Current Biology, Vol. 12, R360–R362, May 14, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S0960-9822(02)00860-6

GTPase Signaling: Bridging the GAP between ARF and Rho

Dispatch

Lorraine C. Santy and James E. Casanova

Membrane traffic and actin cytoskeleton dynamics are intimately linked, and GTPases of the Rho and ARF families may work together to regulate both. Recent studies have identified a family of GTPase activating proteins (GAPs) that contain both ARF-GAP and Rho-GAP domains, providing the first direct link between these two signaling pathways.

Dynamic reorganization of the actin cytoskeleton is required for a wide variety of cellular functions, including adhesion, motility, cytokinesis, phagocytosis and some aspects of vesicular transport. GTPases of the Rho family — Rho, Rac and Cdc42 — are important regulators of actin dynamics (for review, see [1]). In most experimental systems, the active, GTP-bound forms of Rac, Cdc42 and RhoA stimulate the formation of lamellipodia, filopodia or stress fibers, respectively [2]. Importantly, Rho family GTPases may also regulate membrane traffic; mutants of RhoA and Rac inhibit internalization of transferrin receptors in HeLa cells [3], while Cdc42 regulates endocytosis in immature dendritic cells [4], and polarized secretion in MDCK cells [5].

Unlike the Rho GTPases, the ARF family of GTPases, comprising ARF1–6, are best known for their role in vesicular transport. Most attention has focused on ARF1 and ARF6 function. ARF1 localizes primarily to the Golgi complex, where it initiates carrier vesicle formation by nucleating the assembly of coat protein complexes [6]. In contrast, ARF6 has been localized to the plasma membrane/endosomal system, where it regulates the recycling of a subset of membrane proteins [7]. Recent evidence indicates that ARFs also play an important role in regulating cytoskeleton assembly. ARF1 stimulates recruitment of the scaffolding protein paxillin to focal adhesions [8], and activation of ARF6 leads to the disassembly of actin stress fibers and the formation of structures resembling lamellipodia [9,10]. Similarly, expression of a dominant-negative ARF6 mutant inhibits membrane ruffling and formation of lamellipodia in response to epidermal growth factor (EGF), colony-stimulating factor 1 (CSF-1) and bombesin, presumably by inhibiting the activation of Rac [11–13]. In support of this hypothesis, activation of ARF6 has recently been found to stimulate the activation of endogenous Rac1 in MDCK cells [14]. Together, these findings suggest that significant crosstalk exists between ARF and Rho family members.

GTPase activating proteins (GAPs), which stimulate the hydrolysis of bound GTP, regulate the activities of both Rho and ARF GTPases. Until recently, these proteins could be divided into two classes, each possessing either Rho-GAP or ARF-GAP activity, but not both. However, the laboratories of Paul Randazzo [15] and Phillip Hawkins [16] have now identified three new proteins, ARAP1, ARAP2 and ARAP3, that each contain both ARF-GAP and Rho-GAP domains (Figure 1).

An additional feature of these proteins is the presence of five pleckstrin homology (PH) domains. These domains typically mediate the interaction of proteins with phosphoinositides and, in fact, ARAP3 was isolated on the basis of its affinity for immobilized PtdIns(3,4,5)P_3 [16]. ARAP3 binds to PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 with high affinity via its amino-terminal PH domain [16]. Consistent with this binding specificity, the ARF-GAP activity of both ARAP1 and ARAP3 is dependent upon the presence of PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2 [15,16]. Other ARF-GAPs, including ASAP1 [17], PAPα/PAG3 [18], GIT1/CAT1 [19], ACAP1 and ACAP2 [20] have similar phosphoinositide-dependent catalytic activity, although the activating phosphoinositide species varies, and does not always require the presence of a PH domain. In contrast, the Rho-GAP activity of both ARAP1 and ARAP3 is clearly phosphoinositide-independent [15,16]. What, then, is the function of the other four PH domains? One or more of these may prove to be involved in membrane targeting, but this remains an important area for future study.

Like other ARF-GAPs, ARAP1, ARAP2 and ARAP3 each contain several ankyrin repeats immediately carboxy-terminal to the ARF-GAP domain, and these repeats may function in protein–protein interactions (Figure 1). These proteins also have several important structural differences. First, ARAP2 and ARAP3 contain an additional amino-terminal protein interaction module, a sterile-α motif (SAM), which is lacking in ARAP1. Second, sequence analysis predicts the presence of a Ras-interacting domain in ARAP1 that is absent in ARAP2 and ARAP3. Whether ARAP1 interacts with Ras directly remains to be determined. Third, ARAP2 contains a unique region with homology to the switch 2 domain of the small GTPase Rab13. Finally, and importantly, the Rho-GAP domain of ARAP2 lacks the key catalytic arginine residue found in most GAPs (the so-called 'arginine finger') and is therefore unlikely to possess Rho-GAP activity. Although ARAP2 has not been characterized further, ARAP1 and ARAP3 have important functional differences. ARAP1 displayed a clear substrate preference for ARF1 and ARF5 *in vitro*, whereas ARAP3 appeared to be specific for ARF6. Similarly, ARAP1 Rho GAP activity was directed primarily towards RhoA, while ARAP3 used RhoA, Rac1 or Cdc42 without preference [15,16].

