 3' end of TR_S allow the molecule to circularize before the replication phase in the nucleus

(Davison, 1984). This allows the expression of a more recently discovered gene the ORF S/L located astride both ends (Kemble et al., 2000). This brings the number of distinct proteins expressed during VZV lytic cycle to 70 (figure 4). The functional role of many of them is still to be characterized. Most of them have clearly identified homologs in HSV-1, which has been much more thoroughly studied. Consequently, the role of some highly conserved proteins can be partially inferred from HSV-1 data. Of note, six VZV genes are absent from HSV-1 genome: ORFS/L,ORF1, ORF2, ORF13, ORF32 and ORF57 (figure 5). Conversely, nine genes present in HSV-1 are missing in VZV: UL56, UL45, RL1, US2, US5, US6, US11, US12 and LAT). The transcription level of each gene is highly variable from a very low level (ORF5, 16, 46 and 52) to the most abundant transcripts (ORF9, 33/33.5, 49 and 64) (Cohrs et al., 2003b).

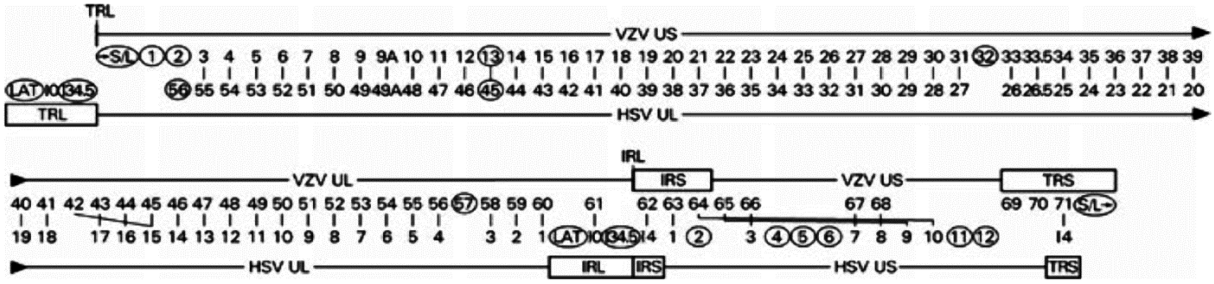


Figure 5. Comparison of HSV-1 and VZV genomes. VZV and HSV-1 genes are shown by numbers. Encircled numbers represent genes that do not have homologs in the corresponding virus (adapted from Cohen 2010).

Five other repeated regions of variable size are present within VZV genome. The repeat length is characteristic of the considered viral strain. Three of them are within gene coding sequences (R1 in ORF11, R2 in ORF14, R3 in ORF22), giving rise to variants, not only at the genomic level, but also at the protein level (Straus et al., 1983). Note also that U_S, and to a much lesser extent U_L can be present in both orientation, giving rise to four different isoforms, two of which representing 95% of the isolated molecules (Davison and Scott, 1986; Dumas et al., 1981; Kinchington et al., 1985; Straus et al., 1981; Straus et al., 1984).

Viral genes and proteins designation has changed several times and is not consistent throughout the scientific literature. Conventionally, VZV genes and corresponding proteins are generally named after their position within the genome, no matter the region of the genome (ORF1, ORF2,...). A "p" is sometimes added to distinguish the protein from the gene. An exception concerns the immediate early (IE) proteins that are named IE(x) where x is the number of the ORF and the glycoproteins, gB, gC, gI, gK, gN, gH, gE, gM, gL, where the letter is assigned according to homology with HSV-1. In HSV-1, genes and corresponding proteins are

now mostly named from their position within the genome (UL1 to UL56, US1 to US12). The prefix "p" has recently been added, in order to distinguish the gene from the protein and might be universally adopted in the future (Mettenleiter et al., 2006). Proteins encoded by the repeated regions continue to be designated as Infected Cell Proteins (ICP0, ICP4 and ICP34.5). The older nomenclature that was established on the basis of decreasing apparent molecular weight remains largely used (for example, VP1/2, "Viral Protein 1/2" is the largest protein and is encoded by UL36). This confusing nomenclature has led some team to suggest the adoption of a more generalized naming, at least for the core genes that are shared by all *herpesviridae* (table 3) (Mocarski, 2007). To help the reading of this document a "conversion table" has been generated.

Common name[a]	**Abbrev. name**	**HSV**	**VZV**	**HCMV**	**HHV 6/7**	**EBV**	**KSHV**	**Key function**
Capsid								
major capsid protein[c]	**MCP**	UL19	ORF40	UL86	U57	BcLF1	ORF25	hexon, penton, capsid structure
triplex monomer[c]	**TRI1**	UL38	ORF20	UL46	U29	BORF1	ORF62	TRI1 and TRI2 assoc to form TRI complex,
triplex dimer[c]	**TRI2**	UL18	ORF41	UL85	U56	BDLF1	ORF26	capsid structure
small capsid protein[b]	**SCP**	UL35	ORF23	UL48A	U32	BFRF3	ORF65	capsid transport
portal protein	**PORT**	UL6	ORF54	UL104	U76	BBRF1	ORF43	penton for DNA encapsidation
portal capping protein[c]	**PCP**	UL25	ORF34	UL77	U50	BVRF1	ORF19	covers portal in mature virions
Tegument and cytoplasmic egress								
virion protein kinase	**VPK**	UL13	ORF47	UL97	U69	BGLF4	ORF36	phosphorylation, regulation
largest tegument protein[c]	**LTP**	UL36	ORF22	UL48	U31	BPLF1	ORF64	uncoating, secondary envelopment
LTP binding protein[b]	**LTPbp**	UL37	ORF21	UL47	U30	BOLF1	ORF63	
encapsidation and egress protein[c]	**EEP**	UL7	ORF53	UL103	U75	BBRF2	ORF42	nuclear egress
cytoplamsic egress tegument protein[b]	**CETP**	UL11	ORF49	UL99	U71	BBLF1	ORF38	secondary envelopment, cytoplasmic egress
CETP binding protein[b]	**CETPbp**	UL16	ORF44	UL94	U65	BGLF2	ORF33	
cytoplasmic egress facilitator-1[b]	**CEF1**	UL51	ORF7	UL71	U44	BSRF1	ORF55	cytoplasmic egress
encapsidation chaperone protein[b]	**ECP**	UL14	ORF46	UL95	U67	BGLF3	ORF34	TERbp chaperone
capsid transport tegument protein[b]	**CTTP**	UL17	ORF43	UL93	U64	BGLF1	ORF32	capsid transport in the nucleus
cytoplasmic egress facilitator-2[b]	**CEF2**	UL21	ORF38	UL88	U59	BTRF1	ORF23	egress, interact with CETPbp
		UL24	ORF35	UL76	U49	BXRF1	ORF20	putative membrane or tegument
Envelope								
glycoprotein B[c]	**gB**	UL27	OEF31	UL55	U39	BALF4	ORF8	heparan-binding, fusion
glycoprotein H[c]	**gH**	UL22	ORF60	UL75	U48	BXLF2	ORF22	gH assoc, fusion

glycoprotein L[c]	**gL**	UL1	ORF37	UL115	U82	BKRF2	ORF47	gL assoc, fusion
glycoprotein M[b]	**gM**	UL10	ORF50	UL100	U72	BBRF3	ORF39	gN assoc
glycoprotein N[b]	**gN**	UL49A	ORF9A	UL73	U46	BLRF1	ORF53	gM assoc
Regulation								
multifunctional regulator of expression[c]	**MRE**	UL54	ORF4	UL69	U42	BSLF1 BMLF1	ORF57	transcriptional, RNA transport regulation
DNA Replication, recombination and metabolism								
DNA polymerase[c]	**POL**	UL30	ORF28	UL54	U38	BALF5	ORF9	DNA synthesis
DNA polymerase processivity subunit[c]	**PPS**	UL42	ORF16	UL44	U27	BMRF1	ORF59	POL processivity
helicase-primase ATPase subunit[c]	**HP1**	UL5	ORF55	UL105	U77	BBLF4	ORF44	
helicase-primase RNA pol subunit B[b]	**HP2**	UL52	ORF6	UL70	U43	BSLF1	ORF56	HP1, HP2 and HP3 assoc to form HP, unwinding and primer synthesis
helicase-primase subunit C[c]	**HP3**	UL8	ORF52	UL102	U74	BBLF2 BBLF3	ORF40 ORF41	
single strand DNA binding protein[c]	**SSB**	UL29	ORF29	UL57	U41	BALF2	ORF6	DNA fork, recombination
alkaline deoxyribonuclease[b]	**NUC**	UL12	ORF48	UL98	U70	BGLF5	ORF37	recombination
deoxyuridine triphosphatase[b]	**dUTPase**	UL50	ORF8	UL72	U45	BLLF3	ORF54	reduce dUTP
uracil-DNA glycosidase[b]	**UNG**	UL2	ORF59	UL114	U81	BKRF3	ORF46	remove uracil from DNA
ribonucleotide reductase large subunit[b]	**RR1**	UL39	ORF19	UL45	U28	BORF2	ORF61	active enzyme only in viruses with RR2
Capsid assembly, DNA encapsidation and nuclear egress								
maturational protease[c]	**PR**	UL26	ORF33	UL80	U53	BVRF2	ORF17	capsid assembly, scaffolding, DNA encapsidation
assembly protein[c]	**AP (NP)[d]**	UL26.5 (UL26)	ORF33.5	UL80.5 (UL80)	U53.5 (U53)	BdRF1 (BVRF2)	ORF17.5 (ORF17)	
capsid transport nuclear protein[c]	**CTNP**	UL32	ORF26	UL52	U36	BFLF1	ORF68	capsid transport to sites of DNA replication
terminase ATPase subunit 1[c]	**TER1**	UL15	ORF42-45	UL89	U66	BGRF1 BDRF1	ORF29	TER1 and TER2 form TER, packaging machinery
terminase DNA binding subunit 2[c]	**TER2**	UL28	ORF30	UL56	U40	BALF3	ORF7	
terminase binding protein[c]	**TERbp**	UL33	ORF25	UL51	U35	BFRF1A	ORF67	TER assoc
nuclear egress membrane protein[c]	**NEMP**	UL34	ORF24	UL50	U34	BFRF2	ORF66	nuclear egress, primary envelopment
nuclear egress lamina protein[c]	**NELP**	UL31	ORF27	UL53	U37	BFLF2	ORF69	

a, proposed
b, required for replication in some viruses or some settings
c, required for replication in all viruses and settings tested.
d, AP and NP are related proteins derived from different primary translation products.

Table 3. Identity, function and proposed nomenclature for known and putative functions of herpesvirus-common gene products of human herpesviruses (adapted from Mocarski 2007)

1.6 Morphology

VZV virion has an approximate size of 200 nm and, similarly to all *herpesviridae*, is composed of three distinct structural elements: the external envelope, in which are inserted viral proteins among which the glycoproteins, the internal nucleocapsid and the protein layer in between, that is called the tegument (figure 6).

The nucleocapsid measures about 100-120nm. Its symmetry is icosahedral with T=16, meaning that it is made of 12 pentameric (figure 6, blue structures) and 150 hexameric capsomers (figure 6, light green structures). Capsomers are multimers of the Major Caspid Protein (MCP), encoded by VZV ORF40, and are linked together by triplexes (figure 6, dark green structures) composed of two molecules of VP23 (VZV ORF41) and one molecule of VP19C (VZV ORF20). The pentameric capsomers represent the twelves vertices while the hexameric capsomers form the facets. One vertex is occupied by a dodecameric complex of the portal protein pUL6 (VZV ORF54) forming a little hole through which the DNA is inserted within the capsid and through which, most probably, the DNA will get out to enter the nucleus of a newly infected cell.

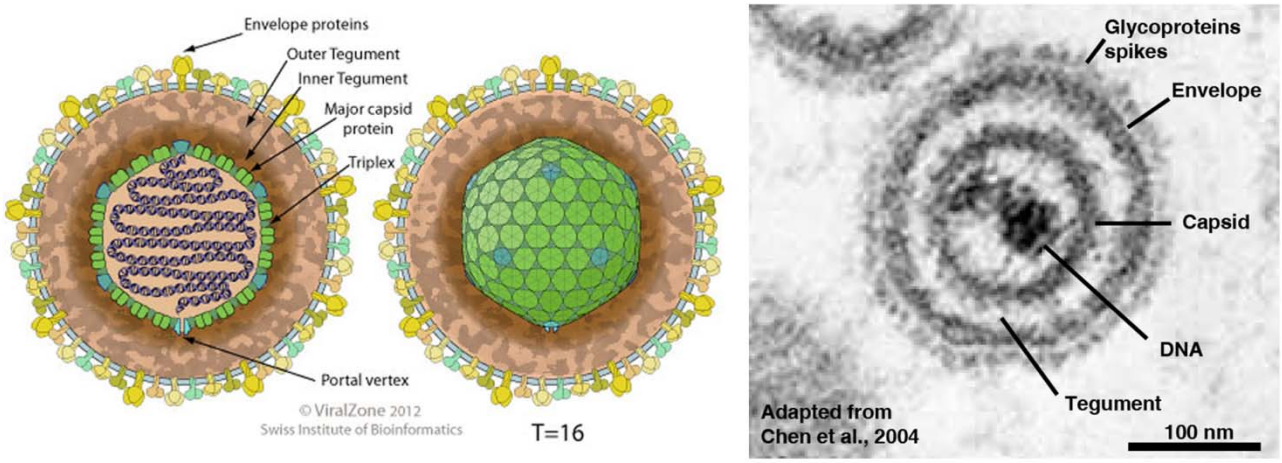


Figure 6. Virion Morphology. (left panel) Schematic representation of an herpesvirus showing the capsid symmetry with pentons at the vertices (blue structures) and hexons forming the faces (light green structures). (right panel) VZV virion in TEM.

In HSV-1, a recent study has identified, via mass spectrometry, the presence of 23 distinct viral proteins and 49 cellular proteins within the tegument (Loret et al., 2008). The inner layer is strongly linked to the capsid and plays a role in the transport of the capsid towards the nucleus during the entry process (see below). In HSV-1, the main components of the inner tegument are pUL36, pUL37 and pUS3. The outer layer is more easily extracted after the treatment of virions with detergent and has been shown to dissociate from the capsid soon after virus entry. The role of the

tegument for the viral life cycle will be discussed in the following paragraphs (for review (Kelly et al., 2009)).

The envelope, acquired during the egress of the viral particle is a lipid bilayer in which are inserted many viral proteins. In electron microscopy, it displays protrusions of several nanometres that correspond to the external, glycosylated domains of a particular class of virally encoded membrane proteins, the glycoproteins. As described below, the glycoproteins are important for the initial binding of the virion at the cell surface and for the fusion of the envelope with the cell membrane to deliver the capsid within the cytoplasm.

2. Herpesvirus lytic cycle

The lytic cycle, represented at figure 7, starts when a viral particle enters in contact with an uninfected host cell (1). The virus enter the cell when its envelope fuses with the plasma membrane (2), the capsid is partially de-tegumented (3) and is

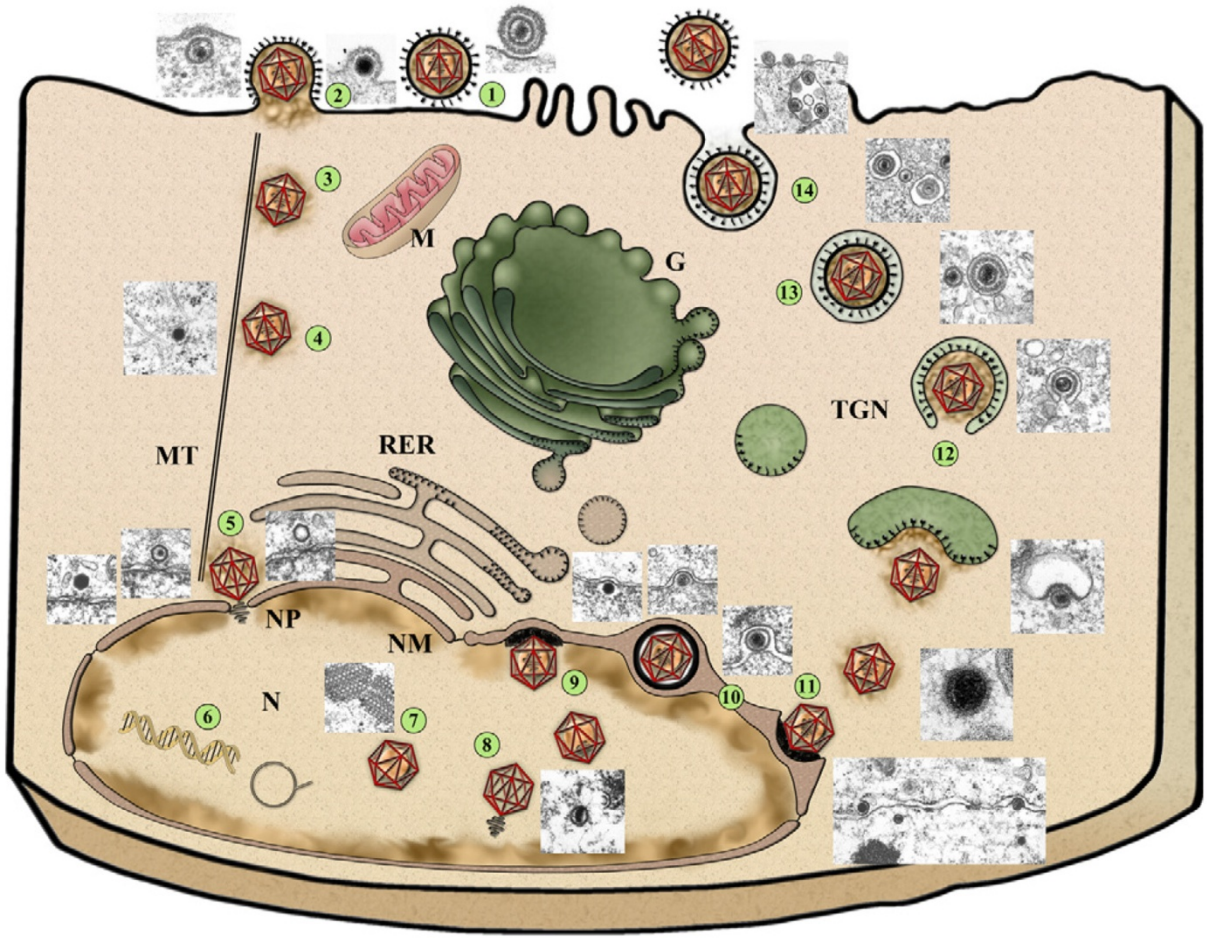


Figure 7. Replication cycle of an alphaherpervirus (pictures are from PRV). See detailed description in the text. G, Golgi apparatus; MT, microtubles; N, nucleus; NM, nuclear membrane; NP, nuclear pore; RER, rough endoplasmic reticulum, TGN: trans-Golgi network (from Mettenleiter et al., 2009)

transported to the nuclear envelope via the microtubule network (4). The capsid docks to the nuclear pore and the viral genomic DNA is transferred in the nucleus (5). Several successive waves of viral gene expression will lead to the replication of viral DNA (6) and the neo-production of procapsids within the nucleus (7). The insertion of the DNA together with the maturation of the procapsids generates mature capsids (8) that exit the nucleus through an envelopment/de-envelopment process (9-11). Naked capsids acquire the tegument and their final envelope by budding through cytoplasmic vesicles in the membrane of which viral glycoproteins are inserted (12). The enveloped particles are released from the cell when the transport vesicle fuse with the plasma membrane.

2.1. Viral entry and delivery to the nucleus

The entry of the virus in the host cell is a multistep process involving specific receptors present at the cell surface and proteins present in the viral envelope (figure 8). The binding at the cell surface is divided in an initial tethering step and a following strong interaction step that constitutes a trigger for the fusion of the

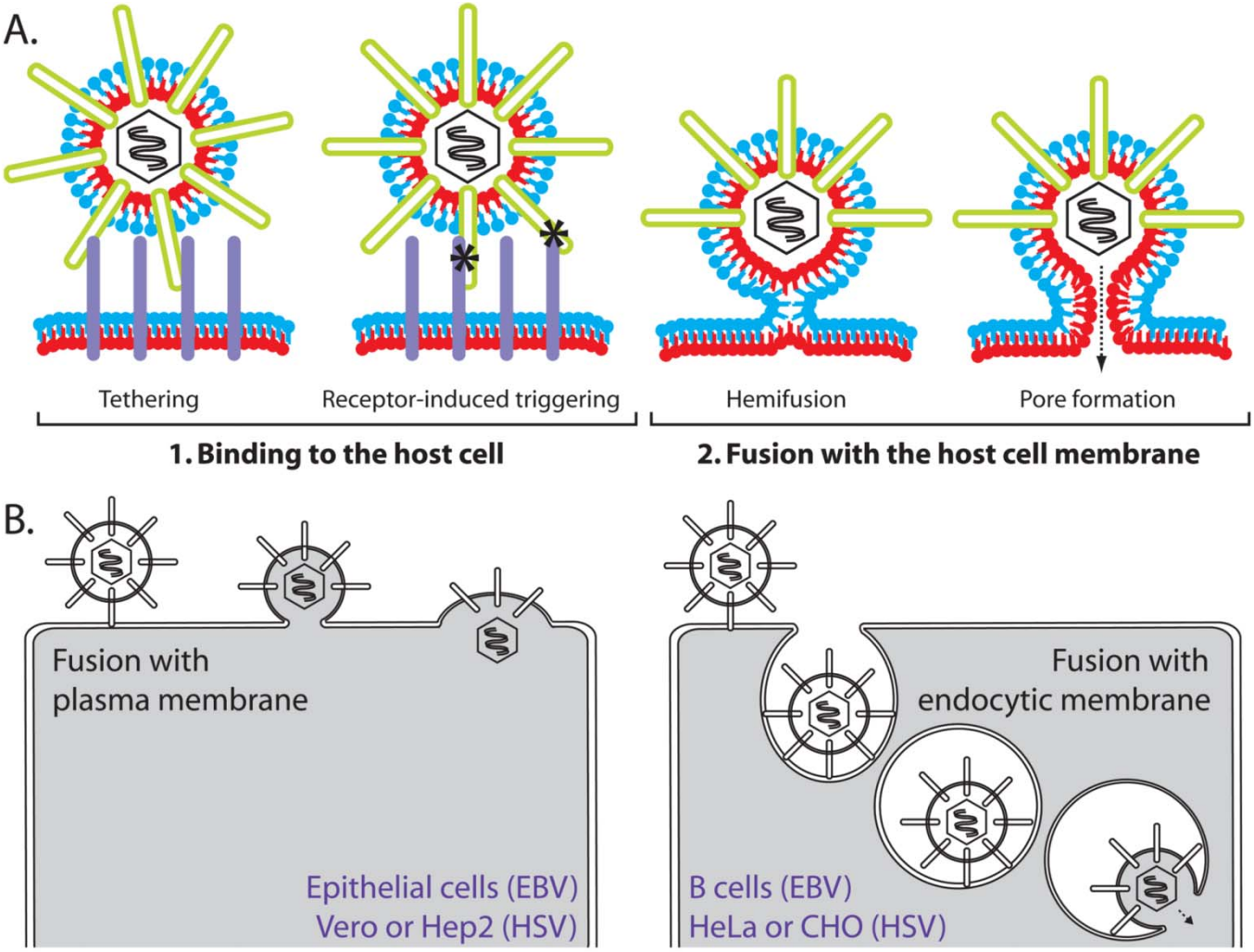


Figure 8. Herpesvirus entry. (A) Steps of viral entry. 1. The virus binds to cellular receptors at the cell surface; eventually, a specific glycoproteins-receptor binding will trigger the subsequent fusion. 2. The fusion of viral and cellular membranes progresses through an hemifusion intermediate followed by a complete fusion with the formation of a fusion pore. (B) Routes of entry. Depending upon cell type, both HSV and EBV can enter cells by fusion at the plasma membrane or with the endocytic membrane after endocytosis.

envelope with the cellular membrane, delivering the capsid in the cytoplasm (figure 8 A1). The viral envelope can either fuse with the plasma membrane or with an endosome after endocytosis (figure 8B).

2.1.1. Attachment to the cell surface

The first step of the viral life cycle is the tethering of the viral particle at the host cell surface (Figure 8A, 1). In alphaherpesviruses, this initial contact is principally mediated by the interaction of gB and gC with heparan sulafte proteoglycan (HSPG), but also mannose-6-phosphate receptor (MPR^{ci}) for VZV. Accordingly, heparin seems to block the attachment of the virions at the cell surface (Zhu et al., 1995a) and MPR^{ci}-deficient cells present a defect in viral entry by cell free VZV but not cell-associated VZV (Chen et al., 2004). In HSV-1 an interaction of gH/gL with integrins also plays a role in this step that serves to concentrate the virus at the cell surface but does not specifically trigger membrane fusion.

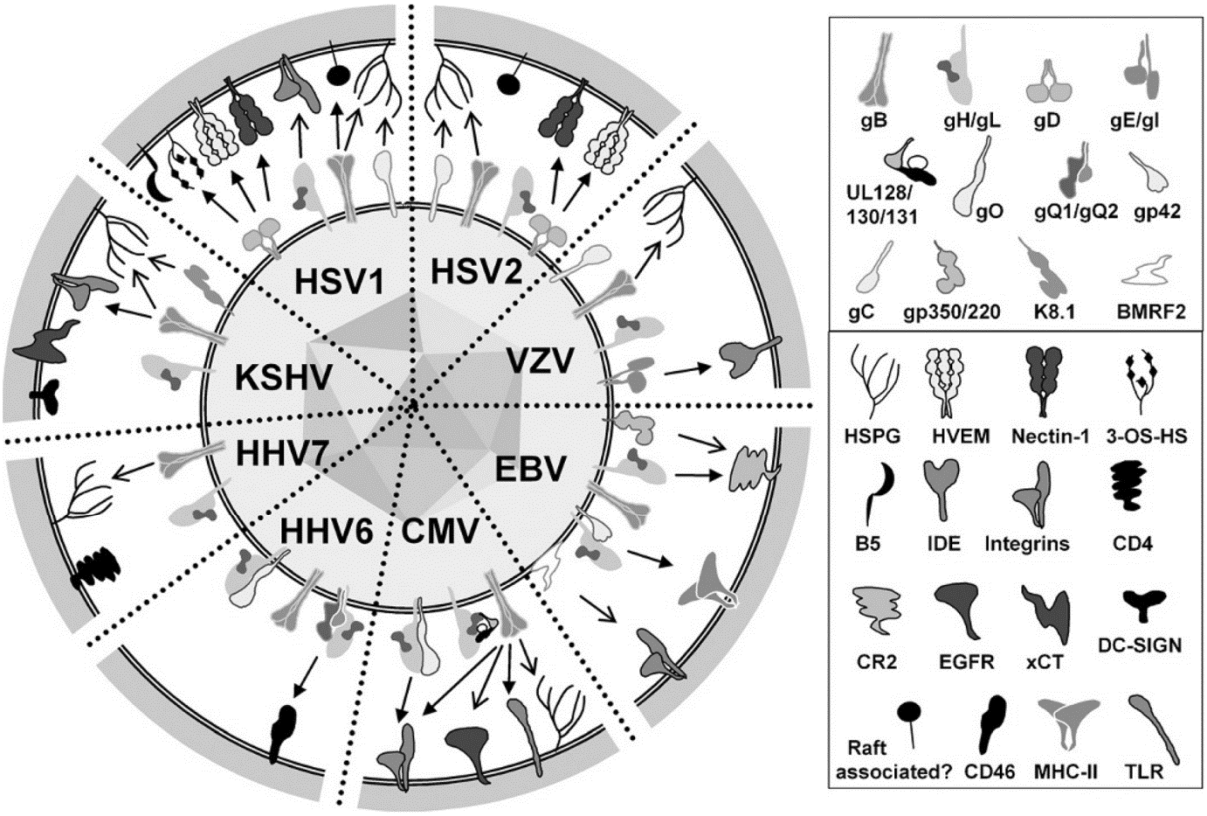


Figure 9. interaction between viral glycoproteins and cellular receptor mediating the entry of the human herpesviruses. Interactions resulting in initial attachment are indicated by open arrowheads and interactions with entry receptors by filled arrowheads. For entry to occur, all the documented interactions do not necessarily take place on the same cell or at the same time (adapted from Heldwein and Krummenacher, 2008).

2.1.2. Interaction with an entry receptor

The second step of viral entry differs between the considered cell type and consist of a strong interaction of the virion with a specific receptor. This interaction defines the host cell range of a particular virus and constitutes a signal to trigger membrane fusion (Figure 9). For both HSV-1 and PRV, gD is the main viral glycoprotein involved in this triggering step. Depending on the considered cell type, HSV-1 gD has been shown to interact with Herpesvirus-Entry-mediator-A (HVEM), a receptor belonging to TNF receptor family (Montgomery et al., 1996), nectin-1 and -2 (Geraghty et al., 1998), and 3-O-sulfated heparan sulfate (Shukla et al., 1999). Even though VZV does not express a gD homolog, a role of these receptors in its entry is not entirely excluded. Nevertheless, for VZV, gE is likely to play the major role in this triggering step. In contrast with HSV-1 and PRV where the absence of gE greatly reduces viral spread but is not lethal, VZV gE is essential for the viral replication. It has been shown to interact with Insulin Degrading Enzyme (IDE) to mediate viral entry. The downregulation of IDE reduces both cell free and cell-associated infectivity and its upregulation has the opposite effect, whilst no impact on HSV-1 entry is observed (Li et al., 2006; Li et al., 2007). Interestingly, gE and gD are thought to derive from duplicated genes (McGeoch, 1990) and share some similarities at the structural level, notably.

2.1.3. Membrane fusion

The fusion of the viral envelope with a cellular membrane that results in the delivery of the capsid within the cytoplasm can occur either at the cell surface or with the endocytic vesicle after an endocytosis event (Figure 8B) (for review, (Connolly et al., 2011; Heldwein and Krummenacher, 2008; Krummenacher et al., 2013)). In the case of the endocytosis pathway, the entry can either require the acidification of the endocytic vesicle or can be low pH independent. The route of entry depends on the virus-cell type combination but can also coexist in one given combination. The core fusion machinery, that is shared among *herpesviridae* is composed of gB, and gH/gL glycoproteins. It remains to be elucidated why and how the same set of glycoproteins (gD, gB, gH/gL) can mediate the three different entry pathways of HSV-1. In the case of VZV, the route of entry in the different cell types remains to be thoroughly characterized, but seems to be dependent on clathrin-mediated endocytosis and on the membrane lipid composition (Hambleton et al., 2007).

HSV-1 and EBV gB have been shown to share structural similarities with known fusion proteins. Fusion proteins have the properties to insert into target membranes and to, through major conformational changes, draw viral and cell membrane together, thereby inducing the formation of fusion pores. Initially thought to be exclusively a fusion protein, recent studies have identify several gB receptors able to trigger the fusion: paired immunoglobulin-like type 2 receptor alpha (PILR-α), non-muscle myosin heavy chain II a and myelin-associated glycoprotein (Arii et al., 2010; Satoh et al., 2008; Suenaga et al., 2010). In VZV, cotranfection experiments showed that the simultaneous expression of gB and gE can drive cell fusion and syncytia formation (Maresova et al., 2001).

The gH/gL complex has originally been described as a fusogen, possibly mediating the hemifusion step (Subramanian and Geraghty, 2007). gH possesses a large exctodomain and one transmembrane anchor while gL lacks any transmembrane domain and is thought to serve as a gH chaperone. gH displays several features typical of type I fusion glycoproteins: five putative fusion motifs and three heptad repeats have been identified in HSV-1 gH (Galdiero et al., 2005; Galdiero et al., 2006; Gianni et al., 2006a; Gianni et al., 2006b). When expressed alone via recombinant vaccinia virus, VZV gH/gL are able to mediate cell-cell fusion (Duus et al., 1995). Nevertheless, the recently described crystal structure of gH/gL ectodomains has revealed no homology with known fusion proteins (Chowdary et al., 2010) and more recent data failed to demonstrate the gH/gL property to induce hemifusion (Jackson and Longnecker, 2010). Accordingly, in a system of exogenous expression exempt of vaccinia virus, the cell-cell fusion was observed only if VZV gB was expressed together with VZV gH/gL (Suenaga et al., 2010). The gH/gL is thus now considered as a fusion regulator through its interaction with gB.

2.1.4. Capsid transport to the nuclear pore and transfer of the DNA to the nucleus

After the fusion of the viral envelope with the cellular membrane, most of the outer tegument is disassembled (Aggarwal et al., 2012; Granzow et al., 2005). A specific set of proteins remains attached to the capsid and is thought to mediate its transport to the nuclear pores via the microtubules (MT) network (Luxton et al., 2005). It is important to note that, to reach the nucleus, the capsid must first move towards the microtubules organizing center (MTOC), then from the MTOC towards the nucleus. It is then not so surprising that both interactions with dynein motors responsible for minus-end directed transport (Dohner et al., 2002) but also with kinesin motors mediating the plus-end transport are crucial for capsid docking to the

nuclear pores (Radtke et al., 2010). Even though it has been shown to bind specifically to dynein light chain RP3 and tctex1 (Douglas et al., 2004), the minor capsid protein VP26 of both PRV and HSV-1 has been demonstrated to be dispensable for the transport of the capsid to the nucleus (Antinone et al., 2006; Dohner et al., 2006). The major role in capsid transport is likely to be played by the tegument. In fact, not only most of the tegument is dispensable for the capsid transport towards the nucleus, but the disassembly of the outer layer is important to expose the inner part, pUL36 and pUL37 in particular, that have been demonstrated to drive the motion of the capsids along MTs (Antinone et al., 2006; Luxton et al., 2006; Wolfstein et al., 2006). Note that, recently, an interaction of pUL37 with dystonin, belonging to another class of Mictrotubule Associated Protein (MAP) has been shown to be important for the transport from the MTOC to the nuclear pore (McElwee et al., 2013).

2.2. Gene expression

Once the capsid is docked at the nuclear outer membrane, a proteolytic cleavage is necessary to release the DNA in the nucleus through the nuclear pore. In HSV-1, this is mediated by pUL36 (Jovasevic et al., 2008). The entry of the DNA within the nucleus is thought to induce the formation of PML-like bodies in very close proximity of the DNA deposition site. Whether this is a prerequisite for the subsequent viral gene expression or an intrinsic cellular defence is still a matter of debate (Everett, 2013). In HSV-1, the expression of ICP0 will subsequently degrades PML protein leading to PML bodies disruption and the progression through the next phase of the lytic cycle.

A characteristic of all herpesviruses is the presence of three distinct and successive phases of viral gene expression. Immediate Early (IE), also called α genes are the first transcribed and allow the expression of Early (E) or β genes. Finally, Late genes (L), or γ genes whose expression is dependent on the viral DNA replication are expressed.

2.2.1. Immediate Early genes

The genes belonging to this class are transcribed by cellular RNA polymerase II, without *de novo* viral protein production. They represent major transcription regulators that are necessary to stimulate the expression of the viral repertoire. They also have been shown to impact positively or negatively the expression level of some cellular genes. Three VZV genes have been formally classified as IE: ORF4 (IE4),

ORF62 (IE62) and ORF63 (IE63). All three are phosphoproteins and belong to the tegument (Kinchington et al., 1995; Kinchington et al., 1992).

IE62, an essential protein, is the major transactivator of the virus, homologous to HSV-1 ICP4 and is crucial to initiate viral gene transcription (Sato et al., 2003a). In HSV-1, the initiation of the viral transcription cascade strongly relies on UL48 gene product, the protein VP16 (VZV ORF10 homolog). VP16 is part of the viral tegument and is brought to the nuclear compartment rapidly after cell entry, independently of the capsid. It forms a complex with cellular factors (principally HCF-1 and Oct-1) which binds to a consensus sequence present in the promoter of all IE genes. The formation of this complex will initiate the transcription of the IE genes (recently reviewed in (Kristie et al., 2010)). In VZV, only IE62 promoter has been shown to possess a similar consensus sequence that, indeed is able to bind a complex containing VZV ORF10, HCF-1 and Oct-1. The presence of this complex is able to enhance the transcription of ORF62 (Moriuchi et al., 1995; Narayanan et al., 2005). Nevertheless, contrarily to HSV VP16, VZV ORF10 is not essential for VZV replication and obviously, the expression of IE63 and IE4 must rely on another mechanism (Che et al., 2006; Kost et al., 1995; Michael et al., 1998). IE62 is known to activate its own promoter and it has been shown that HCF-1 is also implicated in IE62 gene transactivation properties (Disney et al., 1990; Narayanan et al., 2005; Perera et al., 1992b). Both proteins have been shown to interact, maybe through a common binding partner, the transcription factor Sp-1 (Kristie et al., 2010; Peng et al., 2003). Since all three IE promoter contain Sp-1 binding site, the current model therefore suggests that the expression of all IE genes relies on a complex containing HCF-1, Sp-1 and IE62 (Ruyechan et al., 2003). The presence of large amount of IE62 in the viral tegument is compatible with its role in the initiation of the transcription program, similarly to VP16 in HSV-1 (Kinchington et al., 1992). Of note, IE62 has also been shown to bind to the Mediator, TBP/TFIID and RNA pol II and more than half of the VZV genes contain Sp-1 binding sites in their promoter (Narayanan et al., 2007; Peng et al., 2003; Ruyechan et al., 2003). The mechanism by which IE62/Sp-1 recruits the Mediator co-activator, the basal apparatus of transcription as well as HCF-1, itself able to recruit chromatin remodelling factors (MLL histone H3K4 methyltransferase) is likely to play the major role in the expression of the entire VZV genome (for review, (Ruyechan, 2010)).

IE4, also essential for the viral replication (Sato et al., 2003b), was originally considered as a transcription factor (de Maisieres et al., 1998; Defechereux et al., 1993; Perera et al., 1994). We have shown recently that it actually acts principally at a

post-transcriptional level, by binding to RNA and SR proteins and enhancing the export of mRNAs (Defechereux et al., 1997; Ote et al., 2009).

The situation of IE63 is more complicated. Its HSV-1 homolog (ICP22) has been implicated not only in the transactivation of late viral genes, mainly through its association with ICP4 (IE62 homolog), but principally in the repression on IE genes and on many cellular genes (Bastian and Rice, 2009; Leopardi et al., 1997; Long et al., 1999; Poffenberger et al., 1993; Rice et al., 1995; Sears et al., 1985). Conflicting reports on VZV IE63 are present in the literature concerning both its dispensability for the viral replication (Baiker et al., 2004; Cohen et al., 2004; Sommer et al., 2001) and its regulatory properties (Jackers et al., 1992; Kost et al., 1995; Lynch et al., 2002). Our laboratory has shown that VZV IE63 is able to repress all class of viral genes as well as cellular genes by acting on the basal transcription machinery, that it is mainly phosphorylated by the cellular CKII and that its phosphorylation status impacts its cellular localization as well as its repressive properties (Bontems et al., 2002; Di Valentin et al., 2005; Habran et al., 2005).

A last protein might also belong to the IE class, the HSV ICP0 homolog, encoded by ORF61. Even though its strict IE status has not been confirmed, a recent report showed that it is express as early as IE62, namely 1 hour post infection. It is dispensable for viral replication but displays some gene regulatory properties (Inchauspe et al., 1989; Moriuchi et al., 1993; Nagpal and Ostrove, 1991; Perera et al., 1992a; Reichelt et al., 2009). It possesses a RING finger domain with a E3-Ubiquitin ligase property, like ICP0. Its putative role in the regulation of PML bodies during the lytic cycle will be discussed in the following paragraphs.

2.2.2. Early genes

The expression of these genes relies on the presence of newly synthesized IE proteins. Most of the E genes code for enzymes: ORF8 is a dUTPase (Ross et al., 1997), ORF13 a thymidylate synthetase (Cohen and Seidel, 1993; Spector et al., 1989)), ORF18 and ORF19 ribonucleotide reductase (Spector et al., 1989), ORF36 a thymidine kinase (Sawyer et al., 1986) and ORF59 an uracil DNA glycosylase (Reddy et al., 1998b). The two viral kinases encoded by ORF47 and ORF66 and the proteins implicated in the replication of the genomic DNA also belong to this class. Based on homology with HSV-1, seven enzymes are required for the genome replication (Boehmer and Lehman, 1997). VZV DNA polymerase is composed of the catalytic (ORF28) and the processivity (ORF16) subunit whereas the primase/helicase complex contains three subunits (ORF6, the primase, ORF55, the helicase and ORF 52, an

accessory protein specific to alphaherpesviruses) (Mar et al., 1978; Miller and Rapp, 1977). ORF29 is a ssDNA binding protein and, from immunofluorescence study has been shown to also localize as its HSV-1 homolog (ICP8) in punctuated intranuclear regions of infected cells (Cohrs et al., 2002; Kinchington et al., 1988). These domains initially appearing as several small regions are thought to represent areas where viral DNA replication takes place. As the infection progresses, the small spots enlarge and fuse together giving rise to what has been called the "replication compartment" (RC). ORF29 could also modulate the expression of the late gene (Boucaud et al., 1998). The last protein essential for the replication is encoded by ORF51 and correspond to the origin-binding protein (see table 3).

ORF9, the most expressed VZV gene during the lytic cycle is thought to be expressed at the early phase. In contrast to most of its homologs, it is essential for the viral replication. ORF9 is one on the major component of the tegument and interacts with IE62, gE and ORF11 (Che et al., 2008; Cilloniz et al., 2007; Tischer et al., 2007). We recently demonstrated its role in the viral egress (Riva et al., 2013).

2.2.2.1 Viral kinases

ORF66 is a ser/thr kinase specific to alphaherpervirus and presents homology with cellular CDKI (Moffat et al., 2004b). It is essential for viral growth in T cells while dispensable in skin cells or fibroblasts (Ku et al., 2005; Moffat et al., 1998). ORF66 is expressed during latency but is not essential for its establishment (Cohrs et al., 2003a; Sato et al., 2003c). It phosphorylates IE62, which impact the cellular localization and the virion incorporation of this latter (Eisfeld et al., 2006; Erazo et al., 2011; Kinchington et al., 2001; Ng et al., 1994; Walters et al., 2009). As it has been described for its HSV-1 and PRV homolog (US3), ORF66 also has cellular targets: HDAC1 and HDAC2 as well as matrin 3, a nuclear matrix protein (Erazo et al., 2011; Walters et al., 2009).

ORF47 is a CKII-like ser/thr kinase (Kenyon et al., 2003). It belongs to the Conserved Herpesvirus-encoded Protein Kinase (CHPK) family. CHPKs share some features and biological roles. They are part of the tegument and phosphorylate some tegument proteins allowing the formation, maintenance and/or disassembly of the virion (for review (Gershburg and Pagano, 2008)). They were reported to interact with the DNA polymerase processivity subunit, thereby playing a role in the DNA replication. They also were shown to play a role in the hyperphosphorylation of the cellular translation factor EF-1δ which facilitate the protein synthesis. CHPK have been implicated in the innate immune evasion by interfering principally with the IRF3

pathway (Hwang et al., 2009; Shibaki et al., 2001; Vandevenne et al., 2011; Wang et al., 2009). VZV ORF47 is dispensable for *in vitro* viral growth but is determinant for the T cells tropism and for the infection of immature dendritic cells (Heineman and Cohen, 1995; Hu and Cohen, 2005; Moffat et al., 1998). It has been shown to phosphorylate IE62, IE63, gE, gI and ORF32 (Kenyon et al., 2002; Kenyon et al., 2001; Ng et al., 1994; Reddy et al., 1998a). We also showed recently that it phosphorylates ORF9 and strongly interacts with it and that this phosphorylation is important for the viral egress (Riva et al., 2013).

2.2.3. Late genes

The genes belonging to this class are only expressed after the initiation of the genome replication. Most late genes encode structural proteins: proteins forming the capsid as well as the scaffold proteins and maturing protease, all envelope proteins synthesized in the RER, but also some tegument proteins like ORF10. VZV expresses nine distinct glycoproteins, 5 of which presenting conserved homologs among all herpesvirus subfamilies (table 4).

Gene	glycoprotein	Mw	Essential?	function
ORF5	gK	40 kDa	yes	Virion morphogenesis and virus entry
ORF9A	gN	10 kDa		unknown
ORF14	gC	95-105 kDa	no	Adsorption and virus entry
ORF31	gB	125-140 kDa	yes	Adsorption and virus entry
ORF37	gH	105-118 kDa	yes	Membrane fusion
ORF50	gM	42-48 kDa		Cell-to cell spread
ORF60	gL	18 kDa	yes	Membrane fusion
ORF67	gI	50-55 kDa	no	Envelopment
ORF68	gE	85-110 kDa	yes	Virus entry and cell-to-cell spread

Table 4. VZV glycoproteins

2.3. DNA replication

VZV genome as well as HSV-1 genome circularizes prior the replication onset. In the case of HSV-1, the cellular DNA ligase IV has been shown to mediate this step, even though a parallel mechanism involving a recombination between the short direct repeats at the ends of HSV-1 genome is not excluded (Muylaert and Elias, 2007; Strang and Stow, 2005).

The current model of HSV-1 DNA replication involves an initial bidirectional theta-type of replication followed by a switch to a rolling-circle mechanism, generating

head-to-tail concatemers. The existence of a first theta-type replication has not yet been strictly proved nor has the mechanism that would trigger the switch, but the requirement of the circularization and the existence of specific genomic sequences described as origins of replication as well as a viral protein displaying all the properties of an initiator protein are strong arguments in favour of this model. The generation of DNA concatemers has been shown to be strictly required for the subsequent DNA packaging into the preformed capsid (see paragraph 2.4) (Boehmer and Lehman, 1997; Lehman and Boehmer, 1999; Muylaert et al., 2011; Weller and Coen, 2012). The proteins implicated in VZV DNA replication and their HSV-1 counterparts are listed in table 5.

The first step of the DNA replication is the recognition of replication origins (Ori) by the origin-binding protein (OBP), UL9 in HSV-1, ORF51 in VZV. Three Ori sequences are present in the HSV-1 genome, two within the repeats flanking the U_S (OriS) which are conserved in VZV and one in the U_L region (OriL) (Ruyechan, 2010). In HSV-1, the deletion of the two OriS or of the OriL are both compatible with viral growth, showing that the presence of a single functional Ori is sufficient to initiate the DNA replication (Igarashi et al., 1993; Polvino-Bodnar et al., 1987). OriS of both HSV-1 and VZV are organized around a conserved AT-Rich palindrome essential for the genome replication (figure 10). In HSV-1, three OBP binding sites (designated Boxes I, II and III) are present close to the AT-rich region. Mutational analyses have shown that the minimal functional origin contains the AT-rich region and Boxes I and II (Nguyen-Huynh and Schaffer, 1998; Weir and Stow, 1990). In VZV, there are also three complete OBP binding sites, but they are all located upstream the AT-rich region and orientated in the same direction. Box A is essential for the replication, box B is dispensable and the presence of box C increases the replication efficiency (Stow and Davison, 1986; Stow et al., 1990).

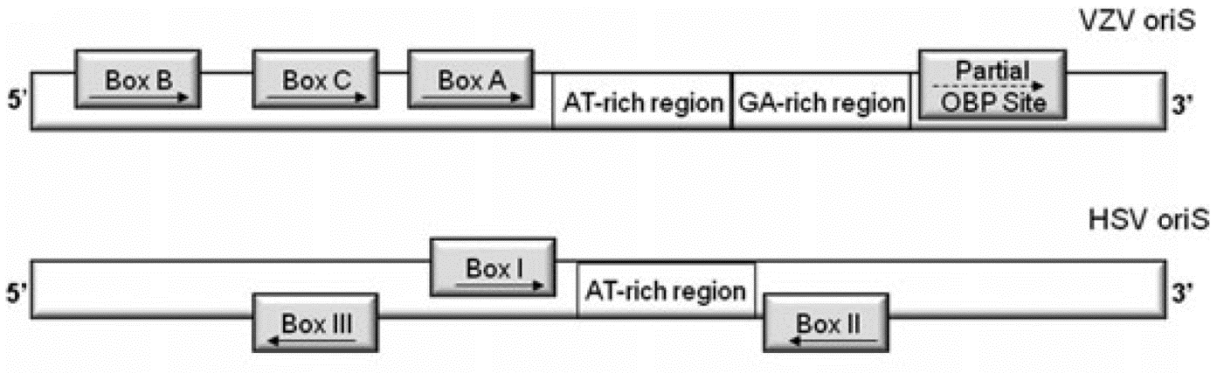


Figure 10. Architecture of VZV and HSV OriS. Orientaton and position of OBP binding sites are represented by grey boxes (adapted from Ruyechan, 2010).

The binding of pUL9 dimers on the Ori sequence will result in a bending of the DNA at the AT-rich region and the association of OBP with the ssDNA binding protein ICP8 (VZV ORF29). The arrangement of OBP binding sites symmetrically on both sides of the AT-region will generate in the case of HSV-1 a characteristic hairpin structure (Muylaert et al., 2011). For VZV, the precise mechanism is so far unknown but possibly involves the binding of a cellular factor or a viral protein downstream the AT-rich region (Ruyechan, 2010; Stow et al., 1990).

HSV	VZV	Abbreviation	Function in genome replication
UL9	ORF51	**OBP**	Origin binding protein, implicated in replication initiation
UL30	ORF28	**POL**	DNA polymerase, catalytic subunit
UL42	ORF16	**PPS**	DNA polymerase, processivity subunit
UL5	ORF55	**H**	DNA helicase
UL52	ORF6	**P**	DNA primase
UL8	ORF52		Accessory factor, binds to ORF55 and ORF6 to form the primase/helicase complex
ICP8 (UL29)	ORF29	**SSB**	Single-strand binding protein

Table 5. DNA replication enzymes.

Together, pUL9 and ICP8 will trigger the initial unwinding of the dsDNA and the recruitment of the trimeric helicase-primase complex, composed of pUL5, pUL8 and pUL52 (VZV ORF55, 52 and 6). The helicase UL5 is responsible for the subsequent DNA unwinding while the primase activity is carried by UL52 which synthesizes the RNA primers necessary to generate the lagging strand. UL8 does not possess any enzymatic activity but seems to be important for the formation and the maintenance of the complete replisome (Cavanaugh et al., 2009; Tenney et al., 1994; Tenney et al., 1995). Its presence has been shown to be crucial for the binding of pUL5/pUL52 with ICP8 and it also interacts with the catalytic subunit of the DNA polymerase (Falkenberg et al., 1997; Marsden et al., 1997; McLean et al., 1994). Recent data suggest that two triplexes are required for the efficient subsequent DNA synthesis (Chen et al., 2011b) and a model has been proposed in which the leading strand and the lagging strand are synthesized simultaneously (figure 11). In this model, each primase/helicase would recruit one DNA polymerase through an interaction with the catalytic subunit, encoded by pUL30 (VZV ORF28).

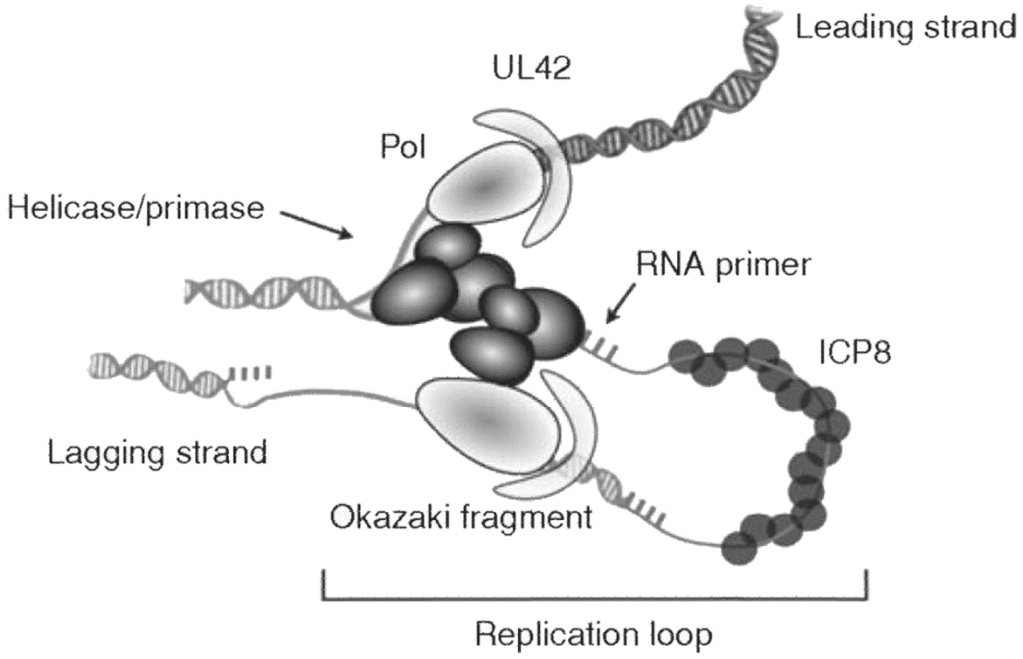


Figure 11. Proposed model of herpesvirus replication fork. In this recent model, each replication fork would contain two trimeric helicase complexes, able to recruit two dimeric polymerases. A replication loop is formed in the lagging strand, allowing the simulataneous synthesis of the leading and the lagging strands (adapted from Weller and Coen, 2012).

The catalytic subunit presents the classical domains of described polymerase thumb, palm and fingers structures but also an extra N-terminal domain containing a motif resembling the RNA-binding motif found in ribonucleoproteins (Liu et al., 2006). pUL30 is a proof reading enzyme possessing a 3'-5' exonuclease activity (Song et al., 2004) and also displays an RNAseH function (Crute et al., 1989; Marcy et al., 1990), which could play a role in the degradation of the RNA primers on the lagging strand. The processivity subunit, pUL42 (VZV ORF16) possesses both an interaction domain with pUL30 and with the DNA. It acts as a clamp able to move along the DNA strand (Gottlieb et al., 1990; Hernandez and Lehman, 1990; Randell and Coen, 2001), which, rather than sliding along the DNA, somehow "hops" by dissociation reassociation events (Komazin-Meredith et al., 2008).

As DNA replication is obviously an obligatory step for viral propagation, almost all the first generation of anti-viral drugs were targeting this step of the viral cycle. Nucleoside analogs (acyclovir, valaciclovir,,..) that most of the time require a phophorylation by the viral thymidine kinase to become the active drugs were developed 30 years ago. They are still currently used. Nevertheless, over the years, resistant strains, that principally display mutation in the TK gene, have emerged. This has led to the development of nucleoside analogs that do not require the phosphorylation by the viral TK. The novel drugs that are under clinical trials target

the ribonucleotide reductase, the Helicase/primase complex, but also the DNA encapsidation step (for review,(Field and Vere Hodge, 2013; Piret and Boivin, 2014)) .

