Structural Elements of ADP-ribosylation Factor 1 Required for Functional Interaction with Cytohesin-1*

(Received for publication, December 29, 1998, and in revised form, February 15, 1999)

ADP-ribosylation factor 1 (ARF1) is a 20-kDa guanine nucleotide-binding protein involved in vesicular trafficking. Conversion of inactive ARF-GDP to active ARF-GTP is catalyzed by guanine nucleotide exchange proteins such as cytohesin-1. Cytohesin-1 and its Sec7 domain (C-1Sec7) exhibit guanine nucleotide exchange protein activity with ARF1 but not ARF-like protein 1 (ARL1), which is 57% identical in amino acid sequence. With chimeric proteins composed of ARF1 (F) and ARL1 (L) sequences we identified three structural elements responsible for this specificity. Cytohesin-1 increased $[^{35}S]_g$uanosine 5'-($\gamma$-thio)triphosphate binding to L28/F (first 28 residues of L, remainder F) and to a much lesser extent F139/L, and mut13F139/L (F139/L with random sequence in the first 13 positions) but not A13ARF1 that lacks the first 13 amino acids; therefore, a nonspecific ARF N terminus was required for cytohesin-1 action. The N terminus was not, however, required for that of C-1Sec7. Both C-1Sec7 and cytohesin-1 effectively released guanosine 5'-($\gamma$-thio)triphosphate from ARF1, but only C-1Sec7 displaced the nonhydrolyzable GTP analog bound to mut13F139/L, again indicating that structure in addition to the Sec7 domain is involved in cytohesin-1 interaction. Some element(s) of the C-terminal region is also involved, because replacement of the last 42 amino acids with ARL sequence in F139L decreased markedly the interaction with cytohesin-1. Participation of both termini is consistent with the crystallographic structure of ARF in which the two terminal a-helices are in close proximity. ARF1 residues 28–50 are also important in the interaction with cytohesin-1; replacement of Lys-38 with Gln, the corresponding residue in ARL1, abolished the ability to serve as substrate for cytohesin-1 or C-1Sec7. These studies have defined multiple structural elements in ARF1, including switch 1 and the N and C termini, that participate in functional interactions with cytohesin-1 (or its catalytic domain C-1Sec7), which were not apparent from crystallographic analysis.

Proteins synthesized at the endoplasmic reticulum are, in part, transported among intracellular compartments by membrane vesicles with COPI, COPII, or clathrin-coats (1–3). COPI-coated vesicles participate in trafficking within the Golgi system and bidirectionally between the endoplasmic reticulum and Golgi (4). COPII-coated vesicles mediate anterograde transport from the endoplasmic reticulum to the Golgi (2), whereas clathrin-coated vesicles function in transport between plasma membrane and trans-Golgi compartments (2). Vesicle budding at a donor membrane is initiated by specific guanine nucleotide-binding proteins (5), the so-called “GTP-dependent molecular switches,” ADP-ribosylation factor (ARF)3 for clathrin- and COPI-coated vesicles and Sar1 for the COPII-coated vesicles (2, 3).

Mammalian ARFs and ARF-like proteins (ARLs) are ~20-kDa guanine nucleotide-binding proteins that are very similar in structure. ARFs were grouped into class I (ARFs 1–3), class II (ARFs 4 and 5), and class III (ARF6), based on size, amino acid sequence, and phylogenetic analysis (6). Class I ARFs were initially identified as components of vesicles that originate in the Golgi (7, 8) and the endoplasmic reticulum (9), whereas class III ARF ARF6 was more recently implicated in endocytosis and exocytosis at the plasma membrane (10–12). ARLs, like ARFs, are widely distributed in eukaryotic organisms; five human ARL cDNAs have been cloned (13). Based on studies of Saccharomyces cerevisiae (13) and rat kidney cells (14), ARLs may also play a role in vesicular trafficking. ARFs are known additionally as activators of phospholipase D and cholaert toxin (CTA) ADP-ribosyltransferase, properties that ARFs were believed to lack (15, 16).

