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Clathrin Adaptors Really Adapt

Minireview

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Summary

The clathrin pathway is the principal route for receptor-mediated endocytosis and growth factor downregulation. Heterotetrameric clathrin adaptors directly link the clathrin coat with cargo transmembrane proteins that are sorted into coated pits and vesicles. A paper in this issue of *Cell* (Collins et al., 2002) describes the atomic structure of the adaptor-protein 2 (AP-2) core, the portion that makes contacts with the membrane and cytosolic tails of cargo proteins.

Membrane traffic is the dynamic process responsible for biogenesis and organization of membrane-bound organelles and for communication among them. Traffic typically requires controlled formation of vesicles and vesiculo-tubular structures from a donor membrane, directed movement of these vesicles to a target, and fusion to the acceptor membrane. The clathrin-dependent traffic pathway was the first to be recognized and studied. A series of contributions from many laboratories has led to the current picture of how a clathrin coat forms, how sorting of trafficking transmembrane cargo proteins by adaptors is achieved, and how many other different proteins are involved in the regulation of the budding, uncoating, trafficking, and fusion steps (reviewed in Kirchhausen, 2000).

Some History

Barbara Pearse discovered clathrin nearly 25 years ago (Pearse, 1976). By far the most abundant protein in coated vesicles, it was proposed to be the scaffold that makes the coat and to be the direct recognition structure that sorts cargo transmembrane proteins into the vesicles. With purified clathrin in hand, it was quickly realized that the scaffold concept was correct, as it self assembled in vitro into cages of similar size as coated vesicles and with the same honeycomb-like latticework making up the coat. The conditions of assembly were far from physiological, however, which prompted investigators to look for additional factors that might facilitate coat formation. This search led to the discovery of what at the time was called the clathrin assembly factor, a heterotetrameric protein complex found in coated vesicles, with the capacity to bind clathrin and facilitate its in vitro assembly into coats (Keen et al., 1979). Soon thereafter, it was realized that there were at least two classes of complexes colocalizing with clathrin in cells—one predominantly at the trans-Golgi network (called AP-1) and the other at the plasma membrane (AP-2). The specificity of these intracellular localizations was unexpected. This observation, together with the realization that different cargo proteins concentrated in clathrin-coated vesicles derived from the trans-Golgi network or the plasma membrane, led to the suggestion that AP complexes, rather than clathrin, were responsible for cargo sorting. Indeed, it turns out that these complexes interact with short peptide sequences found in the cytosolic tails of cargo transmembrane proteins. These sequences are required to direct the traffic of the cargo proteins into clathrin pathways. Because of their key role in linking sorted proteins with the clathrin coat, the assembly factors are now referred to as heterotetrameric clathrin adaptors.

Considerable progress has occurred during the past five years. Novel adaptors have been identified, such as the heterotetrameric AP-3 and AP-4 adaptor protein complexes (which resemble AP-1 and AP-2), the monomeric GGAs (Golgi-localized, γ-ear-containing, ARF-binding proteins) and members of the β-arrestin family (reviewed in Boehm and Bonifacino, 2001). Although the functional relationships among these disparate components remain obscure in many cases, high resolution structures of parts of clathrin, adaptors and ancillary proteins, and lower resolution images of assembled coats, determined by electron cryomicroscopy (Musacchio et al., 1999), now provide the information necessary to integrate various lines of evidence and to design experiments that test specific mechanistic notions. In this issue of *Cell*, Collins and coworkers (2002) add the endocytic AP-2 core, the portion of AP-2 that sorts cargo proteins and makes contact with the membrane, to the list of known atomic structures, thus providing us with a new structural tool to understand how adaptors work.