2.4. Capsid assembly and DNA encapsidation

Capsid assembly is a nuclear process, which implies that capsid proteins must first reach this cellular compartment. Because many of them do not possess a nuclear localization signal (NLS), it has been demonstrated that several cytoplasmic protein-protein interactions are required in order to import all the capsid proteins within the nucleus (figure 12). In HSV-1, the scaffold protein, encoded by UL26.5 is thought to mediate the transport of the major capsid protein (MCP) (VP5, pUL9), whereas VP29C (pUL38) is responsible for the nuclear import of VP23 (pUL18) but can also relocate VP5 (Nicholson et al., 1994; Rixon et al., 1996). The small capsid protein VP26 (pUL35) only reaches the nuclear compartment through its interaction with VP5. In VZV, only the scaffold protein/maturation protease (ORF33/33.5) and the small capsid protein (ORF23) display a functional NLS. Both have been shown to relocate the major capsid protein (ORF40) to the nucleus but neither one nor the other is mediating the transport of ORF20 (pUL38) or ORF41 (pUL18) (Chaudhuri et al., 2008). It is then likely that complexes of higher order must from in the cytoplasm prior the nuclear import.

HSV	HSV	HSV	VZV	Abbre-viation	Function in genome replication
VP5	UL19	ICP5	ORF40	**MCP**	Major capsid protein, forms the pentameric and hexameric capsomers
VP19C	UL38	ICP32	ORF20		Form an heterotrimer $VP23_2$/VP19C called the triplex, linking the capsomers
VP23	UL18		ORF41		
VP11-12	UL6		ORF54	**Portal**	Localize as a dodecamer at one vertex forming a pore through which the DNA is inserted in the capsid
VP26	UL35		ORF23	**SCP**	Small capsid protein
	UL25		ORF34		Bind to the exterior of the capsid, stabilize the DNA within the capsid
VP24+pre-VP21	UL26		ORF33		Scaffold protein
Pre-VP22a	UL26.5	ICP35	ORF33.5		Assembly scaffold protein

Table 6. VZV capsid and assembly proteins and their HSV-1 counterparts.

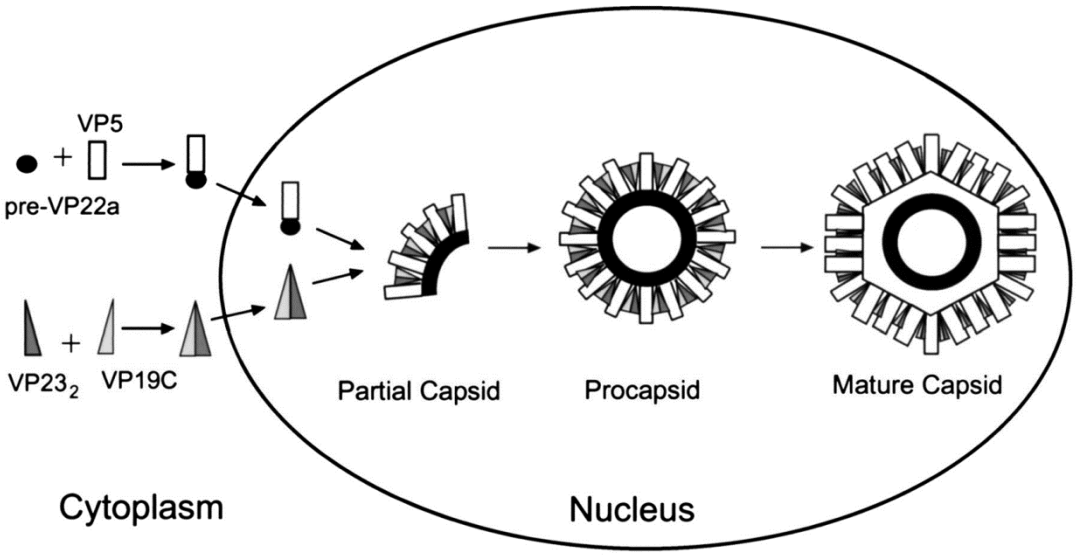


Figure 12: Sequence of events of capsid assembly. (adapted from Spencer et al., 1998) (see description in the text)

Once in the nucleus, the MCP-scaffold complex will assemble into 5-fold and 6-fold caspomers (Kim et al., 2011a; Spencer et al., 1998). The protein scaffold is composed of two proteins encoded by in-frame overlapping genes, UL26 an UL26.5 (VZV ORF33 and 33.5). As described in paragraph 1.5, both proteins share their C-terminal parts but pUL26 possesses an extra N-terminal domain containing a protease motif and a linker (Thomsen et al., 1995). The amount of the pUL26 is ten times lower than the amount of pUL26.5 and the production of capsids is possible but very strongly diminished if the expression of pUL26.5 is impaired (by mutating the initiating methionine) (Matusick-Kumar et al., 1994).The dimerization motif is present in the common part leading to homo- and hetero-dimerization. The association of capsomers by the triplexes and the coalescence of the scaffold protein dimers will first generate partial capsid that will grow and combine to form the spherical, closed, procapsid (figure 12)(Homa and Brown, 1997; Newcomb et al., 2005).

As described at paragraph 1.6, one of the twelve vertices, instead of being occupied by a VP5 penton is occupied by a structure crucial for the maturation steps of the procapsid into a functional, DNA containing capsid. This ring-shaped structure is a

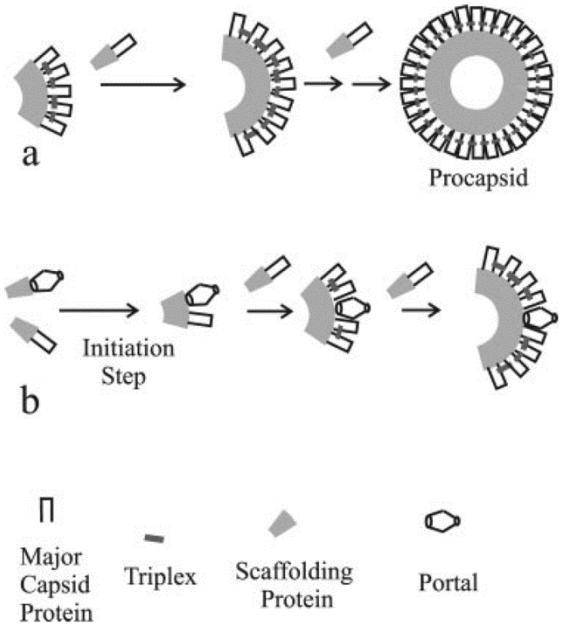


Figure 13. Procapsid formation model in the absence (a) or the presence (b) of the portal protein. (adapted from Newcomb et al., 2005)

multimer containing twelve molecules of the portal protein, pUL6 (VZV ORF54)(Rochat et al., 2011). Even though its presence is not strictly required for the assembly process, to be incorporated in the final capsid, pUL6 has to be present from the initial step. It is then postulated that, in vivo, the nucleation process leading to procapsid formation might, in fact, start from the portal vertex (Huffman et al., 2008; Newcomb et al., 2005) (Figure 13). This would explain how only a single vertex is systematically occupied by the portal.

The procapsid is thought to be the precursor of all forms of capsids found in the nucleus of an infected cell. Once assembled, it will go through several maturation steps required to generate the mature DNA-containing capsid (Church and Wilson, 1997) (figure 14). Even though they are generally seen as simultaneous, the first step corresponds to the activation of the protease motif of pUL26, called VP24, that will free itself from the scaffold via an autocatalytic cleavage (Thomsen et al., 1995). Once activated, VP24 cleaves the scaffold proteins in the common C-terminal region removing the 25 last amino acids and generating the mature VP22a from pUL26.5 (also called "pre-VP22a") and mature VP21 from pUL26, a slightly longer protein containing the remaining linker at the N-terminal side (for review, (Cardone et al., 2012)). The scaffold will then be degraded and expelled together with the DNA insertion. In the same time, the capsid will rigidify itself and become more angular (Newcomb et al., 1996; Roos et al., 2009; Trus et al., 1996). It is unknown how the protease remains inactive until the procapsid is fully formed but this is currently thought that is might simply be a kinetics issue. It is possible that, once initiated, the procapsid assembly is very rapid and that the protease is only slowly activated. VP26, the minor capsid protein encoded by UL25 (VZV ORF23) that decorates the exterior part of the capsid is thought to be associated during the maturation process, as it is not present on the procapsid (Newcomb et al., 2000; Zhou et al., 1995). A-capsids are empty capsids thought to arise when the insertion of the DNA is initiated but not completed leading to the loss of the packaged DNA (Perdue et al., 1976). The case of B-capsids is more controversial, they contain a partially processed scaffold but no DNA. Some authors consider them as a by-product, incidentally produced (Baines, 2011); others have demonstrated that a certain amount of them are able to subsequently mature into C-capsids and see them as an intermediate stage, between procapsid and C-capsids (Perdue et al., 1976; Thomsen et al., 1995) (red arrow at figure 14).

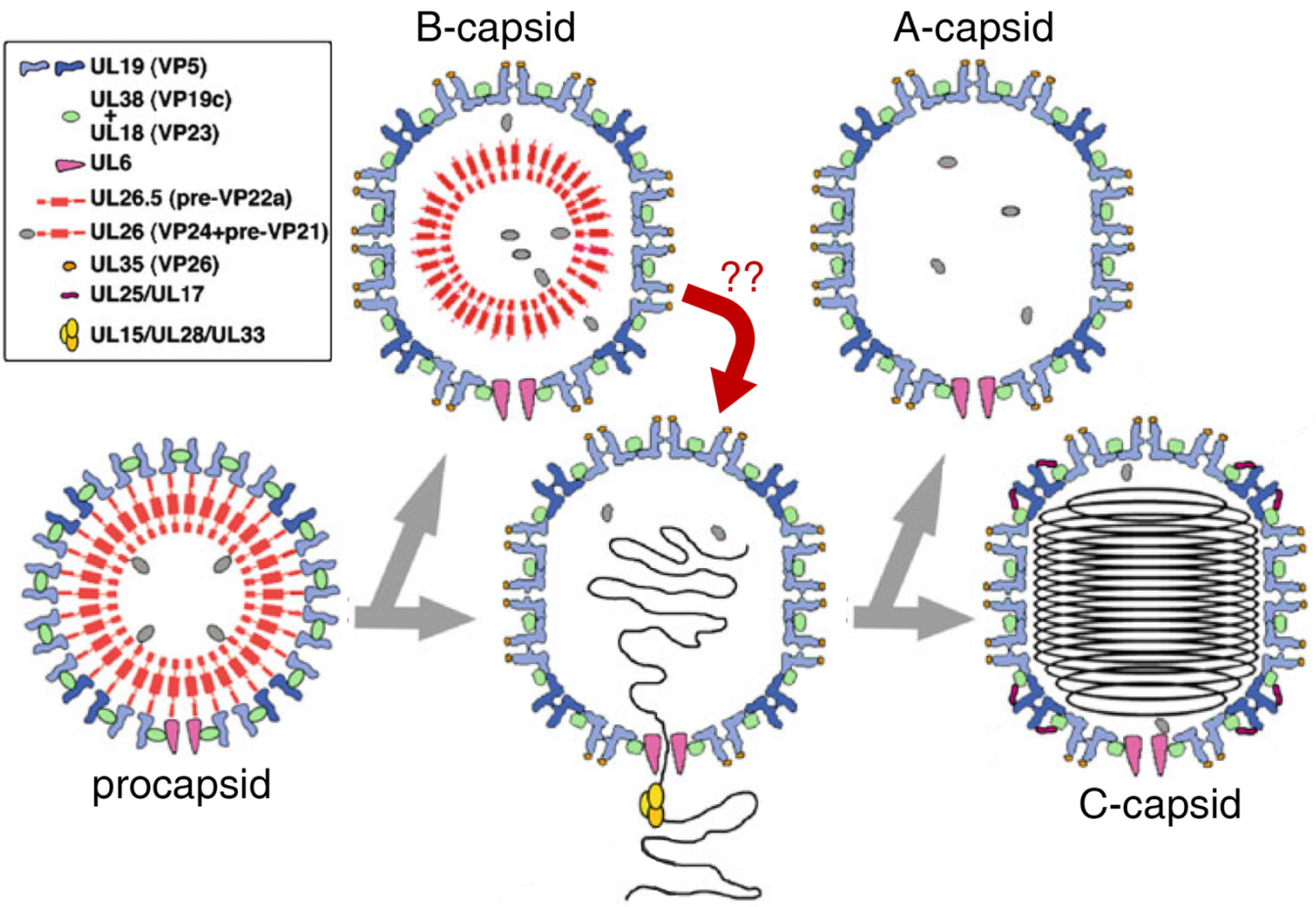


Figure 14. Maturation of procapsids leading to A- , B- an C-capsids formation. See detailed description in the text (adapted from Cardone et al., 2012).

Several proteins have been implicated in the DNA encapsidation process: the portal pUL6 (VZV ORF54), pUL15 (VZV ORF42/45), pUL17 (VZV ORF43), pUL25 (VZV ORF34), pUL28 (VZV ORF30), pUL32 (VZV ORF26), pUL33 (VZV ORF25). Is has been shown that actually, most, if not all, of them already associate with procapsids (Sheaffer et al., 2001). The terminase complex, responsible for the unit size cleavage of the genomic DNA is trimeric and composed of pUL15, pUL28 and pUL33 (Addison et al., 1990; Heming et al., 2014; Yang and Baines, 2006). It has been proposed that the three proteins interact in the cytoplasm and are transported in the nucleus as a complex (Higgs et al., 2008). All three terminase subunits have been shown to interact with capsid and/or the portal (Salmon and Baines, 1998; Yang et al., 2007; Yang et al., 2009). Of note, pUL15 and pUL28 have been shown to be less abundant on C-capsid than on procapsid or B-capsids (Yu and Weller, 1998).

The role of pUL32 in the encapsidation process is still obscure. In its absence DNA is replicated but is neither cleaved nor packaged and capsids accumulate at the periphery of the replication compartment (Fuchs et al., 2009; Lamberti and Weller, 1998). A somewhat similar phenomenon can be observed with a pUL17-defective

virus which presents arrays of immature capsids at the periphery of the replication compartment and display a mislocalization of pUL6 (Taus et al., 1998). Moreover, pUL17 has been shown to bind to the capsid as an heterodimer with pUL25 (Salmon et al., 1998), another protein important for the DNA encapsidation process (McNab et al., 1998; Stow, 2001). The heterodimer actually binds to capsid triplexes through an interaction with pUL18 and forms a structure called "capsid vertex specific complex" (CVSC) that is especially enriched in C-capsid, presumably favouring their egress (Toropova et al., 2011; Trus et al., 2007). In fact, in PRV, pUL25 has been shown to play an essential role in capsid nuclear egress while its presence only strongly favour DNA cleavage and encapsidation (Klupp et al., 2006). It binds the capsid through its interaction with pUL17, and both only associate with C-capsids (Klupp et al., 2005b).

2.5. Nuclear Egress

To produce the infectious virion, the newly produced nucleocapsid has now to acquire a tegument and an envelope. It is now generally accepted that the final envelope acquisition takes place in the cytoplasm via the budding of naked capsid through a cellular membrane either derived from endocytic vesicles or the Trans-Golgi Network (TGN). To reach the cytoplasm, the nuclocapsid has to cross the nuclear envelope. This is achieved through a envelopment-deenvelopment process (figures 15 and 16). The capsid bud through the inner nuclear membrane (INM), acquiring a primary envelope, which is lost by its fusion with the outer nuclear membrane, releasing the naked capsid in the cytoplasm. The primary envelopment necessitates the presence of at least two viral proteins conserved among herpesviruses, pUL31 and pUL34 (VZV ORF27 and ORF24 respectively), that have been called the "nuclear egress complex"(NEC). It is not entirely understood how the nucleocapsid is able to reach the inner nuclear membrane, but possibly via an active transport and the formation of a virally-induced nucleoskeleton (Feierbach et al., 2006; Forest et al., 2005).

pUL31 is a nuclear phosphoprotein maintained in close apposition to the INM through its interaction with pUL34, a type II integral membrane protein localized in the INM (Chang and Roizman, 1993; Reynolds et al., 2002; Shiba et al., 2000). The NEC is important for both the budding step but also for the local disruption of the nuclear lamina necessary for the capsid to get access to the INM. In fact, NEC is able to recruit several kinases that will phosphorylate lamin A/C and lamin B and promote the lamina disassembly. Among those kinases, some are cellular, like PKCδ and PKCα (Leach and Roller, 2010; Muranyi et al., 2002; Park and Baines, 2006) and some are

viral. US3 (VZV ORF66), a kinase conserved among alphaherpesviruses has been shown to phosphorylate lamin A and C whereas the CHPK (encoded by UL13 in HSV, ORF47 in VZV) is crucial for nuclear egress in beta- and gamma-herpesviruses that do not encode a US3 homolog (Gershburg et al., 2007; Krosky et al., 2003; Mou et al., 2007) and has been shown to phorphorylate lamins leading to lamina disruption (Cano-Monreal et al., 2009; Lee et al., 2008a; Marschall et al., 2005).

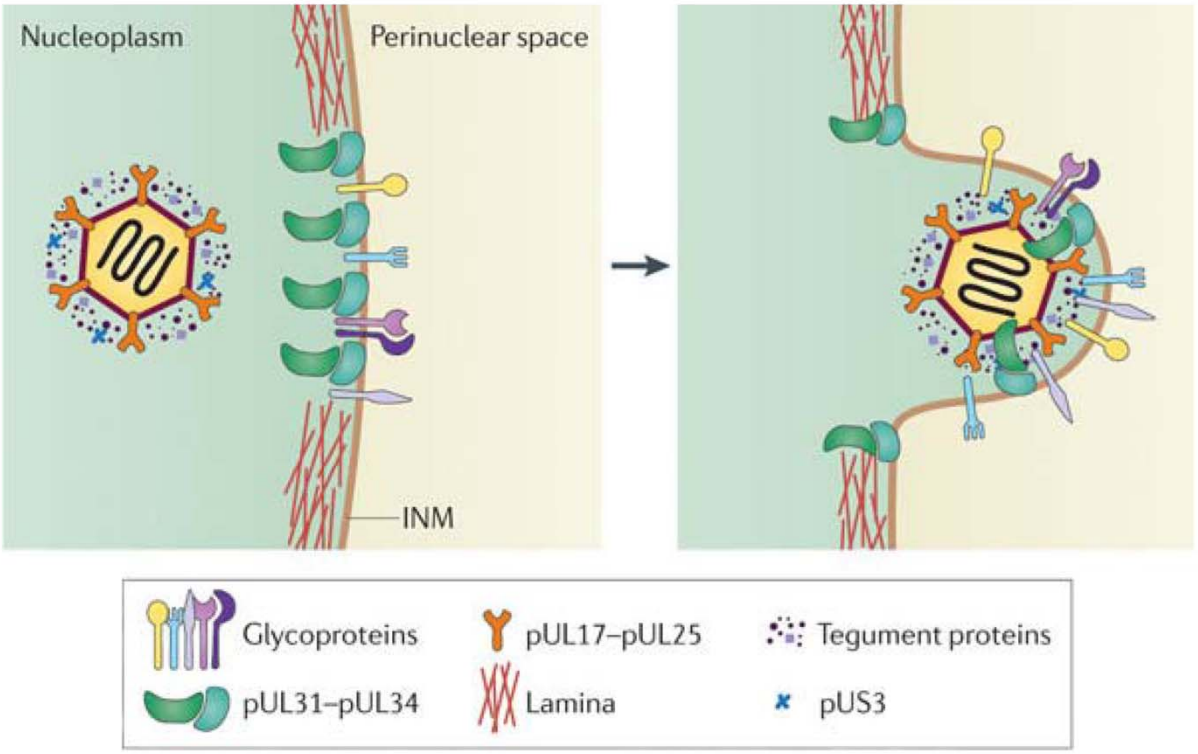


Figure 15. Primary envelopment. The herpes simplex virus (HSV) nuclear envelopment complex (NEC), including pUL31 and pUL34, induces a thinning of the nuclear lamina. The NEC additionally recruits other viral proteins that are incorporated into the enveloping particle. Viral proteins pUL17 and pUL25 form a complex on the surface of the capsid that is important in selecting mature, DNA-filled capsids for primaryenvelopment (from johnson and Baines, 2011).

Despite tremendous efforts to find the protein linking capsid to the NEC, a lot a controversy remains. Because C-capsids are preferentially selected for further egress, a component specific of (or enriched in) them would be a good candidate. Of course, the interaction between the capsids and the NEC has to be transient which may explain why it has not yet been definitely assessed. Large scale yeast-two hybrid experiments have identified pUL33 as a potential candidate, because it binds several capsid and capsid bond proteins (pUL18, VP26, pUL15, pUL28, pUL32) and the NEC (pUL31). But this has not yet been validated *in vivo* (Fossum et al., 2009; Vizoso Pinto et al., 2011). Besides, in the absence of pUL34, PRV pUL31 has recently been shown to binds capsids independently of pUL6, pUL25 or pUL33, whereas another study in

HSV-1 has shown that pUL31 binds to the pUL17/pUL25 (the CVSC) independently of pUL34 and pUS3 (Leelawong et al., 2011; Yang and Baines, 2011).

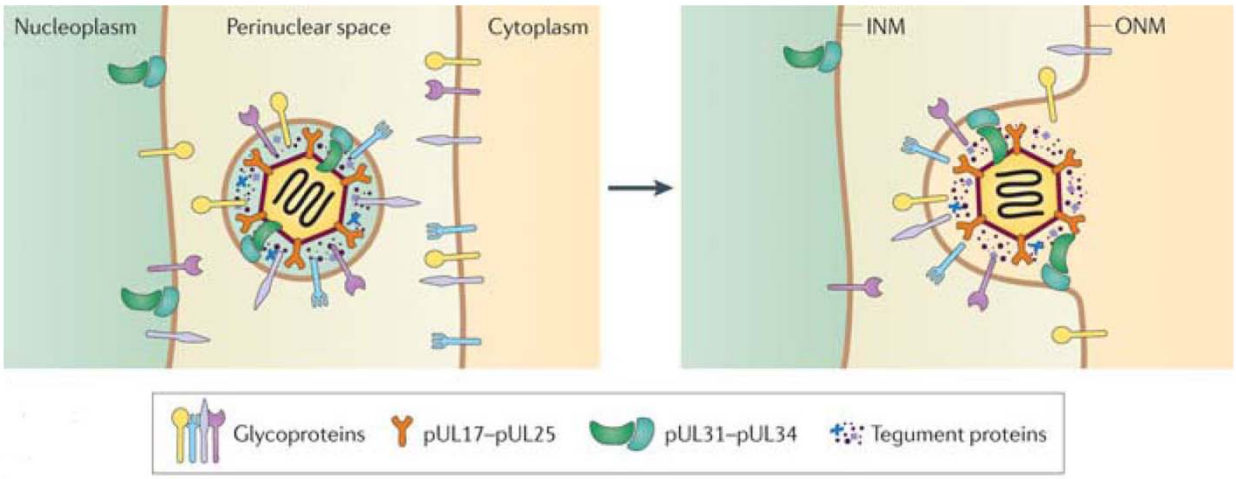


Figure 16. De-envelopment model. After primary envelopment, herpes simplex virus (HSV) particles, with the primary envelope containing some viral glycoproteins and pUL31 and pUL34, reside in the perinuclear space. HSV glycoproteins gB and gH–gL may mediate fusion of the viral envelope with the outer nuclear membrane (ONM), delivering naked capsids into the cytoplasm and leaving pUL31 and pUL34 behind in the ONM (from Johnson and Baines, 2011).

The de-envelopment step that will deliver naked capsids in the cytosol can be compared to the viral entry process and was first thought to rely on the glycoproteins present in the primary envelope (figure 16). In HSV-1, the presence of several viral glycoproteins in the INM, many of which (gD, gB, gH/gL) directly implicated in the viral entry process is a strong argument in favour of this theory (Baines et al., 2007; Farnsworth et al., 2007; Jensen and Norrild, 1998; Stannard et al., 1996; Torrisi et al., 1992). Accordingly, the deletion of them, solely or in combination, has shown to impact the nuclear egress to various extend, resulting in the accumulation of enveloped particles in the perinuclear space or inside herniations of the inner nuclear membrane (Farnsworth et al., 2007; Krishnan et al., 2005; Lee and Longnecker, 1997; Wright et al., 2009). Interestingly, an HSV-1 mutant strain lacking pUS3 kinase displays the same INM herniations showing that this protein could also play a role in the de-envelopment process (Ryckman and Roller, 2004). In fact, HSV-1 gB tail pointing towards the cytoplasm or the nucleoplasm has been shown to be phophorylated by pUS3 at threonine 887 and the mutation of this threonine impairs the fusion at the ONM (Wisner et al., 2009).

Nevertheless, none of the glycoproteins is strictly required for the nuclear egress and even if enveloped virions accumulate in the perinuclear space, many of them are still able to reach the cytoplasm. In fact, in PRV, the glycoproteins implicated in the

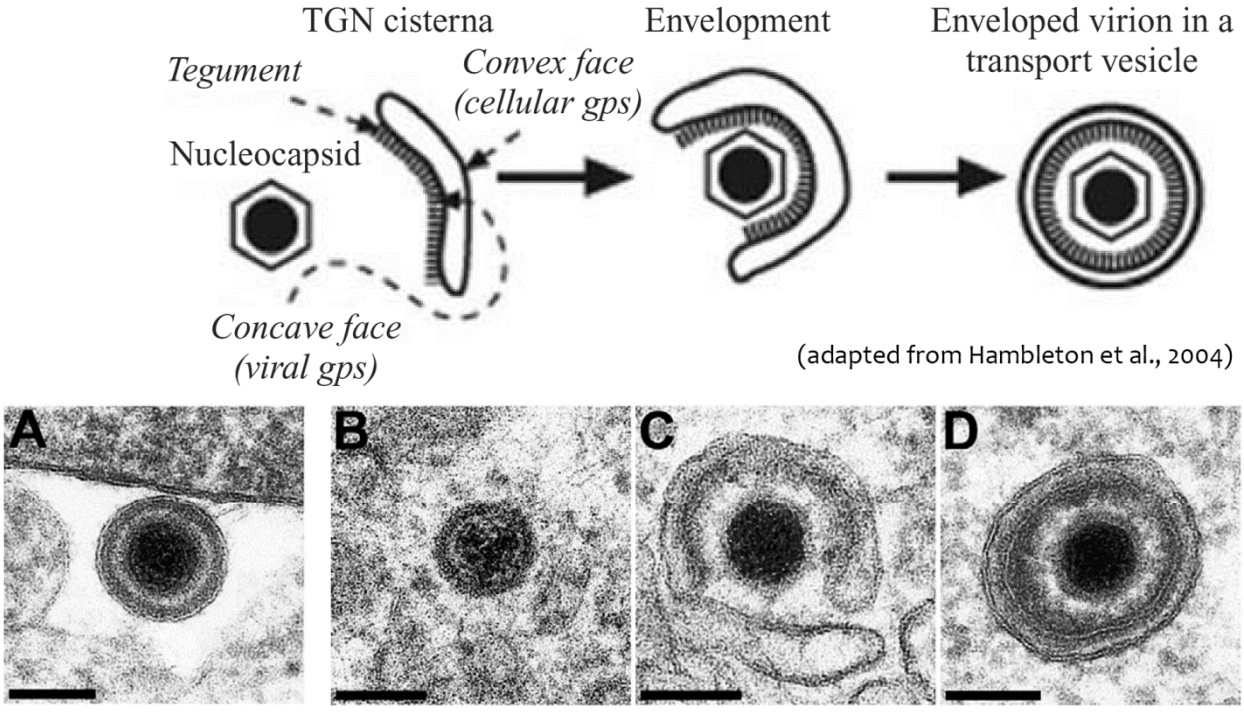
entry process, neither localize in the nuclear membranes, nor play a role in the nuclear egress, arguing for two completely distinct mechanisms, or at least for the existence of an alternative de-envelopment pathway (Klupp et al., 2008). The discovery that the coexpression of pUL31 and pUL34 is sufficient to induce the formation of vesicles in the perinuclear space and that at least some of them are able to fuse with the ONM suggest that the NEC could be one of this pathways (Klupp et al., 2007). Giving the fact that the portion of pUL34 protruding in the perinuclear represents only 5 a. a. it is hard to understand how they could, by themselves, mediate the fusion of those vesicles with the ONM. It is then likely that some cellular proteins are implicated in this phenomenon. Actually, as professional cell hijackers, herpeviruses have been suspected for quite some time to cross the nuclear envelope via an existing cellular process, which remained to be discovered. A recent work in Drosophila has revealed the existence of a, so far completely unknown, mechanism of cytoplasmic transfer of large nuclear RNP complex through a initial budding in the INM and subsequent fusion of the vesicle with the ONM. Further studies need to confirm the existence of such a nuclear budding transport in mammals but it is evident that unravelling this potentially conserved mechanism will have an important impact in the herpesvirus field (Speese et al., 2012).

2.6. Tegumentation and secondary envelopment

The tegument is a protein layer residing between the nucleocapsid and the envelope. It is now described as an inner layer and an outer one. The inner tegument is structurally more organized and more tightly bound to the capsid (Zhou et al., 1999). This internal tegument is mainly constituted of pUL36 (VZV ORF22) and pUL37 (VZV ORF21), two highly conserved proteins and of the pUS3 kinase (VZV ORF66). The place where it is acquired is still a matter of debate but a thin layer of inner tegument is possibly already present at the nuclear envelopment stage and stay attached to the capsid after the de-envelopment process (Bucks et al., 2007; Henaff et al., 2013). An accumulation of inner tegument proteins on the capsid surface most likely takes place in the cytoplasm. This can occur possibly prior to the capsid transport in cytoplamic areas where secondary envelopment takes place but possibly also directly at the TGN where pUL36 and pUL37 have be shown to accumulate (Desai et al., 2008). The binding of the inner tegument to the capsid is a prerequisite for the subsequent acquisition of the outer tegument. This step is consequently crucial for the final envelopment and in the absence of pUL36 or pUL37, major defects in the egress process are observed (Desai et al., 2008; Desai et al., 2001; Desai, 2000; Klupp et al., 2001). pUL36 is a large multifunctional protein able

via its essential C-terminal part to bind the major capsid protein as well as the capsid-bond pUL25 (VZV ORF34)(Coller et al., 2007; Lee et al., 2006). The N-terminal part contains the pUL37 interaction motif (Kelly et al., 2012; Klupp et al., 2002; Lee et al., 2008b) as well as a conserved deubiquitine activity which disruption in PRV impacts virion morphogenesis (Bottcher et al., 2008).

The outer tegument contains many different proteins that are added to the particle during the secondary envelopment process. Surprisingly, the presence of the capsid is not absolutely necessary for the tegument to bud through a vesicle carrying the envelope viral proteins. This leads to the formation and release at the cell surface of capsid-less enveloped particles that are called "light-particles". The light particles have been shown to possess the complete set of tegument and envelope proteins (Irmiere and Gibson, 1983; McLauchlan and Rixon, 1992). The presence of the inner tegument proteins is not required for their formation which indicates that the outer tegument self-assembles by interacting with the cytoplasmic domain of the envelope proteins in a peculiar cellular compartment.



(adapted from Hambleton et al., 2004)

(adapted from Mettenleiter et al., 2009)

Figure 17. Herpesvirus secondary envelopment. Upper part, model depicting the accumulation of tegument proteins at the concave side of the TGN-derived vesicle though which the cytoplamic capsid will bud. The lower part, transmission electron microscopy pictures of HSV-1 viral particle at different stages of the egress process. (A) primary enveloped virion in the perinuclear space, (B) naked capsid in the cytoplasm, after de-envelopment, (C) capsid undergoing secondary envelopment and (D) enveloped particle in its transport vesicle. Note the quite different aspect of the teguement between A and D. bars= 100nm

The vesicles through which capsids buds to acquire the final envelope are thought to derived from the TGN, even though another model has been described in which they rather originate from late endosomes (see below). Nevertheless, because the lipid content of the viral envelope present some similarities with TGN vesicles and because most of the glycoproteins and many outer tegument proteins colocalize in this cellular compartment, the TGN is generally considered as the site of secondary envelopment (Harley et al., 2001; McMillan and Johnson, 2001; Sanchez et al., 2000; Sugimoto et al., 2008; Turcotte et al., 2005; Zhu et al., 1995b). Of note, herpesviruses are well-known to drastically remodel the internal membrane network and many cellular proteins used as marker for these cellular subcompartments are dispersed or relocalized during the infection (Campadelli et al., 1993; Wisner and Johnson, 2004). This may explain why the exact nature of the cellular membrane meant to become the viral envelope is still controversial.

Extensive and redundant protein-protein interactions have been identified between outer tegument proteins, but also between tegument protein and the cytoplasmic tail of the viral glycoproteins (for review (Johnson and Baines, 2011; Mettenleiter, 2002; Mettenleiter et al., 2009)). This leads to the formation of polarized vesicles in which one side (the concave one) is enriched in viral glycoproteins and tegument proteins, the other only containing cellular glycoproteins. After the completion of the envelopment, the concave side becomes the viral envelope whereas the convex one becomes the transport vesicles (figure 17). Some particular interest must be demonstrated for the highly interconnected proteins that might play a more important role. This is the case of pUL48 (VP16, VZV ORF10) and pUL49 (VP22, VZV ORF9). As shown at figure 18, they both interact with gM, either directly or indirectly. pUL49 interacts as well with gE/gI but also with gD while pUL48 interacts with the gH/gL complex ((Maringer et al., 2012) and references therein). The deletion of pUL48 in HSV or PRV results in a strong impairment of the viral egress and the accumulation of non enveloped particles in the cytoplasm (Fuchs et al., 2002; Mossman et al., 2000). A UL49-null HSV is viable but releases 10^5 to 10^6 less viral particles than the wild-type virus and the extracellular virions have been shown to possess decreased amount of gD, gE/gI and ICP0 (Duffy et al., 2006; Elliott et al., 2005). In VZV, some of these interactions have been confirmed and the deletion of pUL49 (ORF9) is not compatible with viral growth (Che et al., 2013b; Che et al., 2008). Moreover, we recently demonstrated a role of ORF9 in the viral egress process (Riva et al., 2013). Another bridge described between the capsid and the envelope is mediated by the pUL11-pUL16-pUL21 complex. pUL11 is a conserved small

protein anchored in the cytoplasmic face of Golgi and TGN vesicles through a myristate and a palmitate lipid motifs, it bind pUL16, which has been shown to bind the capsids but also pUL21 (Baines et al., 1995; Harper et al., 2010; Klupp et al., 2005a; Loomis et al., 2003; Meckes and Wills, 2007; Yeh et al., 2008).

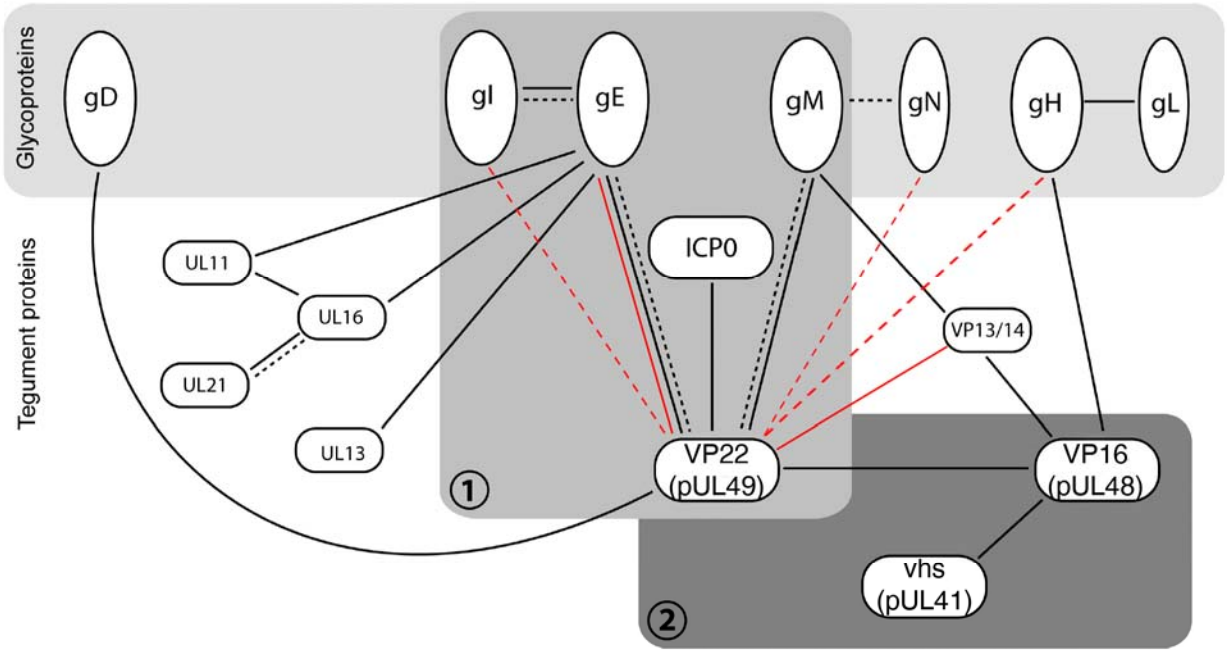


Figure 18. Network of protein-protein interactions around alphaherpesvirus pUL49 and pUL48 tegument proteins. solid black lines show HSV-1 reported interactions, discontinued black lines show PRV described interactions, solid red lines show VZV clearly demontrated interactions and discontinued red lines VZV interactions recently described in a yeast two-hybrid screen (adapated from Maringer et al., 2012).

Several glycoproteins have been implicated in the secondary envelopment process. As in the case for outer tegument proteins, some functional redundancy exists explaining why the deletion of a single glycoprotein only moderately impacts viral egress. Nevertheless, a simultaneous deletion of either gE/gI and gM or gE/gI and gD results in a strong accumulation of unenveloped particles in the cytoplasm (Brack et al., 2000; Farnsworth et al., 2003). The cytoplasmic tail of the glycoprotein is likely to play the major role, since it promotes the binding with the tegument layer. Indeed, mutants lacking only the cytoplasmic domain of gE, together with either gM or gD, present similar defects in the secondary envelopment (Brack et al., 2000; Farnsworth et al., 2003). In addition, cytoplasmic regions of glycoproteins carry important functional domains, among which Yxxφ motifs (where Y is a tyrosine, x any amino acid and φ an hydrophobic residue) that play a major role in endocytosis and cell sorting that may strongly impact the envelopment process (for review (Favoreel, 2006)). It is known for quite some time that VZV gE possesses in its

cytoplasmic tail several Tyrosine-based motifs important for its endocytosis from the cell surface where it accumulates during the infection (Alconada et al., 1996; Olson and Grose, 1997; Zhu et al., 1996). A similar functional motif is present in HSV and PRV gE homologs but also in the cytoplasmic tail of gB and gH (Alconada et al., 1999; Favoreel et al., 2002; Heineman and Hall, 2001; Pasieka et al., 2003; Tirabassi and Enquist, 1999). In VZV, is has been demonstrated that gE, gB and gH must be endocytosed to be incorporated in the virion (Maresova et al., 2005). Accordingly, the deletion of the endocytosis motif in VZV gE strongly impacts viral growth (Moffat et al., 2004a). Even though the deletion of the corresponding motif in PRV only results in a minor viral growth defect (small plaques phenotype), one should remember that while gE is essential for VZV, it is known to be dispensable in HSV-1 and PRV (Tirabassi and Enquist, 1999). It is also possible that the endocytosis of glycoproteins is not equally important for the different herpesviruses.

As mentioned above, the cellular origin of the membrane that will become the final envelope is still a controversial issue. HSV-1 viral envelope is enriched in sphyngomyelin and phosphatidylserine, lipids present in large amount in the Golgi apparatus and the plasma membrane (van Genderen et al., 1994). A recent very elegant study has proposed that endocytic tubules might represent the true source of membranes in which secondary envelopment occur (Hollinshead et al., 2012) (figure 19). By culturing the cells in a medium containing soluble horseradish

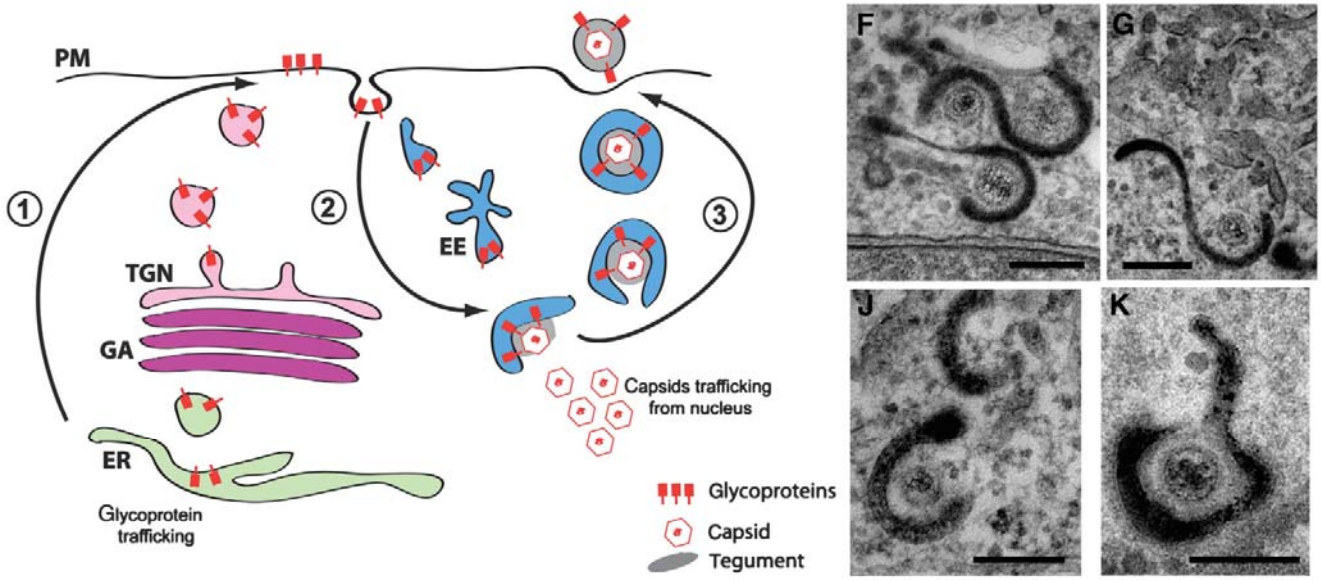


Figure 19. Alternative model of secondary envelopment. (left panel) The model suggests that viral glycoproteins translated in the RER and glycosylated in the RE and the Golgi must reach the cell surface (1) to be present in the endocytic vesicles that will subsequently engulf viral caspids (2). The enveloped virion is released at the cell surface when the transport vesicle fuse back with the cell membrane (3). (right panels) HFFF-2 cells infected with HSV-1 were cultured wih 10 mg/ml of HRP for 60 (F and G), 10 (J) and 2 (K) minutes, fixed, stained with DAB to reveal the presence of HRP and then processed for TEM. The dark curves represent endocytic vesicles. Capsids budding through labeled vesicles are easily detectable even after very short period of incubation (adapted from Hollinshead et al., 2012).

peroxydase, they were able to label those endocytic tubules. TEM analysis with the apporiate HRP substrate demonstrated the wrapping of HSV-1 capsids by labelled tubules as early as 2 minutes after the addition of the peroxidase in the medium (figure 19 right panels).

Cargo movement through the endocytic pathway is regulated at multiple stages by several Rab GTPases among which Rab5 and Rab11 that are involved in the transition from plasma membrane to early endosomes and the way back, respectively. The fact that the simultaneous depletion of rab5 and rab11 reduces the virus yield by 99% is another clue to demonstrate the implication of the endocytic pathway in the final envelopment process. This model could finally explain the importance of the endocytosis motifs in the cytoplamic tails of the envelope proteins.

2.7. Final egress

Once in its transport vesicle, the enveloped virion is thought to be released at the cell surface via the cellular exocytic pathway. This part of the viral life cycle has not been studied in details so whether it is the constitutive secretory pathway or a regulated secretion remains unknown (figure 20). Nevertheless, in the case of VZV a diversion of the transport vesicles to the late endosomal compartment has been described and provide a possible explanation for the high amount of aberrant particles released as the cell surface that characterizes VZV-infected cells (Chen et al., 2004).

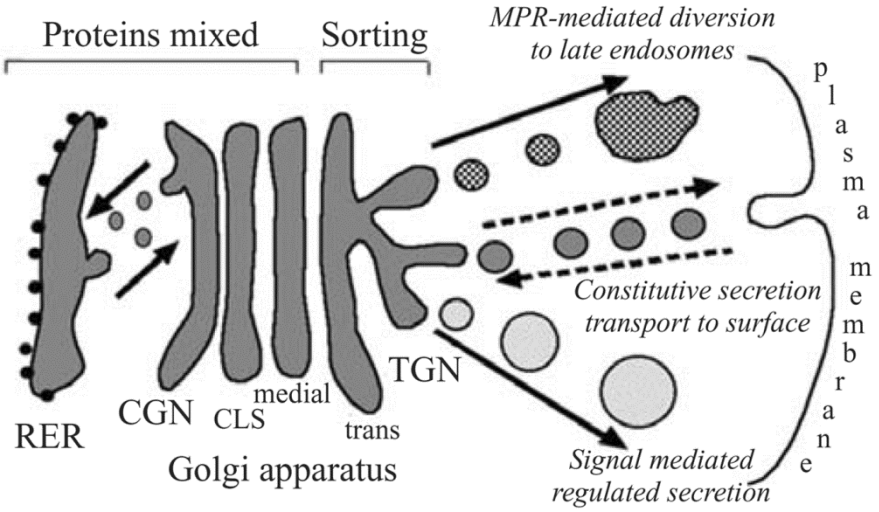


Figure 20. Protein sorting from the TGN. The constitutive secretion is the default pathway. The presence of specific signals may diverte some molecules to the regulated secretory pathway. The mannose-6-phosphate receptor present in the membrane of some vesicles can redirect the cargo to the late endosomal compartment.

According to this model, the presence of mannose-6-phosphate receptor (MPR^{ci}) in the transport vesicle would redirect them toward the late endosomal pathways.

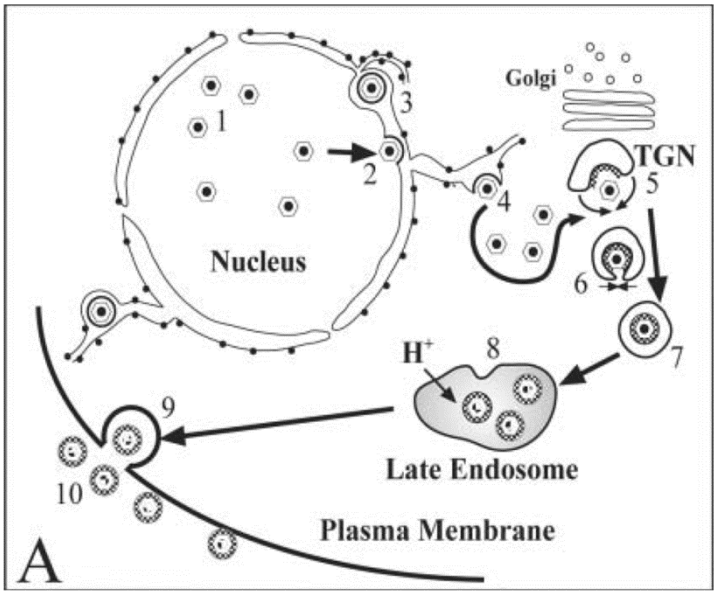


Figure 21. A proposed pathway for VZV egress. After the secondary envelopment (5 and 6), the MPR^{ci}-positive transport vesicles are redirected to the late endocytic compartment in which the low pH environment will partially degrades the particles (8) until the late endosome bring them to the cell surface (9). Most of the released viral particles are aberrant and stay attached to the plasma membrane (10) (adapted from Chen et al., 2004).

The acidic content of this cellular compartment would be responsible for the partial degradation of the particles (figure 21). A natural downregulation of this receptor accompany the keratinocyte maturation into corneocytes or squames. This would explain why virtually no cell free infectious particles are released in the supernatant in cultured cells or in a host naturally experiencing varicella, except in the squamous epithelium characteristic of the epidermis in the varicella vesicles (Gershon and Gershon, 2010). Of note, is has been shown more recently that the vast majority of the aberrant particles present at the cell surface actually represent light-particles suggesting that the diversion towards the late endosomes, if present, is not responsible for the very low amount of complete and potentially infectious particles able to reach the plasma membrane of a VZV-infected cell (Carpenter et al., 2008). It is conceivable that the phenotypical difference observed between keratinocytes of the squamous epithelium and all other cell types in their capacity to release free particles is attributable to the downregulation of MPR^{ci} but that the high level of aberrant particles present at the cell surface comes from a completely independent phenomenon.

3. VZV latent cycle

Besides the lytic cycle that ultimately leads to the infected cell death, herpesviruses are able to enter a latent stage in some specific host cells (table 2). The latency is a life-long stage from which the virus can reactivate, causing recurrent infections. The host cell type in which latency is taking place is a characteristic of the subfamilies, neurons in the case of alphaherpesviruses. Because VZV only infects

productively human and primates cells, no small animal model is able to recapitulate the different steps of a natural infection. Actually, besides chimpanzee and gorillas, so far, no animal placed in contact with VZV, no matter the inoculation technique used, has developed a vesicular skin rash (Cohen et al., 1996; Myers et al., 1987; Willer et al., 2012). Some imperfect but still useful models of latency in rodents have been developed, including in our laboratory, but, so far, none of them has been able to reconstitute the reactivation process (Cohen, 2010a; Sadzot-Delvaux et al., 1990). It is then not surprising that only few data are available in the literature compared to HSV. Unfortunately, even though some characteristics of HSV latency seem to be shared by VZV, on this precise matter, both viruses seem quite divergent (recently reviewed in (Eshleman et al., 2011)).

During the latent stage, several circular copies of HSV or VZV genome are present in the cells but no infectious particle is released (Clarke et al., 1995; Jackson and DeLuca, 2003; Kinchington, 1999; Mellerick and Fraser, 1987). The exact cell type in which VZV resides within the ganglion has been a long debate but is currently considered to be the neurons themselves. (Croen et al., 1988; Kennedy et al., 1998; LaGuardia et al., 1999; Levin et al., 2003; Wang et al., 2005) Upon reactivation, satellite cells become infected as well.

The onset of latency, even for HSV-1, is still an obscure process that may involve the PML bodies. As mentioned above in paragraph 2.2., HSV-1 genomes colocalize upon nuclear entry with the PML bodies. The expression of ICP0 is necessary to disperse PML bodies that otherwise represses viral gene expression. It appears that the balance between PML-induced gene silencing and ICP0-induced PML degradation will deeply impact the outcome of the infection (Everett et al., 2007; Everett et al., 2006). The maintenance of the latent stage is associated with the expression of a unique portion of the HSV-1 genome whose transcript has been termed LAT for Latency-Associated Transcript. This gene has been described as an unstable non-coding transcript of 8.3kb at the origin of several shorter stable RNAs (Farrell et al., 1991; Kang et al., 2006; Kent et al., 2003; Zabolotny et al., 1997). Recently, a potential translation product was identified within the first 1.5 kb of this RNA (Henderson et al., 2009). Nevertheless, the maintenance of the latency driven by the LAT is thought to principally occur at the RNA level. Several miRNAs have been identified within the LAT, one of which has been shown to downregulate ICP0 (Peng et al., 2008; Umbach et al., 2008). Interestingly, other miRNAs have later been discovered in the HSV-1 genome, and at least one is

expressed during latency and downregulates ICP4, a major transcriptional regulator of HSV-1 (Shen et al., 2009).

LAT is present in a HSV genome portion that is missing in VZV genome (see figure 5) and no specific transcript, expressed exclusively during VZV latency, has been identified so far. In fact, some transcripts (ORF4, ORF21, ORF29, ORF62, ORF63 and ORF66) expressed during the lytic cycle are present in latently infected human ganglia, and also, except ORF66, in ganglia of latently infected rodents. Originally identified at the RNA level, their corresponding proteins all have been later detected, at least in a certain proportion of isolated ganglia. Interestingly, ORF4, 21, 29, 62 and 63 have all been detected in the cytoplasm of latently infected neurons whereas they are distributed between the nucleus and the cytoplasm during the lytic infection (Brunell et al., 1999; Cohrs et al., 2003a; Grinfeld and Kennedy, 2004; Kennedy et al., 2000; Kennedy et al., 2001; Lungu et al., 1998; Mahalingam et al., 1996; Sadzot-Delvaux et al., 1995).

VZV gene	Mutation	Phenotype in latency	References
ORF1	Stop	Dispensable	Sato et al. (2003b)
ORF2	Del	Dispensable	Sato et al. (2002b)
ORF4	Del	Impaired	Cohen et al. (2005b)
ORF10	Del	Dispensable	Sato et al. (2003b)
ORF13	Stop	Dispensable	Sato et al. (2003b)
ORF14	Del	Dispensable	Grinfeld et al. (2004)
ORF17	Del	Dispensable	Sato et al. (2002a)
ORF21	Del	Dispensable[a]	Xia et al. (2003)
ORF29	Del	Impaired	Cohen et al. (2007)
	Ectopic promoter	Impaired	Cohen et al. (2007)
ORF32	Del	Dispensable	Sato et al. (2003b)
ORF47	Stop	Dispensable	Sato et al. (2003b)
ORF57	Del	Dispensable	Sato et al. (2003b)
ORF61	Del	Dispensable	Sato et al. (2003a)
ORF63	Del	Impaired	Cohen et al. (2004)
	Carboxy terminal truncations	Dispensable or impaired	Cohen et al. (2005a)
	Phosphorylation sites	Impaired	Cohen et al. (2005a)
ORF66	Stop	Dispensable	Sato et al. (2003b)
ORF67	Stop	Dispensable	Grinfeld et al. (2004)

Stop stop codon; *del* deletion; *ectopic promoter* ORF29 expressed under a cytomegalovirus promoter
[a]Virus was not passaged in non-complementing cells before testing for latency

Table 7. VZV genes implicated or not in the establishment of latency (from Cohen, 2010a).

Rodent models have been used to define the viral genes that are necessary for the establishment of the latency (table 7 and, reviewed in (Cohen, 2010a)). So far, no deletion mutant has proven to be completely unable to establish latency, even though strong defect can be observed. Among the genes expressed during latency, ORF21, even though essential for the lytic cycle is dispensable, while ORF4 and ORF29, both also essential for the lytic cycle are important for the

establishment of the latency. ORF63, which is the most expressed during latency has been shown to be important for the entry in the latent stage and has been shown to possess an anti-apoptotic activity. Finally, other genes that are not expressed during latency have been evaluated for their role in its establishment. Some mutants presenting strong growth defect in cell culture (ORF17 and ORF61 deletion mutants) establish latency at a normal level. ORF10, gI and gC, that are dispensable for viral growth in fibroblast but important for some specific target cells, are not required either to establish latency.

