Arf1-GTP-induced Tubule Formation Suggests a Function of Arf Family Proteins in Curvature Acquisition at Sites of Vesicle Budding

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ADP-ribosylation factor (Arf) and related small GTPases play crucial roles in membrane traffic within the exo- and endocytic pathways. Arfs in their GTP-bound state are associated with curved membrane buds and tubules, frequently together with effector coat proteins to which they bind. Here we report that Arf1 is found on membrane tubules originating from the Golgi complex where it colocalizes with COPI and GGA1 vesicle coat proteins. Arf1 also induces tubulation of liposomes in vitro. Mutations within the amino-terminal amphipathic helix (NTH) of Arf1 affect the number of Arf1-positive tubules. Our data indicate that GTP-controlled local induction of high curvature membranes is an important property of Arf1 that might be shared by a subgroup of Arf/Arl family GTPases.

Membrane traffic in eukaryotic cells involves the formation of curved membrane buds and tubules that carry cargo from a donor to an acceptor compartment (1–3). Frequently areas of positive membrane curvature are stabilized by cytoplasmic coat proteins including COPI, COPII, and clathrin polymers (2, 4, 5). Recruitment of coat proteins is regulated by small Ras-related GTPases (6–8). Arf1, the best characterized member of the Arf subfamily, facilitates recruitment of COPI to Golgi membranes and of clathrin to late Golgi and endosomal compartments through its direct association with heterotetrameric (AP1, AP3, AP4) and monomeric (GGA1–3) adaptors (7, 9) and by activating lipid-modifying enzymes including phospholipase D and phosphatidylinositol-specific (PI)4 kinases (10). Thus, Arf1 might act as a master regulator of coated vesicle formation. Membrane targeting of Arf1 involves guanine-nucleotide exchange factor-mediated GTP loading on Arf1 (11) that is coupled to a conformational change resulting in membrane association of its N-myristoylated amino-terminal helix (NTH) (12) and the co-recruitment of Arf-GTP-binding effector proteins (compare also Fig. 1A). Additional interactions with acidic phospholipids (13) and membrane proteins including SNAREs may contribute to the spatiotemporal regulation of Arf1 recruitment to Golgi and endosomal membranes. Other Arf and Arl family members such as Arf6 may function similarly at other stations of membrane traffic (7, 8).

Arf-triggered coat recruitment correlates with the induction of positive membrane curvature (2, 4) leading to the generation of free coated vesicles or tubules (14, 15). Based on the homology between Arf family proteins and the distantly related small GTPase Sar1 (16), one might speculate that nucleotide exchange and subsequent hydrolysis on Arf could serve as a driving force to complete the cycle of membrane deformation and budding. In the case of Sar1p it has been shown that exposure of its amphipathic NTH induced by GTP binding initiates membrane curvature during budding of COPII-coated vesicles (17). Additional support of this idea comes from the observation that many membrane-deforming proteins such as endophilin (18, 19), epsin (20), and amphiphysin (21, 22) contain amphipathic helices, which may insert into the lipid bilayer.

Here we report that Arf1 is found on phosphatidylinositol 4-phosphate (P14P)-positive membrane tubules in vivo where it partially colocalizes with COPI and GGA1 vesicle coat proteins.

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The abbreviations used are: PI, phosphatidylinositol; NTH, amino-terminal amphipathic helix; PI4P, phosphatidylinositol 4-phosphate; PI(4,5)P2, phosphoinositide 4,5-bisphosphate; eGFP, enhanced green fluorescent protein; WT, wild type; TGN, trans-Golgi network; BFA, brefeldin A; GTPγS, guanosine 5′-O-(thiotriphosphate); HA, hemagglutinin; COPI, coat protein complex I; GGA, Golgi-localized γ-ear containing adaptor; PH, pleckstrin homology.

