Clathrin-associated adaptor protein complexes

Hiroshi Ohno

Laboratory for Epithelial Immunobiology, Research Center for Allergy and Immunology (RCAI), RIKEN, and International Graduate School of Arts and Sciences, Yokohama City University, Yokohama, Japan

Author for correspondence
(e-mail: ohno@rcai.riken.jp)

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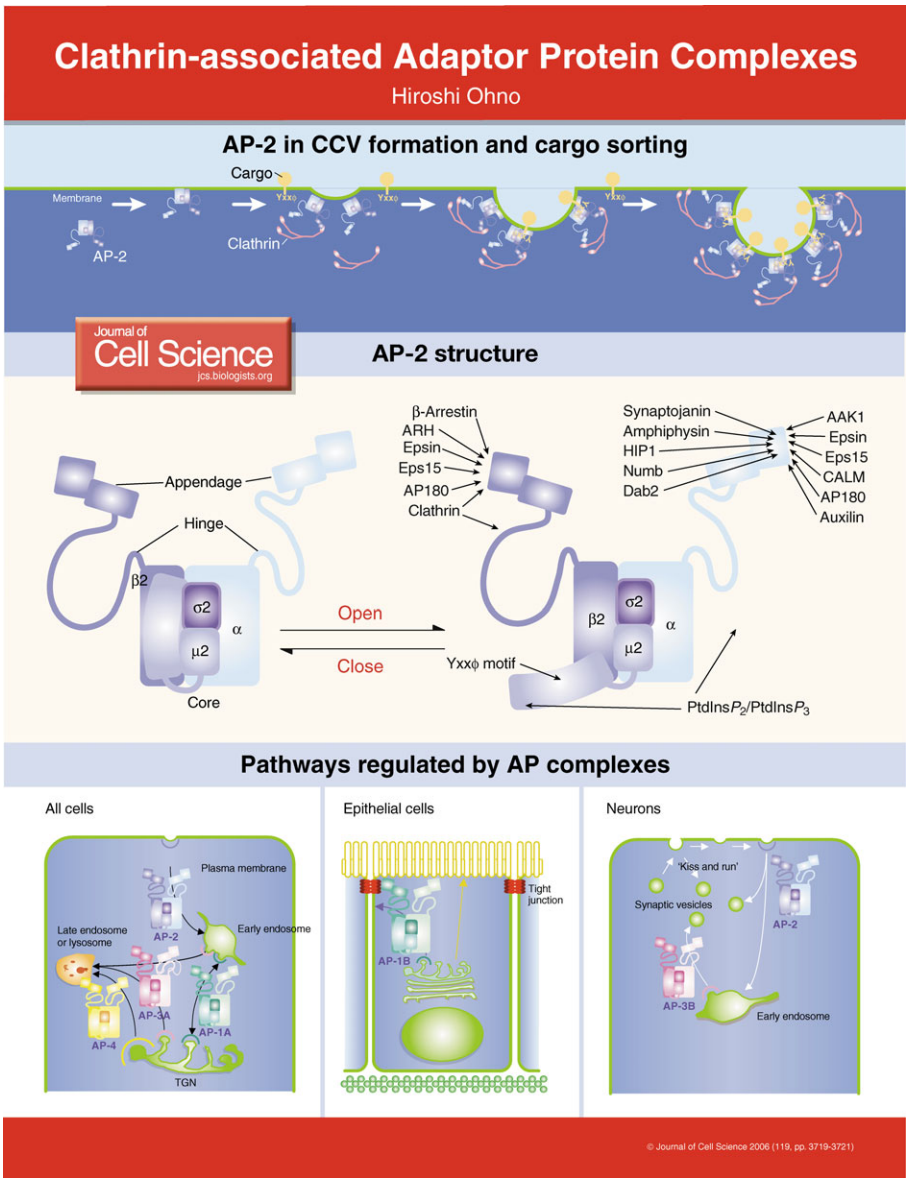
Membrane traffic among organelles of the secretory and endocytic pathways is mediated by small, transport vesicles that are classified according to the protein coat used in their formation and the cargo they contain (Bonifacino and Glick, 2004; Bonifacino and Lippincott-Schwartz, 2003). Clathrin-coated vesicles (CCVs) are involved in the transport between organelles, such as the trans-Golgi network (TGN), the endosome, the lysosome and the plasma membrane (hereafter referred to as the 'post-Golgi network') (Nakatsu and Ohno, 2003; Robinson, 2004). Clathrin, a large complex composed of three heavy and three light chains, self-assembles to form a basket-like 'clathrin lattice' made-up of pentagons and hexagons (Kirchhausen, 2000; Wilbur et al., 2005). The lattice serves as a scaffold but cannot directly bind to membranes. Binding is mediated by clathrin adaptors that can bind directly to both clathrin and the lipid and/or protein components of membranes (Kirchhausen, 2000; Owen et al., 2004). Clathrin-associated adaptor protein (AP) complexes are main clathrin adaptors contributing to the formation of CCVs (Kirchhausen, 2000; Nakatsu and Ohno, 2003; Owen et al., 2004; Robinson, 2004).



