Guanine Nucleotide Exchange on ADP-ribosylation Factors Catalyzed by Cytohesin-1 and Its Sec7 Domain*

(Received for publication, June 19, 1998, and in revised form, July 27, 1998)

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ADP-ribosylation factors (ARFs) are 20-kDa guanine nucleotide-binding proteins that require specific guanine nucleotide-exchange proteins (GEPs) to accelerate the conversion of inactive ARF-GDP to active ARF-GTP. Cytohesin-1, a 46-kDa ARF GEP, contains a central Sec7 domain of 188 amino acids similar in sequence to a region of the yeast Sec7 protein. Cytohesin-1 and its 22-kDa Sec7 domain (C-1 Sec7), synthesized in Escherichia coli, were assayed with recombinant non-myristoylated ARFs and related proteins to compare their GEP activities. Both were effective with native mammalian ARFs 1 and 3. Cytohesin-1 accelerated GTPγS (guanosine 5′-3-O-(thio)triphosphate) binding to recombiant human ARF1 (rARF1), yeast ARF3, and ARD1 (a 64-kDa guanine nucleotide-binding protein containing a C-terminal ARF domain). In contrast, C-1 Sec7 enhanced GTPγS binding to recombinant human ARFs 1, 5, and 6; yeast ARFs 1, 2, and 3; ARD1; two ARD1 mutants that contain the ARF domain; and Δ53ARF1, which lacks the N-terminal α-helix. Neither C-1 Sec7 nor cytohesin-1 increased GTPγS binding to human ARF-like ARL proteins 1, 2, and 3. Thus, ARLs, initially differentiated from ARFs because of their inability to activate cholera toxin, differ also in their failure to interact functionally with C-1 Sec7 or cytohesin-1. As C-1 Sec7 was much less substrate-specific than cytohesin-1, it appears that structure outside of the Sec7 domain is important for ARF specificity. Data obtained with mutant ARF constructs are all consistent with the conclusion that the ARF N terminus is an important determinant of cytohesin-1 specificity.

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determinants of substrate specificity. We, therefore, compared the activities of full-length cytohesin-1 (46-kDa) and its 22-kDa Sec7 domain (C-1 Sec7) with several ARF and ARL protein substrates. As reported here, cytohesin-1 accelerated GTP\(\gamma\)S binding by rARF1, yeast ARF3, and ARD1 only, whereas C-1 Sec7 was effective with a much broader range of ARFs and related proteins. The N terminus of ARF1 was shown to be important for its effective interaction with cytohesin-1.

**EXPERIMENTAL PROCEDURES**

**Materials—**t-c, a-Phosphatidyl-t-serine, β-NAD\(^+\), and aminoguan were purchased from Sigma; GTP\(\gamma\)S, GDP, and GTP from Boehringer Mannheim; [\(\gamma\)S]GTP\(\gamma\)S (12.5 μCi/μmol), [\(\gamma\)H]GDP (10 Ci/μmol), and [adeno-9-]β-NAD\(^+\) from NEN Life Science Products. Sources of other reagents have been published elsewhere (35).

**Preparation of Recombinant ARFs and Related Guanine Nucleotide-binding Proteins—**Synthesis of recombinant proteins has been described for hARF1 (36), hARF5 (37), hARF6 (38), Δ13hARF1 (36), hARL1 (39), and yARFS (40). hARLS 2 and 3 and yARFS 1 and 2 were cloned in a PET7 (Novagen) vector (36). Cloning of hARD1 (6) and synthesis of hARD1, the ARF domain (p3) of hARD1 (7), and N387 hARD1 (41) have been reported. General methods for purification of the several ARF-related proteins have been published elsewhere (36). Single colonies expressing recombinant proteins were grown overnight at 37 °C in 25 ml of Luria-Bertani medium containing ampicillin (100 μg/ml), and then added to 500 ml of the same medium. Incubation with 1 mM isopropyl-1-thio-β-galactopyranoside was started at an A\(_{600}\) of 0.6 and continued for 2 h. Following incubation of the cell pellet with lysozyme followed by sonication and centrifugation (100,000 \(\times\) g, 35 min), the supernatant was applied to a column (2.5 × 200 cm) Ultrogel AcA 54, which was eluted with Buffer A (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM Na\(_2\)SO\(_4\), 200 mM sucrose, 100 mM NaCl, 5 mM MgCl\(_2\)).

