

Of Yeast, Mice, and Men: Rab Proteins and Organelle Transport

Sean W. Deacon and Vladimir I. Gelfand

Department of Cell and Structural Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

A typical eukaryotic cell contains many types of membrane organelles with characteristic distributions within the cytoplasm. This distribution is facilitated in part by the organelle binding to a specific subset of motor proteins. Bound motors then transport the organelles to their proper destinations in the cell by moving them along microtubules or actin filaments. A key question of modern cell biology is how motor proteins recognize their target organelles. A number of recent articles address this question by showing that Rab proteins on the surface of organelles can function as a part of the recognition complex for motor proteins.

Rab proteins form the largest branch of the Ras superfamily of GTPases. They are found in organisms from yeast to human, and have been implicated in various functions within the cell including growth, protein trafficking, and the targeting and fusion of membrane bound organelles (Chavrier and Goud, 1999; Pereira-Leal and Seabra, 2000). The works of Bahadoran et al. (2001), Haddad et al. (2001), Hume et al. (2001), Raposo et al. (2001), and Stinchcombe et al. (2001), published in this issue, address the possibility that Rab proteins may also mediate motor/cargo interactions. They present compelling evidence to support this idea, while at the same time providing important information about some of the human disorders associated with organelle transport.

The most comprehensive evidence for the involvement of Rab proteins in intracellular transport is provided by recent studies demonstrating interactions between Rab proteins and the organelle-transporting motors of the myosin-V class in several experimental systems (Pruyne et al., 1998; Schott et al., 1999). Class V myosins are processive plus-end directed motors that have been implicated in intracellular transport in all eukaryotes from yeast to humans. They consist of two identical heavy chains and twelve light chains. The heavy chains contain an NH₂-terminal motor domain, a neck region with binding sites for light chains, a coiled-coil stalk, and a COOH-terminal globular domain that is involved in cargo binding (Reck-Peterson et al., 2000).

The first suggestion of a Rab/myosin-V interaction was provided by Pruyne et al. (1998) who showed that Myo2p, a yeast class V myosin, and Sec4p, a yeast Rab protein,

colocalized to the growing bud in yeast cells (Pruyne et al., 1998). By using yeast with a conditional mutation in the MYO2 gene they were able to show that the localization of Sec4p was Myo2p dependent. Genetic evidence of an interaction between these two proteins in yeast was demonstrated by Schott et al. (1999) who showed that a mutation in MYO2 was synthetically lethal with a mutation in SEC4.

A direct physical interaction between Rab proteins and myosin-V was first demonstrated by Kumar et al. (Kumar, R., J. Navarre, and J.R. Goldenring. 1999. *Mol. Biol. Cell.* 10S:163a [abstr.]) who studied Rab11a, a Rab protein involved in the plasma membrane recycling system. By immunofluorescent staining of cultured cells, they showed that Rab11a is colocalized with myosin-Vb on vesicles containing the transferrin receptor. More importantly, by using a yeast two-hybrid system they demonstrated that Rab 11a binds the tail domain of myosin-Vb, suggesting that Rab11a may play a role in docking the motor to vesicles.

Rab-myosin-V interactions have been studied carefully in a series of recent papers that demonstrate a role for Rab proteins in myosin-Va-driven organelle transport in pigment cells of mice and humans (Menasche et al., 2000; Wilson et al., 2000; Bahadoran et al., 2001; Hume et al., 2001). In mammals, pigment melanin deposited in skin and hair is produced in melanocytes and transported to the cell periphery by the combined action of microtubule motors and myosin-Va (Wu et al., 1998). At the periphery of melanocytes, the pigment is secreted and then endocytosed by the adjacent epithelial cells. The genetics of pigmentation in mice has long been a subject of extensive study. One result attained by this analysis was the identification of myosin-Va as the protein encoded by *dilute*, a gene required for the transfer of pigment organelles from melanocytes to keratinocytes (Mercer et al., 1991). In addition to *dilute*, there are a number of other mutations that are characterized by similar phenotypes, and it is likely that the products of the genes affected by these mutations also work in the pigment organelle transport pathway. One of these genes, *ashen*, was found to encode Rab27a (Wilson et al., 2000), a Rab protein expressed in eye, lung, spleen, intestine, melanocytes, platelets, and pancreas (Seabra et al., 1995; Chen et al., 1997). Positional cloning has demonstrated that *ashen* mice have a mutation that encodes a nonfunctional version of Rab27a, and that the mutant phenotype can be rescued by addition of a BAC containing Rab27a (Wilson et al., 2000).

