Review

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How calcium makes endocytic receptors attractive

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Nutrients, biological waste-products, toxins, pathogens, and other ligands for endocytosis are typically captured by multidomain receptors with multiligand specificity. Upon internalization, the receptor–ligand complex segregates, followed by lysosomal degradation of the ligand and recycling of the receptor. Endosomal acidification and calcium efflux lead to the essential ligand–receptor affinity switch and separation. Recent data, including crystal structures of receptor–ligand complexes, now reveal how calcium, in different types of domain scaffolds, functions in a common way as a removable 'lynchpin' that stabilizes favorable positioning of ligand-attractive receptor residues. In addition to explaining how calcium depletion can cause ligand–receptor dissociation, the new data add further insight into how acidification contributes to dissociation through structural changes that affect the receptor calcium sites.

Receptor-mediated endocytosis

The cellular uptake of protein complexes by receptor-mediated endocytosis is an efficient and selective process for delivery of nutrients (e.g., vitamins and cholesterol), endogenous biological waste-products (e.g., enzyme–inhibitor complexes), and endogenous substances such as toxins and pathogens to the endolysosomal pathway. Classical receptor-mediated endocytosis, as described in the canonical studies of the uptake of cholesterol and low-density lipoprotein (LDL) particles by Brown and Goldstein [1], is characterized by ligand binding to receptors clustered in clathrin-coated pits of the membrane (Figure 1). These regions invaginate, leading to formation of intracellular clathrin-coated vesicles entrapping the ligand–receptor complex. The clathrin coating is subsequently lost, and the vesicles fuse with early endosomes in which the ligand and receptors are separated. The ligands are sorted for vesicular transport to the lysosomes whereas the receptors can be recycled to the plasma membrane. Extensive studies have characterized the function of adaptor proteins and other important molecular processes in the cytosol and on the cytosolic side of the membranes controlling this internalization and vesicular traffic [2]. Usually, the entire ligand ends up in the lysosome for degradation. However, receptor-mediated uptake of iron-bound transferrin (holotransferrin) is an exception. In this case only iron is released in the endosome, and the transferrin–receptor complex recycles back to the surface where apotransferrin is released [3]. The endocytic receptors, the focus of the present review, primarily serve a constitutive transport function that contrasts with ligand-induced signaling by signaling receptors such as hormone and cytokine/chemokine receptors. It should be added that these two classes of receptors do share overlapping functionality in the sense that some endocytic receptors mediate signaling and some signaling receptors can be internalized.

The pinocytosed fluid in the endosomes undergoes dramatic changes in ion composition owing to the activity of ion transporters in the endosomal membrane. Most significant are the increase in proton concentration and the decrease in calcium concentration. The increasing acidification along the endolysosomal pathway and the outgoing flux of the calcium in the early endosome are important for diverse processes such as vesicular enzyme activity, signaling, and vesicular trafficking and fusion events [4]. Furthermore, the weak acidification of the early endosome and the decrease in calcium concentration are important for ligand–receptor segregation, an essential event preceding receptor recycling and transport of the ligand into the catabolic lysosomal pathway. Several structural and functional studies of receptor–ligand complexes have now provided new insight into how endosomal changes in calcium concentrations can, in a common way, regulate specialized structural receptor elements that function as delicate on/off switches for ligand–receptor binding.

The modular domains of ligand-binding regions

The extracellular regions of the endocytic receptors typically consist of several extracellular domains arranged in tandem (Figure 2A). Trimeric scavenger receptor AI (SR-AI) and macrophage receptor with collagenous structure (MARCO), as well as the asiologlycoprotein receptor, are exceptions of the tandem arrangement. In these receptors, a single domain is placed on top of each of three collagen-like chains that associate into a trimeric receptor complex in which the ligand-binding domains are clustered at the membrane-distal end (Figure 2A).

The receptor domains (in the literature and in the domain nomenclature these are also designated as 'modules' and 'repeats') serve important functions in the

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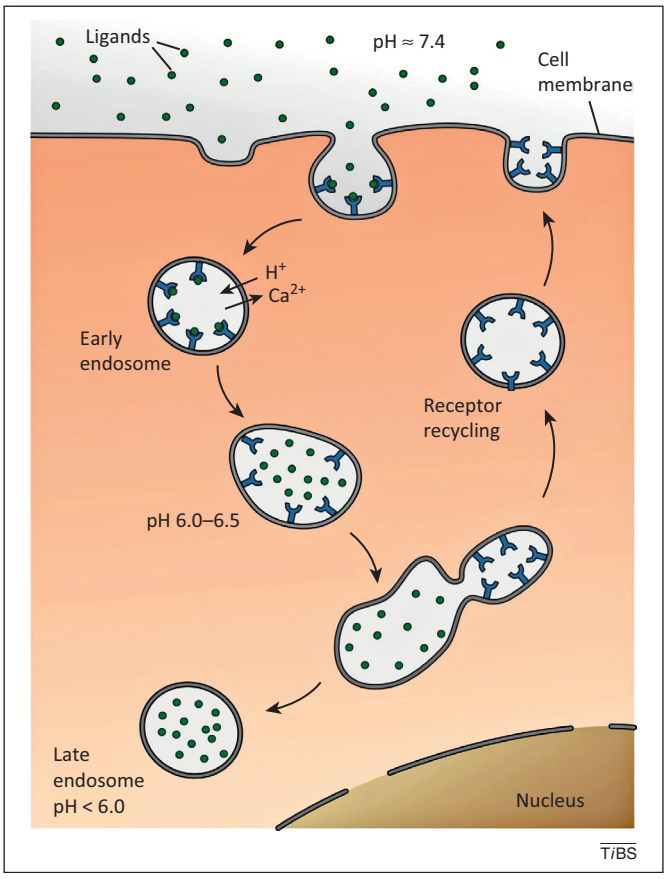

