Ral is a small GTPase involved in critical cellular signaling pathways. The two isoforms, RalA and RalB, are widely distributed in different tissues, with RalA being enriched in brain. The best characterized RalA signaling pathways involve RalBP1 and phospholipase D. To investigate RalA signaling in neuronal cells we searched for RalA-binding proteins in brain. We found at least eight proteins that bound RalA in a GTP-dependent manner. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) identified these as the components of the exocyst complex. The yeast exocyst is a regulator of polarized secretion, docking vesicles to regions of the plasma membrane involved in active exocytosis. We identified the human FLJ10893 protein as the mammalian homologue of the yeast exocyst protein Sec3p. The exocyst complex did not contain the previously identified exocyst component rSec15, but a new homologue of both yeast Sec15p and rSec15, called KIAA0919. Western blots confirmed that two rat exocyst proteins, rSec6 and rSec8, bound active RalA in nerve terminals, as did RalBP1. Phospholipase D bound RalA in a nucleotide-independent manner. This places the RalA signaling system in mammalian nerve terminals, where the exocyst may act as an effector for activated RalA in directing sites of exocytosis.

The small GTPase RalA is a member of the Ras superfamily of monomeric 20–30-kDa GTP-binding proteins, which cycle between active GTP-bound and inactive GDP-bound states. In the GTP-bound state they interact with target proteins (effectors) to initiate downstream responses. Their signal transduction is terminated by an intrinsic GTPase activity, returning them to the GDP-bound state. Two Ral genes exist in vertebrates, RalA and RalB, sharing 85% amino acid identity (1). Both proteins have a wide, partly overlapping, tissue distribution, with highest expression of RalA in brain and platelets and of RalB in platelets, kidney, and adrenal medulla (2). Both isoforms are found predominantly on the plasma membrane (3) and on the membranes of secreting vesicles, and RalA is found particularly on the membranes of synaptic vesicles in brain (4).

The enrichment of RalA in brain and its localization to synaptic vesicles suggests a role in vesicle trafficking events in the nerve terminal.

Ral activation has mainly been characterized in the downstream signaling pathways from activated receptor-linked tyrosine kinases and seven-transmembrane domain receptors, which lead to the activation of Ras (5). Activation of small GTPases is achieved via guanine nucleotide exchange factors (GEFs) that facilitate exchange of GDP for GTP. The best known Ral activating pathway is via Ras activating the Ral-GEF and Ras effector protein Ral guanine nucleotide dissociation stimulator (RalGDS), but Ras-independent mechanisms for Ral activation are likely (5, 6). RalA can also be regulated by intracellular Ca²⁺ (7). Ca²⁺ activates calmodulin, and the complex directly binds to a polybasic region near the C terminus of RalA (8). Ca²⁺-calmodulin binding stimulates GTP binding to RalA, suggesting that it is a RalA activator (9). In vitro studies suggest that Ca²⁺-calmodulin controls RalA subcellular localization, since binding dissociates RalA from synaptic vesicles (10).

RalA downstream signaling is mediated by two additional protein-protein interaction sites. The first binds phospholipase D1 (PLD1) via an N-terminal 11 amino acid sequence. RalA weakly stimulates PLD1 activity but operates synergistically with another small GTPase, Arf1 (11). PLD1 binding to RalA is independent of the nucleotide binding status of RalA, but signaling pathways that lead to RalA activation also lead to phosphatidic acid production by PLD1 (12). The second is an effector binding loop, which mediates interaction of proteins with RalA in a GTP-dependent manner. Two proteins are known to interact with RalA in this fashion, leading to RalA-dependent cellular effects. Ral-binding protein 1 or RalBP1 (13) is involved in receptor-mediated endocytosis through binding to two key endocytic proteins (14, 15). The second is filamin, an actin filament cross-linking protein that mediates filopodia formation in neurites (16).

