CellPress

Review

Emerging Roles for the Unfolded Protein Response in the Developing Nervous System

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The unfolded protein response (UPR) is a homeostatic signaling pathway triggered by protein misfolding in the endoplasmic reticulum (ER). Beyond its protective role, it plays important functions during normal development in response to elevated demand for protein folding. Several UPR effectors show dynamic temporal and spatial expression patterns that correlate with milestones of the central nervous system (CNS) development. Here, we discuss recent studies suggesting that a dynamic regulation of UPR supports generation, maturation, and maintenance of differentiated neurons in the CNS. We further highlight studies supporting a developmental vulnerability of CNS to UPR dysregulation, which underlies neurodevelopmental disorders. We believe that a better understanding of UPR functions may provide novel opportunities for therapeutic strategies to fight ER/UPR-associated human neurological disorders.

The UPR Is a Guardian of Cellular Homeostasis in the Central Nervous System

The central nervous system (CNS) of vertebrates includes two main structures: the brain and the spinal cord. The early stages of neural development are similar across all vertebrate species and start with the closure of the neural tube (NT). The morphogenetic events that shape the brain occur later with the swelling and folding of the anterior part of the NT into three vesicles: the forebrain, midbrain, and hindbrain. The posterior part of the NT becomes the spinal cord. The forebrain further splits into two additional vesicles, one becoming the telencephalon whose dorsal part generates the cerebral cortex. The cortex is an exquisite product of vertebrate evolution that computes higher cognitive functions and whose complex cytoarchitectonics reflect the great diversity of neurons and their migratory behaviors that take place during its formation [1]. In mammals, the laminar organization of the cortex arises inside-out as progenitors generate successive waves of pyramidal neurons in the cortical wall [2] and interneurons in subpallial regions [the medial and caudal ganglionic eminences (MGE and CGE, respectively) and the preoptic area (POA)] [3,4]. Projection neurons travel short distances along radial glial fibers and interneurons navigate along tangential paths to settle in the cortical plate [5]. While poor neuron survival is the hallmark of neurodegenerative disorders, disrupting the production, migration, or differentiation of cortical neurons can lead to cortical malformations often associated with the etiology of psychiatric or neurological disorders [6–8].

During neurogenesis, protein synthesis increases in progenitors to meet the cellular demand imposed by the proliferation and maturation of neurons. Activation of protein quality control

Trends

The unfolded protein response (UPR) is an evolutionary conserved mechanism whose function goes beyond regulation of cellular proteostasis.

Several UPR effectors are expressed during central nervous system (CNS) development and recent studies have highlighted critical roles for UPR during neurodevelopment as well as neuronal maintenance in the CNS.

Cellular and molecular conditions that enhanced ER stress also deregulate UPR signaling during development and can interfere with the myelination process as well as lead to brain malformations.

Dynamically regulated UPR finely tunes the neurogenesis process as well as the morphological maturation of neurons. However, its upstream regulators remain to be identified.

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 http://dx.doi.org/10.1016/j.tins.2016.04.002

pathways plays a critical role to maintain cellular proteostasis during this process. Among these mechanisms, the evolutionary conserved unfolded protein response (UPR) adjusts the endoplasmic reticulum (ER) environment upon detection of misfolded/unfolded proteins to ensure that protein folding capacity is balanced with needs. The ER is responsible for the synthesis and folding of secretory proteins as well as others dedicated to either the cell surface or the membrane of other intracellular organelles. Different cell stress conditions, including dysregulation of calcium homeostasis or redox status, elevated rate of secretory protein synthesis or their altered glycosylation can interfere with their proper folding leading to a 'collectively named' ER stress. This stress is further managed by activation of the UPR that either restores ER homeostasis (the UPR adaptive pathway) or triggers cell death (the UPR apoptosis pathway) [9].

The UPR pathway plays a critical role in synthesis, folding, and structural maturation of approximately one-third of all proteins produced in the cell, most of them being dedicated to secretion or membrane integration [10]. When the protein folding capacity of cells is overwhelmed, they experience ER stress and activate UPR. This situation occurs chronically in disease condition or in 'professionally' secreting cells such as pancreatic β cells where adaptive UPR ensures homeostasis [11]. In higher eukaryotes, UPR transduction involves the activation of ER membrane receptors that contain an ER lumenal domain that senses the accumulation of misfolded proteins, including the inositol-requiring enzyme 1 (Ire-1), the protein kinase (PKR)-like ER kinase (Perk), and the activating transcription factor 6 (Atf6) (Box 1). The activation of the ER stress response is a prosurvival mechanism, which expands the ER and reduces translation to limit ER protein loading. In addition, it promotes chaperone expression to help client proteins to refold properly. The UPR also clears some misfolded proteins through activation of the ER-associated degradation (ERAD) process that promotes ubiquitylation and degradation of these proteins by the 26S proteasome [12]. However, when the UPR cannot cope with the overload of misfolded proteins, then, the cells activates a 'terminal' UPR, which finally leads to apoptosis [13] (Box 2).

This review sums up the current knowledge about the physiological roles played by UPR in cellular homeostasis during CNS development and how disrupting its activity underlies pathology (Figure 1, Key Figure).

