A model for Rab GTPase localization

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Abstract

The human genome encodes almost 70 Rab GTPases. These proteins are C-terminally geranylgeranylated and are localized to the surfaces of distinct membrane-bound compartments in eukaryotic cells. This mini review presents a working model for how Rabs achieve and maintain their steady-state localizations. Data from a number of laboratories suggest that Rabs participate in the generation of macromolecular assemblies that generate functional microdomains within a given membrane compartment. Our data suggest that these complex interactions are important for the cellular localization of Rab proteins at steady state.

Introduction

The human genome encodes almost 70 Rabs and Rab-like proteins [1,2], and members of this large family of Ras-like GTPases are localized to distinct membrane-bound compartments [2]. While some of the Rabs are tissue-specific, many are ubiquitous in their expression. Rabs are versatile catalysts: (i) they participate in receptor cargo collection during transport vesicle formation, (ii) they enable motor proteins to interact with membranes to drive vesicle motility, and (iii) they interact with more additional components to mediate the complex events of accurate docking and fusion of transport vesicles with their targets [2,3].

Within the endocytic pathway, Rab GTPases serve to organize microdomains by recruiting specific sets of effector proteins to distinct regions [2–5]. These collections of effector proteins act in concert to mediate endosomal functions such as endosome–endosome fusion, receptor segregation for recycling to the plasma membrane, or packaging of cargo into vesicles bound for another compartment. Zerial and coworkers [6] have shown that early endosomes are comprised of at least three distinct domains containing Rab4, Rab5 or Rab11. Using video microscopy of living cells, we have shown that Rab9 and Rab7 are segregated in discrete late endosome microdomains [5].

Rab delivery to membranes

Rabs are delivered to membranes by a protein named GDI (GDP dissociation inhibitor; Figure 1) [7,8]. GDI binds with strong preference to prenylated Rabs in their inactive, GDP-bound conformations, and complexes of Rab bound to GDI possess all of the information needed to deliver Rabs to their appropriate targets. Our current model for Rab delivery is that Rab–GDI complexes are recognized by membrane-associated proteins that we have named GDFs (GDI displacement factors). GDFs have the capacity to displace the Rab from GDI and permit it to hop into the adjacent membrane. GDFs do not represent stable binding sites – rather, they have the capacity to act catalytically and should be viewed as 'entry sites' that enable a Rab to become membrane associated. Once on the membrane, a Rab can be activated by a specific GEF (guanine nucleotide-exchange factor) and subsequently bind to Rab-specific effectors to achieve its steady-state localization. If the Rab fails to encounter its GEF and cognate effectors, it can be extracted from the membrane by unoccupied GDI for redelivery to another membrane [8].

A chicken and egg problem

How are Rabs delivered to different locations within the cell? The limited information currently available indicates that GDFs are present either in the early secretory pathway or within the endocytic pathway [8]. In addition, the one GDF that has been analysed biochemically shows specificity for multiple Rabs of the endocytic pathway [8]. Thus we have proposed that Rabs are delivered to either the early secretory pathway or the endocytic pathway. Once delivered, the Rab will diffuse within the plane of the membrane and be stabilized by specific effector binding. In the endocytic pathway, the Rab may move between endosome types by normal trafficking routes, until it finds its binding partners.

This model requires the pre-existence of a membrane microdomain in which Rabs are already clustered in association with Rab effectors. But how would the first Rab become localized? Most Rab effectors are not integral membrane proteins – they are cytosolic proteins that associate with membranes by virtue of multiple protein–protein or protein–lipid interactions. For example, EEA1 (early endosome antigen-1) binds to early endosome membranes via phosphatidylinositol 3-phosphate and Rab5 [2,3]. Thus if there were no pre-existing microdomains, Rab5 could enter the endocytic pathway via GDF. It could then recruit from the cytosol, the kinase that generates phosphatidylinositol 3-phosphate; this would in turn drive the membrane association of EEA1. Other effectors would then bind, and

Key words: endosome, GDP dissociation inhibitor (GDI), GTPase, mannose 6-phosphate receptor, membrane microdomain, Rab.

