an actin-like trimer[6–10]. But we do not know how a WASP-bound actin monomer would be positioned to form this trimeric nucleus. Understanding the rearrangements of the complex needed for activation, as well as the precise site of WASP binding, will reveal the location and position relative to the Arp proteins of the final actin monomer required for rapid and efficient actin polymerization.

These three papers create a picture of the Arp2/3 complex as a molecular machine with multiple parts that can exist in several conformations, and it is the relative orientations of the parts that determine the efficiency of the assembly. It is now clear that 'ignition' of this machine will require concerted actions of multiple activators to maximize the interplay of the parts, and to allow rapid, tightly regulated actin rearrangements within the cell. □

Mara Kreishman-Deltrick and Michael K. Rosen are in the Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas 75390, USA.
e-mail: rosen@mrnmr1.mskcc.org

1. Cooper, J. A., Buhle, E. L., Jr., Walker, S. B., Tsong, T. Y. & Pollard, T. D. *Biochemistry* **22,** 2193–2202 (1983).
2. Tobacman, L. S. & Korn, E. D. *J. Biol. Chem.* **258,** 3207–3214 (1983).
3. Frieden, C. *Proc. Natl Acad. Sci. USA* **80,** 6513–6517 (1983).
4. Machesky, L. M., Atkinson, S. J., Ampe, C., Vandekerckhove, J. & Pollard, T. D. *J. Cell Biol.* **127,** 107–115 (1994).
5. Mullins, R. D., Heuser, J. A. & Pollard, T. D. *Proc. Natl Acad. Sci. USA* **95,** 6181–6186 (1998).
6. Higgs, H. N. & Pollard, T. D. *Ann. Rev. Biochem.* **70,** 649–676 (2001).
7. Takenawa, T. & Miki, H. *J. Cell Sci.* **114,** 1801–1809 (2001).
8. Mullins, R. D. *Curr. Opin. Cell. Biol.* **12,** 91–96 (2000).
9. Welch, M. D. *Trends Cell Biol.* **9,** 423–427 (1999).
10. Carlier, M. F., Ducruix, A. & Pantaloni, D. *Chem. Biol.* **6,** R235-R240 (1999).
11. Svitkina, T. M. & Borisy, G. G. *J. Cell Biol.* **145,** 1009–1026 (1999).
12. Volkmann, N. *et al. Science* **293,** 2456–2459 (2001).
13. Gournier, H., Goley, E. D., Niederstrasser, H., Trinh, T. & Welch, M. D. *Mol. Cell* **8,** 1041–1052 (2001).
14. Robinson, R. C. *et al. Science* **294,** 1679–1684 (2001).
15. Kelleher, J. F., Atkinson, S. J. & Pollard, T. D. *J. Cell Biol.* **131,** 385–397 (1995).
16. Le Clainche, C., Didry, D., Carlier, M. F. & Pantaloni, D. *J. Biol. Chem.* **276,** 46689–46692 (2001).
17. Dayel, M. J., Holleran, E. A. & Mullins, R. D. *Proc. Natl Acad. Sci. USA* **98,** 14871–14876 (2001).
18. Zalevsky, J., Grigorova, I. & Mullins, R. D. *J. Biol. Chem.* **276,** 3468–3475 (2001).
19. Marchand, J. B., Kaiser, D. A., Pollard, T. D. & Higgs, H. N. *Nature Cell Biol.* **3,** 76–82 (2001).
20. Amann, K. J. & Pollard, T. D. *Nature Cell Biol.* **3,** 306–310 (2001).
21. Bailly, M. *et al. Curr. Biol.* **11,** 620–625 (2001).
22. Machesky, L. M. *et al. Proc. Natl Acad. Sci. USA* **96,** 3739–3744 (1999).

'Rab'ing up endosomal membrane transport

Magda Deneka and Peter van der Sluijs

Early endosomes are the first sorting station from which endocytosed materials are targeted to various intracellular destinations. Recent work has identified the FYVE-domain protein rabenosyn-5 as a bifunctional effector of the GTPases rab5 and rab4, physically connecting entry and recycling sites on early endosomes.