Other small GTPases like Rac, Cdc42 have about 30 identified potential GTP-dependent binding proteins (17); however, only two are presently known for RalA. To better understand RalA-mediated signaling pathways in neuronal cells our aim was to identify new RalA-binding proteins in brain. We report on the identification of eight specific GTP-dependent

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† To whom correspondence should be addressed: Cell Signalling Unit, Children’s Medical Research Institute, Locked Bag 23, Wentworthville 2145, NSW, Australia. Tel.: 61-2-9687-2800; Fax: 61-2-9687-2120; E-mail: phrobin@mail.usyd.edu.au.

‡ From the ¶Children’s Medical Research Institute, 214 Hawkesbury Road, Westmead NSW 2145, Australia, the ¶Australian Proteome Analysis Facility, Level 4, Building F7B, Macquarie University, New South Wales 2109, Australia, and the $Faculty of Pharmacy, A15, University of Sydney, New South Wales 2006, Australia.

1 The abbreviations used are: GEF, guanine nucleotide exchange factor; PLD, phospholipase D; GST, glutathione S-transferase; MALDITOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; PAGE, polyacrylamide gel electrophoresis.
RalA-binding proteins, which are proteins that comprise the mammalian exocyst complex. The exocyst was first described in yeast as a complex of eight proteins (Sec5p, Sec6p, Sec7p, Sec8p, Sec10p, Sec15p, Exo70p, and Exo84p) required for targeted exocytosis (18, 19). At least three of these proteins are able to associate directly with three different small GTPases in yeast (20–22). The mammalian equivalent of seven of the eight yeast exocyst proteins have been cloned (23–25). Our results are the first to demonstrate that the mammalian exocyst also interacts with small GTPases and suggests that, in addition to an established role in endocytosis (14, 26) activated RalA may play a central role in directing sites of exocytosis.

**EXPERIMENTAL PROCEDURES**

**Recombinant RalA**—The bacterial expression vector containing the wild-type RalA sequence fused to glutathione S-transferase (GST), pGEX-2T-RalA, was provided by Yoshito Kaziro (Yokohama, Japan). This vector was transformed into *Escherichia coli* by heat shock. GST-RalA bound to GSH beads (Amersham Pharmacia Biotech) was prepared according to the manufacturer’s instructions with the inclusion of 2.5 mM MgCl₂ in all buffers.

**Pull-down Experiments**—Crude synaptosomes (P2) were isolated from three rats as described previously (27) and lysed with Triton X-100 by resuspension in ice-cold lysis buffer (1% v/v Triton X-100, 25 mM Tris, pH 7.4, 150 mM NaCl, 2.5 mM MgCl₂, 1 mM EGTA, 20 μg/ml leupeptin, and 1 μM phenylmethylsulfonyl fluoride). Whole rat tissue lysates were prepared by mincing 2 g of tissue, washing in phosphate-buffered saline, and homogenizing equal tissue weights in lysis buffer. All homogenates were centrifuged at 75,600 × g for 30 min at 4 °C. Protein–bound aliquots of the supernatant were precleared by the addition of GSH-Sepharose for 30 min, pelleted at 50 × g for 5 min at 4 °C, and the supernatant was collected. GST-RalA beads were loaded with guanine nucleotides using established methods (13). A volume of GSH beads containing ∼10 μg of GST-RalA was incubated with an equal volume of 20 μl Tris, pH 7.4, 10 mM EDTA, 25 mM NaCl, and 1 mM GDP or GTP at 37 °C for 20 min. The buffer was adjusted to 10 mM MgCl₂ then the tissue lysates were added and incubated at 4 °C for 1 h. The beads were isolated by centrifugation at 50 × g for 5 min, transferred to small empty spin columns (ProbeQuant G-50 Micro-columns, Amersham Pharmacia Biotech) and washed three times with ice-cold lysis buffer followed by three washes with 20 mM Tris, pH 7.4, containing 2.5 mM MgCl₂. The samples were heated to 85 °C in SDS sample buffer for 5 min and collected into fresh tubes by centrifugation at 17,000 × g for 1 min. Samples were run on SDS-PAGE and stained with Coomassie Brilliant Blue. For some experiments a cytosolic extract was prepared from 70 g of sheep brain. Diced brain was washed with 20 mM Tris, pH 7.7, and homogenized in 20 mM Tris, pH 7.7, 1 mM CaCl₂, 2 mM dithiothreitol, 10 μg/ml leupeptin, 1 μM phenylmethylsulfonyl fluoride using an Ultra Turrax T25 homogenizer. The homogenate was centrifuged at 18,500 × g for 30 min at 4 °C. The extract was adjusted to 3 mM EGTA (to chelate the Ca²⁺).