ARF activity is determined by the identity of the nucleotide bound. Activation of inactive ARF-GDP, which is soluble, is accelerated by guanine nucleotide-exchange proteins (GEPs) that promote GDP release and thereby GTP binding (16, 17). Inactivation, which results from the hydrolysis of bound GTP, is dependent on GTPase-activating proteins (18, 19). ARF GEPs fall into two groups based on their susceptibility to inhibition by brefeldin A. The brefeldin A-sensitive GEPs, which are larger molecules (~200 kDa), include p200 (19), Gea1 (20), and the yeast Sec7 protein (21). Among the ~50-kDa brefeldin A-insensitive forms are cytohesin-1 (22), ARNO or cytohesin-2 (23), and GRP1 or cytohesin-3 (24). All known ARF GEPs contain a Sec7 domain, which is responsible for the catalytic activity and its brefeldin A inhibition (21).

The cytohesin-1 molecule comprises a coiled coil N terminus, a central Sec7 domain (C-1Sec7), and a C-terminal pleckstrin homology domain, the last ~100 residues of which are required for phospholipid binding (23, 24). The solution structure of the C-1Sec7 has been recently solved by NMR spectroscopy (25). It

* The costs of publication of this article 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

¶ Present address: College of Pharmacy, Chungbuk National University, 48 Gaesin-dong, Cheongju 361-763, Korea.

§ Present address: SmithKline Beecham Pharmaceuticals, UEO 435, P.O. Box 1539, 709 Swedeland Rd., King of Prussia, PA 19406.

1 The abbreviations used are: ARF, ADP-ribosylation factor; ARL, ARF-like protein; C-1Sec7, cytohesin-1 Sec7 domain; GEP, guanine nucleotide exchange protein; GTP-\(\gamma\)S, guanosine 5'-($\gamma$-thio)triphosphate; CTA, cholera toxin A subunit; h, human.
ARF1 Elements Involved in Cytohesin-1 Interactions

consists of two domains, each with five α-helices. The active site of C-1Sec7 resides in the C-terminal portion and sequences critical for catalysis include motifs 1 and 2, which had been assigned that role in crystal structures of the closely related ARNO Sec7 domain (26, 27).

All members of the ARF family contain the GTPase fold or G-domain composed of a six-stranded β-sheet surrounded by five α-helices with five loops that are involved in guanine nucleotide binding (28). ARF1 differs from other members of the ras superfamily in containing an amphipathic N-terminal α-helix and an extra β-sheet (29, 30). ARF1 in the GDP-bound form was crystallized as a dimer, with the extra β-sheet described as a region for protein-protein interactions. The N-terminal glycine of native ARF1 is myristoylated, which influences its membrane association (31).

ARFs interact with several regulatory proteins (e.g., GEPs, GTPase-activating proteins, and arfaptins), effector proteins (coatomer, phospholipase D, and G protein βγ subunits), as well as cholera toxin and phospholipids. The N- and C-terminal regions of ARF1 differ from those of ARL1 in several specific amino acids that are required for phospholipase D and CTA activation, respectively (32). In ARF1, Lys-15, Lys-16, Lys-181, and Arg-178 are critical for phosphatidylinositol 4,5-bisphosphate binding, and the N-terminal amphipathic α-helix participates in membrane association (33). Information about other GTPase-activating proteins suggests that switches I and II of ARF1 are also involved in guanine nucleotide exchange.

We recently reported that cytohesin-1 catalyzed guanine nucleotide exchange on nonmyristoylated ARF1, and its Sec7 domain accelerated GTP·S binding by ARF1, ARF5, and ARF6 (34). To define structural elements of ARF required for catalysis of guanine nucleotide exchange by cytohesin-1 and C-1Sec7, we took advantage of the observation that both intact cytohesin-1 and its Sec7 domain accelerated GTP·S binding by nonmyristoylated ARF1, and both failed to alter binding by ARL1. Chimeric proteins composed of sequences from hARF1 and hARL1 were synthesized and tested for exchange activity with C-1Sec7 and cytohesin-1.

**EXPERIMENTAL PROCEDURES**

Materials—t-α-Phosphatidyl-t-serine, cardiolipin, β-NAD+, and agmatine were purchased from Sigma; GTP·S was from Roche Molecular Biochemicals; and [35S]GTP·S and [adenine-32P]βNAD were from Du Pont. Sources of other reagents have been published (34, 35).

