**Arf1 Dissociates from the Clathrin Adaptor GGA Prior to Being Inactivated by Arf GTPase-activating Proteins**

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The effectors of monomeric GTP-binding proteins can influence interactions with GTPase-activating proteins (GAPs) in two ways. In one case, effector and GAP binding to the GTP-binding protein is mutually exclusive. In another case, the GTP-binding protein bound to an effector is the substrate for the GTP-activating protein. Here predictions for these two mechanisms were tested for the Arf1 effector GGA and ASAP family Arf GAPs. GGA inhibited Arf GAP activity of ASAP1, AGAP1, ARAP1, and Arf GAP1 and inhibited binding of Arf1-GTP/S to AGAP1 with Ki values correlating with the Kd for the GGA-Arf1 complex. ASAP1 blocked Arf1-GTP/S binding to GGA with a KI similar to the Kd for the ASAP-Arf1-GTP/S complex. No interaction of GGA with ASAP1 was detected. Consistent with GGA sequestering Arf from GAPs, overexpression of GGA slowed the rate of Arf dissociation from the Golgi apparatus following treatment with brefeldin A. Mutational analysis revealed the amino-terminal α-helix and switch I of Arf1 contributed to interaction with both GGA and GAPs. These data exclude the mechanism previously documented for Arf GAP1/coatomer in which Arf1 is inactivated in a tripartite complex. Instead, termination of Arf1 signals mediated through GGA require that Arf1-GTP dissociates from GGA prior to interaction with GAP and consequent hydrolysis of GTP.

The Arfs are a family of GTP-binding proteins within the Ras superfamily (1, 2). The Arfs include Arf1–6 and the Arf-like proteins. Of these two groups, the Arf proteins have been the most extensively studied. The Arf proteins were first identified as cofactors for cholera toxin-catalyzed ADP-ribosylation of the heterotrimeric GTP-binding protein (3). Subsequently, physiological roles for the Arfs have been found as regulators of membrane traffic and the actin cytoskeleton (for reviews see Refs. 1, 2, and 4–7). Like other GTP-binding proteins, Arf functions as a switch. Arf-GDP is inactive. Arf-GTP binds effectors to mediate signals. A number of Arf-GTP-binding proteins have been identified including vesicle coat proteins (coatomer, AP1, AP3, AP4, GGA1/2/3), phospholipase D, phosphatidylinositol 4-phosphate 5-kinase, arfaptin, and arfophilin (5, 7). The function of coat proteins requires that Arf cycles between the active and inactive forms (1, 4, 6). Arf-GTP binds to and recruits coat proteins to a donor membrane, and the hydrolysis of GTP to form Arf-GDP is necessary for coat proteins to dissociate from vesicles. The physiologic function of Arf with other effectors presumably also requires a cycle of GTP binding and hydrolysis.

Effectors have been found to affect the interaction of Ras family GTP-binding proteins with their cognate GAPs in one of two ways (8). First, some effectors have been found to inhibit GAP activity. For instance, Ras GAPs and effectors compete for an overlapping binding site on Ras-GTP; therefore, Ras effectors inhibit GAPs, and Ras must be dissociated from the effector in order to be inactivated by GAP (8–12). The Ran effector importin is also inhibitory (8, 13–15). In other cases, effectors have been found to facilitate interaction of GTP-binding proteins with GAPs. One example is Ran-binding protein (RanBP) (8, 15–17). A complex of Ran with RanBP is the substrate for Ran GAP. As for other GTP-binding proteins, the conversion of Arf-GTP to Arf-GDP is facilitated by GAPs (18–25). Arf1 inactivation is thought to involve a tripartite complex of Arf GAP, Arf1, and the Arf-binding protein coatomer (26). However, different from that found for other Ras-like GTP-binding proteins, Arf GAP1 activity has been found to be stimulated 2–3 orders of magnitude by the effector coatomer (26). Based on these results, coatomer has been proposed to contribute a catalytic residue into the GTP-binding site of Arf1, consequently inducing the hydrolysis of GTP, while simultaneously bound to Arf1-GTP and Arf GAP1.

Here we tested whether the regulatory interactions described for Arf GAP1 and coatomer extrapolate to the Arf effector GGA. Our results exclude the formation of tripartite complex of GAP-Arf1-GGA as occurs with coatomer and Arf GAP1. Instead, the data are consistent with Arf1 regulation being similar to that of Ras. GGA and GAPs compete for overlapping sites on Arf1. Therefore, Arf1 must dissociate from the coat protein GGA prior to inactivation by Arf GAPs.