Fractions containing ARF activity were pooled and stored at −20 °C.

**Preparation of Cytohesin-1 and C-1 Sec7—**Recombinant cytohesin-1 (B2–1) was prepared as described by Meacci et al. (26). To prepare the Sec7 domain (DIII) as a His, fusion protein, B2–1 DNA was digested with restriction enzymes KpnI and HindIII. The product was subcloned in plasmid pQE30, which was then integrated into the Escherichia coli M15 host strain carrying the pREP4 repressor plasmid. Single colonies, selected by resistance to ampicillin and kanamycin, were grown overnight at 37 °C in 25 ml of Luria-Bertani medium containing ampicillin (100 μg/ml), and then added to 500 ml of the same medium. Incubation with 1 mM isopropyl-1-thio-β-galactopyranoside was started at an A\(_{600}\) of 0.6 and continued for 2 h. Following incubation of the cell pellet with lysozyme followed by sonication and centrifugation (100,000 \(\times\) g, 35 min), the supernatant was applied to a column (2.5 × 200 cm) Ultrogel AcA 54, which was eluted with Buffer A (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM Na\(_2\)SO\(_4\), 200 mM sucrose, 100 mM NaCl, 5 mM MgCl\(_2\)). Fractions containing ARF activity were pooled and stored at −20 °C.

**RESULTS**

**Effects of Cytohesin-1 and C-1 Sec7 on GTP\(\gamma\)S Binding by ARFs 1, 5, and 6**—The purified recombinant ARF proteins (non-myristoylated) were functional in their ability to stimulate cholera toxin ADP-ribosyltransferase activity. hARF1 and hARF5 required GTP for activity, whereas hARF6 was similarly active with GDP and GTP, presumably due to the presence of tightly bound nucleotide, which did not exchange with that in the medium (data not shown) as had been found previously (42). The rate of GTP\(\gamma\)S binding to hARF1 was proportional to the amount of GEP below −2.2 pmol and was maximal with ~23 pmol of C-1 Sec7 or 11 pmol of cytohesin-1 (Fig. 1). GEP activity of both these molecules is relatively low, and data demonstrating GTP\(\gamma\)S binding in excess of the amount of GEP present are shown only in Figs. 3, 5, and 8. (Such data for cytohesin-1 are also found in Ref. 26.) Cytohesin-1 increased GTP\(\gamma\)S binding by hARF1 in a time-dependent manner, but had little or no effect on hARF5 or hARF6 (Figs. 2 and 3). hARF1 appeared to be the preferred substrate for cytohesin-1 with K\(_{\text{on}}\)K\(_{\text{off}}\) of 6.7 ± 3.2 × 10\(^{−6}\) min\(^{−1}\)m. C-1 Sec7 increased GTP\(\gamma\)S binding to 1 μm hARF1, 5, and 6 by 8-, 4-, and 3-fold, respectively (Fig. 4). The K\(_{\text{on}}\)/K\(_{\text{off}}\) values for ARFs 1, 5, and 6 were, respectively, 2.2 ± 1.2 × 10\(^{−6}\), 5.4 ± 1.6 × 10\(^{−4}\), and 3.3 ± 1.1 × 10\(^{−4}\) min\(^{−1}\)m (Fig. 5). C-1 Sec7 was less substrate-specific than cytohesin-1 and also more efficient in catalyzing nucleotide exchange on hARF1, consistent with the conclusion that structural elements outside of the Sec7 domain contribute to substrate specificity and serve also to restrain catalytic activity.

**Release of GDP and GTP from ARFs 1, 5, and 6 Catalyzed by Cytohesin-1 or C-1 Sec7**—In the absence of GEP, release of GTP\(\gamma\)S was faster from hARF5 (K\(_{\text{off}}\) = 0.108 ± 0.009) than from hARF1 (K\(_{\text{off}}\) = 0.032 ± 0.003) (Fig. 6). Cytohesin-1 clearly accelerated release from hARF1 (K\(_{\text{off}}\) = 0.056 ± 0.000) but had minimal effect on hARF5 (K\(_{\text{off}}\) = 0.144 ± 0.006). C-1 Sec7, on the other hand, increased K\(_{\text{off}}\) for hARF5 (K\(_{\text{off}}\) = 0.36 ± 0.036) > hARF1 (K\(_{\text{off}}\) = 0.115 ± 0.006). There was no release of [\(\gamma\)S]GTP\(\gamma\)S from hARF6 with or without cytohesin-1 or C-1 Sec7 (Fig. 6). Although cytohesin-1 did not, C-1 Sec7 did accelerate the release of bound GDP from hARF6 (Fig. 7), consistent with its ability to accelerate GTP\(\gamma\)S binding (Figs. 4 and 5).
native ARFs or by recombinant hARF1 and C-1 Sec7 increased binding only 3.3-fold. Both accelerated binding dramatically in the presence of PS (Table I). GTP\textsuperscript{gS} binding to 1 \mu{}M hARF1 was significantly less than to 0.4 \mu{}M native ARF, despite its higher concentration.