Preparation of Recombinant ARFs and Related Guanine Nucleotide-binding Proteins—DNA constructs for hARF1, hARL1, and related proteins (Δ13ARF1, L28/F, L50/F, F50/L, F73/L, F139/L, mut13F139/L, and ARFK38Q) were prepared in the pET7 vector and purified as described by Hong et al. (36) and Zhang et al. (32). Recombinant ARF proteins were obtained as follows. A single colony expressing recombinant protein was incubated overnight at 37 °C in 25 ml of Luria-Bertani medium containing ampicillin (100 μg/ml) before it was added to 500 ml of the same medium. Incubation with 1 mM isopropyl-1-thio-β-D-galactopyranoside was started at an A600 of 0.6 and continued for 2 h. After incubation of the cell pellet with lysozyme, followed by sonification and centrifugation (100,000 × g, 35 min), the supernatant was applied to a column (2.5 cm × 100 cm) of Ultrogel AcA54, which was eluted with buffer A (200 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM NaN3, 200 mM sucrose, 100 mM NaCl, 5 mM MgCl2). Fractions containing ARF activity were pooled and stored at −20 °C.

Preparation of Cytohesin-1 and C-1Sec7—Cytohesin-1 and its Sec7 domain were prepared as described previously (22, 34). Briefly, single colonies expressing the desired protein were incubated in 5 ml of Luria-Bertani medium containing ampicillin (100 μg/ml) and kanamycin (25 μg/ml) for 3–4 h at 37 °C and transferred to 100 ml of the same medium with antibiotics. After reaching an A600 of 0.5, cells were incubated with 2 mM isopropyl-1-thio-β-D-galactopyranoside for 4 h at 37 °C and then harvested for protein purification as described by Meacci et al. (22). GEP activities of cytohesin-1 and C-1Sec7 were regularly assayed with a mixture of purified native bovine brain ARFs (chiefly ARFs 1 and 3) as a basis for comparison of activities with other substrates.

**FIG. 1.** Effect of cytohesin-1 or C-1Sec7 on [35S]GTP·S binding to ARF1 and ARL1. Recombinant hARF (●, □, and ▲) or hARL1 (○, ●, and ○), 1 μg or 50 pmol each, was incubated without (●) or with 0.2 μg of cytohesin-1 (● and ○) or C-1Sec7 (▲ and ○). Data are means ± S.E. of values from triplicate assays in a representative experiment. Error bars smaller than symbols are not shown. Inset, GTP·S binding to ARL1. Similar observations were made with three different preparations of ARL1.

[35S]GTP·S Binding Assay—GTP·S binding and GEP activity assays have been published (34). In brief, 50 μl of a mixture containing ARF or related protein (1 μg unless otherwise indicated), 40 μg of bovine serum albumin, 10 μg of t-α-phosphatidyl-t-serine, 0.1 μg of each protease inhibitor (aprotinin, leupeptin, soybean trypsin inhibitor, and lima bean trypsin inhibitor), 0.5 mM 4-(2-aminoethyl)-benzamidylfluoride, cytohesin-1 or C-1Sec7 as indicated, and 4 μM [35S]GTP·S (~2.5 × 105 cpm) was incubated for the indicated time at 37 °C, unless otherwise indicated. Tubes were then placed in ice, and contents were transferred to nitrocellulose filters followed by washing of the incubation tube and filter with buffer B (25 mM Tris-HCl, pH 8.0, 5 mM MgCl2, 100 mM NaCl, 1 mM dithiothreitol). Scintillation fluid was added to dried filters before radioassay of bound [35S]GTP·S. Other details are described in figure legends.

**Release of Bound Guanine Nucleotides from Recombinant ARF Proteins**—ARF1 or related protein (usually 50 pmol or ~1 μg) was incubated for 40 min at 37 °C in a mixture (~50 μl) containing 20 mM Tris-HCl, pH 8.0, 1 mM NaN3, 10 mM dithiothreitol, 200 mM sucrose, 10 μg of t-α-phosphatidyl-t-serine, 40 μg of bovine serum albumin, protease inhibitors as used in binding assays, 2 mM MgCl2, 1 mM EDTA, 40 mM NaCl, and 4 μM [35S]GTP·S (~2.5 × 105 cpm). Nucleotide release with or without cytohesin-1 (1 μg) or C-1Sec7 (1 μg) was then determined after addition of a 1 mM concentration of the indicated nonradio labeled nucleotide and 3 mM MgCl2 (final concentrations in 100 μl of reaction mixture) and incubation at 37 °C for the indicated time (100 μl). [35S]GTP·S bound to ARFs was quantified as described for the binding assay.

