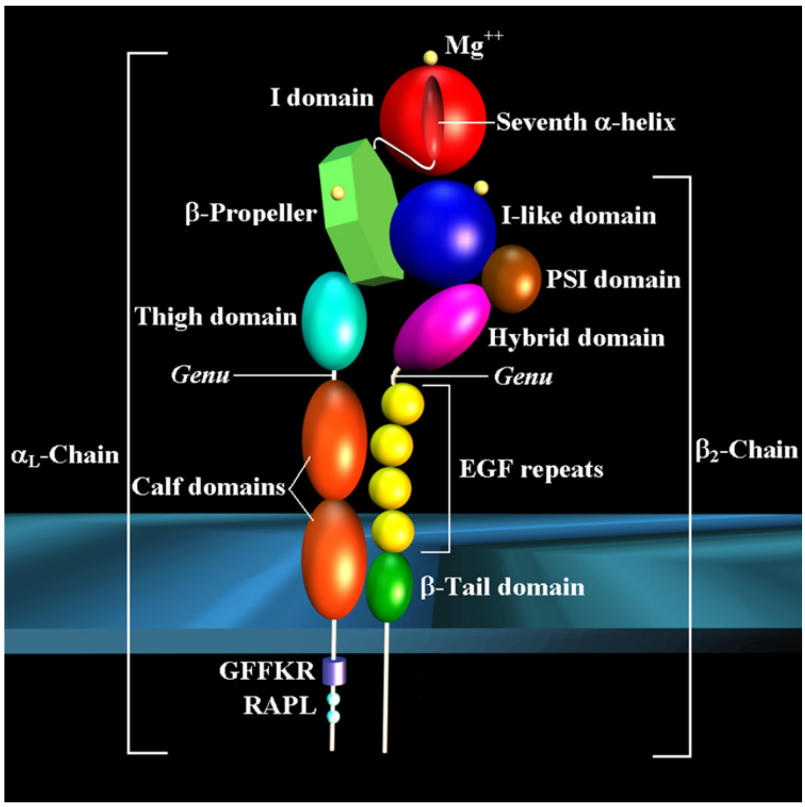

Fig. 1. Schematic representation of structural domains of LFA-1. The CD11a and CD18 subunits each possesses a large extracellular domain and short transmembrane and cytoplasmic parts. In the CD11a subunit, the ligand-binding I domain (red) binds ICAMs in a manner that depends on the presence of a magnesium ion (light yellow) in the MIDAS. The seventh α-helix of the I domain links to the β-propeller region (green), that continue by the thigh domain (turquoise) and the calf domains (orange), which extend toward the plasma membrane. In the CD18 subunit, the PSI domain is colored chocolate and is disulphide bonded to the hybrid domain (mallow) in the center of the subunit that links the I-like domain to 4 EGF-like repeats (yellow). The fourth module extends by the β-tail (green) and then toward the membrane. The hinge motif (GFFKR) and the site that mediates the formation of complexes with regulator of cell adhesion and polarization enriched in lymphoid tissues (KK) are indicated, respectively, by a light mallow cylinder and light blue spheres. Bivalent cation-binding sites are shown as small yellow spheres. The picture was made with Ulead Cool 3D 3.5 and it should be noted that the various domains are not exactly drawn to scale.

altered by changes in metal ion binding, suggesting that the cation-dependence of ligand binding is due to the fact that the metal ion is either involved in direct interaction with ligand or required to promote a favorable quaternary arrangement of the integrin [54]. Furthermore, a crystal form of the CD11b I domain with bound manganese was also reported [48] and comparison with the crystal form of the CD11b I domain with bound magnesium [49] reveals a change in metal coordination, which is linked to a large (10 Å) shift of the C-terminal helix and the burial of 2 phenylalanine residues into the hydrophobic core of the Mn^{2+} form. These structural changes, analogous to those seen in the signal-transducing G-proteins, alter the electrophilicity of the metal, reducing its ability to bind ligand-associated acidic residues, and dramatically alter the surface of the protein implicated in binding ligand. So the authors propose that the Mg^{2+} form represents the conformation of the domain in the