The UPR Contributes to Cell Fate Acquisition during CNS Development

UPR signaling is activated during neurogenesis in various animal models [14–21] (Table 1). Its activation is indicative of a physiological function in neuronal commitment and cell fate acquisition. The first line of evidence comes from *in vitro* studies. First, at least two of the three arms of UPR (Perk and Ire-1) are turned on during neuronal differentiation of mouse embryonic stem (mES) cells [22]. Second, ER stress induction by thapsigargin, tunicamycin, or brefeldin A facilitates neuronal differentiation and inhibits the glial differentiation of mES cells [22]. Third, ER stress inducers also favor neurogenesis at the expense of gliogenesis after differentiation of mouse embryonic carcinoma P19 cells using retinoic acid [23]. These initial studies suggest that UPR is an active factor for neuronal commitment and differentiation. This section describes *in vivo* studies supporting noncanonical roles for UPR signaling in regulating: (i) the lateral inhibition and (ii) cell fate during neurogenesis.

The UPR Functions in Lateral Inhibition

Lateral inhibition is a mechanism that governs cell fate decision between neighboring cells. The first evidence of UPR contribution to lateral inhibition comes from the *Drosophila* neurectoderm, where it dictates the cell fate choice between neural and epidermal progenitors. The *Drosophila* ER-resident protein pecanex (pcx) controls the fate of neuroblasts [24]. Disruption of pcx function in neuroblasts impairs lateral inhibition and results in the increased generation of neuroblasts at the expense of epidermoblasts. This phenotype likely results from defective ER activity as it is suppressed by ectopic expression of two UPR inducers, the activated Xbp1 or

Box 1. The UPR Is Required for the Maintenance of ER Homeostasis

The ER is a specialized organelle involved in the post-translational modification and folding of newly synthetized secretory and membrane proteins. The overloading of misfolded proteins that occurs when the ER folding capacity is overwhelmed triggers a stress that activates UPR signaling to deal with the alteration of ER functions. The three membranous ER stress sensors are Ire-1, Perk, and Atf6 (Figure I). Their activation engages the UPR [51,52]. Activation of Ire-1 leads to Xbp1 splicing, which generates a transcriptional factor (Xbp1s) that controls UPR target gene expression [53]. Moreover, Ire-1 activation can also mediate mRNA degradation by a specific process called RIDD (regulated Ire-1-dependent decay) to limit the ER protein load [54]. Atf6 activation induces its own intramembrane cleavage for translocation to the nucleus [Atf6 (N)] where it binds to the regulatory region of UPR target genes [55]. Perk is activated by dimerization and autophosphorylation. It induces eIF2α phosphorylation at serine 51 that prevents the recycling of the inactive GDP bound eIF2α to its active GTP bound form by eIF2B. This further prevents the formation of $tRNA^{Met}$ ternary initiation complexes and results in general translation attenuation. However, some mRNAs that harbor an internal ribosomal entry site or a small open reading frame in their 5′UTR can bypass the translation inhibition [56]. This is the case for Atf4, whose upregulation upon Perk activation promotes UPR target gene induction [51]. A negative feedback loop can further be activated downstream Atf4 to promote the dephosphorylation of eIF2α by the GADD34/PP1 C phosphatase complex, which restores protein synthesis (reviewed in [57]).

Activation of the UPR sensors induces the expression of various classes of proteins. Among them, the chaperones ensure proper protein folding to prevent protein aggregation and apoptosis. Beside its intrinsic roles as suppressor of apoptosis and for the maintenance of ER integrity (reviewed in [58]), the chaperone Grp78 (also named Bip) controls the activation of the three ER stress sensors: Ire-1, Perk, and Atf6 [59]. Under normal conditions, Grp78 binds to ER sensors to prevent their signaling cascades. But, upon ER stress, Grp78 is released from the membrane and induces the dimerization of Perk and Ire-1 and the translocation of Atf6 from the ER to the Golgi where it is cleaved for its further translocation to the nucleus. Meanwhile, the cell also progressively activates the ERAD, ubiquitin/proteasome, and autophagy signaling pathways to get rid of the misfolded proteins that accumulate in the ER [60]. Nevertheless, a prolonged UPR activation is often associated with the incapacity of the cell to cope with ER stress and ultimately lead to cell death by apoptosis [61].

