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Review

Rab Proteins, Connecting Transport and Vesicle Fusion

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Small GTPases of the Rab family control timing of vesicle fusion. Fusion of two vesicles can only occur when they have been brought into close contact. Transport by microtubule- or actin-based motor proteins will facilitate this process *in vivo*. Ideally, transport and vesicle fusion are linked activities. Active, GTP-bound Rab proteins dock on specific compartments and are therefore perfect candidates to control transport of the different compartments. Recently, a number of Rab proteins were identified that control motor protein recruitment to their specific target membranes. By cycling through inactive and active states, Rab proteins are able to control motor protein-mediated transport and subsequent fusion of intracellular structures in both spatial and timed manners.

Key words: dynactin, dynein, endosomes, GLUT4, Golgi, KIF, kinesin, lysosomes, myosin, Rab proteins, transport

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Rab Proteins and Their Interacting Partners

Over 60 mammalian Rab proteins have been identified, and each Rab protein regulates a distinct intracellular transport step (1,2), through its temporal and spatial association with various interacting proteins. One group of interacting proteins comprises the regulatory proteins. These include guanine-exchange factors (GEFs), GTPase-activating proteins (GAPs), GDP-dissociation inhibitors (GDIs) and GDP-displacement factors (GDFs). Together, these proteins control the switch between active GTP-bound and inactive GDP-bound Rab proteins. Moreover, they might be involved in targeting the Rab proteins to their specific membrane (3), a process which is still poorly understood.

The second group of interacting partners are the effector proteins, a rapidly expanding list of proteins that specifically interact with active, GTP-bound Rab proteins (4). One Rab protein can interact with multiple effector proteins that either function in a larger complex or provide the basis for distinct downstream functions. However, effector proteins can also be shared between related Rab proteins, providing concerted action of different Rab proteins within one pathway (5–7).

Rab proteins are recognized for their key roles in both membrane transport and fusion. Whereas the role of Rab proteins in vesicle fusion is relatively well-understood (8), the exact role of Rab proteins in transport was obscure until recently. This review will focus on the emerging role of Rab proteins in vesicle transport with multiple examples of Rabs controlling motor protein recruitment and activation on defined vesicles (Table 1) (Figure 1).

Intracellular Transport

The presence of many different intracellular compartments requires a high order of regulation of transport to ensure delivery at the correct destination. Two networks support motor protein-driven intracellular transport, the microtubule and the actin network. In animal cells, microtubules provide high-speed, long-range transport, whereas the actin network usually facilitates slower and short-range local transport events. Actin-mediated transport occurs via the members of the myosin family (9). Myosins consist of a motor head, a neck and a variable tail domain that mediates interaction with specific cargoes (10). Besides myosin II, a number of unconventional myosins are involved in organelle transport. These include I, V, VI and VII, of which the class V myosins are the most efficient and processive members (11,12).

Most intracellular transport, however, occurs via the microtubule network. Two families of motor proteins use microtubules, kinesins and dyneins (13,14). There are 14 defined families of kinesin motors based on phylogenetic tree analyses (15). Most kinesin motors transport cargo towards the plus end of the microtubules located in the cell periphery, with a few exceptions (15). Kinesins have a domain structure relatively similar to myosins. They consist of a heavy chain [kinesin heavy chain (KHC)] and a light chain [kinesin light chain (KLC)]. Conventional KHCs have three subdomains: the globular motor head, a stalk and a globular tail domain. The non-motor domains are thought to be involved in cargo and microtubule binding and regulation of the motor unit (16–18).