Figure 1. A simplified model of receptor-mediated endocytosis. Ligands are recognized by receptors on the extracellular side of the cell membrane and are subsequently internalized via a clathrin-dependent pathway. Upon calcium efflux and proton influx in endosomes, ligands are released from their receptors. The receptors cluster in segments of the endosomes, pinch off and recycle to membrane. The endosomal content containing the ligand fuses with late endosomes/lysosomes leading to further acidification and ligand digestion by lysosomal enzymes.

structural integrity of the receptors as well as in internal and external interactions. Some receptors have only a few domains, whereas others have multiple domains. The largest single-chain receptor, megalin/LDL receptor (LDLR)-related protein (LRP2), has several clusters that each contains dozens of domains. These multidomain proteins are also often referred to as mosaic proteins because multiple types of domains are used as building blocks for the extracellular part of the receptors. The exact role of each specific domain is for most proteins not entirely defined, but it seems clear that only a few domains in a multidomain receptor make direct contact with the ligands. The identified ligand-binding domain types are complement-type repeats (CR), CUB domains (for complement C1r/C1s, Uegf, Bmp1), C-type lectin-like domains, scavenger receptor cysteine-rich repeats (SRCR), and epidermal growth factor-like (EGF-like) domains (Figure 2B) (see references in the individual domain-type sections). These domains have little structural similarity except that they are rather small compact domains that in many cases harbor a calcium-binding site where two or three acidic aspartate and/or glutamate residues together with the backbone carbonyl groups coordinate the calcium ion.

The CR domain

The CR domain [alias the LDLR type A repeat (LA)] constitutes the ligand-binding part of the LDLR family, where it is often present in multiple cassettes of 7–10 CR domains separated by β-propeller domains and EGF-like domains [5] The CR domains are largely confined to endocytic receptors in the LDLR family, but they also appear in some other non-receptor proteins such as complement factor C9 and complement factor I, hence the designation of this domain as 'complement-type'. The CR domain has a fold consisting of approximately 40 amino acids which is stabilized by three disulfide bridges (Figure 2B) [6]. A β-hairpin motif forms the N-terminal part of the domain and a highly conserved calcium-binding site is present in its C-terminal part.

The interaction between receptor-associated protein (RAP) and the LDLR is expected to resemble receptor–ligand interactions. Hence, the structure of the CR domains 3 and 4 of LDLR in complex with RAP initially revealed the role of calcium in receptor–ligand interaction (Figure 3A) [7]. RAP is an endoplasmic reticulum protein that binds to many of the CR domains in the LDLR family receptors. It is suggested to function as a type of chaperone protecting these receptors against binding of other ligands in the endoplasmic reticulum and Golgi [8]. In each of the RAP-binding CR domains, a calcium ion coordinates two aspartate residues (Asp108/Asp112 and Asp147/Asp151), which engage in electrostatic interactions with positively charged lysine residues (Lys256 and Lys 270) of RAP. Moreover, structures of the CR domains of LRP8 [9] and the VLDLR [10] in complex with their respective ligands reelin (Figure 3B) and human rhinovirus Vp1 (Figure 3C) have demonstrated nearly identical interactions, with the one exception being that the lysine residue from Vp1 only interacts with one calcium-coordinated aspartate residue (D139) and an adjacent glutamate residue (Glu137). The residues coordinating the calcium ion are conserved in almost all CR domains. This supports the finding that calcium binding also serves a function in maintaining the structural integrity of the single small CR domains [11].

The CUB domain

Compared to the CR domain, the CUB domain is a less abundant and much larger structure composed of approximately 110 amino acids that fold into a compact β-sandwich structure (Figure 2B) [12]. Two disulfide bridges flank the domain and stabilize its structure. A calcium-binding site is often located between two loops at one end of the β-sandwich. The acidic residues forming the calcium-binding site are not conserved in all CUB domains [13], indicating that only a subset of CUB domains may be involved in calcium-dependent ligand binding. Cubilin is a large (460 kDa) CUB domain-containing receptor subunit linked to the membrane via the integral membrane protein amnionless [14]. The receptor is responsible for intestinal uptake of the intrinsic factor/vitamin B_{12} complex and for renal uptake of a range of filtered proteins [15]. The calcium-binding CUB domains 5–8 are responsible for binding of intrinsic factor/vitamin B_{12} [16]. The binding-sites of other ligands are not yet fully defined.