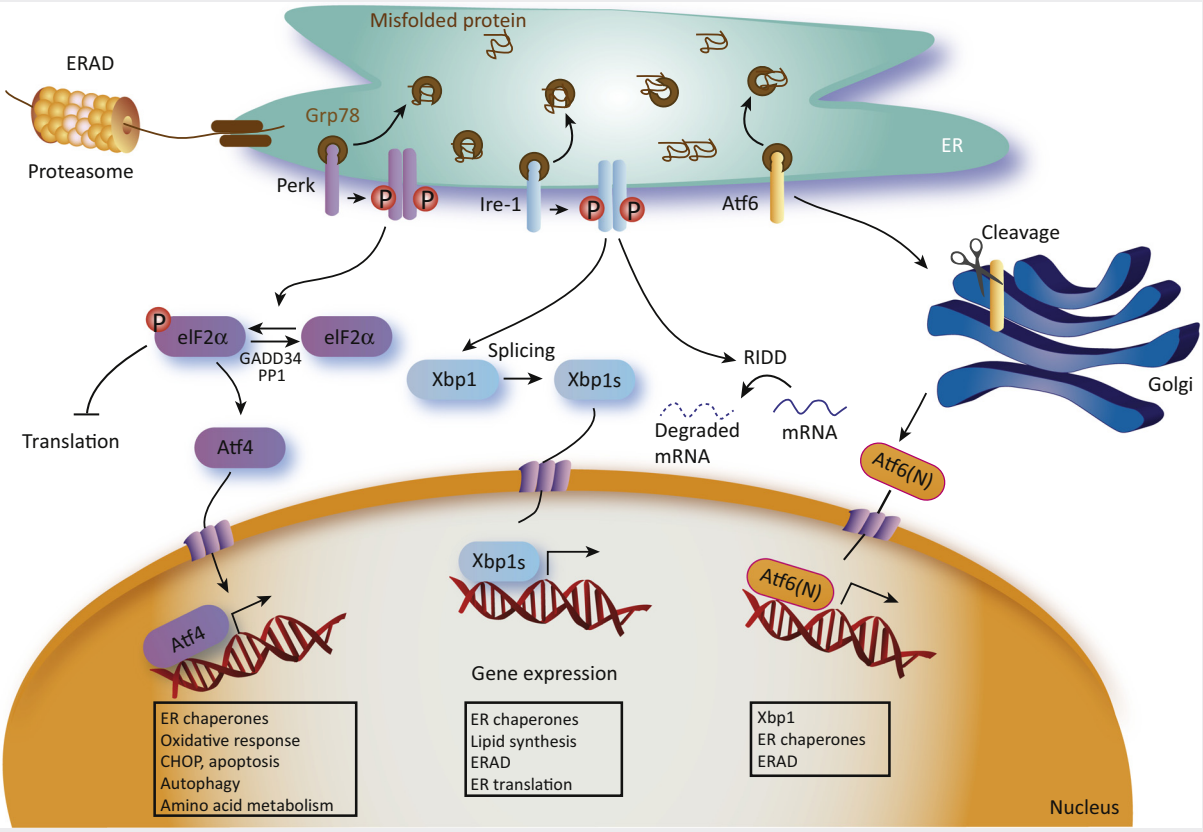

Figure I. UPR Signalling Pathways in Mammals

Box 2. Impairment of UPR Signaling Underlies CNS Disorders

The maintenance and regulation of proteostasis is important and prolonged UPR activation induces cell death and has been more widely associated with neurodegenerative diseases [62,63]. Indeed, chronic ER stress and sustained UPR signaling are emerging as key contributors to several human pathologies ranging from cancers, heart diseases, and pulmonary fibrosis, to neurological disorders (reviewed in [11]). One of the pathological hallmarks of neurodegenerative diseases is the toxic accumulation of misfolded proteins and their aggregation that interfere with neuron viability [64]. Accumulation of protein aggregates correlates with UPR activation and cell death in Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS) [65–68]. The causal role of ER stress and terminal UPR activation in neurodegenerative disorders is further supported by recent data obtained with experimental mouse models [69]. In addition, neurodevelopmental disorders of familial origin, such as Wolfram syndrome and autism-related *CNTNP2*, or induced by prenatal chronic alcohol exposure (fetal alcohol syndrome) and characterized by microcephaly also involve the induction of ER stress and UPR components as a putative trigger of neuronal cell death [70–72]. Recent molecular data also incriminate UPR effectors as downstream targets of DISC1 variants in schizophrenia [73,74].

In the CNS, the oligodendrocytes are the glial cells that produce the greater amount of plasma membrane to support the myelinating process, making them very susceptible to ER stress. Along this line, recent observations suggest that the UPR and its downstream targets are activated during the demyelination process observed in patient suffering from either multiple sclerosis [75] or Pelizaeus–Merzbacher disease (PMD) [76]. PMD is an X-linked dysmyelinating disease characterized by ER accumulation of misfolded PLP proteins that further triggers ER stress and UPR in oligodendrocytes [77]. Correlation between ER stress and poor myelination is also exemplified by the autosomal-recessive hypomyelinating disorder named vanishing white matter disease, which is caused by a point mutation in the protein synthesis factor eIF2B that impairs its response to phosphorylated eIF2α in cell stress condition and triggers UPR activation [47,78].

a dominant negative form Hsc70-3 (Heat-shock cognate 70-3), the *Drosophila* homolog of Grp78 [24]. How activation of UPR compensates for proper Notch signaling in the absence of pcx remains to be established. Interestingly ER stress inducers do not affect Notch signaling during the retinoic acid (RA)-induced neuronal differentiation of P19 cells [23], suggesting that

Key Figure

The Unfolded Protein Response (UPR) Functions in the Developing Central Nervous System (CNS)