Table 1: Rab GTPases interacting with motor proteins

Rab GTPase	Motor	Compartment	Interaction	Effector	Reference
Rab4	KIF3B	EE	IP		(55)
	LIC	EE	Y2H		(61)
Rab5	DIC	EE	IP		(57)
	KIF16B	EE	No	PI3K	(62)
Rab6	$p150^{GLUED}$	Golgi	Y2H	BicD1/2	(49,50)
	$p50^{dynamitin}$	Golgi	Pull down		(50)
	RB6K/MKLP2	Golgi	Y2H, pull down		(45)
Rab7	Dynein/dynactin	LE/Lys	Unknown	RILP	(64)
Rab8	MyoVI	Golgi	Y2H, direct interaction	Optineurin	(89,90)
Rab11	MyoVb	Recycling compartment	Y2H		(85)
			Y2H	FIP-2	(86)
Rab27a	MyoVa	Melanosome	Y2H, IP	Melanophilin/Slac2	(74–78)
	MyoVIIa	Melanosome	Pull down	MyRIP	(80,81)

DIC, dynein intermediate chain; FIP-2, family interacting protein-2; IP, immunoprecipitation; LIC, light intermediate chain; RILP, Rab7-interacting lysosomal protein; Y2H, yeast two-hybrid.

There are only two dynein motor isoforms mediating vesicular transport. These are cytoplasmic dynein 1 and cytoplasmic dynein 2, of which the latter is mainly involved in intraflagellar movement but also functions in Golgi organization (19,20). Dynein 1 (further on referred to as dynein) is the commonly known dynein involved in many cellular processes (21). Dynein only mediates cargo transport towards the minus end located at the Microtubule Organising Centre (MTOC) (22–24). Dynein motors are massive multimeric complexes composed of two bulky heavy chains and a variety of

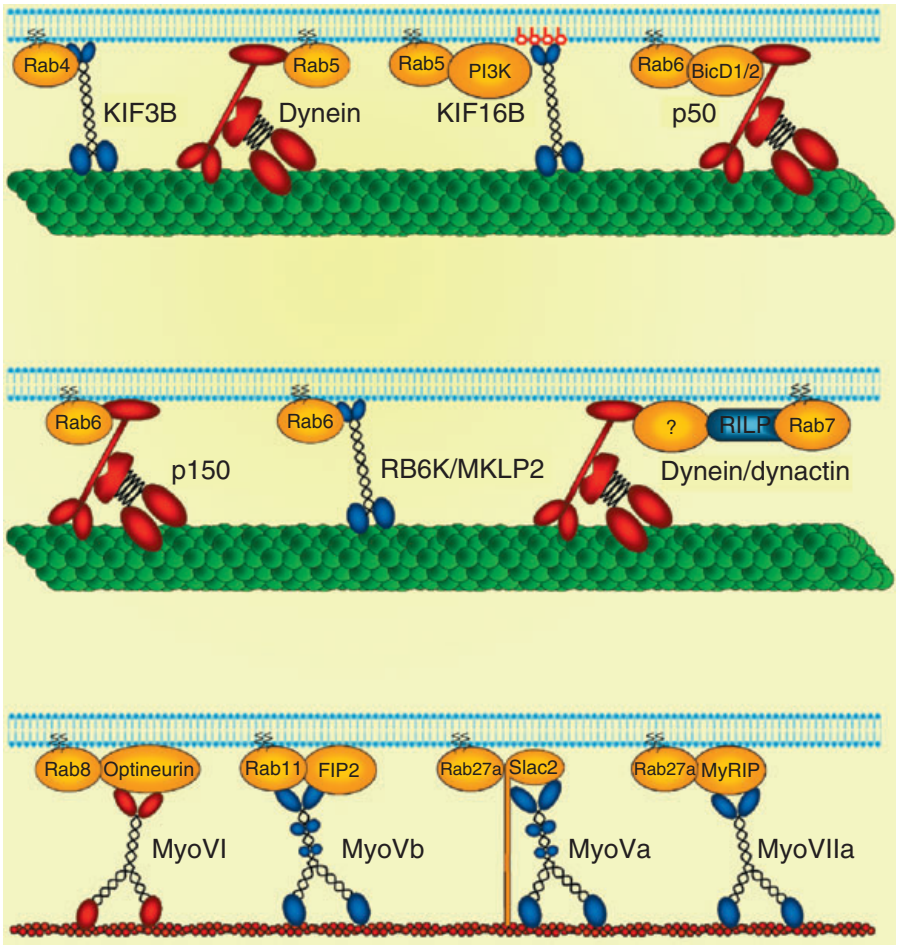

Figure 1: Microtubule- and actin-based motor proteins involved in Rab-dependent motility. Red and blue indicate, respectively, minus-end- and plus-end-directed motors. See text for details. RILP, Rab7-interacting lysosomal protein. ? = unknown protein or protein complex.

