The N-terminal Coiled Coil Domain of the Cytohesin/ARNO Family of Guanine Nucleotide Exchange Factors Interacts with the Scaffolding Protein CASP

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Cytohesin is a guanine nucleotide exchange factor that regulates members of the ADP-ribosylation factor (ARF) family of small GTPases. All of the members of the cytohesin family (including ARNO, ARNO3, and the newly characterized cytohesin-4) have a similar domain distribution consisting of a Sec7 homology domain, a pleckstrin homology domain, and an N-terminal coiled coil. In this study, we attempt to identify proteins that interact specifically with the coiled coil motif of cytohesin. Yeast two-hybrid screening of a B cell library using the cytohesin N terminus as bait, identified CASP, a scaffolding protein of previously unknown function, as a binding partner. CASP contains an internal coiled coil motif that is required for cytohesin binding both in vitro and in COS-1 cells. The specificity of the coiled coil of CASP is not restricted to cytohesin, however, because it is also capable of interacting with other members of the cytohesin/ARNO family, ARNO and ARNO3. In immuno-fluorescence experiments, CASP localizes to perinuclear tubulovesicular structures that are in close proximity to the Golgi. These structures remain relatively undisturbed when the cells are treated with brefeldin A. In epidermal growth factor-stimulated COS-1 cells over-expressing cytohesin and CASP, cytohesin recruits CASP to membrane ruffles, revealing a functional interaction between the two proteins. These observations collectively suggest that CASP is a scaffolding protein that facilitates the function of at least one member of the cytohesin/ARNO family in response to specific cellular stimuli.

The cytohesin/ARNO family of guanine nucleotide exchange factors (GEFs),¹ characterized by an N-terminal coiled coil, a Sec7 homology domain, and a C-terminal pleckstrin homology (PH) domain, have emerged as regulators of the ARF family of small GTPases (1–3). ARF GTPases are divided into three classes based on their gene structure. Class I ARFs (ARFs 1–3) are Golgi-associated GTPases regulating vesicle formation (4–6). Little is known about class II ARFs (ARFs 4 and 5), except that ARF5 may be involved in brefeldin A (BFA)-resistant Golgi/ER retrograde traffic (7). ARF6, the only member of class III ARFs, associates with cell membranes and is involved in endocytosis and actin rearrangements (8–10). The study of ARF function has been focusing primarily on the ER and Golgi where different anterograde and retrograde vesicle trafficking pathways occur. It is generally accepted that coat protein complex (COP) II-coated vesicles budding from the ER carry cargo proteins to the ER/Golgi intermediate compartment where they are replaced by coat protein complex (COP) I-coated vesicles (11). Sar1 is the major small GTPase implicated in the formation of these vesicles (12, 13), whereas the ARFs control COP-I as well as clathrin-coated vesicle formation and traffic in and around the Golgi (14–16).

The cytohesin/ARNO GEFs regulate ARFs through the Sec7 homology domain by facilitating a GDP/GTP exchange, converting inactive GDP-bound ARFs to their active GTP-bound state (1–3). There are currently four known members of the cytohesin/ARNO family. The first was originally cloned in our laboratory and was designated B2-1 (17). It was later renamed by others as cytohesin-1 (18). ARNO is also known as cytohesin-2, and ARNO3 is the human homolog of mouse GRP1 (19). Another member of the cytohesin/ARNO family, cytohesin-4, was recently identified in blood cells (20). To simplify nomenclature, we will follow the designations published in GenBank®: cytohesin-1, ARNO, ARNO3, and cytohesin-4. The specificity of cytohesin/ARNO members to the various ARFs appears to be mediated primarily by the Sec7 domain. All cytohesin/ARNO members activate ARF-1 (19, 20), whereas cytohesin-1, ARNO, and ARNO3 (but not cytohesin-4) activate ARF6 (20–22). Cytohesin-1 can activate ARF3 (2, 23), whereas both cytohesin-1 and -4 can activate ARF-5.

All four members of the family are highly similar on a structural basis. In addition to the Sec7 homology domain, the C-terminal PH domain allows cytohesin/ARNO interactions with membranes by binding to various polyphosphoinositides (24–27). Although the PH domains of cytohesin-1 and ARNO seem to bind nonselectively to various phosphoinositides, ARNO3 shows increased affinity to phosphatidylinositol 3,4,5-trisphosphate, a product of phosphatidylinositol 3-kinase activation (26, 28). Generally, whereas the PH domain anchors the cytohesin/ARNO GEFs to membrane structures, the Sec7 domain facilitates the function of ARF in vesicle formation.