4. The PML bodies

PML bodies (also called ND10 nuclear bodies) are nuclear subdomains belonging to the nuclear matrix. They have been implicated in a large variety of cell processes, including DNA repair, apoptosis, transcriptional regulation, senescence and intrinsic defence against viruses (Bernardi and Pandolfi, 2007; Everett, 2006; Everett and Chelbi-Alix, 2007; Regad and Chelbi-Alix, 2001; Salomoni et al., 2008; Takahashi et al., 2004; Tavalai and Stamminger, 2008). They were discovered in the sixties in transmission electron microscopy as spherical structures of 0,1 to 1 µm, presenting a high degree of diversity but a common electron-dense shell and an inner core. They are present in most cell types and their number is usually between 5 to 15 per nucleus, which explains why they were initially called ND10, for Nuclear Domains 10, where 10 is their approximate number in one nucleus. More than 70 proteins have so far been identified within those structures, either constitutively or under specific conditions. A common feature among these diverse proteins is that they all seem to bear SUMOylation motifs and their SUMOylation status is important for their incorporation in PML bodies. Among them, only the PML protein seems to be strictly required for their formation and maintenance (Lallemand-Breitenbach and de The, 2010). This protein presents more than 14 isoforms with highly variable C-terminal parts attributable to alternative splicing of the mRNA. Some isoforms are exclusively cytoplasmic suggesting that PML might display very diverse biological role. Two other proteins are constitutively present in PML bodies, SP100 and Daxx. All three genes are upregulated upon interferon treatment leading to an increased number of PML bodies in the treated cells (Chelbi-Alix et al., 1995; Gongora et al., 2001; Stadler et al., 1995). Several other exogenous agents are known to perturb the formation or organization of this nuclear subcompartment, among which arsenic trioxide that

has been extensively used to deeply characterize the different steps in the formation of PML bodies (figure 22).

The exact mechanism through which PML bodies are able to play a role in so many divergent biological processes is still under extensive study and several models currently coexist. The first one designate the PML bodies as specific dynamic depot where nuclear proteins are stocked, awaiting some specific stimuli to be relocalized where they are needed (Lehembre et al., 2001; Li et al., 2000; Lin et al., 2006; Negorev and Maul, 2001; Yamada et al., 2001). A second model, a variation of the first, suggests that they represent places where excessive proteins are sequestered, maybe to be subsequently degraded (Lafarga et al., 2002). Because PML bodies are especially enriched in protein-modifying enzymes, the third model envisages them as a favourite place for post-translational modifications (SUMOylation phosphorylation, acetylation,...) of some nuclear proteins (D'Orazi et al., 2002; Pearson et al., 2000). The last model proposes that they may represent sites specific for specific nuclear processes, such as transcriptional regulation of DNA replication (Boisvert et al., 2000; Eskiw et al., 2003; Wang et al., 2004).

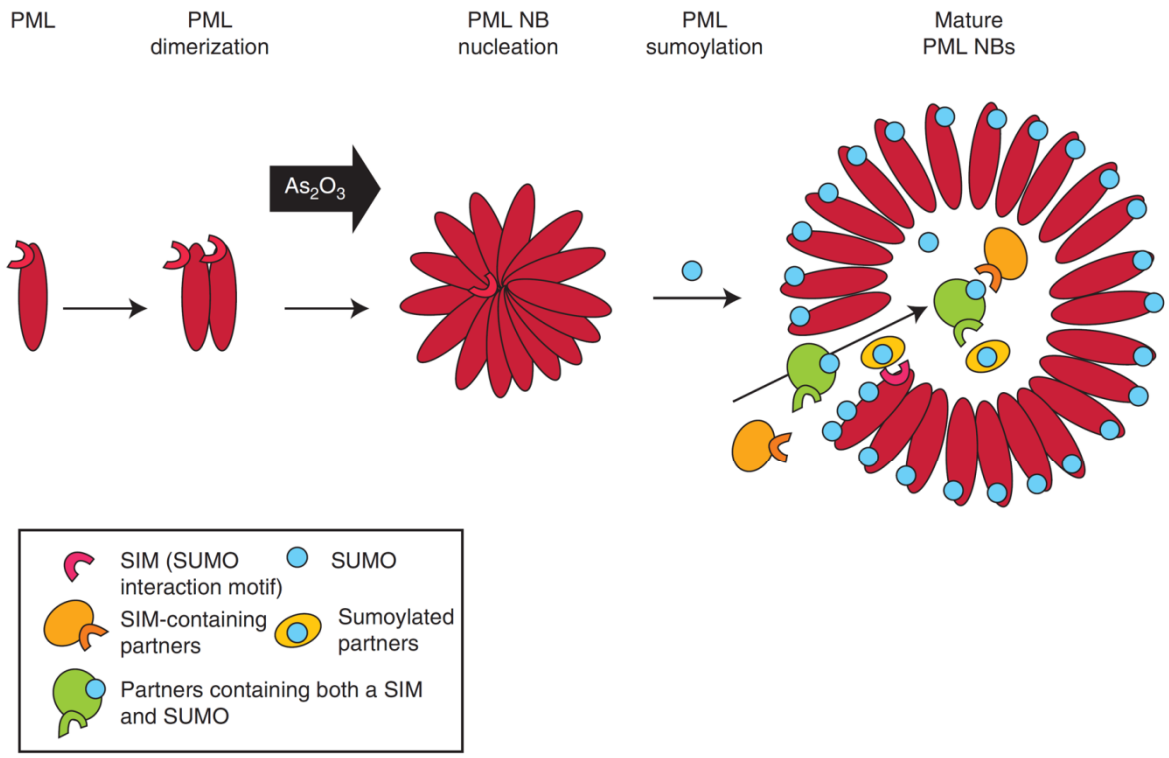


Figure 22. Schematic representation of PML Bodies biogenesis. PML proteins first dimerize through the RBCC motifs and then multimerize to form the nuclear bodies. The SUMOylation of PML and of the different partners is important for the subsequent maturation of the NB and the sequestration of the associated proteins.

4.1 PML bodies and viral infection

PML bodies seem to play a role in the life cycle of nuclear replicating DNA viruses. Incoming genomes of at least three families of viruses (*polyomaviridae*, *adenoviridae* and *herpesviridae*) have been shown to localize in very close proximity to those nuclear bodies ((Ishov and Maul, 1996), and for review (Everett, 2006)). Is has been later shown that it is probably not the genome that moves until it reaches these particular places but a *de novo* ND10 formation at the site of genome deposition (Everett et al., 2004). Moreover, many viruses, among which papillomaviruses (Day et al., 2004; Roberts et al., 2003), adenoviruses (Doucas et al., 1996; Schreiner et al., 2010), polyomaviruses (Erickson et al., 2012; Shishido-Hara et al., 2004), and herpesviruses from the three subfamilies (Adamson and Kenney, 2001; Ahn and Hayward, 1997; Everett and Maul, 1994; Kim et al., 2011b; Lee et al., 2004; Sivachandran et al., 2008) have been shown either to disrupt PML bodies or to modify their content in order to favour gene expression, or, conversely, to require some ND10 component to replicate efficiently.

ICP0 from HSV-1 has been shown to colocalize with PML Bodies very early upon infection and, via its E3 ubiquitin ligase activity, to induce the proteasomal degradation of the sumoylated forms of PML. This will ultimately lead to the degradation of all PML isoforms and of the sumoylated forms of SP100 leading to the dispersal of the PML bodies. This phenomenon is thought to play an important role in the initiation of the viral transcription program. ICP0 is dispensable for viral growth but its absence strongly impacts the viral propagation and it plays an important role in the reactivation of quiescent genome (Boutell et al., 2003; Everett et al., 1998; Everett and Maul, 1994; Everett et al., 2007; Maul et al., 1993; Sourvinos and Everett, 2002).

Whereas all ICP0 homologs that have been tested share, at least *in vitro*, this E3 ubiquitin ligase activity, they differ in their ability to efficiently lead to the degradation of PML and SP100 isoforms and ultimately lead to PML disruption (Everett et al., 2010; Gaudreault and Jones, 2011; Parkinson and Everett, 2000).

4.2 PML bodies and VZV

In contrast to observations in HSV-1, VZV infection is not characterized by PML protein degradation and SP100 level is only very slightly diminished (Kyratsous and Silverstein, 2009). Immunofluorescence with anti-PML antibodies has demonstrated that even though the number of PML bodies present in the nucleus slightly

decreases as the infection progresses, they remain present, even at late time points (Kyratsous and Silverstein, 2009; Reichelt et al., 2011; Wang et al., 2011). The staining of SP100 in infected cells is dispersed at late time points, indicating that the virus might be able to modulate the composition of the PML bodies (Kyratsous and Silverstein, 2009). The downregulation of the major components of PML bodies (PML, SP100 and Daxx) via shRNA has shown a minor impact on viral replication, with a slight increase in viral titers at early times (Kyratsous and Silverstein, 2009). Actually, PML bodies seem to exert an antiviral effect on a later step of VZV viral replication cycle. Indeed, arrays of newly assembled capsids entrapped inside PML cages have recently been identified in infected cells. These structures have been postulated to impede the egress of those particles (Reichelt et al., 2011). It has also been demonstrated recently that the decreased number of PML bodies present in infected cells might be attributable to ORF61 (Wang et al., 2011).

AIM OF THE WORK

The exact location where the replicated DNA is cleaved and encapsidated and where the capsid acquires its inner and outer tegument is still a matter of debate. Immunofluorescence using specific antibodies and, more recently, the fusion of viral genes with genes coding for fluorescent proteins coupled with confocal microscopy have significantly helped to decipher the complex process of virion formation from the nuclear steps to the final egress at the cell surface. Early in infection, capsid proteins are frequently found in the replication compartment (RC), whereas later time points are characterized by the appearance of very dense structures localized at the periphery of the RC. These structures comprise both mature and immature capsid proteins as well as some tegument proteins and have been called "assemblons" (Ward et al., 1996). Their potential role in the infection process is still controversial, as they could either be a functional compartment highly specialized in nucleocapsid formation or a depot of dead-end products resulting from a protein excess (Abaitua and O'Hare, 2008; Hutchinson et al., 2002; Lee et al., 2006; Markovitz and Roizman, 2000; Ward et al., 1996).

Little is known about VZV nucleocapsid formation, but, based on HSV-1 homology, a similar process is expected, even though some peculiar features, like the nuclear import of capsid subunits, have already been described (Chaudhuri et al., 2008).

In the first part of this study, we have created a recombinant VZV whose capsid protein ORF23 (homolog to VP26) is fused to the green fluorescent protein, as well as the first described VZV dually fluorescent viruses where a tegument protein (ORF9, ORF21, ORF22, ORF38 and OR63/70 respectively) is additionally fused to a red fluorescent protein. With these viruses, we have demonstrated that (i) nuclear structures located at the periphery of the RC are formed during VZV infection; (ii) they appear in all cell types around 8 to 12 hours post-infection, as soon as the RC starts to form; (iii) they contain capsid/procapsid proteins as well as some but not all tegument proteins, whereas they do not contain any proteins associated with DNA replication and encapsidation; (iv) they are mainly constituted of fully assembled capsids of the three types, but they also contain many procapsids, as well as a few partial capsids; and (v) they are highly dynamic. We believe that the observed structures might represent a place where capsids are preferentially assembled and/or accumulate after or during their formation, before their egress towards the cytoplasm.

In the second part of this study, we aimed to characterize further the VZV assemblons. We tried to relate them to existing structures in homologous virus and to identify a potential link between them and pre-existing known nuclear structures. Strikingly, we found an association of VZV assemblons with the PML bodies. As VZV capsid aggregates thought to be entrapped in some "PML-cages" have been recently identified in the nucleus of VZV-infected cells, we tried to determine whether the VZV assemblons were somehow related to these PML-cages (Reichelt et al., 2011).

RESULTS

PART I. Formation of dynamic capsid aggregates in the nucleus of VZV-infected cells.

1. Construction of an eGFP-ORF23 VZV.

In order to study the nuclear steps of VZV virion assembly, we generated a virus whose minor capsid protein ORF23 (HSV-1 VP26 homolog) is fused to the green fluorescent protein (eGFP). ORF23 was naturally chosen because HSV-1 and PRV VP26 had been demonstrated to tolerate quite well the presence of a fluorescent tag. Homologous recombination in *Escherichia coli* was used to modify a BAC containing the entire pOka genome of VZV. The BAC-VZV (pOka), kind gift from Dr. Zhu (Zhang et al., 2007) was containing a "CMV-GFP" cassette in its backbone that was used to monitor the transfection efficiency, the subsequent infection progression and, when needed, the correct removal of backbone sequence via the transfection of a Cre recombinase expressing vector. The first cloning step was then the removal of the GFP gene in the BAC-VZV. This was achieved via homologous recombination in bacteria, using the DY380 strain, an *E.Coli* strain containing in its genome a defective copy of the λ prophage where the three genes (Gam, Bet and Exo) allowing the homologous recombination are under the control of a temperature-sensitive repressor (figure 1 and details in the materials and methods section). Briefly, the DY380 bacterial strain containing the BAC-VZV was induced to express the λ prophage recombination proteins and transformed with a PCR-

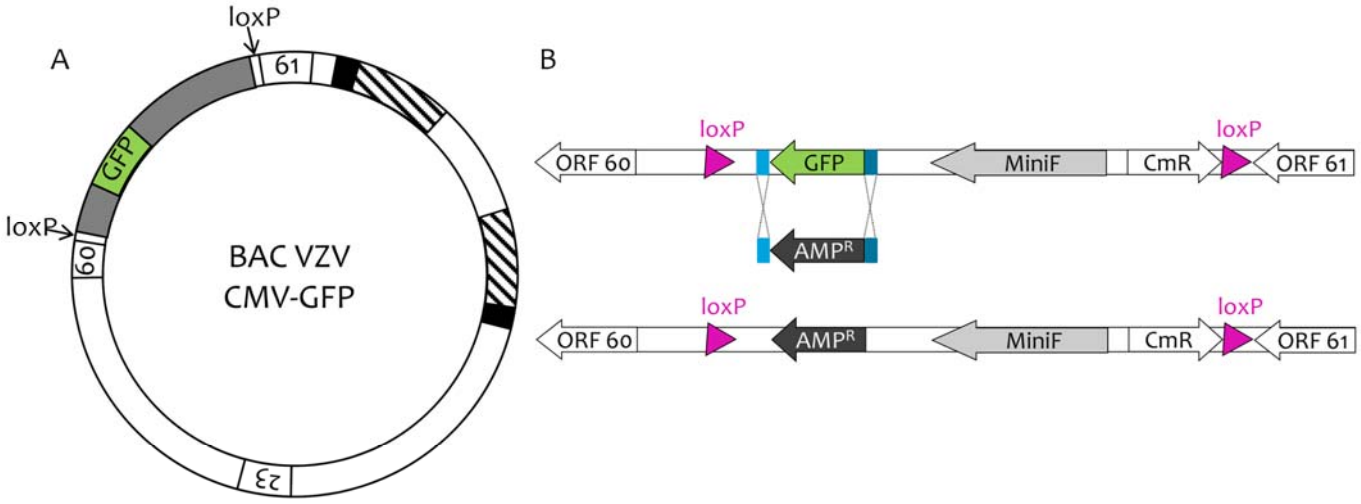
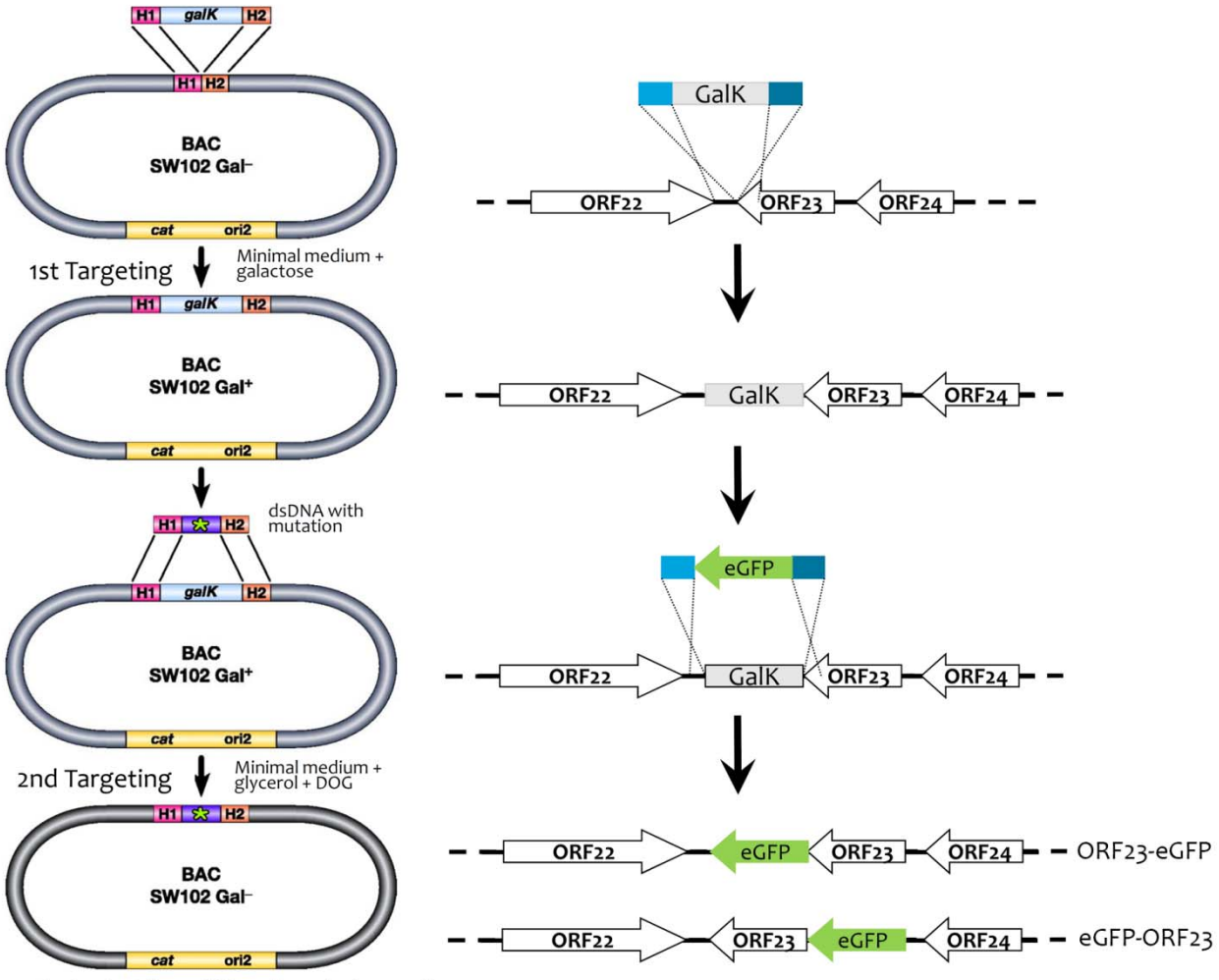


Figure 1. Removal of the GFP cassette from the original BAC-VZV. (A) Scheme of the BAC-VZV received from Dr. Zhu, the backbone is inserted in the intergenic region between ORF60 and ORF61, ▮ represent the repeats flanking U_L and ▨ the repeats flanking U_S. LoxP sites are present at both ends. (B) Replacement of the GFP by an Ampicillin resistance gene via homologous recombination with DY380 bacterial strain.

amplified Ampicillin expression cassette, flanked by 50 bp stretches homologous to the regions bordering the GFP in the BAC. Transformants were PCR–screened and the integrity of the construct verified by the analysis of restriction digestion patterns and sequencing of the modified region.

The second step of the cloning, the insertion of an eGFP gene upstream or downstream ORF23 required a seamless system. The BAC-VZV ΔGFP was then transferred in the SW102 strain. This strain, derived from the DY380, contains, in addition, an incomplete galactose operon which miss, to be fully functional, the galK gene. Two-steps are necessary to bring the desire modification in the BAC of interest. A first step during which the galK gene is inserted at the right location and a second step during which the galK gene is replaced by the desired mutation or insertion (see figure 2 and details in the material and methods section). Strains and



(adapted from Warming et al. 2005)

Figure 2. Insertion of the eGFP gene in frame with VZV ORF23 in the BAC via two-step homologous recombination. (left part) Scheme showing the principle of the technique; the first step consists in the insertion of a galK expression cassete in the genomic region of interest, the second is the replacement of the galK cassette by the desired modification. (right part) The corresponding genomic changes brought to the BAC VZV to insert the eGFP in frame with the ORF23 gene.

materials were obtained at the Biology Research Branch at the NCI and used according to their protocol (Warming et al., 2005).

In two separate experiments, we inserted the eGFP coding sequence either upstream or downstream ORF23 (Figure 2). The two BACs were transfected in a human melanoma cell line (MeWo), which was passaged near confluence until typical infection foci appeared. The cells, once infected have the property to fuse, forming syncytia of sometimes gigantic size. In three successive experiments, transfection of the BAC ORF23-eGFP failed to give rise to infection foci, even four weeks after transfection, whereas transfection of the BAC eGFP-ORF23 led to a productive infection.

The growth property of the fluorescently-labeled virus was compared with a pOka strain obtained after the transfection of the unmodified BAC-VZV. Even though the recombinant virus is viable and infectious, the expression of a fluorescently-tagged ORF23 alters the viral growth (Figure 3A). The expression of the eGFP-fusion protein was verified by western-blotting on total cell extracts of infected MeWo cells with an antibody against GFP (Figure 3B). The fusion protein is detected as a doublet, slightly higher than expected from the predicted molecular weight (51kDa). This is consistent with previously published results suggesting that ORF23 might present phosphorylation-dependent isoforms (Chaudhuri et al., 2008).

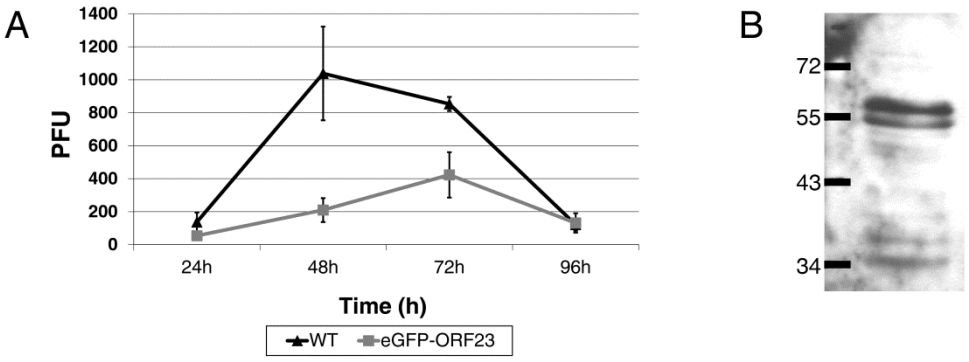


Figure 3. Analysis of the eGFP-ORF23 VZV. (A) Growth curve of the eGFP-ORF23 VZV compared to the pOka parental strain (WT). At day 0, four 25 cm^2 flasks were prepared by mixing 3 x 10^6 uninfected MeWo cells with 200 VZV infected cells. At the indicated periods of time, the number of plaque forming units (PFU) was assessed as described in the material and methods section. Means of a representative experiment out of three are depicted; error bars represent the standard deviation. (C) Western-blotting on total cell extract of MeWo infected with eGFP-ORF23 VZV with an antibody against GFP (expected size of the fusion protein: 51 kDa)

2. Intense nuclear fluorescent spots

Living MeWo cells asynchronously infected with eGFP-VZV display various fluorescence patterns (Figure 4). The green fluorescence actually characterizes their stage of infection. At early stages, infected cells present arrays of incoming capsids docked at the nuclear membrane (Figure 4, cell 1). At the beginning of the late phase of gene expression, ORF23, a late gene, starts to be expressed. This is characterized by a diffuse eGFP signal both in the cytoplasm and the nucleus (Figure 4, cell 2). At later stages, the assembly of progeny virions initiates and bright dots corresponding to single capsids show up in the nucleus (Figure 4, cell 3), after which very intense larger nuclear fluorescent spots begin to appear (Figure 4, cell 4). These spots looked quite similar to the structures described in HSV-1 by Ward et al.(1996) and that were termed "assemblons" because they were suspected to play a role in the assembly of viral capsids. This study and the notion of "assemblons" had been a matter of debate ever since their discovery. One of the major criticisms of the cons was the fact that the structures do not appear in every cell type and that they appear only at very late time points. Of important note, this original study was carried out with a wild-type HSV-1 and the analysis of the localization of several structural proteins during the lytic cycle by the means of immunofluorescence (Ward et al., 1996).

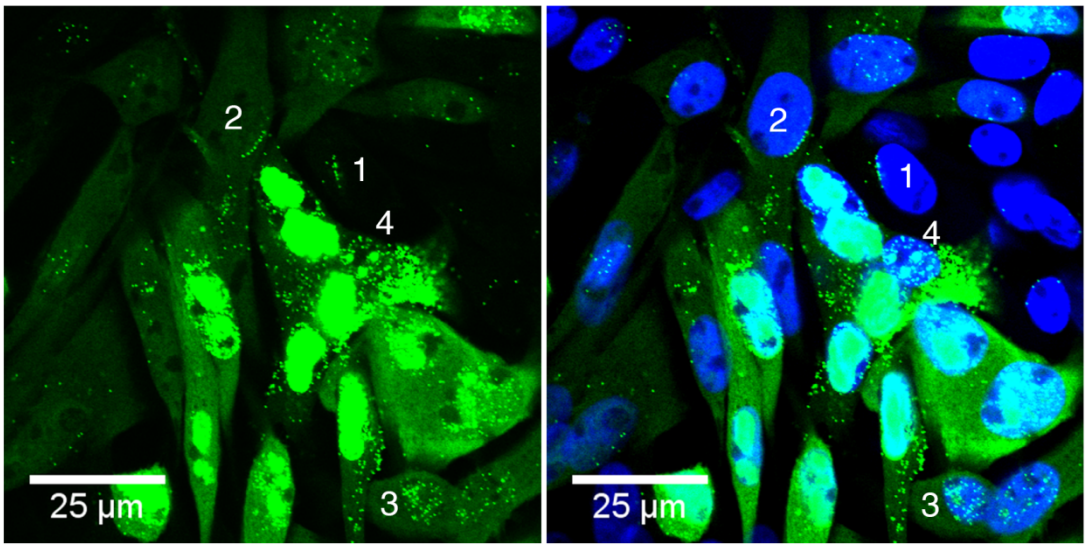


Figure 4. Nuclear dense structures form in eGFP-ORF23 VZV infected cells. Live visualization of MeWo cells infected for 24h with eGFP-ORF23 VZV. Nuclei were stained with Hoechst 33342 and confocal microscope images were captured with a 63x oil-objective. Numbering represents gradual stages of infection.

Before a deep characterization of these nuclear structures, we then decided to analyze several other cell types and to determine at which time post infection these structures appear. Human primary foreskin fibroblasts (HFF) were infected via a 20 min cell contact with eGFP-ORF23-infected MeWo cells, followed by extensive washing, and a coverslip was fixed every hour in a 24h time frame. The cells were immunostained against beta-tubulin and analyzed by confocal microscopy. Selected time points are presented at figure 5. Incoming capsids docked at the nuclear envelope were observed 3 hours p.i. (this stage corresponds to cell 1 in Figure 4), but the diffused green fluorescence resulting from *de novo* ORF23 expression only became detectable at around 6h p.i. (this stage correspond to cell 2 in Figure 4). At 8h p.i., newly assembled capsids appeared throughout the nucleus (like cell 3 in Figure 4), sometimes in tiny aggregates. Brighter and larger fluorescence dots, similar to those seen in MeWo cells (cell 4, Figure 4), were present from 12h p.i. and grew in size as the infection progressed (Figure 5).

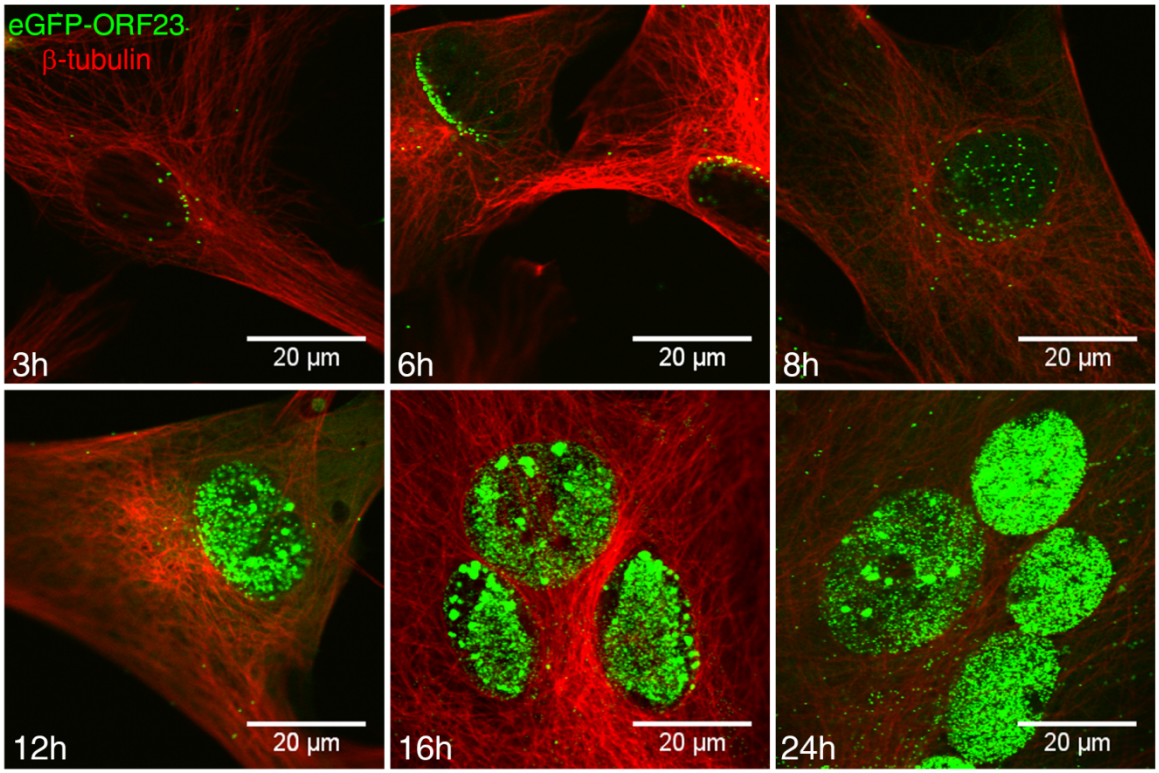


Figure 5. Nuclear dense structures form in primary HFF cells. Human primary foreskin fibroblasts (HFF) were infected with eGFP-ORF23 VZV via a 20-minute contact with MeWo cells followed by extensive washing. HFF were fixed at indicated times p.i. and immunostained against beta-tubulin (Alexa568 secondary antibody).

In a parallel experiment, time-lapse video imaging was performed and confirmed the successive pattern (1 to 4) of the green fluorescence in the course of the infection (movie S1). The brightness of the eGFP enables the detection of single capsids inside infected cells and the analysis of several movies showed that it takes about three hours from the emergence of the diffused eGFP signal until newly-formed capsids show up in the nucleus (a 2h30 time frame separates stage 2 and 3 in movie S1), and only one more hour until the larger fluorescent spots begin to form (Figure 6A compare t0+30' and t0+75' or t0+90').

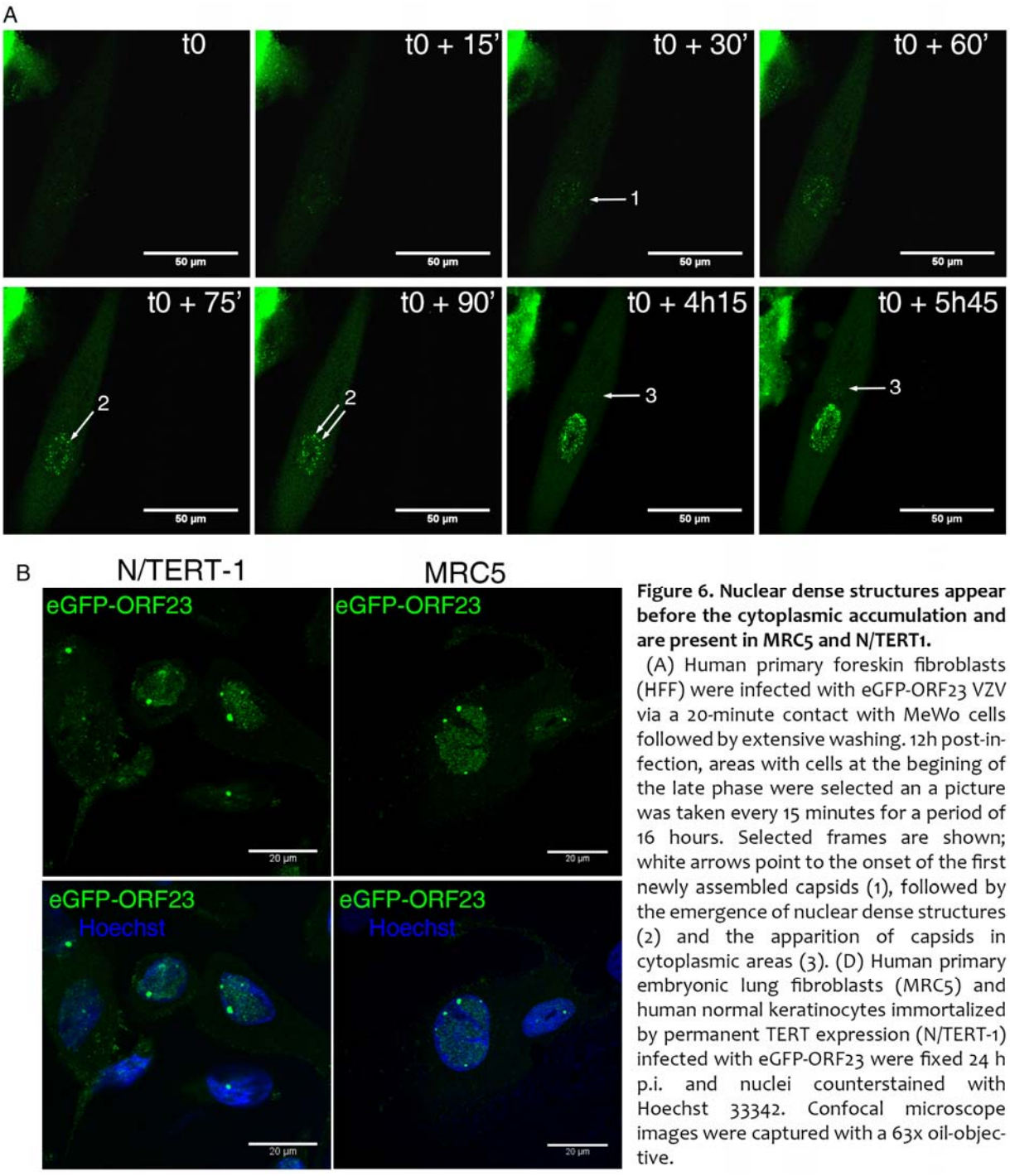


Figure 6. Nuclear dense structures appear before the cytoplasmic accumulation and are present in MRC5 and N/TERT1.
(A) Human primary foreskin fibroblasts (HFF) were infected with eGFP-ORF23 VZV via a 20-minute contact with MeWo cells followed by extensive washing. 12h post-infection, areas with cells at the begining of the late phase were selected an a picture was taken every 15 minutes for a period of 16 hours. Selected frames are shown; white arrows point to the onset of the first newly assembled capsids (1), followed by the emergence of nuclear dense structures (2) and the apparition of capsids in cytoplasmic areas (3). (D) Human primary embryonic lung fibroblasts (MRC5) and human normal keratinocytes immortalized by permanent TERT expression (N/TERT-1) infected with eGFP-ORF23 were fixed 24 h p.i. and nuclei counterstained with Hoechst 33342. Confocal microscope images were captured with a 63x oil-objective.

We noticed that the larger fluorescent spots actually appeared before the massive accumulation of capsids in the cytoplasm, where secondary envelopment occurs (movie S1, cell on the left, figure 6A compare t0+75' with t0+4h15 or t0+5h45 and movie S2, arrows). Such nuclear dense fluorescent structures also formed in human primary embryonic lung fibroblasts (MRC5) and human keratinocytes immortalized via permanent TERT expression (N/TERT-1) (Figure 6B). The latter cell line had been shown to maintain most of the characteristics of primary keratinocytes and constituted a model closer to the preferential natural host of the virus.

We next wanted to exclude the possibility that the structures observed were artefactually induced by the presence of the fusion protein eGFP-ORF23. A recent publication on HSV-1 had demonstrated that the fusion of the small capsid protein with different fluorescent tags could have various outcomes and sometimes could lead to the formation of big protein aggregates and non viable recombinant strains. The nature of the fluorescent protein as well as the way the fusion gene was organized seemed to deeply impact the formation of those aggregates and they concluded that monomeric tag should be preferred (Nagel et al., 2012). For another project, we had in mind to construct a triple fluorescent virus in which the capsid, the tegument and the envelope could be tracked simultaneously. For this purpose we had generated a virus expressing ORF23 fused to tagRFP-T, which is a true monomeric red fluorescent protein. MeWo cells infected with this virus were then analyzed by confocal microscopy (figure 7A left panel) and we observed exactly the same fluorescence pattern. We also needed to check if the intense fluorescent spots would be present with a wild-type infection. Mewo cells were infected in parallel with eGFP-ORF23 and wild-type VZV, fixed and immunostained with an antibody against ORF23, as well as with an antibody against ORF40, the VZV major capsid protein. The presence of similar intense fluorescent spots in both conditions proved that the dense structures are not a consequence of an improper tagging of ORF23 (figure 7B). The fact that these structures appear very early, before the massive accumulation of capsids in cytoplasmic areas, and are present in all tested cell lines contrast somewhat with the described HSV-1 assemblons. We then decided to further characterize those structures.

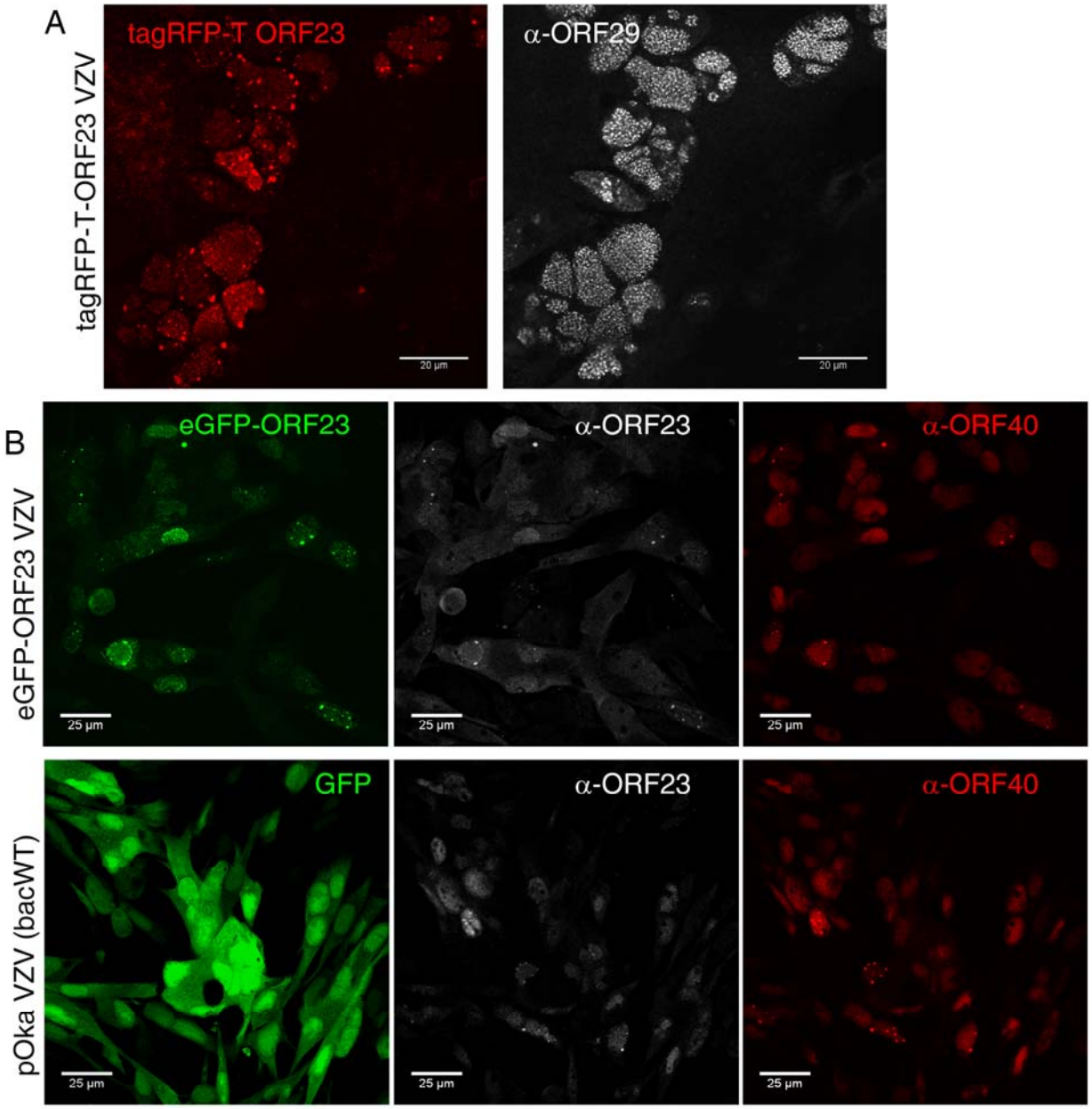


Figure 7. Nuclear dense structures also appear in tagRFP-T-ORF23 VZV- and pOka WT VZV-infected cells. (A) MeWo cells infected with tagRFP-T-ORF23 VZV for 48h were fixed and immunostained with an anti-ORF29 rabbit primary antibody followed by an Alexa633-anti-rabbit secondary antibody. (B) MeWo cells infected for 24h with eGFP-ORF23 in parallel with pOka (unfloxed bacWT) VZV were fixed and immunostained with both an anti-ORF23 rabbit antiserum and an anti-ORF40 mouse antibody. Alexa568-anti-mouse and Alexa633-anti-rabbit were used as secondary antibodies.

3. Characterization of the nuclear structures

3.1. Protein composition and localization regarding the replication compartment.

As we wanted to determine the nature and the potential functionality of these nuclear structures, we first wondered whether other viral proteins implicated in the different nuclear steps of virion formation (capsid and procapsid proteins, DNA replication and encapsidation proteins, proteins implicated in capsid nuclear egress), were present in these discrete nuclear compartments. Unfortunately, at that time, the recently characterized bank of VZV antibodies (Lenac Rovis et al., 2013) was not developed yet and the only commercially available antibody for these class of viral proteins was the antibody against the major capsid protein, ORF40. Thankfully, some teams had produced antibodies of interest and were willing to share their materials. We received an antibody against the single-strand DNA binding protein (ORF29) (from Dr. R. Cohrs, USA), an antibody against the common C-terminal part of the scaffold and maturing protease (ORF33/33.5) (from Dr. F. Rixon and V. Preston, UK), an antibody against the subunit 1 of the DNA packaging terminase (ORF42/45) and an antibody against the portal (ORF54) (both from Dr. Visalli, USA).

MRC5 cells were infected by a 20 minutes contact with eGFP-ORF23 VZV-infected MeWo cells followed by extensive washing. Coverslips were then fixed at 8, 12, 16, 24 and 36h p.i. and immunostained with all mentioned antibodies. Selected time points are presented in Figures 8, 9, and 10. The expression of ORF29 (ICP8 homolog) allowed us to delineate the RC (Figure 8, dashed line), which started to form around 8h p.i. as several spots that enlarged and fused in a single wide area around 24h p.i. The ORF29 labelling partially overlapped the eGFP signal (Figure 8, cyan arrows) but the co-localization area remained rather discrete and did not grow with the infection. We have to mention that anti-ICP8 labelling has been extensively used to locate what is called the replication compartment. This compartment was originally viewed as a nuclear area where viral genomic DNA is synthetised. More recent data have demonstrated that the formation of this compartment is strictly required for late gene expression and that it could well be subdivided in several subcompartments. Nevertheless, as a first approach, this result suggests that, if there is any, viral DNA replication can only occur in a very small part of this nuclear compartment, corresponding to the periphery of the structures. Small isolated eGFP-dots corresponding to individual capsids could be found sparsely within, as well as outside, the RC, albeit the vast majority of the eGFP larger spots were located at the

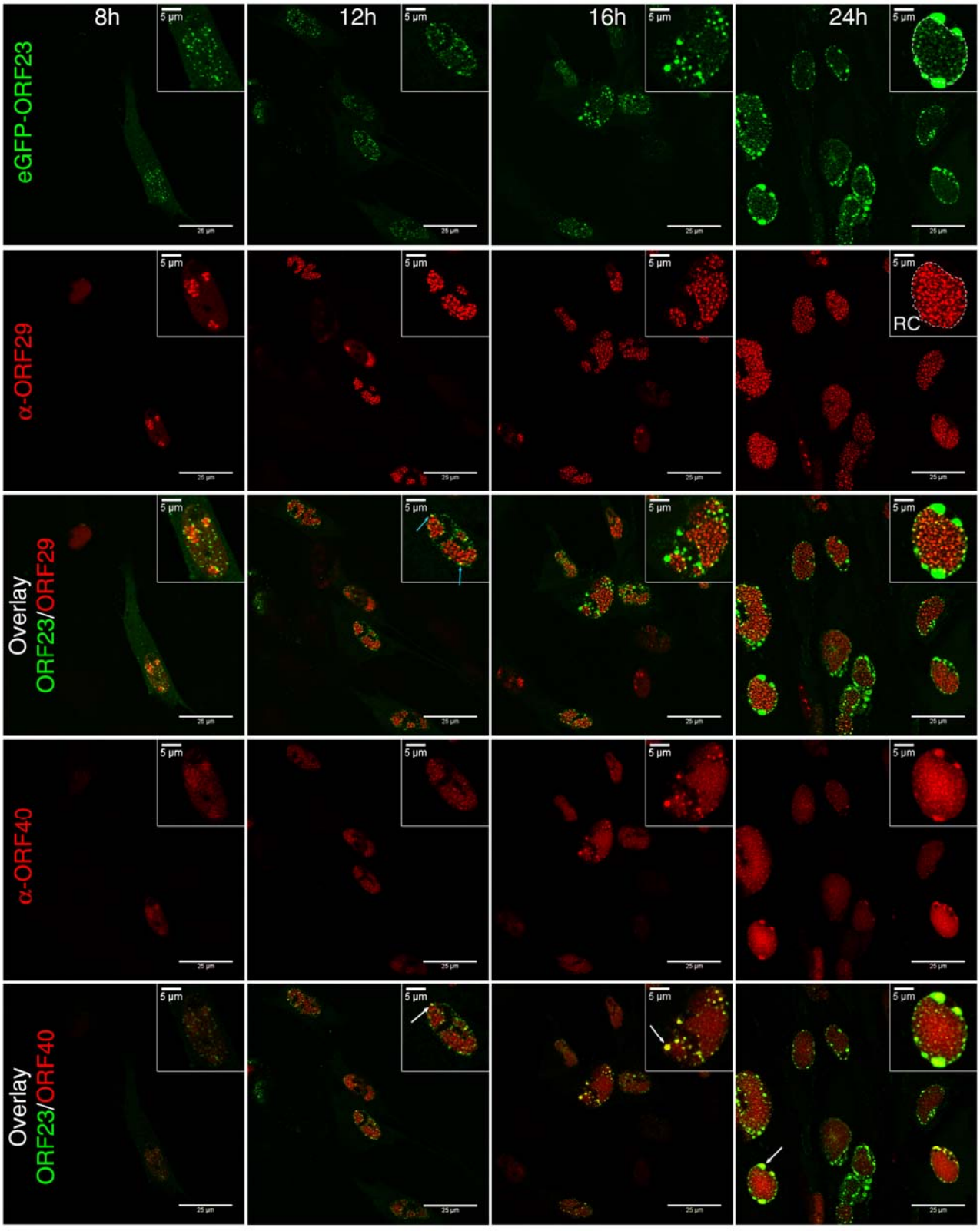


Figure 8. The nuclear dense structures contain the major capsid protein and partially overlap with ORF29. MRC5 infected with eGFP-ORF23 VZV were fixed at indicated times p.i. and immunostained with both mouse anti-ORF40 and rabbit anti-ORF29 primary antibodies. Alexa568-anti-rabbit and Alexa633-anti-mouse were used as secondary antibodies and nuclei were counterstained with Hoechst 33342. Confocal microscope images were captured with a 63x oil-objective. The ORF29 staining was used to draw the dashed line representing the RC, which was then reported on the eGFP picture. Cyan arrows show partial overlapping between eGFP-ORF23 and ORF29 signals. White arrows point to structures where ORF40 colocalizes with eGFP-ORF23.

periphery of the RC. The same localization of the larger fluorescent spots was

observed with the tagRFP-T-ORF23 VZV (figure 7), which corresponds to the location described for HSV-1 assemblons.

The major capsid protein (ORF40) was present in the nuclear structures from 12h p.i. and started to strongly accumulate at 16h p.i. (Figure 8, white arrows), whereas the presence of ORF33/33.5 corresponding to the viral procapsid scaffold was more delayed and was only substantially visible at late time points (24 and 36 hours p.i.) and in only certain very large structures (Figure 9). Compared to anti-ORF40 or anti-29, this antibody was clearly less sensitive, so we do not know whether this appearance only in late time points reflects a late accumulation or a late detection.

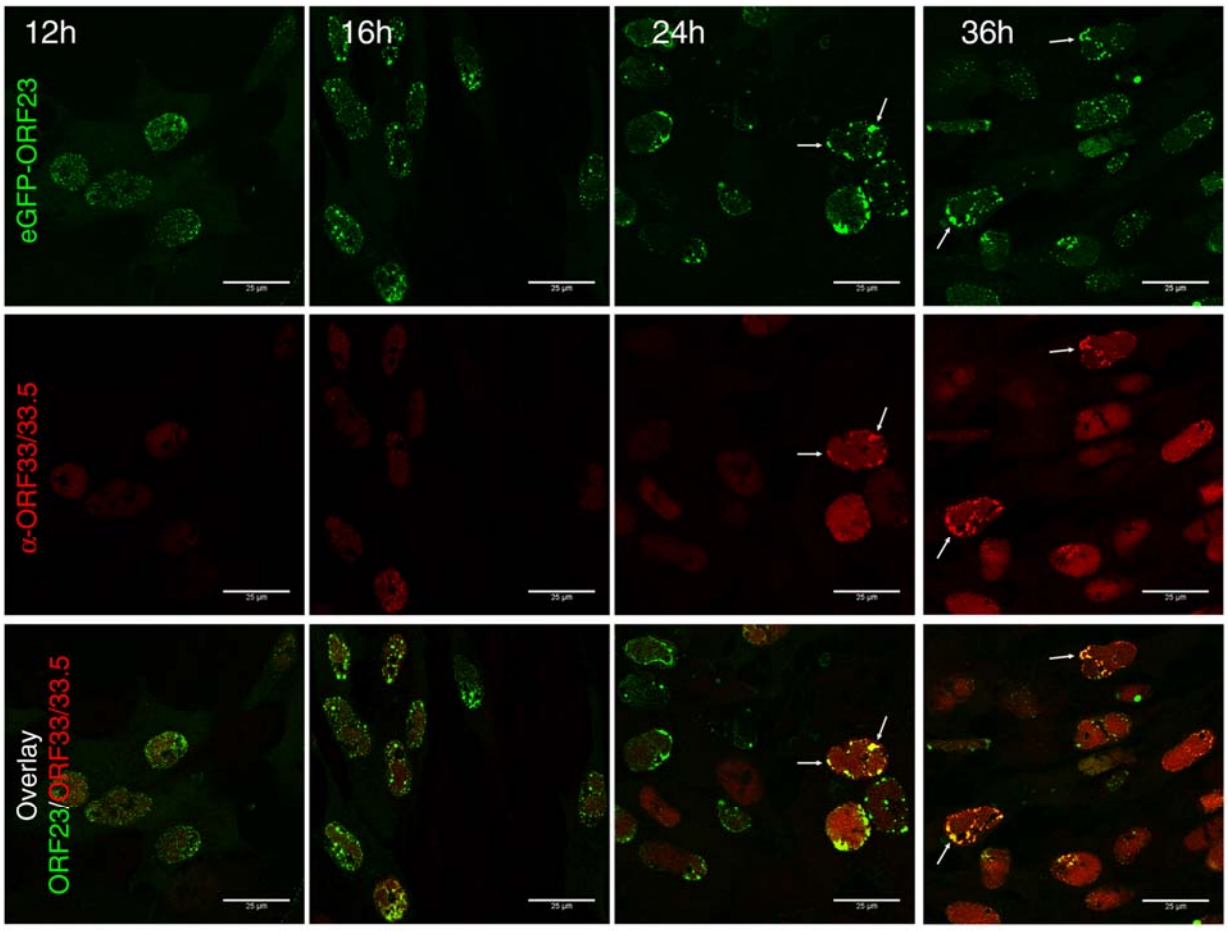


Figure 9. The procapsid scaffold protein is present in the nuclear dense structures. MRC5 infected with eGFP-ORF23 VZV were fixed at the indicated times p.i. and immunostained with an anti-ORF33/33.5 (scaffold protein and maturing protease) primary antibody and an Alexa568 secondary antibody. Confocal microscope images were captured with a 63x oil-objective. White arrows point to co-localization.

The DNA terminase subunit 1, encoded by ORF45/42 (UL15 homolog) never seemed to accumulate in the dense structures, even at very late time points (Figure 10). Rather, even if we cannot demonstrated it (both antibodies are from a rabbit origin), it seems to be distributed in the viral RC.