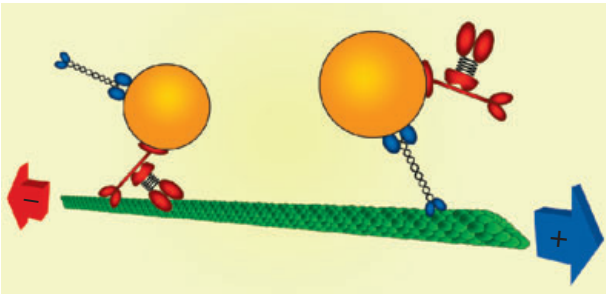

Figure 2: Many cargoes move in a bidirectional manner due to the presence of both kinesin and dynein/dynactin on the same cargo. The alternating activities of the motors determine the direction.

accessory subunits including two intermediate chains [dynein intermediate chains (DICs)], four light intermediate chains (LICs) and several light chains. The heavy chains form the motor domains, whereas the accessory subunits are involved in the interaction with the cargo and regulatory proteins (21). Via the intermediate chains, dynein interacts with its activator, dynactin (25,26). Dynactin is a multisubunit complex composed of $p150^{glued}$, p62, $p50^{dynamitin}$, Arp11, Arp1,β-actin, CapZ α/β, p27, p25 and p24 that links dynein to most cargoes (27).

How motor proteins find their correct vesicular destination is still unclear. Several kinesins directly interact with their specific cargoes via their variable tail domains or their associated light chains, which have been shown to interact with multiple membrane-associated proteins (28,29). Recently, the group of Gelfand (30) identified at least three different dynein LICs, of which one is involved in the targeting of dynein to melanosomes. Although some direct dynein–cargo interactions have been described, efficient dynein processivity and membrane recruitment requires dynactin (31,32). Dynactin by itself is unable to directly interact with lipids in membranes, but via Arp1, dynactin can interact with the spectrin network on vesicular membranes (33–35). Moreover, Muresan et al. (35) showed that spectrin specifically interacts with acidic phospholipids, indicating that the lipid composition of the membrane might be important for the association of motor proteins.

Both, plus-end and minus-end-directed motors can be present on the same cargo (36–38). As a result, many intracellular vesicles like endosomes, lysosomes, secretory vesicles, melanosomes, peroxisomes and mitochondria move along microtubules in a bidirectional manner (37–42). However, these organelles are never observed to be torn apart by two oppositely directed motor proteins. A recent study on bidirectional transport by dynein and kinesin-1 motors of peroxisomes revealed that opposite motor activities do not act simultaneously but are temporarily controlled (37). Apparently, there is an agreement between the motors on when to be active (Figure 2).

Rab Proteins Controlling Motor Proteins

Rab proteins would be ideal to regulate specific recruitment of motor proteins to defined vesicles, because they mark all the different compartments. The first indications that Rab proteins might be directly linked to the cytoskeleton came from Peranen et al. and Deretic et al. (43,44). They observed a striking actin localization and actin-mediated movement of Rab8-positive vesicles, which was largely dependent on Rab8 activity. Since then, a growing number of examples were reported of Rab proteins controlling recruitment of motor proteins onto defined compartments.