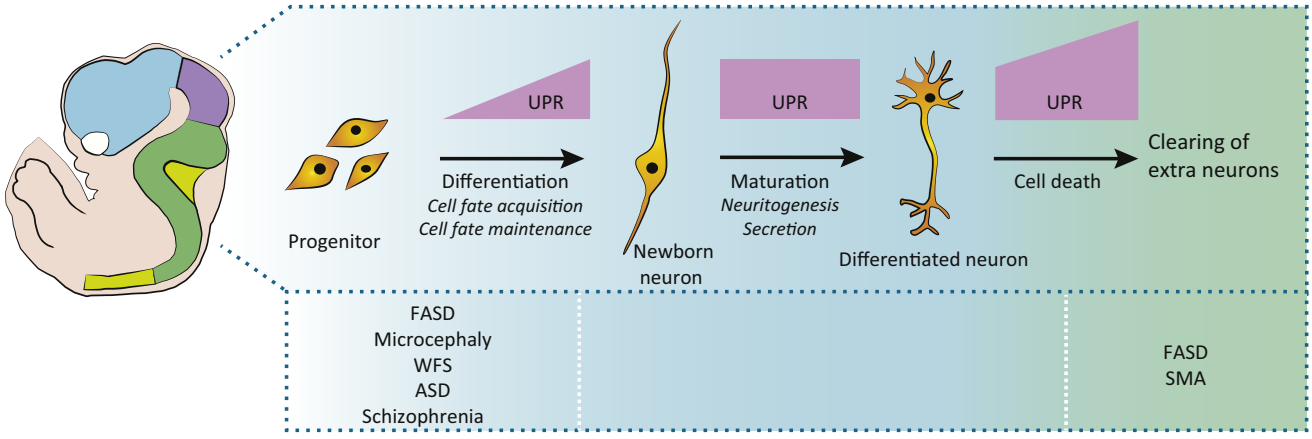

Figure 1. Emerging physiological roles of UPR signaling at milestone stages of brain development: (i) generation and differentiation of neurons, (ii) maturation of newborn neurons, and (iii) maintenance of mature neurons. Effectors of the three UPR signaling cascades (Box 1) are dynamically expressed during CNS development [14–16,79]. Chemical induction of UPR in progenitors favors neuronal differentiation [18,22,23], while genetic ablation of key UPR effectors impairs neurogenesis and cell fate commitment [18,30,33,35]. Altogether, these studies suggest that a progressive increase of UPR is required for normal differentiation. Neurons further require a permanent UPR signaling to mature: both reduction and elevation of UPR signal lead to defect in neuritogenesis [17,19,23,31]. Finally, elevated UPR signaling during CNS development is likely required to eliminate the excess of neurons by apoptosis [30,79,80]. In the lower panel, neurological disorders linked to deregulation of UPR are indicated, supporting a particular vulnerability of the CNS to UPR dysfunction [18,63,70,72–74,81,82] (Box 2). Abbreviations: FASD, fetal alcohol spectrum disorder; WFS, Wolfram syndrome; ASD, autism spectrum disorder; SMA, spinal muscular atrophy.

Table 1. Distribution of Principal UPR Effectors during CNS Development

UPR Effector	Animal Model	Special and Temporal Distribution	Refs
Xbp1	Mouse brain	Embryo >> adult	[79]
	Mouse retina	Embryo >> adult	[79]
	Mouse forebrain	E12 >> E18	[17]
	Drosophila photoreceptor	Larvae and early pupal stage	[33,36]
	C. elegans	Embryo and larvae Neurons >> non-neuronal cells	[20]
Atf4	Mouse cortex	E12 > E14 > E16 Progenitor > postmitotic neurons	[16,18]
	Mouse OSNs	Low level	[30]
	Drosophila photoreceptor	From early pupal stages	[34]
Atf5	OSNs	High expression (Atf5 >>> Atf4)	[30]
	Mouse cortex	E12 > E14 > E16	[18]
Atf6	Mouse brain	Embryo >> adult	[79]
	Mouse retina	Embryo >> adult	[79]
	Mouse OSNs	Low expression	[30]

UPR rather functions *in vivo* to control the lateral inhibition process. Notch-mediated lateral inhibition is also required for maintenance of neural progenitors in the developing mammalian nervous system [25]. It is thus tempting to speculate that the UPR signaling could also mediate lateral inhibition in the developing cerebral cortex or spinal cord.

The UPR Promotes Neurogenesis during Cerebral Cortex Development

The cerebral cortex contains layers of neurons sequentially generated by distinct lineage-related progenitors [26,27]. At the onset of corticogenesis, the first-born progenitors (apical progenitors, APs) divide asymmetrically to give birth directly to neurons. Later, they switch to indirect neurogenesis and generate intermediate progenitors (IPs), which then give rise to projection neurons of all cortical layers (Figure 2) [26]. Dynamic regulation of UPR signals is proposed to govern the switch from direct to indirect neurogenesis (Figure 2) [18]. Laguesse and colleagues present multiple lines of evidence supporting this idea. First, there is an inverse correlation between the intensity of UPR signaling and the rate of indirect neurogenesis [16,18], with a progressive reduction of Atf4 signaling in APs as corticogenesis proceeds [18]. Second, the induction of ER stress with tunicamycin at midcorticogenesis (when indirect neurogenesis predominates) promotes direct neurogenesis at the expense of IPs. Third, depletion of Atf4 at the onset of corticogenesis (when direct neurogenesis is prominent) increases the generation of IPs at the expense of neurons [18]. Those results demonstrate that a progressive down-regulation of UPR in cortical progenitors acts as a physiological signal to amplify IPs and promotes indirect neurogenesis (Figure 2). The physiological relevance of this pathway is indicated by experiments showing neurodevelopmental phenotypes in mice with exacerbated UPR signals. Indeed, mice deficient for the Elongator complex maintain a high level of Perk–eIF2α–Atf4 signaling throughout the cortical development [18]. This elevated UPR results from defective protein translation and/or folding resulting from lack of specific tRNA modifications [18,28]. Those mice display a severe microcephaly that results from a decreased rate of indirect neurogenesis (Figure 2). Importantly, downregulation of Atf4 level in Elongator deficient progenitors rescues the balance between direct and indirect neurogenesis [18]. This suggests that UPR dysregulation may underlie neurodevelopmental disorder (Box 2). Accordingly, Grp78 mutant mice are also microcephalic [19]. As such, it is worth testing the level of UPR signaling in those mice. Interestingly, only the Perk–eIF2α–Atf4 branch of UPR is strengthened in Elongator