**Western Blotting**—Samples were subjected to SDS-PAGE on 12% acrylamide minigels (Bio-Rad) and transferred to nitrocellulose membranes (28). Proteins were detected by chemiluminescence (LumiLight from Roche).

**In-gel Digestion and MALDI-TOF MS**—Protein bands were excised from Coomassie Blue-stained gels, rinsed with water, and destained with several washes of 25 mM ammonium bicarbonate in 50% acetonitrile. The gel pieces were dehydrated with 100% acetonitrile and dried with SpeedVac. The gel pieces were resuspended in ice-cold lysis buffer (1% v/v Triton X-100, 25 mM Tris, pH 7.4, 10 mM EDTA, 25 mM NaCl, and 1 mM EGTA, 20 μg/ml leupeptin, and 1 μM phenylmethylsulfonyl fluoride). Whole rat tissue lysates were prepared by mincing 2 g of tissue, washing in phosphate-buffered saline, and homogenizing equal tissue weights in lysis buffer. All homogenates were centrifuged at 75,600 × g for 30 min at 4 °C. Protein–bound aliquots of the supernatant were precleared by the addition of GSH-Sepharose for 30 min, pelleted at 50 × g for 5 min at 4 °C, and the supernatant was collected. GST-RalA beads were loaded with guanine nucleotides using established methods (13). A volume of GSH beads containing ∼10 μg of GST-RalA was incubated with an equal volume of 20 μl Tris, pH 7.4, 10 mM EDTA, 25 mM NaCl, and 1 mM GDP or GTP at 37 °C for 20 min. The buffer was adjusted to 10 mM MgCl₂ then the tissue lysates were added and incubated at 4 °C for 1 h. The beads were isolated by centrifugation at 50 × g for 5 min, transferred to small empty spin columns (ProbeQuant G-50 Micro-columns, Amersham Pharmacia Biotech) and washed three times with ice-cold lysis buffer followed by three washes with 20 mM Tris, pH 7.4, containing 2.5 mM MgCl₂. The samples were heated to 85 °C in SDS sample buffer for 5 min and collected into fresh tubes by centrifugation at 17,000 × g for 1 min. Samples were run on SDS-PAGE and stained with Coomassie Brilliant Blue. For some experiments a cytosolic extract was prepared from 70 g of sheep brain. Diced brain was washed with 20 mM Tris, pH 7.7, and homogenized in 20 mM Tris, pH 7.7, 1 mM CaCl₂, 2 mM dithiothreitol, 10 μg/ml leupeptin, 1 μM phenylmethylsulfonyl fluoride using an Ultra Turrax T25 homogenizer. The homogenate was centrifuged at 18,500 × g for 30 min at 4 °C. The extract was adjusted to 3 mM EGTA (to chelate the Ca²⁺).