The N-terminal coiled coil motif, reminiscent of leucine zipper domains, is a signature domain of all the cytohesin/ARNO members and still the most elusive. Recently, we showed that this domain targets the cytohesin/ARNO proteins to the Golgi (29, 30). The coiled coil motif most likely interacts with at least one adaptor protein that contains a similar domain and facilitates the higher architecture of signaling complexes that regulate vesicle formation. The only protein known to interact

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¹The abbreviations used are: GEF, guanine nucleotide exchange factor; PH, pleckstrin homology; BFA, brefeldin A; ER, endoplasmic reticulum; a.a., amino acid(s); GST, glutathione S-transferase; CMV, cytomegalovirus; HA, hemagglutinin; PBS, phosphate-buffered saline; EGF, epidermal growth factor; ARF, ADP-ribosylation factor.
with the N terminus of a cytohesin/ARNO protein (mouse homolog of ARNO3, GRP1), is GRASP, a scaffolding protein of unknown function containing a coiled coil domain (31). Here we report the interaction of cytohesin/ARNO proteins, particularly cytohesin, with a GRASP-related scaffolding protein, CASP, originally cloned in our laboratory from Natural Killer-enriched human lymphocytes (32). CASP and GRASP share a similar domain profile, with an N-terminal PDZ domain, a central coiled coil motif, and a C-terminal domain of unknown function.

**EXPERIMENTAL PROCEDURES**

**Plasmids and Cells—**A cytohesin fragment coding for residues 1–68 (cytohesin-N) (29) was subcloned in frame into the NcoI site of the plasmid vector pAS2-1 (CLONTECH) downstream from sequences encoding the Gal4 DNA-binding domain (Gal4 BD). A human B cell cDNA library subcloned into the XhoI site of the activation domain plasmid pACT2 (CLONTECH) and the PJ69-4A yeast strain for the yeast two-hybrid analysis were generous gifts from Dr. C. McMaster (Biochemistry Department, Dalhousie University, Halifax, Canada).

A cytohesin-N BamHI fragment coding for a.a. 1–54 was subcloned in pRSET A (Invitrogen) for generating recombinant His6-cytohesin-N fusion protein. Plasmids for generating recombinant GST/CASP fusions (CASP a.a. 151–201 and a.a. 151–214) were prepared by amplifying the CASP cDNA region coding for the coiled coil motif, PCRII cloning of the PCR fragments (Invitrogen), and then subcloning into the appropriate pGEX vector (Amersham Biosciences). The sense primer used for amplifying the coiled coil region was 5′-AAGCTTACGATGTCG-GAAACCTGCTAAC-3′. Antisense primers were AS5 (5′-AGACAGTTCTCT-GTAATGC-3′) and bish2 (5′-TGATATACGATCCGTCGCTC-3′). Recombinant GST/CASP proteins lacking a significant portion of the coiled coil domain (a.a. 179–195) were generated in a similar manner using a CASP cDNA with an internal PstI deletion.

CASP cDNA with the stop codon removed and CASP cDNA coding for the coiled coil domain (CASP (CC)) were subcloned into a modified (leader sequence removed) Sec Tag vector (Invitrogen), designated Sec CMV. In these constructs, the CASP full cDNA and the CASP coiled cDNA portion were cloned in frame with downstream sequences encoding Myc and His6 tags and was under the control of the CMV promoter. PstI deletion mutants of CASP and CASP (CC) lacking the majority of the coiled coil motif (CASP* and CASP (CC*), respectively) were subcloned into Sec CMV in a similar manner. Primers used for amplifying the CASP (CC) and (CC*) cDNA were ZipATG (5′-GACCTGATGCACAGTGAA-3′) and GAGATCGTCCGGAAACCTGCTAAC-3′. Cytohesin-N, ARNO-N, or ARNO3-N fusion proteins were detected by monoclonal His6 antibodies (Santa Cruz Biotechnology) and ECL (Amersham Biosciences).