Abbreviations used: EEA1, early endosome antigen-1; GDI, GDP dissociation inhibitor; GDF, GDI displacement factor; GEF, guanine nucleotide-exchange factor; MPR, mannose 6-phosphate receptor; siRNA, small interfering RNA; TIP47, tail-interacting protein of 47 kDa.

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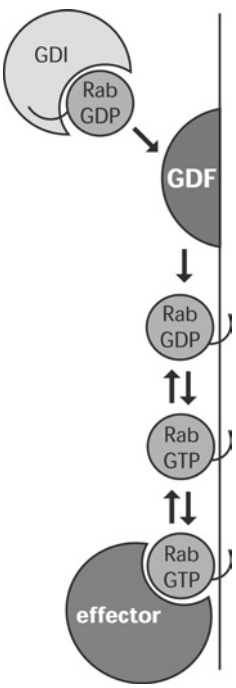
Figure 1 | A model for Rab recruitment on to membranes
Prenylated Rabs in their GDP-bound conformations are present in cytosol bound to GDI. GDI presents the Rab to the membrane; according to our model, a GDF displaces the GDI and allows the prenyl Rab to bind to the membrane. The Rab can then be activated by a specific GEF, converting the Rab into its GTP-bound form. The GTP-bound Rab is then able to bind effectors, and will be stabilized on the membrane by effector binding. If a Rab is not activated, it can be re-extracted by GDI for redelivery to another membrane.



whatever membrane began this assembly process would become an early endosome. Of course, Rabs are always present in cells, so such *de novo* organelle formation would seldom if ever be required.

According to this model, initial Rab delivery to the endocytic or secretory pathways would require selectivity in their recognition by GDF; for overall steady-state localization, selective recognition by microdomain constituents would also be essential. The importance of microdomain interactions for Rab stability was highlighted by recent work from this laboratory. To our surprise, we found that cellular depletion of a Rab9 effector led to a significant destabilization of Rab9 protein [9]. This was not expected because investigators in this research area generally think of prenylated Rabs as either existing in the outer leaflet of an organelle membrane or bound to the carrier protein, GDI, in the cytosol. Indeed, prenylated Rabs are considered independent entities, despite their ability to interact with a variety of important effector proteins [2,4]. These findings suggest that Rab–effector interactions are key to Rab localization. Much work needs to be done to understand the determinants of each Rab that are recognized by effectors, and which effector interactions will be most important for a given Rab protein. We believe that certain interactions will be more important than others in terms of Rab stability and localization.

Rab9 localization

Rab9 is present on late endosomes and is required for the transport of MPRs (mannose 6-phosphate receptors) from late endosomes to the *trans*-Golgi network [10,11]. Rab9 is segregated from Rab7 in late endosomes and appears to form a microdomain that also contains MPRs [5]. TIP47 (tail-interacting protein of 47 kDa) is a soluble protein that binds with high specificity to the cytosolic domains of MPRs [12–14]. In addition, TIP47 binds with even higher affinity to Rab9 [9,15]. The binding of Rab9 to TIP47 enhances its affinity for MPR cytoplasmic domains [16]. In this way, Rab9 facilitates MPR cargo collection during the process of transport vesicle formation.

To gain insight into interactions responsible for microdomain formation, we took an siRNA (small interfering RNA) approach to deplete cells systematically of individual microdomain constituents and then investigate the consequences [9]. We detected minimal changes in the steady-state levels and localizations of MPRs and Rab9 proteins in cells depleted of TIP47. However, significant changes in protein stability were identified, as determined by pulse-chase labelling of the proteins and examination of their turnover. In a previous study, antisense depletion to reduce TIP47 protein levels by approx. 50% led to MPR mis-sorting to the lysosome and it reduced the CI-MPR half-life 2-fold [12]. Thus, as expected, TIP47 siRNA decreased the stability of CI-MPR 1.9-fold, reducing the protein half-life from 26 to 14 h. The change in turnover was not due to a general increase in protein turnover because the LDL (low-density lipoprotein) receptor lifetime was unchanged. Unexpectedly, the half-life of Rab9 protein decreased from 32 to 8 h, a 4-fold decrease in stability due to loss of TIP47.