Effects of Cytohesin-1 or C-1 Sec7 on GTP\textsuperscript{gS} Binding by Other ARF-related Molecules—The initial rate of GTP\textsuperscript{gS} binding to D\textsubscript{13}ARF1, which lacks the first 13 amino acids and the N-terminal myristate, was markedly increased by C-1 Sec7 (Fig. 8). Cytohesin-1 was, however, without effect (Fig. 8).

ARD1 is a 64-kDa protein that contains an 18-kDa C-terminal ARF domain (p3), which is 60% identical to the corresponding sequence of ARF1. The mutant D\textsubscript{387}ARD1 which lacks the first 387 amino acids of ARD1, corresponds to the ARF domain plus a 15-amino acid N-terminal extension. GTP\textsuperscript{gS} binding to ARD1, D\textsubscript{387}ARD1, and p3 was similarly accelerated by C-1

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Effects of Cytohesin-1 or C-1 Sec7 on the release of bound [\textsuperscript{3H}]GDP—The effects of cytohesin-1 or C-1 Sec7 on the release of bound [\textsuperscript{3H}]GDP from hARF6 was similar (Fig. 7). The experiment was repeated twice with similar results. Data are presented as in Fig. 1. Two independent experiments with similar results were performed.
TABLE I

| GEP (1 μg) | PS (10 μg) | GTPγS bound (pmol) |
|------------|------------|---------------------|
| None       | 0          | 1.4 ± 0.43          |
| Cytohesin-1| +          | 1.3 ± 0.06          |
| C-1 Sec7   | +          | 7.3 ± 0.37          |
| C-1 Sec7   | +          | 4.6 ± 0.11          |
| C-1 Sec7   | +          | 6.2 ± 0.29          |
| C-1 Sec7   | +          | 10 ± 0.39           |

* In parentheses, binding relative to that with no GEP = 1.0. The experiment was repeated twice, with similar results.

Sec7, although the effects were smaller than that on hARF1 (Table II, Experiment 1). Cytohesin-1 had effects similar to those of C-1 Sec7 on ARD1 and Δ387ARD1 but was without effect on p3, which lacks sequence corresponding to the ARF N terminus that appears to be important for interaction with cytohesin-1.

C-1 Sec7 increased GTPγS binding to yARFs 1 and 2 approximately 9-fold and to yARF 4-fold (Table II, Experiment 2). Cytohesin-1, which had no effect with yARFs 1 and 2, did significantly increase binding to yARF3, albeit to a lesser degree than did C-1 Sec7 (Table II, Experiment 2). Although ARLs (ARF-like) proteins are very similar to ARFs in size and structure, neither C-1 Sec7 nor cytohesin-1 accelerated GTPγS binding to hARLs 1, 2, or 3 (Table II, Experiment 3).

FIG. 8. Time course of [35S]GTPγS binding to Δ13hARF1. Δ13hARF1 (~50 pmol) was incubated for the indicated time at 37 °C with 0.1 μg of cytohesin-1 (closed squares) or C-1 Sec7 (closed triangles) or vehicle (empty squares). Data are presented as in Fig. 1. Two independent experiments were performed with similar results.