**COS-1 Transfection and Protein Binding Assays—**COS-1 cells seeded in 6-well plates were transfected the following day with 0.5 μg of the appropriate CASP/Sec CMV construct (CASP (CC) or CASP (CC*)), 0.5 μg of either cytohesin-N, ARNO-N, or ARNO3-N/CMV/HA/Myc construct, and 4 μl of Superfect (Qiagen). 0.5 μg of CASP or CASP* were also doubly transfected with 0.5 μg of cytohesin in the same manner. 22 h post-transfection, the cells were seeded for 2 h in a balanced salt solution (136 mM NaCl, 4.7 mM KCl, 1.25 mM MgSO4, 1.25 mM CaCl2, 5 mM sodium phosphate, 2 mM NaHCO3, and 25 mM Hepes, pH 7.4) and then stimulated with 1 μg/ml anti-HA antibodies or 0.5 μg/ml anti-Myc antibodies for 3 min at 37 °C. Unstimulated and EGF-stimulated cells were fixed in 4% paraformaldehyde in PBS (pH 7.2) for 20 min, permeabilized with 0.1% Triton X-100 in PBS, and blocked with goat serum at 1:100 dilution in PBS with 0.1% Triton X-100. Primary and secondary antibody (1:1000 in PBS with 0.1% Triton X-100) incubations were 20 min each at room temperature. secondary antibody (1:1000 in PBS with 0.1% Triton X-100) incubations were 20 min each at room temperature. Receptor grade murine EGF was purchased from Sigma. Anti-Myc antibodies were from Santa Cruz. Polyclonal anti-cytohesin antibodies were a generous gift from Dr. Bourgoin (Laval). CY3-conjugated anti-rabbit and anti-mouse antibodies were purchased from Sigma. Alexa488-conjugated anti-rabbit and anti-mouse antibodies were purchased from Molecular Probes.

**RESULTS**

**The N Terminus of Cytohesin Interacts with CASP in Yeast—**Yeast two-hybrid screening based on the Gal4 system and using cytohesin a.a. 1–254 as bait identified three potential clones. Interaction of all three clones with the N terminus of cytohesin was confirmed in yeast by patching using a repeated temperature β-galactosidase screenings. All three clones were sequenced, two of which corresponded to CASP, a gene originally cloned in our laboratory from a human Natural Killer-enriched population of lymphocytes (32). The third clone corresponded to a tRNA gene and was unlikely to be a true binding partner of cytohesin. Both isolated CASP clones were identical.
The N terminus of cytohesin harbors a coiled coil motif that most likely interacts with another coiled coil domain. The presence of such a domain in the truncated CASP protein expressed in yeast prompted us to confirm the interaction of cytohesin with the CASP coiled coil in vitro. We were unable to produce recombinant CASP protein efficiently in *Escherichia coli* because it had a tendency to precipitate. We therefore made shorter GST fusion proteins that specifically included the coiled coil domain of CASP (Fig. 2, A and B). These proteins were more soluble, particularly if they were used shortly after they were produced. We also circumvented the solubility problem by keeping the GST recombinant proteins coupled to the glutathione beads before performing the binding assays. Additionally, we produced deletion mutants of the same CASP proteins lacking a significant portion of the coiled coil. In addition to the removal of key elements of the coiled coil, this deletion also affected the secondary structure of the remaining α-helix. Recombinant N-terminal cytohesin (cytohesin-N) corresponding to a.a.1–54 and fused to a His6 tag was produced in *E. coli* and tested for its interaction with the CASP/GST proteins. Recombinant cytohesin-N could only be captured in vitro by CASP bound to glutathione beads when the coiled coil of CASP remained intact. The deletion mutants of CASP (CC) and CASP (CC*) used in the yeast two hybrid screening failed to interact with cytohesin-N (Fig. 2C).