**RESULTS**

**RalA-binding Proteins in Rat Tissues**—To identify possible new RalA effector proteins, recombinant GST-RalA, coupled to GST-Sepharose, was loaded with either GDP or GTP and used as an affinity matrix to isolate RalA-binding proteins from various rat tissue Triton X-100 lysates (Fig. 1). This approach isolates GDP- or GTP-dependent RalA-binding proteins, as well as proteins that might associate with these binding proteins. Recombinant GST coupled to GST-Sepharose was used as controls in parallel experiments to provide a further degree of specificity (data not shown). Four main proteins interacted specifically with GTP-bound GST-RalA, compared with GDP-bound RalA in a preparation of rat brain nerve terminals (synaptosomes, Fig. 1A, lanes 1 and 2). These were also abundant in whole brain lysates (lanes 3 and 4), but were much less evident in testis, lung, liver, and kidney (lanes 5–12). Protein 4, however, was present in testis and lung (lanes 6 and 8). While at least three proteins appeared to specifically associate with GDP-bound RalA compared with GTP-RalA in synaptosomes or brain (lanes 1 and 3) and in kidney (lane 11), the GDP dependence of this association was not reproducible in other experiments. Further pull-down experiments using larger amounts of whole brain lysate yielded a number of additional proteins that bound in a GTP-dependent manner and which were previously undetectable by Coomassie Blue (Fig. 1B). Apart from the four major bands six minor GTP-dependent bands were detectable (marked with smaller arrows). Note that bands 1 and 2 were not fully resolved on this gel, but their identities were later...
A number of the other signals in the spectrum were attributed a significant match with a probability of greater than 99% (Table I). A large proportion of the peptide signals were contributed by rSec8, yielding a statistically significant binding complex by MALDI-TOF MS was confirmed by Western blotting with specific antibodies to exocyst complex proteins (Fig. 3). Western blots of proteins from pull-down experiments using rat synaptosome lysates revealed that rSec8 and rSec6 bound to GST-RalA in a GTP-dependent manner (lanes 2 and 3). Neither was detectable in the control pull-down using only GST coupled to GSH-beads (lane 1). rSec6 was also detected in samples of whole brain or testis lysate. To validate our method for the detection of RalA-binding proteins, we used immunoblots to detect two previously reported major RalA-binding proteins. RalBP1 is a known GTP-dependent RalA-binding protein (13), and PLD is known to associate with RalA independently of its nucleotide-bound state (30). Both proteins were appropriately detected in the pull-down experiments using rat brain synaptosomes (Fig. 3).

Protein FLJ10893 Is a Mammalian Homologue of Yeast Sec3p—The sequence of the mammalian exocyst protein that is homologous to yeast sec3p has not previously been reported. However, it was proposed to represent the rat brain exocyst p106 protein, and limited amino acid sequence for p106 was previously published (24). In our study the hypothetical human protein FLJ10893 (GenBank™ accession number NP_060731) was identified as part of the complex binding to RalA in a GTP-dependent manner. FLJ10893 is the only protein in the complex we identified that was not recognized as a homologue of one of the yeast exocyst proteins, but has a predicted mass corresponding to p106. Therefore, we used bioinformatic tools to determine whether FLJ10893 could be the mammalian homologue of yeast sec3p.

Alignment of amino acid sequences of FLJ10893 and yeast sec3p (which is 442 amino acids longer (144 kDa (18)) yielded significant identity (14% identical residues using the PAM250 matrix, Fig. 4). Other yeast-mammalian exocyst proteins exhibit about 12–21% overall identity. FLJ10893 is the only human protein in the nonredundant data base with significant identity to yeast Sec3p. BLAST searches were used to establish an evolutionary relationship between FLJ10893 and yeast Sec3p. Significant matches were obtained to uncharacterized gene products from several species: *Mus musculus* AK013041 (E value = e^{-129}, 97% identity and 98% similarity), *Drosophila melanogaster* CG3885 (E value = e^{-180}, 40% identity and 60% similarity), *Caenorhabditis elegans* F52E4.7 (E value = e^{-125}, 33% identity and 52% similarity), and *Arabidopsis thaliana* A007519 (E value = e^{-53}, 23% identity and 42% similarity). In each case, no other gene products were related. We conclude that each of these proteins represents a FLJ10893 homologue in each of these species. We next investigated the relationship between these genes and the entire yeast genome. Only one significant match was found for each of these genes, yeast Sec3p (for *D. melanogaster* CG3885: E value = 6e^{-7}, 19% identity and 38% similarity, Fig. 4). Finally, we searched for

confirmed by mass spectrometry to be the same as bands 1 and 2 of panel A (data not shown). This results in a total of 10 GTP-dependent RalA-binding proteins in whole rat brain.

**RalA Associates with the Exocyst Complex**—MALDI-TOF MS was utilized to identify the proteins that associated with GTP-bound RalA. Unambiguous identification was achieved with all of the four major RalA-binding proteins from synaptosomes and rat brain (Fig. 1A); however, none of the minor bands yielded unambiguous identification at this stage. Proteins 1–4 were identified as rat Sec8 (rSec8), human hypothetical protein FLJ10893, rSec5, and rSec6, respectively. Three of these were previously found to be components of the mammalian exocyst complex (23–25).