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4 This work was supported in part by German Research Funding Agency DFG Grants HA2686/4-1, SFB 740/TP C6 (to V.H.), and SFB 638/TP A10 (to F.T. W.) and European Molecular Biology Organization (EMBO) YIP Programme (to V.H.). The costs of publication of this article were defrayed in part by the payment of page charges. The on-line version of this article (available at http://www.jbc.org) contains supplemental methods, supplemental Figs. 1–3, and supplemental videos 1–3.
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We also show that Arf1 can deform lipid bilayers into tubules in vitro. The property of Arf1-GTP to associate with membrane tubules appears to be encoded within its NTH. Mutations within the NTH that affect association with membrane tubules in living cells lead to closely correlated changes in the ability of purified Arf1 to deform lipid membranes in vitro. Based on these data and on the observation that the NTHs of other Arf/Arl family members also associate with tubular membranes in living cells we suggest that GTP-controlled local induction of high curvature membranes is an important property of Arf1 and related small GTPases.

EXPERIMENTAL PROCEDURES

Live Cell Imaging and Immunofluorescence Analysis—Confocal images of transfected HeLa or Cos7 cells (15–24 h post-transfection) were acquired using a Zeiss Axiovert 200M-based PerkinElmer Life Sciences UltraView ERS dual spinning disc system. Data were processed using Velocity software (Improvision). For indirect immunofluorescence microscopy cells were fixed in 2% paraformaldehyde, further processed, and analyzed using a Zeiss Axiovert 200M under control of the Stallion System (Intelligent Imaging). For quantification of Arf1-eGFP-decorated tubules living cells expressing modest levels of the transfected proteins were monitored for at least 5 min, and images were taken every 3 s. Ten different time points were chosen, distributed over the entire time of monitoring. For these the numbers of tubules displayed were determined, and an average number of tubules per cell was calculated. Data from at least three different cells were collected and averaged.

Electron Microscopy—Liposomes (26% palmitoyloleoylphosphatidylserine, 26% phosphatidylethanolamine (brain-purified), 4.5% PI (liver purified), 26% palmitoyloleoylphosphatidylcholine, 4.5% phosphoinositol 4,5-bisphosphate (PI(4,5)P2, 13% cholesterol) were prepared by dehydration/rehydration in GTPase buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, 1 mM MgCl2) at 1 mg/ml. To fully resuspend the spontaneously grown liposomes, the tube was shortly vortexed at full power before removing the suspension. Samples for electron microscopy analysis were prepared essentially as described previously (18, 22).

Differential Interference Contrast Videomicroscopy of Membrane Sheets—Differential interference contrast video microscopy of membrane sheets was done essentially as described in Refs. 23 and 24.

COPI-Vesicle Budding Assay—200 µg of rat liver Golgi membranes (pretreated with 500 mM KCl), 10 µg of myristoylated Arf1 protein, and 25 µg of coatamer were incubated for 10 min at 37 °C in a total volume of 250 µl in assay buffer (25 mM HEPES, pH 7.4, 2.5 mM magnesium acetate, 50 mM KCl, 1.2 mM GTP, 300 mM sucrose, and 0.25 mM dithiothreitol), and 1% of the input was taken for Western blot analysis. The sample was subjected to 250 mM KCl to dissociate tethered COPI vesicles from the donor Golgi membranes, which were pelleted by a 10-min centrifugation at 14,000 rpm at 4 °C. The supernatant, containing COPI vesicles, was layered on top of two sucrose cushions in a Beckman SW55-minitube over 37.5% (50 µl) and 45% (5 µl) sucrose. After centrifugation for 50 min at 100,000 × g, the vesicles were taken from the 45–37.5% sucrose inter-phase, and 50% of the collected material was analyzed by immunoblotting.

Multiple Sequence Alignments—Multiple protein sequence alignments were performed using the ClustalW program.

Supplemental methods are available online for plasmids, transfection, antibodies, and reagents, protein expression, purification, nucleotide exchange, and determination of protein N-myristoylation, liposome and effector binding experiments, yeast transformation, and analysis of complementation.

