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Motor Protein Receptors: Moonlighting on Other Jobs

Minireview

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The eukaryotic cytoplasm is a highly ordered, yet dynamic environment. Within the cell, large membrane-bound organelles create compartments that carry out distinct biochemical processes, while smaller vesicles act as carriers that deliver proteins and lipids between these compartments. As examples, proteins destined for secretion are inserted into the endoplasmic reticulum (ER), processed in the Golgi, and then delivered to the cell surface; endocytosed vesicles, on the other hand, are transported centripetally from the plasma membrane to the cell interior for fusion with lysosomes. Mitochondria are also very dynamic organelles and are transported to sites of high ATP consumption in the cytoplasm.

Over the past decade, it has become apparent that overall membrane organization and the shuttling of vesicles both require cytoskeletal filaments and molecular motor proteins (Allan and Schroer, 1999). All three motor protein superfamilies are involved in membrane transport: several types of kinesin motors transport vesicular cargo toward the microtubule plus-end, cytoplasmic dynein transports membranes toward the microtubule minus-end, and certain classes of unconventional myosins convey cargo along actin filaments. Many of these motor proteins coinhabit the same cells (perhaps more than 20 in neurons), and yet a given type of organelle appears to be transported by only one or a few types of motors. Thus, some type of law and order must exist that ensures that specific motor proteins are linked to particular cargoes. The nonmotor "tail" domains of motor polypeptides or associated subunits are thought to impart the information for proper cargo selection. On the organelle side, "receptor" proteins that interact with the motor tail domains are assumed to exist.

While discoveries of motor proteins involved in organelle transport have been plentiful, the identification of motor receptors has, until recently, been slow in coming. Findings presented in this issue of *Cell* (Bowman et al., 2000; Nakagawa et al., 2000) and elsewhere, have begun to uncover proteins that may dock molecular motors onto organelles. These receptors are, for the most part, not uncharacterized proteins looking for a function. Instead, they turn out to be familiar faces with previously identified functions. While the molecular details have yet to take form, these results hint at the close connections between the regulation of membrane trafficking and the docking of motor proteins. In this review, we present five recently discovered classes of proteins that link motors to membranes: coat proteins, modular scaffold proteins, transmembrane proteins, small regulatory GTPases, and other motor proteins (Figure 1).

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Coat Proteins

Transport of cargo proteins between membrane-bound organelles is generally thought to occur via carrier vesicles. The first step in this process is the self-assembly of coat proteins on the cytoplasmic side of the membrane, which acts to recruit cargo proteins and catalyze the formation of a budding vesicle. Clathrin and coatomer proteins (COPs) are the principal coat proteins that function in this capacity, and they are used to shuttle cargo between membranes in distinct steps of the secretory pathway. Coat subunits interact with the surface of an organelle through "adaptor" proteins that provide additional specificity and are needed for the coating and budding processes.

In this issue of *Cell*, the AP-1 clathrin-associated adaptor complex, which helps to mediate the transport of clathrin-coated vesicles from the trans-Golgi network (TGN) to plasma membrane, was found to bind to the *Ki*nesin-super*f*amily motor KIF13A (Nakagawa et al., 2000). This finding emerged from affinity purifications of AP1-β1-adaptin with recombinant KIF13A-GST fusion proteins, and coimmunprecipitations from extracts. This interaction was further confirmed by two-hybrid interactions that showed that the carboxy-terminal tail domain of KIF13A bound to the "ear" domain of AP1-β1-adaptin. KIF13A also partially colocalizes with AP-1, as both show prominent immunofluorescence staining on the Golgi apparatus. Overexpression of KIF13A causes mislocalization of AP-1 as well as a reduction in the plasma membrane appearance of the mannose-6-phosphate receptor (M6PR), suggesting that a KIF13A-AP-1 interaction occurs in vivo. AP-1 is currently thought to transport many types of cargo from the TGN. Therefore, whether KIF13A carries all types of TGN-derived vesicles or is relatively specific for M6PR (perhaps by interacting with additional proteins besides AP-1) remains an open question. These findings suggest that motors can directly interact with coat proteins facilitating vesicle transport and delivery between organelles.