Recognition of Sorting Signals

Genetic and cell biological experiments have clearly established that intracellular traffic of many membrane-bound proteins requires sequences facing the cytosol. In many instances, the sorting information is encoded in short peptide motifs, typically 4–6 amino acids, referred to as "sorting signals." These motifs determine which vesicular traffic pathway is used to transport a particular molecule, and hence determine its final destination. It took a while, however, to establish firmly a direct association between tetrameric adaptors and cargo proteins. An important step was the demonstration, by yeast two-hybrid experiments, that the C-terminal domain of μ2-adaptin of AP-2 recognizes the tyrosine-based endocytic sorting signal of the form YppØ (where Y denotes tyrosine, p tends to be a polar or positively charged residue, and Ø is an amino acid with a bulky hydrophobic side group) (Ohno et al., 1995). More recently, the molecular basis for this sorting signal recognition has been revealed by X-ray crystallography (Owen and Evans, 1998). The structure shows that the YppØ motif contacts an unpartnered β strand in μ2-adaptin. μ1, μ3, and μ4, the related subunits in AP-1, AP-3, and AP-4 recognize related YxxØ motifs (where x denotes a non-positively charged amino acid), and they do so with specificities that correspond to the in vivo sorting pattern of cargo proteins mediated by these

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adaptors, presumably with a mechanism of recognition similar to that of μ2.

A second example of an extended peptide to protein-surface interaction used for recognition of sorting signals is provided by the recent X-ray structures of the N-terminal VHS domains of the monomeric GGA1 and GGA3 adaptors bound to peptides containing the acidic-cluster-dileucine sorting motif DxxLLxx from the mannose 6-phosphate receptors (Misra et al., 2002; Shiba et al., 2002). The structures show that the VHS domain is composed of a right-handed super helix of eight α helices, with the peptides bound in an extended conformation along a groove between helices 6 and 8. The dileucine motif of the form (−)xxxLL and the FDNPVY motif are two other sorting signals recognized by APs, but the structural explanations remain to be determined (Kirchhausen, 2000).

Molecular Anatomy of Adaptors

The heterotetrameric adaptors contain a pair of large chains (α and β1 in AP-1, α and β2 in AP-2, δ and β3 in AP-3, and ε and β4 in AP-4), a medium chain and a small chain (μ1-4 and σ1-4 in AP1-4, respectively). Because of the extensive similarities among the four heterotetrameric AP complexes, and because AP-2 is most abundant, most structural studies have been done with AP-2. Electron microscopic images of rotary shadowed AP-2 adaptors showed two approximately 30 Å globular appendages or "ears" flanking a 90 × 70 × 70 Å globular core or "head." The ears correspond to the C-terminal domains of α and β, and the core contains the complete μ2 and σ2 subunits in tight association with the N-terminal domains of α and β. The stalk or hinge that joins the head to the ears is thought to be flexible, because the disposition of the ears with respect to the head can vary significantly in different images of AP-2. The clathrin box, a short sequence motif of the form LØ[D/E]Ø[D/E], is found in a number of proteins that interact with clathrin. This motif binds in the groove between two blades of the β-propeller N-terminal domain of clathrin (Ter Haar et al., 2000). Clathrin-box motifs are found in the β-hinge, and they are clearly required for the functional association of clathrin with AP-1 and AP-2.

The ears of the adaptors act as platforms to provide sites of interaction with such proteins as amphiphysin, AP180, auxilin, epsin, Eps15, AAK, γ-synergin, and synaptojanin, all involved in the regulation of vesicle formation and disassembly, and/or cargo sorting. In many cases, these proteins contain short DPØ sequence motifs (where Ø is F or W) recognized by the ears through relatively weak peptide/protein interactions. Atomic structures of the α and β2 ear domains of AP-2 have been determined (referenced in Collins et al., 2002 [this issue of *Cell*]). The ears of α and β2 have similar two-domain structures, with a noticeable interdomain cleft.

The heart of the matter lies in the core. It is here that a number of coordinated events permit regulated recruitment of the adaptor to a target membrane and modulated recognition of sorting signals. The work of Collins et al. reveals the molecular organization of these subunits and provides clues for some aspects of the regulation.