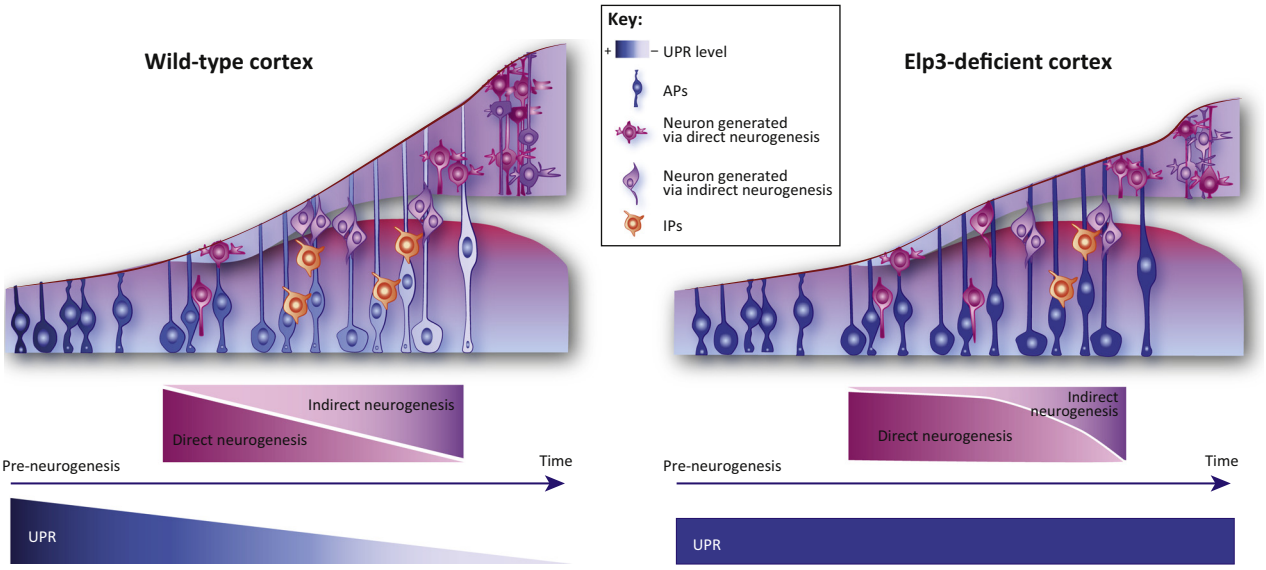

Figure 2. The Unfolded Protein Response Regulates the Balance between Direct and Indirect Neurogenesis in the Developing Cortex. Wild-type corticogenesis (left panel) is characterized by a progressive decrease of UPR signaling levels in apical progenitors (APs–depicted in blue–dark blue and light blue showing intense and low UPR signals, respectively). At the early steps of corticogenesis, APs generate neurons by direct neurogenesis: they divide to give rise to one AP and one neuron (magenta). As development progresses, APs produce neurons (purple) through the generation of proliferative intermediate progenitors (IPs–orange cells). Indirect neurogenesis contributes to the increased rate of neuron production as corticogenesis proceeds. Upon *elp3* deletion (right panel), the maintenance of high UPR level throughout development favors direct neurogenesis at the expense of indirect neurogenesis and therefore leads to a reduction of the neuronal output and microcephaly (Adapted from [83]).

deficient mice, suggesting that Atf6 and Ire-1 pathways only play minor roles in this pathological context. However, one cannot exclude a physiological role of the two other branches. This remains to be tested.

Besides its contribution to the regulation of cortical neurogenesis, Atf4 also controls cell cycle progression of the earliest progenitors, the neuroepithelial progenitors. Those progenitors divide symmetrically to self-renew and a small percentage divides asymmetrically to give birth to the early born neurons. Stabilization of Atf4 (by overexpressing a form that cannot be degraded) in those progenitors leads to cell cycle arrest in G1 and positioning defects [16]. Interestingly, Cyclin D expression rescues the Atf4-dependent proliferation phenotype but not the migration phenotype. This implies that Atf4 plays a dual role in early corticogenesis [16]: (i) it controls cell proliferation through regulation of Cyclin D promoter activity and (ii) it controls migration of earliest born neurons by unknown mechanisms. It is not clear whether this function of Atf4 requires upstream activation of the UPR. However, the ability of Atf4 to potently suppress proliferation and elicit G1 arrest may require Perk activation as it has been shown to trigger G1 arrest through repression of Cyclin D translation in fibroblast [29]. Altogether, those studies show that neuronal progenitors are acutely sensitive to Atf4 dosage and that proper level of Atf4 is required for efficient neurogenesis in the developing mouse brain.