TIP47 depletion destabilized both Rab9 and MPRs, but not Rab7: the half-life of Rab7 protein was completely unchanged [9]. Although a significant fraction of Rab9-containing compartments contain Rab7, loss of Rab9 had no effect on the steady-state level or turnover of Rab7. This suggests that the Rab9 microdomain is regulated independent of neighbouring Rab7 protein and, presumably, Rab7-interacting proteins.

Rab9 gene expression was induced upon TIP47 depletion to compensate for its increased rate of degradation [9]. How TIP47 depletion triggers Rab9 transcription is completely unknown. These experiments demonstrate that we cannot think of Rabs as truly independent constituents; their interactions with effectors can influence greatly their cellular fates.

Rab hypervariable domains and localization

The C-terminal approx. 30 amino acid residues of Rab proteins are called hypervariable domains as they represent

the most divergent elements of Rab sequences [17]. These domains are primarily unstructured [18,19]. Because of their unique sequence signatures, hypervariable domains have been postulated to be key for Rab protein localization [20]. However, more recent studies have suggested that determinants of Rab localization are likely to be more complex [21,22].

Rab C-termini differ not only in sequence, but also in length (from 27 to 43 residues based on early sequence alignments [17]) and they usually terminate with two prenylated cysteine residues. Sequence variability between Rab proteins begins within the last α-helix (so-called helix 5) and extends to the C-termini of the proteins. It is noteworthy that the length differences between Rab C-termini include the length of the last α-helix and the remaining unstructured sequences. Because Rab C-termini are unstructured, they have not been detected in Rab crystal structures. The first structure of a hypervariable domain comes from the work of Goody and co-workers [23] who determined the structure of a monoprenylated Rab protein (yeast Ypt1p) in complex with GDI. The Rab hypervariable domain extends down the side of GDI in an extended conformation. Two key hydrophobic residues in the Rab make important contacts with GDI, and appear to attach the hypervariable domain on to the face of GDI. Other Rab hypervariable domains retain hydrophobic residues near this position that can serve this interaction role [23,24]. Finally, the monoprenyl group lies in a pocket at the bottom of GDI [23]. Thus hypervariable domains provide a polypeptide extension between the Rab prenylation site and the globular GTPase domain. This feature suggests further that Rabs may extend some distance from membranes by virtue of a long, unstructured C-terminal 'tether'. A final noteworthy feature of hypervariable domain sequences is their high content of proline and glycine residues which probably contribute to helix breakage and generation of the extended structure that is important for both GDI binding [23], Rab geranylgeranylation [24] and probably also other protein interactions.

If hypervariable domains are targeting 'address tags', they should be recognized by targeting receptors. To date, the only proteins that have been identified that interact with hypervariable domains are GDI and REP (Rab escort protein) and neither of these shows any Rab specificity. Very recently, Rab1 was shown to be phosphorylated in its hypervariable domain during mitosis [25]. This modification was needed for interaction of Rab1 with Polo-like kinase 1, but this specific interaction is not responsible for Rab1 localization [25].

More research work is needed to test whether Rabs possess binding partners that recognize their hypervariable domain sequences, a requirement of a model in which these domains serve as 'address-tags'. A number of effectors have been shown to interact with Rabs using entirely non-hypervariable domain interactions. For example, Rabaptin-5 binds to Rab5A, Rab5B and Rab5C using non-hypervariable domain sequences [26], and EEA1 does not need hypervariable domain sequences to bind Rab5 [27]. Finally, effectors such as Rim1, Rim2, Noc2 and Rabphilin interact with numerous Rab proteins that contain entirely distinct C-termini (see [28]). Most importantly, Rabs with very different hypervariable domains share both effectors and localizations.

A new concept: 'key' effectors

To fully understand Rab localization, it will be essential to determine the protein–protein interactions that are most important for the localization of each Rab in mammalian cells. For Rab9, TIP47 seems to be most important for Rab9 stability and localization. But TIP47 is not a receptor for Rab9 because it is a predominantly soluble protein [12]. Thus TIP47 appears to be key for the formation of a microdomain into which Rab9 becomes stabilized [9]. We propose that each Rab has its own 'key' effector that is essential for its steady-state localization. Key effector interactions will have the capacity to localize a Rab protein and to stabilize that Rab. Key effectors may or may not recognize hypervariable domain sequences; nevertheless, their identities will be important to determine.