The Atomic Structure of the AP-2 Core

The core is a highly compact structure (Figure 1); the N-terminal domains of α and β are composed of 29 and

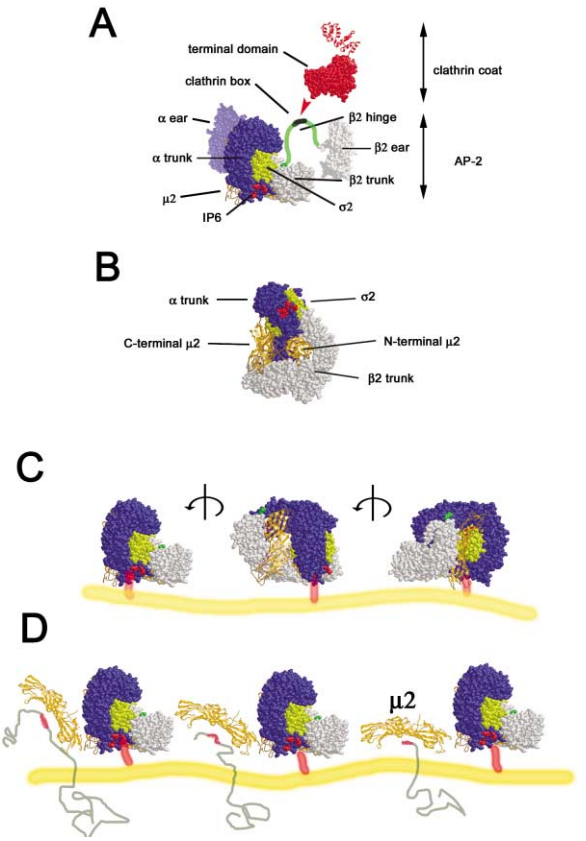

Figure 1. Coat Formation and Cargo Recognition

(A) Cross-section of a clathrin-coated vesicle showing the major interactions involved in cargo sorting. The atomic structures correspond to the N-terminal domain of clathrin (red) making contact with the clathrin box (black) at the flexible β-hinge (green) between the C-terminal ear and N-terminal trunk (gray) of β-adaptin. The AP-core contains σ2 (yellow), μ2 (orange), and the N-terminal head of α-adaptin (dark blue). The α-ear is depicted in the back of the core (light blue). The positions of ears and hinges are not known with certainty.

(B) Bottom view of the AP-2 core seen from the plane of the membrane.

(C) Several side views of the core in the inactive or closed conformation, rotated with respect to an axis perpendicular to the membrane (yellow). The orientation of the core is as proposed by Collins et al. (2002); it locates the binding site in α-adaptin (red) for the phosphate groups of the membrane-bound phosphoinositides (light red) on a position close to the inner leaflet of the membrane (yellow).

(D) The YppØ sorting motif (red) within different locations in the cytosolic tail of transmembrane cargo proteins (gray) can make contact with the C-terminal domain of μ2; depicted are possible different active or open conformations, from partial (left) to total (right) extension. When totally extended, a region of μ2 would also interact with a second membrane-bound phosphoinositide.

28 α helices, respectively, of 4–6 turns each, arranged like the armadillo repeats found in β-catenin, and in the ENTH and VHS domains of some proteins involved in membrane traffic. These domains lie parallel to each other and define the pocket where μ2 and σ2 lie; both superhelices contain a sharp bend or elbow such that the N-terminal domains of α and β2 hook around σ2 and the N-terminal domain of μ2, respectively (Figures 1A and 1B). It was known that σ2 and the N-terminal domain of μ2 have significant sequence identity, and indeed,

the structure clearly shows that these globular regions are almost identical to each other. The C-terminal region of μ2 (containing the binding site for the YppØ sorting motif) is a platform-shaped, all-β domain, whose atomic structure has been determined earlier (Owen and Evans, 1998). In the core, its structure is maintained, and the domain lies on one face of the complex, in a hollow groove between α and β (Figure 1C). A linker between the N- and C-terminal domains of μ2 is exposed to solvent and is poorly visible.