UPR and Olfactory Receptor Choice in Mammalian Olfactory Sensory Neurons

Mammalian olfactory sensory neurons (OSNs) fate choice is guided by the expression of a single olfactory receptor (OR) allele and a feedback signal that locks the OR choice. Recent findings suggest that UPR components participate to the OR feedback process by detecting OR proteins in the ER [30]. *Perk* and *Atf5* knockout (KO) mice, as well as eIF2α phosphorylation mutants exhibit unstable OR expression, suggesting that the Perk/eIF2α/Atf5 pathway is

required to stabilize OR expression and ultimately maintain the fate of OSNs [30]. Ire-1 and Atf6 branches are unlikely to be involved in this feedback process as no phenotype has been observed in *Xbp1* mutants and as Atf6 is only slightly expressed in OSNs. Mechanistically, the authors nicely showed that increased eIF2α-dependent translation (translation initiation function, Box 1) of nuclear Atf5 after OR expression initiates transcription of Adenylate cyclase 3, which then signals to lock OR expression [30]. Noteworthy, *Atf4* KO mice do not exhibit OR instability phenotype, indicating the use of a noncanonical Perk pathway in developing OSNs.

The UPR Facilitates Various Neuronal Differentiation Processes

The UPR plays an important physiological function to cope with the considerable demand for protein folding that accompanies neuronal branching and the production of secretory factors during cell differentiation. While the full spectrum of downstream targets of the UPR pathway remains to be discovered in differentiating neurons, they converge toward membrane production and vesicular trafficking (Box 3).

The UPR Signals in Neuritogenesis

During development, dendrites acquire their morphology by considerable branch sprouting, which comes with an increased need of protein production. Interestingly, in *Caenorhabditis elegans*, loss of *ire-1* impairs dendritic morphogenesis of the highly branched neurons but has no impact on neurons with fewer dendritic or axonal branches [31], suggesting a preferential role for Ire-1 in large and complicated dendritic arbor development. In accordance, increased UPR activity in low-branched neurons efficiently induces ectopic branches. Noteworthy, Ire-1 mutants do not show any axonal defect. There is an apparent discrepancy with results obtained in cultured mouse cortical neurons, showing a reduction of axonal branches in $Xbp1^{-/-}$ neurons [17]. The role of Xbp1 on axogenesis may depend on the physiological context and needs further investigation. Both Xbp1 mRNA splicing and RIDD (Ire-1-dependent decay of mRNA, Box 1) pathway regulate dendritic branching of worm sensory neurons, showing that the entire Ire-1 arm of the UPR pathway is involved in dendritic morphogenesis. Remarkably, the two others arms of the UPR pathway (Atf6 and Perk) only contribute marginally to dendritogenesis. The authors nicely showed that UPR activity was specifically induced during the time of dendritic branching. The physiological increase of the Ire-1/Xbp1 pathway upregulates specific chaperones, such as HSP-4, that helps to fold DMA-1, whose overexpression is required for dendritic branching. The physiological role of the RIDD pathway and the nature of its targets in dendritic morphogenesis of worm sensory neurons are less clear.

The role of UPR signaling in dendritogenesis is further supported by experiments with cultured mouse hippocampal neurons, demonstrating that both Xbp1 and Eif2α are activated in neurites in response to BDNF (brain-derived neurotrophic factor) [17]. Indeed, BDNF-induced neurite

Box 3. UPR Signaling Converges toward Membrane Synthesis in Differentiating Neurons

While the downstream targets of the UPR pathway are not always known in differentiating neurons, mechanisms often converge toward membrane requirement and vesicular trafficking. We discussed that Ire-1 contributes to Rhodopsin delivery via degradation of fatp mRNA [33]. How does elevated fatp level lead to rhabdomere morphogenesis defects in *Ire-1* mutants? Fatp mediates the uptake of fatty acids into cells and fatty acids are precursors for the biosynthesis of phosphatidic acids, a major component of the plasma membrane. Increased levels of phosphatidic acid were shown to impair photoreceptor apical membrane transport and disrupt rhabdomere morphogenesis [84], causing a phenotype similar to that of *Ire-1* mutant photoreceptor. It is therefore proposed that increased fatp elevates phosphatidic acid and thus prevents membrane-associated Rhodopsin delivery to the rhabdomere. In line with this, the impairment of BDNF-induced neurite outgrowth in $Xbp1^{-/-}$ neurons may also rely on defective membrane synthesis [17]. Indeed, Xbp1 is reported to promote lipid biosynthesis in fibroblast cells [85] and secretory organs [86]. One can envisage that UPR is used to cope with increased demand by the exocytosis pathway. Further studies are required to determine whether UPR may control exocytosis in developing neurons; this could have many consequences on vesicular transport, an indispensable process for proper neuronal differentiation and maturation.

outgrowth is strongly impaired in Xbp1$^{-/-}$ neurons. BDNF-induced protein synthesis in neurites could initiate Xbp1 splicing. Spliced Xbp1 is then transported to the nucleus where it serves as a signal transducer for neurite outgrowth [17]. However, molecular targets downstream of Xbp1 signaling for neurite outgrowth remain to be elucidated (Box 3). While there are no *in vivo* studies supporting this hypothesis, the ERAD pathway may also promote neuronal maturation. Indeed, downregulation of Hrd-1 level, one well-known ERAD-associated E3 ubiquitin ligase, counteracts the deleterious effect of mild ER stress on dendrite extension during RA-induced neuronal differentiation of P19 cells [23].