RESULTS

Arf1 Association with Membrane Tubules in Living Cells Is Modulated by Its NTH—GTP binding to Sar1p has been shown to result in exposure of its NTH to initiate membrane curvature acquisition during budding of COPII-coated vesicles at ER exit sites (17). We thus hypothesized that in analogy to Sar1p the conformational transition triggered by GTP loading on Arf1 via insertion of its amphipathic NTH (12) would result in its association with tubular membranes, perhaps by direct induction of positive membrane curvature (Fig. 1A). To determine if Arf1 is indeed associated with tubular membrane structures in living cells we used spinning disc confocal microscopy. Arf1-eGFP localized to the perinuclear area and to puncta and tubules throughout the cytoplasm (Fig. 1B). Arf1-eGFP-decorated tubular structures were seen to emanate from the Golgi and frequently underwent fission (supplemental Fig. 1A and supplemental video 1). Arf1-eGFP-positive tubules were also seen in cells expressing GTP-locked Arf1 (Q71L) (Fig. 1B), whereas Arf1-GDP (T31N) remained largely cytosolic (supplemental Fig. 1C). To determine the contribution of the NTH to its association with membrane tubules we created Arf1 mutants in which hydrophobic amino acids Ile-4 and Phe-5 (lying on one side of the predicted amphipathic helix, compare with Fig. 4A) had been replaced by hydrophilic Arf1 (I4Q/F5E) or even more hydrophobic Arf1 (F5W) residues. Analogous mutants have been introduced previously into other amphipathic helix-containing proteins to analyze their role in membrane bending (18–22). Arf1-eGFP (I4Q) showed a reduced association with the Golgi area and with membrane tubules (Fig. 1, B and C; supplemental video 2), suggesting that the hydrophobic face of the NTH, perhaps in cooperation with the N-myristoyl group, stabilizes Arf1 at membranes. The reduced membrane association of Arf1 (I4Q) could be overcome by locking the protein in the GTP-bound state Arf1 (I4Q/Q71L). However, even in this case mutant Arf1-eGFP (I4Q/Q71L) was rarely observed on membrane tubules (Fig. 1, B and C). By contrast, Arf1-eGFP (F5W) and Arf1-eGFP (F5W/Q71L) (Fig. 1, B and C; supplemental Fig. 1B) decorated numerous membrane tubules originating from Golgi donor compartments (supplemental video 3) at a frequency about 3 times higher than that seen in cells expressing Arf1-eGFP (WT) (Fig. 1C). All Arf1 mutants underwent N-myristoylation similar to the WT protein (Fig. 1D). Neither Arf1 (I4Q) nor Arf1 (F5W) interfered with the overall Golgi structure, as monitored by double-labeling experiments with the Golgi matrix protein GM130 or p230, a peripheral protein associating with the trans-Golgi network (supplemental Fig. 2). These data suggest that Arf1 (I4Q) does not act as a dominant-negative mutant. This is underscored by our obser-
membrane tubules (data not shown). In conclusion, our data suggest that Arf1 associates with and perhaps induces the formation of PI(4)P-positive membrane tubules containing GGA1 and COPI coat proteins.

Given that Arf1-eGFP (F5W) (and to some extent also the WT) was associated with GGAs on numerous membrane tubules, we wanted to test whether the reduced number of Arf1 (I4Q)- or Arf1 (I4Q/Q71L)-eGFP-decorated membrane tubules might be an indirect effect of an impaired association of mutant Arf1 with coat proteins, which might induce and/or stabilize tubules. To test this possibility, cells were treated with the fungal metabolite brefeldin A (BFA), an inhibitor of Arf-specific guanine nucleotide exchange factors, which prevents GTP loading of Arf1. Such treatment results in a rapid redistribution of endogenous GGAs to the cytosol (7, 8), a defect that can be rescued by overexpressed Arf1 (Q71L) locked in its GTP-bound state (Fig. 3A). As seen in Fig. 3B, tubulation-defective Arf1-eGFP (I4Q/Q71L) was capable of stably recruiting GGA3 to the TGN of BFA-treated cells, similar to the activity of its WT counterpart in the absence of BFA. Consistent with these findings in living cells Arf1 (I4Q), Arf1 (I4Q/F5E), and Arf1 (F5W) interacted with COPI and AP-3 adaptor proteins in vitro with an efficiency indistinguishable from that of wild-type Arf1 (Fig. 3C). By contrast, purified Rab11 did not associate with any of these proteins. Thus, mutations within the NTH of Arf1 do not impair its ability to interact with coat proteins in vitro or in living cells. These results also rule out that mutations within the Arf1 NTH cause any gross folding defects or altered GTP binding. We note that BFA, which inhibits nucleotide exchange on Arf1, was also shown to induce membrane tubulation (26). However, BFA-induced tubules are driven by motors that pull the membrane along microtubular tracks (27), whereas GTP-Arf1 appears to act directly by producing membrane deformation.