**RESULTS**

To identify structural elements of ARF1 required for functional GEP interaction, we took advantage of our observations that cytohesin-1 and C-1Sec7 accelerated GTP·S binding to ARF1 (34), but both failed to enhance GTP·S binding to ARL1 (Fig. 1). The amino acid sequences of ARF1 and ARL1 are overall 57% identical (32), including the conserved residues.
involved in GTP binding. We therefore prepared the ARF(F) and ARF(L) chimeric proteins shown diagrammatically in Fig. 2 and tested these as substrates for cytohesin-1 and C-1Sec7 GEP activity.

Role of N terminus of ARF1 in Cytohesin-catalyzed Guanine Nucleotide Exchange—Cytohesin-1, as well as C-1Sec7, increased \(^{[35S]}\)GTP\(_S\) binding to recombinant L28/F composed of the first 28 amino acids of ARL1 and the remainder of ARF1 (Fig. 3). Although binding at 37 °C was too rapid to permit meaningful comparison of initial rates, it seemed clear at 25 °C that C-1Sec7 was much more active than cytohesin-1. Conclusions were similar when \(^{[35S]}\)GTP\(_S\) release from L28/F in the presence of both cytohesin-1 or C-1Sec7 was measured (Fig. 4). Thus, cytohesin-1 and C-1Sec7 can use an ARF with ARL1 sequence at its N terminus, consistent with the conclusion that the ARF1 N terminus can replace that of ARF1 for cytohesin-1-catalyzed guanine nucleotide exchange.

To investigate further effects of the ARF1 N terminus, rates of guanine nucleotide exchange catalyzed by 4.3 pmol of cytohesin-1 or C-1Sec7 were compared using as substrates native ARF1/3, ARF1 and Δ13ARF1. Cytohesin-1 and C-1Sec7 increased the initial rate of GTP\(_S\) binding by native ARF (−4- and 10-fold), respectively (Fig. 5). Thus, C-1Sec7 was the more efficient in catalyzing guanine nucleotide exchange, as had been reported for nonmyristoylated ARF1 (34). Binding by Δ13ARF1 was >10 times that by ARF1 in the absence of GEP and was accelerated by C-1Sec7 >40-fold at 2 min, whereas binding to ARF1 was increased much less (Fig. 6). Thus, although the N terminus of ARF1 was not required for C-1Sec7-catalyzed guanine nucleotide exchange, it did appear to influence guanine nucleotide exchange. The rates of release of bound \(^{[35S]}\)GTP\(_S\) from native ARF1/3 and ARF1 in the presence of C-1Sec7 were similar, whereas release from Δ13ARF1 was twice as fast (Fig. 7). In addition, cytohesin-1 failed to stimulate release GTP\(_S\) from Δ13ARF1.

Role of the C terminus of ARF1 in Cytohesin-1-catalyzed Guanine Nucleotide Exchange—Because the N- and C-terminal helices of ARF1 are in close proximity on the same side of the ARF1 molecule (29, 30), we wondered whether parts of both might interact with cytohesin-1 to influence guanine nucleotide exchange. To address this question, the last 42 residues of ARF1 were replaced with those of ARL1 in F139/L, which bound GTP\(_S\) much more slowly than did ARF1 (Fig. 1). Rates of binding in the presence of cytohesin-1 or C-1Sec7 were also considerably less (Fig. 8) than they were with ARF1. In fact, 10-fold more cytohesin-1 was required to obtain a rate of GTP\(_S\) binding to F139/L similar to that of ARF1 (Figs. 6 and 8). It appears that the C terminus of ARF1 may influence basal as well as catalyzed guanine nucleotide exchange, consistent with, but not in itself proof of, the presence of an interaction site outside of the Sec7 domain. GTP\(_S\) binding to mut13F139/L, with a random sequence (2-GNISPTSSRAFLA-13) at the N terminus replacing the ARF1 sequence (2-GNIFANFLPKGFL-13), was much more rapid than that to ARF1 (Fig. 1) and was only minimally affected by cytohesin-1. C-1Sec7, however, dramatically accelerated GTP\(_S\) binding, which was almost 50% of maximal at zero time (Fig. 8).