Altogether, these studies suggest that UPR signals need to be temporally and spatially fine-tuned during neuronal differentiation. Its levels are indeed critical for neuronal maturation, neurites being vulnerable to both low and high signals. UPR may therefore be a homeostat regulating neuronal differentiation.

Drosophila Photoreceptor Differentiation

In the developing *Drosophila* photoreceptor cell, the growth of the rhabdomeres requires the delivery of large amounts of membrane and proteins, including Rhodopsin-1 (Rh1), into this structure, imposing a high demand for protein folding and membrane production in the ER. Ire-1 accommodates proper rhodopsin-1 delivery to the rhabdomeres through the regulation of Fatp (fatty acid transporter protein) mRNA, a previously described regulator of Rh1 levels [32], by RIDD [33]. Another arm of the UPR might also be involved in normal photoreceptor development, as Atf4 reporter activity has been detected in *Drosophila* photoreceptor from early pupal stages [34]. Further investigations are required to define the precise contribution of this pathway to *Drosophila* photoreceptor differentiation. In addition, the Perk/Atf4/CHOP pathway may have a role in mammalian retinas [35]. Indeed, using CHOP$^{-/-}$ mouse embryonic fibroblasts, Behrman and colleagues identified rhodopsin as a target of miR-708, a microRNA whose expression is controlled by CHOP [35]. The physiological relevance of this mechanism still remains to be validated *in vivo* in retinas where it could represent an additional mechanism to cope with the excessive load of rhodopsin and facilitate photoreceptor differentiation. In *Drosophila*, the role of Ire-1 in rhabdomere morphogenesis is independent of Xbp1 but rather involves the RIDD mechanism [33,36]. However, owing to several Xbp1–EGFP reporters, it has been shown that the activation of Xbp1 starts well before rhabdomere growth that occurs at midpupal stage, suggesting that Ire-1/Xbp1 signaling might be required at early developmental stages [33,36]. Accordingly, ER differentiation defects have been observed in Ire-1 mutant photoreceptor at early pupal stage before Rhodopsin expression and the massive secretion that builds the rhabdomere [36]. While normal photoreceptor shows ER amplification with rough morphology, Ire-1 mutant photoreceptor shows an abnormal ER proliferation with tangled tubular shape [36]. This tubular shape is unlikely to be reminiscent of accumulation of misfolded proteins [37] or of rhodopsin accumulation [38]. Remarkably, Xbp1 hypomorphic photoreceptors show normal ER morphology and expansion, evidencing an Xbp1-independent role of Ire-1 in this context [36]. The mechanism by which Ire-1 supports photoreceptor ER differentiation remains to be determined (Box 3). Altogether, one can speculate that Ire-1 activity is required to shape a dynamic ER to anticipate a peak of protein synthesis. As Ire-1 mutants do not display any defects in specification or polarity at third-instar larval stage [33], one can speculate that if Ire-1/Xbp1 participates in early steps of photoreceptor development, redundant mechanisms may exist, through other branches of the UPR, for instance. In accordance, Atf4 upregulation has been observed in Ire-1 mutants [33]. In the *Drosophila* ommatidia, Ire-1 also mediates the secretion of Spacemaker/eyes shut into the inter-rhabdomeral space (IRS) by an Xbp1-independent mechanism [33,36]. Whether the RIDD pathway also regulates formation of IRS has not been analyzed yet. In conclusion, Ire-1-dependent pathways are critical to facilitate the folding and processing of the high load of secreted proteins by the ER as the *Drosophila* receptor differentiates.

Reelin Secretory Cells in Developing Mammalian Brain

During brain development, Cajal–Retzius (CR) cells, found in the marginal zone of the cortex and in the dentate gyrus (DG) of the hippocampus, secrete reelin that diffuses and ensures correct neuronal polarization and positioning. Loss of reelin expression results in cortical layer inversion [39]. Mimura and colleagues showed that Grp78 mutant mice display an outside-in pattern of layer formation in the cerebral cortex and migration defects in cerebellum where reelin is also secreted [19]. Cultured cortical mutant neurons fail to secrete reelin but respond correctly to reelin stimuli, suggesting that the impaired secretion of reelin by CR cells rather than defective responsiveness is responsible for the cortical malformation of Grp78 mutant mice [19]. Several studies point toward a physiological role of Grp78 in reelin folding: (i) transcription of reelin is normal in mutant CR cells; (ii) reelin protein levels are decreased in mutant brains; (iii) Grp78 colocalizes with reelin; and (iv) overexpression of Grp78 greatly enhances expression of reelin protein. Grp78 mutant mice do not display migration phenotype in the hippocampus [19] suggesting that: (i) Grp78 does not facilitate reelin folding in the DG; or (ii) compensatory mechanisms overcome the loss of Grp78; or/and (iii) other ER molecular chaperones are required for proper reelin secretion. Reelin also ensures the correct positioning of several types of neurons in the developing spinal cord [40,41]. It would be interesting to test whether UPR function could be extended to most of the reelin-sensitive neurons. It is noteworthy that Grp78 mutant mice display additional phenotype compared with the reeler mice [19]. These include microcephaly and scattering of CR cells throughout the cortex. Mutant Grp78 may therefore interfere with other crucial factors for brain development. Although precise activation of UPR signaling has not been assessed in this biological context, it is conceivable that UPR is required in CR cells to enhance ER protein folding capacity to cope with specific protein demand during development.