Endogenous ARAP1 localized primarily to the Golgi complex, and could also be observed at the plasma membrane during cell spreading. Consistent with the

Department of Cell Biology, University of Virginia, Health Sciences Center, P.O. Box 800732, Charlottesville, Virginia 22908-0732, USA. E-mail: jec9e@virginia.edu

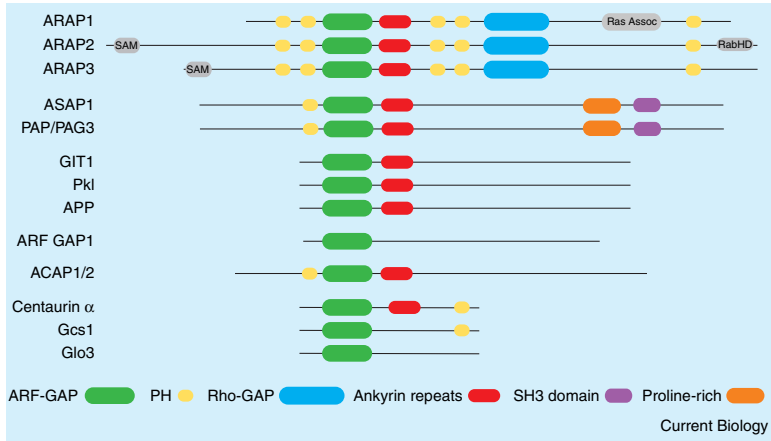

Figure 1. Domain organization of ARF GAP family members.

Domains with well-characterized functions are colored as described in the key at bottom. Abbreviations: Ras Assoc, putative Ras-association domain; SAM, sterile-α motif; RabHD, Rab13 homology domain.

idea that ARAP1 may regulate ARF1 function at the Golgi, overexpression of ARAP1 induced redistribution of some, but not all, Golgi markers, suggesting that the effects of ARAP1 were specific to subregions of the Golgi. ARAP1 also slowed reassembly of the Golgi after exposure to brefeldin A, a drug that inhibits ARF activation. These actions of ARAP1 at the Golgi required its ARF-GAP activity, but were independent of Rho-GAP function [15]. Overexpression of ARAP1 in NIH3T3 cells also affected the actin cytoskeleton, inducing a loss of stress fibers and cell rounding (which required Rho-GAP activity) and increased numbers of filopodia (which required ARF-GAP activity). Direct measurement of GTPase activation in transfected HEK293 cells revealed a two-fold increase in active Cdc42, consistent with the observed increase in filopodia. Surprisingly, however, no corresponding decrease in Rho activity was detected [15]. One plausible explanation for this is that the interaction of ARAP1 with RhoA may be highly localized, affecting only a small fraction of the total RhoA pool.

In contrast, ARAP3 did not localize to the Golgi, but was instead diffusely cytosolic in resting cells. Association of ARAP3 with the plasma membrane was enhanced by agents that increase cellular levels of PtdIns(3,4,5)P_3, such as EGF and platelet-derived growth factor (PDGF). This translocation was inhibited by the phosphatidylinositol 3-kinase inhibitor wortmannin, suggesting that plasma membrane localization of this protein is mediated by direct binding to PtdIns(3,4,5)P_3. Overexpression of ARAP3 in resting cells led to a reduction in the number of stress fibers, and a dramatic reduction in membrane ruffling induced by PDGF, which could be reversed by mutation of the amino-terminal PH domain, or simultaneous mutation of both ARF-GAP and Rho-GAP domains. Mutants lacking either ARF-GAP or Rho-GAP activity also inhibited membrane ruffling, but less potently than double mutants, suggesting that both ARF6 and a Rho family member (presumably Rac) act cooperatively in this process [16]. This is consistent with our recent observations that ARF6 and Rac function in a signaling cascade leading to the formation of lamellipodia in MDCK cells [14].

Together, these observations suggest that the ARAPs can directly couple phosphoinositide signaling to both vesicular transport and organization of the actin cytoskeleton. However, numerous questions remain to be answered. What is the substrate specificity of these proteins *in vivo*? Why so many PH domains, and what role does each play in ARAP function? Does the Rho-GAP domain of ARAP1 function in any aspect of Golgi transport? Does ARAP2 serve as a Rho effector rather than a GAP? These two reports provide a solid foundation from which to pursue the answers to such questions.