Spectrin is another type of coat protein that has been implicated in membrane organization. Spectrin was originally identified as a component of the erythrocyte plasma membrane, where it binds directly to lipids and bridges filamentous actin with other membrane proteins to establish a two-dimensional meshwork that imparts mechanical rigidity to the cell. More recently, several isoforms of spectrin have been discovered to interact with membraneous organelles. While the functions of these organelle-associated spectrins have not been clarified, these proteins are believed to participate in membrane trafficking in some fashion (De Matteis and Morrow, 2000).

This notion is supported by the finding that spectrin binds to the multiprotein dynactin complex, an adaptor thought to link dynein to its cargoes. One component

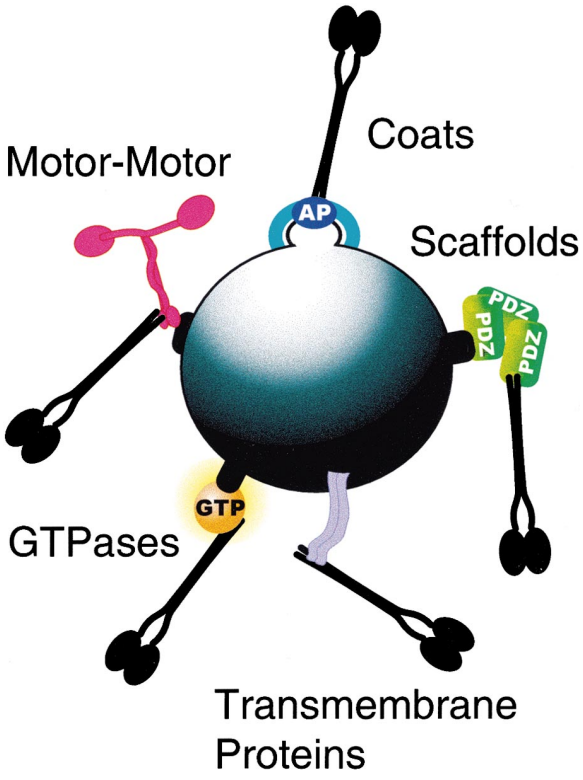

Figure 1. A Summary of the Mechanisms by which Molecular Motor Proteins Attach to Membranes

The classes of multifunctional receptors and their interactions are discussed in the text.

of dynactin, the actin-related protein Arp1, assembles into a short filament at the base of the dynactin complex. When Arp1 was overexpressed, Holleran et al. (1996) found it to form long, cytoplasmic actin-like filaments that recruit dynactin subunits, Golgi membranes, and several isoforms of spectrin. Direct interactions between spectrin and dynactin were further demonstrated by biochemical analysis. As the Golgi apparatus is known to rely upon dynein for its localization to the cell center, the authors postulated a model in which the dynein-dynactin complex associates with this organelle via interactions between the Arp1 filament and membrane-bound spectrin.

Dynein may not be the only motor that associates with spectrin. Takeda et al. (2000) showed that fodrin, a neuronal isoform of spectrin, might act as a binding partner for the heterotrimeric kinesin KIF3. In a two-hybrid assay, fodrin was found to interact with KAP3, the accessory subunit of KIF3 that has been suspected to be involved in organelle binding. The authors also demonstrated that KIF3 and fodrin are present on the same population of vesicles by immunoprecipitation and immunolocalization and also showed that both proteins are transported within axons at similar rates.