To identify the minor bands we made two changes to the pull-down experiments (Fig. 2A). First, to obtain a much larger quantity of extract from brain, we changed species to sheep. Second, we used only a cytosolic extract rather than whole brain, as preliminary experiments suggested the GTP-dependent binding proteins were predominantly cytosolic (data not shown). Note that the migration of some of these proteins in SDS-PAGE did not exactly match that found in rat brain. Eight distinct GTP-dependent bands were detected. MALDI-TOF MS identified all eight proteins as components of the exocyst complex (Fig. 2A). A representative MALDI-TOF MS peptide mass map obtained for one of the mammalian exocyst proteins, rSec8, is shown in Fig. 2B. A large proportion of the peptide signals were contributed by rSec8, yielding a statistically significant match with a probability of greater than 99% (Table I). A number of the other signals in the spectrum were attributed to tryptic peptides from FLJ10893 (the adjacent protein to rSec8 on the gel), GST-RalA, trypsin autolytic peptides, and keratin. Similar high quality spectra were achieved for all eight gel bands (data not shown), and probability scores for the identities of the protein bands are shown in Table I. The purified exocyst complex is known to contain at least eight proteins (23–25), and all were identified by MALDI-TOF MS as associated with GTP-bound RalA. However, the identification of Sec3 and Sec15 required further analysis (see below). A number of the proteins that bound RalA in a nucleotide-independent manner were identified as polymers of GST-RalA (94 and 112 kDa, Fig. 2A, and four additional proteins migrating as high as 150 kDa (data not shown)). The basis for the appearance of the polymers is unknown, but polymers have been reported by others (29).

The identification of the exocyst as a GTP-dependent RalA binding complex by MALDI-TOF MS was confirmed by Western blotting with specific antibodies to exocyst complex proteins (Fig. 3). Western blots of proteins from pull-down experiments using rat synaptosome lysates revealed that rSec8 and rSec6 bound to GST-RalA in a GTP-dependent manner (lanes 2 and 3). Neither was detectable in the control pull-down using only GST coupled to GSH-beads (lane 1). rSec6 was also detected in samples of whole brain or testis lysate. To validate our method for the detection of RalA-binding proteins, we used immunoblots to detect two previously reported major RalA-binding proteins. RalBP1 is a known GTP-dependent RalA-binding protein (13), and PLD is known to associate with RalA independently of its nucleotide-bound state (30). Both proteins were appropriately detected in the pull-down experiments using rat brain synaptosomes (Fig. 3).

**Fig. 2. RalA associates with the exocyst complex in brain.** A, a GST-RalA pull-down experiment was performed as in Fig. 1, but from sheep brain cytosol. Each indicated band was analyzed by MALDI-TOF mass spectrometry. The protein identified by MALDI-MS in each band is indicated. B, MALDI-MS peptide mass map of rSec8. This spectrum is representative of data obtained by MALDI-MS for all the marked bands in panel A. Peptide signals that correspond to theoretical in silico digests of each protein component are indicated.
The GTP-dependent RalA-binding protein that corresponds to mammalian homologue of the yeast Sec3p protein. An eighth protein of 106 kDa has also been identified (24). Two of the six peptides from rat brain exocyst complex protein p96 from Hsu et al. (24) with hSec15B/KIAA0919, three out of five peptides matched better to the hSec15B/KIAA0919 sequence than to the sequence of rSec15. We conclude that there are two human orthologues of yeast Sec15p and that we have identified hSec15B/KIAA0919 as the Sec15 component of the exocyst complex that interacts with RalA.