DISCUSSION

The data reported here establish that C-1 Sec7 can act as a GEP for a variety of ARF-related proteins. Determinants of the much more restricted substrate specificity of cytohesin-1 must, therefore, lie outside of the Sec7 domain. C-1 Sec7 accelerated GTPγS binding to hARFs 1, 5, and 6; yARFs 1, 2, and 3; ARD1 and its ARF domain; and Δ18ARF1 (lacking the first 13 amino acids). Cytohesin-1, on the other hand, was active only with hARF1 (or native class I ARFs), yARF3, ARD1, and Δ387ARD1. Its ability to serve as a GEP for yARF3 was somewhat surprising, as yARF3 is in toto more similar to ARF6 (60% identical) than to any other of the mammalian ARFs (40). At the N terminus, however, it is not. In the first 12 positions, there are eight differences, including four residues missing in ARF6. Thus, the N terminus of hARF6 differs markedly from that of yARF3, as it does also from the five other mammalian ARFs. hARF1 differs also from yARF3 in 8 of the first 12 positions, but some of those are conservative replacements, and there are no “missing” amino acids so that the N-terminal structure of yARF3 overall probably resembles that of ARF1 more than it does that of hARF6. The failure of ARLs to serve as substrates for cytohesin-1 or its Sec7 domain parallels their relative inability to activate cholera toxin. It may be related to differences in structure of the switch 2 regions (4 of 11 residues different in ARF1 and ARL1), which in ARF is believed to interact with the ARNO Sec7 domain (33, 34).

Although there was no release of bound GTPγS from hARF6 without or with cytohesin-1 or C-1 Sec7, the latter did accelerate release of bound [3H]GDP, consistent with its ability to accelerate GTPγS binding by hARF6 (as well as several other ARF-related molecules). The mechanism of action of the ARF GEPs remains to be established. Certain GEPs for other GTPases of the Ras superfamily, however, are believed to act by stabilizing the protein in a nucleotide-free state and the association of ARNO with a nucleotide-free ARF mutant was shown directly by gel filtration (43). The intrinsic rate of GDP release from ARF1 has been reported to range over 2 orders of magnitude depending on the specific phospholipid present, whereas rates of release of GTPγS varied less than 20% (44). In the presence of PIP2, for example, the GDP off-rate was 20 times that for GTPγS (44). Preferential interaction with the nucleotide-free conformation of hARF6 could be consistent with the effect of C-1 Sec7 on release of bound GDP, but not GTPγS.

Cytohesin-1 failed to accelerate GTPγS binding by the ARF domain of ARD1 (p3), which lacks sequence corresponding to the first 15 amino acids of ARF1, and thus resembles Δ13ARF1 (6, 7). It did, however, function with Δ387ARD1, which corresponds to p3 with an N-terminal extension of 15 amino acids, demonstrating again the importance of the ARF N terminus in the functional interactions with cytohesin-1. Both of these ARFs with N-terminal deletions were, however, substrates for C-1 Sec7. hARF6, although it does not have such a large deletion, may be another example of an ARF N terminus unsuitable for cytohesin-1 interaction, but functional with C-1 Sec7.

Other workers (43) demonstrated the importance of the ARF N terminus in nucleotide exchange catalyzed by ARNO, which is 82% identical in amino acid sequence to cytohesin-1. Two groups recently reported crystal structures of the ARNO Sec7 domain (33, 34). Paris et al. (43) had earlier investigated the interactions of intact ARNO or its Sec7 domain with myristoylated ARF1 or Δ17ARF1 (lacking the first 17 amino acids as well as the N-terminal myristate) and the effects of several phospholipids on them. Although GTPγS binding to myristoylated ARF1 in the presence of ARNO was markedly accelerated by the addition of phospholipid vesicles containing PIP2, the phospholipids had no effect on ARNO-catalyzed GTPγS binding to Δ17ARF1. In addition, in the absence of phospholipid, the activities of intact ARNO and its Sec7 domain toward Δ17ARF1 were identical (43). Thus, PIP2 binding to the PH domain of ARNO does not modify its catalytic activity but serves to promote its association with membranes. Similarly, Paris et al. (43) found that ARF1-GDP was a poor substrate for ARNO in the absence of PIP2 and concluded that binding of PIP2 by both the PH domain of ARNO and the terminal α-helix of ARF is needed to concentrate the two proteins at a membrane surface, thereby facilitating their interaction. Conformational change at the N terminus resulting from interaction of the myristate and/or the α-helix with membrane lipids probably contributes to the release of bound GDP (intrinsically or GEP-catalyzed) and thereby GTP binding.

The effect of PS, which was required for cytohesin-1 activity and also enhanced that of C-1 Sec7, is an example of the importance of acidic phospholipids in the action of these ARF...
The development of in vitro assays of GEP activity that will accurately reflect ARF specificity is of major importance, along with improved detection of individual ARFs in intact cells.

Acknowledgments—We thank Dr. Walter A. Patton for the ARFs utilized in initial studies, Ronald Adamik for the native ARF, Dr. Makoto Sata for the yARFs, and Carol Kosh for expert manuscript preparation.