To assess further the role of the ARF1 C terminus, \(^{[35S]}\)GTP\(_S\) release from mut13F139/L without and with cytohesin-1 or C-1Sec7 was determined. Although C-1Sec7 acceler-
ated GTPγS release from mut13F139/L, intact cytohesin-1 did not (Fig. 9), consistent with the notion that the C terminus of ARF1 does restrain release of bound nucleotide and is involved in the functional interaction with cytohesin-1 but is not as critical for C-1Sec7. The experiments with mut13F139/L are also consistent with other evidence that cytohesin-1 does not require a specific sequence at the ARF1 N terminus.

Role of the Central Region of ARF1 in Cytohesin-1-catalyzed Guanine Nucleotide Exchange—The observation that C-1Sec7 increased GTPγS binding to ARFs 1, 5, and 6 suggested that it interacts with structure conserved in all three classes of ARFs. Because the region, between residues 21 and 100, has the least variability, we first prepared L50/F in which the first 50 amino acids of ARF1 were replaced with the corresponding ARL1 sequence. Both cytohesin-1 and C-1Sec7 failed to accelerate [35S]GTPγS binding to L50/F (Fig. 10). Because the protein was essentially as effective as ARF1 in activating cholera toxin ADP-ribosyltransferase activity in a GTP-dependent manner (data not shown), it appeared that residues 28–50 of ARF1 contain elements critical for functional interaction with cytohesin-1 or its Sec7 domain. In this region, ARF and ARL are 83% identical, and lysine 38 of ARF1 is the only amino acid conserved among mammalian and yeast ARFs that has a non-conservative replacement (glutamine) in ARL (Fig. 11). Both cytohesin-1 and C-1Sec7 failed to increase [35S]GTPγS binding to ARF1K38Q in which lysine 38 was replaced with glutamine (Fig. 10), although the mutant protein was as effective as ARF1 in stimulating cholera toxin ADP-ribosyltransferase activity in a GTP-dependent manner (Fig. 12). It appears that lysine 38 is involved in the functional interaction with cytohesin-1, but its role in the GTPase activity seems to be less critical.
critical either for the proper structure of this region of ARF1 or for its effective interaction with GEP.

The involvement of other regions of ARF1 in guanine nucleotide exchange catalyzed by cytohesin-1 or C-1Sec7 was investigated with chimeric proteins F50/L and F73/L. The latter had been shown to activate phospholipase D (32). Neither was a substrate for cytohesin-1 or C-1Sec7, consistent with the conclusion that residues between 73 and 139 of ARF1 are involved in functional interaction with these GEPs (Table I).

DISCUSSION

The data reported here support the view that multiple regions of both ARF1 and cytohesin-1 are involved in guanine nucleotide exchange. Structural elements outside of the Sec7 domain of cytohesin-1 appear to interact with a common ARF structure. Our data also support the idea that the region of ARF1 between amino acids 28 and 139 includes that minimum required structure. The three-dimensional structures of ARF1 and ARL1 are probably very similar, because both contain the conserved sequences that form the guanine nucleotide binding and GTPase catalytic sites. Indeed, the demonstration that hARL1 can activate CTA and phospholipase D under specific assay conditions led to the suggestion that the ARFs and ARLs are members of a continuum of proteins (37). It was for this reason that chimeric proteins composed of ARF1 and ARL1 sequences were used for our studies. Similar approaches to identify functional regions have been used to define interactions between other GTPases and their specific guanine nucleotide-exchange factors (38–40).

Although the N terminus of ARF1 was clearly important in its interaction with cytohesin-1 (34), the observation that cytohesin-1 increased GTP bound to L28/F and mut13F139/L appeared to indicate that the specific ARF sequence is not required. The association of native ARF-GDP or ARF-GTP with membranes involves the N-terminal myristoyl moiety and is reinforced by further interaction of the N-terminal amphipathic a-helix of ARF-GTP with membrane lipid (31, 37, 41). It is possible that when membrane bound, myristoylated ARF1-GTP exposes regions similar to those exposed on nonmyristoylated ARF1-GTP in solution. Therefore, the GEP might recognize the solution-exposed motifs, which could explain the similar rates of GTP release from native ARF1/3 and recombinant ARF1.

