Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 1445–1449, February 1994
Cell Biology

n-Sec1: A neural-specific syntaxin-binding protein

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Communicated by James E. Rothman, December 2, 1993 (received for review November 17, 1993)

ABSTRACT **We have identified n-Sec1, a rat brain homolog of the yeast Sec1p protein that participates in the constitutive secretory pathway between the Golgi apparatus and the plasma membrane. The rat brain cDNA is predicted to encode a 68-kDa protein with 65% amino acid identity to *Drosophila* rop, 59% identity to *Caenorhabditis elegans* unc-18, and 27% identity to *Saccharomyces cerevisiae* Sec1p. By RNA blot analysis, n-Sec1 mRNA expression is neural-specific. An anti-peptide antiserum directed against the n-Sec1 carboxyl terminus detects a 68-kDa protein in rat brain cytosol and membranes, but not in peripheral tissues. In the presence of syntaxin 1a, a plasma membrane protein implicated in synaptic vesicle docking, n-Sec1 becomes membrane-associated. n-Sec1 binds to syntaxin 1a, 2, and 3 fusion proteins coupled to agarose beads, but not to syntaxin 4 fusion protein or beads coupled to a variety of other proteins. These findings indicate that n-Sec1 is a neural-specific, syntaxin-binding protein that may participate in the regulation of synaptic vesicle docking and fusion.**

Within the secretory pathway, proteins and other cargo are transferred from one compartment to another by vesicular traffic. Transport vesicles bud from donor membranes and dock to specific acceptor compartments (1, 2). Following vesicle docking, membrane fusion results in the transfer of vesicular constituents. This highly ordered process is common to all eukaryotic cells and underlies such diverse functions as constitutive and regulated secretion, as well as endocytosis. The specificity of vesicular trafficking is thought to be guaranteed by the proteins that reside on particular donor and acceptor membranes.

A well-characterized example of vesicular trafficking is the neurotransmitter-containing synaptic vesicle which, upon calcium entry into the presynaptic nerve terminal, fuses with the presynaptic plasma membrane at a specialized region called the active zone (3, 4). Two membrane-spanning proteins of the synaptic vesicle, VAMP (vesicle-associated membrane protein) and synaptotagmin, have been shown to interact in a 7S particle with syntaxin 1a and the 25-kDa synaptosomal protein SNAP-25, two proteins localized to the presynaptic plasma membrane of neurons (5). Furthermore, a 20S particle forms upon addition of the soluble fusion-promoting factors NSF (*N*-ethylmaleimide-sensitive factor) and αSNAP (soluble NSF attachment protein) (6). This particle is dissociated upon hydrolysis of ATP by NSF, and this series of protein assembly and dissembly steps is likely to be central to the vesicle docking and fusion reaction. According to the SNAP receptor (SNARE) hypothesis (5, 6), the specificity of vesicle docking is provided by the binding of vesicular SNAREs such as VAMP with their cognate target SNAREs such as syntaxin and SNAP-25, followed by the action of the general fusion factors NSF and SNAP.

VAMP and syntaxin have small multigene families, with members localized to distinct intracellular compartments (7–9). Thus synaptic transmission is a specialized form of vesicular trafficking. This hypothesis is supported by the description of yeast proteins homologous to those defined in synaptic transmission (2, 10). Studies of yeast secretory mutants (11) have also defined a series of components for which no mammalian homologs have been identified. These include the *SEC2*, *SEC8*, and *SEC15* genes, whose products are likely to be critical in mediating the function of low molecular weight GTPases such as Sec4p (12). Three related yeast proteins involved in transport from endoplasmic reticulum to Golgi apparatus, from Golgi apparatus to plasma membrane, and from Golgi apparatus to vacuole are Sly1p, Sec1p, and Slp1p, respectively (13). These proteins are about 20% identical and have no apparent membrane anchor. In this report we identify a rat brain homolog of the yeast Sec1p protein, which we call neural (n)-Sec1.† This molecule is of particular interest because the yeast syntaxin homologs *SSO1* and *SSO2* were identified as multicopy suppressors of the *sec1* mutation (14).