REFERENCES

1. Rothman, J. E. (1994) Nature 372, 55–63
2. Cosson, P., and Letourneur, F. (1997) Curr. Opin. Cell Biol. 9, 484–487
3. Tsuchiya, M., Price, S. R., Tsai, S.-C., Moss, J., and Vaughan, M. (1991) J. Biol. Chem. 266, 2772–2777
4. Moss, J., and Vaughan, M. (1995) J. Biol. Chem. 270, 12327–12330
5. Tsai, S.-C., Adamik, R., Kahn, R. A., Kissinger, M., Brinzelu, B. J., Rulka, C., Scott, M. P., and Kennison, J. A. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3120–3124
6. Mitchima, K., Tsuchiya, M., Nightingale, M. S., Moss, J., and Vaughan, M. (1993) J. Biol. Chem. 268, 8801–8807
7. Vitale, N., Moss, J., and Vaughan, M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1941–1944
8. Rothman, J. E., and Wieland, F. T. (1996) Science 272, 227–234
9. Scheekman, R., and Orci, L. (1996) Science 271, 1536–1533
10. Zhao, L., Helms, J. B., Brugger, B., Harker, C., Martoglio, B., Graf, R., Brunner, J., and Wieland, F. T. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4418–4423
11. Brown, H. A., Gutowski, S., Moomaw, C. R., Slaughter, C., and Sternweis, P. C. (1993) Cell 75, 1137–1145
12. Cockcroft, S., Thomas, G. M. H., Fensome, A., Geny, B., Cunningham, E., Gout, I., Hiles, I., Totty, N. F., Truong, O., and Hsuan, J. J. (1994) Science 263, 523–526
13. Roth, M. G., and Sternweis, P. C. (1987) Curr. Opin. Cell Biol. 9, 519–526
14. Lisovicvitch, M., Chalifa, V., Pertile, P., Chen, C.-S., and Cantley, L. C. (1994) J. Biol. Chem. 269, 21403–21406
15. Makler, V., Cukierman, E., Rotman, M., Admon, A., and Cukierman, D. (1995) J. Biol. Chem. 270, 5232–5237
16. Cukierman, E., Huber, I., Rotman, M., and Cukierman, D. (1995) Science 269, 2270–2272
17. Ding, M., Vitale, N., Tsai, S.-C., Adamik, R., Moss, J., and Vaughan, M. (1996) J. Biol. Chem. 271, 24005–24009
18. Poon, P. P., Wang, X., Rotman, M., Huber, I., Cukierman, E., Cassel, D., Singer, R. A., and Johnstone, G. C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 10074–10077
19. Donaldson, J. G., Finazzi, D., and Klausner, R. D. (1992) Nature 359, 350–352
20. Helms, J. B., and Rothman, J. E. (1992) Nature 359, 352–354
21. Morinaga, N., Moss, J., and Vaughan, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12327–12330
22. Meacci, E., Tsai, S.-C., Adamik, R., Moss, J., and Vaughan, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12856–12860
23. Peyroche, A., Paris, S., and Jackson, C. L. (1996) Nature 384, 479–481
24. Sata, M., Donaldson, J. G., Moss, J., and Vaughan, M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 4204–4208
25. Chardin, P., Paris, S., Antonny, B., Robinoue, S., Berou-Dufour, S., Jackson, C. L., and Chahre, M. (1996) Nature 384, 481–484
26. Tsai, S.-C., Adamik, R., Moss, J., and Vaughan, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3063–3066
27. Tsai, S.-C., Adamik, R., Moss, J., and Vaughan, M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 305–309