**MATERIALS AND METHODS**

DNA—The open reading frame for a His6-tagged Arf GAP1 in pCDNA3 was the gift from Drs. Victor Hsu and Dan Cassel. POW12 for the expression of Arf1 in bacteria has been described previously (27). Constructs for the bacterial expression of fusions of GST and the VHS protein were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: GAPs, GTPase-activating proteins; GTPS, guanosine 5′-O-(thiotriphosphate); PiP(4,5)P2, phosphatidylinositol 4,5-bisphosphate; DTT, dithiothreitol; PA, phosphatidic acid; BFA, brefeldin A; GST, glutathione S-transferase; GFP, green fluorescent protein; VHS, Vps27, Hrs, STAM1 homology; GAT, GGA and TOM1 homology; GGA, Golgi-associated, γ-adaptin homologous, Arf-interacting.

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were gifts from Drs. Rosa Puertollano and Juan Bonifacino (NICHD, National Institute of Health, Bethesda). The reading frames expressing the VHS, VHS GAT, and GAT domains of GGA1 were amplified from the plasmid provided by Dr. Bonifacino using Vent polymerase and inserted into pET19 to provide a vector for expression the His$_{10}$-tagged proteins. GFP fused to the VHS and to the VHS GAT domains from GGA3 and yellow fluorescent protein fused to GGA1 were gifts from Drs. Juan Bonifacino and Rosa Puertollano. Mutations were introduced using the Quikchange kit (Stratagene).

Proteins—Arf proteins were expressed in bacteria and purified as described (27). The proteins were not myristoylated. In preliminary studies we found that GGA inhibited GAP activity against myristoylated Arf and nonmyristoylated Arf to the same extent. Therefore, we used nonmyristoylated Arf to ensure homogeneity of the proteins as the extent of myristoylation of recombinant Arfs varied between preparations and between the mutants used in these studies. A fusion protein of GST and the VHS GAT domains of GGA3 was expressed and purified using glutathione beads (Amersham Biosciences) by standard methods. FLAG-tagged ARAP1 and ASAP1b were expressed in and purified from HEK293T cells (20, 30). [35S]GTP$_S$ was expressed in as a GST fusion protein, was titrated into the GAP reaction. The proteins were not myristoylated. In preliminary measurements were determined by the Bio-Rad Dye binding assay. GAP activity measurements were fit to a single exponential decay equation to determine $k_{obs}$.

Immunofluorescence—NIH 3T3 fibroblasts were grown in Dulbecco's Modified Eagle's medium with 10% fetal bovine serum at 37°C. The cells were transfected with the indicated plasmids using FuGENE (Roche Molecular Biochemicals) according to the manufacturer's protocol. Twenty four hours after transfection the cells were treated for 1 min with 56 μm brefeldin A (BFA), fixed with 2% formaldehyde, and immunostained with an antibody to Arf (1D9 mouse monoclonal, ABR). The secondary antibody for visualizing Arf was Alexa 594-conjugated goat ant-mouse.

Miscellaneous—Nitrocellulose filter binding to determine protein-bound nucleotide was performed as described (27). Protein concentrations were determined by the Bio-Rad Dye binding assay. GAP activity

![Fig. 1. GGA inhibits Arf GAP activity. The effect of GGA on the activity of 7.5 μM Flag-AGAP1 was determined using Arf1 as a substrate and in the presence of 360 μM PA and 45 μM PI(4,5)P$_2$. A, inhibition of AGAP1 activity by VHS-GAT-GGA. VHS-GAT of GGA3, expressed as a GST fusion protein, was titrated into the GAP reaction. B, effect of fragments of GGA1 on GAP activity of AGAP1. Proteins derived from GGA1 expressed as His$_{10}$ fusions were titrated into the GAP reaction measuring the dissociation rate, $k_{obs}$, at varying concentrations of GGA and fitting the data to Equation 4 (12).