Cytoshein and CASP Coiled Coils Domains Interact in COS-1 Cells—We attempted to confirm the validity of the cytoshein/CASP interaction that we observed in vitro by co-transfecting COS-1 cells with cytohesin-N and CASP coiled coil domain (CASP (CC)) and testing for an interaction in COS-1 lysates. CASP expressed in eukaryotic cells cannot be detected with our anti-CASP antibodies generated against recombinant protein, possibly as a result of fundamental differences in CASP protein folding and/or post-translational modifications in eukaryotic cells. It was therefore necessary to fuse CASP (CC) with a Myc tag for detection by Western blotting. A His6 tag was also fused to CASP (CC) in the hope of using nickel beads on COS-1 lysates to co-purify cytohesin-N, but the nickel beads showed high nonspecific affinity to COS-1 lysates. We therefore turned to co-immunoprecipitating cytohesin-N and CASP (CC) using anti-HA and anti-cytohesin antibodies. Cytohesin-N was also fused to a Myc tag for detection by Western blotting using anti-Myc antibodies. CASP (CC) readily co-precipitated with cytohesin-N from transfected COS-1 lysates using anti-HA antibodies (Fig. 3). This interaction is specific to the coiled coil domain of CASP because the deletion mutant CASP (CC*) lacking the same portion of the coiled coil domain as the recombinant mutant CASP constructs used in the *in vitro* assay showed no interaction with cytohesin-N (Fig. 3B). Furthermore, CASP (CC) was not precipitated with protein A-agarose beads and antibodies without the presence of cytohesin-N. Expression of the appropriate proteins in COS-1 cells was confirmed by immunoprecipitating Myc-labeled proteins from lysates of the same transfections using anti-Myc antibodies (Fig. 3C).

CASP Interacts with Other Members of the Cytoshein/ARNO GEFs Family—All members of the cytoshein/ARNO family are characterized by an N-terminal coiled coil motif. We therefore examined the binding specificity of CASP to the various members of this family of GEFs, including ARNO and ARNO3. Recombinant proteins corresponding to the N termini of ARNO and ARNO3 (ARNO-N and ARNO3-N) harboring the coiled coil motif were produced in *E. coli* and tested for their ability to interact with GST/CASP recombinant proteins in vitro. Both ARNO-N and ARNO3-N were capable of interacting with an intact CASP coiled coil domain but not with the deletion variant of the same protein (Fig. 4). These interactions were confirmed in COS-1 cells by co-transfecting HA-tagged ARNO-N or ARNO3-N with either CASP (CC) or CASP (CC*) and co-immunoprecipitation with anti-HA antibodies. CASP (CC) but not the deletion variant co-precipitated with both ARNO-N and ARNO3-N from COS-1 lysates (Fig. 5A). CASP shows no differential specificity to the various members of the cytoshein/GRASP proteins.

The complete deduced amino acid sequence of CASP was aligned with GRASP, the only other known member of the CASP family, using Clustal W. Asterisks denote matching amino acids, colons denote conserved substitutions, and dots represent semi-conserved substitutions. The yellow box represents the N-terminal PDZ domain, and the green box represents the C-terminal mystery domain of unknown function. The coiled coil region of CASP is shown as an open box (CC). The greatest sequence divergence between CASP and GRASP is present at the N terminus and within the C-terminal domain. The arrow shows the start of the partial CASP protein interacting with the cytohesin N terminus bait used in the yeast two hybrid screening.
ARNO family in both our in vitro and COS-1 binding assays.

CASP Intracellular Localization Is Perinuclear in COS-1 Cells—We have previously shown that the cytohesin, ARNO, and ARNO3 localize to the Golgi through their coiled coil motifs. We suspected that CASP may be a Golgi protein because it interacts with all three members of the cytohesin/ARNO family. Immunolocalization of CASP in transfected COS-1 cells clearly shows a perinuclear signal that is characteristic of the Golgi. To our surprise, however, CASP did not co-localize with the Golgi marker mannosidase II (Fig. 6) or giantin (not shown), nor did it co-localize with the ER marker GRP78 (data not shown). It did, however, partially overlap with the ER/Golgi intermediate marker ERGIC-53. This partial overlap was more evident when COS-1 cells were treated with BFA, causing the redistribution of both ERGIC-53 and CASP into similar tubular structures (Fig. 7). BFA caused the relocation of mannosidase II into the ER as expected (33, 34). The CASP-stained tubules were in proximity to, but clearly distinct from, the Golgi and the ERGIC-53-associated structures.