MATERIALS AND METHODS

Cloning and Sequence Analysis of n-Sec1 cDNA. Two degenerate oligonucleotide primers were synthesized corresponding to amino acids 226–232 and 304–311 of Sec1p, 221–227 and 291–298 of rop, and 290–296 and 362–369 of unc-18: 5′-GAYGANRAAYGAYGAYYTNTGGGT-3′ and 5′-GAYGCNTAYAARGCNGAYGA-3′. These primers were used to isolate a 230-bp cDNA fragment by using 5 ng of rat brain cDNA as a template in the polymerase chain reaction (PCR). Samples were amplified for 30 cycles with conditions of denaturing (94°C, 30 sec), annealing (45°C, 45 sec), and extension (72°C, 1 min) by using *Taq* polymerase (Boehringer Mannheim). The DNA product was isolated from a 2% agarose gel, radiolabeled with random hexamer primers, and used to screen a cDNA library derived from adult rat cerebral cortex (Stratagene). Thirty-eight positive clones were isolated, and 26 were sequenced by random chain termination with the Sequenase polymerase (United States Biochemical). Clone K17 (nucleotides 1–2104; open reading frame from 86 to 1870) was sequenced on both strands. The entire sequence, including overlapping clone K1 (nucleotides 82–2369), has been deposited in GenBank.†

RNA Blot Analysis. The coding region was isolated from clone K5 (nucleotides 17–1876) by digestion with *Nco* I and *Hin*dIII, radiolabeled by random hexamer priming, and used to probe RNA blots containing 2 μg of poly(A)$^+$ RNA from various rat tissues (Clontech) or 10 μg of total RNA from rat brain at various developmental stages. Blots were hybridized at 68°C as described (Clontech), washed at 50°C in 15 mM NaCl/1.5 mM sodium citrate, pH 7.0/0.1% SDS, and exposed to film at −70°C. Methylene blue staining confirmed that equal amounts of RNA were on the blots.

Antiserum Production and Western Analyses. A peptide was synthesized corresponding to the predicted 20 carboxyl-

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Abbreviation: GST, glutathione *S*-transferase.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. L26264).

terminal amino acids of n-Sec1, coupled to keyhole limpet hemocyanin via cysteine residues with sulfosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (Pierce) and injected into a rabbit to generate polyclonal antiserum. The antibodies were affinity purified on a column consisting of synthetic peptide coupled to Affi-Gel 10 and Affi-Gel 15 (Bio-Rad), with elution by 0.1 M glycine (pH 2.5). For Western blotting, rats were decapitated and tissues were homogenized in 10 mM Hepes, pH 7.5/0.32 M sucrose/1 mM EGTA/1 mM EDTA/0.1 mM phenylmethanesulfonyl fluoride. Brains were homogenized with eight strokes of a glass/Teflon homogenizer, and the homogenate was centrifuged at 1000 × *g* for 15 min to prepare a postnuclear supernatant. This was centrifuged in a Beckman Ti70 rotor for 1 hr at 100,000 × *g* to prepare cytosol (S2) and membrane (P2) fractions. Protein samples (15 μg) were electrophoresed in SDS/10% or 12% polyacrylamide gels. Proteins were transferred to nitrocellulose, and the blots were blocked in 0.15 M NaCl/5% (wt/vol) milk powder/50 mM Tris, pH 8.0/0.1% Tween 20/2% normal goat serum and probed with affinity-purified antibodies (1:500 in blocking buffer). Bound antibodies were visualized by enhanced chemiluminescence (ECL system, Amersham).