All eukaryotic cells internalize cell-surface proteins and material from their environment by endocytosis. The pathway is used, amongst others, for the uptake of nutrients, regulation of cell surface receptors and for the recycling of proteins used in the secretory pathway. Typically, receptors and bound ligand are targeted to early endosomes called sorting endosomes. Most ligands then dissociate from their receptors at the mildly acidic internal pH of the early endosome and are delivered to degradative late endocytic compartments. Uncoupling receptor–ligand complexes allows receptors to be recycled to the cell surface via either a direct 'fast' track or a parallel 'slow' route through recycling endosomes and possibly the *trans*-Golgi network (TGN) (Fig. 1). Unlike the initial steps of clathrin- and AP-2-dependent endocytosis and transport to early endosomes, little is known about the recycling pathway from early endosomes back to the cell surface and the proteins that regulate it. In this issue of *Nature Cell Biology*, de Renzis and colleagues[1] uncover a connection between fusion of early endosomes and receptor recycling to the cell surface. They document that the FYVE-finger protein rabenosyn-5 is a bifunctional effector of rab5 and rab4 that serves to link the activities of these sequentially acting GTPases on membrane traffic through early endosomes.

Recycling endosomes can be distinguished morphologically by their propensity to retain recycling transferrin receptors in a characteristic location close to the microtubule-organizing center (MTOC) in Chinese hamster ovary (CHO) and human choriocarcinoma-derived BeWo cells. But this property is not shared by all cells, and the dynamic pleiomorphic membrane organization of early endocytic organelles has so far precluded an ultrastructural characterization and definition of boundaries between early endosomes and recycling endosomes. Colocalization of proteins such as rab11 and RME-1 (ref. 2) with endocytosed transferrin at a late stage of its intracellular itinery nevertheless implies some form of biochemical heterogeneity in early endocytic organelles. In this respect it is immaterial whether this heterogeneity reflects the existence of separate compartments with their surrounding membranes or dynamic assemblies of tubulovesicular membranes with distinct physicochemical properties.

Food for the latter thought derives from several pieces of evidence. Early endosomes contain phosphatidylinositol-3-phosphate (PI(3)P) which specifically interacts with FYVE- and PX-domain containing peripheral membrane proteins[3,4]. PI(3)P is present on internal vesicles and the limiting membrane of early endocytic organelles[5], and is involved in multivesicular body (MVB) biogenesis[6]. On the other hand, recycling endosomes have a relatively high content of the raft-organizing lipids cholesterol and glycosphingolipids[7]. Important conceptual progress has also been provided by recent observations from Marino Zerial's laboratory. They found that rab5, which controls fusion of endocytic vesicles with early endosomes[8], and rab4 and rab11, which regulate recycling from early endosomes[9,10], sequentially organize the endocytic/recycling pathway of transferrin in domains within a continuous membrane structure. The distinct nature of these domains is reflected by their differential sensitivity to pharmacological tools such as brefeldin A and the phosphatidylinositol kinase inhibitor wortmannin. Interestingly, these domains are distinct for rab5-controlled entry into the early endosomal system, and rab4/rab11-dependent departure to the cell surface[11]. The two gateways are connected by an intermediate domain defined by rab5/rab4, from which fast transferrin recycling occurs. These domains are probably generated through the recruitment of specific effectors by the activated membrane-bound GTPases. In this sense, rab proteins might serve as nucleation sites for the assembly of oligomeric complexes. For example, rab5 has been shown to functionally recruit the phosphatidylinositol 3-kinase hVPS34, and at least two PI(3)P-binding proteins,

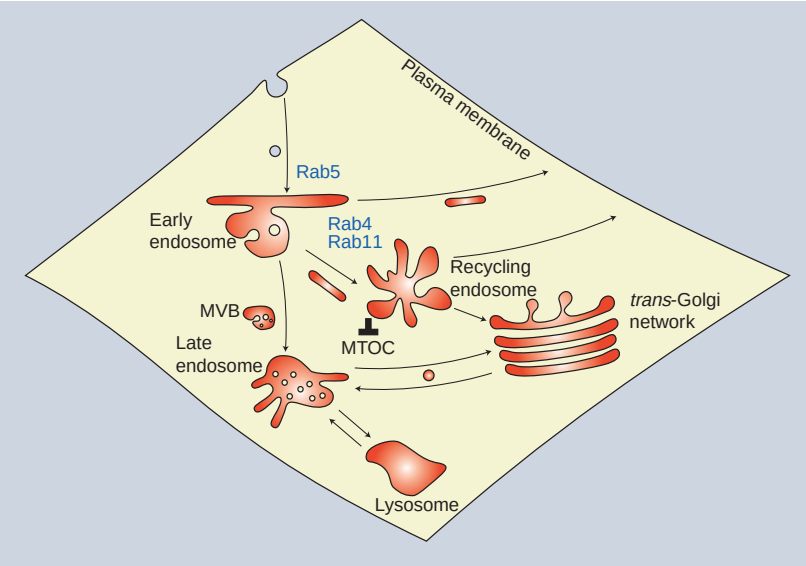


Figure 1 **Overview of endocytic pathways and compartments (see text for details).**