Does Glial Differentiation Require UPR Signaling?

Besides its activity in neuronal cells, UPR may also regulate the biology of glial cells. Indeed, canonical UPR transducers Atf6, Ire-1, and Perk are expressed in glial cells in various animal or cellular models [23,42–45]. However, several studies point toward a low or mild contribution of UPR to gliogenesis: (i) ER stress inducers inhibit glial differentiation *in vitro* [22,23]; (ii) while the three UPR branches are slightly activated upon *in vitro* astrocytes differentiation, this induction is not necessary for astrogenesis [43]; and (iii) Perk is dispensable for oligodendrocyte normal development [45]. Together with many studies showing a strong induction and need of UPR signaling in pathological conditions [45–48], one can suggest that canonical UPR mainly protects glial cells from injury rather than contributing to their development. Interestingly, a noncanonical UPR pathway has been proposed to promote astrocyte differentiation [43]. OASIS is an alternative UPR inducer, which is exclusively activated in astrocytes [49,50]. Similarly to Atf6, OASIS is cleaved by S1P and S2P in response to ER stress (Box 1). OASIS fragment translocates in the nucleus to activate transcription of well-known UPR target genes such as Grp78 [50]. OASIS knockout mice exhibit impaired astrocyte differentiation with a large decrease of astrocyte number, indicating a role for a noncanonical UPR signaling in developing astrocytes [43]. At the molecular level, OASIS promotes the transcription of *gcm1* (glia cell missing 1), a critical gene for astrocyte differentiation [43]. While OASIS-dependent transcription of *gcm1* is increased upon ER stress [43], it not clear if Grp78 or other UPR target genes are activated upon astrocyte differentiation.

Concluding Remarks and Future Directions

While initially identified as a regulatory element of homeostatic processes, recent data support that the UPR functions beyond ER proteostasis. By driving some critical aspects of the development and the maintenance of the nervous system, UPR signaling must be seen as a toolbox rather than just a homeostatic regulator. Moreover, accumulating data indicate that the activation profile of UPR signaling is unique in every single cell and employs different branches and thus arrays of effectors to control specific processes such as neurite outgrowth or cell fate

Outstanding Questions

Why are some branches of the UPR selectively activated, depending on the physiological context? Is the activation of the UPR signaling during CNS development exclusively dependent on elevated ER stress? Can we envisage that other stress could trigger UPR in the developing CNS? Can UPR effectors be activated independently of ER stress in that context?

Could deregulation of UPR signaling induce microcephaly phenotype in mouse after depletion of other tRNA modification genes? Would UPR signals be elevated in those models? Would the same mechanism, that is, an impaired balance between direct and indirect neurogenesis, be involved? Could we envisage UPR effector level as biomarkers for neurodevelopmental disorders? Would selective UPR inhibitors be promising therapeutically compounds to treat such diseases?

What is the physiological function of UPR signaling in neuronal migration? To what extent and how does any alteration of this function contribute to neurodevelopmental pathologies characterized by impaired migration (lissencephaly, polymicrogyria, pachygyria)?

Could UPR signaling be as important in adult neurogenesis than it is in embryonic neurogenesis? Would the same branches of the UPR be involved?

Is any UPR effector expressed in the other type of progenitors, exclusively found in primates, the outer radial glial cells (oRGs)? What would be the function of UPR in human oRGs? What would be the consequence of altered UPR signals for human neurodevelopment?

specification. Despite lack of apparent ER stress, the UPR can be active but in most cases its upstream regulators remain to be identified (see Outstanding Questions). Thus, it is an exciting time for researchers to better understand the role and the regulation of UPR pathways that are not only at play during CNS development but that are also incriminated in neurodegenerative disorders, making them attractive targets for therapeutic development.

Acknowledgments

The authors thank Sandra Bour and the IGBMC communication service. L.N. and C.C. are, respectively, research associate and postdoctoral researcher from the FRS-FNRS (National Scientific Research Fund). J.D.G. is funded by the ATIP-Avenir joint CNRS-INSERM program, the French National Research Agency [ANR-10-LABX-0030-INRT (programme Investissements d'Avenir ANR-10-IDEX-0002-02)] and the Fondation Fyssen. L.N. is funded by FRS-FNRS, the Fonds Léon Fredericq, the Fondation Médicale Reine Elisabeth, the Fondation Simone et Pierre Clerdent, the Belgian Science Policy (IAP-VII network P7/20), and the ARC (ARC11/16-01).

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