***In Vitro* Transcription and Translation.** Full-length cDNAs coding for rab3A, rabphilin, synaptotagmin, VAMP, SNAP-25, and n-Sec1 were linearized from pBluescript plasmids (Stratagene), transcribed *in vitro* with T3 or T7 phage polymerase, and translated with reagents as described (Promega). Translations were in a rabbit reticulocyte lysate supplemented with [^{35}S]methionine (DuPont/NEN) in the presence of canine pancreatic microsomes. The translated proteins were analyzed by SDS/polyacrylamide gel electrophoresis (12% polyacrylamide/0.8% *N,N'*-methylene bisacrylamide) and the gels were treated with Amplify (Amersham) for fluorography. Signals were quantitated by a PhosphorImager (Molecular Dynamics).

For glycerol gradients, translated n-Sec1 (5 μl of lysate), syntaxin 1a (associated with microsomes), and n-Sec1 associated with microsome-bound syntaxin 1a were solubilized in 0.5% Triton X-100 in 25 ml of 20 mM Hepes, pH 7.4/140 mM KCl/0.1 mM EGTA (gradient buffer). The solubilized samples were centrifuged at 20,000 × *g* for 15 min at 4°C and layered on 1-ml linear 4–17.5% (vol/vol) glycerol gradients containing 0.1% Triton X-100 in the gradient buffer. The gradients were centrifuged in a Beckman TLS 55 rotor at 41,000 rpm for 16 hr at 4°C. Fractions (75 μl) were collected and analyzed by fluorography.

***In Vitro* Binding Assay.** [^{35}S]Methionine-labeled n-Sec1 (35 μl) was incubated for 1 hr at 4°C with 20 μl of glutathione-agarose beads to which were coupled glutathione *S*-transferase (GST) or GST fusion proteins purified from bacterial lysates. Samples were washed three times in 0.5 ml of 10 mM Hepes, pH 7.4/150 mM NaCl with brief centrifugation. The beads were boiled in 15 μl of SDS sample buffer and proteins were electrophoresed in SDS/10% polyacrylamide gels and processed for fluorography.

RESULTS

Cloning and Sequence Analysis of n-Sec1. Amplification of rat brain cDNA with degenerate oligonucleotides corresponding to amino acids conserved between Sec1p and its homologs *Drosophila* rop (15) and *Caenorhabiditis elegans* unc-18 (16) produced a 230-bp DNA fragment, similar in size to fragments generated from *Drosophila* head cDNA. This amplified DNA fragment was used to screen 350,000 plaques from a rat cerebral cortex cDNA library. Thirty-eight positive clones were isolated and 26 were partially sequenced. cDNA clone K17 includes an open reading frame of 1785 bp predicted to encode a 67,568-Da protein. The calculated isoelectric point of the protein is 7.0. The protein includes potential sites for phosphorylation by protein kinase A, protein kinase C, casein kinase II, and protein-tyrosine kinase (Fig. 1).

Comparison of the predicted n-Sec1 protein with the GenBank data base (release 80.0) revealed 65% amino acid identity to *Drosophila* rop (4 gaps in the alignment), 59% identity to *C. elegans* unc-18 (5 gaps), 27% identity to yeast Sec1p (20 gaps), 22% identity to yeast Sly1 (19 gaps), and 20% identity to yeast Slp1 (26 gaps). Additionally, n-Sec1 has 100% identity to a human hippocampal cDNA predicted protein (24 amino acids, 0 gaps) that was isolated in a screen for brain-specific cDNAs (17).

A comparison of n-Sec1 with these other protein sequences indicates that their similarities are distributed throughout (Fig. 1 and data not shown). Neither Sec1p (18) nor n-Sec1 contains predicted transmembrane domains, although each contains several clusters of up to nine hydrophobic amino acids that may serve as peripheral membrane attachment sites.