These results suggest that, in addition to their roles in mechanical support and molecular organization of membranes, spectrin family members may also function as motor binding sites for at least two classes of microtubule motor proteins. The cellular pool of spectrin exhibits a high degree of heterogeneity owing to multiple genes and alternative mRNA splicing (De Matteis and Morrow, 2000). Some isoforms of spectrin also have the ability to oligomerize in different combinations, thereby further increasing the number of possible structural combinations. This diversity may be important for recruiting particular motors or combinations of motors to organelles.

Scaffold Proteins

Signal transduction cascades utilize multifunctional proteins with several protein–protein interaction modules (scaffold proteins) to assemble large protein–protein complexes at the plasma membrane. Sequence analyses have revealed that the tail domains of several motors also contain protein interaction modules, such as SH2, SH3, pleckstrin homology (PH), tetratricopeptide, and PDZ domains. These observations have suggested that perhaps multiprotein complexes also link motors to organelle surfaces.

Recently, a kinesin protein (KIF17) has been found to associate with a large PDZ-containing complex that forms on neuronal membranes. PDZ domains are protein–protein interaction motifs of ~90 amino acids that were originally discovered as organizers of pre- and postsynaptic plasma membrane structures. Using a two-hybrid screen and GST-pulldown experiments, Setou et al. (2000) showed that KIF17 binds directly to the C-terminal PDZ domain of LIN-10 and that mutants in the PDZ domain abolish this motor-cargo interaction. In *C. elegans*, LIN-10 is part of a complex (LIN-10/2/7) with two other PDZ domain–containing proteins, and this complex was shown to be necessary for the basolateral sorting of the EGF-receptor in vulval epithelial cells (Kaech et al., 1998). In mouse, a similar heterotrimeric LIN complex docks onto membranes by binding to the cytoplasmic domain of NMDA-receptor subunit 2B (Jo et al., 1999). In the experiments of Setou et al., immunoprecipitation of KIF17 brought down the heterotrimeric LIN complex as well as the NMDA-receptor. The authors also showed that NMDA receptor–containing vesicles were transported along microtubules in vitro and this process was inhibited by addition of soluble KIF17 tail peptide. Based upon these results, it was postulated that KIF17, by interacting with the LIN complex, targets NMDA receptors to the dendritic plasma membrane. This hypothesis can be further explored by testing whether disruption or deletion of KIF17 results in NMDA-receptor mislocalization.

A remarkable attribute of KIF17 is that it appears to deliver its cargo selectively to dendrites instead of axons. A similar selectivity may exist in other polarized cells, since the work in *C. elegans* implicates the LIN10/2/7-attachment complex in protein sorting/delivery to the basolateral membrane in epithelial cells. Since KIF17 is expressed only in neurons, another kinesin motor may interact with this same LIN scaffold complex in epithelial cells. These results also suggest the intriguing possibility that kinesin–LIN complexes execute polarized delivery by traveling preferentially along dendritic or basolateral-directed microtubules.

Another scaffold protein that may act as a motor receptor has emerged from studies of KIF1C, which belongs to a class of largely monomeric kinesin motors. KIF1C was previously implicated in membrane traffick-

ing from the Golgi to the ER. A two-hybrid screen to identify proteins that interact with the KIF1C C-terminal domain produced four isoforms of the 14-3-3 family (β, γ, ϵ, and ζ) (Dorner et al., 1999). This conserved family of ubiquitously expressed molecules serves as scaffolds for a diverse group of signaling proteins such as phosphatases, kinases, and transmembrane receptors. 14-3-3 proteins recognize a specific ligand consensus sequence that is present in the tail of KIF1C. The authors focused on the interactions between the C terminus of KIF1C and 14-3-3γ, a phospholipid binding isoform. Using coimmunoprecipitation, they demonstrated that the two proteins interact only when a particular serine in the motor's C-terminal domain was phosphorylated by casein kinase II. Whether this phosphorylation occurs in vivo or is germane to its role in vesicle trafficking, however, has not been established. These findings suggest that components of signaling cascades are recruited to scaffold assemblages along with downstream target motors in order to drive the regulated movement of attached cargo.