$$k_{obs} = \frac{k_1}{[GGA] + K_d}$$

In these experiments, [35S]GTP$_S$-Arf1 was prepared by incubating 1 μM Arf1 with 1 μM [35S]GTP$_S$ in 25 mM Tris, 100 mM NaCl, 0.5 mM MgCl$_2$, 1 mM EDTA, 0.1% Triton X-100, and 1 mM dithiothreitol (DTT) for 60 min at 30°C. Arf1-[35S]GTP$_S$ was then incubated with the immobilized GGA at constant temperature (30°C) in 20 mM Tris, 100 mM NaCl, 1 mM GTP, 2 mM MgCl$_2$, 1 mM DTT, 0.1% Triton X-100 and, unless otherwise indicated, 90 μM phosphatidylinositol 4,5-bisphosphate (PI(4,5)P$_2$) and 360 μM phosphatidic acid in a total volume of 50–100 μl. The beads were separated from bulk solution by a brief (5–10 s) centrifugation. Eighty percent of the total volume was removed as supernatant. GTP$_S$ bound to Arf1 both in the supernatant and in the solution containing the beads (pellet) was determined by scintillation spectrometry. With no binding, 20% of the ArfGTP$_S$ should be associated with the beads because this is the relative volume of the beads relative to the total reaction volume. The percent of ArfGTP$_S$ associated with the beads should increase on binding to GGA as described in Equation 1. This same approach was used with GST-AGAP1. The results for AGAP1 agreed with the $K_d$ value determined by competition inhibition of Arf GAP activity by Arf1-GTP$_S$.

$$\frac{\text{[Arf}}{\text{GTP$_S$]_{	ext{pellet}}}}{\text{[Arf}}{\text{GTP$_S$]_{	ext{total}}}} = 0.2 \times \frac{\text{[Arf}}{\text{GTP$_S$]_{	ext{bound}}}}{\text{[Arf}}{\text{GTP$_S$]_{	ext{total}}}} + 0.8 \times \frac{\text{[Arf}}{\text{GTP$_S$]_{	ext{total}}}}{\text{[Arf}}{\text{GTP$_S$]_{	ext{total}}}}$$

where $\text{[Arf}}{\text{GTP$_S$]_{	ext{pellet}}}$ is the amount of ArfGTP$_S$ in the pellet; $\text{[Arf}}{\text{GTP$_S$]_{	ext{total}}}$ is the total amount of ArfGTP$_S$ in the assay, and $B_{\text{max}}$ is the maximum observed binding.

Wittinghofer and colleagues (12) have described another approach to determine affinities. As shown in the scheme in Fig. 2B, which is exactly analogous to that described for Ras (12), GTP dissociates from free Arf1 at a rate of $k_-$, and Arf1 bound to GGA at a rate of $k_+$ as described in Equation 2.

$$-\frac{\text{d}[\text{Arf}}{\text{GTP$_S$]}}{\text{dt}} = k_+ \times [\text{Arf}}{\text{GTP$_S$}] + k_- \times [\text{GGG} \times \text{Arf}}{\text{GTP$_S$}]$$

GTP dissociates much more slowly from Arf1-GGA than from free Arf1 (see Fig. 2C), i.e., $k_- > k_+ > k_{\text{obs}}$, therefore, the rate of dissociation can be estimated by Equation 3.

$$-\frac{\text{d}[\text{Arf}}{\text{GTP$_S$]}}{\text{dt}} = k_- \times [\text{Arf}}{\text{GTP$_S$}]$$

With $k_{\text{obs}} = K_{\text{GGA}}$ for binding GGA, we were able to estimate the $K_d$ by

$^{7}$ Z. Nie, K. Stanley, S. Stauffer, K. Jacques, D. Hirsch, and P. Randazzo, submitted for publication.
RESULTS

As we had found previously (32), GGA inhibited Arf GAP activity (Fig. 1). The inhibition was independent of the GAP being examined. Truncated GGAs consisting of the VHS GAT domains was titrated into GAP reactions using ASAP1, a truncated ASAP1 consisting of the residues 325–724, AGAP1, Arf GAP1, and ARAP1 as enzymes. The inhibition in each case occurred with similar K<sub>d</sub> values for GGA (Fig. 1A and Table I).

Inhibition by GGA was dependent on GAT, the domain of GGA that binds Arf1-GTP. By using either AGAP1 or ASAP1, we found that full-length GGA1, truncated proteins consisting of the VHS GAT domains of either GGA1 or GGAs, and the isolated GAT domain of GGA1 inhibited GAP activity (Table I and Fig. 1, A and B). The isolated VHS domain had little or no effect.