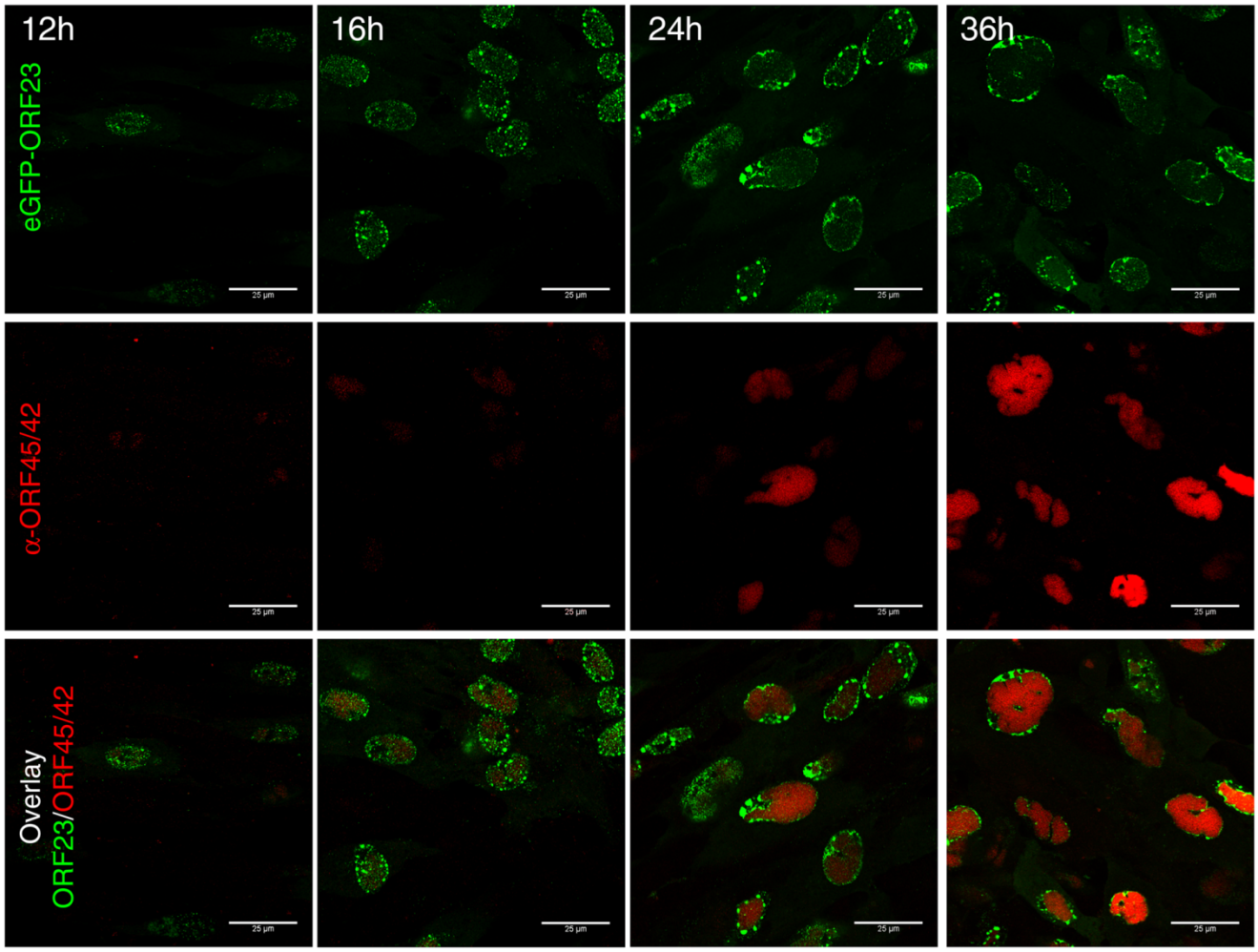


Figure 10. The DNA terminase subunit 1 is absent from the nuclear dense structures. MRC5 infected with eGFP-ORF23 VZV were fixed at the indicated times p.i. and immunostained with an anti-ORF45/42 (DNA terminase subunit 1) primary antibody and an Alexa568 secondary antibody. Confocal microscope images were captured with a 63x oil-objective.

Unfortunately, none of the three anti-ORF54 antibodies appeared to work in immunofluorescence. Two of them had been tested previously by the Visalli team, which reached the same conclusion. The third one was totally uncharacterized but, even after a long set-up only gave unspecific labelling.

We can conclude from these results that, like the HSV-1 assemblons, the observed structures ultimately contain important proteins implicated in capsid assembly but not the proteins that are implicated in DNA replication and encapsidation.

We next wondered whether the structures observed by confocal microscopy would be distinguishable at the ultrastructural level and if they were constituted of fully or partially assembled capsids or of capsid proteins.

3.2 Capsids proteins or fully/partially assembled capsids?

In order to precisely and irrefutably characterize the nature of the nuclear dense structures observed in confocal microscopy, we decided to carry out correlative microscopy.

MeWo cells were grown on a glass-bottom dish, infected for 48h with eGFP-ORF23 VZV, fixed with paraformaldehyde, stained with Hoechst and analyzed by confocal microscopy. A Z-stack image was recorded for each infection focus present on the glass coverslip that was then unglued from the plastic dish via a short contact with methanol. The cells on the coverslip were fixed again with glutaraldhehyde and osmium tetroxide, embedded in epon and processed for transmission electron microscopy (TEM). Ultrathin sections were carefully examined and compared with confocal images to clearly assess a concordance. Pictures of the chosen area were then systematically recorded on all available sections. The image of an infection focus was reconstructed from ten separate TEM images (Figure 11A) and compared with the confocal Z-stack and (Figure 11B).

We directly noticed the presence of capsid aggregates of various size, present most of the time at the periphery of the RC and that seemed to correspond to the intense fluorescent spots observed in confocal (Figure 12). We then wondered if this was the case for all dense fluorescent spots or if some of them would not contain any capsid. In order to properly interpret this experiment, one should remember that the section thickness in TEM is about 80 nm whereas the theoretical thickness of an optical section in laser-scanning confocal microscopy (LSCM) with our 60x-oil objective, even with the narrowest pinhole parameter is ≈ 500nm. Of note, this theoretical axial resolution is almost never reached in reality. This means that the signal detected in LSCM represents the sum of several successive z-sections observed in TEM. This implies that it is essential to take many TEM pictures of the same area on different serial z sections. This is critical to match properly the available pictures recorded originally in LSCM. Taking into account the relative thickness of sections in TEM relative to the optical section in LSCM and the well-known axial distortion, that makes spherical fluorescent objects look ovoid in confocal

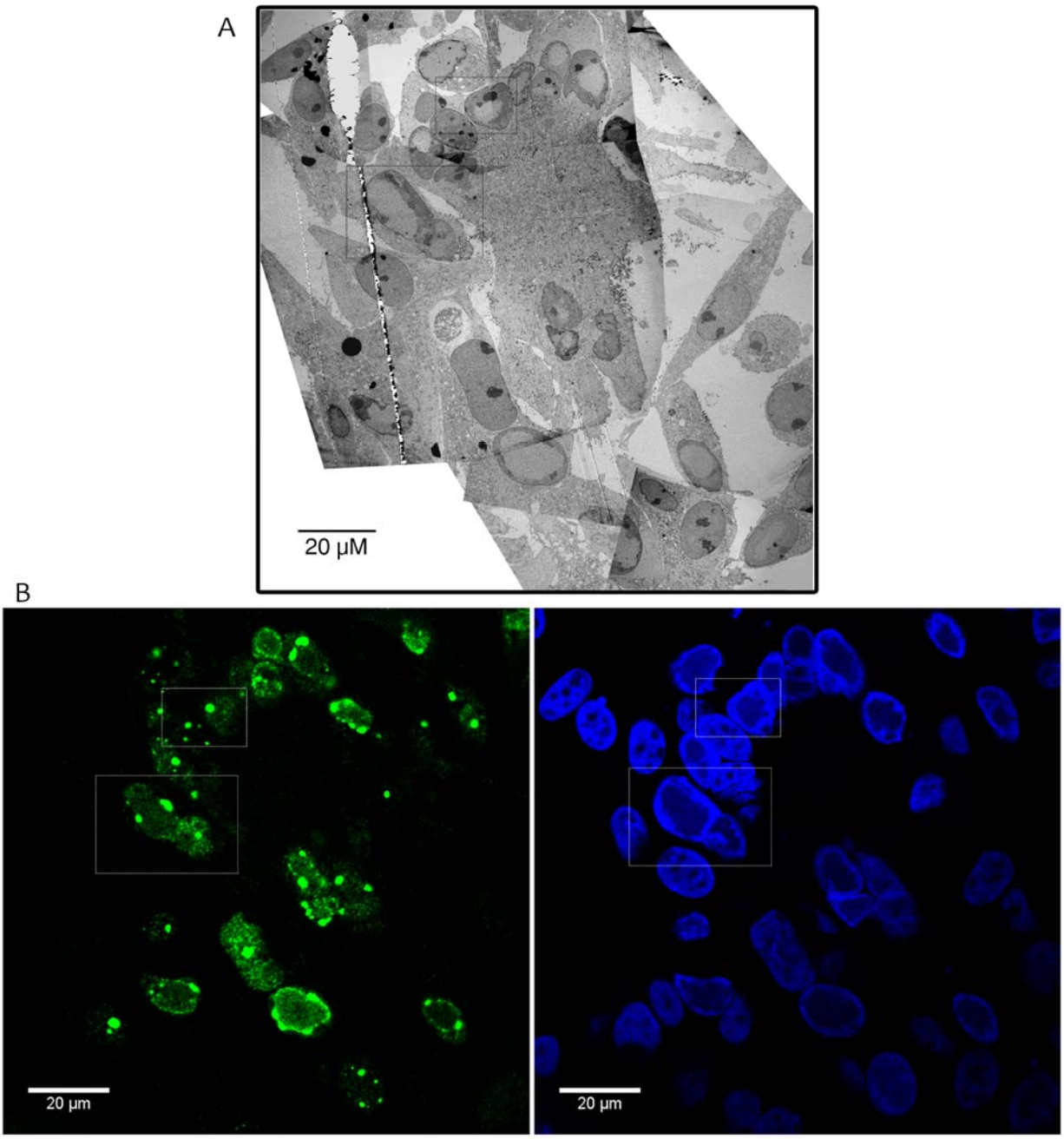


Figure 11. The nuclear dense structures are capsid aggregates. MeWo cells were infected for 48h with eGFP-ORF23 VZV in a glass-bottom dish. Cells were fixed, labeled with Hoechst and analyzed by confocal microscopy. A Z-stack of pictures was recorded for each infection focus. The glass coverslip was then separated from the plastic dish and the cell monolayer was re-fixed and embedded in Epon for analysis by Transmission Electron Microscopy. Several pictures obtained by TEM (A) were merged to reconstitute one of the infection focus identified by confocal microscopy (B); dotted line frames delineate areas chosen for panels at figure 13.

microscopy, the number of aggregates is then expected to be much lower in a TEM picture of one single ultrathin section than in a confocal image of one z-plane. It is also important to notice that the sectioning of the embedded cells for TEM is never perfectly parallel to the surface of the cells originally fixed on the glass, which adds a level of complexity.

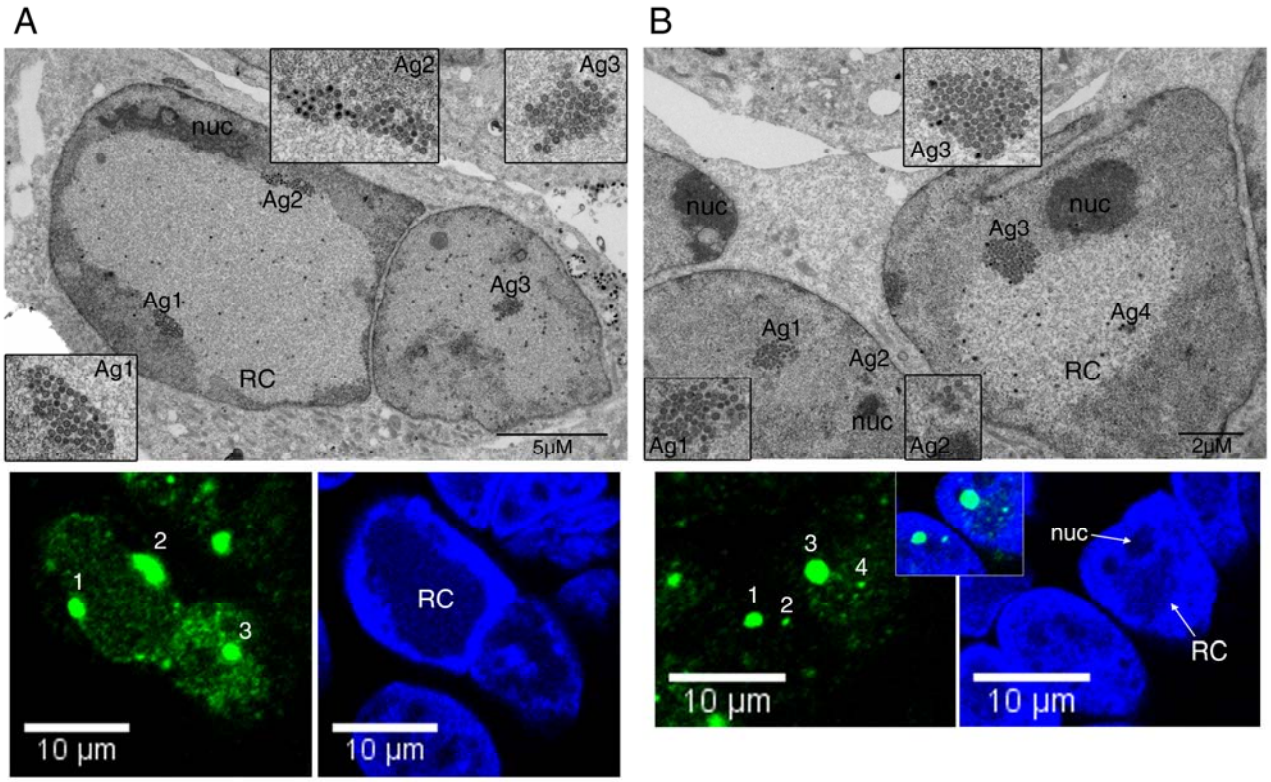


Figure 12. The nuclear dense structures are capsid aggregates. (A) and (B) Higher magnification of field portions from figure 12; Ag: capsid aggregates (numbered to make the correspondance between TEM and confocal images), nuc: nucleolus, RC: replication compartment.

Taking those potential sources of misinterpretation into account, we carefully examined all our pictures and reached the conclusion that each intense fluorescent spot, regardless of its size, corresponds, at the ultrastructural level to an array of capsids, almost always residing at the periphery of the RC. The latter was easily detectable as a paler area in the center of the nucleus. We confirmed that the size of the capsid aggregates increases over the time during the infection process by analyzing ultrathin sections of MeWo cells infected at 8h, 12h, 16h and 24h p.i. (data not shown).

In order to prove that the dense fluorescence structures observed in wild-type VZV-infected cells immunostained for both ORF23 and ORF40 truly reflect the presence of capsid aggregates in a wild-type infection, pOka VZV-infected MeWo cells were analyzed by TEM in parallel of eGFP-ORF23 VZV-infected cells. We indeed detected similar capsid aggregates with the wild-type virus (Figure 13). As for the eGFP-ORF23 virus, the aggregates were found at the periphery of the RC (figure 13B). By looking at the capsid content of the aggregates we were able to distinguish four types of capsids. Empty A-capsid and DNA-containing C-capsids were easily recognizable. A third type, "polka-dotted capsids", very frequent was considered, by

comparison to published data, to B-capsid. The fourth type of capsid comprised an inside ring surrounded by a thinner and more defined outer edge and corresponded, by comparison to what is known about HSV-1, to VZV procapsids (originally identified as "large-core B-capsids) (Newcomb et al., 2000; Thomsen et al., 1995) (Figure 13B).

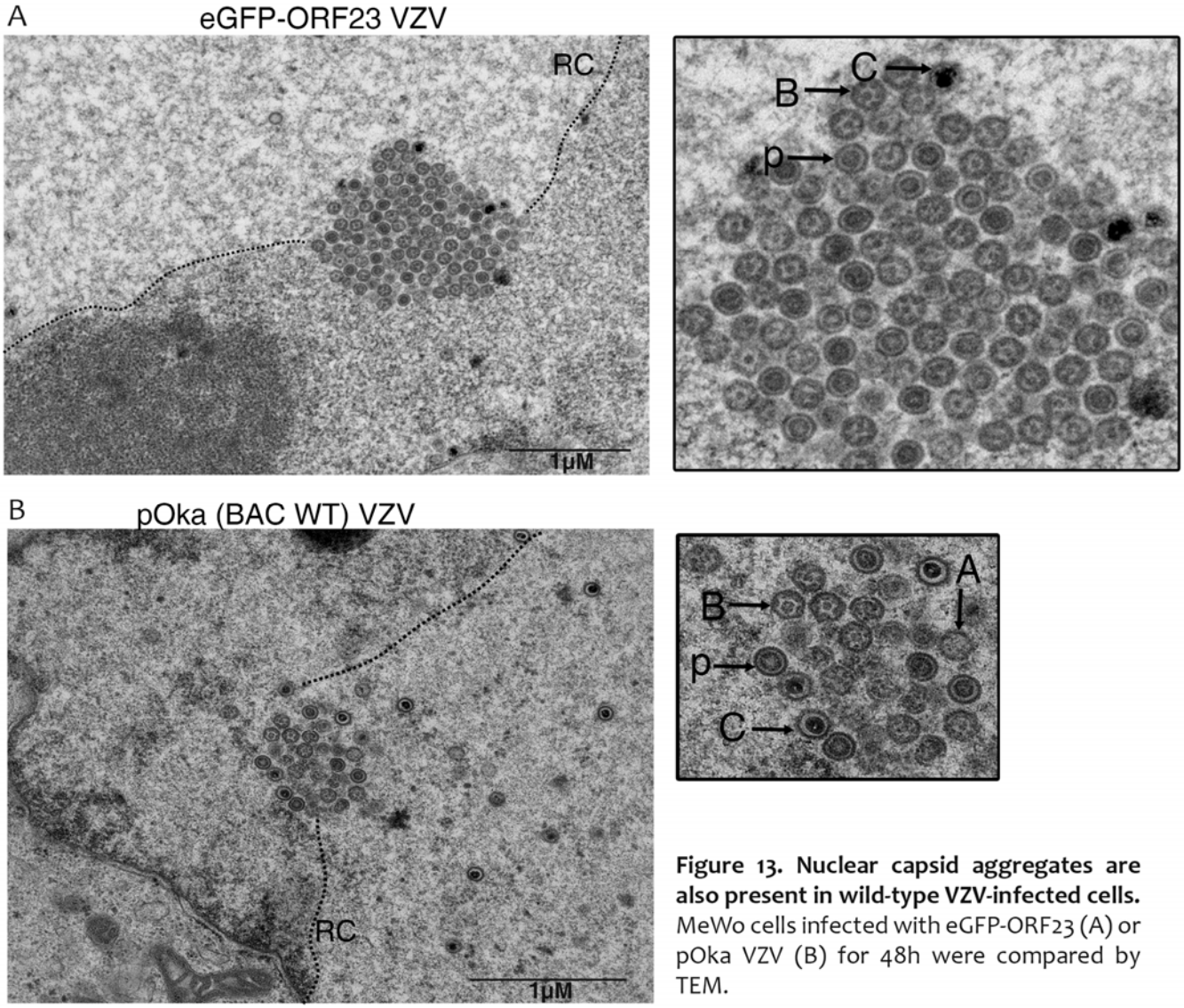
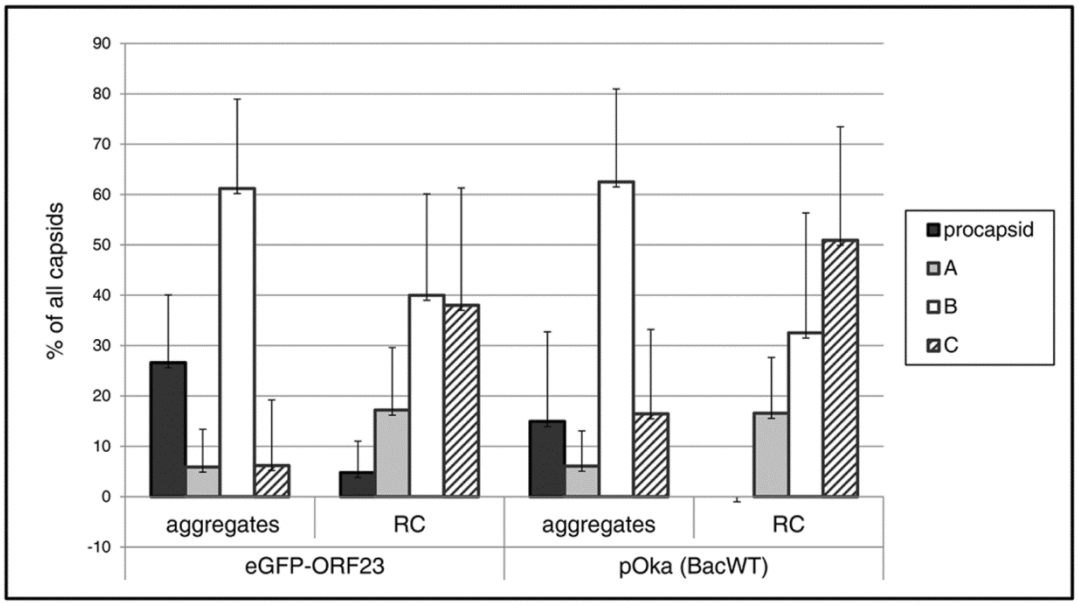


Figure 13. Nuclear capsid aggregates are also present in wild-type VZV-infected cells. MeWo cells infected with eGFP-ORF23 (A) or pOka VZV (B) for 48h were compared by TEM.

We then examined the capsid content of two separate nuclear compartments: the capsid aggregates and the RC. For the latter, we only kept nuclei for which a well-defined RC was present and to avoid bias due to small capsid aggregates and tangent sections of large capsid aggregates, which were present at the periphery of the RC, we excluded a 0.5 µM strip at the edge of the RC from the counting. Over three thousand capsids were counted (within 22 nuclei and 43 capsid aggregates of eGFP-ORF23 VZV-infected cells and 12 nuclei and 14 capsid aggregates for the wild-type infection) and the percentage of the four types calculated for each RC and each

aggregate. We noted that, for both the wild-type and the eGFP-ORF23 virurses, the predominant form of capsid found within the aggregates is the B type. Importantly, capsid aggregates were also enriched in procapsids, which were even totally absent from the central part of the RC in a wild-type infection. Pair-wise t-tests were ran to compare the mean percentage between the two compartment but also between the two viruses. For both viruses, we found that the mean percentage of each type of capsid within the aggregates was statistically different from that within the RC. We also found a significant higher amount of procapsids in the eGFP-ORF23 virus compared to the wild-type, both in the aggregates and in the RC (Figure 14).

A



B

Aggregates vs RC	procapsids	capsids A	capsids B	capsids C
eGFP-ORF23	<0,0001	<0,0001	<0,0001	<0,0001
pOka (BACWT)	0,0079	0,0192	0,0014	0,0002
eGFP-ORF23 vs pOka (BAC WT)	**procapsids**	**capsids A**	**capsids B**	**capsids C**
aggregates	0,012	0,9372 (NS)	0,8118 (NS)	0,0206
RC	0,0127	0,8826 (NS)	0,3390 (NS)	0,1285 (NS)

Figure 14. capsid content in the aggregates and in the the RC are different . MeWo cells infected with eGFP-ORF23 or pOka VZV were analyzed by TEM. The proportion of the four types of capsids (procapsids, A, B and C, black arrows) present within the aggregates and the replication compartment was determined by systematic counting of randomly chosen infected nuclei. Mean percentages are shown in (A); error bars represent the standard deviation. Mean percentages were pairwise compared using a two-tailed t-test in order to determine statistically significant differences; p values are presented in (B), NS: non-signifi-

During this analysis, we noticed that the distribution of C capsids within the aggregates did not seem random. We then decided to classify them regarding to their position within the aggregates. C capsids that were part of the most exterior

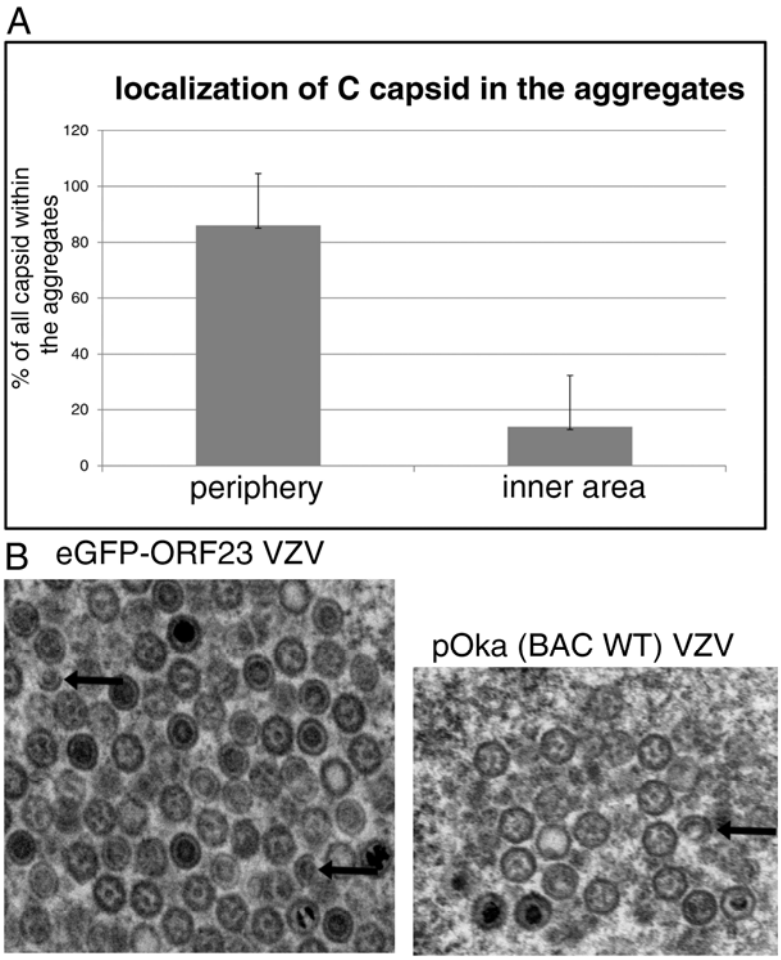


Figure 15. Nuclear capsid aggregates present a characteristic organization and contain late partial procapsids. MeWo cells infected with eGFP-ORF23 or pOka VZV were analyzed by TEM. The C capsids present within the aggregates were divided into two categories on the basis of their localization; periphery was strictly determined as the most exterior layer of capsids and mean percentage are shown, with error bars representing the standard deviation. (D) Late partial capsids can be found in the nuclear capsid aggregates of both viruses

layer of capsids were considered as belonging to the periphery. Based on this criteria, we observed that 86% of C-capsids within the aggregates actually resided at the periphery (Figure 15A). If we considered the periphery as the two most external layers of capsid, this percentage peaked to over 95% (data not shown). Another very interesting point was the presence of some partial capsids that were not observed elsewhere in the infected nuclei (Figure 15B) and which are thought to be precursors of procapsids.

The fact that the capsid composition of the aggregates does not reflect the one found in the RC, the presence of numerous procapsids and few partial capsids as well as the "not-random" organization indicated that those structures might represent a functional compartment possibly implicated in capsid assembly, rather than a simple accumulation over time. This prompted us to characterize the dynamics within the structures.

4. Dynamic or static structures?

We first tried to visualized the initial formation of the aggregates. For this, we infected MRC5 cells via cell contact with eGFP-ORF23 VZV-infected MeWo cells for 12 hours and then selected cells in which isolated capsids were clearly visible in the nucleus but in which not evident aggregate was present. We then took pictures every 2 seconds over a 15 minutes time frame. Most of the capsids were in constant movement but a few static fluorescent dots could also be observed (movie S3). During the 15 minutes time frame, some of those static dots developed into nascent aggregates whose size constantly evolved whilst seemingly preserving their initial nuclear location (movie S3, white arrow). Isolated capsids seemed to constantly move from and towards the aggregates.

We next studied the dynamics inside the aggregates via a Fluorescence Recovery After Photobleaching (FRAP) experiment. Infected MRC5 were analyzed 16h p.i. in order to obtain aggregates of moderate size. We bleached a region of interest (ROI) corresponding to half of the aggregate during 45 seconds (Figure 16 and movie S4) and then took pictures every 15 seconds. Two minutes post-bleaching, the fluorescence already started to reappear in the bleached area, but it took 10 to 15 minutes to return to the original situation where the fluorescence was homogenous overall the entire structure. This speed of fluorescence recovery was not compatible with a fast protein diffusion, like that observed if we bleached a ROI of diffused eGFP in the cytoplasm corresponding to the signal of the fusion protein rather than fully assembled capsid (data not shown). On the contrary, it could be compatible with a movement of capsids within the aggregates, which is expected to be much slower. We concluded from this experiment that the capsids in the aggregates are constantly reorganized.

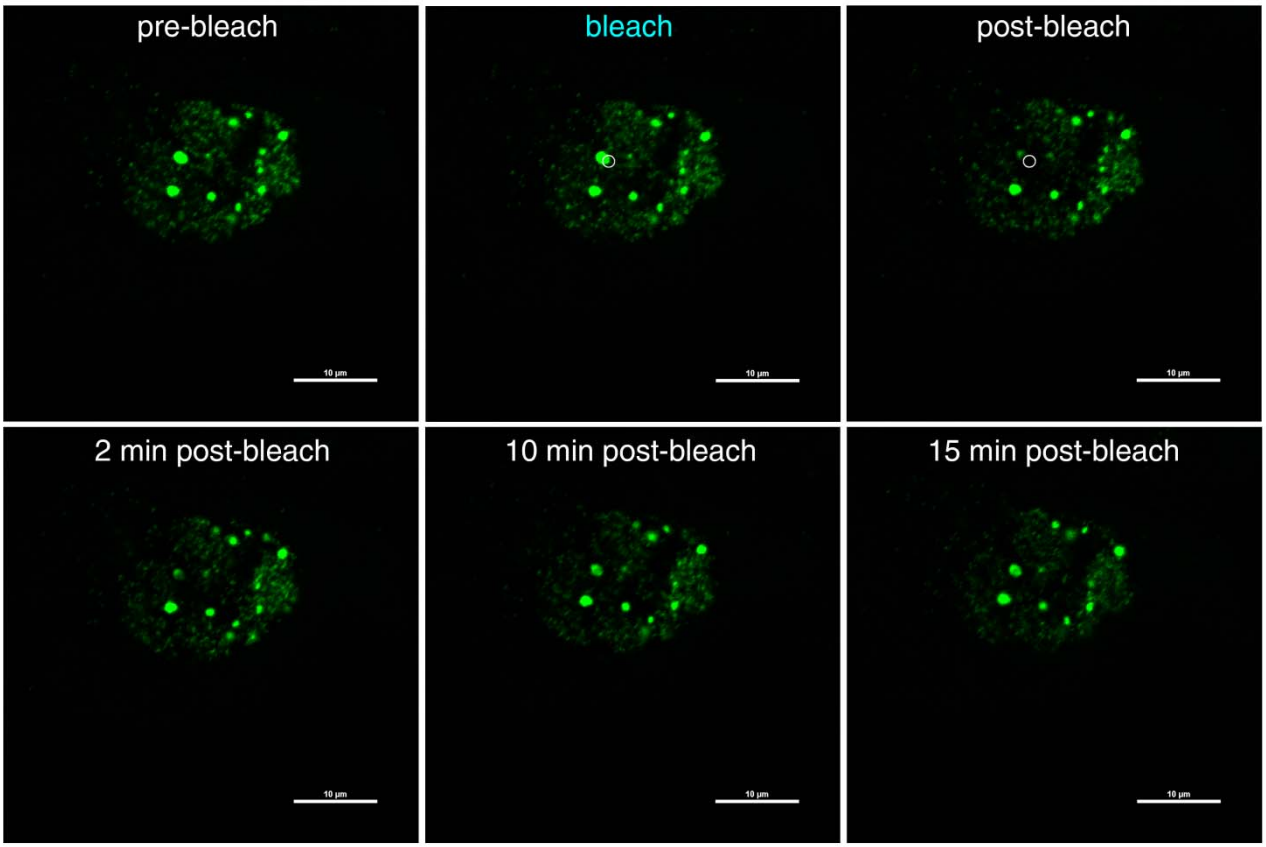


Figure 16. FRAP experiment showing that capsids inside aggregates are not static. MRC5 cells were infected for 16h with eGFP-ORF23 VZV via cell contact followed by extensive washing. A region of interest (ROI) represented by the white circle was bleached for 45 seconds and then an image was recorded every 15 seconds for 15 minutes. Selected time points are shown. Confocal microscope images were captured with a 60x oil-objective equipped with a chamber controlling the temperature and the CO_2 level.

If they were truly assembly sites, we would expect that once assembled, the capsids would leave the structures to move towards the nuclear envelope and finally exit the nucleus. We then decided to see what would happen if the structures were constantly bleached and performed a Fluorescence Loss In Photobleaching (FLIP) experiment. We infected MRC5 for 16h and demonstrated that if the structures were bleached constantly for a certain time, an entire area of the nucleus was bleached (Figure 17 and movie S5). One should note that around 16 hours p.i., the RC was very frequently still divided into several areas separated by a thin layer of cellular genomic DNA and each of these replication sub-compartments contained one to three capsid aggregates.

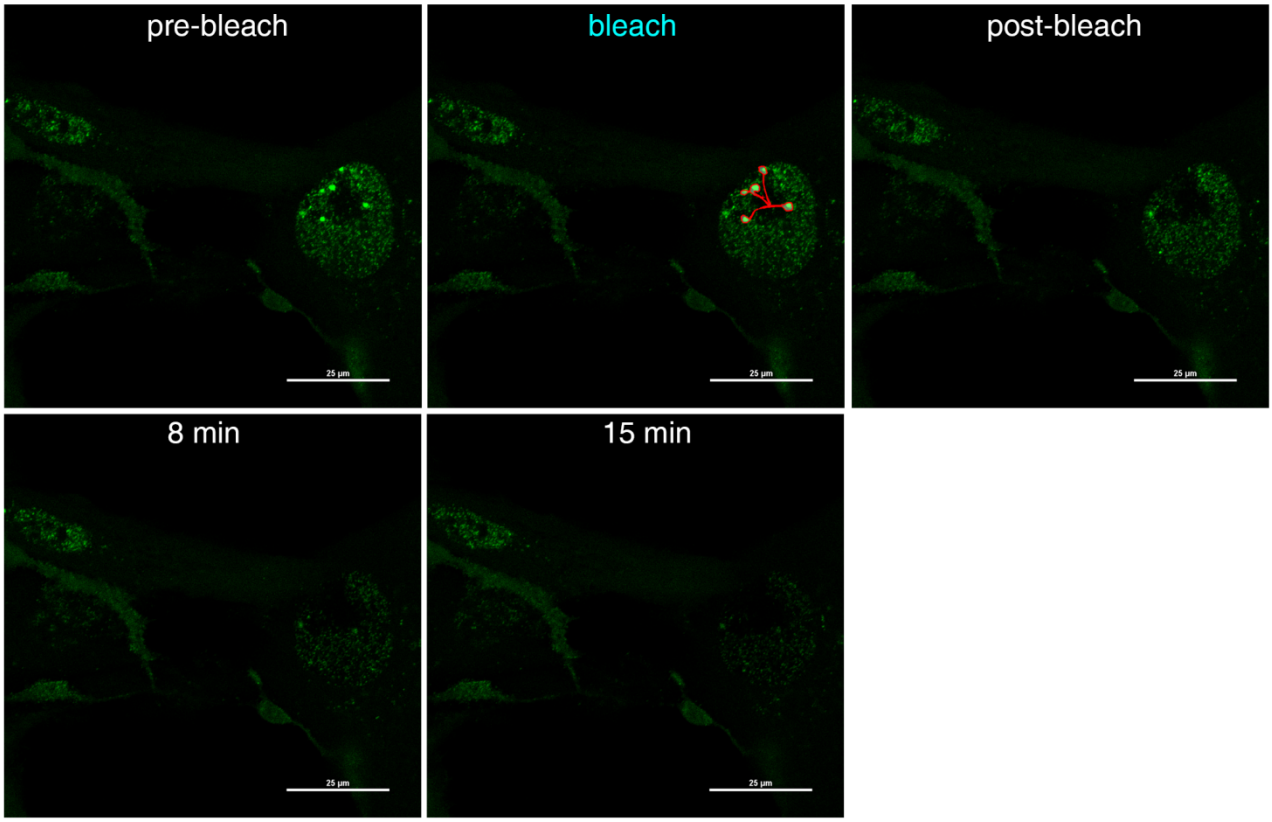


Figure 17. FLIP experiment showing that capsids within aggregates move from there. MRC5 cells were infected for 16h with eGFP-ORF23 VZV via cell contact followed by extensive washing. A ROI represented by the red shape was first bleached for 1 minute before successive steps of bleaching and image acquisition. Selected time points are shown. Confocal microscope images were captured with a 60x oil-objective equipped with a chamber controlling the temperature and the CO_2 level.

Surprisingly, the analysis of several time-lapse imaging experiment carried out over longer periods of time demonstrated that the capsid aggregates could sometimes give rise to a massive simultaneous transfer of a large amount of capsids from the nucleus to the cytoplasm (movie S6, white arrows). Further experiments would be required in order to evaluate the importance of this phenomenon for the VZV lytic cycle and to determine whether this massive transfer occurs via a budding through the inner nuclear membrane or maybe via a transient enlargement of the nuclear pores.

We finally wondered whether some aggregates were the site of some capsid maturation process such as partial tegumentation that potentially would be important for the future nuclear egress.

5. Localization of tegument proteins.

As said above, antibodies against VZV proteins, in particular against structural proteins, were quite sparse and were not always suitable for immunostaining. Moreover, as we aimed to decipher potential tegument association in living cells, dually fluorescent VZV were created, starting from our eGFP-ORF23 BAC construct. Different proteins were chosen: ORF21 and ORF22, homologous to HSV-1 UL37 and UL36, respectively, which are supposed to belong to the inner tegument, ORF38, the VZV pUL21 homolog, ORF9, corresponding to HSV-1 VP22, the most expressed gene during the lytic cycle and supposedly the major tegument protein and ORF63, also well-known to be part of the tegument. As we had already determined from the targeting of ORF23 that the orientation of the fluorescent tag regarding the gene could be of a great importance, we directly tested both possibilities. Moreover, because some of these genes are expressed at a low level, we tested two different red fluorescent tags: DsRed-monomer (referred to as "mDsRed") and tagRFP-T (referred to as "tRFP-T"). Only a few number of "targeted-genes/position of the tag/nature of the tag" combinations gave rise to infectious VZV after transfection of the corresponding BAC in MeWo cells (Table 1). Other constructs either totally failed to lead to infection foci, even four weeks after transfection, or engendered very slowly replicating viruses (data not shown).

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BAC	viral growth
ORF9-DsRed	slow replication
DsRed-ORF9	no
tagRFP-T-ORF9	no
ORF9-tagRFP-T	yes
ORF21-DsRed	no
DsRed-ORF21	yes
tagRFP-T-ORF21	yes
ORF22-DsRed	yes
DsRed-ORF22	no
tagRFP-T-ORF22	no
ORF38-DsRed	yes
DsRed-ORF38	no
ORF38-tagRFP-T	no
ORF63-tagRP-T	yes
tagRFP-T-ORF63	yes
ORF63-tagRP-T/ORF70-tagRFP-T	yes

Table 1. BAC contructs generated to create dually fluorescent VZV.

We evaluated the viral growth of the different recombinant strains The addition of a second fluorescent tag did not seem to deeply impact the replication kinetics of the virus (Figure 18B).

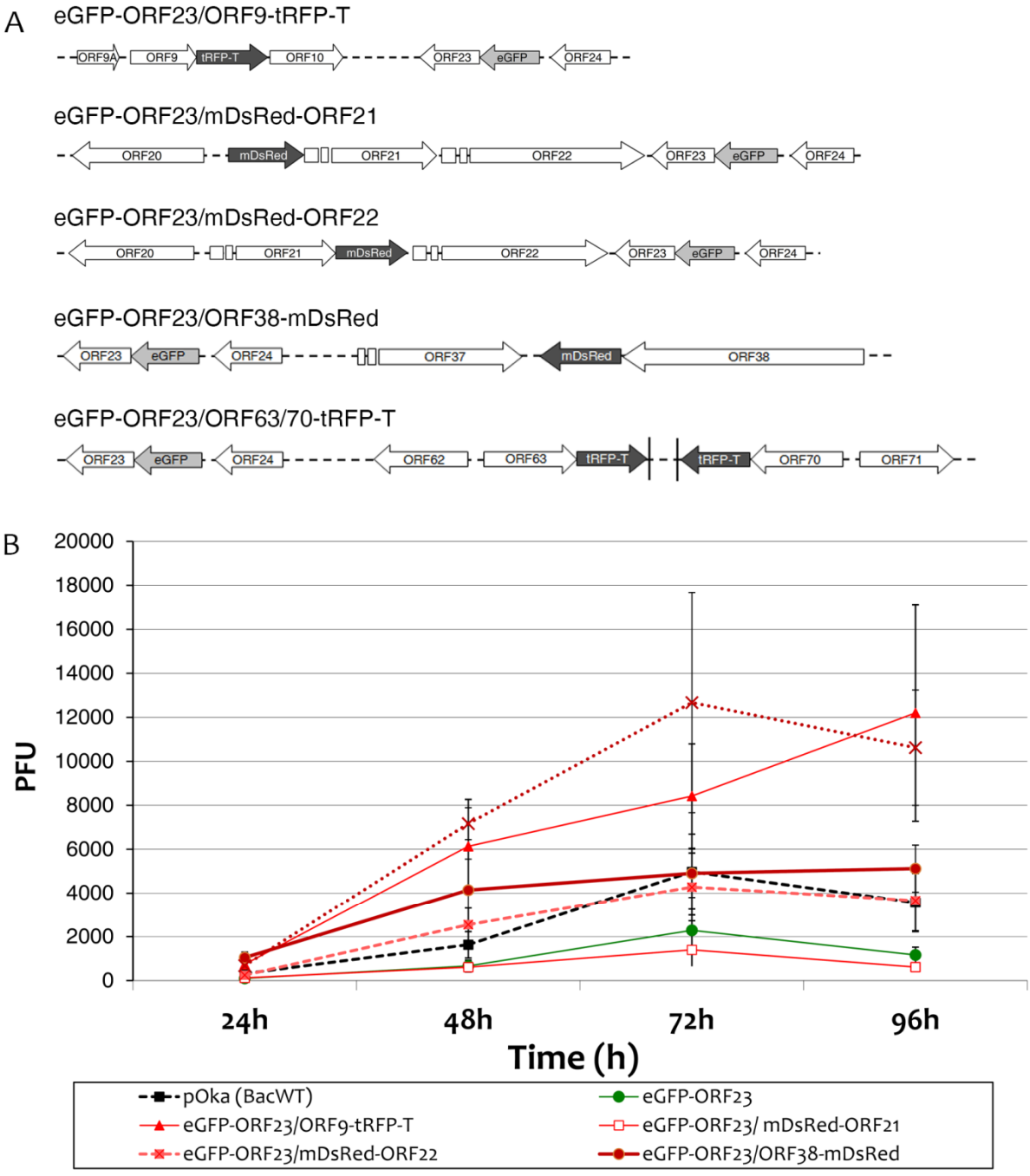


Figure 18. Construction and growth ability of dually fluorescent VZV strains. (A) Schematic representation of the genomic region that has been modified in the BACVZV eGFP-ORF23 to create the dually fluorescent viruses. (B) Growth curve of the dually fluorescent viruses compared to the eGFP-ORF23. The same technique was used as described in Figure 4, except that 500 infected cells were used instead of 200. Means of a representative experiment out of three are depicted; error bars represent the standard deviation.

We analyzed the distribution of the red fluorescence in infected living MeWo cells. Even though ORF21 , ORF22 are mostly cytoplasmic and ORF38 present both in the cytoplasm and the nucleus, all three were present as well in some of the capsid aggregates (Figure 19, white arrows).

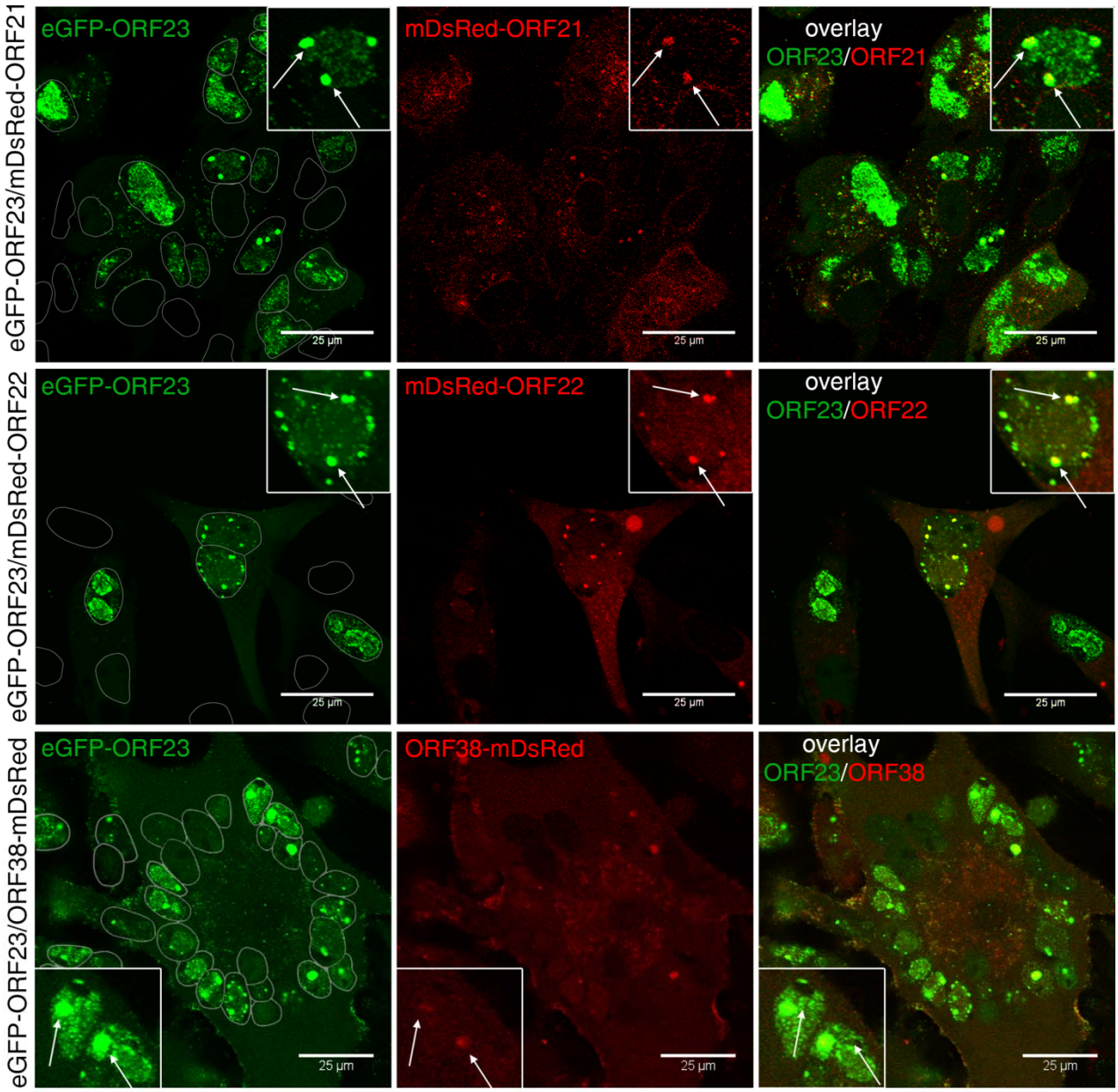


Figure 19. ORF21, ORF22 and ORF38 tegument proteins co-localize with nuclear capsid aggregates . Live visualization of MeWo cells infected for 24h with eGFP-ORF23/mDsRed-ORF21, eGFP-OR23/mDsRed-ORF22 or eGFP-ORF23/ORF38-mDsRed. Nuclei were labeled with Hoechst and confocal microscope images were captured with a 63x oil-objective. White arrows in insets point to co-localization between eGFP-ORF23p and mDsRed-ORF21 or mDsRed-ORF22; solid grey lines delineate nuclei from the Hoechst labeling.

Most of the time though, only a portion of the eGFP-ORF23 area was also positive for either mDsRed-ORF21, mDsRed-ORF22 or ORF38-mDsRed (Fig. 19, insets). In the cytoplasm, ORF38 is accumulating in the TGN area and is also enriched in the viral nuclear RC (data not shown).

The three proteins seem to accumulate and colocalize with the green signal at the cell periphery in strongly infected syncitia and a portion of isolated cytoplasmic caspids appears both green and red, which indicates that they are likely incorporated in the mature virions. Unfortunately, the intensity of the red signal is too weak to reliably study the incorporation of the tegument at the single particle level.

ORF63-tRFP-T is diffusely present in both the cytoplasmic and the nuclear compartment, accumulating in this latter in the RC (Figure 20). This localization reflects the one observed by immunofluorescence on wild-type VZV infected cells labelled with an anti-ORF63 antibody (data not shown). Even though it has been shown to be part of the tegument, neither cytoplasmic nor extracellular viral particles appear dually fluorescent. The amount of incorporated protein might be

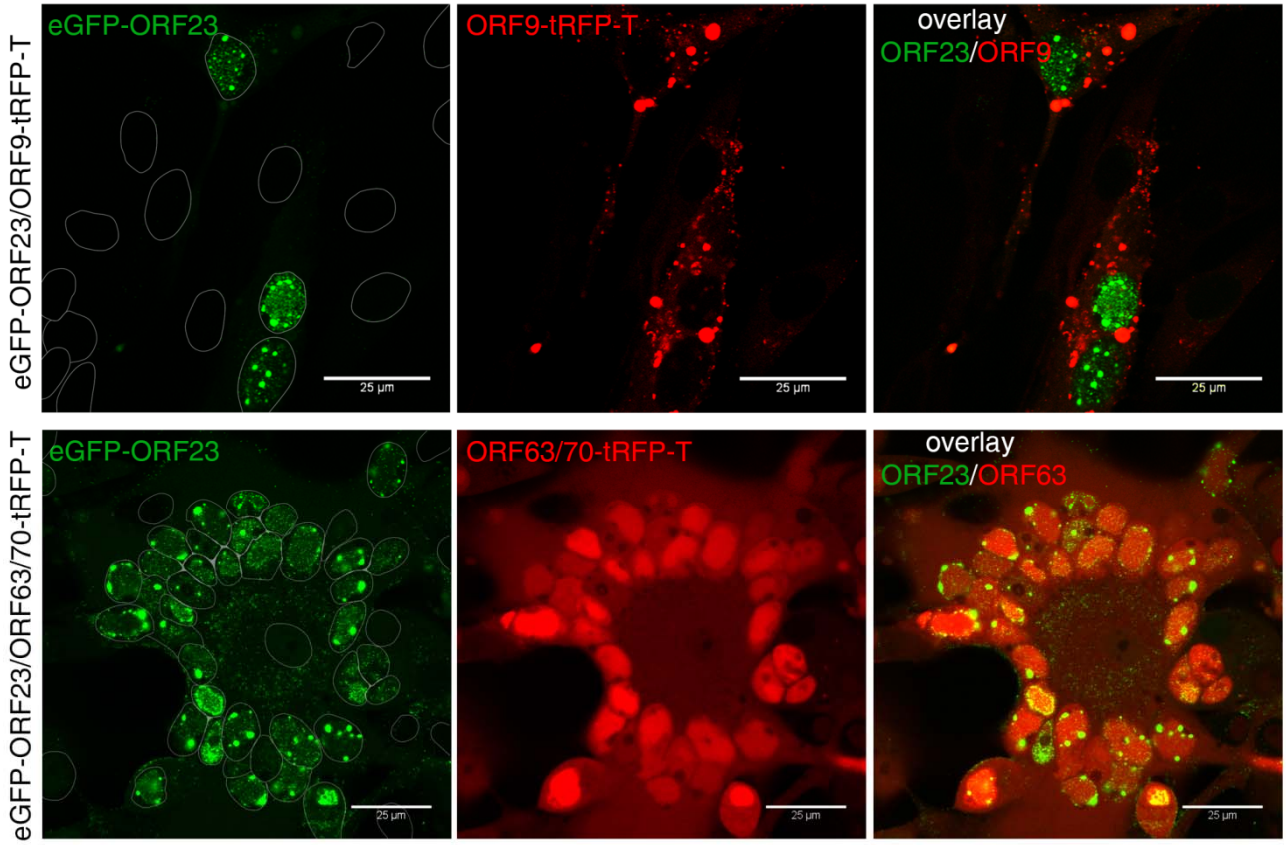


Figure 20. ORF9 and ORF63 tegument proteins do not co-localize with nuclear capsid aggregates. Live visualization of MeWo cells infected for 24h with eGFP-ORF23/ORF9-tRFP-T and for 36h with eGFP-ORF23/ORF63/70-tRFP-T. Nuclei were labeled with Hoechst and confocal microscope images were captured with a 63x oil-objective. White arrows in insets point to co-localization between eGFP-ORF23 and mDsRed-ORF21 or mDsRed-ORF22; solid grey lines delineate nuclei from the Hoechst labeling.

too low compared to the large amount of cytoplasmic protein.

ORF9, which was mostly displayed as cytoplasmic aggregates, was never detected in these nuclear structures.

6. Discussion PartI

The entire process of VZV virion formation and egress is far from being fully elucidated. Nucleocapsid formation in the infected cells leads to dramatic changes in the nuclear architecture, the role of which is not entirely understood.

In order to better understand these processes, we created a virus with the small capsid protein ORF23 fused to the eGFP. With this virus, we identified fluorescent dense nuclear structures emerging around 8 to 12 hours p.i. and enlarging as the infection progressed. As revealed by confocal microscopy, the identified structures contain capsid and procapsid proteins as well as some proteins that supposedly belong to the inner tegument. This prompted us to question whether they were similar to the already described HSV-1 assemblons. These structures were first identified in optical microscopy with antibodies against capsid proteins but the fusion of HSV-1 VP26 with different fluorescent tags has demonstrated that this protein forms peculiar aggregates whose size increases in the course of the infection and likely correspond to the assemblons (de Oliveira et al., 2008; Desai and Person, 1998). Remarkably, the C-terminal region of VP-26, containing stretches of conserved residues, has been demonstrated to carry an interaction motif with the capsids and to redirect VP26 to nuclear spots defined as assembly sites (Desai et al., 2003). Nevertheless, attempts to relate those intense fluorescent spots with some virus-induced structures, discernible on ultrathin sections of infected cells, have remained quite scarce (Feierbach et al., 2006; Nagel et al., 2012). A recent publication has even shown that the fusion of HSV-1 VP26 with some fluorescent tags can lead to the formation of fusion protein aggregates impeding viral growth (Nagel et al., 2012). Here, in VZV-infected MeWo cells, we showed, via correlative microscopy, that the nuclear structures observed in confocal microscopy actually correspond to capsid aggregates identified in electron microscopy. We compared our eGFP-ORF23 VZV with a wild-type virus, and observed similar nuclear dense structures in confocal microscopy corresponding to similar capsid aggregates found in TEM. This proves that the structures described here were not artefactually-induced by the eGFP-ORF23 fusion protein.