Adaptor Regulation

When AP-2 associates with clathrin to form a coat, it undergoes a conformational change. The change is transmitted to the core, evidenced by a dramatic increase in accessibility of the link between the N- and C-terminal domains of μ2, as detected by proteolytic cleavage. Moreover, the ability of μ2 in AP-2 to recognize YppØ sorting signals is increased by the co-assembly of AP-2 with clathrin into coats, establishing a link between cargo selection and coat formation (Rapoport et al., 1997). Similarly, the interaction of AP-2 with PI3P and PI(3,4)P2 phosphoinositides phosphorylated at the D-3 position in the inositol ring, results in an equivalent increase in the recognition of the YppØ motif (Rapoport et al., 1997). These experiments clearly showed a transition in the APs, from an inactive to an active state. More recently, it was discovered that association of AP-2 with peptides containing the YQRL endocytic motif sequence also switches AP-2s into an active state, resulting in an increased binding capacity for synaptotagmin through a contact whose characteristics are not well understood (Haucke and De Camilli, 1999). Phosphorylation is yet another way to regulate the function of APs. Serine/threonine phosphorylation in the hinge region, between the C-terminal ear and N-terminal domains of the β subunits, affects AP-2's ability to interact with clathrin and to associate with membranes (Wilde and Brodsky, 1996). T156 in μ2 is another important target of phosphorylation. It is specifically modified by the recently discovered AAK1 kinase, which copurifies with clathrin-coated vesicles (Conner and Schmid, 2002; Ricotta et al., 2002). Upon phosphorylation of μ2 T156, the binding affinity for the YppØ motif increases.

AP-2 also binds to PI(3,4,5)P3 and inositolhexakisphosphate (IP6) through an interaction that was mapped by mutagenesis to a site at the N terminus of α-adaptin (Gaidarov and Keen, 1999). PI(3,4,5)P3 are phosphoinositides found in the inner leaflet of the plasma membrane and endosomes, two membranes with which AP-2 associates. It is thought that interaction of AP-2 with this lipid provides an anchor for membrane attachment. Expression of mutated α-adaptin unable to bind PI(3,4,5)P3 interferes with proper targeting of AP-2 to the plasma membrane and interferes with endocytosis. Knockout mice lacking synaptojanin (a phosphatase that converts PI(3,4,5)P3 into PI(3,4)P2) exhibit a partial accumulation of coated vesicles, suggesting that the release of AP-2 from the vesicle is retarded (Cremona et al., 1999).

What Does the Structure Reveal?

The structure of the AP-2 core determined by Collins and coworkers includes IP6. Not surprisingly, it binds to the site in α-adaptin predicted by mutagenesis; its location imposes important constraints for the possible orientations of AP-2 with respect to the plane of the membrane, most likely with the μ2 C-terminal domain placed perpendicular to the membrane (Figure 1D). A second binding site for IP6 is found in the C-terminal domain of μ2, although at present it is not clear whether this represents a physiologically relevant interaction or alternatively, whether it simply results from the way AP-2 cores pack in the crystal lattice. Also satisfying is the observation that the link containing T156 between the N- and C-terminal domains of μ2 is exposed to solvent. Unexpected, however, is the partial occlusion by β-adaptin of the adjacent binding site in μ2 for the YppØ motif. This result suggests that the crystal form of the AP-2 core solved by Collins corresponds to the inactive state.

What sorts of structural transitions can the AP-2 core accommodate in order to switch between the inactive and active states? One possibility, favored by Collins et al., involves a dramatic reorientation (about 90°) in the μ2 C-terminal domain, from a position tightly bound to the rest of the core to a conformation projecting away of the core, akin to the release of a spring-loaded blade in a pocket knife (Figure 1D, right). Collins et al. postulate that the C-terminal domain in this new conformation would be oriented parallel to the membrane so that it can make simultaneous contacts with the phosphates in the head group of membrane-bound phosphoinositides and with the YppØ sorting signal in the cytosolic tail of transmembrane proteins. An alternative model (Figure 1D) is to imagine that the C terminus of μ2 is loosely tethered to the core by the μ-linker so that, when released, it can sample many orientations, until it finds and binds to an YppØ sequence in a cytosolic tail. This model can easily accommodate the numerous relative positions of sorting signals with respect to the inner leaflet of the membrane, known to vary from 6 to 7 amino residues to as many as 300 or more residues. The potential adaptability of the activated μ2 platform is a particularly intriguing feature of the atomic structure. Perhaps this adaptor really does adapt.