**DISCUSSION**

The main finding of this study is that the GTPase RalA interacts with at least eight proteins in brain in a GTP-dependent manner. Eight of these were identified either as known components of the mammalian exocyst complex or as homologues of known exocyst proteins. Therefore the exocyst is a novel effector for RalA. We also report the first identification of the mammalian Sec3 protein and show that mammalian Sec15 exists as at least two gene products, hSec15A and hSec15B. Sec3p and Sec15p are two of the three yeast exocyst proteins known to bind small GTPases, these being Rho1p and Sec4p, respectively (20, 22). The exocyst complex was initially characterized in yeast as a multiprotein complex thought to be involved in polarized secretion and which localizes to sites of active exocytosis in bud tips (18). The yeast exocyst complex consists of eight characterized proteins, named Sec3p, Sec6p, Sec15p, Sec5p, Sec10p, Sec6p, Exo84p, and Exo70p, of molecular masses 144, 131, 113, 107, 100, 88, 84, and 70 kDa, respectively (18, 19). Specific mutations in these proteins lead to a phenotypic temperature-sensitive deficiency in invertase secretion and an accumulation of secretory vesicles in the cytoplasm (31). The mammalian exocyst complex was later characterized from rat brain and also found to be composed of a corresponding set of eight proteins (24). Seven of the exocyst genes have been cloned from rat brain, and these were named rSec8, rSec5, rSec15, rSec6, rExo84, rExo70, and rSec10, with molecular masses 110, 102, 96, 86, 84, 79, and 71 kDa, respectively (23, 25). An eighth protein of 106 kDa has also been found in the purified exocyst and is likely to be a mammalian homologue of the yeast Sec15p, but it is yet to be cloned (24).

The finding that the exocyst complex associates with RalA in a GTP-dependent manner was confirmed in three independent approaches in our study. Initially, four major and six minor proteins were detected in whole rat brain lysates. The four major proteins were readily identified by MALDI-TOF MS tryptic peptide mass mapping as rSec8, FLJ10893, rSec5, and rSec6. The six minor proteins have yet to be identified from rat,

### Table I

**Identification of GTP-dependent RalA binding proteins in sheep brain cytosol**

| Mass (kDa) | Identity | GenBank™ accession number | Score | % probability |
|-----------|----------|---------------------------|-------|--------------|
| 103       | rSec8    | AAC52265                  | 99+   | 99           |
| 100       | Hypothetical protein FLJ10893 (human Sec3p homologue) | BAA91868, NP_060731 | 96     |               |
| 97        | hSec5    | CAB54145                  | 97    |               |
| 92        | KIAA0919 protein (human rSec15 homologue) | BAA76763 | 99+   | 99           |
| 85        | hExo84   | CAB81620                  | 99+   |               |
| 84        | rSec6    | AAB55505                  | 98    |               |
| 80        | mExo70   | AAB69345, NP_058553       | 99+   |               |
| 70        | hSec10   | AAB53388, NP_066535       | 99+   |               |

Molecular mass of the proteins from sheep is shown on the left. The preceding letters r, h, and m refer to rat, mouse, and human species, respectively. Statistical significance is determined at a probability of ≥95%.

**Fig. 3.** Exocyst complex proteins are identified as GTP-dependent RalA-binding proteins by Western blotting. Pull-down experiments using GSH beads bound to recombinant GST or to GST-RalA loaded with GDP or GTP were performed from rat brain synaptosomes and whole tissues. Bound proteins were analyzed by Western blot using anti-rSec8 and anti-rSec6 monoclonal antibodies (StressGen) and anti-PLD and anti-RalBP1 polyclonal antibodies (Santa Cruz).
D. melanogaster sequence from sequence of FLJ10893 protein (fly) was aligned with the homologous mammalian homologue of yeast Sec3p.

The amino acid sequence homology, and that amino acid sequences from the mammalian Sec3 was supported by its similar molecular mass, sequence homology, and that amino acid sequences from the rat exocyst protein p106 (24) also closely match FLJ10893. However, this identification needs to be confirmed by cloning the equivalent gene from rat or sheep.