The major question to be asked concerns the potential functionality of these nuclear dense structures. The presence of numerous A, B and C capsids, as well as many procapsids, suggests that they may indeed represent sites where assembly can take place. Procapsids are usually rarely seen in the nucleus of infected cells, due to their rapid maturation into A, B and C capsids (Newcomb et al., 1996; Trus et al., 1996). The high percentage of procapsids within the aggregates, and the identification, only in this nuclear compartment, of partial procapsids, representing earlier stages of assembly (Newcomb et al., 1999), are strong arguments in favour of a functional role of these structures. Of course, these sites are probably not the sole location of capsid formation, since some infected nuclei are devoid of them and the first newly-formed capsids appear randomly distributed within the RC. Of note, while procapsids are completely absent from the RC in a wild-type infection, a significant amount of them can be found in the RC of cells infected with the eGFP-ORF23 VZV. Moreover, the proportion of procapsids within the aggregates is significantly higher for the eGFP-ORF23 compared to the wild-type virus (26.6% vs. 14.9%). We believe that this may reflect a slower maturation rate, probably due to the presence of the eGFP tag, and this could, in part, explain the growth defect of the recombinant virus compared to the wild-type. The presence of the tag could, for example, impact the assembly of DNA encapsidation complex, the accessibility of the portal or the expulsion of the scaffold.

This could partially reconcile the data presented by Nagel et al. with ours. Nagel et al. have demonstrated that the size of the fluorescent foci observed with the different fluorescent-tag-VP26 fusion proteins is inversely correlated with the growth ability of the recombinant viral strain. We think that this could actually reflect a different degree of defect in the capsid maturation process. The TEM picture of their correlative microscopy analysis contains many capsid aggregates similar to the ones we observed but they did not insist on this particular point. It would be very interesting to perform correlative microscopy on cells infected with all the different HSV-1 strains used in their study, in parallel with cells transfected with the BAC constructs that never led to a productive infection, to search for capsid aggregates and, if present, to analyze their size and their capsid content over time. It is conceivable that the nature of the fluorescent tag or the 3-D conformation of the fusion protein differently affects the maturation of procapsids to A, B and C capsids, maybe DNA encapsidation is totally prevented in some cases, which is leading to a non viable viral strain. This could completely explain the fact that the deletion of VP26-encoding gene has a lower impact on viral growth than its fusion with some

fluorescent proteins encoding genes. Of interesting note, Chauduri et al.(2008) had also reached the conclusion that the deletion of VZV ORF23 was compatible with viral growth whereas it fusion at the C-terminal end with a red tag was not. We, accordingly, never obtained infectious virus after the transfection of the BAC ORF23-eGFP. As concluded by Nagel, we believe that the nature of the tag is determining the growth ability of ORF23 fluorescent viral strains. Nevertheless, the fact that we were unable to recover infectious virus from mYFP-ORF23 or mCFP-ORF23 BAC constructs indicates that the dimerization properties of the tag is not the sole parameter to consider (mYFP and mCFP are monomeric variants of the respective YFP and CFP fluorescent proteins).

If this hypothesis is true, the fact that we observed a few procapsids within the RC of eGFP-ORF23 VZV-infected nuclei suggests that, indeed, a few number of capsids are produced in this nuclear compartment but that, in a wild-type infection, the procapsids are very rapidly converted in ulterior maturation stages, rendering their visualization quite difficult.

Another interesting point is that the capsids within the aggregates are not organized randomly. C-capsids are almost always found at their periphery, and, also, the proportion of the different types of capsids does not mirror that found in the rest of the nucleus. If the aggregates were simply due to an excess of capsids at late time points of infection, these observations would not be expected. It is tempting to speculate that DNA encapsidation occurs at the periphery of the aggregates, but our data cannot exclude the possibility that some C capsids produced in the RC could later be sequestered at the periphery of these aggregates. A recent paper on HCMV has demonstrated that the replication of the genomic DNA only occurs at the periphery of the RC, and that replicated molecules would then move towards the centre of the RC. They showed that an active replication is necessary to maintain the architecture of the RC and they suggested that this latter display a much more complex organization that previously anticipated (Strang et al., 2012). We will discuss this particular point in the final discussion.

The presence of numerous B capsids, which are largely thought to be dead end particles, in the aggregates provides an argument for a depot of mostly immature capsids with no function in the viral life cycle. Nevertheless, if this was the case, we would expect within the aggregates a stronger enrichment of this type of capsid compared to the enrichment of procapsids. In fact, B capsids are the most abundant type of capsid produced during the infection. For the eGFP-ORF23 VZV, they

represent 40% of the capsids in the RC and 60% percent of the capsids within the aggregates. This corresponds to a 1,5 fold enrichment. Procapsids represent 5% of the capsids found in the RC and 25% of the capsids present within the aggregates, corresponding to a 5-fold enrichment. Of course, we did not evaluate if the composition of the aggregates was changing in the course of the infection. It would be very interesting to measure the proportion of the different types of capsid within the aggregates over time to evaluate whether their increasing size is mainly attributable to an increasing proportion of B capsids. Of note, the idea that B capsids might represent maturation intermediates rather than dead-end by-product, based on an old publication from Perdue, is still supported by some teams (Perdue et al., 1976). This theory would be compatible with a role for these structures in capsid assembly.

We demonstrated by FRAP that there is a constant movement of capsids within the structures and time-lapse experiments demonstrated that their initial apparition seems to be a dynamic process. Of note, an interesting study on PRV showed that the formation of capsid assembly sites within the nucleus is a dynamic process requiring a virally induced "nucleo-squeleton" (Feierbach et al., 2006). In their study, they also identified capsid aggregates whose size increases when the cells are treated with Jasplakinolide, a drug known to stabilize actin filament. They also demonstrated that the capsids aggregates colocalized with an actin motor, Myosin Va, but not with myosin I or II. We actually performed an immunostaining against MyoVa in VZV-infected cells but found no association, no matter the cell lines used (MeWo, MRC5 or HFF). Of course, as MyoVa is an actin-motor quite specific for neurons, but supposedly also present in melanocytes, it is possible that another actin-base motor is implicated in the cell line we used. It would be interesting to treat eGFP-ORF23 VZV infected cells with several cytoskeleton stabilizing and de-stabilizing agents to evaluate the formation of VZV assemblons in these conditions.

The FLIP technology allowed us to demonstrate that the capsids residing in the structures can subsequently relocalize elsewhere in the replication compartment, which is a crucial point if the capsid aggregates truly represent assembly sites. It would have been of course very interesting to label ORF23 with a photoswitchable tag that would have allow to track the photoswitched capsids further down the egress process.

We cannot exclude the possibility that the dynamic nature and the growing size of the aggregates could also reflect an inability of the capsids to efficiently egress the

nucleus towards the cytoplasm. In that case, aggregates could then be locations where capsids simply accumulate, maybe in order to gain some of the properties that are necessary for the future egress or simply waiting for some processes to be achieved, like the disruption of the nuclear lamina or the maturation of the RC, subsequently giving the capsids access to the inner nuclear membrane through which they need to bud.

The ultimate demonstration that the structures identified here have a crucial role for the virus life cycle would require a system in which we can impede their formation and show that it impacts capsid formation or egress, which is, unfortunately, difficult to set up. Nevertheless, attempts to prove that the nuclear punctuated patterning of VP26 is essential for viral replication have recently been carried out on EBV and KSHV with capsid self-assembly systems. Indeed, VP26 nuclear distribution has been shown to be dependent on its interaction with the major capsid protein and the deletion of the region that is essential for its localization in nuclear dense structures impedes capsid formation (Henson et al., 2009; Perkins et al., 2008).

In this study, we created the first dually fluorescent VZV where, not only ORF23, but also an additional tegument protein was fluorescently labelled.

With these viruses, we showed that ORF9 is mainly displayed as cytoplasmic aggregates that could be related to its role in secondary envelopment which we recently demonstrated (Riva et al., 2013). The localization of ORF63 was the same as the one observed by immunofluorescence with an anti-ORF63 antibody on wild-type infected cells. The protein present a diffuse pattern both in the cytoplasm and the nucleus in which a higher concentration is found in the RC.

VZV ORF38 is largely uncharacterized. A study in which each VZV gene was separately deleted in a BAC construct showed that no infectious virus was recovered after the transfection of a ΔORF38-BAC (Zhang et al., 2010). We had also generated a BAC-VZV-ΔORF38 before the publication of these data and reached the same conclusion. Even though the generation of a complementing cell line expressing ORF38 or a rescued virus expressing ORF38 in another locus should be generated to formally ascertain it, VZV ORF38 seems to be an essential gene. In HSV-1, its homolog pUL21 was shown to be dispensable for viral growth in cultured cells (Baines et al., 1994; Mbong et al., 2012). PRV ΔUL21 PRV strain displays only a minor viral growth defect *in vitro* and in intranasal infection mouse model but is strongly impaired for growth in pig, the natural host of PRV (Klopfleisch et al., 2006; Klupp et al., 2005a;

Klupp et al., 1995). Its absence results in an important decrease in virion incorporation of pUL46, pUL49 and pUS3. Interestingly, a live attenuated PRV strain (Bartha strain) carries, among others, punctual mutations in the pUL21 gene and also fails to package wild-type amount of pUL46, pUL49 and pUS3. The restoration of a wild-type copy of pUL21 in Bartha strain reverts largely this phenotype and the replacement of pUL21 gene with the Bartha mutated version in a wild-type virulent strain (PRV-Ka) leads to a partial defect in incorporation of pUL46, pUL49 and pUS3 (Michael et al., 2007). Of note, the restoration of a wild-type copy of pUL21 in the Bartha strain also increases its virulence (Curanovic et al., 2009; Klupp et al., 1995; Lomniczi et al., 1987). The case of pUL21 of HSV-2 is also of interest. The deletion of HSV-2 pUL21 is not compatible with viral replication and the mutant virus display a delay in the gene expression profile but also a decreased number of produced capsids and a strong accumulation of them within the nucleus, with almost no capsid present in the cytoplasm (Le Sage et al., 2013). In HSV-2, pUL21, as in HSV-1 and PRV, is present both in the nucleus and in the cytoplasm, compatible with a role both in nuclear egress and in tegument incorporation during the secondary envelopment. The analysis of the localization of ORF38-DsRed in infected cells revealed that the protein is also present both in the nuclear and in the cytoplasmic compartments. The strong accumulation of ORF38 at the TGN that we observed is consistent with a role in the secondary envelopment process. In the nucleus, ORF38 is present both in the RC and in the capsid aggregates, which would be compatible with a role in capsid assembly and/or egress. In a recent Yeast-two-hybrid screen, many potential viral proteins have been shown to interact with ORF38 among which: ORF25 (pUL33), ORF26 (pUL32) and ORF42/45 (pUl15), all three implicated in the DNA cleavage/packaging step; ORF24 and ORF27 (VZV NEC complex); ORF22 (pUL36), an interaction conserved in EBV and finally some proteins implicated in the secondary envelopment process, ORF9 (pUL49), ORF11 (pUL47), ORF49 (pUL11) and gI (Stellberger et al., 2010).

The localization of ORF21 and ORF22, belonging to the inner tegument, in the nuclear capsid aggregates revives the debate surrounding where the initial step of tegumentation takes place. The presence of UL36 (ORF22 homolog) in the nucleus of PRV and HSV-1-infected cells is highly controversial. Some reports demonstrating its presence in the nuclear compartment (McNabb and Courtney, 1992), at the nuclear envelope (Morrison et al., 1998) and its association with intranuclear C capsids (Bucks et al., 2007) are corroborated by the presence in its N-terminal region of a recently described functional and highly conserved, Nuclear Localization Signal

(Abaitua and O'Hare, 2008). Moreover, another highly conserved 62 bp sequence, in the C-terminal region of PRV UL36, has been shown to be essential for viral replication and to be able to redirect the GFP to nuclear assemblons upon infection (Lee et al., 2006). This region has since been shown to be functionally conserved in HSV-1 (Coller et al., 2007). On the other hand, some groups failed to detect any UL36 in the nuclear compartment or in association with nuclear capsids, either on ultrathin sections or after extraction on sedimentation gradients (Klupp et al., 2002; Mohl et al., 2009; Trus et al., 2007). The amount of ORF22 found in VZV-infected nuclei is very low compared to the cytoplasmic level and the use of a virus expressing fluorescently-tagged ORF22 circumvents issues linked to antibodies' sensitivity and the possible masking of epitopes, potentially through protein conformation or post-translational modifications. This might, in part, explain the discrepancies between some published data and those reported here, bearing in mind that the VZV ORF22 localization has never been specifically investigated before. ORF21 has already been shown in the nucleus of VZV-infected cells, but not in association with peculiar structures (Cohrs et al., 2002). It is very interesting to note that the previously described UL37-GFP/VP26-mRFP-expressing HSV-1 displays a high degree of co-localization of capsids with UL37 at the Trans-Golgi Network, but also at some particular nuclear sites that strongly resemble the structures that we observed (Desai et al., 2008). The localization of both ORF22 and ORF21 at the nuclear capsid aggregates is also corroborated by a very recent report demonstrating that up to 5 tegument proteins could be added to the HSV-1 particle at the nuclear stage, among which were UL36 and UL37 (Henaff et al., 2013). In order to confirm that the addition of a fluorescent tag on both proteins does not preclude their virion incorporation, we purified VZV virions via two successive gradient steps (sucrose, then potassium tartrate-glycerol) (Kinchington et al., 1995), and, via western-blotting with an antibody against dsRed, confirmed the presence of DsRed-ORF21 in the viral particles (data not shown). However, we were unable to detect the presence of DsRed-ORF22, neither on the infected cell extracts, nor with highly purified virions extracts (data not shown). We also confirmed that the presence of the fluorescent tag, at least for ORF21, does not impact on its apparent cellular localization by generating a V5-ORF21 VZV in an otherwise wild-type viral context (figure 21).

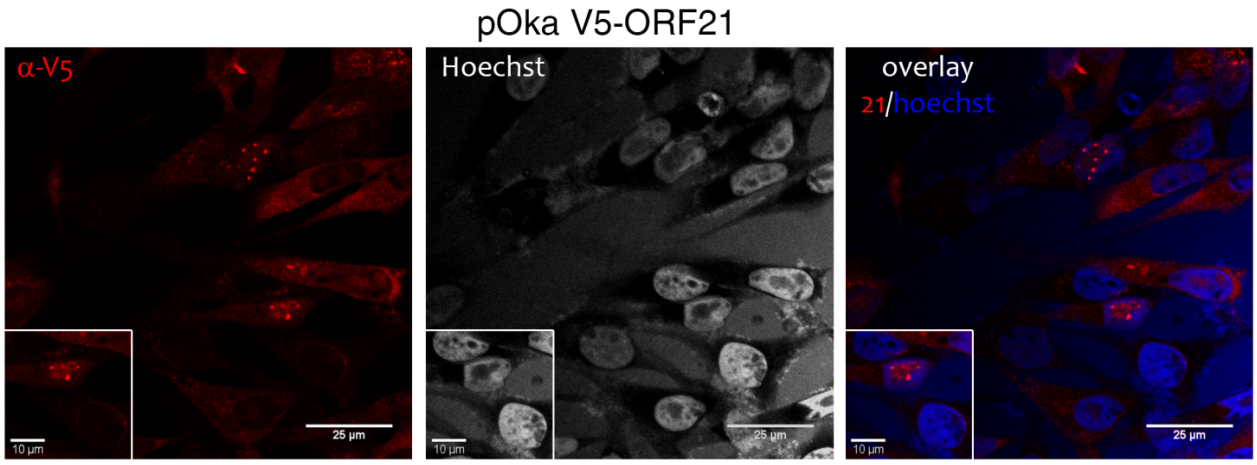


Figure 21. some ORF21 aggregates also appear in pOka V5-ORF21 infected cells. Mewo cells were infected with pOka V5-ORF21 VZV for 24h and immunstained with an antibody against the V5 tag, nuclei were labeled with hoechst. confocal images were captured with a 63x oil-objective.

A recent Yeast-Two-Hybrid screen has identified ORF21 as an interacting partner of both ORF23 and ORF22 (Stellberger et al., 2010) and HSV-1 and PRV UL36 and UL37 are also well known to interact (Kelly et al., 2012; Klupp et al., 2002; Lee et al., 2008b). These interactions could explain the presence of the two tegument proteins in the observed structures.

Of interest, while both UL36 and UL37 of PRV and HSV-1 have been shown to play a role in the viral particle maturation at the cytoplasmic step, PRV UL36 and HSV-1 UL37 specifically have also been shown to be crucial for the nuclear egress (Desai et al., 2001; Desai, 2000; Klupp et al., 2001; Luxton et al., 2006). It is therefore possible that a layer of tegument is necessary for the capsid to bud through the inner nuclear membrane. To date, we do not know why the co-localization of those two tegument proteins is not homogenous within the capsid aggregates and only arise in some of them, but, as revealed by TEM, the capsid content seemed to evolve during the infection process; it is therefore possible that the protein composition is not homogenous between aggregates. This particular point will be discussed in the second part of the results section.

Now that a new bank of antibodies against VZV proteins has been generated (Lenac Rovis et al., 2013), it would be very interesting to define the protein composition of the structures along with capsid type proportions over time in more detail by using both electron and confocal microscopy.

Altogether, this first part shows that VZV induces the formation of nuclear structures containing capsid and tegument proteins, as well as assembled A, B and C

capsids and procapsids. These dynamic capsid piles start to appear at specific and defined locations within the nucleus as early as 8-12 hours post-infection and are located at the periphery of the replication compartment. We believe that those structures could be implicated in capsid assembly and/or maturation before egress towards the cytoplasm. For this reason, and for the clarity of the following paragraphs, we will further refer these structures "VZV assemblons".

PART II. Association of the nuclear capsid aggregates with the PML bodies.

1. VZV assemblons partially colocalize with PML bodies

During the characterization of the VZV assemblons, we noticed that their localization seemed to be determined at early time points. Moreover, a new nuclear subcompartment called VICE (for Virus-Induced Chaperones enriched) domains had been described in HSV-1 infected cells (Burch and Weller, 2004; Livingston et al., 2009). These domains are thought to either play a role in the assembly process, or to sequester several types of protein (viral protein made in excess or misfolded, cellular proteins implicated in the DNA damage response,...). The sequestration of those proteins might provide a way for the virus to impede the activation of stress pathways or premature apoptosis that would be detrimental for the virus (for review, (Wileman, 2007)). We therefore wondered whether some pre-existing nuclear architecture was somehow dictating the places where the VZV assemblons develop, or if the formation of VZV assemblons was recruiting some cellular proteins. We decided to label infected cells for two well-known nuclear subdomains, namely the PML bodies (antibody against PML protein) and the nuclear speckles (antibody against SC35) and for several chaperones and DNA damage proteins which had been demonstrated to be present in the HSV-1 VICE domains (Hsc70, Hsp70, Hsp90, HSF-1, P-RPA, PH_2AX).

The analysis of HFF cells infected with eGFP-ORF23 VZV for 12, 24 and 48 hours revealed that neither chaperones (Hsc70, Hsp70, HSF-1), nor DNA damage proteins (P-RPA, P-H2AX) were co-localizing with VZV assemblons (data not shown). We did not detect any specific signal for Hsp90 labelling. The only potentially interesting information that we got from these experiments was that P-RPA was accumulating in the RC of infected cells.

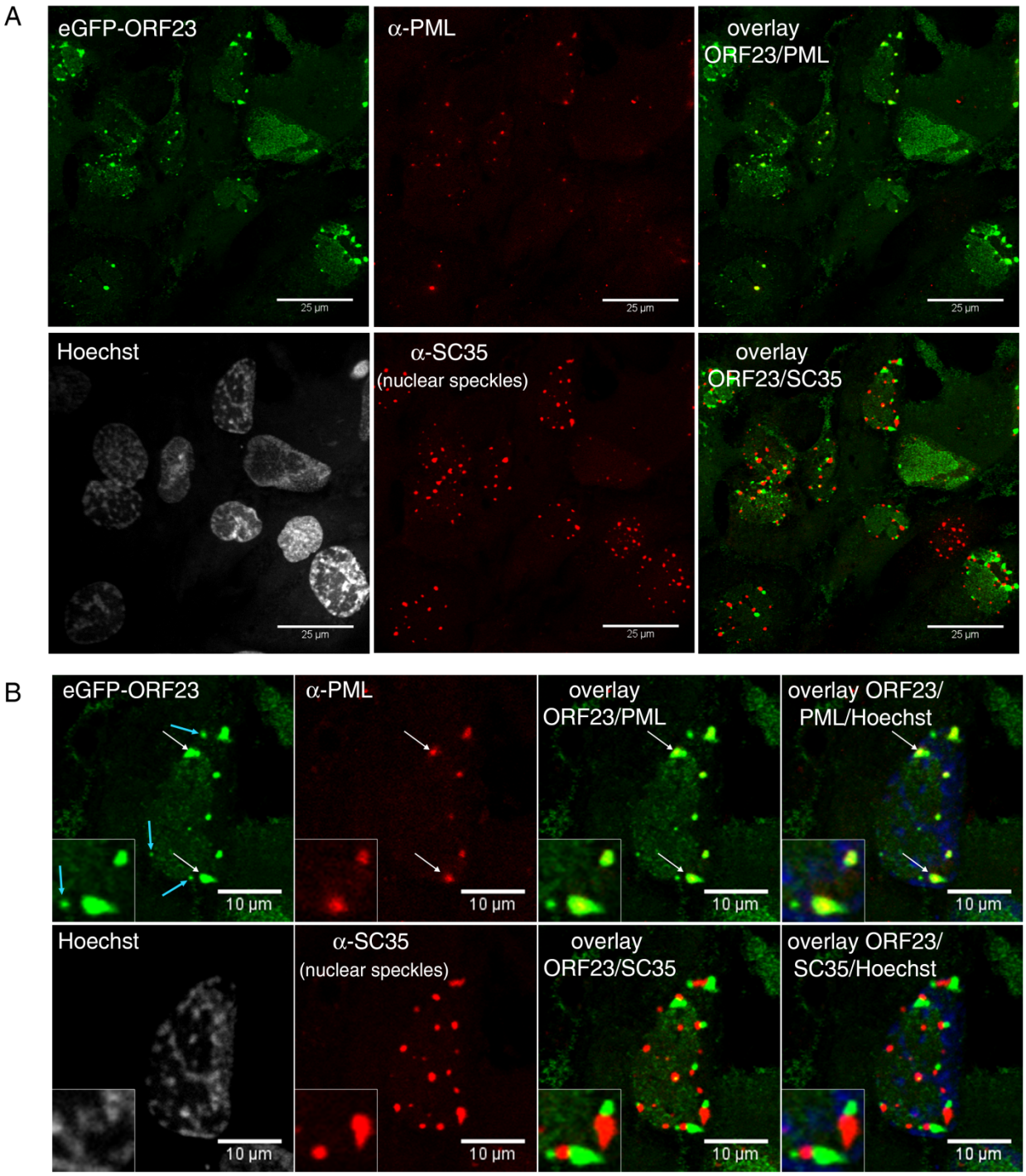


Figure 22. VZV assemblons partially colocalize with PML bodies. (A) HFF cells infected with eGFP-ORF23 VZV for 48h were fixed and immunostained with a rabbit anti-PML and a mouse anti-SC35 primary antibodies (Alexa568 -anti-mouse and Alexa633-anti-rabbit secondary antibodies). Confocal microscope images were captured with a 63x oil-objectif. (B) Higher magnification of field portion. White arrows point to colocalization, cyan arrows point to assemblons that does not colocalize with PML labelling.

HFF infected for 24h with eGFP-ORF23 VZV immunostained for both PML and SC35 demonstrated that a substantial amount of VZV assemblons colocalize with PML

bodies (Figure 22 A and B, upper panels) while they are very frequently juxtaposed to nuclear speckles (Figure 22 A and B, lower panels). At a higher magnification, we can observe that, most of the time, the eGFP-ORF23 positive area is larger than the PML positive one (Figure 22B, white arrows). Using Imaris software, we estimated that around 63 percents of the assemblons colocalize with the PML bodies.

We then looked back to the TEM pictures that we had made with MeWo infected cells and noticed that, indeed, at the ultrastuctural level, VZV assemblons are frequently localized next to nuclear speckles (Figure 23A, B and D).

Knowing their association with PML bodies in confocal microscopy, we searched in TEM for PML bodies in infected MeWo cells, at several time post infection. Even though we were able to detect structures similar at the ultrastructural level to the described PML bodies in non infected MeWo cells, their detection in infected cells proved to be much harder. Instead, we could detect a particular arrangement of some capsids that looked like surrounded by some material (figure 23C, E and F, black arrows). This "spherical" arrangement is sometimes discernible in particular areas of the larger capsid aggregates (figure 23D, G and H, black arrows). In very big assemblons, even though less evident, such peculiar zones can still be distinguished (figure 23I, black arrow).

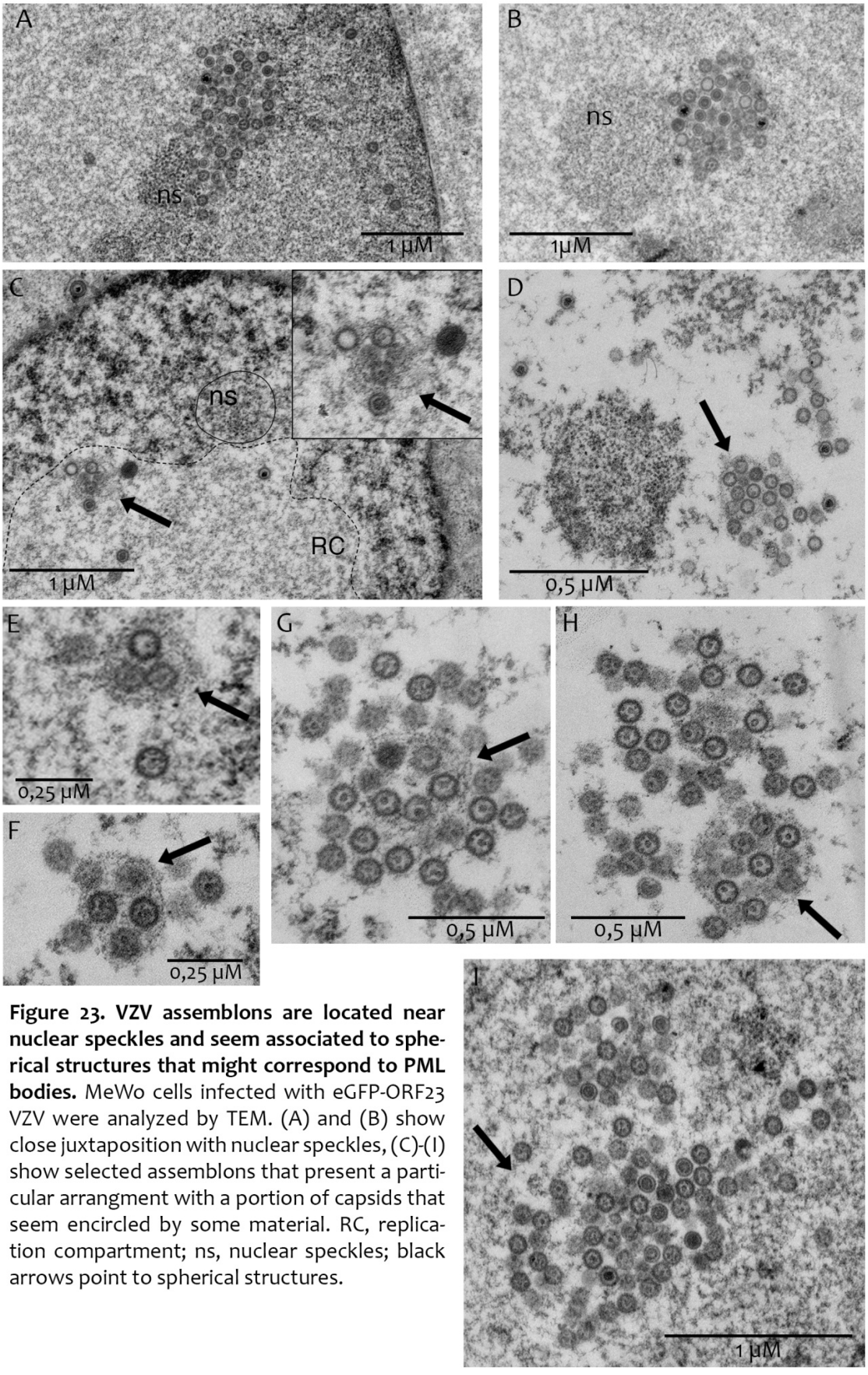


Figure 23. VZV assemblons are located near nuclear speckles and seem associated to spherical structures that might correspond to PML bodies. MeWo cells infected with eGFP-ORF23 VZV were analyzed by TEM. (A) and (B) show close juxtaposition with nuclear speckles, (C)-(I) show selected assemblons that present a particular arrangment with a portion of capsids that seem encircled by some material. RC, replication compartment; ns, nuclear speckles; black arrows point to spherical structures.

2.ORF21- and ORF22-containing assemblons colocalize with PML bodies

When then clearly had an association of some VZV assemblons with the PML bodies. As we had also observed in the first part of this work that inner tegument proteins were present in some but not all VZV assemblons, we wondered whether we were facing two distinct structures: VZV assemblons associated with tegument proteins and VZV assemblons associated with PML bodies.

MeWo cells infected for 48h with eGFP-ORF23/mDsRed-ORF21 (Figure 24A) or eGFP-ORF23/mDsRed-ORF22 (Figure 24B) VZV were immunostained with the anti-PML antibody. The experiment demonstrated that VZV assemblons containing tegumentary proteins are associated with PML bodies. Noteworthily and more evident for ORF21 than for ORF22, signals for the tegument protein and for the PML bodies only partially overlap while are both comprised in the eGFP positive area. This is compatible with the observation made in TEM suggesting that the capsids aggregates are not homogenous and that several distinct parts can be distinguish.

We had reached that point in our reflections when Ann Arvin's team published their data indentifying, at late time points of infection, nascent VZV virions entrapped inside PML bodies. They suggested that those structures represent an intrinsic cellular defence against the viral infection and tried to show that the viral particles present in those structures are unable to proceed with further egress, which decreases the viral infectivity (Reichelt et al., 2011).

We then wondered if the dynamic capsid aggregates appearing around 12h p.i. that we identified represented earlier form of these described PML-cages. We then decided to evaluate the formation of VZV assemblons in the absence of PML bodies.

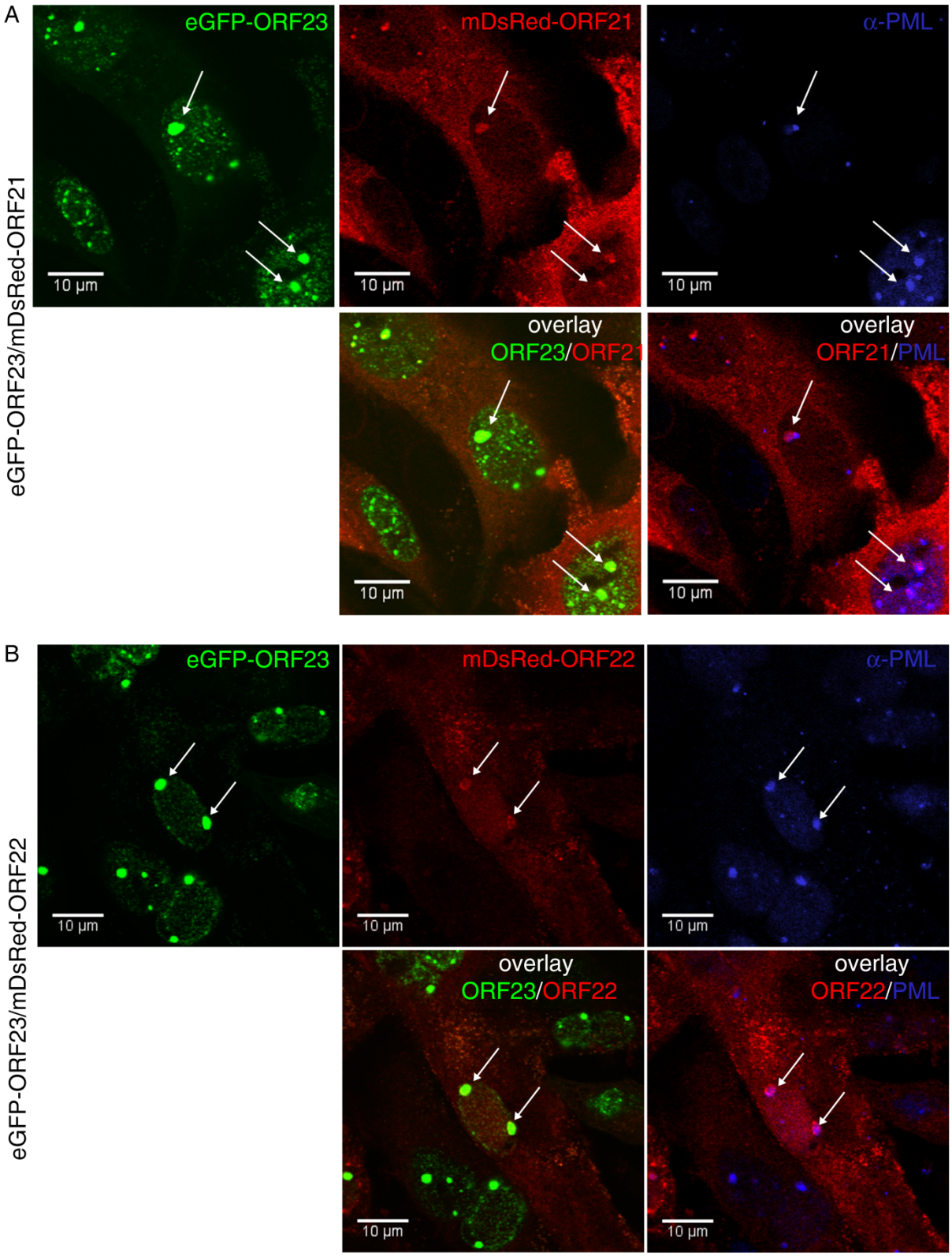


Figure 24. ORF21- and ORF22-containing assemblons colocalize with PML bodies. MeWo cells were infected with eGFP-ORF23/mDsRed-ORF 21 (A) or eGFP-ORF23/mDsRed-ORF22 (B) VZV. 48 hours post-infection, cells were immunostained with a rabbit anti-PML primary antibody and an AMCA anti-rabbit secondary antibody. Confocal microscope images wre captured with a 63x oil-objective. White arrows point colocalization areas.

3. VZV assemblons form independently of the PML bodies

We generated a MeWo cell line stably expressing a shRNA against all PML isoforms via retroviral vectors particles transductions and from an already described plasmid. (pSuper-Retro-Hygro-shPML, a kind gift from Dr. C. Kyratsous (Kyratsous et al., 2009)). Non-transduced MeWo cells in parallel with sh-PML expressing cells were infected with eGFP-ORF23 VZV and fixed 24 hours p.i. Immunostaining against PML was performed to control the knockdown of the gene (Figure 25A). Nuclear dense fluorescent structures were present in both cell lines showing that the PML bodies are not required for their formation (figure 25A). We performed an immunostaining against ORF29, ORF40 and SC35 in infected shPML-MeWo cells and demonstrated that VZV assemblons in this cell line are also mostly localized at the periphery of the RC, frequently juxtaposed to the nuclear speckles and contained ORF40. This indicates that VZV assemblons that form in the absence of PML bodies resemble the ones that form in their presence.

In order to confirm the nature of the fluorescent dense structures, we analyzed by TEM sh-PML MeWo cells infected with eGFP-ORF23 for 48h. It revealed the presence of capsid aggregates similar to those observed in non-transduced MeWo cells (figure 25B).

The presence of VZV assemblons in PML-depleted cells suggested that they may represent a virus-induced nuclear compartment that is important for its life cycle and not solely some intrinsic cellular defence that progressively entraps newly formed capsids, impeding their egress. Of course, both hypotheses are not completely mutually exclusive. At early times the association with PML bodies could be beneficial for the virus and at later time points, maybe when the structures reaches a certain size, capsids present in the structure are no longer able to leave them.

We then wanted to discriminate between a progressive accumulation of capsids at or within PML bodies, as suggested by Reichelt at al., and a recruitment of the latter where the nascent assemblons containing capsids start to form.

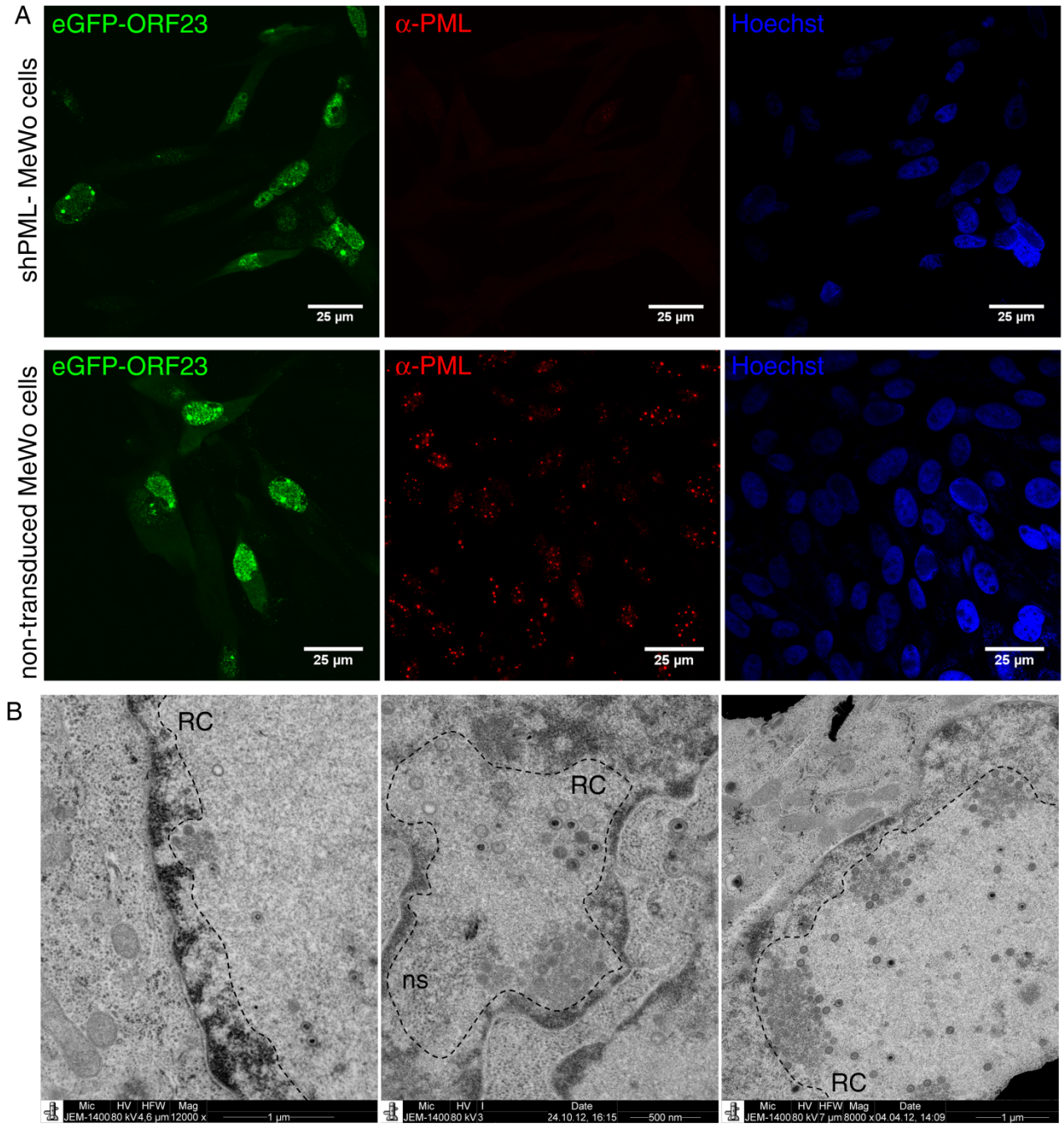


Figure 25. The nuclear dense structures corresponding to capsid aggregates also appear in PML-depleted cells. (A) shPML-MeWo cells as well as non-transduced MeWo cells were infected with eGFP-ORF23 VZV for 24h and analyzed by immunofluorescence using an anti-PML antibody (Alexa568 secondary antibody). Confocal microscope images were captured with a 63x oil-objective. (B) shPML MeWo cells infected with eGFP-ORF23 VZV for 48h were analyzed by TEM. RC, replication compartment; ns, nuclear speckles.

4. Assemblons formation and colocalization with PML bodies is timely orchestrated.

We infected MeWo cells with eGFP-ORF23 VZV by a 20-minutes contact with MeWo infected cells followed by extensive washes with PBS and fixed coverslips after 4, 6, 8, 12, 16, 20 and 24 hours to perform an immunostaining against PML and ORF40. For each time point we scored all the different dots present in the infected nuclei (positively stained for PML only, ORF23 only, ORF40 only and all the possible combinations of colocalization). The Imaris software was used to analyze the data and to help us to make a distinction between the eGFP signal most likely corresponding to individual capsids and the eGFP signal corresponding to assemblons. Of course, some bias is unavoidable, particularly at the early time points where the size of the nascent assemblons barely exceeds the size of single capsids. Representative pictures of selected time points are shown at figure 26 and graphs depicting the numbering results at figure 27 A and B.

Dots positive for ORF40 alone or for ORF40 + PML were absent from all time points. At 4h p.i., neither ORF23 nor ORF40 were expressed, this time point was then excluded from the scoring. The green fluorescence appeared at 6h p.i. as a diffused cytoplasmic and nuclear signal, but no capsid dots were detected inside the nuclei. At that time, ORF40 presented a diffuse nuclear localization. At 8h p.i., newly formed capsids showed up as isolated bright little dots throughout the nucleus. Occasionally, those little dots seemed to be localized close to or at some PML bodies (Figure 26, white arrows) that appeared partially yellow in the overlay. This represented the first colocalization event between the studied proteins. At this time point, PML bodies overlapping partially with the eGFP-ORF23 signal represented 50% of all dots while PML bodies negative for ORF23 accounted for 40%. Later on, as the infection progresses, colocalization of PML and ORF23 increases (Figure 26, cyan arrows) and two other major types of punctuations arise: ORF23 + ORF40 and triple colocalization between ORF23, ORF40 and PML.

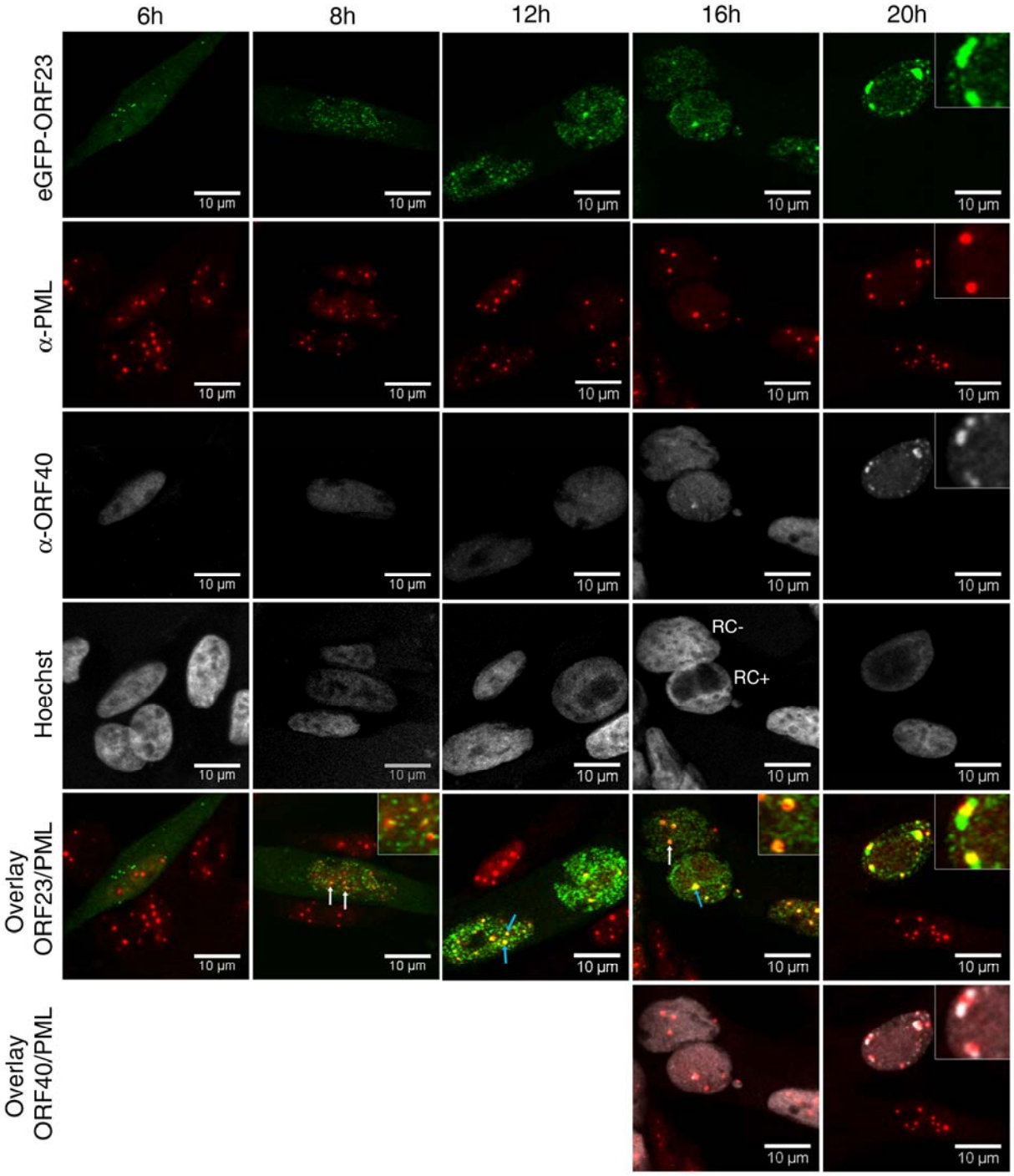


Figure 26. Temporal localization of ORF23, ORF40 and PML during VZV infection. MeWo cells were infected with eGFP-ORF23 VZV via a 20 minutes contact with infected MeWo cells, followed by extensive washing. Cells were fixed after 6h, 8h, 12h, 16h, 20h and 24h and immunostaind against PML and ORF40.

Progressively, the proportions of the different kind of dots evolve towards a majority of the ones where the three proteins colocalize and the ones with ORF23 colocalizing with ORF40 (Figure 27). Starting from 12-16 hours p.i., the population of infected cells becomes more and more heterogeneous because of secondary infections. The presence of a large, well-defined, replication compartment was then

assessed in the infected nuclei and cells were subdivided in two groups, based on this criterion (Figure 27B).

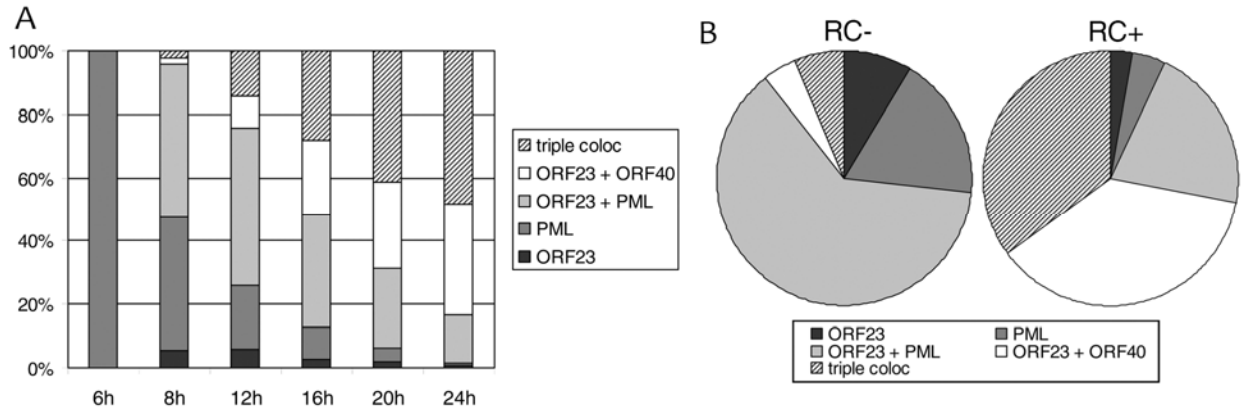


Figure 27. The localization of VZV assemblons regarding the PML bodies is timely orchestrated. (A) Quantification of the different fluorescent spots present in the nuclei of infected cells at the indicated time points, corresponding to the pictures shown at figure 26 (N>100 except for 6h and 8h where N=50). For each time point, mean number of the different types of spots are represented as a percentage of all spots present in the cells. (B) Nuclei from all time points were divived in two groups based on the presence or absence of a well defined RC.

We can clearly observe that before the establishment of the RC more than 75% of all dots are PML only or PML+ ORF23 whereas in cells with a well-defined RC, 75% of the dots represent triple colocalization or ORF40 + ORF23. Structures containing both ORF23 and ORF40 that do not colocalize with PML might argue for two distinct type of VZV assemblons or could only reflect a limited amount of PML proteins available in the infected nuclei. It is also important to note that when the three proteins colocalize, most of the time, ORF40p and PML signals only partially overlap whereas all the area is eGFP positive (Figure 26, insets at 20h p.i.). The fact that the major capsid protein seems absent from nascent assemblons supposedly containing capsid was not expected. The commercial antibody that we used was raised against infected cell extracts and we do not know if it is able to detect ORF40 present in the assembled capsids (and procapsids) or is only detecting the soluble form of the protein. The lack of punctuates at 8h post infection demonstrates that even if the antibody is able to label assembled capsids, its sensitivity is not sufficient to detect single capsids. The fact that VZV assemblons are positive for ORF40 staining at late time points can be explained either by the presence of a very large number of capsids or by the accumulation of capsid proteins in addition to the capsids. It is likey that, at late time points, part of the regions positive for both ORF23 and PML may represent the capsids entrapped in PML bodies described by Reichelt et al. and that could correspond to the structures we observed in TEM (Figure 23). It is possible that those PML cages render ORF40 present in the entrapped capsids hardly

accessible for the antibody, or that PML-cages only contains capsids whereas the surrounding area also contain capsid proteins.

The mean number of PML bodies present in infected nuclei was determined for each time point and compared with the mean number of PML bodies in non infected cells for which 200 nuclei were counted. Pair-wise t-test were run and showed that from 8 hours p.i. the number of PML bodies starts to drop and reaches a two-fold decrease at 24h p.i. (figure28). This drop in the number of PML bodies concomitant with the appearance of the VZV assemblons is in favour of a disassembly-reassembly of the PML bodies.

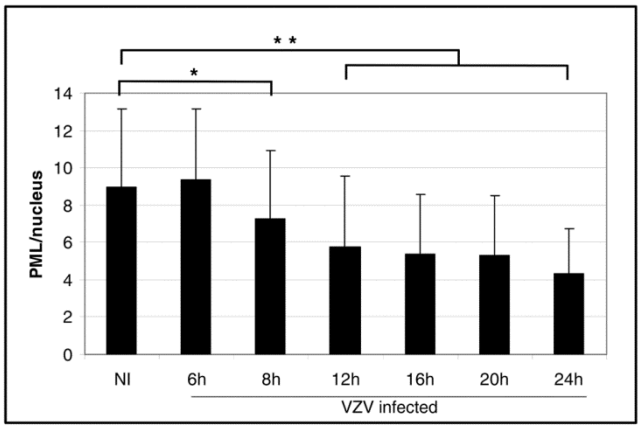


Figure 28. The number of PML bodies decreases during VZV infection. The total number of PML bodies present in the nuclei of infected cells was calculated for each time point of the experiment presented at figure 26 and 27. Histogram represents the means and error bars the standard deviation. The mean number of PML bodies present in non infected cells was determined on >200 nuclei. Pairwise t-tests were run to compare the means, *p value <0,01; ** p value <0,0001.

In order to determine if two distinct VZV assemblons were present in infected cells or if the amount of PML proteins is simply too low to be recruited at these places we wondered what would happen in cells overexpressing PML.

We generated two expression vectors where PML is tagged at its N-terminal side with either mCherry or eGFP. We used gateway cloning and the entry vector pDONR223-PML obtained in the human ORFeome 5.1 bank available at the GIGA-interactomic platform. The two vectors were transfected into MeWo cells that were subsequently infected with either eGFP-ORF23 or tRFP-T-ORF23 VZV. Even though the transfection efficiency was very low despite many unsuccessful set-up, it was possible to find cells that were both transfected and infected (figure 29). In both cases (eGFP-PML with tRFP-T-ORF23 VZV and mCherry-PML with eGFP-ORF23 VZV), all VZV assemblons colocalized with PML-bodies (figure 29, white arrows). Interestingly, it was possible to detect syncytia obviously resulting from the fusion of a cell that was expressing the tagged-PML with cells that were most likely non transfected (figure 29, cyan arrow). In these nuclei the red labelling was very faint, only a portion of VZV assemblons were marked suggesting that PML protein was most likely recruited to existing VZV assemblons. Of course, if would have been

interesting to have recorded time-lapse images on living cells to definitely prove this statement.