Regional Distribution of n-Sec1 mRNA and Protein. By RNA blot analysis, n-Sec1 mRNA was expressed predominantly in brain (Fig. 2*A*) and spinal cord (data not shown), with a message size of 4 kb. Upon long exposures of the autoradiograms, faint signals of the same message size were detectable in all tissues tested. Quantitation by phosphorimaging revealed that the expression in brain was at least 100 times that in other tissues. We also examined the developmental profile of n-Sec1 mRNA expression (Fig. 2*B*). Faint levels were detectable at embryonic day 14, with levels rising at later embryonic ages and peaking at postnatal day 7. This developmental time course parallels synapse formation in the rat and is in contrast to the developmental time course of unc-18 in *C. elegans*, which peaks during early larval and embryonic stages (16).

To further characterize n-Sec1, we generated anti-peptide antibodies which were affinity purified and used to probe nitrocellulose blots of tissue homogenates. The immune serum recognized a protein of 68 kDa, the molecular size predicted from the cDNA (Fig. 3*A*). n-Sec1 was present in both cytosol and a membrane fraction. The specificity of the antibodies was confirmed by preincubation with 1 mg of peptide, which completely blocked the signal (Fig. 3*A*). The brain-specific regional protein distribution (Fig. 3*B*) is consistent with the results of RNA blotting. All brain regions tested contained similar amounts of n-Sec1.

Binding of n-Sec1 to Syntaxin. Genetic evidence indicates an interaction between Sec1p and the syntaxin homologs Sso1p and Sso2p (14). We examined the binding of *in vitro* translated, [^{35}S]methionine-labeled n-Sec1 to *in vitro* translated syntaxin and other proteins localized to the nerve terminal. n-Sec1 was soluble when translated alone or in the presence of SNAP-25, VAMP, or synaptotagmin (Fig. 4*A*), as well as rabphilin and rab3A (data not shown). However, in the presence of syntaxin 1a, n-Sec1 was associated with the microsomal membrane (Fig. 4 *A* and *B*). Increasing concentrations of syntaxin cause an increased amount of n-Sec1 binding to the membrane with a concomitant decrease in soluble n-Sec1 (Fig. 4*B*). The molar ratio of n-Sec1 to syntaxin was 0.8:1, based on quantitation of the pixel density of each protein in lanes 2 and 3 and correction for the 19 methionine residues in n-Sec1 versus 11 in syntaxin. In linear glycerol gradients, translated n-Sec1 migrated with a peak at fractions 7–9, and syntaxin peaked in fraction 5, consistent with their migration as monomers (Fig. 4*C*, upper two autoradiograms). When cotranslated and analyzed on a glycerol gradient, both proteins migrated with peaks further into the gradient (syntaxin, fractions 5–10; n-Sec1, fractions 8–10; Fig. 4*C*). Quantitation of the autoradiograms by PhosphorImager confirmed that syntaxin in the presence of n-Sec1 migrated as a broad peak closer to the bottom of the gradient relative to the migration behavior of syntaxin alone (Fig. 4*C*, bottom autoradiogram). Taken together, the *in vitro* transla-

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n-sec1    MAPIGLKAVVGEKIMHDVIKKVKKKGEWKVLVVDQLSMRMLSSCCKMTDIMTEGITIVEDINKR.R  65
sec1p        MSDLIELQRNYLIGVLNQIETKNNLKFLIIDKTVETILSYLFLTPQELLNNVTSVDLIDSPTR

n-sec1    EPLPSLEAVYLITPSEKSVHSLISDFKDPPTAKYRAAHVFFTDSCPDALFNELVKSR.AAKVIKTL 130
sec1p     KGQSSIEAIYILEPTKYNINCIDADFMVRP.PKYRRCHIRFLPGLTNPIFQFFQSKRYIAQNLESF

n-sec1    TEINIAFLPYESQVY.SLDSADSFQSFYSPHKAQMKNPILERLAEQIATLCATLKEYPAVRYR... 192
sec1p     KPIELGFFVKESQFFETLQMEHSLQVFFNNNCKALIPTNVRKIVGSLVSLCVITGEYPIVRYSVSN