**Association of the NTHs of Select Arf/Arl Family Members with Membrane Tubules**—The experiments described above imply that Arf1 associates with high curvature membranes in vivo and raise the possibility that this association may result from a membrane bending activity of its amphipathic NTH (28) (see below also). We hypothesized therefore that the amphipathic NTHs of Arf and Arl proteins in general (16) might be important determinants for their association with tubular membranes in living cells. Multiple sequence alignments (Fig. 4A) revealed that the NTHs of many Arf/Arl family proteins...
members contain hydrophobic residues that are predicted to lie on one side of an amphipathic helix. Based on these sequence comparisons we predicted that the NTHs of some members of the Arf/Arl family including Arf6 and Arl1 would be able to associate with high curvature membranes, whereas others, such as Arl4D or Arl8a would not. Arl8a lacks the critical Gly residue in position 2 and therefore is predicted not to undergo N-myristoylation. Within the NTH of Arl4D a hydrophilic amino acid (His) is present at position 4 corresponding to Ile-4 within Arf1, a substitution expected to compromise its association with tubules and membranes per se (compare B and C of Fig. 1). To test our hypothesis that the NTHs of Arf/Arl family proteins are indeed important determinants for their association with membrane tubules, we created chimeric proteins comprising the first 16 residues of the NTHs (N) of Arf5 and -6, or Arl1, -4D, -5B, and -8A fused to an Arf1-eGFP truncation mutant lacking its own NTH. These proteins were analyzed for their ability to associate with membrane tubules in fibroblasts. As predicted, expression of Arl1N-Arf1 led to the growth of numerous membrane tubules from the Golgi complex region which were even more numerous than those seen for Arf1 (WT) (Fig. 4, B and C). This might be due to the substitution of Ile-4 within the Arf1-NTH by a much bulkier Phe residue in the NTH of Arl1. Likewise, we saw extensive tubulation in cells expressing fusion proteins comprising the NTHs of Arf5, Arf6, and Arl5B (Fig. 4, B and C). By contrast, Arl4DN- (containing a His in position 4 instead of Ile in Arf1) or Arl8AN-Arf1 (the latter presumably lacking the N-myristate, see above) did not lead to membrane tubulation and instead remained largely cytosolic (Fig. 4, B and C). Thus, the ability to associate with curved membranes might be a property common to a subset of Arf/Arl family proteins. Based on the observed close correlation between the presence of an intact amphipathic NTH and the association of Arf1 with tubular structures in living cells we hypothesized that Arf1 may in fact induce membrane tubulation itself.

**Arf1-GTP Is Sufficient to Induce Membrane Tubulation**

Because of the results shown above (Fig. 4) we decided to directly investigate the membrane tubulating properties of Arf1 in vitro. To this aim we purified untagged N-myristoylated human Arf1 (myrArf1) from bacteria co-expressing human N-myristoyltransferase from a bicistronic plasmid. Resulting Arf1 preparations were more than 90% pure, and about 80% of this protein had also undergone N-myristoylation (not shown (30)). As a premise to test our hypothesis we investigated the membrane binding properties of myrArf1. Liposomes of different compositions were incubated with purified myrArf1, and protein binding was monitored by a cosedimentation assay. MyrArf1 specifically associated with liposomes containing PI(4)P or PI(4,5)P2 (13) and to a lesser extent with other negatively charged phospholipids (data not shown). Membrane association was seen only for GTP-loaded myrArf1 consistent with in vivo data (Fig. 5A), whereas myrArf1-GDP remained largely soluble. To determine if myrArf1 could induce positive membrane curvature, we incubated phosphoinositide-containing liposomes with purified GTP- or GDP-loaded wild-type (WT) or mutant Arf1 proteins and analyzed the samples by negative stain electron microscopy. We observed extensive tubulation of liposomal membranes by Arf1-GTP (WT) (Fig. 5,
Arf1 (I4Q), or Arf1 (I4Q/F5E) in the presence of GTP, and vesicles were incubated with purified coatomer and Arf1 (WT), branes of both Arf1 (I4Q) and Arf1 (I4Q/F5E) (Fig. 5 C).