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Structural basis of the function of the AP-complex family

The AP-complex family (Boehm and Bonifacino, 2001; Nakatsu and Ohno, 2003; Owen et al., 2004; Robinson, 2004) has six members in mammals. AP-1A, AP-2, AP-3A and AP-4 are ubiquitously expressed. The other two members, AP-5 and AP-6, are cell-type-specific isoforms of AP-1A and AP-3A: the epithelium-specific AP-1B and the neuron-restricted AP-3B.

The AP complexes consist of four subunits: one small (σ1-σ4), one medium (μ1-μ4) and two large (α, γ, δ or ϵ; and β1-β4) subunits. These assemble to form a structure in which two appendage domains are connected by flexible hinge regions to the core (Owen et al., 2004; Owen and Luzio, 2000; Robinson, 2004). The large subunits are divided into three domains: the N-terminal domain, which makes up the core with the μ and σ subunits; the hinge domain, and the C-terminal appendage. One of the large subunits (α, γ, δ or ϵ) is implicated in binding to the target membrane (Collins et al., 2002; Nakatsu and Ohno, 2003; Owen et al., 2004; Traub, 2005). AP-2α binds to phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] and/or phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3] lipids enriched in the plasma membrane. Similarly, AP-1A is proposed to bind to Golgi-localized phosphatidylinositol (4)-monophosphate [PtdIns(4)P]. The recruitment of AP-1A, AP-3A and AP-4 is also believed to involve direct interaction with the GTP-bound form of the GTPase Arf1.

The other large subunit (β1-β3) recruits clathrin through a clathrin-binding sequence termed the clathrin box. This has the consensus sequence of LϕxϕD/E, (where ϕ is a bulky hydrophobic residue) and lies in the hinge region (Owen et al., 2004).

Although β4 lacks the clathrin box, one morphological study has suggested that AP-4 can also interact with clathrin (Barois and Bakke, 2005). The appendages interact with various clathrin adaptor and/or accessory proteins (Owen et al., 2004; Owen and Luzio, 2000; Traub, 2005). The β2-appendage also provides an additional clathrin-binding site.

The μ subunits consist of two domains. The N-terminal third of the polypeptide is part of the core. The remaining C-terminal two-thirds dictate cargo selection by directly recognizing the Yxxϕ motif, one of the most common sorting signals present in the cytosolic domains of transmembrane proteins. This recognition can thus mediate the efficient concentration of these proteins in forming CCVs (Ohno et al., 1995). The regulatory mechanisms for Yxxϕ-motif recognition have been characterized in the case of AP-2 μ2 (Nakatsu and Ohno, 2003; Owen et al., 2004; Traub, 2005). In the cytosol, the μ2 C-terminal domain is thought to interact with the core, which keeps the Yxxϕ-binding site at the μ2-β2 interface. A threonine residue is present in the short linker sequence between the N- and C-terminal domains of μ2, and its phosphorylation by AAK1, an Ark family kinase, probably induces a conformational change that exposes the Yxxϕ-binding site. The C-terminal domain also has a PtdIns(4,5)P_2-binding site that probably approaches the plasma membrane when this conformational change occurs, and the interaction of the μ2 C-terminal domain with PtdIns(4,5)P_2 in the plasma membrane may keep the Yxxϕ-binding site open. The kinase activity of AAK1, an α-appendage-binding protein, is activated by clathrin.

The σ subunits are also part of the core and are thought to be involved in the stabilization of the complex (Collins et al., 2002). The N-terminal domain of the μ subunits shows a certain degree of sequence similarity with the σ subunits (Boehm and Bonifacino, 2001), consistent with the notion that it also stabilizes the complex. Another commonly observed sorting signal, the [DE]xxxL[LI]-type di-leucine motif, interacts with the core, although there are still some arguments over the precise binding site(s) (Owen et al., 2004; Traub, 2005).

Post-Golgi and endocytic transport regulated by AP complexes

Each AP complex is involved in distinct transport pathways in the post-Golgi and/or endocytic network. AP-2 mediates the formation of CCVs from the plasma membrane for endocytosis, which are destined for fusion with early endosomes (Owen et al., 2004; Traub, 2005). AP-2 also serves as a cargo receptor to selectively sort transmembrane proteins, such as transferrin receptors, in forming CCVs (Ohno et al., 1995).