n-sec1    .....GEYKDNAL.........LAQLIQDKLDAYKADDFTM.GEGPDKARSQLLILDRGFDPSSPV 243
sec1p     PVEEEDARNGNAVVNANSLTRSIANAFQIAIDTYARNNPDFPPQNTERPRSILIITDRTLDPFAPI

n-sec1    LHELTFQAMSYDLLP...IENDVYKYETSGIGEARVKEV..LLDEDDDLWIALRHKHIAEVSQEVT 304
sec1p     LHDFSYQAMAYDLVANVDTQKDIYHYSAENEAGEQEEKVSKLVDLYDPDWIDLKHQHIMDANEYIQ

n-sec1    RSLKDFSSSKRM..NTGEKTTMRDLSQMLKKMPQYQKELSKYSTHLHLAEDCM.KHYQGTVDKLCR 367
sec1p     GRIKELIAKNPLLVDRSNVKNTTDLLSVVAHLKDFDEERRRLILHKTLVDECLGENAERKLADISA

n-sec1    VEQDL.AMGTDAEGEKIKDPMRAIVPILLDANVSTYDKIRIILLYIFLKNGITEENLNKLIQHAQI 432
sec1p     IEQNLSGFGMDFSGEKIKHIIDDLLPALAMKEPTILDKLRYIIAYALFRGGIIELDFIKLLNFIGV

n-sec1    PPEDSE......IITNMAHLGVPIVTDSTLRRRSKPERKERISE......QTYQLSRWTPIIKDIM 486
sec1p     THEHENFQQYLKIFRNYDLIDFKLIKD...KPKDKPFQKEWFHDTLVNDPNIYHTSRFVPAVGNIL

n-sec1    EDTIEDK..LDTKHYPYISTRSSASF..........STTAVSARYGHWHKNKAPGEYRSG...... 534
sec1p     SKVIANPLLLSEQYFPYLKDKPIELLNEEEFQAGLANTSANSSSSLRNPRHKAAWTTKSSNIKKNI

n-sec1    ..PRLIIFILGGVSLNEMRCAYEVTQANGK.WEVLIGSTHILTPQKLLDTLKKLNKTDEEISS    594
sec1p     PRQRFFYYVIGGISIPEIKAAYD..QSNLKNRDIFIGSDEILTPTKFLDEVERLQNPREFEKFKED

sec1p     QRQQVNPPDFLLREMKPVAQPVSHVHLKSQDNSPKSGTSSPKAAGSLKSEPPEKEKKRSKFSRFLK

sec1p     RKSHHDK
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FIG. 1. Comparison of n-Sec1 and yeast Sec1p protein sequences. Identical amino acid residues are shaded. Conservative amino substitutions (I, L, M, F, and V; K and R; D and E; N and Q; S and T) are underlined. The n-Sec1 amino acids are numbered. There are potential sites for phosphorylation of n-Sec1 by protein kinase A (residues 457–460), tyrosine kinase (residues 467–473), protein kinase C (12 sites), and casein kinase II (13 sites).

tion data are consistent with a model in which syntaxin and n-Sec1 form a 1:1 stoichiometric complex.

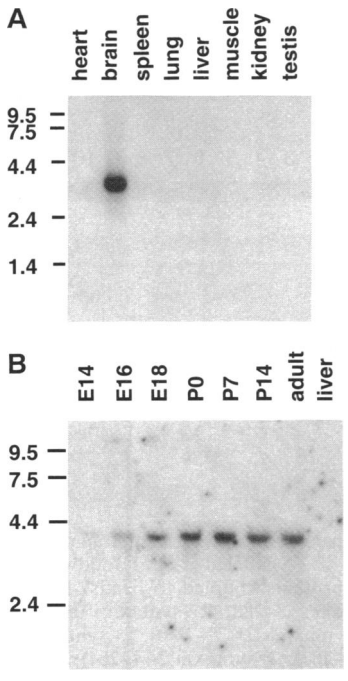

FIG. 2. Northern analysis of n-Sec1 mRNA expression. (*A*) Regional Northern blot analysis using 2 μg of poly(A)$^+$ RNA per lane. Molecular size markers are indicated in kilobases. (*B*) Developmental profile of n-Sec1 expression. E*n*, embryonic day *n*; P*n*, postnatal day *n*. Blots were exposed to x-ray film for 2 hr (*A*) or 18 hr (*B*).