Deletion of the ARF1 N terminus markedly increased the rate and affinity of GTP binding (36, 42), consistent with earlier observations that the N terminus of ARF1 inhibits
ARF1 Elements Involved in Cytohesin-1 Interactions

Table I

| Experiment | ARF protein | [35S]GTP S bound |
|------------|-------------|-----------------|
|            | No GEP      | Cytohesin-1     | C-1Sec7 |
| 1          | ARF1/3      | 0.52 ± 0.03     | 2.82 ± 0.45 (5.4) |
|            | hARF1       | 0.53 ± 0.04     | 1.29 ± 0.05 (2.4) |
|            | F73L        | 0.22 ± 0.01     | 0.24 ± 0.05 (1.1) |
|            | F50L        | 0.15 ± 0.00     | 0.21 ± 0.02 (1.4) |
| 2          | ARF1/3      | 0.58 ± 0.05     | 2.68 ± 0.10 (4.6) |
|            | hARF1       | 1.14 ± 0.30     | 4.21 ± 0.15 (3.7) |
|            | hARF5       | 0.77 ± 0.10     | 1.37 ± 0.10 (1.7) |
|            | hARF6       | 0.60 ± 0.02     | 0.97 ± 0.04 (1.6) |

* In parentheses, binding relative to that with no GEP = 1.0.

Samples (50 pmol) of recombinant ARF or related protein or 20 pmol of mixed native ARF (ARF1/3) without or with 1 μg of cytohesin-1 or C-1Sec7 were incubated for 20 min at 37 °C before measurement of bound [35S]GTP S. Data are means ± S.E.M. of values from triplicate assays. Experiments were repeated three times with similar results.

Guanine nucleotide exchange. Participation of the ARF N terminus in the actin of cytohesin-1 suggests that the latter might disrupt interaction of the amphipathic α-helix with the ARF1 core, which could facilitate GDP release and association of the ARF1 N-terminal helix with a membrane surface. The N terminus of GTP-bound ARF1 in association with phospholipids could be oriented parallel to the membrane surface (43), allowing its interaction with effectors or GTPase-activating proteins.

The inactive state of many guanine nucleotide-binding proteins in the cell is preserved by their association with GDP dissociation inhibitor proteins. Rab proteins, for instance, interact specifically with Rab-GDP dissociation inhibitors, and Gα subunits are inactive when associated with βγ subunits as heterotrimeric bound to G protein-coupled receptors (28). The ARF domain of the 64-kDa ARD1 contains an internal GDP dissociation inhibitory region analogous to the N terminus of ARF1 (44). Our data support the idea that the N terminus of ARF1 functions as a GDP dissociation inhibitor domain. Observations were similar when the rates of GTP binding by the ARF1 N-terminal helix with a membrane surface. The N and C termini of ARF1 are that are conserved among ARFs. Based on our results, it is tempting to speculate that the N and C termini of ARF1 are recognized simultaneously by cytohesin-1, and that the C-1Sec7 domain interacts with an internal sequence common to ARFs. It appears that the model of ARF1|Δ17) interacting with a Sec7 domain, based on a crystal structure of the ARNO Sec7 domain associated with an ARF1 that lacks the N-terminal 17 residues (26, 27, 45), does not reflect completely, or perhaps correctly, interactions in the ARF-cytohesin complex.

Mossessova et al. (26) by protein protection analysis of Δ17ARF1 (which lacks the first 17 residues including the amphipathic α-helix) and the Sec7 domain of ARNO determined that multiple regions were shielded by their interaction. It was concluded that the putative switch I and II segments of ARF1 were the major sites of contact with the Sec7 domain. This approach could not, however, provide information concerning physiological roles of the ARF1 N terminus and regulatory elements of cytohesin-1. In our studies, functional interaction of C-1Sec7 with the internal region of ARF1 appeared to be chiefly via amino acids 28–50, which contain the so-called extra β-sheet, β2E, of ARF1 as seen in the crystal structure of ARF-GDP (29, 30). The finding that cytohesin-1 and C-1Sec7 were unable to accelerate [35S]GTP S binding to the chimeric protein L50/F is consonant with participation of the extra β-sheet in the GEP interaction.