TABLE II

Specificity of Cytohesin-1 for ARFs

| Experiment no. | ARF protein | No GEP | Cytohesin-1 | C-1 Sec7 |
|---------------|-------------|--------|-------------|---------|
| 1             | hARF1       | 0.89 ± 0.03 | 5.0 ± 0.11 (5.6)a | 6.5 ± 0.16 (7.3) |
|               | hARD1       | 0.35 ± 0.01 | 1.3 ± 0.02 (3.7) | 1.7 ± 0.09 (4.9) |
|               | p3          | 0.30 ± 0.02 | 0.29 ± 0.01 (1.0) | 1.2 ± 0.06 (4.0) |
|               | Δ387ARD1    | 0.35 ± 0.01 | 1.4 ± 0.13 (4.0) | 1.8 ± 0.03 (5.1) |
| 2             | yARF1       | 1.3 ± 0.06 | 7.3 ± 0.35 (5.6) | 10.2 ± 0.39 (7.8) |
|               | yARF2       | 3.7 ± 1.5  | 4.2 ± 0.67 (1.1) | 33 ± 0.43 (8.9)  |
|               | yARF3       | 3.9 ± 0.30 | 5.6 ± 0.38 (1.4) | 38 ± 1.7 (9.7)   |
| 3             | hARF1       | 0.98 ± 0.14 | 3.7 ± 0.21 (3.8) | 6.2 ± 0.25 (6.3) |
|               | hARL1       | 0.61 ± 0.21 | 0.74 ± 0.35 (1.2) | 0.29 ± 0.09 (0.5) |
|               | hARL2       | 0.36 ± 0.10 | 0.30 ± 0.10 (0.8) | 0.23 ± 0.03 (0.6) |
|               | hARL3       | 3.2 ± 0.88  | 4.3 ± 0.88 (1.3) | 3.6 ± 0.01 (1.1) |

In parentheses, binding relative to that with no GEP = 1.0. The experiments were carried out twice with similar results.

GEPs. It differs, however, from the effect of phosphatidylinositol 3,4,5-triphosphate on GRP1, which was not seen with the Sec7 domain alone, i.e., it apparently required specifically the PH domain (45). PH domains from GRP1 and cytohesin-1, but not those from IRS-1 or SOS, specifically bound phosphatidylinositol 3,4,5-triphosphate (27). Klarlund et al. (45) suggested that GRP1 and cytohesin-1 might serve to link signaling pathways that involve activation of phosphatidylinositol 3-kinase to ARF function in vesicular trafficking or in cell adhesion, based on the reported interaction of cytohesin-1 with β2 integrin (31).
30. Liu, L., and Pohajdak, B. (1992) Biochim. Biophys. Acta 1132, 75–78
31. Kolanus, W., Nagel, W., Schiller, B., Zeitlmann, L., Godar, S., Stockinger, H., and Seed, B. (1996) Cell 86, 233–242
32. Achstetter, T., Franzusoff, A., Field, C., and Schekman, R. (1988) J. Biol. Chem. 263, 11711–11717
33. Mossessova, E., Gulbis, J. M., and Goldberg, J. (1996) Cell 92, 415–423
34. Cherfils, J., Ménetrey, J., Mathieu, M., Le Bras, G., Robineau, S., Béraud-Dufour, S., Antonny, B., and Chardin, P. (1998) Nature 392, 101–105
35. 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
36. Hong, J.-X., Haun, R. S., Tsai, S.-C., Moss, J., and Vaughan, M. (1994) J. Biol. Chem. 269, 9743–9745
37. Haun, R. S., Tsai, S.-C., Adamik, R., Moss, J., and Vaughan, M. (1993) J. Biol. Chem. 268, 7064–7068
38. Massenburg, D., Han, J.-S., Liyanage, M., Patton, W. A., Rhee, S. G., Moss, J., and Vaughan, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11718–11722
39. 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
40. Lee, F.-J. S., Stevens, L. A., Kao, Y. L., Moss, J., and Vaughan, M. (1994) J. Biol. Chem. 269, 20931–20937
41. Vitale, N., Moss, J., and Vaughan, M. (1997) J. Biol. Chem. 272, 25077–25082
42. Welsh, C. F., Moss, J., and Vaughan, M. (1994) J. Biol. Chem. 269, 15983–15987
43. Paris, S., Béraud-Dufour, S., Robineau, S., Bigay, J., Antonny, B., Chabre, M., and Chardin, P. (1997) J. Biol. Chem. 272, 22221–22226
44. Terui, T., Kahn, R. A., and Randazzo, P. (1994) J. Biol. Chem. 269, 28130–28135
45. Klarlund, J. K., Rameh, L. E., Cantley, L. C., Buxton, J. M., Holik, J. J., Sukels, C., Parki, V., Corvera, S., and Czech, M. P. (1998) J. Biol. Chem. 273, 1859–1862
46. Frank, S., Upender, S., Hansen, S. H., and Casanova, J. E. (1998) J. Biol. Chem. 273, 23–27
47. Radhakrishna, H., and Donaldson, J. G. (1997) J. Cell Biol. 139, 49–61