AP-1A, in conjunction with GGA proteins, regulates vesicular transport of cargos, such as mannose 6-phosphate receptors, between the TGN and endosomes, although the direction of transport is still unclear (Owen et al., 2004; Traub, 2005). AP-1B is involved in polarized sorting of cargo molecules to the basolateral plasma membrane in epithelial cells (Folsch et al., 1999; Nakatsu and Ohno, 2003).

AP-3A is believed to traffic cargo from TGN and/or early endosomes to late endosomes or multivesicular bodies (MVBs), and/or lysosomes and lysosome-related organelles (Nakatsu and Ohno, 2003; Owen et al., 2004). Studies of the neuroendocrine cell line PC12 have indicated that AP-3B is involved in the biogenesis of synaptic vesicles from endosomes (Faundez et al., 1998; Nakatsu and Ohno, 2003). Indeed, AP-3B is preferentially concentrated in neuronal processes in primary cultures of neurons (Seong et al., 2005).

AP-4 mediates the transport of certain lysosomal proteins from the TGN to lysosomes and might be involved in the basolateral transport of low-density lipoprotein receptor (LDLR) in polarized epithelial cells (Nakatsu and Ohno, 2003).

AP complexes in multicellular organisms

Two different AP-1A-deficient mice have been reported: mice lacking the γ subunit (Zizioli et al., 1999) and mice lacking the μ1A subunit (Meyer et al., 2000). The γ-knockout mice die as early as embryonic day 3.5 (E3.5), the blastocyst stage, whereas μ1A-knockout embryos survive until E13.5. Although no direct evidence is available, it seems that the μ1B isoform can compensate, at least in part, for the absence of μ1A in the early stages of development, explaining the difference in timing of death observed between the two knockout genotypes (Ohno, 2006). AP-1A might thus be essential for viability of individual cells. Alternatively, it might be required for a more complicated function in multicellular systems, such as the development of embryos beyond the blastocyst stage or nidation.

Studies of cultured cells using dominant-negative and RNAi approaches have shown that AP-2 is required for rapid internalization but not for cell viability, although small amounts of residual AP-2 in those experiments could have been sufficient to sustain cell viability (Ohno, 2006). Indeed, we have found that μ2-deficient embryos die before E3.5, suggesting that the AP-2 complex is indispensable for cell viability (Mitsunari et al., 2005).

The Hermansky-Pudlak syndrome (HPS) consists of a group of genetically different autosomal recessive disorders that share oculocutaneous albinism, platelet storage pool deficiency, and some degree of ceroid lipofuscinosis (Huizing et al., 2000; Huizing et al., 2002), because the function and/or biogenesis of lysosomes and lysosome-related organelles such as melanosomes and platelet dense granules are impaired (Di Pietro and Dell'Angelica, 2005). One of the HPS-causing mutations affects the *AP3B1* gene, which encodes the β3A subunit of the AP-3A complex (Dell'Angelica et al., 1999). Of the 16 mutant models for HPS, mutations that produce *pearl* and *mocha* mice have been identified in the genes encoding the β3A and δ subunits, respectively, of the AP-3A complex (Li et al., 2004). β3A-null mice generated by gene targeting also showed a similar phenotype to that of *pearl* mice (Li et al., 2004; Ohno, 2006).

The AP-3 δ subunit is shared by the ubiquitous AP-3A and neuron-restricted AP-3B. *mocha* mice lacking both AP-3A and AP-3B additionally suffer from neurological abnormalities (Li et al., 2004; Ohno, 2006). These may be due to the absence of AP-3B. In fact, mice lacking μ3B, a neuron-specific subunit

of AP-3B, exhibit spontaneous epileptic seizures (Nakatsu et al., 2004). Subsequent analyses have revealed that AP-3B plays a crucial role in the formation and function of a subset of synaptic vesicles, which probably explains the impairment of inhibitory GABAergic neurons observed in these mice. This, in turn, could cause an imbalance in excitatory and inhibitory neuronal activities, ultimately leading to recurrent epileptic seizures.

Perspectives

Despite major advances in recent years, many important issues remain to be resolved regarding the AP complexes: the mode of recognition of [DE]xxxL[LI] motifs, the precise transport pathways regulated by AP-1A and AP-3A, and the ambiguous role of AP-4 not only at the physiological level but also at the molecular level. Further studies are needed to help us better understand the roles of these complexes in vesicular traffic and their importance in developmental biology and medicine.

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