Co-localization of Cytohesin and CASP Is Coiled Coil-dependent—Cytohesin localizes to the Golgi when expressed at low levels in COS-1 cells (29, 30) but exhibits cytoplasmic distribution when overexpressed in Chinese hamster ovary and PC-12 cells (25). Furthermore, overexpressed cytohesin can be targeted to Chinese hamster ovary and PC-12 membranes by the appropriate extracellular stimuli. Similarly, redistribution of GRP1 (ARNO-3) was observed by others in COS-1 cells stimulated with EGF (22). We examined the localization of cytohesin in COS-1 cells upon EGF stimulation and found that cytohesin, like GRP1, translocated to the plasma membrane (Fig. 8, A and B). Full-length CASP and CASP*, a deletion mutant of CASP lacking a portion of the coiled coil domain, exhibited perinuclear localization that was unaffected by EGF stimulation (Fig. 8, C–F). When cytohesin was co-expressed with CASP, however, EGF stimulation caused the translocation of both cytohesin and CASP to membrane ruffles (Fig. 9, D–F). In unstimulated cells, both CASP and cytohesin exhibited a diffuse cytoplasmic distribution with little membrane association. CASP perinuclear localization in these cells was disrupted presumably as a result of cytohesin sequestering CASP through the coiled coil-mediated interaction. EGF-in-
duced redistribution of CASP to membrane ruffles in the presence of cytohesin is clearly dependent on the CASP coiled coil motif, because the deletion mutant CASP* failed to relocate under the same conditions (Fig. 9, J–L). Furthermore, CASP recruitment to membrane ruffles is mediated by cytohesin because CASP could not relocate to ruffles when expressed alone (Fig. 8).

DISCUSSION

We took a yeast two-hybrid approach to identify proteins that specifically interact with the coiled coil domain found in the N terminus of cytohesin. Using cytohesin amino acids 1–54 as bait, we identified CASP as a potential binding partner. CASP was originally cloned in our laboratory from a Natural Killer/T cell population. EST database searches suggest the expression of CASP in other cell types such as CD34+/H11001 hematopoietic stem/progenitor cells, germinal center B cells, and activated T cells, as well as a number of cancers including adenocarcinoma, embryonal carcinoma, myeloma, melanoma, and lymphoma. CASP contains at least two known protein interaction domains: an N-terminal PDZ domain and a coiled coil motif. The presence of a coiled coil in CASP suggested to us that the cytohesin/CASP interaction is mediated by this motif. In vitro binding assays with partial CASP recombinant proteins containing primarily the coiled coil motif and deletion mutants of the same protein in which the coiled coil motif is impaired clearly demonstrate that this region of CASP specifically interacts with the coiled coil of cytohesin. Additionally, interaction assays in COS-1 cells expressing coiled coil constructs of cytohesin, CASP, and coiled coil deletion mutants of

![Fig. 4. Interaction of the coiled coil of CASP with cytohesin-N, ARNO-N, and ARNO3-N in vitro. A, multiple alignment of the coiled coil motif found in the N terminus of cytohesin, ARNO, and ARNO3. b, recombinant His6-tagged proteins corresponding to the cytohesin a.a. 1–54 (lane C), ARNO a.a. 1–53 (lane A), and ARNO3 a.a. 1–58 (lane A3) were purified and then visualized by Western blotting using anti-His6 antibodies. C, recombinant cytohesin, ARNO, and ARNO3 proteins interact in vitro with the GST tagged CASP construct LC bound to glutathione beads. No interaction could be detected with the deletion mutant LC*.

![Fig. 5. CASP interaction with ARNO-N and ARNO3-N in vivo is mediated by the coiled coil domain. A, cDNA sequences coding for ARNO a.a. 1–53 (A) and ARNO3 a.a. 1–58 (A3) were cloned downstream from an HA tag sequence and upstream from a Myc tag sequence under the control of the CMV promotor. COS-1 cells were transfected with ARNO, ARNO3, and CASP (CC)/CASP (CC*) plasmids as indicated. ARNO, ARNO3, CASP (CC), and CASP (CC*) proteins were detected by Western blotting using monoclonal anti-Myc antibodies. B, expression of ARNO, ARNO3, CASP (CC) and CASP (CC*) in COS-1 cells was confirmed by immunoprecipitation of Myc-tagged proteins with polyclonal anti-Myc antibodies followed by Western blotting using monoclonal anti-Myc antibodies. ARNO and ARNO3 are indicated with double arrowheads. CASP (CC) and CASP (CC*) are indicated with single arrowheads.](image-url)
CASP show that the cytohesin/CASP interaction is specifically mediated by the coiled coil motifs. CASP was identified by others as a cytohesin-interacting protein by yeast two hybrid screening of a differential expression dendritic cell library and was submitted to GenBank™ as a cytohesin-binding protein (accession number AF068836). In that case, however, the entirety of cytohesin was used as bait, and the protein segments responsible for the interaction were never published. We are the first to confirm such an interaction both in vitro and in a cellular system, as well as to identify the domains responsible for this interaction.