References

1. Van Aelst, L. and D'Souza-Schorey, C. (1997). Rho GTPases and signaling networks. Genes Dev. *11*, 2295–2322.
2. Hall, A. (1998) Rho GTPases and the actin cytoskeleton. Science *279*, 509–514.
3. Lamaze, C., Chuang, T.H., Terlecky, L.J., Bokoch, G.M. and Schmid, S.L. (1996). Regulation of receptor-mediated endocytosis by Rho and Rac. Nature *382*, 177–179.
4. Garrett, W.S., Chen, L.M., Kroschewski, R., Ebersold, M., Turley, S., Trombetta, S., Galan, J.E. and Mellman I. (2000). Developmental control of endocytosis in dendritic cells by Cdc42. Cell *102*, 325–334.
5. Musch, A., Cohen, D., Kreitzer, G. and Rodriguez-Boulan, E. (2001). Cdc42 regulates the exit of apical and basolateral proteins from the trans-Golgi network. EMBO J. *20*, 2171–2179.
6. Donaldson, J.G. and Klausner, R.D. (1994). ARF: a key regulatory switch in membrane traffic and organelle structure. Curr. Opin. Cell Biol. *6*, 527–532.
7. Chavrier, P. and Goud, B. (1999). The role of ARF and Rab GTPases in membrane transport. Curr. Opin. Cell Biol. *11*, 466–475.
8. Norman, J.C., Jones, D., Barry, S.T., Holt, M.R., Cockcroft, S. and Critchley, D.R. (1998). ARF1 mediates paxillin recruitment to focal adhesions and potentiates Rho-stimulated stress fiber formation in intact and permeabilized Swiss 3T3 fibroblasts. J. Cell Biol. *143*, 1981–1995.
9. Radhakrishna, H., Klausner, R.D. and Donaldson, J.G. (1996). Aluminum fluoride stimulates surface protrusions in cells overexpressing the ARF6 GTPase. J. Cell Biol. *134*, 935–947.
10. D'Souza-Schorey, C., Boshans, R.L., McDonough, M., Stahl, P.D. and Van Aelst, L. (1997). A role for POR1, a Rac1-interacting protein, in ARF6-mediated cytoskeletal rearrangements. EMBO J. *16*, 5445–5454.
11. Boshans, R.L., Szanto, S., van Aelst, L. and D'Souza-Schorey, C. (2000). ADP-ribosylation factor 6 regulates actin cytoskeleton remodeling in coordination with Rac1 and RhoA. Mol. Cell Biol. *20*, 3685–3694.
12. Honda, A., Nogami, M., Yokozeki, T., Yamazaki, M., Nakamura, H., Watanabe, H., Kawamoto, K., Nakayama, K., Morris, A.J., Frohman, M.A. and Kanaho, Y. (1999). Phosphatidylinositol 4-phosphate 5-kinase alpha is a downstream effector of the small G protein ARF6 in membrane ruffle formation. Cell *99*, 521–532.
13. Zhang, Q., Calafat, J., Janssen, H. and Greenberg, S. (1999). ARF6 is required for growth factor- and rac-mediated membrane ruffling in macrophages at a stage distal to rac membrane targeting. Mol. Cell Biol. *19*, 8158–8168.

14. Santy, L.C. and Casanova, J.E. (2001) Activation of ARF6 by ARNO stimulates epithelial cell migration through downstream activation of both Rac1 and phospholipase D. J. Cell Biol. *154*, 599–610.

15. Miura, K., Jacques, K.M., Stauffer, S., Kubosaki, A., Zhu, K., Hirsch, D.S., Resau, J., Zheng, Y. and Randazzo, P.A. (2002). ARAP1: a point of convergence for Arf and Rho signaling. Mol. Cell *9*, 109–119.

16. Krugmann, S., Anderson, K.E., Ridley, S.H., Risso, N., McGregor, A., Coadwell, J., Davidson, K., Eguinoa, A., Ellson, C.D., Lipp, P. *et al.* (2002). Identification of ARAP3, a novel PI3K effector regulating both Arf and Rho GTPases, by selective capture on phosphoinositide affinity matrices. Mol. Cell *9*, 95–108.

17. Brown, M.T., Andrade, J., Radhakrishna, H., Donaldson, J.G., Cooper, J.A. and Randazzo, P.A. (1998). ASAP1, a phospholipid-dependent Arf GTPase-activating protein that associates with and is phosphorylated by Src. Mol. Cell Biol. *18*, 7038–7051.

18. Andreev, J., Simon, J.P., Sabatini, D.D., Kam, J., Plowman,G., Randazzo, P.A. and Schlessinger, J. (1999). Identification of a new Pyk2 target protein with Arf-GAP activity. Mol. Cell Biol. *19*, 2338–2350.

19. Vitale, N., Patton, W.A., Moss, J., Vaughan, M., Lefkowitz, R.J. and Premont, R.T. (2000). GIT proteins, A novel family of phosphatidylinositol 3,4, 5-trisphosphate-stimulated GTPase-activating proteins for ARF6. J. Biol. Chem. *275*, 13901–13906.

20. Jackson, T.R., Brown, F.D., Nie, Z., Miura, K., Foroni, L., Sun, J., Hsu, V.W., Donaldson, J.G. and Randazzo, P.A. (2000). ACAPs are arf6 GTPase-activating proteins that function in the cell periphery. J. Cell Biol. *151*, 627–638.