Transmembrane Proteins

An early effort to affinity purify a membrane receptor for conventional kinesin resulted in the identification of kinectin, a transmembrane protein found primarily in the ER. Antibodies against kinectin inhibited both plus- and minus-end directed microtubule motility of organelles in an in vitro assay, suggesting that it anchors dynein as well as kinesin motors (Kumar et al., 1995). However, neither the *Drosophila* and *C. elegans* genomes possess a kinectin gene, although both contain a highly conserved conventional kinesin heavy chain gene. This finding indicated that additional membrane receptors must exist for this organelle transport motor.

In this issue of *Cell*, a novel potential transmembrane receptor for conventional kinesin has emerged from genetic studies on *Drosophila* (Bowman et al., 2000). Previous studies have shown that mutations in conventional kinesin and cytoplasmic dynein both cause larval paralysis. This phenotype is the consequence of neuronal dysfunction caused by impaired axonal transport, and electron microscopy revealed that axons in these mutants became clogged with organelles (Hurd and Saxton, 1996). Bowman et al. (2000) reasoned that proteins interacting with these motors could be identified using a genetic screen for mutant flies that show a similar larval paralysis defect. This screen identified Sunday driver (syd), which is a member of a conserved family of proteins found in *C. elegans*, mice, and humans. The Syd protein exhibits a tripartite structure, consisting of an N-terminal domain possessing two predicted coiled-coil regions, a central, transmembrane domain, and a C-terminal domain. In mammalian tissue culture cells, transfected Syd was localized to tubulovesicular Golgi-derived organelles, where it colocalized with conventional kinesin. Two-hybrid analysis revealed that Syd did not associate with the kinesin heavy chain directly; rather, the N terminus of Syd interacted with the tetratricopeptide domain of the kinesin light chains. This interaction was confirmed by Syd's ability to coimmunoprecipitate with kinesin heavy chain from wild-type mouse brain extracts using KLC1 antibodies, but not from extracts derived from transgenic mice lacking a kinesin light chain. Taken together, these results suggest that Syd attaches kinesin to exocytic organelles through the motor's light chains.

Syd probably has another function beyond its proposed role as a kinesin receptor. Syd was recently identified as a binding partner for several MAP kinases involved in a stress-activated signaling cascade (Kelkar et al., 2000). By binding multiple MAP kinases, Syd may also function as a scaffolding protein that facilitates kinase-to-kinase phosphorylation during signaling. A class of Syd-related proteins also exists in which the N-terminal, kinesin binding domain is replaced with a putative guanine nucleotide-exchange factor domain. The similarity between the C-terminal domains of Syd and the Syd-like proteins, however, suggests this protein family probably performs a common function within the lumen of the organelle. The connection between Syd's kinesin binding activity and its other signaling and membrane functions will be an interesting topic for future investigation.

Organelle-associated transmembrane proteins appear to be a common mechanism for motor attachment. Cytoplasmic dynein appears to bind to rhodopsin, a seven-pass transmembrane protein involved in light-activated signal transduction in photoreceptor cells. In these cells, rhodopsin is synthesized in the neuronal cell body and transported along microtubules to their minus ends at the base of a specialized distal projection termed the outer segment. At this position, the microtubules switch polarity so that transport of cargo into the outer segment would require a plus-end-directed motor protein. In a recent study by Tai et al. (1999), rhodopsin was found to bind a cytoplasmic dynein light chain (Tctex-1) through an interaction that does not require the dynactin complex. Interestingly, some human rhodopsin mutations that cause retinitis pigmentosa, a genetic disease that results in photoreceptor cell death, produce rhodopsin mislocalization, and Tai et al. show that these mutations decrease the affinity of the rhodopsin for Tctex-1. This study illustrates that the cargo molecule itself (in this case rhodopsin) also can perform a second function by acting as a motor receptor to ensure its proper targeting within the cell.