Address correspondence to Vladimir Gelfand, Department of Cell and Structural Biology, University of Illinois at Urbana-Champaign, 601 S. Goodwin Ave., Urbana, IL 61801. Tel.: (217) 333-5972. Fax: (217) 244-1648. E-mail: vgelfand@uiuc.edu

A similar picture of pigment transport mechanisms emerged from the analysis of Griscelli syndrome, a human autosomal recessive disorder that causes pigment abnormalities and neurological defects. Most cases also display immunological disorders that include haemophagocytic syndrome and defective exocytosis of lytic granules from cytotoxic T lymphocytes (Griscelli et al., 1978). Initially, the genetic defect in patients with Griscelli syndrome was mapped to the gene encoding myosin-Va. However, it has been shown recently that Griscelli patients with the immunological abnormalities have a mutation in the gene that encodes Rab27a rather than myosin-Va (Menasche et al., 2000).

These papers (Menasche et al., 2000; Wilson et al., 2000) suggest that Rab27a can work as a component of the pigment transport machinery that uses myosin-Va as a motor. In two recent works, one of which is published in this issue, it was shown that wild-type melanocytes transfected with a dominant negative form of Rab27a showed a perinuclear arrangement of melanosomes, similar to that observed in *ashen* and *dilute* melanocytes (Wilson et al., 2000; Hume et al., 2001; Wu et al., 2001). Conversely, it was demonstrated by Bahadoran et al. (2001) that transfection of wild-type Rab27a into melanocytes from a patient with Griscelli syndrome restored the wild-type distribution of melanosomes to the cell periphery. The same result was obtained in the mouse model using *ashen* melanocytes (Bahadoran et al., 2001; Wu et al., 2001).

In wild-type melanocytes, Rab27a is localized to the cytoplasmic face of melanosomes along with myosin-Va. However, in *ashen* melanocytes, myosin-Va failed to localize to melanosomes (Hume et al., 2001; Wu et al., 2001). These results indicate that not only does Rab27a colocalize with myosin-Va, but it is also required for motor binding to melanosomes. The simplest explanation for this observation is that Rab27a provides a binding site for myosin-Va on the surface of melanosomes. If this is the case, then the two proteins should interact with each other. Evidence for a physical interaction is provided by Hume et al. (2001) who show that antibodies against Rab27a are able to immunoprecipitate myosin-Va from melanocyte extracts. When taken together, the results of these experiments show that Rab27a is involved in targeting myosin-Va to melanosome membranes.

Studies of patients with Griscelli syndrome and *ashen* mice suggest that, in addition to regulating and anchoring myosin-Va, Rab 27a may have another function. It has been shown that patients with Griscelli syndrome caused by the mutation in Rab27a and *ashen* mice are deficient in the ability to secrete lytic granules from their cytotoxic T lymphocytes, resulting in a reduced ability to kill their targets (Menasche et al., 2000; Haddad et al., 2001; Stinchcombe et al., 2001). At the same time, Griscelli patients with the mutation in myosin-Va and *dilute* mice do not display these immunological defects (Menasche et al., 2000; Haddad et al., 2001). It could be that in T-lymphocytes, Rab27a is involved in the anchoring of some other motor required for lytic granule exocytosis, whereas in melanocytes, Rab27a is involved in the targeting of myosin-Va to pigment organelles. If this is the case, a mutation in the Rab27a gene would affect both types of organelles, whereas a mutation in myosin-Va would have no effect in T lymphocytes. Another possibility is that Rab27a is not

involved in motor anchoring to lytic granules, but is required for the targeting and fusion of lytic granules with the plasma membrane. In this case, one may speculate that Rab27a plays a similar role in pigment cells by facilitating melanin exocytosis from melanocytes, a process that is still poorly understood.