Given that Arf1 is required for formation of COPI-coated vesicles in vivo and in vitro we wanted to determine the effect of the mutations in an in vitro budding assay. Isolated Golgi membranes were incubated with purified coatomer and Arf1 (WT), Arf1 (I4Q), or Arf1 (I4Q/F5E) in the presence of GTP, and vesicle formation was assayed by immunoblotting of density gradient-purified COPI vesicle fractions (Fig. 6A). Quantitative analysis revealed that myrArf1 (I4Q) and even more pronounced myrArf1 (I4Q/F5E) displayed a strongly reduced ability to support COPI vesicle formation (Fig. 6B), despite their association with COPI coat proteins (Fig. 3C). In the presence of coatomer and GTPyS, myrArf1 (I4Q) associated with Golgi membranes, albeit with slightly reduced efficiency when compared with myrArf1 (WT) (Fig. 6C), resulting in stable recruitment of COPI coat proteins to the membrane (Fig. 6D).

To further support this finding, we made use of yeast genetics. Yeast mutants lacking yARF1 and yARF2 are non-viable but can be rescued by expressing a plasmid-borne copy of yARF1 from its own promoter (31). Yeast ∆arf1/∆arf2 double knockout cells kept alive by yARF1 encoded on a URA3-containing plasmid were transformed with LEU2-based expression plasmids for yARF1 (WT) or yARF1 (F4Q), a mutation corresponding to I4Q in mammalian Arf1, and spotted onto media containing 5-fluoroorotic acid to counterselect against the URA3-
plasmid. As expected, yARF1 (WT) or a hexahistidine tagged version of it was able to complement loss of the URA3-yARF1 plasmid, but the F4Q mutant was not (supplemental Fig. 3A), although yARF1-His_{6} (WT) and yARF1-His_{6} (F4Q) were expressed to similar levels in the absence of 5-fluoroorotic acid and underwent proper N-myristoylation (supplemental Fig. 3B). Thus, hydrophobic residues within the amphipatic NTH of yARF1 are required for viability of yeast cells in vivo, further suggesting that properties which critically require these residues such as membrane binding and deformation are physiologically important.

**DISCUSSION**

In summary, our data indicate that Arf1-GTP induces positive curvature on membranes, and this property appears to be related to its role in COPI vesicle budding. This conclusion is based on several lines of evidence. First, Arf1-eGFP is associated with membrane tubules that also contain coat proteins such as COPI, GGA1, and the PI(4)P- and Arf-binding adaptor FAPP1. The frequency at which such Arf1-decorated tubules are observed is dependent on hydrophobic residues within the amphipatic NTH of Arf1, suggesting that tubules might at least in part be generated by Arf1 itself. Second, we find that purified untagged myristoylated Arf1 can induce membrane tubules on liposomes, a property affected by mutations in its NTH. Third, an Arf1 mutant, in which a hydrophobic residue found to be critical for the induction of high curvature on membranes has been exchanged for a hydrophilic amino acid (I4Q), is compromised in its ability to facilitate budding of COPI-coated vesicles from native Golgi membranes in vitro, although it is perfectly well able to bind to and recruit coatomer to the membrane.

We therefore propose that Arf1 and, based on our experiments involving chimeric proteins (compare Fig. 4), also other members of the Arf/Arl subfamily of GTPases such as Arf5 and -6 (29) and Arf1 and -5B act as GTP-regulated membrane benders during tubulovesicular membrane traffic. Pre-existing cur-
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Acknowledgment—We thank Dr. A. Nakano for providing the Δarf1/Δarf2 yeast strain.