Inhibition of GAP activity correlated with GGA binding to Arf1-GTP. Binding of Arf1 to GGA has been demonstrated by two-hybrid experiments; however, this approach does not allow affinities to be quantified and, consequently, correlation with the inhibition constants. We used two assays to quantify equilibria binding. In one, Arf1 loaded with [35S]GTPyS was incubated with VHS GAP fused with GST and bound to glutathione-agarose beads. The beads were separated from the bulk solution by centrifugation, and Arf1-GTPyS in the bulk solution and the pellet was measured. By using this assay, we found that GGA bound Arf1 ~10-fold more tightly in the presence of PA and PI(4,5)P<sub>2</sub> (conditions used for the GAP assays) than in the absence of these lipids (Fig. 2A). The K<sub>d</sub> of 0.51 μM ± 0.06 μM correlated closely with the K<sub>d</sub> of 0.5 μM measured for GAP activity of ASAP1, AGAP1, ARAP1, and Arf GAP1.

A second approach to measure the interaction between GGA and Arf1 has been used to examine effector interaction with Ras by Wittinghofer and colleagues (12). As for Ras, dissociation of GTP from Arf1 bound to effector (k<sub>−</sub> in Fig. 2B) is much slower than GTP dissociation from free Arf1 (k<sub>−</sub>). Therefore, the K<sub>d</sub> value for Arf1-GGA could be determined by measuring the GTPyS dissociation rate, k<sub>obs</sub>, in the presence of increasing concentrations of GGA (Fig. 2C) and analyzing the relationship between the k<sub>obs</sub> and effector concentration according to Equation 4. The derived K<sub>d</sub> was similar to the values determined from the direct binding assay (Table I, part B). The results further support the correlation between K<sub>d</sub> for Arf1-effector and K<sub>d</sub> for effector inhibition of GAP activity. The isolated GAT domain had a higher K<sub>d</sub> in the GAP assay and a higher K<sub>d</sub> for Arf1 binding than did the VHS GAT(GGA1) or VHS GAT (GGAs) (Table I, part B).

The similarity between K<sub>d</sub> and K<sub>d</sub> values suggested that GGA inhibited GAP activity by sequestering Arf1-GTP. However, these results do not exclude other mechanisms of inhibition including an inhibitory interaction between the GAP and effector. If GGA sequestered Arf1-GTP, then GGA should inhibit the binding of Arf1-GTP to GAP. To test this prediction, we examined Arf1 bound to the slowly hydrolyzable GTP analog GTPyS. GAP does not induce hydrolysis of GTPyS bound to Arf1. Without hydrolysis of GTP, we were able to measure the direct binding of Arf1-GTPyS to a protein composed of the pleckstrin homology, Arf GAP, and ANK repeat domains of AGAP1 fused to GST with the same assay used to measure binding of Arf1-GTPyS to GGA fused to GST. A K<sub>d</sub> of 0.78 ± 0.16 μM was determined, similar to the K<sub>d</sub> of 1.1 ± 0.05 μM determined by competition of Arf1-GTPyS with Arf1-GTP in the GAP assay. VHS GAP(GGA1) into the binding assay inhibited Arf1-GTPyS binding to AGAP1 with a K<sub>d</sub> of 0.94 ± 0.2 μM (assuming a K<sub>d</sub> for AGAP1-Arf1 of 0.78 μM, Fig. 3A).

If GGA sequesters Arf1-GTP, then, conversely, Arf1-GTP bound to GAP should be sequestered from GGA. To test this, we again examined Arf1 bound to GTPyS so effects could not be attributed to inactivating Arf1. We found that [325–724]ASAP1 was determined as described (31). Statistical analyses were performed with GraphPad Prism®.
GGA Inhibits Arf GAP

FIG. 2. GGA binding to Arf1-GTPγS. A, direct binding assay. The binding of GST-VHS GAT of GGA3 to Arf1-GTPγS was determined in the presence of absence of 59 μM PA and 45 μM Pi(4,5)P2 as described under “Materials and Methods.” B, paths for GTPγS dissociation from Arf1 in the presence of GGA. C, determination of GGA binding to Arf1 by measuring effect on GTPγS dissociation rate. GTPγS dissociation from Arf1 was determined in the presence of the indicated concentrations of His10-VHS GAT (GGA1). The observed rates were plotted against the concentration of VHS GAT to determine the Kd for the Arf1-GTPγS-GGA complex.