Evidence is also emerging that suggests Rab proteins interact with microtubule motors. Echard et al. (1998) have demonstrated that a Golgi-associated Rab protein, Rab6, binds to a member of the kinesin family subsequently termed Rabkinesin-6, which appears to play a role in cytokinesis (Echard et al., 1998; Hill et al., 2000). Results obtained from studies with *gunmetal* mice (Stinchcombe et al., 2001) also suggest an interaction between Rab proteins and microtubule motors. Mice with the *gunmetal* mutation produce a partially functional Rab geranylgeranyl transferase (RGGT), an enzyme responsible for the prenylation and activation of several Rab family members (Detter et al., 2000). It is demonstrated in this issue by Stinchcombe et al. (2001) that lytic granules from *gunmetal* T lymphocytes do not polarize to the immunological synapse correctly, a process known to depend on microtubules (Kupfer et al., 1983). In contrast to this, lytic granule polarization in cytotoxic T lymphocytes from *ashen* mice appears to be normal, suggesting that Rab27a is not involved (Haddad et al., 2001; Stinchcombe et al., 2001). It could be the case that some other Rab protein not prenylated in *gunmetal* T lymphocytes is involved in the transport of lytic granules along microtubules.

The works in this issue (Bahadoran et al., 2001; Haddad et al., 2001; Hume et al., 2001; Raposo et al., 2001; Stinchcombe et al., 2001) indicate that one possible function of Rab proteins is to form a binding site for motor proteins on the surface of organelles. This would allow the motor proteins to bind the organelles, facilitating their transport and localization. The idea is an attractive one, in that Rab proteins are organelle-specific, which makes them well suited for such a function. It is interesting to note that while melanosomes share a number of membrane proteins with lysosomes, Raposo et al. (2001) suggests that there is a unique sorting pathway for their biogenesis. This pathway would allow melanosomes to possess a specific subset of Rab and motor proteins that would regulate their movement and exocytosis separately from lysosomes. Thus, Rab proteins may be one of the keys to unlocking the much bigger question of how motor proteins recognize specific organelles and transport them to their correct intracellular location.

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References

- Bahadoran, P., E. Aberdam, F. Mantoux, K. Bille, N. Yalman, G. de Saint-Basille, R. Casaroli-Marano, J. Ortonne, and R. Ballotti. 2001. Rab27a: a key to melanosome transport in human melanocytes. *J. Cell Biol.* 152:843–849.
- Chavrier, P., and B. Goud. 1999. The role of ARF and Rab GTPases in membrane transport. *Curr. Opin. Cell Biol.* 11:466–475.
- Chen, D., J. Guo, T. Miki, M. Tachibana, and W.A. Gahl. 1997. Molecular cloning and characterization of rab27a and rab27b, novel human rab proteins shared by melanocytes and platelets. *Biochem. Mol. Med.* 60:27–37.
- Detter, J.C., Q. Zhang, E.H. Mules, E.K. Novak, V.S. Mishra, W. Li, E.B. McMurtrie, V.T. Tchernev, M.R. Wallace, M.C. Seabra, et al. 2000. Rab geranylgeranyl transferase alpha mutation in the *gunmetal* mouse reduces Rab prenylation and platelet synthesis. *Proc. Natl. Acad. Sci. USA.* 97:4144–4149.
- Echard, A., F. Jollivet, O. Martinez, J.J. Lacapere, A. Rousset, I. Janoueix-