Comparison of ARF amino acid sequences reveals that lysine 38 is conserved among all of the substrates for both cytohesin-1 and C-1Sec7 (Fig. 11). The failure of ARF1K38Q to serve as a substrate for either one is consistent with involvement of this region in the functional interactions. Although we cannot rule out the possibility of a conformational change induced by the mutation, the protein was essentially as active as ARF1 in activating the cholera toxin ADP-ribosyltransferase. Lysine 38 is located at the N terminus of the L2 loop, which by analogy to the p21 ras structure would be near the effector loop (29, 30). Thus it is tempting to propose that the sequence KKL (residues 36–38) in ARF1 is critical for catalysis of guanine nucleotide exchange.

To our knowledge, the molecular mechanism of guanine nucleotide exchange catalyzed by cytohesin-1 is not yet clear. Although it may be modified by differences in phospholipid requirements for each protein, we might propose that cytohesin-1 interacts with ARF1 in specific membrane environments that have destabilized its molecular structure. The change in conformation might allow interaction of the Sec7 domain with internal sequences of membrane-associated ARF1. Displacement of bound GDP could allow formation of a Sec7 complex with nucleotide-free ARF1, followed by dissociation of the complex triggered by GTP binding. Understanding the regulation of cytohesin-1 function in lymphocytes and other cells (46), as well as the role of ARF-cytohesin events in cell adhesion (47, 48), will require the development of methods to allow monitoring of specific cytohesin-1 interactions in its physiological milieu.

Acknowledgment—We thank Carol Kosh for expert secretarial assistance.

REFERENCES
1. Rothman, J. E. (1994) Nature 372, 55–63
2. Schekman, R., and Orci, L. (1996) Science 271, 1526–1533
ARF1 Elements Involved in Cytohesin-1 Interactions