Two Sec15 genes were identified in this study. Sec15 from rat was previously cloned (25), and we found a human homologue from GenBank™ which we named hSec15A/CAB70736. However, the MS data did not identify the 92-kDa component of the RalA-binding exocyst complex as Sec15A. The statistically significant match was to KIAA0919, a novel homologue of Sec15, which we named hSec15B/KIAA0919. The proteins are 72% identical in their core region, but have distinct N- or C-terminal extensions. Furthermore, aligning the rat tryptic peptide sequences obtained by Hsu et al. (24) with hSec15B/KIAA0919 produces a much better match than to hSec15A/CAB70736 or to rSec15. This raises the possibility that the mammalian Sec15 exists in two forms and that the B form is a component of the exocyst complex that interacts with RalA. Other mammalian exocyst proteins may arise from multiple genes, for example Sec6 and B94 (25).

RalA is the first mammalian protein shown to interact with the exocyst in a GTP-dependent manner. It is unlikely that RalA interacts with all eight proteins, but the specific exocyst protein target for RalA has not been determined. Specific components of the yeast exocyst are known to associate with at least three small GTPases. Sec4p (the yeast equivalent of Rab3A) anchors the complex to secretory vesicles via Sec15p (20), Rho1p anchors it to the plasma membrane via Sec3p (22), and Rho3p binds Exo70p (21). As there is no yeast equivalent of Ral (the closest related proteins being the Ras proteins) it is difficult to speculate on a function for the RalA-exocyst interaction based on the known small GTPase-exocyst interactions in yeast. RalA is localized both on secretory vesicles and on the plasma membrane (3, 4) and, like Rab3A (35), undergoes reversible association with synaptic vesicle membranes (10). Therefore, it will be important to determine whether the mammalian RalA-exocyst interaction might mimic the yeast Sec15p-Exo4p interaction in tethering the complex to synaptic vesicles, the Sec3p-Rho1p interaction in tethering the complex to synaptic vesicles, the Sec15p-Sec4p interaction in tethering the complex to synaptic vesicles, and the Sec10p-Rho3p interaction in tethering the complex to the plasma membrane, or whether there is a novel function.

Our results localize the exocyst and most of the known Ral signaling pathway to nerve terminals, which are regions of neuronal cells highly adapted for secretory roles. RalA regulates receptor-mediated endocytosis in nonneuronal cells via its effector RalBP1 (14). Another protein directly activated by RalA is PLD1, which is also required for endocytosis (12). Our results localize these two signaling pathways to nerve terminals, sites of extremely active synaptic vesicle endocytosis, but it remains to be demonstrated whether RalA has an endocytic role in the nerve terminal. Our finding that the exocyst is another effector for RalA localizes this complex to the mature

**FIG. 4. Identification of human hypothetical protein FLJ10893 as the mammalian homologue of yeast Sec3p.** The amino acid sequence of FLJ10893 protein (human) was aligned with the homologous sequence from D. melanogaster CG3885 (fly) and from yeast Sec3p (yeast) using ClustalW. Identical amino acids are shown in white text on a black background, while similar amino acids are shown against a gray background. Regions matching Edman sequence reported previously (24) for tryptic digests of the rat brain exocyst protein p106 are overlined.
nerve terminal. The complex was previously localized to growth cones of axons and dendrites in developing neurons, in discrete domains along the axon, and in most newly formed nerve terminals (36). The likely role for the mammalian exocyst in the development of nerve terminal exocytosis and the established role for RalA in endocytosis places RalA in a unique situation where it potentially regulates both, although not necessarily during the same stage of development. Exocytosis and endocytosis in nerve terminals is Ca\(^{2+}\)-dependent, and RalA is also activated by Ca\(^{2+}\)-calmodulin (8, 9). Thus, the possible role of RalA in synaptic vesicle recycling in neurons now needs to be more clearly established. A dynamic interaction of the exocyst with synaptic vesicle and plasma membrane-bound small GTPases is compatible with current views of the cellular function of the exocyst. RalA also recruits filamin in a GTP-dependent manner to specific sites on the plasma membrane and has been proposed to thereby induce filopodia formation (16). The exocyst complex is required for exocytosis and neurite outgrowth, and it localizes to filopodia and neurite growth cones. Therefore, RalA might be acting as a control point for the integration of receptor and calcium signaling with neurite outgrowth, endocytosis, and directing sites of exocytosis.

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Adam Brymora, Valentina A. Valova, Martin R. Larsen, Basil D. Roufogalis and Phillip J. Robinson

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