The specificity of the CASP coiled coil domain was tested by examining the interaction of CASP with other members of cytohesin/ARNO family, particularly ARNO and ARNO-3. All three members are associated with the Golgi of COS-1 cells (29) and most likely play specific roles in ARF-mediated vesicle formation. CASP is capable of interacting with all three members of the family, at least in our experimental system. There may be differential specificity with the various ARNOs at a lower expression level than that induced by the CMV promotor, but that remains to be tested. We were unable to test such an interaction by co-immunoprecipitating proteins from normal cell lysates due to the lack of functional CASP antibodies. Nonetheless, our data suggest that CASP may regulate or facilitate a specialized aspect of vesicle transport that involves at least one member of the cytohesin family in hematopoietic cells.

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The interaction of CASP with cytohesin/ARNO/ARNO3 in COS-1 cells suggests an association of CASP with the Golgi complex. Immunofluorescence experiments clearly showed the association of CASP with Golgi proximal structures. Co-localization studies with Golgi markers, on the other hand, showed that CASP was not directly associated with the Golgi. The only marker tested that exhibited partial overlap was ERGIC-53, a well recognized component of the ER-Golgi intermediate region (35). This partial overlap persisted even after BFA treatment.
which caused the redistribution of both CASP and ERGIC-53 into similar but not identical tubulovesicular structures. Others have shown that BFA treatment causes the dissociation of the Golgi stack and the recycling of some Golgi components such as mannosidase II into the ER, whereas other components such as ERGIC-53 and the Golgin GM130 cluster in distinct tubulovesicular structures (34). It appears that CASP is associated with a dynamic compartment that normally interacts with the Golgi and fuses with vesicular Golgi remnants after BFA treatment. This compartment may be part of the ER/Golgi intermediate region but is clearly distinct from the ERGIC-53-associated structures. The physical interaction of CASP with cytohesin and the apparent association of CASP and cytohesin proteins with different but overlapping compartments of the perinuclear region most likely reflect the dynamic or inducible nature of the function of CASP. The physical interaction of endogenous CASP and cytohesin and/or ARNO/ARNO3 at the Golgi may require stimuli that remain unidentified to date.

Intracellular localization studies in EGF-stimulated COS-1 cells overexpressing CASP and cytohesin clearly show the functional interaction of the two proteins. Furthermore, the coiled coil interaction is responsible for the co-localization observed. The translocation of CASP in the presence of cytohesin upon EGF stimulation is likely mediated by the PH domain of cytohesin, a property of cytohesin reported by others in PC-12 cells (25). Cytohesin translocation to membranes is similar to GRP1 and ARNO translocation reported by other groups (24, 36) and is consistent with the ability of all three proteins to activate ARF6 \textit{in vitro} and more importantly membrane bound ARF6 \textit{in vivo} (21, 37). CASP overexpressed alone failed to localize to the membrane, most likely as a result of the overwhelming CASP levels compared with endogenous cytohesin (and potentially ARNO/ARNO3) levels. The inability of CASP to disrupt cytohesin translocation to the membrane in response to EGF is expected because the interaction of cytohesin with CASP and membranes is mediated by two different domains: the coiled coil and the PH domain, respectively. The association of CASP and cytohesin at membranes following EGF stimulation suggests that cytohesin is capable of recruiting CASP to the appropriate site of activity in response to specific stimuli.

Interestingly, CASP is not the only protein capable of interacting with cytohesin/ARNO proteins through their N-terminal coiled coil domain. GRASP (GRP1-associated scaffolding protein), the only other known member of the CASP family, was recently cloned from a mouse library and shown to interact with both ARNO and GRP1 (ARNO3) (31). GRASP expression is induced by \textit{trans}-retinoic acid in embryonal carcinoma PC19 cells, and its interaction with GRP1 occurs at the cell periphery. The ability of GRASP to interact with cytohesin was never established because it is not expressed in PC19 cells. The
lates a novel pathway of vesicle formation and trafficking. CASP may represent a scaffolding protein that regulates the perinuclear compartment targeted by CASP as well as map the region responsible for this perinuclear localization. CASP may represent a scaffolding protein that regulates a novel pathway of vesicle formation and trafficking.

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