3. Rothman, J. E., and Wieland, F. T. (1996) Science 272, 227–234
4. Schekman, R., and Mellman, I. (1997) Cell 90, 197–200
5. Nussler, C., and Balch, W. E. (1994) Annu. Rev. Biochem. 63, 949–990
6. Tsujiya, M., Price, S. R., Tsai, S.-C., Moss, J., and Vaughan, M. (1991) J. Biol. Chem. 266, 2772–2777
7. Stearns, T., Willingham, M. C., Botstein, D., and Kahn, R. A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 1238–1242
8. Serafini, T., Orii, L., Amherdt, M., Brunner, M., Kahn, R. A., and Rothman, J. E. (1991) Cell 67, 239–253
9. Dascher, C., and Balch, W. E. (1994) J. Biol. Chem. 269, 1437–1448
10. D’Souza-Schorey, C., Li, G., Colombo, M. I., and Stahl, P. D. (1995) Science 267, 1175–1178
11. Radhakrishna, H., and Donaldson, J. G. (1997) J. Cell Sci. 110, 209–220
12. Lee, F.-J. S., Huang, C.-F., Yu, W. L., Bui, L. M., Lin, C. Y., Huang, M. C., Moss, J., and Vaughan, M. (1997) J. Biol. Chem. 272, 30998–41005
13. Lowe, S. L., Wong, S. H., and Hong, W. (1996) J. Cell Sci. 109, 209–220
14. Tamkun, J. W., Kahn, R. A., Kissinger, M., Britzelma, B. J., Rulka, C., Scott, M. P., and Kennison, J. A. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3120–3124
15. Moss, J., and Vaughan, M. (1998) J. Biol. Chem. 273, 21431–21434
16. Donaldson, J. G., and Klausner, R. D. (1994) Curr. Opin. Cell Biol. 6, 527–532
17. Makler, V., Cukierman, E., Rotman, M., Admon, D., and Cassel, D. (1995) J. Biol. Chem. 270, 5232–5237
18. Morinaga, N., Moss, J., and Vaughan, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12926–12931
19. Poyreche, A., Paris, S., and Jackson, C. L. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 479–481
20. Sata, M., Donaldson, J. G., Moss, J., and Vaughan, M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 13039–13044
21. Meacci, E., Tsai, S.-C., Adamik, R., Moss, J., and Vaughan, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1745–1748
22. Chardin, P., Paris, S., Antony, B., Robineau, S., Berard-Dufour, S., Jackson, C. L., and Chabre, M. (1996) Nature 384, 481–484
23. Klarlund, J. K., Rameh, J., L., Cantley, L. C., Buxton, J. M., Holik, J. J., Sakelis, C., Patki, V., Corvera, S., and Czech, M. P. (1996) J. Biol. Chem. 271, 8623–8628
24. Bata, S. F., Schnuchel, A., Wang, H., Olegniaczek, E. T., Meadows, R. P., Lipsky, B. P., Harris, K. A. S., Staunton, D. E., and Pesik, S. W. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7909–7914
25. Mosseessaova, E., Gulbis, J. M., and Goldberg, J. (1998) Cell 92, 415–423
26. Cherfils, J., Menuet, J., Mathieu, M., Le Brass, G., Robineau, S., Beraud-Dufour, S., Antony, B., and Chardin, P. (1998) Nature 392, 101–105
27. Sprang, S. R. (1997) Annu. Rev. Biochem. 66, 659–678
28. Greaves, E. S., Jihui, T., Teahan, C., Solari, R., Fensome, A., Thomas, G. M., Cockcroft, S., and Bax, B. (1995) Nat. Struct. Biol. 2, 797–806
29. Amor, J. C., Harrison, D. H., Kahn, R. A., and Ringe, D. (1994) Nature 372, 704–708
30. Antony, B., Berard-Dufour, S., Chardin, P., and Chabre, M. (1997) Biochemistry 36, 4675–4684
31. Zhang, G.-F., Patton, W. A., Lee, F.-J. S., Liyanage, M., Han, J.-S., Rhee, S. G., Moss, J., and Vaughan, M. (1995) J. Biol. Chem. 270, 21–24
32. Randazzo, P. A. (1997) J. Biol. Chem. 272, 7688–7692
33. Pacheco-Rodriguez, G., Meacci, E., Vitale, N., Moss, J., and Vaughan, M. (1998) J. Biol. Chem. 273, 26545–26548
34. Patton, W. A., Zhang, G.-F., Moss, J., and Vaughan, M. (1997) in Bacterial Toxins: Tools in Cell Biology and Pharmacology (Aktories, K., ed) pp. 15–32, Chapman & Hall, Weinheim
35. Hong, J.-X., Haun, R. S., Tsai, S.-C., Moss, J., and Vaughan, M. (1994) J. Biol. Chem. 269, 9743–9745
36. Hong, J.-X., Lee, F.-J. S., Patton, W. A., Lin, C. Y., Moss, J., and Vaughan, M. (1998) J. Biol. Chem. 273, 15872–15876
37. Mosteller, R. D., Han, J., and Brea, D. (1994) Mol. Cell. Biol. 14, 1104–1112
38. Li, R., and Zheng, Y. (1997) J. Biol. Chem. 272, 4671–4679
39. Liang, J. O., Sung, T.-C., Morris, A. J., Frohman, M. A., and Kornfeld, S. (1997) J. Biol. Chem. 272, 33001–33008
40. Paris, S., Berard-Dufour, S., Robineau, S., Bigay, J., Antony, B., Chabre, M., and Chardin, P. (1997) J. Biol. Chem. 272, 22221–22226
41. Kahn, R. A., Rulka, C., Serafini, T., Weiss, O., Rulka, C., Clark, J. A., Amherdt, M., Ringer, M., and Rothman, J. E. (1992) J. Biol. Chem. 267, 13039–13046
42. Lossoane, J. A., and Prestegard, J. H. (1998) Biochemistry 37, 706–716
43. Vitale, N., Moss, J., and Vaughan, M. (1997) J. Biol. Chem. 272, 25077–25082
44. Goldberg, J. (1998) Cell 95, 237–248
45. Liu, L., and Pohlajak, B. (1992) Biochim. Biophys. Acta 1132, 75–78
46. Kalonan, W., Nagel, W., Schiller, B., Zeitlmann, L., Gadar, O., Stockinger, H., and Seed, B. (1996) Cell 86, 233–242
47. Nagel, W., Zeitlmann, L., Schiller, B., Geiger, C., Kolanus, J., and Kolanus, W. (1998) J. Biol. Chem. 273, 14853–14861