REFERENCES

1. Rothman, J. E. (2002) Nat. Med. 8, 1059–1062
2. Bonifacino, J. S., and Glick, B. S. (2004) Cell 116, 153–166
3. Cai, H., Reinsch, K., and Ferro-Novick, S. (2007) Dev. Cell 12, 671–682
4. Antonny, B. (2006) Curr. Opin. Cell Biol. 18, 386–394
5. Lee, M. C., Miller, E. A., Goldberg, J., Orci, L., and Schekman, R. (2004) Annu. Rev. Cell Dev. Biol. 20, 87–123
6. Burd, C. G., Strohlic, T. I., and Gangi Setty, S. R. (2004) Trends Cell Biol. 14, 687–694
7. D’Souza-Schorey, C., and Chavrier, P. (2006) Nat. Rev. Mol. Cell. Biol. 7, 347–358
8. Gillingham, A., and Munro, S. (2007) Annu. Rev. Cell Dev. Biol. 23, 579–611
9. Robinson, M. S. (2004) Trends Cell Biol. 14, 167–174
10. Godi, A., Pertile, P., Meyers, R., Marra, P., Di Tullio, G., Jurisici, C., Luini, A., Corda, D., and De Matteis, M. A. (1999) Nat. Cell Biol. 1, 280–287
11. Bos, J. L., Rehmann, H., and Wittinghofer, A. (2007) Cell 129, 865–877
12. Antonny, B., Beraud-Dufour, S., Chardin, P., and Chabre, M. (1997) Biochemistry 36, 4675–4684
13. Randazzo, P. A. (1997) J. Biol. Chem. 272, 7688–7692
14. McMahon, H. T., and Gallop, J. L. (2005) Nature 438, 590–596
15. Farsad, K., and De Camilli, P. (2003) Curr. Opin. Cell Biol. 15, 372–381
16. Kahn, R. A., Cherfils, E., Elia, M., Lovering, R. C., Munro, S., and Schurmann, A. (2006) J. Cell Biol. 172, 645–650
17. Lee, M. C., Orci, L., Hamamoto, S., Futai, E., Ravazzola, M., and Schekman, R. (2005) Cell 122, 605–617
18. Farsad, K., Ringstad, N., Takei, K., Floyd, S. R., Rose, K., and De Camilli, P. (2001) J. Cell Biol. 155, 193–200
19. Gallop, J. L., Jao, C. C., Kent, H. M., Butler, P. J., Evans, P. R., Langen, R., and McMahon, H. T. (2006) EMBO J. 25, 2898–2910
20. Ford, M. G., Mills, I. G., Peter, B. J., Valls, Y., Praefcke, G. J., Evans, P. R., and McMahon, H. T. (2002) Nature 419, 361–366
21. Peter, B. J., Kent, H. M., Mills, I. G., Valls, Y., Butler, P. J., Evans, P. R., and McMahon, H. T. (2004) Science 303, 495–499
22. Takei, K., Slepnev, V. I., Hauke, V., and De Camilli, P. (1999) Nat. Cell Biol. 1, 33–39
23. Itoh, T., Erdmann, K. S., Roux, A., Habermann, B., Werner, H., and De Camilli, P. (2005) Dev. Cell 9, 791–804
24. Roux, A., Uyhazi, K., Frost, A., and De Camilli, P. (2006) Nature 441, 528–531
25. Polischuck, R. S., San Pietro, E., Di Pentima, A., Tete, S., and Bonifacino, J. S. (2006) Traffic 7, 1092–1103
26. Sciaky, N., Presley, J., Smith, C., Zaal, K. J., Cole, N., Moreira, J. E., Terasaki, M., Sigig, E., and Lippincott-Schwartz, J. (1997) J. Cell Biol. 139, 1137–1155
27. Lippincott-Schwartz, J., Donaldson, J. G., Schweizer, A., Berger, E. G., Hauri, H. P., Yuan, L. C., and Klausner, R. D. (1990) Cell 60, 821–836
28. Losonczy, J. A., and Prestegard, J. H. (1998) Biochemistry 37, 706–716
29. Caplan, S., Naslavsky, N., Hartnell, L. M., Lodge, R., Poliuch, R. S., Donaldson, J. G., and Bonifacino, J. S. (2002) EMBO J. 21, 2557–2567
30. Sun, Z., Ander, F., Koh, K., Zhao, L., Hanke, S., Brugger, B., Wieland, F., and Bethune, J. (2007) Traffic 8, 582–593
31. Yahara, N., Ueda, T., Sato, K., and Nakano, A. (2001) Mol. Biol. Cell 12, 221–238