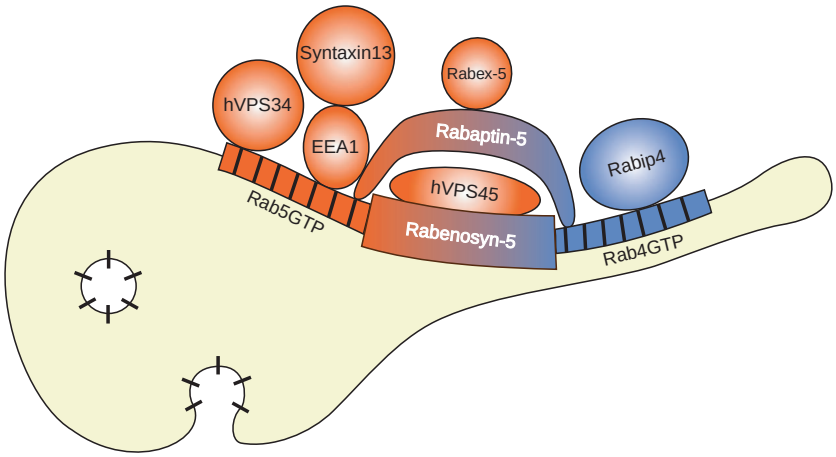


Figure 2 **Proposed model for the bivalent effector function of rabenosyn-5 connecting rab5 and rab4 domains on early endosomes. Activation of rab5 by the guanine-nucleotide-exchange complex rabaptin-5/rabex-5 allows hVPS34 to be recruited and locally increases PI(3)P concentration (indicated by black sticks). Both PI(3)P and rab5GTP are required to recruit EEA1 and rabenosyn-5 to early endosomes. Rabenosyn-5 can then bind to a rab4 domain formed by rab4GTP, its effector rabip4 and PI(3)P to connect the two domains. Rabenosyn-5 and rabaptin-5 may provide spatial control integrating rab5 dependent fusion and rab4-dependent fission events. Alternatively these bivalent linkers may provide structural integrity to the junction between early endosomal vacuoles and tubules, the latter of which are thought to be involved in recycling.**

EEA1 and rabenosyn-5, to early endosomes (Fig. 2). In turn, EEA1 directly binds to the t-SNARE syntaxin 13, which regulates transferrin recycling. Rabenosyn-5 binds to the sec1 homologue hVPS45, which interacts with syntaxins 4, 6, 13, and Tlg2p (ref. 12,13) and thus could function to control pairing of SNARE complexes operating in endosomal recycling and transport to the TGN.

Given the sequential transfer of endocytosed tranferrin from a rab5 domain to the distal domain containing rab5 and rab4, de Renzis *et al.*[1] have now asked how these domains functionally communicate with each other. Guided by the premise that this might occur via proteins that interact directly with both rab5 and rab4, or a linker protein physically connecting their effector complexes, they eluted cytosolic proteins that were specifically bound to a rab5GTP-affinity column, loaded them on a rab4GTP column and determined which of these also bound to rab4GTP. Using this system they confirmed that rabaptin-5 interacts with both rab5 and rab4, and found that two already known rab5 effectors, rabaptin-5β (ref. 14) and rabenosyn-5 (ref. 12; Fig. 2) also directly bound to rab4. Rabaptin-5 and rabaptin-5β are coiled-coil proteins in which the amino- and carboxy-terminal coiled domains are connected by a hinge region that contains a single copy of the tripeptide DPF, which functions as a protein-interaction module. Both proteins are important for early endosome fusion *in vitro*, and rabaptin-5 serves as an anchoring site for the rab5-guanine-nucleotide-exchange protein rabex-5. Gratifyingly, and consistent with their domain model, none of these proteins bound to rab11. Rabenosyn-5 contains an internal FYVE finger required for its association with early endosomes and five copies of the NPF tripeptide repeats that may provide binding sites for Eps15 homology (EH) domain proteins. Because many of the EH proteins act as accessory proteins in cargo selection and clathrin-coated pit formation at the plasma membrane, this suggests that rabenosyn-5 may have a related function in regulating protein sorting and recycling from early endosomes. The presence of the EH domain proteins RME-1 (ref. 2) and possibly γ-synergin[15] on early endosomes/recycling endosomes bears testimony to this exciting notion.