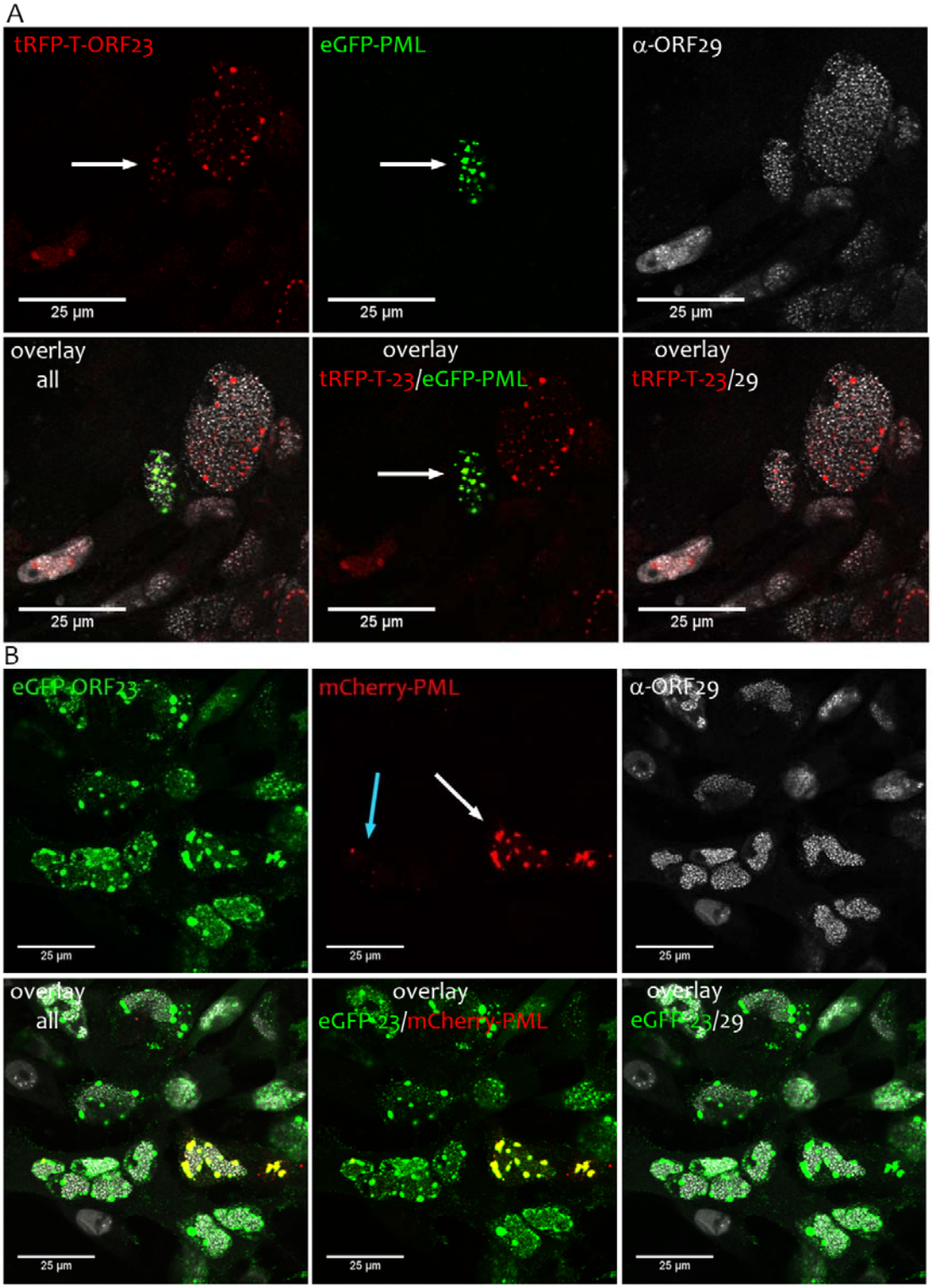


Figure 29. Localization of VZV assemblons in cells overexpressing PML protein. Mewo cells were transfected with a plasmid expressing the PML protein fused to eGFP (A) or to mCherry (B). 24 hours post-transfection, the cells were infected via cell contact with MeWo infected with tRFP-T-ORF23 (A) or eGFP-ORF23 VZV followed by extensive washing. Pictures were recorded 24 hours later with a 63x oil-objective. White arrows point to nuclei in which all VZV assemblons colocalize with tagged-PML, cyan arrows point to secondary association after cell fusion.

If we looked at transfected cells infected at a stage before the emergence of big assemblons, the capsids were evenly distributed in the nucleus and occasionally colocalized with PML bodies (Figure 30). This was also observed with endogenous level of PML protein (Figure 26 8h p.i.). The overexpression of PML protein was then unable to trap all newly produced virions.

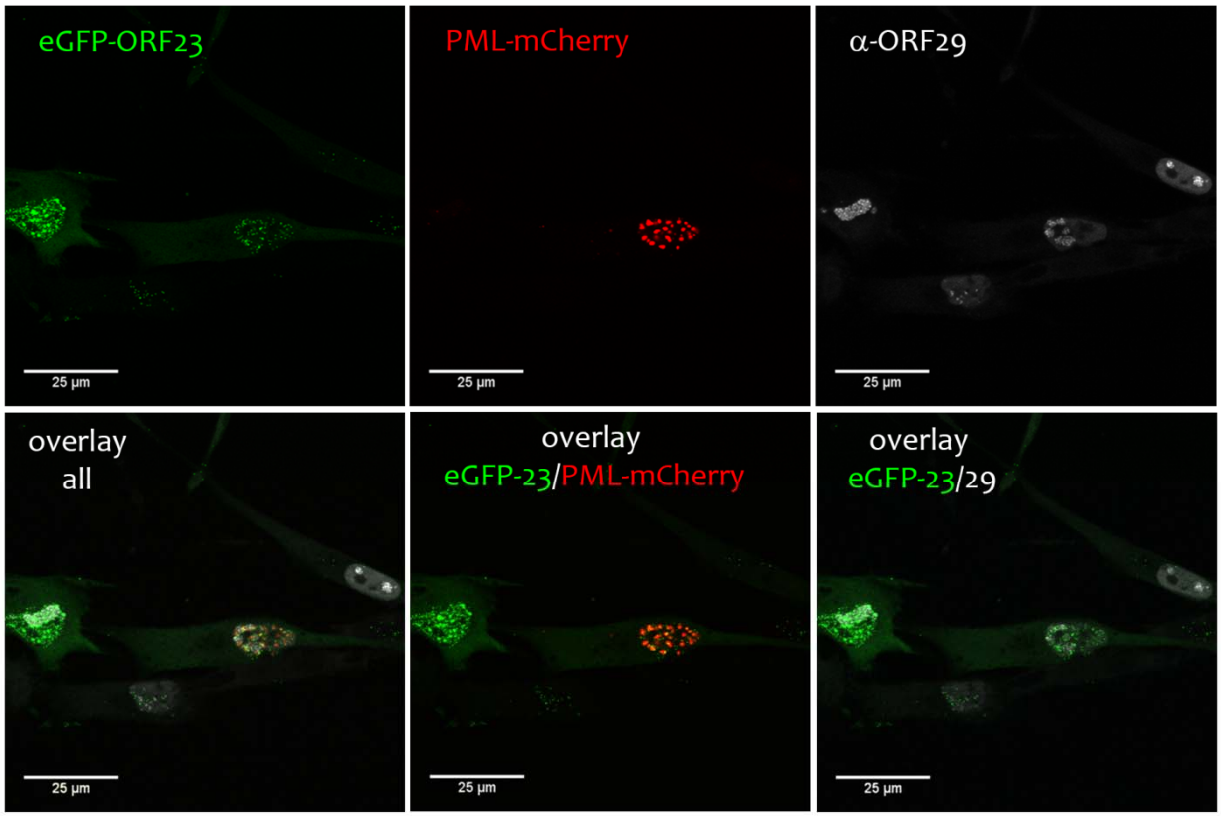


Figure 30. Localization of newly assembled capsids in cells overexpressing PML protein. Mewo cells were transfected with a plasmid expressing the PML protein fused to mCherry. 24 hours post transfection, the cells were infected via cell contact with MeWo infected with eGFP-ORF23 VZV followed by extensive washing. Pictures were recorded 24 hours later with a 63x oil-objective.

Of course, one have to remember that 14 isoforms of the PML protein have been described so far and that Reichelt suggested that the isoform IV (PML-IV) was uniquely able to interact with ORF23 and to trap newly assembled virions. The isoform that was present in the human ORFeome 5.1 is the isoform 13, whose C-terminal part is similar to PML-II and really different from PML-IV. We then decided to repeat the experiment described in Reichelt's study with six different PML isoforms. We received the 6 expression vectors (PML-I, II, III, IV, V, VI-eGP) from Dr. Peter Hemmerich (Leibnitz-Institute of Age Research, Jena, Germany), transfected them in 293 and HeLa cells (to avoid the transfection problems we encountered with MeWo cells) and infected the cells with tRFP-T VZV (data not shown). We actually did not find any major differences between the different isoforms, and the results were

completely similar to the ones obtained with isoform 13 (figure 29 and 30). We so far do not know why we could not reproduce their experiment.

5. Discussion Part II

In this second part of our work, we aimed to determine if VZV assemblons were related to VICE domains described for HSV-1 and if they were associated with particular nuclear subdomains.

VICE domains described for HSV-1 infection are characterized by an accumulation of Heat shock proteins and chaperons such as Hsc70, Hsp70 and Hsp90. We failed to detect chaperones or DNA damage proteins in association with VZV assemblons. Actually, none of the studied proteins seemed to localize into nuclear punctuates like it is described for HSV-1. Even Hsp70, which aggregates have been described by several teams, is diffuse in VZV-infected cells. It is possible that VICE domains do not form in VZV-infected cells or that their composition differs. One should note that in HSV-1 infected cells, most of those proteins are present in the nucloplasm as well, which have been shown to mask their localization in the HSV-1 VICE domains. Accordingly, some published protocols suggest to include a nucleoplasmic extraction step by a short detergent treatment of the cells prior fixation. In order to exclude the possible link between VZV assemblons and VICE domains we should repeat these immunofluorescence experiments with this additional step (Wilkinson and Weller, 2006).

The first interesting point was the apparent frequent juxtaposition of VZV assemblons with the nuclear speckles. Those structures, also called interchromatin granules clusters, represent nuclear area specifically enriched in mRNA maturation proteins (splicing and export factors, among others) and some transcription factors. Nuclear speckles are dynamic and there is a permanent exchange between the nucleoplasma pool and the speckle pool of their constitutive proteins and RNPs. Their role in cellular gene expression is still not fully understood but highly expressed genes have been shown to localize at their very close proximity. This is consistent with the idea that they may represent a supply or a recycling factory for the factors playing crucial role in transcription, pre-mRNA maturation and export (for review, (Spector and Lamond, 2011)). Some studies have demonstrated a relocalization of hnRNP and snRNPs upon HSV-1 infection. hnRNPs become marginalized at the nuclear envelope together with the genomic DNA whereas snRNPs, from a diffuse

nuclear localization pattern in non infected cells, become, in addition, highly concentrated in peculiar punctuates that were later shown to be the nuclear speckles (Martin et al., 1987). The nuclear speckles themselves are reorganized upon HSV-1 infection, their number diminishes while their size increases and from an homogenous distribution throughout the nucleus they are found at the edge of the RC, close to the margin of genomic DNA (Sandri-Goldin et al., 1995). The redistribution of snRNPs and nuclear speckles has been attributed to HSV-1 ICP27 (VZV IE4) which also concentrates in these domains (Phelan et al., 1993; Sandri-Goldin et al., 1995). Since ICP27 was known to inhibit host cell splicing this ICP27-driven nuclear reorganization was logically considered first to contribute to this inhibition (Hardwicke and Sandri-Goldin, 1994; Hardy and Sandri-Goldin, 1994; Sandri-Goldin and Mendoza, 1992; Schroder et al., 1989). Nevertheless, the generation of ICP27 mutants unable to relocalize snRNP and nuclear speckles but still able to inhibit host cell splicing invalidated this hypothesis (Sandri-Goldin et al., 1995). The discovery that those domains are enriched in polyA-RNA (Besse et al., 1995) and that the ICP27 mutants unable to induce snRNP and nuclear speckles reorganization present defects in late gene expression raised the possibility that this phenomenon is important for the export of the late mRNAs (Hardwicke et al., 1989; McMahan and Schaffer, 1990; Rice and Knipe, 1990). A recent study has shown that the viral RC forms by fusion of distinct smaller compartments moving in the nucleus via an active process and that this coalescence occurs at the nuclear speckles and enhances the export of a subset of viral late mRNAs (Chang et al., 2011).

Interestingly, PML bodies and nuclear speckles are known to be frequently juxtaposed in the cell nucleus and a study on HCMV has demonstrated a tight relationship between the deposition site of the incoming genome and the localization of both PML bodies and nuclear speckles. This is thought to create an environment favouring immediate early gene expression. The association of incoming genome with PML bodies will be discussed in more details in the following paragraphs, but this demonstrates that virally-induced nuclear remodelling is likely to play an important role at many steps of the viral life cycle (Ishov et al., 1997).

The fact that VZV assemblons forming around 12h p.i. are found in proximity of the nuclear speckles could be the reflect that the initial localization of the incoming genome is somehow maintained throughout the lytic cycle even after the formation of the RC or could be due to their association with PML bodies that somehow keep their initial juxtaposition with the nuclear speckles.

One of the most intriguing observations of this second part of the results resides in the colocalization of VZV assemblons with the cellular PML bodies. This phenomenon is meant to be quite unique among herpesviruses since most of them have developed different ways to disrupt the PML bodies in the course of the infection. The first and most characterized disruption of PML bodies by a viral infection is probably the way ICP0 from HSV-1, via its E3 ubiquitin ligase activity, induces the proteasomal degradation of all PML isoforms leading to the degradation of the sumoylated forms of SP100, promoting thereby viral gene expression (Everett et al., 1998; Maul et al., 1993). Briefly, in 1993, ICP0 was shown to specifically and transiently localize to PML bodies. Subsequently, HSV-1 genome itself has been identified at this peculiar location (Ishov and Maul, 1996). Several mechanisms leading to this association have been hypothesized but it is now generally admitted that, upon entry, HSV-1 genome, in association with a specific set of viral and/or cellular proteins, is able to recruit specific components of PML bodies, among which some PML isoforms, inducing *de novo* PML bodies formation (Everett and Murray, 2005; Everett et al., 2004). This association is thought to represent an intrinsic antiviral defence that HSV-1 overcomes through ICP0-mediated PML degradation (Everett et al., 2008). This PML bodies-mediated repression is not sufficient to totally impede the viral cycle progression, since ICP0 deletion is not lethal for the virus but is thought to represent a decisive step towards the lytic or the latent cycle (Everett et al., 2006). During latency, the HSV-1 genome has been shown to be surrounded by a shell of PML, which is disrupted upon reactivation, another clue for a role of PML bodies-ICP0 interaction on the fate of the viral infection (Catez et al., 2012; Everett et al., 2007).

ICP0 is conserved among alphaherpesviruses and its E3 ubiquitin ligase activity is crucial for PML proteasome-mediated degradation. Nevertheless, whereas all homologs, including the VZV one, that have been tested share, at least *in vitro*, this E3 ubiquitin ligase activity, they differ in their ability to efficiently lead to the degradation of PML and SP100 isoforms and ultimately provoke PML disruption (Everett et al., 2010; Gaudreault and Jones, 2011; Kyratsous et al., 2009; Parkinson and Everett, 2000). It is possible that the detailed mechanism through which PRV, BoHV-1 and EHV-1 disrupt the PML bodies is slightly different from the one described for HSV-1. The viral proteins leading to PML disruption in beta- and gammaherpesviruses do not possess an E3 ubiquitin ligase activity, which indicates that the means to counteract this intrinsic cellular defence are varied. Among herpesviruses, only KSHV and VZV seem, at a first sight, not to lead to PML bodies

disruption. In fact, when expressed alone, ORF61, the VZV ICP0 homolog, , is able to slightly decrease the SP100 level, but the amount of PML and SP100 remains quite stable during the infection process (Kyratsous and Silverstein, 2009). Accordingly, the PML bodies are maintained throughout the VZV lytic cycle even though their number decreases, their size seems to increase and the labelling looks fainter and surrounded by a halo (Kyratsous and Silverstein, 2009; Reichelt et al., 2011). The silencing of SP100 was shown not to impact VZV infectivity while the silencing of PML increased somewhat the viral titer at early time points as well as the growth kinetic of infection foci (Kyratsous and Silverstein, 2009).

Surprisingly, all these characteristics represent hallmarks of another type of nuclear replicating DNA viruses, the polyomaviruses (Erickson et al., 2012; Gasparovic et al., 2009; Jiang et al., 2011; Shishido-Hara et al., 2004). Even though the relationship between polyomaviruses and PML seems to be quite distinct for each virus, one common property is that the PML bodies (at least the punctuate pattern seen in immunofluorescence with anti-PML antibody) seem to be maintained in infected nuclei. Neither JCV, nor SV40 seem to disrupt PML bodies, even though, SV40 genome and T-antigen localize in these nuclear bodies (Gasparovic et al., 2009; Ishov and Maul, 1996). BKV has been shown not to disrupt but to reorganize the PML bodies, leading to a decrease in their number while their size increases. During BKV infection, Sp100 and Daxx are relocated diffusely within the nucleus but their protein level is unchanged (Jiang et al., 2011). Of important note, a dispersed signal was also observed for Sp100 in VZV-infected cells (Kyratsous et al., 2009). In our study, we found that the total amount of PML bodies decreases by two-fold in VZV-infected cells at 24h post infection. At the ultrastructural level, we were able to distinguish "classical" PML bodies in non-infected cells or cells infected at early stages, whereas, at later time, we could only detect spherical arrangement of capsids, that could potentially represent PML-bodies. We then can hypothesize that VZV, as well, is dramatically reorganizing the PML bodies during the infection.

Another interesting analogy between what we observed with VZV and what has been described for polyomaviruses resides in the observation that JCV minor and major capsid proteins can be found in PML-bodies, not only in transfected cells, but also in brain cells of patients with progressive multifocal leukoencephalopathy, a pathology resulting from JCV infection (Shishido-Hara et al., 2004). PML bodies were hypothesized in this case to constitute platforms for viral assembly. Papillomaviruses represent another interesting example in which capsid proteins colocalize with PML bodies. The HPV minor capsid E2 has been shown to relocalize the major capsid L1

and the early protein E2 to PML bodies. The composition of the latter is modified by the infection, with an accumulation of hDaxx but no more SP100 (Day et al., 1998; Florin et al., 2002). Whether this association is beneficial for the virus or is simply a consequence of the viral infection with no harms and no benefits is still to be confirmed (Day et al., 2004; Narayanan et al., 2007).

Some VZV capsid aggregates entrapped in PML-cages have recently been identified in the nucleus of infected cells (Reichelt et al., 2012; Reichelt et al., 2011), which is believed to limit capsid egress to the cytoplasm. We first thought that the structures described here represented earlier stages of this phenomenon. However, when we silenced PML gene expression via shRNA technology, we still observed eGFP-ORF23 intense fluorescent spots in confocal microscopy, as well as capsid aggregates in TEM. The nuclear polyomavirus virus factories are also found in association with PML bodies in infected cells but, as in our case, the depletion of PML protein does not preclude their formation (Erickson et al., 2012).

As VZV assemblons form in absence of PML bodies, we wondered what was leading to their association. It is conceivable that the formation of VZV assemblons occurs independently of the presence of the PML bodies but that their enlargement as infection progresses constitutes some sort of danger signal that induces *de novo* PML bodies nucleation. This could explain the fact that the number of PML bodies decreases with the infection and that the timing between VZV assemblons formation seems to correlate with this decrease. Another possibility would be that VZV genome recruits PML constituents at its entry site in the nucleus, forming new PML-like bodies (maybe without SP100 protein). Subsequent transcription and replication phases would then occur in close association with these structures, as described for other herpesviruses. It is possible that capsid assembly and DNA encapsidation would also take place in this close vicinity. As no VZV protein is able to efficiently lead to PML degradation or PML disruption, the newly assembled particles would then progressively be entrapped in those PML bodies. The decreased number of PML bodies would then represents the number of initially incoming genomes. However, the timing of the decrease in not coherent with this hypothesis, since VZV IE proteins are expressed as early as 1h to p.i. (Reichelt et al., 2009) and the PML bodies recruitment followed by their disruption has been shown to occur in the first 2 hours of infection and before the formation of prereplicative sites (Maul et al., 1993). Precise kinetics experiments are particularly hard to set up with VZV due to its highly cell-associated nature and we could not label in our experiments ORF29 together with PML because both antibodies are from a rabbit origin. Interestingly, in its 2009

publication, Reichelt showed that IE62 (HSV ICP4 homolog) foci thought to label incoming genomes form at 1-2h p.i. but do not colocalize or seem juxtaposed with PML bodies, as described for HSV, whereas some ORF61 foci colocalizing with PML bodies were observed at this time (Reichelt et al., 2009). Whether ORF61 association with PML bodies has an impact on VZV assemblons formation is unknown.

The major capsid protein, VZV ORF40, seems to localize within VZV assemblons only from 16h p.i. which was of course not expected since they are supposed to contain capsids. It is possible that the sensitivity of the antibody is too low to label single capsids or that the antibody used is unable to detect ORF40 when assembled within the capsid. The fact that ORF40 and PML labelling only overlap in large assemblons is consistent with the TEM pictures showing that some assemblons seem to contain two distinct areas, one of which displaying a spherical arrangement of the capsids and could correspond to the PML positive area observed in confocal microscopy. The lack of ORF40 labelling in the area positive for the PML staining could originate from a poor accessibility of the capsids in this part of the assemblon or from the presence of capsid only in the PML-positive area and a mix of capsids and capsid proteins outside the PML-positive area. Interestingly, the same phenomenon is observed with the localization of mDsRed-OR21 and mDsRed-ORF22 for which sensitivity or accessibility of the antibody cannot be evoked. It is possible that the capsids outside the PML-positive area possess a tegument layer but not the ones accumulating in the PML specific area. Another possibility is that no capsid wouls display a layer of tegument but that ORF21 and ORF22 would have a role to play in this specific part of the nucleus. Since C-capsids are more likely to be tegumented than the other types, determining if is the proportion of the different types of capsids differs between the PML-positive and the PML-negative area using Immunoelelectron microscopy could help us to answer this question.

Our data show that only a proportion of VZV assemblons colocalizes with the PML bodies and that the "PML-negative" ones seem to appear secondarily. If this colocalization indeed corresponds to the PML-cages described by Reichelt, which we think, we have to reconcile the "dynamic capsid aggregates concept" and role in assembly that we have tried to demonstrate with the antiviral defence concept (Reichelt et al., 2011). It is possible that the dynamic character of VZV assemblons is different between PML-positive and PML-negative structures. We tried to answer this question with the transfection of fluorescently-tagged-PML expressing vectors followed by infection with fluorescent VZV. Unfortunately, this experiment proved to be hard to set up. Indeed, we never managed to transfect HFF cells with the

expression vectors and tranfection of MeWo cells was far from efficient (see figure 29). Furthermore the expression of tagged-PML was often leading to very large structures quite different from the endogenous PML. To overcome this issue, we generated a mCherry-SP100 and an eGFP-SP100 expression vectors but it leads to the same inconclusive results. Another explanation that may conciliate Reichelt'data with ours is that over the time, the dynamic capsid aggregates progressively freeze by a progressive solidification of the PML-cage. Indeed, we performed all our dynamic experiments between 16h to 24h p.i. whereas Reichelt observed their PML-cages at 48h p.i. Lastly, one should note that the true sequestration of capsids they described was only observed after the overexpression of PML-IV, an isoform that, endogenously, is only expressed at low level. In other words, whereas the observed association between VZV assemblons with endogenous PML truly impacts further egress or represent simple colocalization remains to be proved.

CONCLUSION AND PERSPECTIVES

Viruses are obligatory parasites. They have developed many ways to subvert the cellular machinery to their own benefit. A fine tuning between the cellular intrinsic antiviral defence and the expression of the viral repertoire leading to virion production and release is meant to be crucial for the outcome of the infection in the host. Herpesviruses are slow evolving viruses, known to have co-evolved with their hosts and possess a large genome. The complexity of their virion necessitates a complex life cycle, not to mention that they all have the property to establish a latent cycle besides the lytic one. The virus-host cells interactions governing the fate of the infection are still largely unknown.

The nuclear architecture is completely remodelled upon herpesvirus infection that induces the progressive formation of a huge compartment termed the Replication compartment (RC). Many cellular and viral proteins have been shown to localize in the RC, among which all the proteins necessary for the replication of the viral genome, which is thought to occur at numerous specific spots present in this compartment and proteins implicated in viral gene expression. It appeared later that the optimal expression of viral late genes strongly relies on the formation of this RC and recent data on HCMV indicate that the area where viral genomic replication takes place might be restricted to the periphery of the RC. Of note, the single strand DNA binding protein (VZV ORF29, HSV-1 ICP8) that has been extensively used to label this nuclear compartment have been shown to play a role in viral late gene expression. The RC is thought to form from smaller entities containing a specific set of viral proteins and that have been called "prereplicative sites". It is now known that only in HSV-1, a specific subset of these prereplicative sites will develop into mature RC and this specific subset is associated with the cellular PML bodies. Proteins of viral and cellular origin have been shown to form colocalized, juxtaposed or distinct foci in infected nuclei and accumulating evidences suggest that the RC is much more compartmentalized than anticipated. Selected examples of this virus-induced territorialisation of the nucleus as well as our suggested model for VZV are presented below.

In 1997, Ishov et al. simultaneously labelled HCMV genome, HCMV IE mRNA and two IE proteins in infected nuclei and suggested that upon entry, the genome associates with two nuclear structures, the PML bodies and the nuclear speckles and initiates the transcription program. Subsequently, IE72 localize to PML bodies, while IE86 accumulates at the junction between PML bodies and nuclear speckles. This is

thought to create an environment favouring IE gene transcription and export and is followed by PML bodies dispersal induced by IE72 that concomitantly relocalizes to a diffuse pattern. The subsequent formation of the replication compartment was thought to initiate at these sites (figure 1).

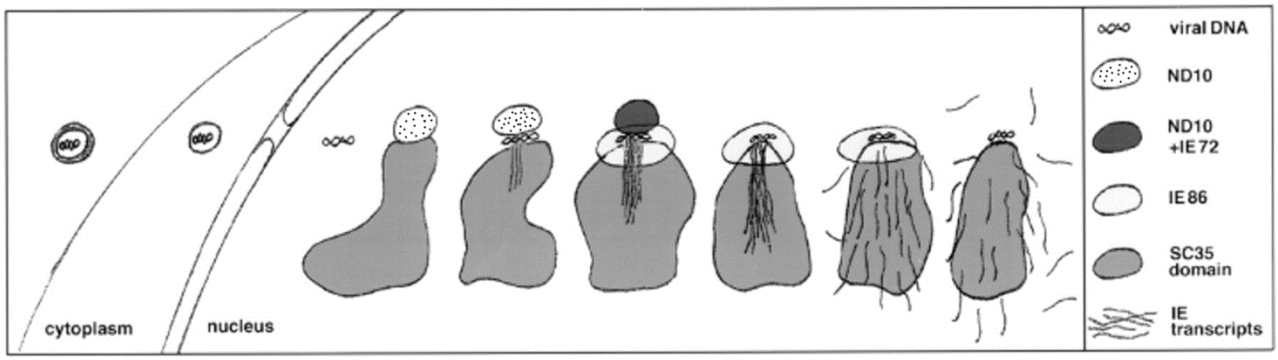


Figure 1. HCMV development and dissolution of the immediate transcript environment. Upon entry in the nucleus, the viral genome will associate with PML bodies (ND10) and nuclear speckles (SC35 domain). After the onset of IE gene expression, IE proteins are specifically recruited to this peculiar nuclear location. IE1 (IE72) will localize to PML bodies and subsequently disperse them whereas IE2 (IE86) wil occupy an area partially overlapping with ND10/IE72 and the SC35 domain (adapted from Ishov et al., 1997).

In 2008, Livingston et al. proposed a model for HSV-1 RC formation (figure 2). In this model, the first step corresponds to the accumulation of ICP4 on incoming HSV-1 genome which leads to the formation of PML bodies adjacent to ICP4 foci (Stage I). The detailed transition from stage I to stage II (figure 2B) would correspond to the ICP0 recruitment to and subsequent diruption of the PML bodies. The disruption of the PML bodies is followed by the formation of the VICE domain, adjacent to the genome-bound ICP4 foci. This complex recruits ICP8 leading to the formation of microfoci (Stage II). The subsequent recruitment of replication proteins (fisrt the helicase/primase trimeric complex pUL5/pUL8/pUL52 and the OBP pUL9, then the DNA pol, pUL30/pUL42) gives rise to the prereplicative foci (stage III a and b). The fusion of the prereplicative foci finally leads to the RC formation.

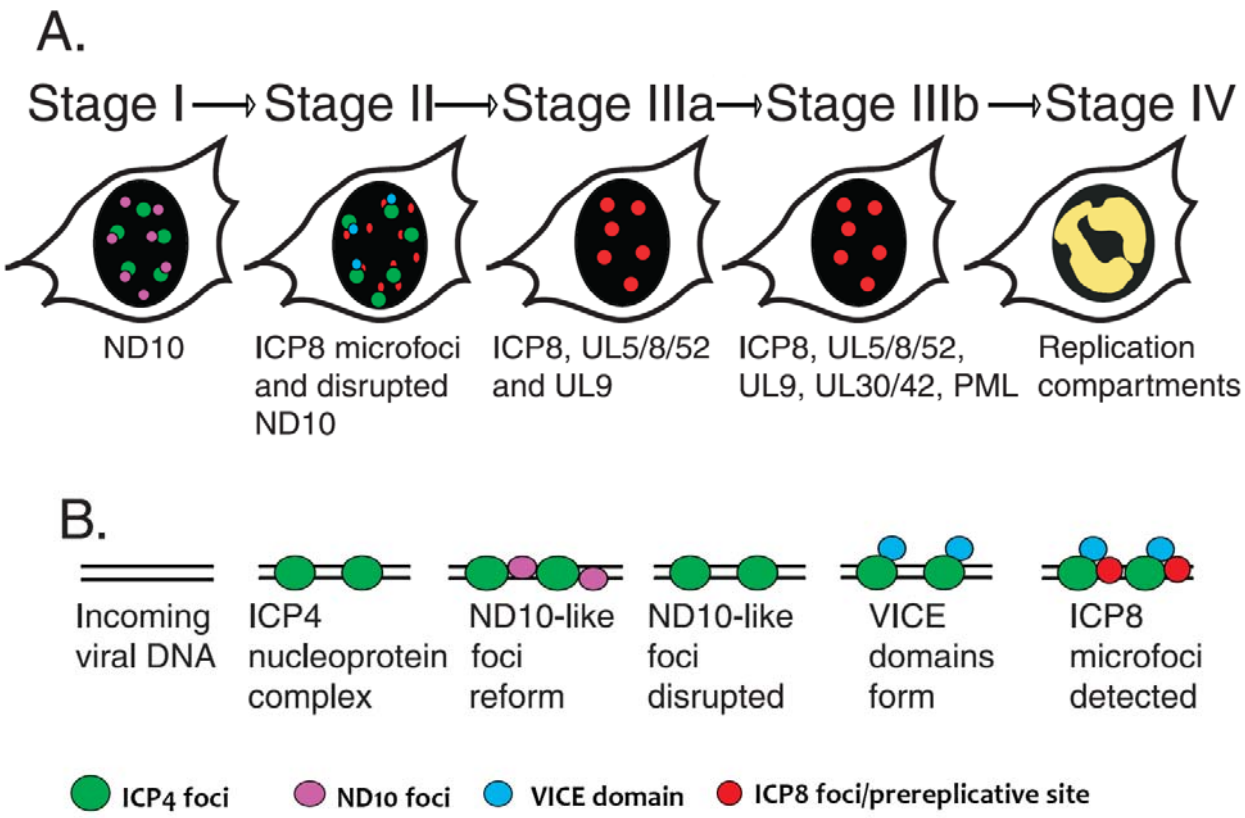


Figure 2. Proposed model of HSV-1 replication compartment formation. (A) sequence of foci formation from initial ICP4 juxtaposition to ND10 (stage I) to the mature RC (stage 4). (B) proposed events sequence that leads to ICP8 microfoci formation (adapted from Livingston et al., 2008)

Such model have not been presented for VZV, mainly because precise kinetics experiments are harder to set up leading to data sometimes difficult to interpret. According to our data and data from the literature, especially a paper published by Reichelt et al. in 2009, the likely sequence of event at early stages of infection is the following. The first visible foci that are dectected are foci of IE61 (HSV-1 ICP0 homolog) and foci of IE62 (HV-1 ICP4), which emerge at distinct locations. IE61 foci are colocalizing with the PML bodies, whereas ICP4 most likely are fixed on VZV genome and consequently label their deposition site (figure 3, 2h p.i.). This represents the first difference between HSV-1 and VZV since no close association of incoming genome with PML is detected. Subsequently, IE61 foci progressively evolves toward a diffuse nuclear signal and ORF29 (HSV-1 ICP8) expression becomes visible as a diffuse signal (4h p.i.). The first ORF29 foci, likely corresponding to prereplicative sites, appear at 6h p.i. juxtaposed to the IE62 signal. The formation of nascent globular domains that will fuse forming the mature RC is evident starting from 8h p.i. At that time, the first newly assembled capsids are produced and are localized inside as well as oustide the globular domain. As seen by video imaging, at that time, capsids are highly motile and seem to be able to move freely in the entire

nuclear space. Some of them colocalize with PML bodies and we believe that, somehow, the capsid assemby process induces de novo PML formation. At 12h p.i.,

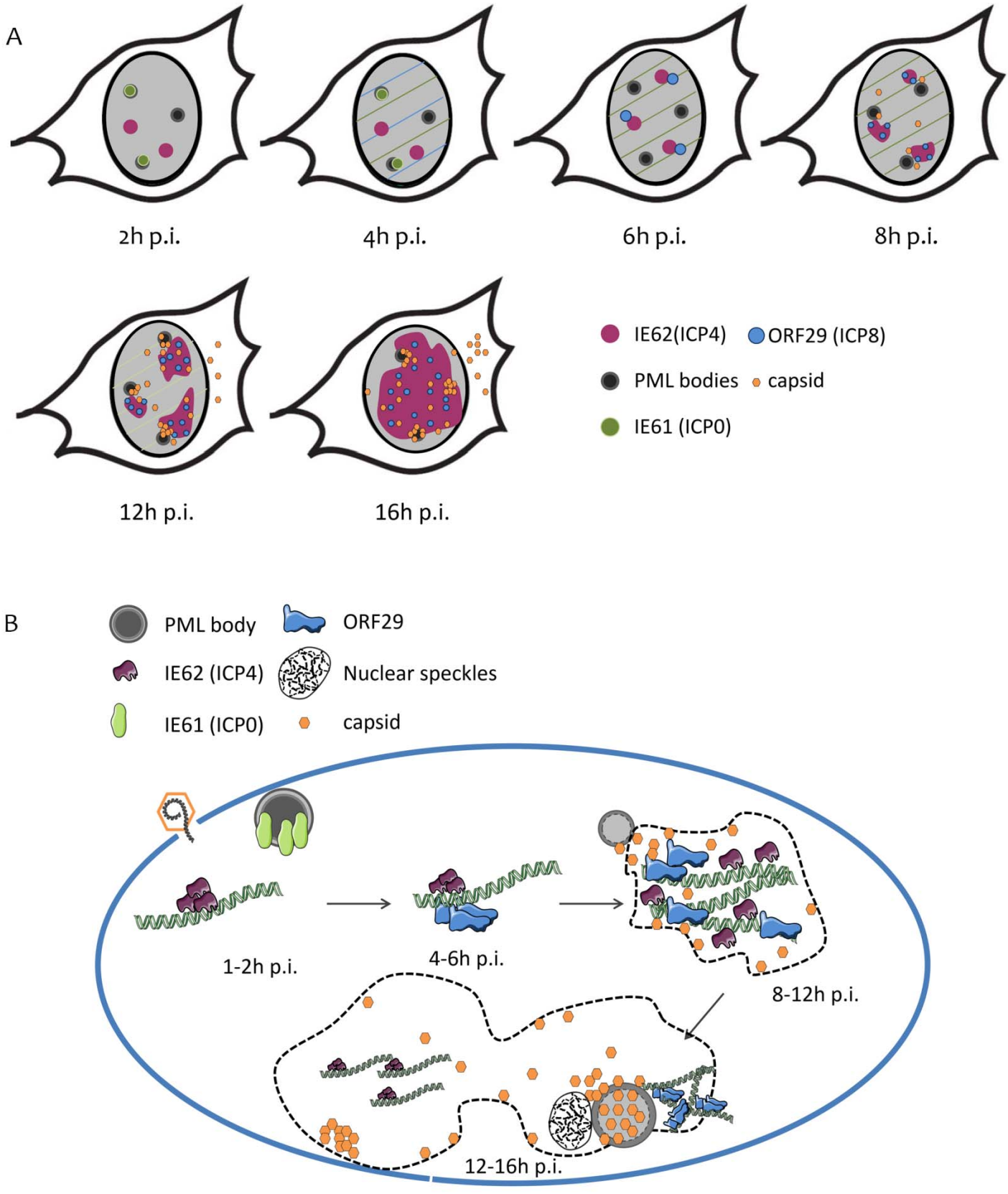


Figure 3. Proposed model of nuclear remodelling upon VZV infection.

while the RC enlarges, the first capsid aggregates appear, most of them in association with the PML bodies, and a few capsids can be observed in cytoplasmic areas. At 16h p.i., large VZV assemblons are present at the periphery of the RC that now occupy almost the entire nucleus. Some of them strongly colocalize with PML bodies and they are frequently juxtaposed to nuclear speckles. At later times, maybe because the capsid assembly slows down, the assemblons also contain soluble capsids and procapsids proteins. In VZV, no VICE domain formation is observed, which is another difference with HSV-1. In HSV-1, the PML dispersal is necessary for the VICE to form. It is then possible that the persistence of PML bodies in VZV-infected cells precludes their formation.

MATERIALS AND METHODS

1. Construction of the BAC-VZVΔGFP

The GFP cassette was removed from the BAC-VZV (kindly provided by Dr. Zhu) and replaced by an ampicillin resistance gene via homologous recombination with the *E. coli* strain DY380. Briefly, the DY380 bacteria containing the BAC-VZV were grown at 32°C to an O.D. of 0.6. The bacteria were then placed in a shaking waterbath at 42°C for 15 minutes, cooled on ice for 5 minutes and made electrocompetent by 3 successive wash steps with ice-cold water. The final bacterial pellet was resuspended in 50 µl of ice-cold 10% glycerol. The bacteria were electroporated in a BioRad Gene pulser, at 1.75 kV, 200 Ohms, 25 µF with 50 ng of an ampicillin resistance cassette, amplified by PCR using the pUC19 as template (table S1). Bacteria were plated on ampicillin LB-agar and the correct recombinant analyzed by PCR and sequencing.

2. Construction of recombinant and mutant viruses

Viruses presenting one (or two) open reading frame(s) fused with fluorescent protein encoding genes were constructed by modifying the BAC-VZV-ΔGFP. This was achieved via homologous recombination using the SW102 bacterial strain and a galK positive and negative selection cassette (Warming et al., 2005)

2.1. Generation of the targeting cassettes

The targeting cassettes were PCR-amplified with the specific set of primers and template as described in supplemental Table 1. After cycling, the PCR products were *Dpn*I-digested and purified after migration on a 1% agarose gel .

2.2. Generation of the recombinant BACs

The BAC-VZVΔGFP was first transferred to SW102 bacterial strain by electroporation. The galK cassettes were inserted into VZV genome as followed: SW102 bacteria containing the target BAC were grown, temperature-induced and made electrocompetent exactly as described above. Bacteria were electroporated with 50 ng of the appropriate galK cassette and recovered with 1 ml of LB at 32°C for 1 hour. After two washes with M9 solution (37 mM, Na_2HPO_4, 22 mM KH_2PO_4, 19 mM NaCl), bacteria were resuspended in 1 ml of M9 and 150 µl of them were plated onto galactose containing minimal medium. Colony-PCR was used to select clones containing the correct galK insertion. Positive clones were streaked onto galactose containing McConkey agar plates and single bright red colonies were further

analysed by restriction enzyme digestion of miniprep DNA. One or two clones were then chosen for the next recombination step: the replacement of the galK expressing cassette by a fluorescent protein encoding gene.

The galK positive SW102 clone selected at the first step was temperature-induced and made electrocompetent as described above. Identical electroporation conditions were used to transform the cells except that 200 ng of the galK replacement cassette were used. After 1h of recovery and two washes with M9, 150 µl of bacteria were plated onto minimal medium containing glycerol as carbon source and 2-deoxygalactose (Sigma-Aaldrich) to select against galK. Colony-PCR using two different sets of primers was used to screen the clones. Clones positive for both PCR were streaked on galactose-containing MacConkey agar plates and single white colonies were cultured overnight in 20 mL of LB to extract BAC DNA. Restriction digestion pattern was analyzed in details to confirm the correct replacement of the galK cassette.

3. Cell culture

Human melanoma cell line (MeWo) (ATCC number HTB-65) and human primary embryonic lung fibroblasts (MRC5) were cultured in Eagle's minimal essential medium supplemented with 1% of non-essential amino acids, 1% glutamine, 1% antibiotic mix (penicillin-streptomycin) and 10% FBS (Fisher Scientifics, GIBCO). Primary Human Foreskin Fibroblasts (HFF) were cultured in Dulbecco's modified Eagle's medium supplemented with 1% glutamine, 1% antibiotic mix (penicillin-streptomycin) and 10% FBS (Fisher Scientifics, GIBCO). Human normal keratinocytes immortalized by permanent TERT expression (N/TERT-1 cells) were obtained from Dr. James Rheinwald (Harvard Skin Disease Research Center) and cultured according to their protocol (available upon request at their cell culture facility).

4. Recombinant virus culture

To reconstitute recombinant viruses, 3 µg of the modified BACs per well of 6-well plates were transfected into MeWo cells using 6 µl of JetPEI (PolyPlus transfection), according to the manufacturer's protocol. Three days later, the cells were transferred to a 25 cm^2 flask and passaged near confluence every 2-3 days until typical infection foci appeared.

To infect HFF/MRC5/N/TERT-1 cells, strongly infected MeWo cells were trypsinized and placed in contact with the cells for 20 minutes. The cells were then washed 3 times with PBS and placed back in the incubator with complete culture medium.

5. Determination of viral growth

In order to determine the growth kinetics of the different viral recombinant strains, infected MeWo cells were trypsinized on day 0 and the proportion of GFP positive cells, corresponding to infected cells, was determined by FACS (BD CantoII). Four 25 cm^2 flasks of MeWo cells for each viral strain were then infected via co-culture with 200 or 500 infected cells (see figure legend). On day 1 to 4, one 25 cm^2 flask for each virus was trypsinized and serially diluted (dilution range 1/36 to 1/1024, performed in triplicates) on 24-well plates containing uninfected MeWo cells. Three days post-seeding, the number of infection foci was recorded in every countable well with an inverted fluorescence microscope. A second measure was carried out on the following day in order to increase accuracy.

6. Western blotting

Protein samples were incubated with sodium dodecyl sulfate (SDS)-loading buffer (125 mM Tris-HCl [pH 6.8], 4% SDS, 20% glycerol, 6% β-mercaptoethanol, 0.03% bromophenol blue) and boiled for 3 min. Proteins were then separated by SDS-PAGE and transferred onto a polyvinylidene difluoride membrane. Membranes were blocked with 5% nonfat dry milk PBS-Tween and then incubated with anti-GFP antibody (Roche, 1/500). After 3 washes, the membrane was incubated with the appropriate secondary antibody and the proteins of interest were detected using the Amersham ECL Western blotting detection reagents kit (GE Healthcare) and the ImageQuant LAS4000 (GE Healthcare).

7. Immunofluorescence

Coverslips were washed with PBS, fixed for 30 minutes in 4% paraformaldehyde-PBS, permeabilized in 0.1% Triton X100-PBS and blocked with 10% FBS-PBS. Coverslips were then incubated for 1-2 hours with the primary antibody diluted in 10% FBS-PBS as follows: mouse anti-beta-tubulin (1/500, Sigma), rabbit anti-PML (1/50, Santa Cruz), mouse anti-ORF40 (1/50, Santa Cruz), rabbit anti-ORF29 (1/200, kind gift from Dr. R. Cohrs), rabbit anti-ORF45/42 (1/200, kind gift from Dr. R. Visalli), rabbit anti-ORF33/33.5 (1/50, kind gift from Dr. F. Rixon), rabbit anti-ORF23 (1/200, kind gift from Dr. M. Sommer), mouse anti-V5 (1/500, Invitrogen), mouse anti-SC35 (1/500, Abcam), After 3 washes in 5% FBS-PBS, coverslips were incubated for 1 hour with the appropriate secondary antibody (coupled to Alexa568 or Alexa633, as described in the figure legend) (Invitrogen) diluted 1/400 in 10% FBS-PBS. After washing and nuclei staining with Hoechst33342 (Acros Organics) diluted 1/100000, coverslips were

mounted on glass slides with mowiol. Images were recorded with an Olympus FV1000 confocal inverted microscope using a 63x oil objective.

8. Live imaging

A Nikon A1R confocal microscope equipped with a motorized platform and a chamber controlling the temperature, the humidity and the CO_2 level was used for all live imaging experiments. For Fluorescence Recovery After Photobleaching (FRAP) experiments, a region of interest (ROI) was bleached via 488-laser illumination for 45 seconds at the laser maximum intensity before recording images every 15 seconds. For Fluorescence Loss in Photobleaching (FLIP), the ROI was first bleached for 1 minute before successive pictures acquisition (2 frames each time) followed by 5-seconds photobleaching.

9. Generation of shPML-MeWo cell line

Retrovirus vectors allowing shPML expression were generated after the transfection of phoenix-Ampho cells (ATCC #SD3443) with the pCKsuper-shPML (kind gift from Dr. C. Kyratsous). The retrovirus vectors were used to transduce MeWo cells that were selected with first 200 and then 500 µg/ml of hygromycin.

10. Electron microscopy

VZV eGFP-23p-infected wild-type MeWo cells and shPML-MeWo cells were washed in Sörensen's buffer and fixed for 1.5 h at 4°C with 2.5% glutaraldehyde in a Sörensen 0.1 M phosphate buffer (pH 7.4) and post-fixed for 30 minutes with 2% osmium tetroxide. After dehydration in graded ethanol, samples were embedded in Epon. Ultrathin sections obtained with a Reichert Ultracut S ultramicrotome were contrasted with uranyl acetate and lead citrate. Observations were made with a Jeol JEM-1400 transmission electron microscope at 80 kV.

11. Correlative microscopy

MeWo cells grown in a 35mm glass-bottom dish were infected via cell contact for **48h with eGFP-ORF23 VZV, washed with PBS and fixed with 4% paraformaldehyde-**PBS. In order to reduce the surface of cells to be analyzed, most of the monolayer was scraped off the dish to leave a central small area which was stained with Hoechst (1/100000 for 5 minutes), washed 3 times with PBS and visualized by confocal microscopy. An image Z-stack was recorded for each infection focus present on the glass coverslip that was then unglued from the plastic dish via a 10

seconds contact with methanol. The cells on the coverslip were fixed again for 1 h at room temperature with 2.5% glutaraldehyde in a Sörensen 0.1 M phosphate buffer (pH 7.4) and post-fixed for 30 minutes with 2% osmium tetroxide. After dehydration in graded ethanol, samples were embedded in Epon. Serial ultrathin sections obtained with a Reichert Ultracut S ultramicrotome were contrasted and observed as described above. Ultrathin sections were carefully scrutinized and compared with confocal images until a concordance was clearly assessed. Pictures of the chosen area were then systematically recorded on all available sections.

12. Generation of PML and SP100 expressing vectors

The pDONR223-PML and the pDONR223-SP100 were obtained in the human ORFeome 5.1 bank. LR reaction with pCS-DEST-mCherry or pCS-DEST-eGFP were performed overnight at room tempeture with 150ng of entry clone and 150ng of destination vector, 1 µl of LRII clonase mix (invitrogen) in a total volume of 10 µl. One µl of proteinase K was added to the LR reaction that was place one hour at 37°C. The reaction mix was dialysed for 1h and then electroporated in electrocompetent DH10B.

13. Cell transfection

MeWo cells were transfected with 1µg of eGFP-PML or mCherry-PML expression vectors with lipofectamine according to the manufacturer's protocol (invitrogen).

BIBLIOGRAPHY

Abaitua, F., and O'Hare, P. (2008). Identification of a highly conserved, functional nuclear localization signal within the N-terminal region of herpes simplex virus type 1 VP1-2 tegument protein. J Virol *82*, 5234-5244.
Adamson, A.L., and Kenney, S. (2001). Epstein-barr virus immediate-early protein BZLF1 is SUMO-1 modified and disrupts promyelocytic leukemia bodies. J Virol *75*, 2388-2399.
Addison, C., Rixon, F.J., and Preston, V.G. (1990). Herpes simplex virus type 1 UL28 gene product is important for the formation of mature capsids. J Gen Virol *71 (Pt 10)*, 2377-2384.
Aggarwal, A., Miranda-Saksena, M., Boadle, R.A., Kelly, B.J., Diefenbach, R.J., Alam, W., and Cunningham, A.L. (2012). Ultrastructural visualization of individual tegument protein dissociation during entry of herpes simplex virus 1 into human and rat dorsal root ganglion neurons. J Virol *86*, 6123-6137.
Ahn, J.H., and Hayward, G.S. (1997). The major immediate-early proteins IE1 and IE2 of human cytomegalovirus colocalize with and disrupt PML-associated nuclear bodies at very early times in infected permissive cells. Journal of virology *71*, 4599-4613.
Alconada, A., Bauer, U., and Hoflack, B. (1996). A tyrosine-based motif and a casein kinase II phosphorylation site regulate the intracellular trafficking of the varicella-zoster virus glycoprotein I, a protein localized in the trans-Golgi network. The EMBO journal *15*, 6096-6110.
Alconada, A., Bauer, U., Sodeik, B., and Hoflack, B. (1999). Intracellular traffic of herpes simplex virus glycoprotein gE: characterization of the sorting signals required for its trans-Golgi network localization. J Virol *73*, 377-387.
Antinone, S.E., Shubeita, G.T., Coller, K.E., Lee, J.I., Haverlock-Moyns, S., Gross, S.P., and Smith, G.A. (2006). The Herpesvirus capsid surface protein, VP26, and the majority of the tegument proteins are dispensable for capsid transport toward the nucleus. J Virol *80*, 5494-5498.
Arii, J., Goto, H., Suenaga, T., Oyama, M., Kozuka-Hata, H., Imai, T., Minowa, A., Akashi, H., Arase, H., Kawaoka, Y., *et al.* (2010). Non-muscle myosin IIA is a functional entry receptor for herpes simplex virus-1. Nature *467*, 859-862.
Arvin, A.M. (1996). Varicella-zoster virus. Clin Microbiol Rev *9*, 361-381.
Arvin, A.M., Moffat, J.F., Sommer, M., Oliver, S., Che, X., Vleck, S., Zerboni, L., and Ku, C.C. (2010). Varicella-zoster virus T cell tropism and the pathogenesis of skin infection. Current topics in microbiology and immunology *342*, 189-209.
Arvin, A.M.G., A. (2000). Varicella-Zoster Virus: virology and clinical management (Cambridge University Press).
Asano, Y., Hirose, S., Iwayama, S., Miyata, T., Yazaki, T., and Takahashi, M. (1982). Protective effect of immediate inoculation of a live varicella vaccine in household contacts in relation to the viral dose and interval between exposure and vaccination. Biken journal *25*, 43-45.
Asano, Y., Yazaki, T., Miyata, T., Nakayama, H., and Hirose, S. (1975). Application of a live attenuated varicella vaccine to hospitalized children and its protective effect on spread of varicella infection. Biken journal *18*, 35-40.
Baiker, A., Bagowski, C., Ito, H., Sommer, M., Zerboni, L., Fabel, K., Hay, J., Ruyechan, W., and Arvin, A.M. (2004). The immediate-early 63 protein of Varicella-Zoster virus: analysis of functional domains required for replication in vitro and for T-cell and skin tropism in the SCIDhu model in vivo. J Virol *78*, 1181-1194.
Baines, J.D. (2011). Herpes simplex virus capsid assembly and DNA packaging: a present and future antiviral drug target. Trends in microbiology *19*, 606-613.
Baines, J.D., Jacob, R.J., Simmerman, L., and Roizman, B. (1995). The herpes simplex virus 1 UL11 proteins are associated with cytoplasmic and nuclear membranes and with nuclear bodies of infected cells. J Virol *69*, 825-833.
Baines, J.D., Koyama, A.H., Huang, T., and Roizman, B. (1994). The UL21 gene products of herpes simplex virus 1 are dispensable for growth in cultured cells. J Virol *68*, 2929-2936.
Baines, J.D., Wills, E., Jacob, R.J., Pennington, J., and Roizman, B. (2007). Glycoprotein M of herpes simplex virus 1 is incorporated into virions during budding at the inner nuclear membrane. J Virol *81*, 800-812.
Bastian, T.W., and Rice, S.A. (2009). Identification of sequences in herpes simplex virus type 1 ICP22 that influence RNA polymerase II modification and viral late gene expression. J Virol *83*, 128-139.
Bernardi, R., and Pandolfi, P.P. (2007). Structure, dynamics and functions of promyelocytic leukaemia nuclear bodies. Nature reviews. Molecular cell biology *8*, 1006-1016.
Besse, S., Vigneron, M., Pichard, E., and Puvion-Dutilleul, F. (1995). Synthesis and maturation of viral transcripts in herpes simplex virus type 1 infected HeLa cells: the role of interchromatin granules. Gene expression *4*, 143-161.
Birlea, M., Arendt, G., Orhan, E., Schmid, D.S., Bellini, W.J., Schmidt, C., Gilden, D., and Cohrs, R.J. (2011). Subclinical reactivation of varicella zoster virus in all stages of HIV infection. Journal of the neurological sciences *304*, 22-24.
Blumenthal, D.T., Shacham-Shmueli, E., Bokstein, F., Schmid, D.S., Cohrs, R.J., Nagel, M.A., Mahalingam, R., and Gilden, D. (2011). Zoster sine herpete: virologic verification by detection of anti-VZV IgG antibody in CSF. Neurology *76*, 484-485.
Boehmer, P.E., and Lehman, I.R. (1997). Herpes simplex virus DNA replication. Annual review of biochemistry *66*, 347-384.
Boisvert, F.M., Hendzel, M.J., and Bazett-Jones, D.P. (2000). Promyelocytic leukemia (PML) nuclear bodies are protein structures that do not accumulate RNA. The Journal of cell biology *148*, 283-292.
Bontems, S., Di Valentin, E., Baudoux, L., Rentier, B., Sadzot-Delvaux, C., and Piette, J. (2002). Phosphorylation of varicella-zoster virus IE63 protein by casein kinases influences its cellular localization and gene regulation activity. J Biol Chem *277*, 21050-21060.