We further characterized the interaction of n-Sec1 and syntaxin with an *in vitro* binding assay. [^{35}S]Methionine-labeled n-Sec1 was incubated with syntaxin GST fusion protein attached to beads, and after 1 hr the beads were washed extensively and the bound radioactive protein was eluted, electrophoresed, and visualized by autoradiography. n-Sec1 bound to syntaxin 1a (Fig. 5*A*, lane 1) but not to GST fusion

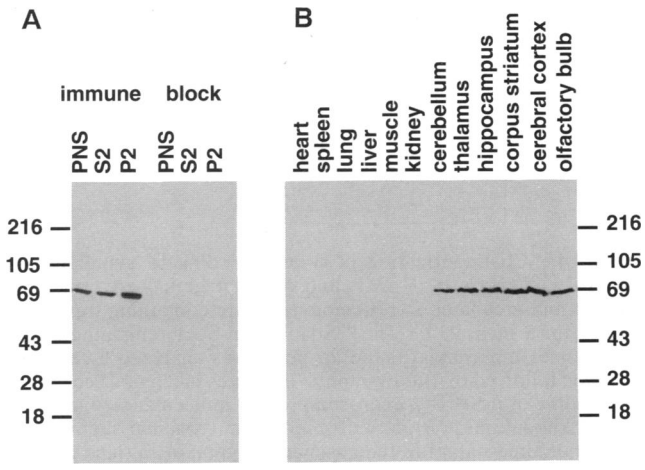

FIG. 3. Western blot analysis of n-Sec1 distribution. (*A*) Samples (15 μg) of rat brain postnuclear supernatant (PNS), cytosol (S2), and membrane (P2) fractions were probed with affinity-purified antibodies (1:500) as described in *Materials and Methods*. Blocking of the antibodies was achieved by preincubation for 30 min with 1 mg of synthetic n-Sec1 peptide. (*B*) Regional distribution of n-Sec1 protein. Samples (15 μg) of total tissue homogenates were probed as in *A*. Molecular size markers are in kilodaltons.

protein (lane 13) or to SNAP-25, synaptotagmin, rab3A, or rabphilin (data not shown). ^{35}S-labeled n-Sec1 bound to a full-length syntaxin 1a construct (Fig. 5*A*, lane 1; amino acids 8–288) or to a form lacking the hydrophobic carboxyl-terminal membrane-spanning domain (lane 2; amino acids 4–267), but only weakly (lane 5) or not at all (lanes 3–6) to other truncated forms of syntaxin 1a. ^{35}S-labeled n-Sec1 bound to syntaxin 2 and syntaxin 3 fusion proteins, but not to syntaxin 4 (Fig. 5*A*, lanes 7, 10, and 11). In all binding experiments, approximately equal amounts of fusion protein attached to beads were employed as judged by Coomassie blue staining of SDS/polyacrylamide gels (Fig. 5*B*).

DISCUSSION

The neural-specific homolog of yeast Sec1p presented here extends the parallel between synaptic transmission in neurons and Golgi apparatus to plasma membrane–vesicular trafficking in yeast. Seven components can now be considered homologous proteins in the two systems. On the synaptic vesicle, VAMP is similar to yeast Snc1p and Snc2p, which are essential membrane constituents of post-Golgi transport vesicles (19, 20). The synaptic vesicle also contains GTP-binding proteins including rab3A (21, 22), and there are homologous yeast GTP-binding proteins such as Sec4p, which is associated with Golgi-to-plasma membrane transport vesicles (23). The acceptor membrane in neurons is demarcated by the proteins syntaxin 1a (24) and SNAP-25 (25), which correspond to the yeast plasma membrane proteins Sso1p/Sso2p (14) and Sec9p (26), respectively. Two soluble factors, αSNAP (27, 28) and NSF (1, 29), in mammalian cells and the corresponding Sec17p (30) and Sec18p (31) in yeast, are thought to be involved in multiple vesicular trafficking steps.