Inhibited Arf1-GTPγS binding to GGA (Fig. 3B). The Kd for ASAP1:Arf1-GTPγS, 4.0 ± 0.6 μM, was similar to the Kd, 4.3 ± 0.31 μM, determined assuming the Kd for GGA:Arf1 is 0.5 μM. No interaction between ASAP1 and GGA was detected when each was present in excess of the concentration of GAP giving half-maximal inhibition of Arf1-GGA interaction (Fig. 3C). [325–724]ASAP1 was incubated with GST-VHS GAT immobilized on glutathione beads. The amount of ASAP1 associated with the beads was the same in the absence or presence of GGA. Taken together, these data exclude the formation of a three part complex of Arf1-GTP-GGA-GAP. The data also exclude the possibility of a direct interaction between GGA and GAP resulting in the inhibition of Arf1-GTP binding to either protein in the complex. Instead, the data are consistent with mutually exclusive binding of GAP and GGA to Arf1.

As a further test for the effect of GGA on GAP activity, the effect of overexpressing GGA and GGA constructs on BFA-induced Arf dissociation from the Golgi was examined. If GGA binding to Arf prevented GAP activity, then GGA should slow Arf dissociation from the Golgi that results from inactivation of exchange factors with BFA. As predicted, the VHS domain of GGA, which does not bind Arf, had no effect on Arf dissociation from the Golgi (not shown). However, the VHS GAT domain and full-length GGA1 protein slowed Arf dissociation (Fig. 4). When untreated, Arf was concentrated around the nucleus in cells that did not express GGA ectopically (open arrows), cells that expressed VHS GAT (solid arrows), or cells that ectopically expressed epitope-tagged GGA1 (arrowheads). After a 1-min BFA treatment, the distribution of Arf had substantially changed from a perinuclear to a diffuse distribution in non-transfected cells (open arrows). In contrast, Arf was still perinuclear in the cells expressing VHS GAT (solid arrows) or GGA1 (arrowheads). These results are similar to those obtained with GFP-tagged Arf1 (32).

If the GAP and effectors have overlapping sites, then mutations in Arf1 affecting the binding site should affect both GAP and GGA interaction. Several mutant Arf1s were chosen to test this prediction. Because of the previously documented critical role of the amino-terminal α-helix (33–35), we examined an Arf1 lacking the first 13 amino acids (34, 36) and Arf1 with residues 3–7 replaced with residues 3–7 of ScArf1 (3–...
We examined Arf1 with mutations in residues of switch I and II. Some of these variants had been shown previously (37, 38) to affect interaction with GGA in two-hybrid studies. The effects of these mutations on nucleotide binding have been documented previously (37, 38). We also examined Arf1 with a mutation at glutamate 105. This variant of Arf1 bound GTP·S to the same extent as did the wild-type protein (not shown).

We first compared the effect of the mutations on interactions with two Arf GAPs, AGAP1 and ASAP1. The amount of GAP necessary to induce hydrolysis of 50% of the GTP bound to Arf1 (we call this amount of GAP "C₅₀" for concentration for 50% hydrolysis) was determined (Table II). The C₅₀ is proportional to K₉₅/K₉₄. Therefore, a large number would indicate a low affinity (large K₉₅), low K₉₄, or both. The relative effects of the mutations in Arf1 on C₅₀ values were similar for AGAP1 and ASAP1. Mutation of residues 3–7 ([3–7LFASK]Arf1), tyrosine 81 ([Y81H]Arf1), or glutamate 105 ([E105A]Arf1) did not affect the C₅₀ for either ASAP1 or AGAP1. Deletion of the amino terminus ([Δ13]Arf1), mutation of isoleucine 49 ([I49T]Arf1), or mutation of phenylalanine 51 ([F51Y]Arf1) affected the C₅₀ values for both Arf GAPs. The C₅₀ values in part reflect changes in affinities. The effects of the mutations on direct binding to AGAP1 correlated with effects on C₅₀ values (Table II). Mutation of residues 3–7, tyrosine 81, and glutamate 105 had no effect on affinity, whereas deletion of the amino terminus, mutation of isoleucine 49, and mutation of phenylalanine 51 decreased the affinity.