- Lerosey, and B. Goud. 1998. Interaction of a Golgi-associated kinesin-like protein with Rab6. *Science*. 279:580–585.
- Griscelli, C., A. Durandy, D. Guy-Grand, F. Daguillard, C. Herzog, and M. Prunieras. 1978. A syndrome associating partial albinism and immunodeficiency. *Am. J. Med.* 65:691–702.
- Haddad, E.K., X. Wu, J.A. Hammer III, and P.A. Henkart. 2001. Defective granule exocytosis in Rab27a-deficient lymphocytes from *ashen* mice. *J. Cell Biol.* 152:835–841.
- Hill, E., M. Clarke, and F.A. Barr. 2000. The Rab6-binding kinesin, Rab6-KIFL, is required for cytokinesis. *EMBO (Eur. Mol. Biol. Organ.) J.* 19:5711–5719.
- Hume, A.N., L.M. Collinson, A. Rapak, A.Q. Gomes, C.R. Hopkins, and M.C. Seabra. 2001. Rab27a regulates the peripheral distribution of melanosomes in melanocytes. *J. Cell Biol.* 152:795–808.
- Kupfer, A., G. Dennert, and S.J. Singer. 1983. Polarization of the Golgi apparatus and the microtubule-organizing center within cloned natural killer cells bound to their targets. *Proc. Natl. Acad. Sci. USA.* 80:7224–7228.
- Menasche, G., E. Pastural, J. Feldmann, S. Certain, F. Ersoy, S. Dupuis, N. Wulfraat, D. Bianchi, A. Fischer, F. Le Deist, and G. de Saint Basile. 2000. Mutations in RAB27A cause Griscelli syndrome associated with haemophagocytic syndrome. *Nat. Genet.* 25:173–176.
- Mercer, J.A., P.K. Seperack, M.C. Strobel, N.G. Copeland, and N.A. Jenkins. 1991. Novel myosin heavy chain encoded by murine dilute coat colour locus. *Nature.* 349:709–713.
- Pereira-Leal, J.B., and M.C. Seabra. 2000. The mammalian Rab family of small GTPases: definition of family and subfamily sequence motifs suggests a mechanism for functional specificity in the Ras superfamily. *J. Mol. Biol.* 301:1077–1087.
- Pruyne, D.W., D.H. Schott, and A. Bretscher. 1998. Tropomyosin-containing actin cables direct the Myo2p-dependent polarized delivery of secretory vesicles in budding yeast. *J. Cell Biol.* 143:1931–1945.
- Raposo, G., D. Tenza, D.M. Murphy, J.F. Berson, and M.S. Marks. 2001. Distinct protein sorting and localization to premelanosomes, melanosomes, and lysosomes in pigmented melanocytic cells. *J. Cell Biol.* 152:809–823.
- Reck-Peterson, S.L., D.W. Provan, Jr., M.S. Mooseker, and J.A. Mercer. 2000. Class V myosins. *Biochim. Biophys. Acta.* 1496:36–51.
- Schott, D., J. Ho, D. Pruyn, and A. Bretscher. 1999. The COOH-terminal domain of Myo2p, a yeast myosin V, has a direct role in secretory vesicle targeting. *J. Cell Biol.* 147:791–808.
- Seabra, M.C., Y.K. Ho, and J.S. Anant. 1995. Deficient geranylgeranylation of Ram/Rab27 in choroideremia. *J. Biol. Chem.* 270:24420–24427.
- Stinchcombe, J.C., D.C. Barral, E.H. Mules, S. Booth, A.N. Hume, L.M. Machesky, M.C. Seabra, and G.M. Griffiths. 2001. Rab27a is required for regulating secretion in cytotoxic T lymphocytes. *J. Cell Biol.* 152:825–833.
- Wilson, S.M., R. Yip, D.A. Swing, T.N. O'Sullivan, Y. Zhang, E.K. Novak, R.T. Swank, L.B. Russell, N.G. Copeland, and N.A. Jenkins. 2000. A mutation in Rab27a causes the vesicle transport defects observed in *ashen* mice. *Proc. Natl. Acad. Sci. USA.* 97:7933–7938.
- Wu, X., B. Bowers, K. Rao, Q. Wei, and J.A.R. Hammer. 1998. Visualization of melanosome dynamics within wild-type and dilute melanocytes suggests a paradigm for myosin V function in vivo. *J. Cell Biol.* 143:1899–1918.
- Wu, X., K. Rao, M.B. Bowers, N.G. Copeland, N.A. Jenkins, and J.A. Hammer III. 2001. Rab27a enables myosin-Va-dependent melanosome capture by recruiting the myosin to the organelle. *J. Cell Sci.* In press.