Golgi-Associated Rab6 and Its Associated Motors

The first description of a Rab protein directly coupling to a motor protein was when Goud and coworkers (45) identified a new kinesin-like protein as an effector of Golgi-associated Rab6, Rabkinesin-6 (RB6K). RB6K specifically interacted with GTP-Rab6, and overexpression resulted in dispersion of the Golgi towards the plus end of microtubules. Interestingly, RB6K has two microtubule-binding sites; one in the N-terminal motor domain and one in the Rab6-binding C-terminus. The functional relevance of these two microtubule-binding sites became apparent when RB6K was shown to be crucial for cytokinesis and was highly upregulated during mitosis (46–48). In addition to its localization to the central spindle, the motor domain of RB6K is highly similar to the mitotic kinesin MKLP1 and is therefore reclassified as MKLP2. During the cell cycle, RB6K/MKLP2 localization is independent of Rab6, which is in contradiction with its role in Golgi transport, and some controversy exists about the latter function. However, RB6K/MKLP2 might have different interaction partners during the various stages of the cell cycle.

Other Rab6 effector proteins were subsequently identified. Short et al. and Matanis et al. isolated two related dynactin-binding proteins, BicD1 and BicD2, that interacted with GTP-Rab6 (49,50). BicD2, as well as BicD1, bind to $p50^{dynamitin}$ (51) and thereby link Rab6 to the dynein/dynactin complex. Overexpression of the Rab6-binding C-terminal portion of BicD2 significantly diminished minus-end transport of Rab6-positive vesicles, most likely by uncoupling the motor from Rab6 (49). In addition, a link between Rab6 and the dynactin subunit $p150^{Glued}$ was observed *in vitro* (50), but the functional relevance of these two modes of interaction remains elusive.

Thus, active Rab6 can interact with both kinesin (RB6K/MKLP2) and the dynein/dynactin complex, thereby regulating both plus-end and minus-end transport of Golgi compartments. Still, directional transport of Golgi vesicles occurs, which cannot occur by simultaneous activity of the

two oppositely directed motor proteins. How the relative activities of both motors are controlled is yet unclear.

Rab4 and Rab5 Regulating Exocytosis and Endocytosis via Co-ordinated Interactions with Kinesin and Dynein Motors

The transport of the insulin-responsive glucose transporter GLUT4 in 3T3-1L adipocytes illustrates the interplay between Rab proteins and motors. GLUT4 proteins are translocated from the perinuclear area to the plasma membrane following insulin stimulation due to both enhanced exocytosis and reduced endocytosis (52–54). Imamura et al. (55) identified Rab4 as a main regulator of exocytosis of GLUT4. They found an interaction between active GTP-Rab4 and KIF3B, a kinesin-2 family member. Insulin activates Rab4 in a PI3K- and PKCγ-dependent manner, which in turn enhances its association to KIF3B. In addition, insulin increased binding of KIF3B to microtubules, again dependent on PI3K- and PKCγ activity. Because phosphorylation of kinesin has been suggested to affect microtubule binding, it is possible that KIF3B is a target of these kinases (56).

The other component of the GLUT4 cycle, endocytosis, is controlled by Rab5. Huang et al. (57) showed that dynein could be coisolated with Rab5. Upon stimulation with insulin, they observed a significant decrease in the levels of GTP-Rab5, which resulted in a concomitant reduction in dynein association and inhibition of minus-end transport. In addition, insulin decreased the interaction of dynein with microtubules in a PI3K-dependent manner. Again, this might be mediated by phosphorylation of the dynein/dynactin complex, which has previously been shown to negatively regulate its microtubule-binding capacity (58). Involvement of a large group of kinases (>207) in endocytosis and exocytosis is further supported by a recent RNAi screen by Zerial and coworkers (59). How these kinases might affect motor protein-based transport remains elusive.

Thus, the insulin-stimulated increase of active GTP-Rab4 leads to enhanced recruitment (by coisolation experiments) and activity of KIF3B resulting in enhanced plus-end-directed transport (i.e. exocytosis). However, insulin-dependent phosphorylation events also inactivate Rab5 and diminish dynein/dynactin function thereby inhibiting minus-end transport (i.e. endocytosis). By these means, insulin stimulation results in efficient delivery of GLUT4 at the plasma membrane.