Similarities in the effects of mutations on binding to GGA and interaction with the GAPs indicated overlapping binding sites. Deletion of the amino terminus ([Δ13]Arf1) and mutations in switch I ([I49T]Arf1 and [F51Y]Arf1) decreased interaction with GGA and the GAPs (Table II). Mutation of residues 3–7 had no effect on GGA or GAP binding. Mutation of arginine 79 affected neither the K₉₅ for GGA nor the C₅₀ for ASAP1. There were differences indicating the binding sites were not completely overlapping. A mutation in switch II, Y81H, affected binding to GGA by 3–4-fold but had no detectable effect on binding to GAP. Also, deletion of the amino terminus had a relatively larger effect on Arf1 interaction with GAP than with GGA.

In summary, the overlap in the GGA and GAP-binding sites on Arf1 is sufficient for their interactions to be mutually exclusive.

**DISCUSSION**

We have examined mechanisms by which signals mediated by Arf1-GTP-GGA are terminated by ASAP family Arf GAPs and Arf GAP1. Another Arf1 effector, coatomer, has been found to bind Arf1 simultaneously with the GAP and to accelerate GTP hydrolysis. In contrast, GGA inhibits GAP activity. The inhibition is a consequence of GGA excluding GAP binding to Arf1-GTP. As a result, Arf1-GTP must dissociate from GGA in order to interact with and be inactivated by GAP. Because the regulation of Arf1 is integral to its function, our results suggest the current model for Arf1 function in coatomer dynamics does not extrapolate to the clathrin coat adaptor GGA.

The relationship of GGA with Arf1 and Arf GAPs is similar to that of a number of Ras effectors with Ras and Ras GAPs. The binding of Ras effectors and Ras GAP is mutually exclusive. Structural studies have shown that effectors and Ras GAP

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**FIG. 4. Effect of GGA overexpression on Arf1 dissociation from the Golgi apparatus.** Cells expressing VHS GAT or GGA1 fused with GFP were treated for 1 min with 56 μM brefeldin A, fixed, and immunostained with an antibody to Arf. Bar = 10 μm. Open arrows point to the perinuclear region of untransfected cells; solid arrows point to cells expressing VHS GAT, and arrowheads point to cells expressing epitope-tagged GGA1.

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**Table II.**

| K₅₀ (μM) | AGAP1 | ASAP1 |
|---------|-------|-------|
| Arf1    | 0.51 ± 0.06 (5) | 0.78 ± 0.16 (8) | 55 ± 26 (5) |
| [Δ13]Arf1 | 3.7 ± 0.95 (3) | NBD* | 0.45 ± 0.17 (19) |
| [3–7LFASK]Arf1 | 0.64 ± 0.03 (2) | 0.62 ± 0.05 (2) | 68 ± 11 (2) |
| [I49T]Arf1 | 1.8 ± 0.71 (3) | 1.1 ± 0.11 (3) | 516 ± 90 (2) |
| [F51Y]Arf1 | 3.0 ± 0.11 (2) | 2.3 ± 0.64 (3) | 615 ± 75 (2) |
| [R79G]Arf1 | 0.45 ± 0.18 (2) | Not done | 6.09 ± 0.26 (3) |
| [Y81H]Arf1 | 1.7 ± 0.3 (2) | 0.53 ± 0.06 (3) | 50 ± 12 (2) |
| [E105A]Arf1 | Not done | 0.63 ± 0.07 (3) | 52 ± 21 (2) |

* NBD, no binding detected.
* NAD, no activity detected.
bind overlapping sites on Ras involving switch I. The structural basis for the exclusionary binding on Arf1 has not been determined. Switch I mutations in Arf1 affected interaction with both GGA and GAP (8, 39). These results raise the possibility that switch I of Arf1 has a role in the interaction with Arf GAP that was not appreciated based on the crystal structure of Arf1-ArfGAP1 (26).

The mechanisms regulating Ras and Arf1 are not exactly analogous given the differences in the intrinsic GTPase rates of Ras and Arf1. Ras does have intrinsic GTPase activity, so dissociation from the effector and interaction with the GAP is not required to terminate the signal mediated by Ras-GTP-effector (12). In contrast, Arf1 has no detectable intrinsic GTPase activity, and GGA has not been examined. Intrinsic dissociation rates may be sufficiently rapid for GGA relative to the rates of the biological processes being controlled and need to be measured. Although we did not detect an effect of clathrin on GTP hydrolysis by GGA in the presence or absence of GGA (32), we cannot rule out that an additional factor may be required. Ongoing studies will determine the significance of these structural and biochemical differences to the cellular functions of the Arf Gaps and coat proteins and to what extent the mechanisms for Arf inactivation converge.

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