Bottcher, S., Maresch, C., Granzow, H., Klupp, B.G., Teifke, J.P., and Mettenleiter, T.C. (2008). Mutagenesis of the active-site cysteine in the ubiquitin-specific protease contained in large tegument protein pUL36 of pseudorabies virus impairs viral replication in vitro and neuroinvasion in vivo. J Virol *82*, 6009-6016.
Boucaud, D., Yoshitake, H., Hay, J., and Ruyechan, W. (1998). The varicella-zoster virus (VZV) open-reading frame 29 protein acts as a modulator of a late VZV gene promoter. The Journal of infectious diseases *178 Suppl 1*, S34-38.
Boutell, C., Orr, A., and Everett, R.D. (2003). PML residue lysine 160 is required for the degradation of PML induced by herpes simplex virus type 1 regulatory protein ICP0. J Virol *77*, 8686-8694.
Brack, A.R., Klupp, B.G., Granzow, H., Tirabassi, R., Enquist, L.W., and Mettenleiter, T.C. (2000). Role of the cytoplasmic tail of pseudorabies virus glycoprotein E in virion formation. J Virol *74*, 4004-4016.
Brisson, M., Gay, N.J., Edmunds, W.J., and Andrews, N.J. (2002). Exposure to varicella boosts immunity to herpes-zoster: implications for mass vaccination against chickenpox. Vaccine *20*, 2500-2507.
Brunell, P.A., Ren, L.C., Cohen, J.I., and Straus, S.E. (1999). Viral gene expression in rat trigeminal ganglia following neonatal infection with varicella-zoster virus. Journal of medical virology *58*, 286-290.
Bucks, M.A., O'Regan, K.J., Murphy, M.A., Wills, J.W., and Courtney, R.J. (2007). Herpes simplex virus type 1 tegument proteins VP1/2 and UL37 are associated with intranuclear capsids. Virology *361*, 316-324.
Burch, A.D., and Weller, S.K. (2004). Nuclear sequestration of cellular chaperone and proteasomal machinery during herpes simplex virus type 1 infection. J Virol *78*, 7175-7185.
Campadelli, G., Brandimarti, R., Di Lazzaro, C., Ward, P.L., Roizman, B., and Torrisi, M.R. (1993). Fragmentation and dispersal of Golgi proteins and redistribution of glycoproteins and glycolipids processed through the Golgi apparatus after infection with herpes simplex virus 1. Proc Natl Acad Sci U S A *90*, 2798-2802.
Cano-Monreal, G.L., Wylie, K.M., Cao, F., Tavis, J.E., and Morrison, L.A. (2009). Herpes simplex virus 2 UL13 protein kinase disrupts nuclear lamins. Virology *392*, 137-147.
Cardone, G., Heymann, J.B., Cheng, N., Trus, B.L., and Steven, A.C. (2012). Procapsid assembly, maturation, nuclear exit: dynamic steps in the production of infectious herpesvirions. Advances in experimental medicine and biology *726*, 423-439.
Carpenter, J.E., Hutchinson, J.A., Jackson, W., and Grose, C. (2008). Egress of light particles among filopodia on the surface of Varicella-Zoster virus-infected cells. J Virol *82*, 2821-2835.
Catez, F., Picard, C., Held, K., Gross, S., Rousseau, A., Theil, D., Sawtell, N., Labetoulle, M., and Lomonte, P. (2012). HSV-1 genome subnuclear positioning and associations with host-cell PML-NBs and centromeres regulate LAT locus transcription during latency in neurons. PLoS pathogens *8*, e1002852.
Cavanaugh, N.A., Ramirez-Aguilar, K.A., Urban, M., and Kuchta, R.D. (2009). Herpes simplex virus-1 helicase-primase: roles of each subunit in DNA binding and phosphodiester bond formation. Biochemistry *48*, 10199-10207.
Chang, L., Godinez, W.J., Kim, I.H., Tektonidis, M., de Lanerolle, P., Eils, R., Rohr, K., and Knipe, D.M. (2011). Herpesviral replication compartments move and coalesce at nuclear speckles to enhance export of viral late mRNA. Proc Natl Acad Sci U S A *108*, E136-144.
Chang, Y.E., and Roizman, B. (1993). The product of the UL31 gene of herpes simplex virus 1 is a nuclear phosphoprotein which partitions with the nuclear matrix. J Virol *67*, 6348-6356.
Chaudhuri, V., Sommer, M., Rajamani, J., Zerboni, L., and Arvin, A.M. (2008). Functions of Varicella-zoster virus ORF23 capsid protein in viral replication and the pathogenesis of skin infection. J Virol *82*, 10231-10246.
Che, H., Lukas, C., Morel, J., and Combe, B. (2013a). Risk of herpes/herpes zoster during anti-tumor necrosis factor therapy in patients with rheumatoid arthritis. Systematic review and meta-analysis. Joint, bone, spine : revue du rhumatisme.
Che, X., Oliver, S.L., Reichelt, M., Sommer, M.H., Haas, J., Rovis, T.L., and Arvin, A.M. (2013b). ORF11 protein interacts with the ORF9 essential tegument protein in varicella-zoster virus infection. J Virol *87*, 5106-5117.
Che, X., Reichelt, M., Sommer, M.H., Rajamani, J., Zerboni, L., and Arvin, A.M. (2008). Functions of the ORF9-to-ORF12 gene cluster in varicella-zoster virus replication and in the pathogenesis of skin infection. J Virol *82*, 5825-5834.
Che, X., Zerboni, L., Sommer, M.H., and Arvin, A.M. (2006). Varicella-zoster virus open reading frame 10 is a virulence determinant in skin cells but not in T cells in vivo. J Virol *80*, 3238-3248.
Chelbi-Alix, M.K., Pelicano, L., Quignon, F., Koken, M.H., Venturini, L., Stadler, M., Pavlovic, J., Degos, L., and de The, H. (1995). Induction of the PML protein by interferons in normal and APL cells. Leukemia *9*, 2027-2033.
Chen, J.J., Gershon, A.A., Li, Z., Cowles, R.A., and Gershon, M.D. (2011a). Varicella zoster virus (VZV) infects and establishes latency in enteric neurons. Journal of neurovirology *17*, 578-589.
Chen, J.J., Zhu, Z., Gershon, A.A., and Gershon, M.D. (2004). Mannose 6-phosphate receptor dependence of varicella zoster virus infection in vitro and in the epidermis during varicella and zoster. Cell *119*, 915-926.
Chen, Y., Bai, P., Mackay, S., Korza, G., Carson, J.H., Kuchta, R.D., and Weller, S.K. (2011b). Herpes simplex virus type 1 helicase-primase: DNA binding and consequent protein oligomerization and primase activation. J Virol *85*, 968-978.
Chowdary, T.K., Cairns, T.M., Atanasiu, D., Cohen, G.H., Eisenberg, R.J., and Heldwein, E.E. (2010). Crystal structure of the conserved herpesvirus fusion regulator complex gH-gL. Nature structural & molecular biology *17*, 882-888.
Church, G.A., and Wilson, D.W. (1997). Study of herpes simplex virus maturation during a synchronous wave of assembly. J Virol *71*, 3603-3612.
Cilloniz, C., Jackson, W., Grose, C., Czechowski, D., Hay, J., and Ruyechan, W.T. (2007). The varicella-zoster virus (VZV) ORF9 protein interacts with the IE62 major VZV transactivator. J Virol *81*, 761-774.
Clarke, P., Beer, T., Cohrs, R., and Gilden, D.H. (1995). Configuration of latent varicella-zoster virus DNA. J Virol *69*, 8151-8154.
Cohen, J.I. (2010a). Rodent models of varicella-zoster virus neurotropism. Current topics in microbiology and immunology *342*, 277-289.
Cohen, J.I. (2010b). The varicella-zoster virus genome. Current topics in microbiology and immunology *342*, 1-14.

Cohen, J.I., Cox, E., Pesnicak, L., Srinivas, S., and Krogmann, T. (2004). The varicella-zoster virus open reading frame 63 latency-associated protein is critical for establishment of latency. J Virol *78*, 11833-11840.
Cohen, J.I., Moskal, T., Shapiro, M., and Purcell, R.H. (1996). Varicella in Chimpanzees. Journal of medical virology *50*, 289-292.
Cohen, J.I., and Seidel, K.E. (1993). Generation of varicella-zoster virus (VZV) and viral mutants from cosmid DNAs: VZV thymidylate synthetase is not essential for replication in vitro. Proc Natl Acad Sci U S A *90*, 7376-7380.
Cohrs, R.J., Gilden, D.H., Kinchington, P.R., Grinfeld, E., and Kennedy, P.G. (2003a). Varicella-zoster virus gene 66 transcription and translation in latently infected human Ganglia. J Virol *77*, 6660-6665.
Cohrs, R.J., Hurley, M.P., and Gilden, D.H. (2003b). Array analysis of viral gene transcription during lytic infection of cells in tissue culture with Varicella-Zoster virus. J Virol *77*, 11718-11732.
Cohrs, R.J., Mehta, S.K., Schmid, D.S., Gilden, D.H., and Pierson, D.L. (2008). Asymptomatic reactivation and shed of infectious varicella zoster virus in astronauts. Journal of medical virology *80*, 1116-1122.
Cohrs, R.J., Wischer, J., Essman, C., and Gilden, D.H. (2002). Characterization of varicella-zoster virus gene 21 and 29 proteins in infected cells. J Virol *76*, 7228-7238.
Coller, K.E., Lee, J.I., Ueda, A., and Smith, G.A. (2007). The capsid and tegument of the alphaherpesviruses are linked by an interaction between the UL25 and VP1/2 proteins. J Virol *81*, 11790-11797.
Connolly, S.A., Jackson, J.O., Jardetzky, T.S., and Longnecker, R. (2011). Fusing structure and function: a structural view of the herpesvirus entry machinery. Nature reviews. Microbiology *9*, 369-381.
Croen, K.D., Ostrove, J.M., Dragovic, L.J., and Straus, S.E. (1988). Patterns of gene expression and sites of latency in human nerve ganglia are different for varicella-zoster and herpes simplex viruses. Proc Natl Acad Sci U S A *85*, 9773-9777.
Crute, J.J., Tsurumi, T., Zhu, L.A., Weller, S.K., Olivo, P.D., Challberg, M.D., Mocarski, E.S., and Lehman, I.R. (1989). Herpes simplex virus 1 helicase-primase: a complex of three herpes-encoded gene products. Proc Natl Acad Sci U S A *86*, 2186-2189.
Curanovic, D., Lyman, M.G., Bou-Abboud, C., Card, J.P., and Enquist, L.W. (2009). Repair of the UL21 locus in pseudorabies virus Bartha enhances the kinetics of retrograde, transneuronal infection in vitro and in vivo. J Virol *83*, 1173-1183.
D'Orazi, G., Cecchinelli, B., Bruno, T., Manni, I., Higashimoto, Y., Saito, S., Gostissa, M., Coen, S., Marchetti, A., Del Sal, G., *et al.* (2002). Homeodomain-interacting protein kinase-2 phosphorylates p53 at Ser 46 and mediates apoptosis. Nature cell biology *4*, 11-19.
Davison, A.J. (1984). Structure of the genome termini of varicella-zoster virus. J Gen Virol *65 (Pt 11)*, 1969-1977.
Davison, A.J. (1991). Varicella-zoster virus. The Fourteenth Fleming lecture. J Gen Virol *72 (Pt 3)*, 475-486.
Davison, A.J., and Scott, J.E. (1986). The complete DNA sequence of varicella-zoster virus. J Gen Virol *67 (Pt 9)*, 1759-1816.
Day, P.M., Baker, C.C., Lowy, D.R., and Schiller, J.T. (2004). Establishment of papillomavirus infection is enhanced by promyelocytic leukemia protein (PML) expression. Proceedings of the National Academy of Sciences of the United States of America *101*, 14252-14257.
Day, P.M., Roden, R.B., Lowy, D.R., and Schiller, J.T. (1998). The papillomavirus minor capsid protein, L2, induces localization of the major capsid protein, L1, and the viral transcription/replication protein, E2, to PML oncogenic domains. J Virol *72*, 142-150.
De Castro, N., Carmagnat, M., Kerneis, S., Scieux, C., Rabian, C., and Molina, J.M. (2011). Varicella-zoster virus-specific cell-mediated immune responses in HIV-infected adults. AIDS research and human retroviruses *27*, 1089-1097.
de Maisieres, P.D., Baudoux-Tebache, L., Merville, M.P., Rentier, B., Bours, V., and Piette, J. (1998). Activation of the human immunodeficiency virus long terminal repeat by varicella-zoster virus IE4 protein requires nuclear factor-kappaB and involves both the amino-terminal and the carboxyl-terminal cysteine-rich region. J Biol Chem *273*, 13636-13644.
de Oliveira, A.P., Glauser, D.L., Laimbacher, A.S., Strasser, R., Schraner, E.M., Wild, P., Ziegler, U., Breakefield, X.O., Ackermann, M., and Fraefel, C. (2008). Live visualization of herpes simplex virus type 1 compartment dynamics. J Virol *82*, 4974-4990.
Defechereux, P., Debrus, S., Baudoux, L., Rentier, B., and Piette, J. (1997). Varicella-zoster virus open reading frame 4 encodes an immediate-early protein with posttranscriptional regulatory properties. J Virol *71*, 7073-7079.
Defechereux, P., Melen, L., Baudoux, L., Merville-Louis, M.P., Rentier, B., and Piette, J. (1993). Characterization of the regulatory functions of varicella-zoster virus open reading frame 4 gene product. J Virol *67*, 4379-4385.
Desai, P., Akpa, J.C., and Person, S. (2003). Residues of VP26 of herpes simplex virus type 1 that are required for its interaction with capsids. J Virol *77*, 391-404.
Desai, P., and Person, S. (1998). Incorporation of the green fluorescent protein into the herpes simplex virus type 1 capsid. J Virol *72*, 7563-7568.
Desai, P., Sexton, G.L., Huang, E., and Person, S. (2008). Localization of herpes simplex virus type 1 UL37 in the Golgi complex requires UL36 but not capsid structures. J Virol *82*, 11354-11361.
Desai, P., Sexton, G.L., McCaffery, J.M., and Person, S. (2001). A null mutation in the gene encoding the herpes simplex virus type 1 UL37 polypeptide abrogates virus maturation. J Virol *75*, 10259-10271.
Desai, P.J. (2000). A null mutation in the UL36 gene of herpes simplex virus type 1 results in accumulation of unenveloped DNA-filled capsids in the cytoplasm of infected cells. J Virol *74*, 11608-11618.
Di Valentin, E., Bontems, S., Habran, L., Jolois, O., Markine-Goriaynoff, N., Vanderplasschen, A., Sadzot-Delvaux, C., and Piette, J. (2005). Varicella-zoster virus IE63 protein represses the basal transcription machinery by disorganizing the pre-initiation complex. Biological chemistry *386*, 255-267.
Disney, G.H., McKee, T.A., Preston, C.M., and Everett, R.D. (1990). The product of varicella-zoster virus gene 62 autoregulates its own promoter. J Gen Virol *71 (Pt 12)*, 2999-3003.
Doerr, H.W. (2013). Progress in VZV vaccination? Some concerns. Medical microbiology and immunology *202*, 257-258.

Dohner, K., Radtke, K., Schmidt, S., and Sodeik, B. (2006). Eclipse phase of herpes simplex virus type 1 infection: Efficient dynein-mediated capsid transport without the small capsid protein VP26. J Virol *80*, 8211-8224.
Dohner, K., Wolfstein, A., Prank, U., Echeverri, C., Dujardin, D., Vallee, R., and Sodeik, B. (2002). Function of dynein and dynactin in herpes simplex virus capsid transport. Mol Biol Cell *13*, 2795-2809.
Doucas, V., Ishov, A.M., Romo, A., Juguilon, H., Weitzman, M.D., Evans, R.M., and Maul, G.G. (1996). Adenovirus replication is coupled with the dynamic properties of the PML nuclear structure. Genes & development *10*, 196-207.
Douglas, M.W., Diefenbach, R.J., Homa, F.L., Miranda-Saksena, M., Rixon, F.J., Vittone, V., Byth, K., and Cunningham, A.L. (2004). Herpes simplex virus type 1 capsid protein VP26 interacts with dynein light chains RP3 and Tctex1 and plays a role in retrograde cellular transport. J Biol Chem *279*, 28522-28530.
Duffy, C., Lavail, J.H., Tauscher, A.N., Wills, E.G., Blaho, J.A., and Baines, J.D. (2006). Characterization of a UL49-null mutant: VP22 of herpes simplex virus type 1 facilitates viral spread in cultured cells and the mouse cornea. J Virol *80*, 8664-8675.
Dumas, A.M., Geelen, J.L., Weststrate, M.W., Wertheim, P., and van der Noordaa, J. (1981). XbaI, PstI, and BglII restriction enzyme maps of the two orientations of the varicella-zoster virus genome. J Virol *39*, 390-400.
Duus, K.M., Hatfield, C., and Grose, C. (1995). Cell surface expression and fusion by the varicella-zoster virus gH:gL glycoprotein complex: analysis by laser scanning confocal microscopy. Virology *210*, 429-440.
Echevarria, J.M., Casas, I., and Martinez-Martin, P. (1997). Infections of the nervous system caused by varicella-zoster virus: a review. Intervirology *40*, 72-84.
Eisfeld, A.J., Turse, S.E., Jackson, S.A., Lerner, E.C., and Kinchington, P.R. (2006). Phosphorylation of the varicella-zoster virus (VZV) major transcriptional regulatory protein IE62 by the VZV open reading frame 66 protein kinase. J Virol *80*, 1710-1723.
Elliott, G., Hafezi, W., Whiteley, A., and Bernard, E. (2005). Deletion of the herpes simplex virus VP22-encoding gene (UL49) alters the expression, localization, and virion incorporation of ICP0. J Virol *79*, 9735-9745.
Erazo, A., Yee, M.B., Banfield, B.W., and Kinchington, P.R. (2011). The alphaherpesvirus US3/ORF66 protein kinases direct phosphorylation of the nuclear matrix protein matrin 3. J Virol *85*, 568-581.
Erickson, K.D., Bouchet-Marquis, C., Heiser, K., Szomolanyi-Tsuda, E., Mishra, R., Lamothe, B., Hoenger, A., and Garcea, R.L. (2012). Virion assembly factories in the nucleus of polyomavirus-infected cells. PLoS pathogens *8*, e1002630.
Eshleman, E., Shahzad, A., and Cohrs, R.J. (2011). Varicella zoster virus latency. Future virology *6*, 341-355.
Eskiw, C.H., Dellaire, G., Mymryk, J.S., and Bazett-Jones, D.P. (2003). Size, position and dynamic behavior of PML nuclear bodies following cell stress as a paradigm for supramolecular trafficking and assembly. Journal of cell science *116*, 4455-4466.
Everett, R.D. (2006). Interactions between DNA viruses, ND10 and the DNA damage response. Cellular microbiology *8*, 365-374.
Everett, R.D. (2013). The spatial organization of DNA virus genomes in the nucleus. PLoS pathogens *9*, e1003386.
Everett, R.D., Boutell, C., McNair, C., Grant, L., and Orr, A. (2010). Comparison of the biological and biochemical activities of several members of the alphaherpesvirus ICP0 family of proteins. Journal of virology *84*, 3476-3487.
Everett, R.D., and Chelbi-Alix, M.K. (2007). PML and PML nuclear bodies: implications in antiviral defence. Biochimie *89*, 819-830.
Everett, R.D., Freemont, P., Saitoh, H., Dasso, M., Orr, A., Kathoria, M., and Parkinson, J. (1998). The disruption of ND10 during herpes simplex virus infection correlates with the Vmw110- and proteasome-dependent loss of several PML isoforms. J Virol *72*, 6581-6591.
Everett, R.D., and Maul, G.G. (1994). HSV-1 IE protein Vmw110 causes redistribution of PML. The EMBO journal *13*, 5062-5069.
Everett, R.D., and Murray, J. (2005). ND10 components relocate to sites associated with herpes simplex virus type 1 nucleoprotein complexes during virus infection. J Virol *79*, 5078-5089.
Everett, R.D., Murray, J., Orr, A., and Preston, C.M. (2007). Herpes simplex virus type 1 genomes are associated with ND10 nuclear substructures in quiescently infected human fibroblasts. J Virol *81*, 10991-11004.
Everett, R.D., Parada, C., Gripon, P., Sirma, H., and Orr, A. (2008). Replication of ICP0-null mutant herpes simplex virus type 1 is restricted by both PML and Sp100. J Virol *82*, 2661-2672.
Everett, R.D., Rechter, S., Papior, P., Tavalai, N., Stamminger, T., and Orr, A. (2006). PML contributes to a cellular mechanism of repression of herpes simplex virus type 1 infection that is inactivated by ICP0. J Virol *80*, 7995-8005.
Everett, R.D., Sourvinos, G., Leiper, C., Clements, J.B., and Orr, A. (2004). Formation of nuclear foci of the herpes simplex virus type 1 regulatory protein ICP4 at early times of infection: localization, dynamics, recruitment of ICP27, and evidence for the de novo induction of ND10-like complexes. Journal of virology *78*, 1903-1917.
Falkenberg, M., Bushnell, D.A., Elias, P., and Lehman, I.R. (1997). The UL8 subunit of the heterotrimeric herpes simplex virus type 1 helicase-primase is required for the unwinding of single strand DNA-binding protein (ICP8)-coated DNA substrates. J Biol Chem *272*, 22766-22770.
Farnsworth, A., Goldsmith, K., and Johnson, D.C. (2003). Herpes simplex virus glycoproteins gD and gE/gI serve essential but redundant functions during acquisition of the virion envelope in the cytoplasm. J Virol *77*, 8481-8494.
Farnsworth, A., Wisner, T.W., Webb, M., Roller, R., Cohen, G., Eisenberg, R., and Johnson, D.C. (2007). Herpes simplex virus glycoproteins gB and gH function in fusion between the virion envelope and the outer nuclear membrane. Proc Natl Acad Sci U S A *104*, 10187-10192.
Farrell, M.J., Dobson, A.T., and Feldman, L.T. (1991). Herpes simplex virus latency-associated transcript is a stable intron. Proc Natl Acad Sci U S A *88*, 790-794.
Favoreel, H.W. (2006). The why's of Y-based motifs in alphaherpesvirus envelope proteins. Virus research *117*, 202-208.
Favoreel, H.W., Van Minnebruggen, G., Nauwynck, H.J., Enquist, L.W., and Pensaert, M.B. (2002). A tyrosine-based motif in the cytoplasmic tail of pseudorabies virus glycoprotein B is important for both antibody-induced internalization of viral glycoproteins and efficient cell-to-cell spread. J Virol *76*, 6845-6851.

Feierbach, B., Piccinotti, S., Bisher, M., Denk, W., and Enquist, L.W. (2006). Alpha-herpesvirus infection induces the formation of nuclear actin filaments. PLoS pathogens *2*, e85.
Field, H.J., and Vere Hodge, R.A. (2013). Recent developments in anti-herpesvirus drugs. British medical bulletin *106*, 213-249.
Fleisher, G., Henry, W., McSorley, M., Arbeter, A., and Plotkin, S. (1981). Life-threatening complications of varicella. American journal of diseases of children *135*, 896-899.
Florin, L., Schafer, F., Sotlar, K., Streeck, R.E., and Sapp, M. (2002). Reorganization of nuclear domain 10 induced by papillomavirus capsid protein l2. Virology *295*, 97-107.
Forest, T., Barnard, S., and Baines, J.D. (2005). Active intranuclear movement of herpesvirus capsids. Nature cell biology *7*, 429-431.
Fossum, E., Friedel, C.C., Rajagopala, S.V., Titz, B., Baiker, A., Schmidt, T., Kraus, T., Stellberger, T., Rutenberg, C., Suthram, S.*, et al.* (2009). Evolutionarily conserved herpesviral protein interaction networks. PLoS pathogens *5*, e1000570.
Fuchs, W., Granzow, H., Klupp, B.G., Kopp, M., and Mettenleiter, T.C. (2002). The UL48 tegument protein of pseudorabies virus is critical for intracytoplasmic assembly of infectious virions. J Virol *76*, 6729-6742.
Fuchs, W., Klupp, B.G., Granzow, H., Leege, T., and Mettenleiter, T.C. (2009). Characterization of pseudorabies virus (PrV) cleavage-encapsidation proteins and functional complementation of PrV pUL32 by the homologous protein of herpes simplex virus type 1. J Virol *83*, 3930-3943.
Galdiero, S., Falanga, A., Vitiello, M., Browne, H., Pedone, C., and Galdiero, M. (2005). Fusogenic domains in herpes simplex virus type 1 glycoprotein H. J Biol Chem *280*, 28632-28643.
Galdiero, S., Vitiello, M., D'Isanto, M., Falanga, A., Collins, C., Raieta, K., Pedone, C., Browne, H., and Galdiero, M. (2006). Analysis of synthetic peptides from heptad-repeat domains of herpes simplex virus type 1 glycoproteins H and B. J Gen Virol *87*, 1085-1097.
Garland, J. (1943). Varicella Following Exposure to Herpes Zoster. New England Journal of Medicine *228*, 336-337.
Gasparovic, M.L., Maginnis, M.S., O'Hara, B.A., Dugan, A.S., and Atwood, W.J. (2009). Modulation of PML protein expression regulates JCV infection. Virology *390*, 279-288.
Gaudreault, N., and Jones, C. (2011). Regulation of promyelocytic leukemia (PML) protein levels and cell morphology by bovine herpesvirus 1 infected cell protein 0 (bICP0) and mutant bICP0 proteins that do not localize to the nucleus. Virus research *156*, 17-24.
Geraghty, R.J., Krummenacher, C., Cohen, G.H., Eisenberg, R.J., and Spear, P.G. (1998). Entry of alphaherpesviruses mediated by poliovirus receptor-related protein 1 and poliovirus receptor. Science *280*, 1618-1620.
Gershburg, E., and Pagano, J.S. (2008). Conserved herpesvirus protein kinases. Biochimica et biophysica acta *1784*, 203-212.
Gershburg, E., Raffa, S., Torrisi, M.R., and Pagano, J.S. (2007). Epstein-Barr virus-encoded protein kinase (BGLF4) is involved in production of infectious virus. J Virol *81*, 5407-5412.
Gershon, A.A. (2011). The history and mystery of VZV in saliva. The Journal of infectious diseases *204*, 815-816.
Gershon, A.A., Chen, J., Davis, L., Krinsky, C., Cowles, R., Reichard, R., and Gershon, M. (2012). Latency of varicella zoster virus in dorsal root, cranial, and enteric ganglia in vaccinated children. Transactions of the American Clinical and Climatological Association *123*, 17-33; discussion 33-15.
Gershon, M.D., and Gershon, A.A. (2010). VZV infection of keratinocytes: production of cell-free infectious virions in vivo. Current topics in microbiology and immunology *342*, 173-188.
Gianni, T., Fato, R., Bergamini, C., Lenaz, G., and Campadelli-Fiume, G. (2006a). Hydrophobic alpha-helices 1 and 2 of herpes simplex virus gH interact with lipids, and their mimetic peptides enhance virus infection and fusion. J Virol *80*, 8190-8198.
Gianni, T., Forghieri, C., and Campadelli-Fiume, G. (2006b). The herpesvirus glycoproteins B and H.L are sequentially recruited to the receptor-bound gD to effect membrane fusion at virus entry. Proc Natl Acad Sci U S A *103*, 14572-14577.
Gilden, D., Cohrs, R.J., Mahalingam, R., and Nagel, M.A. (2010). Neurological disease produced by varicella zoster virus reactivation without rash. Current topics in microbiology and immunology *342*, 243-253.
Gilden, D.H., Gesser, R., Smith, J., Wellish, M., Laguardia, J.J., Cohrs, R.J., and Mahalingam, R. (2001). Presence of VZV and HSV-1 DNA in human nodose and celiac ganglia. Virus genes *23*, 145-147.
Glesby, M.J., Moore, R.D., and Chaisson, R.E. (1995). Clinical spectrum of herpes zoster in adults infected with human immunodeficiency virus. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America *21*, 370-375.
Goldman, G., and King, P. (2013a). Vaccination to prevent varicella: Goldman and King's response to Myers' interpretation of Varicella Active Surveillance Project data. Human & experimental toxicology.
Goldman, G.S. (2005). Universal varicella vaccination: efficacy trends and effect on herpes zoster. International journal of toxicology *24*, 205-213.
Goldman, G.S., and King, P.G. (2013b). Review of the United States universal varicella vaccination program: Herpes zoster incidence rates, cost-effectiveness, and vaccine efficacy based primarily on the Antelope Valley Varicella Active Surveillance Project data. Vaccine *31*, 1680-1694.
Gongora, R., Stephan, R.P., Zhang, Z., and Cooper, M.D. (2001). An essential role for Daxx in the inhibition of B lymphopoiesis by type I interferons. Immunity *14*, 727-737.
Goodpasture, E.W., and Talbot, F.B. (1921). COncerning the nature of "protozoan-like" cells in certain lesions of infancy. American journal of diseases of children *21*, 415-425.
Goodpasture, E.W., and Teague, O. (1923). Transmission of the Virus of Herpes Febrilis along Nerves in experimentally infected Rabbits. The Journal of medical research *44*, 139-184 137.
Gottlieb, J., Marcy, A.I., Coen, D.M., and Challberg, M.D. (1990). The herpes simplex virus type 1 UL42 gene product: a subunit of DNA polymerase that functions to increase processivity. J Virol *64*, 5976-5987.

Gourishankar, S., McDermid, J.C., Jhangri, G.S., and Preiksaitis, J.K. (2004). Herpes zoster infection following solid organ transplantation: incidence, risk factors and outcomes in the current immunosuppressive era. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons *4*, 108-115.
Granzow, H., Klupp, B.G., and Mettenleiter, T.C. (2005). Entry of pseudorabies virus: an immunogold-labeling study. J Virol *79*, 3200-3205.
Grinfeld, E., and Kennedy, P.G. (2004). Translation of varicella-zoster virus genes during human ganglionic latency. Virus genes *29*, 317-319.
Grose, C. (1981). Variation on a theme by Fenner: the pathogenesis of chickenpox. Pediatrics *68*, 735-737.
Guess, H.A., Broughton, D.D., Melton, L.J., 3rd, and Kurland, L.T. (1986). Population-based studies of varicella complications. Pediatrics *78*, 723-727.
Habran, L., Bontems, S., Di Valentin, E., Sadzot-Delvaux, C., and Piette, J. (2005). Varicella-zoster virus IE63 protein phosphorylation by roscovitine-sensitive cyclin-dependent kinases modulates its cellular localization and activity. J Biol Chem *280*, 29135-29143.
Hambleton, S., Steinberg, S.P., Gershon, M.D., and Gershon, A.A. (2007). Cholesterol dependence of varicella-zoster virion entry into target cells. J Virol *81*, 7548-7558.
Hardwicke, M.A., and Sandri-Goldin, R.M. (1994). The herpes simplex virus regulatory protein ICP27 contributes to the decrease in cellular mRNA levels during infection. J Virol *68*, 4797-4810.
Hardwicke, M.A., Vaughan, P.J., Sekulovich, R.E., O'Conner, R., and Sandri-Goldin, R.M. (1989). The regions important for the activator and repressor functions of herpes simplex virus type 1 alpha protein ICP27 map to the C-terminal half of the molecule. J Virol *63*, 4590-4602.
Hardy, W.R., and Sandri-Goldin, R.M. (1994). Herpes simplex virus inhibits host cell splicing, and regulatory protein ICP27 is required for this effect. J Virol *68*, 7790-7799.
Harley, C.A., Dasgupta, A., and Wilson, D.W. (2001). Characterization of herpes simplex virus-containing organelles by subcellular fractionation: role for organelle acidification in assembly of infectious particles. J Virol *75*, 1236-1251.
Harper, A.L., Meckes, D.G., Jr., Marsh, J.A., Ward, M.D., Yeh, P.C., Baird, N.L., Wilson, C.B., Semmes, O.J., and Wills, J.W. (2010). Interaction domains of the UL16 and UL21 tegument proteins of herpes simplex virus. J Virol *84*, 2963-2971.
Heineman, T.C., and Cohen, J.I. (1995). The varicella-zoster virus (VZV) open reading frame 47 (ORF47) protein kinase is dispensable for viral replication and is not required for phosphorylation of ORF63 protein, the VZV homolog of herpes simplex virus ICP22. J Virol *69*, 7367-7370.
Heineman, T.C., and Hall, S.L. (2001). VZV gB endocytosis and Golgi localization are mediated by YXXphi motifs in its cytoplasmic domain. Virology *285*, 42-49.
Heininger, U., and Seward, J.F. (2006). Varicella. Lancet *368*, 1365-1376.
Heldwein, E.E., and Krummenacher, C. (2008). Entry of herpesviruses into mammalian cells. Cellular and molecular life sciences : CMLS *65*, 1653-1668.
Heming, J.D., Huffman, J.B., Jones, L.M., and Homa, F.L. (2014). Isolation and characterization of the herpes simplex virus 1 terminase complex. J Virol *88*, 225-236.
Henaff, D., Remillard-Labrosse, G., Loret, S., and Lippe, R. (2013). Analysis of the early steps of herpes simplex virus 1 capsid tegumentation. J Virol *87*, 4895-4906.
Henderson, G., Jaber, T., Carpenter, D., Wechsler, S.L., and Jones, C. (2009). Identification of herpes simplex virus type 1 proteins encoded within the first 1.5 kb of the latency-associated transcript. Journal of neurovirology *15*, 439-448.
Henson, B.W., Perkins, E.M., Cothran, J.E., and Desai, P. (2009). Self-assembly of Epstein-Barr virus capsids. J Virol *83*, 3877-3890.
Hernandez, T.R., and Lehman, I.R. (1990). Functional interaction between the herpes simplex-1 DNA polymerase and UL42 protein. J Biol Chem *265*, 11227-11232.
Higgs, M.R., Preston, V.G., and Stow, N.D. (2008). The UL15 protein of herpes simplex virus type 1 is necessary for the localization of the UL28 and UL33 proteins to viral DNA replication centres. J Gen Virol *89*, 1709-1715.
Hill, G., Chauvenet, A.R., Lovato, J., and McLean, T.W. (2005). Recent steroid therapy increases severity of varicella infections in children with acute lymphoblastic leukemia. Pediatrics *116*, e525-529.
Hollinshead, M., Johns, H.L., Sayers, C.L., Gonzalez-Lopez, C., Smith, G.L., and Elliott, G. (2012). Endocytic tubules regulated by Rab GTPases 5 and 11 are used for envelopment of herpes simplex virus. The EMBO journal *31*, 4204-4220.
Homa, F.L., and Brown, J.C. (1997). Capsid assembly and DNA packaging in herpes simplex virus. Reviews in medical virology *7*, 107-122.
Hope-Simpson, R.E. (1965). The Nature of Herpes Zoster: A Long-Term Study and a New Hypothesis. Proceedings of the Royal Society of Medicine *58*, 9-20.
Hu, H., and Cohen, J.I. (2005). Varicella-zoster virus open reading frame 47 (ORF47) protein is critical for virus replication in dendritic cells and for spread to other cells. Virology *337*, 304-311.
Huffman, J.B., Newcomb, W.W., Brown, J.C., and Homa, F.L. (2008). Amino acids 143 to 150 of the herpes simplex virus type 1 scaffold protein are required for the formation of portal-containing capsids. J Virol *82*, 6778-6781.
Hutchinson, I., Whiteley, A., Browne, H., and Elliott, G. (2002). Sequential localization of two herpes simplex virus tegument proteins to punctate nuclear dots adjacent to ICP0 domains. J Virol *76*, 10365-10373.
Hwang, S., Kim, K.S., Flano, E., Wu, T.T., Tong, L.M., Park, A.N., Song, M.J., Sanchez, D.J., O'Connell, R.M., Cheng, G., *et al.* (2009). Conserved herpesviral kinase promotes viral persistence by inhibiting the IRF-3-mediated type I interferon response. Cell host & microbe *5*, 166-178.
Igarashi, K., Fawl, R., Roller, R.J., and Roizman, B. (1993). Construction and properties of a recombinant herpes simplex virus 1 lacking both S-component origins of DNA synthesis. J Virol *67*, 2123-2132.

Inchauspe, G., Nagpal, S., and Ostrove, J.M. (1989). Mapping of two varicella-zoster virus-encoded genes that activate the expression of viral early and late genes. Virology *173*, 700-709.
Irmiere, A., and Gibson, W. (1983). Isolation and characterization of a noninfectious virion-like particle released from cells infected with human strains of cytomegalovirus. Virology *130*, 118-133.
Ishov, A.M., and Maul, G.G. (1996). The periphery of nuclear domain 10 (ND10) as site of DNA virus deposition. The Journal of cell biology *134*, 815-826.
Ishov, A.M., Stenberg, R.M., and Maul, G.G. (1997). Human cytomegalovirus immediate early interaction with host nuclear structures: definition of an immediate transcript environment. The Journal of cell biology *138*, 5-16.
Jackers, P., Defechereux, P., Baudoux, L., Lambert, C., Massaer, M., Merville-Louis, M.P., Rentier, B., and Piette, J. (1992). Characterization of regulatory functions of the varicella-zoster virus gene 63-encoded protein. J Virol *66*, 3899-3903.
Jackson, J.O., and Longnecker, R. (2010). Reevaluating herpes simplex virus hemifusion. J Virol *84*, 11814-11821.
Jackson, S.A., and DeLuca, N.A. (2003). Relationship of herpes simplex virus genome configuration to productive and persistent infections. Proc Natl Acad Sci U S A *100*, 7871-7876.
Jensen, H.L., and Norrild, B. (1998). Herpes simplex virus type 1-infected human embryonic lung cells studied by optimized immunogold cryosection electron microscopy. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society *46*, 487-496.
Jiang, M., Entezami, P., Gamez, M., Stamminger, T., and Imperiale, M.J. (2011). Functional reorganization of promyelocytic leukemia nuclear bodies during BK virus infection. mBio *2*, e00281-00210.
Johnson, D.C., and Baines, J.D. (2011). Herpesviruses remodel host membranes for virus egress. Nature reviews. Microbiology *9*, 382-394.
Jovasevic, V., Liang, L., and Roizman, B. (2008). Proteolytic cleavage of VP1-2 is required for release of herpes simplex virus 1 DNA into the nucleus. J Virol *82*, 3311-3319.
Kang, W., Mukerjee, R., Gartner, J.J., Hatzigeorgiou, A.G., Sandri-Goldin, R.M., and Fraser, N.W. (2006). Characterization of a spliced exon product of herpes simplex type-1 latency-associated transcript in productively infected cells. Virology *356*, 106-114.
Kelly, B.J., Fraefel, C., Cunningham, A.L., and Diefenbach, R.J. (2009). Functional roles of the tegument proteins of herpes simplex virus type 1. Virus research *145*, 173-186.
Kelly, B.J., Mijatov, B., Fraefel, C., Cunningham, A.L., and Diefenbach, R.J. (2012). Identification of a single amino acid residue which is critical for the interaction between HSV-1 inner tegument proteins pUL36 and pUL37. Virology *422*, 308-316.
Kemble, G.W., Annunziato, P., Lungu, O., Winter, R.E., Cha, T.A., Silverstein, S.J., and Spaete, R.R. (2000). Open reading frame S/L of varicella-zoster virus encodes a cytoplasmic protein expressed in infected cells. J Virol *74*, 11311-11321.
Kennedy, P.G., Grinfeld, E., and Bell, J.E. (2000). Varicella-zoster virus gene expression in latently infected and explanted human ganglia. J Virol *74*, 11893-11898.
Kennedy, P.G., Grinfeld, E., Bontems, S., and Sadzot-Delvaux, C. (2001). Varicella-Zoster virus gene expression in latently infected rat dorsal root ganglia. Virology *289*, 218-223.
Kennedy, P.G., Grinfeld, E., and Gow, J.W. (1998). Latent varicella-zoster virus is located predominantly in neurons in human trigeminal ganglia. Proc Natl Acad Sci U S A *95*, 4658-4662.
Kent, J.R., Kang, W., Miller, C.G., and Fraser, N.W. (2003). Herpes simplex virus latency-associated transcript gene function. Journal of neurovirology *9*, 285-290.
Kenyon, T.K., Cohen, J.I., and Grose, C. (2002). Phosphorylation by the varicella-zoster virus ORF47 protein serine kinase determines whether endocytosed viral gE traffics to the trans-Golgi network or recycles to the cell membrane. J Virol *76*, 10980-10993.
Kenyon, T.K., Homan, E., Storlie, J., Ikoma, M., and Grose, C. (2003). Comparison of varicella-zoster virus ORF47 protein kinase and casein kinase II and their substrates. Journal of medical virology *70 Suppl 1*, S95-102.
Kenyon, T.K., Lynch, J., Hay, J., Ruyechan, W., and Grose, C. (2001). Varicella-zoster virus ORF47 protein serine kinase: characterization of a cloned, biologically active phosphotransferase and two viral substrates, ORF62 and ORF63. J Virol *75*, 8854-8858.
Kim, H.S., Huang, E., Desai, J., Sole, M., Pryce, E.N., Okoye, M.E., Person, S., and Desai, P.J. (2011a). A domain in the herpes simplex virus 1 triplex protein VP23 is essential for closure of capsid shells into icosahedral structures. J Virol *85*, 12698-12707.
Kim, Y.E., Lee, J.H., Kim, E.T., Shin, H.J., Gu, S.Y., Seol, H.S., Ling, P.D., Lee, C.H., and Ahn, J.H. (2011b). Human cytomegalovirus infection causes degradation of Sp100 proteins that suppress viral gene expression. Journal of virology *85*, 11928-11937.
Kinchington, P.R. (1999). Latency of varicella zoster virus; a persistently perplexing state. Front Biosci *4*, D200-211.
Kinchington, P.R., Bookey, D., and Turse, S.E. (1995). The transcriptional regulatory proteins encoded by varicella-zoster virus open reading frames (ORFs) 4 and 63, but not ORF 61, are associated with purified virus particles. J Virol *69*, 4274-4282.
Kinchington, P.R., Fite, K., Seman, A., and Turse, S.E. (2001). Virion association of IE62, the varicella-zoster virus (VZV) major transcriptional regulatory protein, requires expression of the VZV open reading frame 66 protein kinase. J Virol *75*, 9106-9113.
Kinchington, P.R., Hougland, J.K., Arvin, A.M., Ruyechan, W.T., and Hay, J. (1992). The varicella-zoster virus immediate-early protein IE62 is a major component of virus particles. J Virol *66*, 359-366.
Kinchington, P.R., Inchauspe, G., Subak-Sharpe, J.H., Robey, F., Hay, J., and Ruyechan, W.T. (1988). Identification and characterization of a varicella-zoster virus DNA-binding protein by using antisera directed against a predicted synthetic oligopeptide. J Virol *62*, 802-809.

Kinchington, P.R., Reinhold, W.C., Casey, T.A., Straus, S.E., Hay, J., and Ruyechan, W.T. (1985). Inversion and circularization of the varicella-zoster virus genome. J Virol *56*, 194-200.
Klopfleisch, R., Klupp, B.G., Fuchs, W., Kopp, M., Teifke, J.P., and Mettenleiter, T.C. (2006). Influence of pseudorabies virus proteins on neuroinvasion and neurovirulence in mice. J Virol *80*, 5571-5576.
Klupp, B., Altenschmidt, J., Granzow, H., Fuchs, W., and Mettenleiter, T.C. (2008). Glycoproteins required for entry are not necessary for egress of pseudorabies virus. J Virol *82*, 6299-6309.
Klupp, B.G., Bottcher, S., Granzow, H., Kopp, M., and Mettenleiter, T.C. (2005a). Complex formation between the UL16 and UL21 tegument proteins of pseudorabies virus. J Virol *79*, 1510-1522.
Klupp, B.G., Fuchs, W., Granzow, H., Nixdorf, R., and Mettenleiter, T.C. (2002). Pseudorabies virus UL36 tegument protein physically interacts with the UL37 protein. J Virol *76*, 3065-3071.
Klupp, B.G., Granzow, H., Fuchs, W., Keil, G.M., Finke, S., and Mettenleiter, T.C. (2007). Vesicle formation from the nuclear membrane is induced by coexpression of two conserved herpesvirus proteins. Proc Natl Acad Sci U S A *104*, 7241-7246.
Klupp, B.G., Granzow, H., Karger, A., and Mettenleiter, T.C. (2005b). Identification, subviral localization, and functional characterization of the pseudorabies virus UL17 protein. J Virol *79*, 13442-13453.
Klupp, B.G., Granzow, H., Keil, G.M., and Mettenleiter, T.C. (2006). The capsid-associated UL25 protein of the alphaherpesvirus pseudorabies virus is nonessential for cleavage and encapsidation of genomic DNA but is required for nuclear egress of capsids. J Virol *80*, 6235-6246.
Klupp, B.G., Granzow, H., Mundt, E., and Mettenleiter, T.C. (2001). Pseudorabies virus UL37 gene product is involved in secondary envelopment. J Virol *75*, 8927-8936.
Klupp, B.G., Lomniczi, B., Visser, N., Fuchs, W., and Mettenleiter, T.C. (1995). Mutations affecting the UL21 gene contribute to avirulence of pseudorabies virus vaccine strain Bartha. Virology *212*, 466-473.
Komazin-Meredith, G., Mirchev, R., Golan, D.E., van Oijen, A.M., and Coen, D.M. (2008). Hopping of a processivity factor on DNA revealed by single-molecule assays of diffusion. Proc Natl Acad Sci U S A *105*, 10721-10726.
Kost, R.G., Kupinsky, H., and Straus, S.E. (1995). Varicella-zoster virus gene 63: transcript mapping and regulatory activity. Virology *209*, 218-224.
Krishnan, H.H., Sharma-Walia, N., Zeng, L., Gao, S.J., and Chandran, B. (2005). Envelope glycoprotein gB of Kaposi's sarcoma-associated herpesvirus is essential for egress from infected cells. J Virol *79*, 10952-10967.
Kristie, T.M., Liang, Y., and Vogel, J.L. (2010). Control of alpha-herpesvirus IE gene expression by HCF-1 coupled chromatin modification activities. Biochimica et biophysica acta *1799*, 257-265.
Krosky, P.M., Baek, M.C., and Coen, D.M. (2003). The human cytomegalovirus UL97 protein kinase, an antiviral drug target, is required at the stage of nuclear egress. J Virol *77*, 905-914.
Krummenacher, C., Carfi, A., Eisenberg, R.J., and Cohen, G.H. (2013). Entry of herpesviruses into cells: the enigma variations. Advances in experimental medicine and biology *790*, 178-195.
Ku, C.C., Besser, J., Abendroth, A., Grose, C., and Arvin, A.M. (2005). Varicella-Zoster virus pathogenesis and immunobiology: new concepts emerging from investigations with the SCIDhu mouse model. J Virol *79*, 2651-2658.
Kuter, B., Matthews, H., Shinefield, H., Black, S., Dennehy, P., Watson, B., Reisinger, K., Kim, L.L., Lupinacci, L., Hartzel, J.*, et al.* (2004). Ten year follow-up of healthy children who received one or two injections of varicella vaccine. The Pediatric infectious disease journal *23*, 132-137.
Kyratsous, C.A., and Silverstein, S.J. (2009). Components of nuclear domain 10 bodies regulate varicella-zoster virus replication. Journal of virology *83*, 4262-4274.
Kyratsous, C.A., Walters, M.S., Panagiotidis, C.A., and Silverstein, S.J. (2009). Complementation of a herpes simplex virus ICP0 null mutant by varicella-zoster virus ORF61p. Journal of virology *83*, 10637-10643.
Lafarga, M., Berciano, M.T., Pena, E., Mayo, I., Castano, J.G., Bohmann, D., Rodrigues, J.P., Tavanez, J.P., and Carmo-Fonseca, M. (2002). Clastosome: a subtype of nuclear body enriched in 19S and 20S proteasomes, ubiquitin, and protein substrates of proteasome. Mol Biol Cell *13*, 2771-2782.
LaGuardia, J.J., Cohrs, R.J., and Gilden, D.H. (1999). Prevalence of varicella-zoster virus DNA in dissociated human trigeminal ganglion neurons and nonneuronal cells. J Virol *73*, 8571-8577.
Lallemand-Breitenbach, V., and de The, H. (2010). PML nuclear bodies. Cold Spring Harbor perspectives in biology *2*, a000661.
Lamberti, C., and Weller, S.K. (1998). The herpes simplex virus type 1 cleavage/packaging protein, UL32, is involved in efficient localization of capsids to replication compartments. J Virol *72*, 2463-2473.
Le Sage, V., Jung, M., Alter, J.D., Wills, E.G., Johnston, S.M., Kawaguchi, Y., Baines, J.D., and Banfield, B.W. (2013). The herpes simplex virus 2 UL21 protein is essential for virus propagation. J Virol *87*, 5904-5915.
Leach, N.R., and Roller, R.J. (2010). Significance of host cell kinases in herpes simplex virus type 1 egress and lamin-associated protein disassembly from the nuclear lamina. Virology *406*, 127-137.
Lee, C.P., Huang, Y.H., Lin, S.F., Chang, Y., Chang, Y.H., Takada, K., and Chen, M.R. (2008a). Epstein-Barr virus BGLF4 kinase induces disassembly of the nuclear lamina to facilitate virion production. J Virol *82*, 11913-11926.
Lee, H.R., Kim, D.J., Lee, J.M., Choi, C.Y., Ahn, B.Y., Hayward, G.S., and Ahn, J.H. (2004). Ability of the human cytomegalovirus IE1 protein to modulate sumoylation of PML correlates with its functional activities in transcriptional regulation and infectivity in cultured fibroblast cells. Journal of virology *78*, 6527-6542.
Lee, J.H., Vittone, V., Diefenbach, E., Cunningham, A.L., and Diefenbach, R.J. (2008b). Identification of structural protein-protein interactions of herpes simplex virus type 1. Virology *378*, 347-354.
Lee, J.I., Luxton, G.W., and Smith, G.A. (2006). Identification of an essential domain in the herpesvirus VP1/2 tegument protein: the carboxy terminus directs incorporation into capsid assemblons. J Virol *80*, 12086-12094.
Lee, S.K., and Longnecker, R. (1997). The Epstein-Barr virus glycoprotein 110 carboxy-terminal tail domain is essential for lytic virus replication. J Virol *71*, 4092-4097.

Leelawong, M., Guo, D., and Smith, G.A. (2011). A physical link between the pseudorabies virus capsid and the nuclear egress complex. J Virol *85*, 11675-11684.
Lehembre, F., Muller, S., Pandolfi, P.P., and Dejean, A. (2001). Regulation of Pax3 transcriptional activity by SUMO-1-modified PML. Oncogene *20*, 1-9.
Lehman, I.R., and Boehmer, P.E. (1999). Replication of herpes simplex virus DNA. J Biol Chem *274*, 28059-28062.
Lenac Rovis, T., Bailer, S.M., Pothineni, V.R., Ouwendijk, W.J., Simic, H., Babic, M., Miklic, K., Malic, S., Verweij, M.C., Baiker, A.*, et al.* (2013). Comprehensive analysis of varicella-zoster virus proteins using a new monoclonal antibody collection. J Virol *87*, 6943-6954.
Leopardi, R., Ward, P.L., Ogle, W.O., and Roizman, B. (1997). Association of herpes simplex virus regulatory protein ICP22 with transcriptional complexes containing EAP, ICP4, RNA polymerase II, and viral DNA requires posttranslational modification by the U(L)13 proteinkinase. J Virol *71*, 1133-1139.
Leung, J., Harpaz, R., Baughman, A.L., Heath, K., Loparev, V., Vazquez, M., Watson, B.M., and Schmid, D.S. (2010). Evaluation of laboratory methods for diagnosis of varicella. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America *51*, 23-32.
Levin, M.J., Cai, G.Y., Manchak, M.D., and Pizer, L.I. (2003). Varicella-zoster virus DNA in cells isolated from human trigeminal ganglia. J Virol *77*, 6979-6987.
Li, H., Leo, C., Zhu, J., Wu, X., O'Neil, J., Park, E.J., and Chen, J.D. (2000). Sequestration and inhibition of Daxx-mediated transcriptional repression by PML. Molecular and cellular biology *20*, 1784-1796.
Li, Q., Ali, M.A., and Cohen, J.I. (2006). Insulin degrading enzyme is a cellular receptor mediating varicella-zoster virus infection and cell-to-cell spread. Cell *127*, 305-316.
Li, Q., Krogmann, T., Ali, M.A., Tang, W.J., and Cohen, J.I. (2007). The amino terminus of varicella-zoster virus (VZV) glycoprotein E is required for binding to insulin-degrading enzyme, a VZV receptor. J Virol *81*, 8525-8532.
Lin, D.Y., Huang, Y.S., Jeng, J.C., Kuo, H.Y., Chang, C.C., Chao, T.T., Ho, C.C., Chen, Y.C., Lin, T.P., Fang, H.I.*, et al.* (2006). Role of SUMO-interacting motif in Daxx SUMO modification, subnuclear localization, and repression of sumoylated transcription factors. Molecular cell *24*, 341-354.
Liu, S., Knafels, J.D., Chang, J.S., Waszak, G.A., Baldwin, E.T., Deibel, M.R., Jr., Thomsen, D.R., Homa, F.L., Wells, P.A., Tory, M.C.*, et al.* (2006). Crystal structure of the herpes simplex virus 1 DNA polymerase. J Biol Chem *281*, 18193-18200.
Livingston, C.M., Ifrim, M.F., Cowan, A.E., and Weller, S.K. (2009). Virus-Induced Chaperone-Enriched (VICE) domains function as nuclear protein quality control centers during HSV-1 infection. PLoS pathogens *5*, e1000619.
Lolekha, S., Tanthiphabha, W., Sornchai, P., Kosuwan, P., Sutra, S., Warachit, B., Chup-Upprakarn, S., Hutagalung, Y., Weil, J., and Bock, H.L. (2001). Effect of climatic factors and population density on varicella zoster virus epidemiology within a tropical country. The American journal of tropical medicine and hygiene *64*, 131-136.
Lomniczi, B., Watanabe, S., Ben-Porat, T., and Kaplan, A.S. (1987). Genome location and identification of functions defective in the Bartha vaccine strain of pseudorabies virus. J Virol *61*, 796-801.
Long, M.C., Leong, V., Schaffer, P.A., Spencer, C.A., and Rice, S.A. (1999). ICP22 and the UL13 protein kinase are both required for herpes simplex virus-induced modification of the large subunit of RNA polymerase II. J Virol *73*, 5593-5604.
Loomis, J.S., Courtney, R.J., and Wills, J.W. (2003). Binding partners for the UL11 tegument protein of herpes simplex virus type 1. J Virol *77*, 11417-11424.
Loret, S., Guay, G., and Lippe, R. (2008). Comprehensive characterization of extracellular herpes simplex virus type 1 virions. J Virol *82*, 8605-8618.
Lungu, O., Panagiotidis, C.A., Annunziato, P.W., Gershon, A.A., and Silverstein, S.J. (1998). Aberrant intracellular localization of Varicella-Zoster virus regulatory proteins during latency. Proc Natl Acad Sci U S A *95*, 7080-7085.
Luxton, G.W., Haverlock, S., Coller, K.E., Antinone, S.E., Pincetic, A., and Smith, G.A. (2005). Targeting of herpesvirus capsid transport in axons is coupled to association with specific sets of tegument proteins. Proc Natl Acad Sci U S A *102*, 5832-5837.
Luxton, G.W., Lee, J.I., Haverlock-Moyns, S., Schober, J.M., and Smith, G.A. (2006). The pseudorabies virus VP1/2 tegument protein is required for intracellular capsid transport. J Virol *80*, 201-209.
Lynch, J.M., Kenyon, T.K., Grose, C., Hay, J., and Ruyechan, W.T. (2002). Physical and functional interaction between the varicella zoster virus IE63 and IE62 proteins. Virology *302*, 71-82.
Mahalingam, R., Wellish, M., Cohrs, R., Debrus, S., Piette, J., Rentier, B., and Gilden, D.H. (1996). Expression of protein encoded by varicella-zoster virus open reading frame 63 in latently infected human ganglionic neurons. Proc Natl Acad Sci U S A *93*, 2122-2124.
Mahalingam, R., Wellish, M., Wolf, W., Dueland, A.N., Cohrs, R., Vafai, A., and Gilden, D. (1990). Latent varicella-zoster viral DNA in human trigeminal and thoracic ganglia. N Engl J Med *323*, 627-631.
Mar, E.C., Huang, Y.S., and Huang, E.S. (1978). Purification and characterization of varicella-zoster virus-induced DNA polymerase. J Virol *26*, 249-256.
Marcy, A.I., Olivo, P.D., Challberg, M.D., and Coen, D.M. (1990). Enzymatic activities of overexpressed herpes simplex virus DNA polymerase purified from recombinant baculovirus-infected insect cells. Nucleic acids research *18*, 1207-1215.
Maresova, L., Pasieka, T.J., and Grose, C. (2001). Varicella-zoster Virus gB and gE coexpression, but not gB or gE alone, leads to abundant fusion and syncytium formation equivalent to those from gH and gL coexpression. J Virol *75*, 9483-9492.
Maresova, L., Pasieka, T.J., Homan, E., Gerday, E., and Grose, C. (2005). Incorporation of three endocytosed varicella-zoster virus glycoproteins, gE, gH, and gB, into the virion envelope. J Virol *79*, 997-1007.
Maringer, K., Stylianou, J., and Elliott, G. (2012). A network of protein interactions around the herpes simplex virus tegument protein VP22. J Virol *86*, 12971-12982.
Markovitz, N.S., and Roizman, B. (2000). Small dense nuclear bodies are the site of localization of herpes simplex virus 1 U(L)3 and U(L)4 proteins and of ICP22 only when the latter protein is present. J Virol *74*, 523-528.