Earlier, Nielsen et al. showed that Rab5 activity is required for minus-end-directed transport of early endosomes isolated from A431 cells. But surprisingly, antibodies directed against the dynein motor did not affect minus-end transport. In contrast, MC44 antibodies directed against multiple KHCs blocked both minus-end and plus-end transport of early endosomes (60). This suggests a link between Rab5 and a minus-end directed kinesin motor protein. Moreover, yeast two-hybrid data indicate that Rab4 interacts with the dynein LIC (61). Apparently, Rab proteins can adapt to the diverse motor requirements of distinct (sub)-compartments in different cell types, but various interactions still have to be confirmed by techniques other than yeast two-hybrid and coisolation experiments.

Rab5 Creates a Membrane Patch for KIF16B Motor Association

Whereas the previous examples described direct interactions between microtubule-based motor proteins and Rab proteins, Zerial and colleagues recently showed that Rab5 can create a local environment for motor recruitment without the need for a direct interaction. They identified a new kinesin family member, KIF16B, which was recruited to early endosomes in a Rab5-dependent manner (62). KIF16B can be classified as a plus-end-directed kinesin-3 family member (15). Together with Unc104, another kinesin-3, it is the only known kinesin that contains a lipid-binding domain. Hoepfner et al. showed that KIF16B interacts with PI(3)P-loaded vesicles via its C-terminal PhoX homology (PX) domain. Vps34 is a PI3 kinase and an effector protein of Rab5 and is responsible for the local production of PI(3)P on the endosomal membrane. Rab5 thus regulates KIF16B recruitment by creating a PI(3)P-containing microdomain suitable for KIF16B binding, thereby stimulating plus-end transport of the endosomes. However, Rab5 and PI3-kinase activity also appeared to regulate minus-end transport of early endosomes (60); therefore, the question remains how the opposite activities are temporarily controlled by the same Rab5 protein and possibly the same PI3 kinase.

Rab7 and Its Effector Rab7-Interacting Lysosomal protein Regulate Dynein/Dynactin-Motor Recruitment to Late Endosomes

Rab7 is present on late endosomal and lysosomal structures that – like other structures – move along microtubules in a bidirectional manner due to the alternating activities of dynein and kinesin motors (42). Rab7 requires its effector protein, Rab7-interacting lysosomal protein (RILP) (63,64), to recruit the dynein/dynactin motor to late endosomes and lysosomes resulting in the accumulation of these structures at the extreme minus end of microtubules, the MTOC (64). Overexpression of the (dominant-negative) C-terminal Rab7-binding portion of RILP prevents recruitment of the dynein/dynactin complex (64) and results in the relocation of late endosomal compartments towards the cell periphery by kinesin (63,64). A direct interaction of the dynein/dynactin motor with either Rab7 or RILP was not seen.

Lebrand et al. (65) have shown that the lipid composition of late endosomes is of major importance for the membrane localization of Rab7. Interestingly, spectrin is also found on Rab7-containing compartments and is required for the perinuclear retention of these compartments, thereby making spectrin a potential mediator of motor recruitment (unpublished observations). Thus, lipids and possibly spectrin might play a role in Rab7-mediated motor recruitment. Interestingly, Vps34 and its adaptor protein p150 also form a complex with inactive GDP-bound Rab7 (66). Rab7 activation results in the release of Vps34, which raises the possibility that Vps34 is cycling between early and late endosomes. Collectively, these data suggest that the recruitment of the dynein/dynactin motor to late endosomal compartments might be the consequence of a Rab7-/RILP-mediated alteration of the late endosomal membrane (by affecting the lipid composition resulting in spectrin binding – for example) analogous to the recruitment of KIF16B to early endosomes by Rab5/Vps34 (62).

The Control of Myosin Motors by Rab Proteins

There are also many examples of Rab proteins interacting with the actin-based myosin motors [for review (67)]. The earliest finding with respect to these interactions was the association of Rab27a with the actin-based motor myosin Va (68). This link became apparent with the similar phenotypes of two-coat colour mutants in mice, *dilute* and *ashen*, which have mutations in, respectively, the *MYOVA* and the *RAB27A* genes (69,70). As a result, melanocytes of these mutant mice show a striking depletion of melanin-loaded melanosomes in their dendrites (71,72). A third colour-coat mutant, *leaden*, has a defective gene encoding for Melanophilin/Slac-2a (73), a Rab27a effector that was later identified as the MyoVa receptor by several groups (74–78). Recently, Rab27a was also found to interact with another myosin motor, MyoVII (79), in retina-pigment epithelial cells. This interaction was dependent on a different Rab27a effector, MyRIP (80,81), which illustrates the cell-type-specific interactions between Rab proteins, their effectors and motors.