Small GTPases

Small G proteins participate in a wide variety of regulatory and signaling events. One of their essential cellular roles is in controlling trafficking in the endocytic and secretory pathways by directing vesicle budding and membrane fusion reactions. Effects of nonhydrolyzable GTP analogs on organelle transport assays in vitro have also suggested that G proteins may regulate microtubule-based motors (Fullerton et al., 1998). However, the molecular mechanisms tying GTPases to organelle transport and molecular motors have not been characterized.

A recent study, however, has described a direct interaction between a kinesin-related protein and the Rab6 GTPase. Rab6, one of the best-characterized members of the small G protein family, is found on the cytoplasmic face of the Golgi, where it is thought to direct retrograde trafficking of protein and lipids to the ER. In an effort to identify Rab6 effector molecules, Echard et al. (1998) performed a two-hybrid screen using the active, GTP-bound form of Rab6 as bait. Unexpectedly, a novel member of the kinesin superfamily, named rabkinesin-6, was

obtained from this screen. This motor coimmunoprecipitated with constitutively active Rab6-GTP, but not Rab6-GDP. Moreover, overexpression of the Rab-6 binding site of this kinesin attenuated the hypersecretory phenotype produced by transfected GTP-Rab6. Overexpression of the full-length rabkinesin-6 caused Golgi dispersion, implicating a function in controlling the dynamics and structure of this organelle. Although the exact function of rabkinesin-6 remains to be defined, these results suggest that members of the Rab family can associate directly with motors during specific phases of their GTPase cycle. Such interactions may serve to recruit and release the motor from the organelle in a manner that is controlled by the nucleotide-state of the GTPase switch. However, these cyclic associations, as well as the nature of the Rab6 receptor on the Golgi membrane, remain to be determined.

The interactions between Rab6 and rabkinesin-6 represent a tantalizing connection between membrane trafficking pathways and a molecular motor. Given that the Rab family has numerous members, it is tempting to speculate that other Rab–motor partnerships also may exist within the cell. Interestingly, mutations in Rab27a result in a coat color defect in mice that appears to result from defective melanosome transport in the melanocytes (Wilson et al., 2000). Since microtubule- and actin-based motors are both necessary to transport these organelles, Rab27a might act as a motor attachment factor.

Piggybacking on Other Motors

Motors may also dock onto a membrane by piggybacking onto other motor proteins. This possibility was suggested recently by the finding that myosin V, an actin-based organelle motor, interacts with conventional kinesin as demonstrated in a two-hybrid assay and in coimmunoprecipitation experiments (Huang et al., 1999). This interaction was shown to involve the AF-6/cno homology domain in the myosin V tail, a protein module that is commonly involved in forming protein–protein interactions. At this point, it is unclear whether myosin V binds another organelle-associated protein and carries kinesin to the surface or vice versa. Nevertheless, these results raise the possibility that actin-based and microtubule-based motors may exist as a complex on organelle surfaces, which may facilitate the switching of an organelle between microtubule and actin tracks (Goode et al., 2000).

Conclusions

The recent work described here suggests that cytoskeletal-based transport is intimately coupled to other events in membrane trafficking. During membrane budding, for example, the export of secretory proteins from the TGN is likely achieved by simultaneously packaging them at the membrane and recruiting motor proteins to immediately transport nascent vesicles or to extend cargo-containing membrane tubules. Indeed, active motors have been observed to attach selectively to membrane domains at sites of concentrated cargo protein (Allan and Vale, 1994). The connection between motors and Rabs also suggests a coordination between vesicle formation/fusion and the control of motor-based transport. Such "integration points" may also transduce information arriving from different signaling pathways to regulate the flow of vesicle formation and delivery in the secretory and endocytotic pathways. These findings on motor receptors likely mark a new era in which the molecular machinery linking motor proteins to cargo selection and delivery will be defined.

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