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References

1 Colicelli, J. (2004) Science STKE 2004, re13
2 Zerial, M. and McBride, H. (2001) Nat. Rev. Mol. Cell Biol. **2**, 107–117
3 Miaczynska, M. and Zerial, M. (2002) Exp. Cell Res. **272**, 8–14
4 Pfeffer, S. (2003) Cell (Cambridge, Mass.) **112**, 507–517
5 Barbero, P., Bittova, L. and Pfeffer, S.R. (2002) J. Cell Biol. **156**, 511–518
6 Sonnichsen, B., De Renzis, S., Nielsen, E., Rietdorf, J. and Zerial, M. (2000) J. Cell Biol. **149**, 901–914
7 Soldati, T., Riederer, M.A. and Pfeffer, S.R. (1993) Mol. Biol. Cell **4**, 425–434
8 Pfeffer, S.R. and Aivazian, D. (2004) Nat. Rev. Mol. Cell Biol. **5**, 1–12
9 Ganley, I., Carroll, K., Bittova, L. and Pfeffer, S.R. (2004) Mol. Biol. Cell **15**, 5420–5430
10 Lombardi, D., Soldati, T., Riederer, M.A., Goda, Y., Zerial, M. and Pfeffer, S.R. (1993) EMBO J. **12**, 677–682
11 Riederer, M.A., Soldati, T., Shapiro, A.D., Lin, J. and Pfeffer, S.R. (1994) J. Cell Biol. **125**, 573–582
12 Diaz, E. and Pfeffer, S.R. (1998) Cell (Cambridge, Mass.) **93**, 433–443
13 Krise, J.P., Sincock, P.M., Orsel, J.G. and Pfeffer, S.R. (2000) J. Biol. Chem. **275**, 25188–25193
14 Orsel, J.G., Sincock, P.M., Krise, J.P. and Pfeffer, S.R. (2000) Proc. Natl. Acad. Sci. U.S.A. **97**, 9047–9051
15 Hanna, J., Carroll, K. and Pfeffer, S.R. (2002) Proc. Natl. Acad. Sci. U.S.A. **99**, 7450–7454
16 Carroll, K.S., Hanna, J., Simon, I., Krise, J., Barbero, P. and Pfeffer, S.R. (2001) Science **292**, 1373–1376
17 Chavrier, P., Vingron, M., Sander, C., Simons, K. and Zerial, M. (1990) Mol. Cell. Biol. **12**, 6578–6585
18 Neu, M., Brachvogel, V., Oschkinat, H., Zerial, M. and Metcalf, P. (1997) Proteins **27**, 204–209
19 Ostermeier, C. and Brunger, A.T. (1999) Cell (Cambridge, Mass.) **96**, 363–374
20 Chavrier, P., Gorvel, J.P., Stelzer, E., Simons, K., Gruenberg, J. and Zerial, M. (1991) Nature (London) **353**, 769–772
21 Stenmark, H., Valencia, A., Martinez, O., Ullrich, O., Goud, B. and Zerial, M. (1994) EMBO J. **13**, 575–583
22 Ali, B.R., Wasmeier, C., Lamoreux, L., Strom, M. and Seabra, M.C. (2004) J. Cell Sci. **117**, 6401–6412

23 Rak, A., Pylypenko, O., Durek, T., Watzke, A., Kushnir, S., Brunsveld, L., Waldmann, H., Goody, R.S. and Alexandrov, K. (2003) Science **302**, 646–650
24 Rak, A., Pylypenko, O., Niculae, A., Pyatkov, K., Goody, R.S. and Alexandrov, K. (2004) Cell (Cambridge, Mass.) **117**, 749–760
25 Preisinger, C., Körner, R., Wind, M., Lehmann, W.D., Kopajtich, R. and Barr, F.A. (2005) EMBO J. **24**, 753–765
26 Zhu, G., Zhai, P., Liu, J., Terzyan, S., Li, G. and Zhang, X.C. (2003) Nat. Struct. Mol. Biol. **11**, 975–983
27 Merithew, E., Stone, C., Eathiraj, S. and Lambright, D.G. (2003) J. Biol. Chem. **278**, 8494–8500
28 Fukuda, M. (2003) J. Biol. Chem. **278**, 15373–15380

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