The binding sites for rab5 and rab4 on rabenosyn-5 mapped to distinct regions in the effector, consistent with the idea that rabenosyn-5 might act as a linker between the two rab proteins. Direct proof for this was found in a biochemical *in vitro* binding assay, which revealed that rab5 and rab4 bound simultaneously to rabenosyn-5, and by semi-quantitative triple label fluorescence microscopy where rabenosyn-5 nearly completely colocalized with rab5. About 50% of these structures also contained rab4, and these often segregated in a more elongated subdomain, suggesting that rabenosyn-5 serves to cluster rab5 and rab4 domains. Indeed, moderate overexpression of rabenosyn-5 or rabaptin-5, greatly enhanced colocalization of rab4 and rab5 endosomes. This phenotype was not seen with rabenosyn-5 mutants that lacked either the rab4-binding region or the FYVE domain. These results convincingly show that the rab4-binding region and the FYVE domain are required for the coalescence of rab4 into rab5 domains. At the same time the number of rab4/rab11-containing endosomes decreased, suggesting that the additional rab4 molecules now associating with the rab5/rabenosyn-5 were relocated from the rab4/rab11 domain. That these rabenosyn-5-induced changes in domain organization were of functional consequence was documented in transferrin-transport assays. Overexpression of rabenosyn-5 caused rapid recycling of transferrin and strongly reduced transfer of transferrin to recycling domains,

consistent with earlier observations seen after overexpression of rab4 (ref. 16). This effect required that rabenosyn-5 interact with rab4, as a rabenosyn-5 mutant lacking a rab4-binding site, or co-expression of a dominant-negative rab4 mutant and rabenosyn-5 did not elicit this phenotype. Because the transport assays were done *in vivo*, two scenarios could explain the effects seen after overexpression of rabenosyn-5. Either reduced transport to recycling endosomes is a consequence of enhanced rapid recycling, which would reduce the pool of transferrin available for the slow-recycling pathway. Alternatively, the primary effect of overexpressed rabenosyn-5 is a less efficient 'slow' recycling pathway, that might lead to compensatory upregulation of the 'fast' recycling route.

The interesting findings reported in this paper, as often, raise many additional questions. For instance, we would like to know whether effector-linking of rab protein domains can be extrapolated to the degradative branch of the endocytic pathway, or even heterotypic transport routes such as between endosomes and the Golgi complex. Intriguingly, rabaptin-5 also binds to rab33b, which is involved in retrograde transport through the Golgi complex[17]. On a different level, why are there at least three ubiquitously expressed bivalent effectors of rab5 and rab4. Perhaps rabenosyn-5, rabaptin-5 and rabaptin-5β act cooperatively to ensure a sufficiently strong physical link between the rab4 and rab5 domains. Alternatively, redundancy might be required because bifunctional rab effectors may act in parallel transport steps running from a given domain. Rabenosyn-5 is also known to be involved in delivering of newly synthesized cathepsin D from the Golgi complex to lysosomes[12]. It will be interesting to learn from further studies about this other function of rabenosyn-5. The exciting findings of de Renzis *et al.*[1] are of considerable interest, as they provide an appealing and testable model for the spatio-temporal coupling of entry to and exit from early endosomes, an endocytic sorting station that determines the fate of many biologically important proteins. □

Magda Deneka and Peter van der Sluijs are in the Department of Cell Biology and Institute of Biomembranes, University Medical Center, 3584 CX Utrecht, The Netherlands.
e-mail: pvander@knoware.nl