Any detailed mechanism will need to explain why a number of different conditions lead to activation of AP-2. For example, how does phosphorylation of the μ2 link facilitate exposure of the YppØ binding site? And how do AP-2 clathrin contacts in the coat result in a similar opening of the occluded site in μ2? Inspection of the AP-2 core structure does not yet explain how binding of PI3P or PI(3,4)P2 to AP-2 increases its ability to interact with the endocytic YppØ motif, nor does it provides direct structural clues concerning where in the β subunit the dileucine sorting motif binds. Noteworthy, however, is the structural similarity between the superhelical fold in β-adaptin and the VHS domain of GGAs (Misra et al., 2002; Shiba et al., 2002), raising the possibility for similar rules of engagement in the recognition of dileucine and acidic cluster dileucine sorting motifs by APs and GGAs, respectively. And the structure does rule out the model by which a region in the N-terminal domain of μ2 involving the sequence F_{118}GYPQ was proposed to recognize dileucine motifs (Bremnes et al., 1998), since this segment is buried inside the AP-2 core and therefore cannot be available for binding dileucine signals.

Another form of modulation is found with AP-1, and its cousins AP-3 and AP-4, which need the presence of membrane-bound ARF-GTP in order to be recruited to the endosomal and trans-Golgi membranes (Boehm et

al., 2001 and references therein). The large subunits of these adaptors make contact with the activated ARF, but the location of the interaction, and possible changes in affinity of interaction for sorting signals or for clathrin remains to be established. Soon, we hope, their structures will be available, helping us understand this additional form of regulation.

Selected Reading

Boehm, M., and Bonifacino, J.S. (2001). Mol. Biol. Cell *12*, 2907–2920.

Boehm, M., Aguilar, R.C., and Bonifacino, J.S. (2001). EMBO J. *20*, 6265–6276.

Bremnes, T., Lauvrak, V., Lindqvist, B., and Bakke, O. (1998). J. Biol. Chem. *273*, 8638–8645.

Collins, B.M., McCoy, A.J., Kent, H.M., Evans, P.R., and Owen, D.J. (2002). Cell *109*, this issue, 523–535.

Conner, S.D., and Schmid, S.L. (2002). J. Cell Biol. *156*, 921–929.

Cremona, O., Di Paolo, G., Wenk, M.R., Luthi, A., Kim, W.T., Takei, K., Daniell, L., Nemoto, Y., Shears, S.B., Flavell, R.A., et al. (1999). Cell *99*, 179–188.

Gaidarov, I., and Keen, J.H. (1999). J. Cell Biol. *146*, 755–764.

Haucke, V., and De Camilli, P. (1999). Science *285*, 1268–1271.

Keen, J.H., Willingham, M.C., and Pastan, I.H. (1979). Cell *16*, 303–312.

Kirchhausen, T. (2000). Nat. Rev. Mol. Cell. Biol. *1*, 187–198.

Misra, S., Puertollano, R., Kato, Y., Bonifacino, J.S., and Hurley, J.H. (2002). Nature *415*, 933–937.

Musacchio, A., Smith, C.J., Roseman, A.M., Harrison, S.C., Kirchhausen, T., and Pearse, B.M.F. (1999). Mol. Cell *3*, 761–770.

Ohno, H., Stewart, J., Fournier, M.C., Bosshart, H., Rhee, I., Miyatake, S., Saito, T., Gallusser, A., Kirchhausen, T., and Bonifacino, J.S. (1995). Science *269*, 1872–1875.

Owen, D.J., and Evans, P.R. (1998). Science *282*, 1327–1332.

Pearse, B.M. (1976). Proc. Natl. Acad. Sci. USA *73*, 1255–1259.

Rapoport, I., Miyazaki, M., Boll, W., Duckworth, B., Cantley, L.C., Shoelson, S., and Kirchhausen, T. (1997). EMBO J. *16*, 2240–2250.

Ricotta, D., Conner, S.D., Schmid, S.L., von Figura, K., and Honing, S. (2002). J. Cell Biol. *156*, 791–795.

Shiba, T., Takatsu, H., Nogi, T., Matsugaki, N., Kawasaki, M., Igarashi, N., Suzuki, M., Kato, R., Earnest, T., Nakayama, K., and Wakatsuki, S. (2002). Nature *415*, 937–941.

Ter Haar, E., Harrison, S.C., and Kirchhausen, T. (2000). Proc. Natl. Acad. Sci. USA *97*, 1096–1100.

Wilde, A., and Brodsky, F.M. (1996). J. Cell Biol. *135*, 635–645.