Marschall, M., Marzi, A., aus dem Siepen, P., Jochmann, R., Kalmer, M., Auerochs, S., Lischka, P., Leis, M., and Stamminger, T. (2005). Cellular p32 recruits cytomegalovirus kinase pUL97 to redistribute the nuclear lamina. J Biol Chem *280*, 33357-33367.
Marsden, H.S., McLean, G.W., Barnard, E.C., Francis, G.J., MacEachran, K., Murphy, M., McVey, G., Cross, A., Abbotts, A.P., and Stow, N.D. (1997). The catalytic subunit of the DNA polymerase of herpes simplex virus type 1 interacts specifically with the C terminus of the UL8 component of the viral helicase-primase complex. J Virol *71*, 6390-6397.
Martin, T.E., Barghusen, S.C., Leser, G.P., and Spear, P.G. (1987). Redistribution of nuclear ribonucleoprotein antigens during herpes simplex virus infection. The Journal of cell biology *105*, 2069-2082.
Matusick-Kumar, L., Hurlburt, W., Weinheimer, S.P., Newcomb, W.W., Brown, J.C., and Gao, M. (1994). Phenotype of the herpes simplex virus type 1 protease substrate ICP35 mutant virus. J Virol *68*, 5384-5394.
Maul, G.G., Guldner, H.H., and Spivack, J.G. (1993). Modification of discrete nuclear domains induced by herpes simplex virus type 1 immediate early gene 1 product (ICP0). J Gen Virol *74 (Pt 12)*, 2679-2690.
Mbong, E.F., Woodley, L., Frost, E., Baines, J.D., and Duffy, C. (2012). Deletion of UL21 causes a delay in the early stages of the herpes simplex virus 1 replication cycle. J Virol *86*, 7003-7007.
McElwee, M., Beilstein, F., Labetoulle, M., Rixon, F.J., and Pasdeloup, D. (2013). Dystonin/BPAG1 promotes plus-end-directed transport of herpes simplex virus 1 capsids on microtubules during entry. J Virol *87*, 11008-11018.
McGeoch, D.J. (1990). Evolutionary relationships of virion glycoprotein genes in the S regions of alphaherpesvirus genomes. J Gen Virol *71 (Pt 10)*, 2361-2367.
McLauchlan, J., and Rixon, F.J. (1992). Characterization of enveloped tegument structures (L particles) produced by alphaherpesviruses: integrity of the tegument does not depend on the presence of capsid or envelope. J Gen Virol *73 (Pt 2)*, 269-276.
McLean, G.W., Abbotts, A.P., Parry, M.E., Marsden, H.S., and Stow, N.D. (1994). The herpes simplex virus type 1 origin-binding protein interacts specifically with the viral UL8 protein. J Gen Virol *75 (Pt 10)*, 2699-2706.
McMahan, L., and Schaffer, P.A. (1990). The repressing and enhancing functions of the herpes simplex virus regulatory protein ICP27 map to C-terminal regions and are required to modulate viral gene expression very early in infection. J Virol *64*, 3471-3485.
McMillan, T.N., and Johnson, D.C. (2001). Cytoplasmic domain of herpes simplex virus gE causes accumulation in the trans-Golgi network, a site of virus envelopment and sorting of virions to cell junctions. J Virol *75*, 1928-1940.
McNab, A.R., Desai, P., Person, S., Roof, L.L., Thomsen, D.R., Newcomb, W.W., Brown, J.C., and Homa, F.L. (1998). The product of the herpes simplex virus type 1 UL25 gene is required for encapsidation but not for cleavage of replicated viral DNA. J Virol *72*, 1060-1070.
McNabb, D.S., and Courtney, R.J. (1992). Characterization of the large tegument protein (ICP1/2) of herpes simplex virus type 1. Virology *190*, 221-232.
Meckes, D.G., Jr., and Wills, J.W. (2007). Dynamic interactions of the UL16 tegument protein with the capsid of herpes simplex virus. J Virol *81*, 13028-13036.
Mehta, S.K., Tyring, S.K., Gilden, D.H., Cohrs, R.J., Leal, M.J., Castro, V.A., Feiveson, A.H., Ott, C.M., and Pierson, D.L. (2008). Varicella-zoster virus in the saliva of patients with herpes zoster. The Journal of infectious diseases *197*, 654-657.
Mellerick, D.M., and Fraser, N.W. (1987). Physical state of the latent herpes simplex virus genome in a mouse model system: evidence suggesting an episomal state. Virology *158*, 265-275.
Mettenleiter, T.C. (2002). Herpesvirus assembly and egress. J Virol *76*, 1537-1547.
Mettenleiter, T.C., Klupp, B.G., and Granzow, H. (2006). Herpesvirus assembly: a tale of two membranes. Current opinion in microbiology *9*, 423-429.
Mettenleiter, T.C., Klupp, B.G., and Granzow, H. (2009). Herpesvirus assembly: an update. Virus research *143*, 222-234.
Michael, E.J., Kuck, K.M., and Kinchington, P.R. (1998). Anatomy of the varicella-zoster virus open-reading frame 4 promoter. The Journal of infectious diseases *178 Suppl 1*, S27-33.
Michael, K., Klupp, B.G., Karger, A., and Mettenleiter, T.C. (2007). Efficient incorporation of tegument proteins pUL46, pUL49, and pUS3 into pseudorabies virus particles depends on the presence of pUL21. J Virol *81*, 1048-1051.
Miller, R.L., and Rapp, F. (1977). Varicella-zoster virus-induced DNA polymerase. J Gen Virol *36*, 515-524.
Mocarski, E. (2007). Comparative analysis of herpesvirus-common proteins. In Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis, C.-F.G. Arvin A, Mocarski E, et al., ed. (Available from: http://www.ncbi.nlm.nih.gov/books/NBK47403/: Cambridge University Press).
Moffat, J., Mo, C., Cheng, J.J., Sommer, M., Zerboni, L., Stamatis, S., and Arvin, A.M. (2004a). Functions of the C-terminal domain of varicella-zoster virus glycoprotein E in viral replication in vitro and skin and T-cell tropism in vivo. J Virol *78*, 12406-12415.
Moffat, J.F., McMichael, M.A., Leisenfelder, S.A., and Taylor, S.L. (2004b). Viral and cellular kinases are potential antiviral targets and have a central role in varicella zoster virus pathogenesis. Biochimica et biophysica acta *1697*, 225-231.
Moffat, J.F., Zerboni, L., Sommer, M.H., Heineman, T.C., Cohen, J.I., Kaneshima, H., and Arvin, A.M. (1998). The ORF47 and ORF66 putative protein kinases of varicella-zoster virus determine tropism for human T cells and skin in the SCID-hu mouse. Proc Natl Acad Sci U S A *95*, 11969-11974.
Mohl, B.S., Bottcher, S., Granzow, H., Kuhn, J., Klupp, B.G., and Mettenleiter, T.C. (2009). Intracellular localization of the pseudorabies virus large tegument protein pUL36. J Virol *83*, 9641-9651.
Montgomery, R.I., Warner, M.S., Lum, B.J., and Spear, P.G. (1996). Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. Cell *87*, 427-436.
Moriuchi, H., Moriuchi, M., and Cohen, J.I. (1995). Proteins and cis-acting elements associated with transactivation of the varicella-zoster virus (VZV) immediate-early gene 62 promoter by VZV open reading frame 10 protein. J Virol *69*, 4693-4701.

Moriuchi, H., Moriuchi, M., Straus, S.E., and Cohen, J.I. (1993). Varicella-zoster virus (VZV) open reading frame 61 protein transactivates VZV gene promoters and enhances the infectivity of VZV DNA. J Virol *67*, 4290-4295.
Morrison, E.E., Stevenson, A.J., Wang, Y.F., and Meredith, D.M. (1998). Differences in the intracellular localization and fate of herpes simplex virus tegument proteins early in the infection of Vero cells. J Gen Virol *79 (Pt 10)*, 2517-2528.
Mossman, K.L., Sherburne, R., Lavery, C., Duncan, J., and Smiley, J.R. (2000). Evidence that herpes simplex virus VP16 is required for viral egress downstream of the initial envelopment event. J Virol *74*, 6287-6299.
Mou, F., Forest, T., and Baines, J.D. (2007). US3 of herpes simplex virus type 1 encodes a promiscuous protein kinase that phosphorylates and alters localization of lamin A/C in infected cells. J Virol *81*, 6459-6470.
Muranyi, W., Haas, J., Wagner, M., Krohne, G., and Koszinowski, U.H. (2002). Cytomegalovirus recruitment of cellular kinases to dissolve the nuclear lamina. Science *297*, 854-857.
Muylaert, I., and Elias, P. (2007). Knockdown of DNA ligase IV/XRCC4 by RNA interference inhibits herpes simplex virus type I DNA replication. J Biol Chem *282*, 10865-10872.
Muylaert, I., Tang, K.W., and Elias, P. (2011). Replication and recombination of herpes simplex virus DNA. J Biol Chem *286*, 15619-15624.
Myers, M.G. (1979). Viremia caused by varicella-zoster virus: association with malignant progressive varicella. The Journal of infectious diseases *140*, 229-233.
Myers, M.G. (2013). Vaccination to prevent varicella. Vaccine *31*, 1695.
Myers, M.G., Kramer, L.W., and Stanberry, L.R. (1987). Varicella in a gorilla. Journal of medical virology *23*, 317-322.
Nagel, C.H., Dohner, K., Binz, A., Bauerfeind, R., and Sodeik, B. (2012). Improper tagging of the non-essential small capsid protein VP26 impairs nuclear capsid egress of herpes simplex virus. PloS one *7*, e44177.
Nagler, F.P., and Rake, G. (1948). The Use of the Electron Microscope in Diagnosis of Variola, Vaccinia, and Varicella. Journal of bacteriology *55*, 45-51.
Nagpal, S., and Ostrove, J.M. (1991). Characterization of a potent varicella-zoster virus-encoded trans-repressor. J Virol *65*, 5289-5296.
Narayanan, A., Nogueira, M.L., Ruyechan, W.T., and Kristie, T.M. (2005). Combinatorial transcription of herpes simplex virus and varicella zoster virus immediate early genes is strictly determined by the cellular coactivator HCF-1. J Biol Chem *280*, 1369-1375.
Narayanan, A., Ruyechan, W.T., and Kristie, T.M. (2007). The coactivator host cell factor-1 mediates Set1 and MLL1 H3K4 trimethylation at herpesvirus immediate early promoters for initiation of infection. Proc Natl Acad Sci U S A *104*, 10835-10840.
Negorev, D., and Maul, G.G. (2001). Cellular proteins localized at and interacting within ND10/PML nuclear bodies/PODs suggest functions of a nuclear depot. Oncogene *20*, 7234-7242.
Newcomb, W.W., Homa, F.L., and Brown, J.C. (2005). Involvement of the portal at an early step in herpes simplex virus capsid assembly. J Virol *79*, 10540-10546.
Newcomb, W.W., Homa, F.L., Thomsen, D.R., Booy, F.P., Trus, B.L., Steven, A.C., Spencer, J.V., and Brown, J.C. (1996). Assembly of the herpes simplex virus capsid: characterization of intermediates observed during cell-free capsid formation. Journal of molecular biology *263*, 432-446.
Newcomb, W.W., Homa, F.L., Thomsen, D.R., Trus, B.L., Cheng, N., Steven, A., Booy, F., and Brown, J.C. (1999). Assembly of the herpes simplex virus procapsid from purified components and identification of small complexes containing the major capsid and scaffolding proteins. J Virol *73*, 4239-4250.
Newcomb, W.W., Trus, B.L., Cheng, N., Steven, A.C., Sheaffer, A.K., Tenney, D.J., Weller, S.K., and Brown, J.C. (2000). Isolation of herpes simplex virus procapsids from cells infected with a protease-deficient mutant virus. J Virol *74*, 1663-1673.
Ng, T.I., Keenan, L., Kinchington, P.R., and Grose, C. (1994). Phosphorylation of varicella-zoster virus open reading frame (ORF) 62 regulatory product by viral ORF 47-associated protein kinase. J Virol *68*, 1350-1359.
Nguyen-Huynh, A.T., and Schaffer, P.A. (1998). Cellular transcription factors enhance herpes simplex virus type 1 oriS-dependent DNA replication. J Virol *72*, 3635-3645.
Nguyen, H.Q., Jumaan, A.O., and Seward, J.F. (2005). Decline in mortality due to varicella after implementation of varicella vaccination in the United States. N Engl J Med *352*, 450-458.
Nicholson, P., Addison, C., Cross, A.M., Kennard, J., Preston, V.G., and Rixon, F.J. (1994). Localization of the herpes simplex virus type 1 major capsid protein VP5 to the cell nucleus requires the abundant scaffolding protein VP22a. J Gen Virol *75 (Pt 5)*, 1091-1099.
Olson, J.K., and Grose, C. (1997). Endocytosis and recycling of varicella-zoster virus Fc receptor glycoprotein gE: internalization mediated by a YXXL motif in the cytoplasmic tail. J Virol *71*, 4042-4054.
Ote, I., Lebrun, M., Vandevenne, P., Bontems, S., Medina-Palazon, C., Manet, E., Piette, J., and Sadzot-Delvaux, C. (2009). Varicella-zoster virus IE4 protein interacts with SR proteins and exports mRNAs through the TAP/NXF1 pathway. PloS one *4*, e7882.
Ozaki, T. (2013). Varicella vaccination in Japan: necessity of implementing a routine vaccination program. Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy *19*, 188-195.
Ozaki, T., Matsui, T., Ichikawa, T., Asano, Y., Yamanishi, K., and Takahashi, M. (1984). Clinical trial of the Oka strain of live attenuated varicella vaccine on healthy children. Biken journal *27*, 39-42.
Park, R., and Baines, J.D. (2006). Herpes simplex virus type 1 infection induces activation and recruitment of protein kinase C to the nuclear membrane and increased phosphorylation of lamin B. J Virol *80*, 494-504.
Parkinson, J., and Everett, R.D. (2000). Alphaherpesvirus proteins related to herpes simplex virus type 1 ICP0 affect cellular structures and proteins. Journal of virology *74*, 10006-10017.
Pasieka, T.J., Maresova, L., and Grose, C. (2003). A functional YNKI motif in the short cytoplasmic tail of varicella-zoster virus glycoprotein gH mediates clathrin-dependent and antibody-independent endocytosis. J Virol *77*, 4191-4204.

Pearson, M., Carbone, R., Sebastiani, C., Cioce, M., Fagioli, M., Saito, S., Higashimoto, Y., Appella, E., Minucci, S., Pandolfi, P.P*., et al.* (2000). PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. Nature *406*, 207-210.

Peng, H., He, H., Hay, J., and Ruyechan, W.T. (2003). Interaction between the varicella zoster virus IE62 major transactivator and cellular transcription factor Sp1. J Biol Chem *278*, 38068-38075.

Peng, W., Vitvitskaia, O., Carpenter, D., Wechsler, S.L., and Jones, C. (2008). Identification of two small RNAs within the first 1.5-kb of the herpes simplex virus type 1-encoded latency-associated transcript. Journal of neurovirology *14*, 41-52.

Penkert, R.R., and Kalejta, R.F. (2011). Tegument protein control of latent herpesvirus establishment and animation. Herpesviridae *2*, 3.

Perdue, M.L., Cohen, J.C., Randall, C.C., and O'Callaghan, D.J. (1976). Biochemical studies of the maturation of herpesvirus nucleocapsid species. Virology *74*, 194-208.

Perera, L.P., Kaushal, S., Kinchington, P.R., Mosca, J.D., Hayward, G.S., and Straus, S.E. (1994). Varicella-zoster virus open reading frame 4 encodes a transcriptional activator that is functionally distinct from that of herpes simplex virus homology ICP27. J Virol *68*, 2468-2477.

Perera, L.P., Mosca, J.D., Ruyechan, W.T., and Hay, J. (1992a). Regulation of varicella-zoster virus gene expression in human T lymphocytes. J Virol *66*, 5298-5304.

Perera, L.P., Mosca, J.D., Sadeghi-Zadeh, M., Ruyechan, W.T., and Hay, J. (1992b). The varicella-zoster virus immediate early protein, IE62, can positively regulate its cognate promoter. Virology *191*, 346-354.

Perkins, E.M., Anacker, D., Davis, A., Sankar, V., Ambinder, R.F., and Desai, P. (2008). Small capsid protein pORF65 is essential for assembly of Kaposi's sarcoma-associated herpesvirus capsids. J Virol *82*, 7201-7211.

Phelan, A., Carmo-Fonseca, M., McLaughlan, J., Lamond, A.I., and Clements, J.B. (1993). A herpes simplex virus type 1 immediate-early gene product, IE63, regulates small nuclear ribonucleoprotein distribution. Proc Natl Acad Sci U S A *90*, 9056-9060.

Piret, J., and Boivin, G. (2014). Antiviral drug resistance in herpesviruses other than cytomegalovirus. Reviews in medical virology.

Poffenberger, K.L., Raichlen, P.E., and Herman, R.C. (1993). In vitro characterization of a herpes simplex virus type 1 ICP22 deletion mutant. Virus genes *7*, 171-186.

Polvino-Bodnar, M., Orberg, P.K., and Schaffer, P.A. (1987). Herpes simplex virus type 1 oriL is not required for virus replication or for the establishment and reactivation of latent infection in mice. J Virol *61*, 3528-3535.

Radtke, K., Kieneke, D., Wolfstein, A., Michael, K., Steffen, W., Scholz, T., Karger, A., and Sodeik, B. (2010). Plus- and minus-end directed microtubule motors bind simultaneously to herpes simplex virus capsids using different inner tegument structures. PLoS pathogens *6*, e1000991.

Rake, G., Blank, H., and et al. (1948). The relationship of varicella and herpes zoster; electron microscope studies. Journal of bacteriology *56*, 293-303.

Randell, J.C., and Coen, D.M. (2001). Linear diffusion on DNA despite high-affinity binding by a DNA polymerase processivity factor. Molecular cell *8*, 911-920.

Reddy, S.M., Cox, E., Iofin, I., Soong, W., and Cohen, J.I. (1998a). Varicella-zoster virus (VZV) ORF32 encodes a phosphoprotein that is posttranslationally modified by the VZV ORF47 protein kinase. J Virol *72*, 8083-8088.

Reddy, S.M., Williams, M., and Cohen, J.I. (1998b). Expression of a uracil DNA glycosylase (UNG) inhibitor in mammalian cells: varicella-zoster virus can replicate in vitro in the absence of detectable UNG activity. Virology *251*, 393-401.

Regad, T., and Chelbi-Alix, M.K. (2001). Role and fate of PML nuclear bodies in response to interferon and viral infections. Oncogene *20*, 7274-7286.

Reichelt, M., Brady, J., and Arvin, A.M. (2009). The replication cycle of varicella-zoster virus: analysis of the kinetics of viral protein expression, genome synthesis, and virion assembly at the single-cell level. J Virol *83*, 3904-3918.

Reichelt, M., Joubert, L., Perrino, J., Koh, A.L., Phanwar, I., and Arvin, A.M. (2012). 3D reconstruction of VZV infected cell nuclei and PML nuclear cages by serial section array scanning electron microscopy and electron tomography. PLoS pathogens *8*, e1002740.

Reichelt, M., Wang, L., Sommer, M., Perrino, J., Nour, A.M., Sen, N., Baiker, A., Zerboni, L., and Arvin, A.M. (2011). Entrapment of viral capsids in nuclear PML cages is an intrinsic antiviral host defense against varicella-zoster virus. PLoS Pathog *7*, e1001266.

Reynolds, A.E., Wills, E.G., Roller, R.J., Ryckman, B.J., and Baines, J.D. (2002). Ultrastructural localization of the herpes simplex virus type 1 UL31, UL34, and US3 proteins suggests specific roles in primary envelopment and egress of nucleocapsids. J Virol *76*, 8939-8952.

Rice, S.A., and Knipe, D.M. (1990). Genetic evidence for two distinct transactivation functions of the herpes simplex virus alpha protein ICP27. J Virol *64*, 1704-1715.

Rice, S.A., Long, M.C., Lam, V., Schaffer, P.A., and Spencer, C.A. (1995). Herpes simplex virus immediate-early protein ICP22 is required for viral modification of host RNA polymerase II and establishment of the normal viral transcription program. J Virol *69*, 5550-5559.

Riva, L., Thiry, M., Bontems, S., Joris, A., Piette, J., Lebrun, M., and Sadzot-Delvaux, C. (2013). ORF9p phosphorylation by ORF47p is crucial for the formation and egress of varicella-zoster virus viral particles. J Virol *87*, 2868-2881.

Rixon, F.J., Addison, C., McGregor, A., Macnab, S.J., Nicholson, P., Preston, V.G., and Tatman, J.D. (1996). Multiple interactions control the intracellular localization of the herpes simplex virus type 1 capsid proteins. J Gen Virol *77 (Pt 9)*, 2251-2260.

Roberts, S., Hillman, M.L., Knight, G.L., and Gallimore, P.H. (2003). The ND10 component promyelocytic leukemia protein relocates to human papillomavirus type 1 E4 intranuclear inclusion bodies in cultured keratinocytes and in warts. Journal of virology *77*, 673-684.

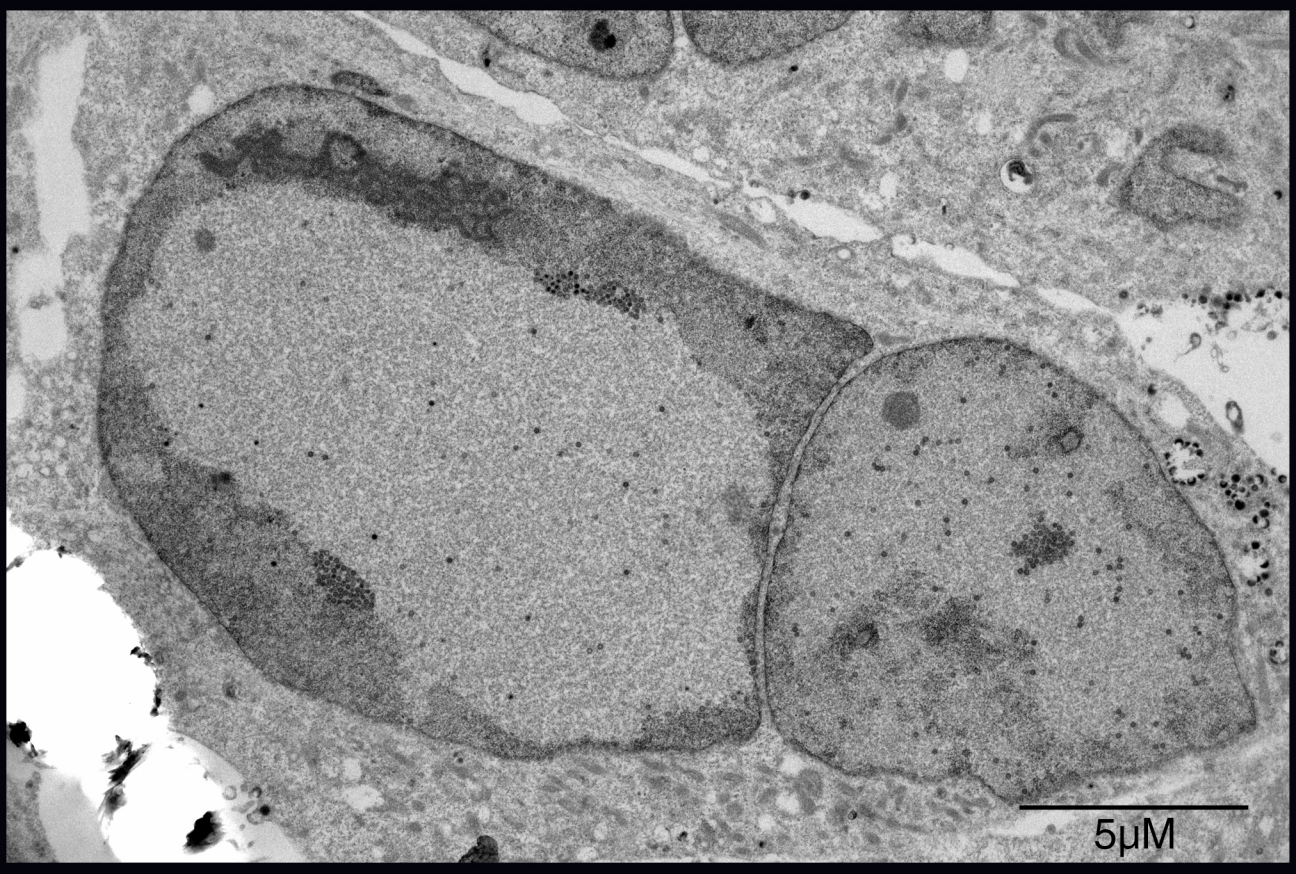
Rochat, R.H., Liu, X., Murata, K., Nagayama, K., Rixon, F.J., and Chiu, W. (2011). Seeing the portal in herpes simplex virus type 1 B capsids. J Virol *85*, 1871-1874.
Roos, W.H., Radtke, K., Kniesmeijer, E., Geertsema, H., Sodeik, B., and Wuite, G.J. (2009). Scaffold expulsion and genome packaging trigger stabilization of herpes simplex virus capsids. Proc Natl Acad Sci U S A *106*, 9673-9678.
Ross, J., Williams, M., and Cohen, J.I. (1997). Disruption of the varicella-zoster virus dUTPase and the adjacent ORF9A gene results in impaired growth and reduced syncytia formation in vitro. Virology *234*, 186-195.
Ruyechan, W.T. (2010). Roles of cellular transcription factors in VZV replication. Current topics in microbiology and immunology *342*, 43-65.
Ruyechan, W.T., Peng, H., Yang, M., and Hay, J. (2003). Cellular factors and IE62 activation of VZV promoters. Journal of medical virology *70 Suppl 1*, S90-94.
Ryckman, B.J., and Roller, R.J. (2004). Herpes simplex virus type 1 primary envelopment: UL34 protein modification and the US3-UL34 catalytic relationship. J Virol *78*, 399-412.
Sadzot-Delvaux, C., Debrus, S., Nikkels, A., Piette, J., and Rentier, B. (1995). Varicella-zoster virus latency in the adult rat is a useful model for human latent infection. Neurology *45*, S18-20.
Sadzot-Delvaux, C., Merville-Louis, M.P., Delree, P., Marc, P., Piette, J., Moonen, G., and Rentier, B. (1990). An in vivo model of varicella-zoster virus latent infection of dorsal root ganglia. Journal of neuroscience research *26*, 83-89.
Salmon, B., and Baines, J.D. (1998). Herpes simplex virus DNA cleavage and packaging: association of multiple forms of U(L)15-encoded proteins with B capsids requires at least the U(L)6, U(L)17, and U(L)28 genes. J Virol *72*, 3045-3050.
Salmon, B., Cunningham, C., Davison, A.J., Harris, W.J., and Baines, J.D. (1998). The herpes simplex virus type 1 U(L)17 gene encodes virion tegument proteins that are required for cleavage and packaging of viral DNA. J Virol *72*, 3779-3788.
Salomoni, P., Ferguson, B.J., Wyllie, A.H., and Rich, T. (2008). New insights into the role of PML in tumour suppression. Cell research *18*, 622-640.
Sanchez, V., Greis, K.D., Sztul, E., and Britt, W.J. (2000). Accumulation of virion tegument and envelope proteins in a stable cytoplasmic compartment during human cytomegalovirus replication: characterization of a potential site of virus assembly. J Virol *74*, 975-986.
Sandri-Goldin, R.M., Hibbard, M.K., and Hardwicke, M.A. (1995). The C-terminal repressor region of herpes simplex virus type 1 ICP27 is required for the redistribution of small nuclear ribonucleoprotein particles and splicing factor SC35; however, these alterations are not sufficient to inhibit host cell splicing. J Virol *69*, 6063-6076.
Sandri-Goldin, R.M., and Mendoza, G.E. (1992). A herpesvirus regulatory protein appears to act post-transcriptionally by affecting mRNA processing. Genes & development *6*, 848-863.
Sato, B., Ito, H., Hinchliffe, S., Sommer, M.H., Zerboni, L., and Arvin, A.M. (2003a). Mutational analysis of open reading frames 62 and 71, encoding the varicella-zoster virus immediate-early transactivating protein, IE62, and effects on replication in vitro and in skin xenografts in the SCID-hu mouse in vivo. J Virol *77*, 5607-5620.
Sato, B., Sommer, M., Ito, H., and Arvin, A.M. (2003b). Requirement of varicella-zoster virus immediate-early 4 protein for viral replication. J Virol *77*, 12369-12372.
Sato, H., Pesnicak, L., and Cohen, J.I. (2003c). Use of a rodent model to show that varicella-zoster virus ORF61 is dispensable for establishment of latency. Journal of medical virology *70 Suppl 1*, S79-81.
Satoh, T., Arii, J., Suenaga, T., Wang, J., Kogure, A., Uehori, J., Arase, N., Shiratori, I., Tanaka, S., Kawaguchi, Y.*, et al.* (2008). PILRalpha is a herpes simplex virus-1 entry coreceptor that associates with glycoprotein B. Cell *132*, 935-944.
Sawyer, M.H., Ostrove, J.M., Felser, J.M., and Straus, S.E. (1986). Mapping of the varicella zoster virus deoxypyrimidine kinase gene and preliminary identification of its transcript. Virology *149*, 1-9.
Schmid, D.S., and Jumaan, A.O. (2010). Impact of varicella vaccine on varicella-zoster virus dynamics. Clin Microbiol Rev *23*, 202-217.
Schmidt-Chanasit, J., Bleymehl, K., Rabenau, H.F., Ulrich, R.G., Cinatl, J., Jr., and Doerr, H.W. (2008). In vitro replication of varicella-zoster virus in human retinal pigment epithelial cells. Journal of clinical microbiology *46*, 2122-2124.
Schreiner, S., Wimmer, P., Sirma, H., Everett, R.D., Blanchette, P., Groitl, P., and Dobner, T. (2010). Proteasome-dependent degradation of Daxx by the viral E1B-55K protein in human adenovirus-infected cells. Journal of virology *84*, 7029-7038.
Schroder, H.C., Falke, D., Weise, K., Bachmann, M., Carmo-Fonseca, M., Zaubitzer, T., and Muller, W.E. (1989). Change of processing and nucleocytoplasmic transport of mRNA in HSV-1-infected cells. Virus research *13*, 61-78.
Sears, A.E., Halliburton, I.W., Meignier, B., Silver, S., and Roizman, B. (1985). Herpes simplex virus 1 mutant deleted in the alpha 22 gene: growth and gene expression in permissive and restrictive cells and establishment of latency in mice. J Virol *55*, 338-346.
Sheaffer, A.K., Newcomb, W.W., Gao, M., Yu, D., Weller, S.K., Brown, J.C., and Tenney, D.J. (2001). Herpes simplex virus DNA cleavage and packaging proteins associate with the procapsid prior to its maturation. J Virol *75*, 687-698.
Shen, W., Sa e Silva, M., Jaber, T., Vitvitskaia, O., Li, S., Henderson, G., and Jones, C. (2009). Two small RNAs encoded within the first 1.5 kilobases of the herpes simplex virus type 1 latency-associated transcript can inhibit productive infection and cooperate to inhibit apoptosis. J Virol *83*, 9131-9139.
Shiba, C., Daikoku, T., Goshima, F., Takakuwa, H., Yamauchi, Y., Koiwai, O., and Nishiyama, Y. (2000). The UL34 gene product of herpes simplex virus type 2 is a tail-anchored type II membrane protein that is significant for virus envelopment. J Gen Virol *81*, 2397-2405.
Shibaki, T., Suzutani, T., Yoshida, I., Ogasawara, M., and Azuma, M. (2001). Participation of type I interferon in the decreased virulence of the UL13 gene-deleted mutant of herpes simplex virus type 1. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research *21*, 279-285.
Shishido-Hara, Y., Ichinose, S., Higuchi, K., Hara, Y., and Yasui, K. (2004). Major and minor capsid proteins of human polyomavirus JC cooperatively accumulate to nuclear domain 10 for assembly into virions. Journal of virology *78*, 9890-9903.

Shukla, D., Liu, J., Blaiklock, P., Shworak, N.W., Bai, X., Esko, J.D., Cohen, G.H., Eisenberg, R.J., Rosenberg, R.D., and Spear, P.G. (1999). A novel role for 3-O-sulfated heparan sulfate in herpes simplex virus 1 entry. Cell *99*, 13-22.
Sivachandran, N., Sarkari, F., and Frappier, L. (2008). Epstein-Barr nuclear antigen 1 contributes to nasopharyngeal carcinoma through disruption of PML nuclear bodies. PLoS pathogens *4*, e1000170.
Sloutskin, A., Kinchington, P.R., and Goldstein, R.S. (2013). Productive vs non-productive infection by cell-free varicella zoster virus of human neurons derived from embryonic stem cells is dependent upon infectious viral dose. Virology *443*, 285-293.
Sommer, M.H., Zagha, E., Serrano, O.K., Ku, C.C., Zerboni, L., Baiker, A., Santos, R., Spengler, M., Lynch, J., Grose, C.*, et al.* (2001). Mutational analysis of the repeated open reading frames, ORFs 63 and 70 and ORFs 64 and 69, of varicella-zoster virus. J Virol *75*, 8224-8239.
Song, L., Chaudhuri, M., Knopf, C.W., and Parris, D.S. (2004). Contribution of the 3'- to 5'-exonuclease activity of herpes simplex virus type 1 DNA polymerase to the fidelity of DNA synthesis. J Biol Chem *279*, 18535-18543.
Sourvinos, G., and Everett, R.D. (2002). Visualization of parental HSV-1 genomes and replication compartments in association with ND10 in live infected cells. The EMBO journal *21*, 4989-4997.
Spector, D.L., and Lamond, A.I. (2011). Nuclear speckles. Cold Spring Harbor perspectives in biology *3*.
Spector, T., Harrington, J.A., Morrison, R.W., Jr., Lambe, C.U., Nelson, D.J., Averett, D.R., Biron, K., and Furman, P.A. (1989). 2-Acetylpyridine 5-[(dimethylamino)thiocarbonyl]-thiocarbonohydrazone (A1110U), a potent inactivator of ribonucleotide reductases of herpes simplex and varicella-zoster viruses and a potentiator of acyclovir. Proc Natl Acad Sci U S A *86*, 1051-1055.
Speese, S.D., Ashley, J., Jokhi, V., Nunnari, J., Barria, R., Li, Y., Ataman, B., Koon, A., Chang, Y.T., Li, Q.*, et al.* (2012). Nuclear envelope budding enables large ribonucleoprotein particle export during synaptic Wnt signaling. Cell *149*, 832-846.
Spencer, J.V., Newcomb, W.W., Thomsen, D.R., Homa, F.L., and Brown, J.C. (1998). Assembly of the herpes simplex virus capsid: preformed triplexes bind to the nascent capsid. J Virol *72*, 3944-3951.
Stadler, M., Chelbi-Alix, M.K., Koken, M.H., Venturini, L., Lee, C., Saib, A., Quignon, F., Pelicano, L., Guillemin, M.C., Schindler, C.*, et al.* (1995). Transcriptional induction of the PML growth suppressor gene by interferons is mediated through an ISRE and a GAS element. Oncogene *11*, 2565-2573.
Stannard, L.M., Himmelhoch, S., and Wynchank, S. (1996). Intra-nuclear localization of two envelope proteins, gB and gD, of herpes simplex virus. Arch Virol *141*, 505-524.
Stellberger, T., Hauser, R., Baiker, A., Pothineni, V.R., Haas, J., and Uetz, P. (2010). Improving the yeast two-hybrid system with permutated fusions proteins: the Varicella Zoster Virus interactome. Proteome science *8*, 8.
Stow, N.D. (2001). Packaging of genomic and amplicon DNA by the herpes simplex virus type 1 UL25-null mutant KUL25NS. J Virol *75*, 10755-10765.
Stow, N.D., and Davison, A.J. (1986). Identification of a varicella-zoster virus origin of DNA replication and its activation by herpes simplex virus type 1 gene products. J Gen Virol *67 (Pt 8)*, 1613-1623.
Stow, N.D., Weir, H.M., and Stow, E.C. (1990). Analysis of the binding sites for the varicella-zoster virus gene 51 product within the viral origin of DNA replication. Virology *177*, 570-577.
Strang, B.L., Boulant, S., Chang, L., Knipe, D.M., Kirchhausen, T., and Coen, D.M. (2012). Human cytomegalovirus UL44 concentrates at the periphery of replication compartments, the site of viral DNA synthesis. J Virol *86*, 2089-2095.
Strang, B.L., and Stow, N.D. (2005). Circularization of the herpes simplex virus type 1 genome upon lytic infection. J Virol *79*, 12487-12494.
Straus, S.E., Aulakh, H.S., Ruyechan, W.T., Hay, J., Casey, T.A., Vande Woude, G.F., Owens, J., and Smith, H.A. (1981). Structure of varicella-zoster virus DNA. J Virol *40*, 516-525.
Straus, S.E., Hay, J., Smith, H., and Owens, J. (1983). Genome differences among varicella-zoster virus isolates. J Gen Virol *64*, 1031-1041.
Straus, S.E., Reinhold, W., Smith, H.A., Ruyechan, W.T., Henderson, D.K., Blaese, R.M., and Hay, J. (1984). Endonuclease analysis of viral DNA from varicella and subsequent zoster infections in the same patient. N Engl J Med *311*, 1362-1364.
Subramanian, R.P., and Geraghty, R.J. (2007). Herpes simplex virus type 1 mediates fusion through a hemifusion intermediate by sequential activity of glycoproteins D, H, L, and B. Proc Natl Acad Sci U S A *104*, 2903-2908.
Suenaga, T., Satoh, T., Somboonthum, P., Kawaguchi, Y., Mori, Y., and Arase, H. (2010). Myelin-associated glycoprotein mediates membrane fusion and entry of neurotropic herpesviruses. Proc Natl Acad Sci U S A *107*, 866-871.
Sugimoto, K., Uema, M., Sagara, H., Tanaka, M., Sata, T., Hashimoto, Y., and Kawaguchi, Y. (2008). Simultaneous tracking of capsid, tegument, and envelope protein localization in living cells infected with triply fluorescent herpes simplex virus 1. J Virol *82*, 5198-5211.
Takahashi, M., Otsuka, T., Okuno, Y., Asano, Y., and Yazaki, T. (1974). Live vaccine used to prevent the spread of varicella in children in hospital. Lancet *2*, 1288-1290.
Takahashi, Y., Lallemand-Breitenbach, V., Zhu, J., and de The, H. (2004). PML nuclear bodies and apoptosis. Oncogene *23*, 2819-2824.
Taus, N.S., Salmon, B., and Baines, J.D. (1998). The herpes simplex virus 1 UL 17 gene is required for localization of capsids and major and minor capsid proteins to intranuclear sites where viral DNA is cleaved and packaged. Virology *252*, 115-125.
Tavalai, N., and Stamminger, T. (2008). New insights into the role of the subnuclear structure ND10 for viral infection. Biochimica et biophysica acta *1783*, 2207-2221.
Tenney, D.J., Hurlburt, W.W., Micheletti, P.A., Bifano, M., and Hamatake, R.K. (1994). The UL8 component of the herpes simplex virus helicase-primase complex stimulates primer synthesis by a subassembly of the UL5 and UL52 components. J Biol Chem *269*, 5030-5035.
Tenney, D.J., Sheaffer, A.K., Hurlburt, W.W., Bifano, M., and Hamatake, R.K. (1995). Sequence-dependent primer synthesis by the herpes simplex virus helicase-primase complex. J Biol Chem *270*, 9129-9136.

Thomas, S.L., Wheeler, J.G., and Hall, A.J. (2002). Contacts with varicella or with children and protection against herpes zoster in adults: a case-control study. Lancet *360*, 678-682.
Thomsen, D.R., Newcomb, W.W., Brown, J.C., and Homa, F.L. (1995). Assembly of the herpes simplex virus capsid: requirement for the carboxyl-terminal twenty-five amino acids of the proteins encoded by the UL26 and UL26.5 genes. J Virol *69*, 3690-3703.
Tirabassi, R.S., and Enquist, L.W. (1999). Mutation of the YXXL endocytosis motif in the cytoplasmic tail of pseudorabies virus gE. J Virol *73*, 2717-2728.
Tischer, B.K., Kaufer, B.B., Sommer, M., Wussow, F., Arvin, A.M., and Osterrieder, N. (2007). A self-excisable infectious bacterial artificial chromosome clone of varicella-zoster virus allows analysis of the essential tegument protein encoded by ORF9. J Virol *81*, 13200-13208.
Toropova, K., Huffman, J.B., Homa, F.L., and Conway, J.F. (2011). The herpes simplex virus 1 UL17 protein is the second constituent of the capsid vertex-specific component required for DNA packaging and retention. J Virol *85*, 7513-7522.
Torrisi, M.R., Di Lazzaro, C., Pavan, A., Pereira, L., and Campadelli-Fiume, G. (1992). Herpes simplex virus envelopment and maturation studied by fracture label. J Virol *66*, 554-561.
Trus, B.L., Booy, F.P., Newcomb, W.W., Brown, J.C., Homa, F.L., Thomsen, D.R., and Steven, A.C. (1996). The herpes simplex virus procapsid: structure, conformational changes upon maturation, and roles of the triplex proteins VP19c and VP23 in assembly. Journal of molecular biology *263*, 447-462.
Trus, B.L., Newcomb, W.W., Cheng, N., Cardone, G., Marekov, L., Homa, F.L., Brown, J.C., and Steven, A.C. (2007). Allosteric signaling and a nuclear exit strategy: binding of UL25/UL17 heterodimers to DNA-Filled HSV-1 capsids. Molecular cell *26*, 479-489.
Turcotte, S., Letellier, J., and Lippe, R. (2005). Herpes simplex virus type 1 capsids transit by the trans-Golgi network, where viral glycoproteins accumulate independently of capsid egress. J Virol *79*, 8847-8860.
Umbach, J.L., Kramer, M.F., Jurak, I., Karnowski, H.W., Coen, D.M., and Cullen, B.R. (2008). MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. Nature *454*, 780-783.
van Genderen, I.L., Brandimarti, R., Torrisi, M.R., Campadelli, G., and van Meer, G. (1994). The phospholipid composition of extracellular herpes simplex virions differs from that of host cell nuclei. Virology *200*, 831-836.
van Hoek, A.J., Melegaro, A., Gay, N., Bilcke, J., and Edmunds, W.J. (2012). The cost-effectiveness of varicella and combined varicella and herpes zoster vaccination programmes in the United Kingdom. Vaccine *30*, 1225-1234.
Vandevenne, P., Lebrun, M., El Mjiyad, N., Ote, I., Di Valentin, E., Habraken, Y., Dortu, E., Piette, J., and Sadzot-Delvaux, C. (2011). The varicella-zoster virus ORF47 kinase interferes with host innate immune response by inhibiting the activation of IRF3. PloS one *6*, e16870.
Visalli, R.J., Nicolosi, D.M., Irven, K.L., Goshorn, B., Khan, T., and Visalli, M.A. (2007). The Varicella-zoster virus DNA encapsidation genes: Identification and characterization of the putative terminase subunits. Virus research *129*, 200-211.
Vizoso Pinto, M.G., Pothineni, V.R., Haase, R., Woidy, M., Lotz-Havla, A.S., Gersting, S.W., Muntau, A.C., Haas, J., Sommer, M., Arvin, A.M.*, et al.* (2011). Varicella zoster virus ORF25 gene product: an essential hub protein linking encapsidation proteins and the nuclear egress complex. J Proteome Res *10*, 5374-5382.
Walters, M.S., Erazo, A., Kinchington, P.R., and Silverstein, S. (2009). Histone deacetylases 1 and 2 are phosphorylated at novel sites during varicella-zoster virus infection. J Virol *83*, 11502-11513.
Wang, J., Shiels, C., Sasieni, P., Wu, P.J., Islam, S.A., Freemont, P.S., and Sheer, D. (2004). Promyelocytic leukemia nuclear bodies associate with transcriptionally active genomic regions. The Journal of cell biology *164*, 515-526.
Wang, J.T., Doong, S.L., Teng, S.C., Lee, C.P., Tsai, C.H., and Chen, M.R. (2009). Epstein-Barr virus BGLF4 kinase suppresses the interferon regulatory factor 3 signaling pathway. J Virol *83*, 1856-1869.
Wang, K., Lau, T.Y., Morales, M., Mont, E.K., and Straus, S.E. (2005). Laser-capture microdissection: refining estimates of the quantity and distribution of latent herpes simplex virus 1 and varicella-zoster virus DNA in human trigeminal Ganglia at the single-cell level. J Virol *79*, 14079-14087.
Wang, L., Oliver, S.L., Sommer, M., Rajamani, J., Reichelt, M., and Arvin, A.M. (2011). Disruption of PML nuclear bodies is mediated by ORF61 SUMO-interacting motifs and required for varicella-zoster virus pathogenesis in skin. PLoS Pathog *7*, e1002157.
Ward, P.L., Ogle, W.O., and Roizman, B. (1996). Assemblons: nuclear structures defined by aggregation of immature capsids and some tegument proteins of herpes simplex virus 1. Journal of virology *70*, 4623-4631.
Warming, S., Costantino, N., Court, D.L., Jenkins, N.A., and Copeland, N.G. (2005). Simple and highly efficient BAC recombineering using galK selection. Nucleic acids research *33*, e36.
Weinberg, A., and Levin, M.J. (2010). VZV T cell-mediated immunity. Current topics in microbiology and immunology *342*, 341-357.
Weinberg, J.M. (2007). Herpes zoster: epidemiology, natural history, and common complications. Journal of the American Academy of Dermatology *57*, S130-135.
Weir, H.M., and Stow, N.D. (1990). Two binding sites for the herpes simplex virus type 1 UL9 protein are required for efficient activity of the oriS replication origin. J Gen Virol *71 (Pt 6)*, 1379-1385.
Weller, S.K., and Coen, D.M. (2012). Herpes simplex viruses: mechanisms of DNA replication. Cold Spring Harbor perspectives in biology *4*, a013011.
Weller, T.H. (1953). Serial propagation in vitro of agents producing inclusion bodies derived from varicella and herpes zoster. Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine *83*, 340-346.
Weller, T.H. (1996). Varicella: historical perspective and clinical overview. The Journal of infectious diseases *174 Suppl 3*, S306-309.
Weller, T.H., and Stoddard, M.B. (1952). Intranuclear inclusion bodies in tissue cultures inoculated with varicella vesicle fluid. A.M.A. American journal of diseases of children *83*, 75-76.

Weller, T.H., and Witton, H.M. (1958). The etiologic agents of varicella and herpes zoster; serologic studies with the viruses as propagated in vitro. J Exp Med *108*, 869-890.
Wileman, T. (2007). Aggresomes and pericentriolar sites of virus assembly: cellular defense or viral design? Annual review of microbiology *61*, 149-167.
Wilkinson, D.E., and Weller, S.K. (2006). Herpes simplex virus type I disrupts the ATR-dependent DNA-damage response during lytic infection. Journal of cell science *119*, 2695-2703.
Willer, D.O., Ambagala, A.P., Pilon, R., Chan, J.K., Fournier, J., Brooks, J., Sandstrom, P., and Macdonald, K.S. (2012). Experimental infection of Cynomolgus Macaques (Macaca fascicularis) with human varicella-zoster virus. J Virol *86*, 3626-3634.
Wisner, T.W., and Johnson, D.C. (2004). Redistribution of cellular and herpes simplex virus proteins from the trans-golgi network to cell junctions without enveloped capsids. J Virol *78*, 11519-11535.
Wisner, T.W., Wright, C.C., Kato, A., Kawaguchi, Y., Mou, F., Baines, J.D., Roller, R.J., and Johnson, D.C. (2009). Herpesvirus gB-induced fusion between the virion envelope and outer nuclear membrane during virus egress is regulated by the viral US3 kinase. J Virol *83*, 3115-3126.
Wolfstein, A., Nagel, C.H., Radtke, K., Dohner, K., Allan, V.J., and Sodeik, B. (2006). The inner tegument promotes herpes simplex virus capsid motility along microtubules in vitro. Traffic *7*, 227-237.
Wright, C.C., Wisner, T.W., Hannah, B.P., Eisenberg, R.J., Cohen, G.H., and Johnson, D.C. (2009). Fusion between perinuclear virions and the outer nuclear membrane requires the fusogenic activity of herpes simplex virus gB. J Virol *83*, 11847-11856.
Yamada, M., Sato, T., Shimohata, T., Hayashi, S., Igarashi, S., Tsuji, S., and Takahashi, H. (2001). Interaction between neuronal intranuclear inclusions and promyelocytic leukemia protein nuclear and coiled bodies in CAG repeat diseases. The American journal of pathology *159*, 1785-1795.
Yang, K., and Baines, J.D. (2006). The putative terminase subunit of herpes simplex virus 1 encoded by UL28 is necessary and sufficient to mediate interaction between pUL15 and pUL33. J Virol *80*, 5733-5739.
Yang, K., and Baines, J.D. (2011). Selection of HSV capsids for envelopment involves interaction between capsid surface components pUL31, pUL17, and pUL25. Proc Natl Acad Sci U S A *108*, 14276-14281.
Yang, K., Homa, F., and Baines, J.D. (2007). Putative terminase subunits of herpes simplex virus 1 form a complex in the cytoplasm and interact with portal protein in the nucleus. J Virol *81*, 6419-6433.
Yang, K., Wills, E., and Baines, J.D. (2009). The putative leucine zipper of the UL6-encoded portal protein of herpes simplex virus 1 is necessary for interaction with pUL15 and pUL28 and their association with capsids. J Virol *83*, 4557-4564.
Yeh, P.C., Meckes, D.G., Jr., and Wills, J.W. (2008). Analysis of the interaction between the UL11 and UL16 tegument proteins of herpes simplex virus. J Virol *82*, 10693-10700.
Yu, D., and Weller, S.K. (1998). Herpes simplex virus type 1 cleavage and packaging proteins UL15 and UL28 are associated with B but not C capsids during packaging. J Virol *72*, 7428-7439.
Zabolotny, J.M., Krummenacher, C., and Fraser, N.W. (1997). The herpes simplex virus type 1 2.0-kilobase latency-associated transcript is a stable intron which branches at a guanosine. J Virol *71*, 4199-4208.
Zhang, Z., Rowe, J., Wang, W., Sommer, M., Arvin, A., Moffat, J., and Zhu, H. (2007). Genetic Analysis of Varicella-Zoster Virus ORF0 to ORF4 by Use of a Novel Luciferase Bacterial Artificial Chromosome System

10.1128/JVI.02666-06. J. Virol. *81*, 9024-9033.
Zhang, Z., Selariu, A., Warden, C., Huang, G., Huang, Y., Zaccheus, O., Cheng, T., Xia, N., and Zhu, H. (2010). Genome-wide mutagenesis reveals that ORF7 is a novel VZV skin-tropic factor. PLoS pathogens *6*, e1000971.
Zhou, Z.H., Chen, D.H., Jakana, J., Rixon, F.J., and Chiu, W. (1999). Visualization of tegument-capsid interactions and DNA in intact herpes simplex virus type 1 virions. J Virol *73*, 3210-3218.
Zhou, Z.H., He, J., Jakana, J., Tatman, J.D., Rixon, F.J., and Chiu, W. (1995). Assembly of VP26 in herpes simplex virus-1 inferred from structures of wild-type and recombinant capsids. Nature structural biology *2*, 1026-1030.
Zhu, Z., Gershon, M.D., Ambron, R., Gabel, C., and Gershon, A.A. (1995a). Infection of cells by varicella zoster virus: inhibition of viral entry by mannose 6-phosphate and heparin. Proc Natl Acad Sci U S A *92*, 3546-3550.
Zhu, Z., Gershon, M.D., Hao, Y., Ambron, R.T., Gabel, C.A., and Gershon, A.A. (1995b). Envelopment of varicella-zoster virus: targeting of viral glycoproteins to the trans-Golgi network. J Virol *69*, 7951-7959.
Zhu, Z., Hao, Y., Gershon, M.D., Ambron, R.T., and Gershon, A.A. (1996). Targeting of glycoprotein I (gE) of varicella-zoster virus to the trans-Golgi network by an AYRV sequence and an acidic amino acid-rich patch in the cytosolic domain of the molecule. J Virol *70*, 6563-6575.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