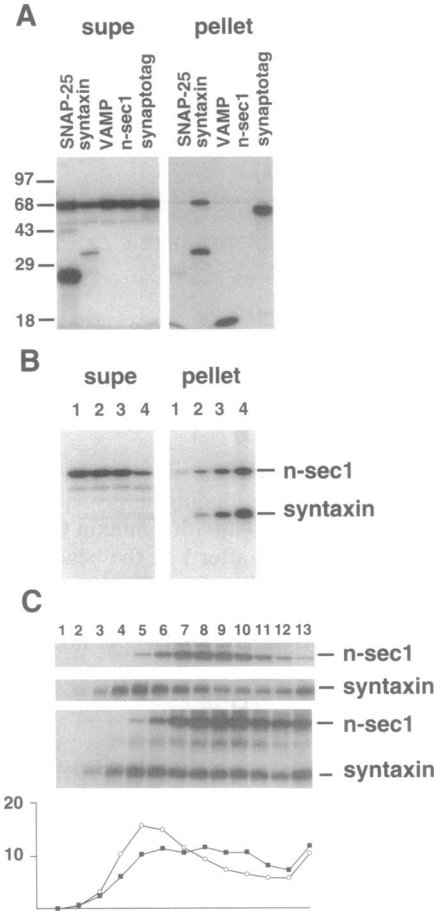

FIG. 4. Characterization of n-Sec1 binding to syntaxin. (*A*) SNAP-25, syntaxin 1a, VAMP, and synaptotagmin were translated *in vitro* and incubated in reticulocyte lysate containing translated n-Sec1 for 1 hr at 23°C. The ^{35}S-labeled n-Sec1 remaining in the microsomal supernatant (supe) or pellet was analyzed by electrophoresis followed by fluorography. The lanes labeled n-Sec1 show the binding of n-Sec1 to microsomes. The molecular size markers are in kilodaltons. (*B*) n-Sec1 binding microsomal membranes (pellet) or supernatant in the absence of syntaxin 1a (lane 1) or in the presence of syntaxin 1a (1, 3, or 6 pg; lanes 2–4). (*C*) Glycerol gradient analysis of a syntaxin 1a/n-Sec1 complex. Fraction 1 corresponds to the top of the glycerol gradient. The autoradiogram was quantitated by PhosphorImager analysis and the percent of the total pixel intensity across all fractions (*y* axis) is plotted versus fraction number for syntaxin alone (○) or syntaxin centrifuged in the presence of n-Sec1 (■). Markers were cytochrome *c* (1.7 S, fraction 4), ovalbumin (3.5 S, fraction 7), and yeast alcohol dehydrogenase (6.7 S, fraction 13).

The direct binding of n-Sec1 to syntaxin is consistent with genetic studies which demonstrate that the yeast syntaxins Sso1p and Sso2p are suppressors of *sec1* mutations (14). It is interesting that n-Sec1 binds syntaxins 1a, 2, and 3 but not the more divergent syntaxin 4. These data suggest that additional members of the n-Sec family remain to be identified in mammalian systems, including homologs for the related proteins Sly1p and Slp1p involved in other transport steps in yeast (13). The binding of n-Sec1 to syntaxin is likely to require the full cytoplasmic domain. This suggests that n-Sec1 recognizes a series of domains which are folded into proximity or that the two proteins bind along an extended region of interaction.