Two other Rabs have been shown to couple to myosins, Rab11 and Rab8. Rab11 is associated to the recycling compartment and regulates recycling of the transferrin receptor and several G protein-coupled receptors (82–84). A direct interaction was found between Rab11 and myosin Vb via a yeast two-hybrid screen (85), yet the Rab11 effector *'Rab11 family interacting protein-2'* (FIP-2) also interacts with myosin Vb (86). Rab8 regulates the biosynthetic pathway from the Golgi towards the plasma membrane (87,88). Recently, myosin VI has been observed to directly interact with optineurin (89), an interaction partner of Rab8 (90), which may explain the Rab8-dependent actin-based movements observed earlier (43,44).

In conclusion, these data illustrate how various Rab proteins recruit – either directly or indirectly – specific microtubule- or actin-based motor proteins to their target membranes. The factors regulating the GTPase cycle of Rab proteins (but also other proteins like kinases and probably also phosphatases) then control the timing of motor activities on specific vesicles.

Rab Proteins Combining Two Activities: Transport and Vesicle Fusion

Rab proteins are best known for their role in regulating most – if not all – intracellular fusion and fission events (2,8,91). They can create membrane subdomains via their interaction with various effector proteins to sort cargo (5,92). In addition, Rab proteins play a major role in the process prior to fusion with the target membrane by recruiting tethering and docking factors (8). Rab proteins are thus able to connect membrane fission/fusion and transport and, given their defined intracellular location, provide specificity.

Wolkoff and coworkers (93) already showed 5 years ago, in an *in vitro* reconstituted system, that fission of early endosomes requires the action of microtubule-based motor proteins. They observed bidirectional movement of vesicles, which was blocked by a general antibody against the KHC. Inhibiting Rab4 activity on these endosomes stimulated minus-end transport by the minus-end-directed kinesin protein KIF2C and increased the fission events *in vitro*, consistent with Rab4 acting as a switch between plus- and minus-end transport.

The interaction of Rab5 with Vps34 that leads to local early endosomal production of PI(3)P is another illustrative example of a direct link between transport and fusion. Local increase of PI(3)P not only leads to the recruitment of KIF16B but also attracts FYVE domain containing proteins like EEA1 which are part of the docking and fusion machinery (94).

Another example of a Rab protein controlling fusion via motor proteins is found in the phagocytic pathway. Phagosomes are lysosome-related organelles and largely regulated by both Rab5 and Rab7 (95,96). In the end stage of the phagocytic pathway, pathogens are degraded in the phagolysosomes due to the fusion with lysosomes and the introduction of various hydrolases (97,98). *Salmonella* is one of the pathogens that is able to survive intracellularly in a so-called *Salmonella*-containing vacuole (SCV). Like nascent phagosomes, the SCV acquires Rab7, which is required for its maturation (99). But the SCV is able to prevent its fusion with mature lysosomes. Recently, Guignot et al. (100) have shown that both kinesin and dynein motor activities are required for the formation and maintenance of the SCV. Interestingly, by increasing the amount of the Rab7 effector RILP on SCVs or nascent

phagosomes, the motor balance can be shifted towards dynein/dynactin, which subsequently leads to enhanced fusion with mature lysosomes (101,102). As a result, intracellular *Salmonella* replication is blocked (102).

Thus Rab proteins orchestrate membrane fusion not only by recruiting the components of the fusion machinery, but also by controlling the process prior to fusion, motor-based vesicle transport. This once again proves Rab proteins are master regulators of intracellular membrane trafficking by controlling two independent but tightly linked processes: fusion and transport.

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