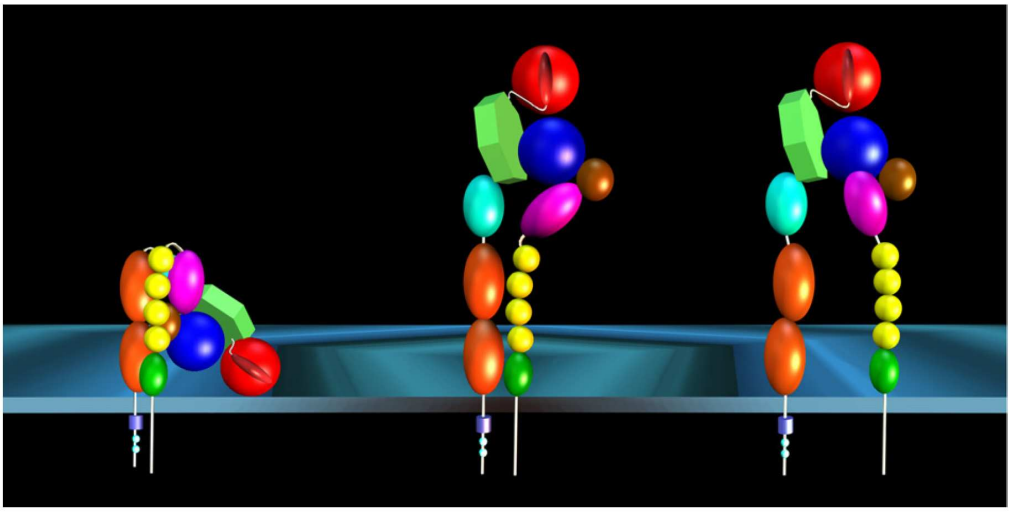

Fig. 2. Schematic representation of affinity regulation of LFA-1. Structural analyses has led to a general model in which conformational changes affecting integrin affinity occur after ligand binding or when triggered by inside-out signaling. In the low-affinity conformation (on the left), the stalk region is acutely bent at the *genu*, with the ligand-binding headpiece in close proximity to the membrane-proximal stalk region. A switch-blade–like extension of the stalk regions shifts the molecule to intermediate-affinity conformation, rendering the headpiece accessible to ligands. Separation of the stalk, transmembrane, and hinge regions are translated through the I-like domain into a downward movement of the seventh α-helix of the I domain, which induces a change in the conformation of the MIDAS that results in the high-affinity conformation of LFA-1. The intermediate- and high-affinity forms display ~500- and ~10,000-fold increase in affinity, when compared with the low-affinity state. The picture was made with Ulead Cool 3D 3.5 and it should be noted that the various domains are not exactly drawn to scale.

clustering of LFA-1 in absence of ligand and (2) binding of monomeric soluble ICAM-1 induce profound changes in LFA-1 conformation but do not alter clustering, whereas binding of ICAM-1 oligomers induces significant microclustering (Fig. 3) [135]. Clustered LFA-1 is also involved in "cross talk" with growth factor receptors through activation of mitogen-activated protein kinase signaling [136].

4.3. Inside-out signaling

The inside-out signaling regroups either affinity- or valency-based regulatory events that precede, or occur independently of, ligand binding, and serve to enhance the propensity to bind ligand efficiently [116,131]. The inside-out signal can be produced via activation of other cell surface molecules such as T-cell receptor and CD2, which generate intracellular second messengers that ultimately modulate integrin adhesiveness through affinity modulation (in which ligand-binding affinity is altered) and avidity modulation (in which integrin cell surface diffusion and clustering are modified) [72,116,137–139]. This rapid up-regulation of integrin adhesiveness enables circulating leukocytes to interact avidly with the endothelium and antigen-presenting cells [140,141]. Separation of the integrin α and β subunit transmembrane and cytoplasmic domains has emerged as the critical trigger for initiation of inside-out conformational signaling (Fig. 2) [116]. As it is key to integrin function, there

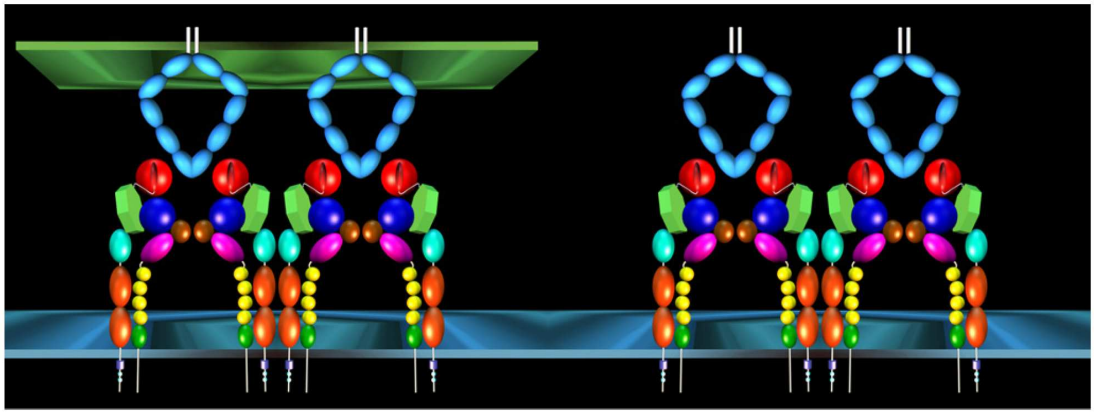

Fig. 3. Schematic representation of substrate-induced valency regulation of LFA-1. Clustering of integrins on the cell surface is induced by binding of membrane-bound (on the left) or soluble (on the right) multimeric ICAM-1 complexes. The picture was made with Ulead Cool 3D 3.5 and it should be noted that the various domains are not exactly drawn to scale.

has been an intense effort to elucidate its molecular mechanism, leading to identification of cytoplasmic partners and characterization of signal transduction pathways [142–149].

4.4. Internalization

The spontaneous internalization of ectopically expressed LFA-1 in CHO cells is a very rapid process, considering that >30% of surface-expressed receptors are internalized in 30–40 min, with a half-time of internalization of 10–15 min. However, the rate of internalization of endogenously expressed integrins may differ widely depending on the cell type and individual members of the family as broad variations in the efficiency of internalization of LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) have been observed, which suggest that α subunits may play a fundamental role in the endocytic cycle of these receptors [117]. LFA-1 in particular was shown to have a relatively slow turnover in lymphoid cell lines compared with the other members of the leukocyte integrin family. Conceivably, integrin dynamics at the cell surface may be subjected to variations depending on the activation state of the cell and the phenotype, that is, nonadherent, statically adherent, or locomoting, of the cell being studied [91].

4.5. Cytoskeletal association

The active form of LFA-1 regulates its own function on primary human T-cells by directing the remodeling of the F-actin cytoskeleton to strengthen T-cell adhesion to ICAM-1 as confocal microscopy revealed that both F-actin bundling and overall levels of F-actin are increased in the ICAM-1–adhering T-cells [134].

The complete deletion and truncations or point mutations of the CD18 cytoplasmic region (mutants Δ731, Δ744, Y735A, F754A, and T758TT/AAA) has also a tendency to increase basal levels of adhesion, whereas any increase in ligand-binding affinity was detected [65,150]. This increase in basal adhesion may in fact reflect a release of cytoskeletal linkage