Previous studies of the docked 7S particle (VAMP, synaptotagmin, SNAP-25, and syntaxin) or the 20S particle formed with αSNAP and NSF (5, 6) did not identify n-Sec1 as a major constituent of these complexes. Further studies with the n-Sec1 antibodies will allow us to further address this issue. Several possibilities could account for the absence of n-Sec1 from these complexes. First, n-Sec1 might dissociate during the relatively stringent washing used in their isolation. Second, n-Sec1 could act later in the pathway, so that the binding occurs after the dissociation of the complex. Finally, we favor the hypothesis that n-Sec1 is displaced from the 7S complex either prior to or during vesicle docking. n-Sec1 might therefore function to regulate the formation of the docking complex, possibly through interaction with GTP-

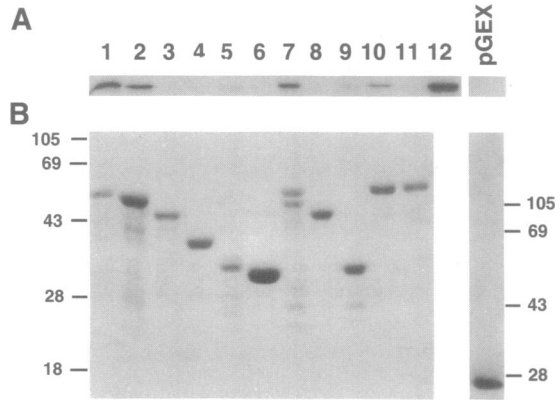

FIG. 5. Binding of n-Sec1 to syntaxin fragments and isoforms. (*A*) Autoradiograms of ^{35}S-labeled n-Sec1 following binding to GST–syntaxin fusion proteins coupled to beads. Lanes: 1, syntaxin 1a amino acid residues 8–288; 2, syntaxin 1a-(4–267); 3, syntaxin 1a-(4–193); 4, syntaxin 1a-(77–288); 5, syntaxin 1a-(194–288); 6, syntaxin 1a-(194–267); 7, syntaxin 2-(4–264); 8, syntaxin 2-(4–191); 9, syntaxin 2-(192–264); 10, syntaxin 3-(4–264); 11, syntaxin 4-(5–274); 12, 1 μl of ^{35}S-labeled n-Sec1 probe; pGEX, the GST protein coupled to beads. (*B*) Coomassie brilliant blue profile of the syntaxin proteins used in *A*. Proteins on 20 μl of beads were solubilized in sample buffer, electrophoresed in an SDS/12% polyacrylamide gel, and stained.

binding proteins. Genetic interactions have been observed between both *SEC1* and *SEC4* (32), as well as *SLY1* and the GTPase gene *YPT1* (33). Furthermore, the *rop* gene, a *Drosophila SEC1* homolog, is adjacent to the *Ras2* gene and the two are coordinately expressed under the control of a bidirectional promoter (15). Thus there is a variety of indirect evidence for an interaction between n-Sec1 and low molecular weight GTPases.

Recent experiments suggest that VAMPs 1 and 2 directly bind syntaxins 1 and 4 but not syntaxins 2 and 3 (unpublished data). The data presented here demonstrate an interaction of n-Sec1 with syntaxins 1, 2, and 3 but not syntaxin 4. These studies may provide a glimpse of a set of combinatorial protein–protein interactions which regulate and define the specificity of vesicular trafficking. We hypothesize that a set of specific protein complexes must assemble to mediate the docking and fusion of transport vesicles at each compartment. Expression of specific combinations of VAMPs, syntaxins, n-Sec1 isoforms, rab isoforms, and other proteins is likely to define the character of the secretory pathway within particular cell types.

We thank Robert Embacher for help with the initial isolation of n-Sec1, Dr. Mark Bennett for syntaxin constructs, Dr. Michael Ferns for providing a developmental RNA blot, and Dr. Thomas Söllner for a SNAP-25 cDNA. R.H.S. is supported by a grant from the National Institute of Mental Health.

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