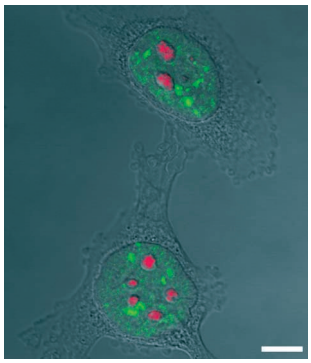
1. de Renzis, S., Sönnichsen, B. & Zerial, M. *Nature Cell Biol.* **4,** 124–133 (2002).
2. Lin, S. X., Grant, B., Hirsch, D. & Maxfield, F. *Nature Cell Biol.* **3,** 567–572 (2001).
3. Patki, V., Lawe, D. C., Corvera, S., Virbasius, J. V. & Chawla, A. *Nature* **394,** 433–434 (1998).
4. Simonsen, A. & Stenmark, H. PX domains: *Nature Cell Biol.* 3, E179–E182 (2001).
5. Gillooly, D. J. *et al. EMBO J.* **19,** 4577–4588 (2000).
6. Fernandez-Borja, M. *et al. Curr. Biol.* **9,** 55–58 (1999).
7. Gruenberg, J. *Nature Rev. Mol. Cell Biol.* **2,** 721–730 (2001).
8. Bucci, C. *et al. Cell* **70,** 715–728 (1992).
9. de Wit, H. *et al. Mol. Biol. Cell* **12,** 3703–3715 (2001).
10. Ullrich, O., Reinsch, O., Urbe, S., Zerial, M. & Parton, R. *J. Cell Biol.* **135,** 913–924 (1996).
11. Sönnichsen, B., De Renzis, S., Nielsen, E., Rietdorf, J. & Zerial, M. *J. Cell Biol.* **149,** 901–913 (2000).
12. Nielsen, E. *et al. J. Cell Biol.* **151,** 601–612 (2000).
13. Bryant, N. J. & James, D. E. *EMBO J.* **13,** 3380–3388 (2001).
14. Gournier, H., Stenmark, H., Rybin, V., Lippe, R. & Zerial, M. *EMBO J.* **17,** 1930–1940 (1998).
15. Page, L., Sowerby, P. J., Lui, W. W. Y. & Robinson, M. S. *J. Cell Biol.* **146,** 993–1004 (1999).
16. van der Sluijs, P. *et al. Cell* **70,** 729–740 (1992).
17. Valsdottir, R. *et al. FEBS Lett.* **508,** 201–209 (2001).

Proteomics of the nucleolus

Despite our increased understanding of the dynamic nature of the nucleus, and the heterogeneity of the suborganelles it contains, our knowledge of the factors they contain and the functions they mediate is still far from clear. One of the best-characterized suborganelles is the nucleolus, a nuclear body that mediates ribosome subunit biogenesis, and more recently has been implicated in other roles, such as transport of RNA. To determine if we have overlooked other equally critical components and functions of the nucleolus, the laboratories of Lamond and Mann have taken on the Herculian task of elucidating the proteome of this still-mysterious body (*Curr. Biol.* **12,** 1–11, 2002).

From purified nucleoli, Andersen and colleagues identified 271 proteins, of which over 30% were previously unidentified or uncharacterized. Identification of the isolated proteins were facilitated by directed searches of the draft human genome sequence. The nucleolar proteome that emerges is more complex than previously thought and supports additional roles for this suborganelle. For example, translation factors such as EIF5A, EIF4A1 and ETF1, which had not previously been shown to pre-assemble in the nucleolus, showed up in searches. This may support a role for nucleolar translation activity. To confirm that these were indeed *bona fide* nucleolar components, the authors constructed YFP fusion proteins with 18 of these factors. Of the 18 examined, 15 localized to nucleoli, in a variety of localization patterns. Next, Andersen and colleagues repeated the screen on cells in which transcription had been inhibited. They found that a subset of factors became enriched in nucleoli, suggesting that the nucleolus is a highly dynamic suborganelle whose molecular composition adapts to the metabolic state of the cell.

In addition, this work has also identified a new suborganelle – paraspeckles. In an accompanying paper from the same lab (*Curr. Biol.* **12,** 13–25, 2002), Fox and colleagues characterize one of the



novel factors, PSP1 (paraspeckle 1). PSP1 is recruited to the nucleolus after inhibition of transcription, but is localized to paraspeckles at steady state (the figure shows YFP–PSP1 in green and nucleolin, which localizes to the nucleolus, in red; scale bar 5 μm). Paraspeckles are found in the interchromatin space, generally at 10–20 copies per cell. Two other factors identified in the screen, PSP2 and p54/nrb are also localized here. So what is the function of this new body? The authors have observed that paraspeckles often colocalize with splicing speckles. Furthermore, when transcription is inhibited, paraspeckle factors relocalize to the nucleolar periphery. This suggests that, in common with the nucleolus, paraspeckles are dynamic bodies that respond to the changing environment of the cell. From the results so far, the authors speculate that paraspeckles may have a role in transcriptional regulation, although more work will be required to show that this is indeed the true function of this new body.

ALISON SCHULDT