A further consideration is that, although five dopamine receptor subtypes have been identified by molecular cloning techniques, only D1-like (D1, D5) and D2-like (D2, D3, D4) receptors can be readily distinguished pharmacologically[23]. Consequently, the potential involvement of the D5 receptor cannot be excluded.

The D1-mediated facilitation of GABA transmission at $GABA_B$ receptors suggests a simple cellular model for the D1 facilitation of D2-mediated responses observed in the whole animal [24–26]. Dopamine acting at D2 receptors hyperpolarizes midbrain dopamine neurons by increasing potassium conductance[27]. Similarly, GABA acting at $GABA_B$ receptors can act by the same mechanism in the same cells[28]. Thus, the costimulation of D1 and D2 receptors may result in a larger increase in cellular potassium conductance than that afforded by stimulation of the D2 receptor alone.

Defining of the role of D1 receptors in the midbrain may also have important implications for understanding movement disorders, such as Parkinson's disease and the motor side-effects caused by dopamine receptor antagonists in the form of antipsychotic drugs. □

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Synaptic vesicle fusion complex contains *unc-18* homologue bound to syntaxin

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THREE synaptic proteins, syntaxin, SNAP-25 and synaptobrevin, were recently identified as targets of clostridial neurotoxins that irreversibly inhibit synaptic vesicle fusion[1–4]. Experiments searching for membrane receptors for *N*-ethylmaleimide-sensitive fusion protein (NSF), which has an important role in membrane fusion, revealed an ATP-dependent interaction of the same three synaptic proteins with NSF and its soluble attachment proteins[5]. Thus, two independent approaches identify syntaxin, synaptobrevin and SNAP-25 as components of the synaptic vesicle fusion machinery, but their mode of action is unclear[6]. We have now discovered a brain protein of relative molecular mass 67,000 (67K) which binds stably to syntaxin. Amino-acid sequencing and complementary DNA cloning revealed that the 67K protein is encoded by the mammalian homologue of the *Caenorhabditis elegans* gene *unc-18*. In *C. elegans*, *unc-18* belongs to a group of genes defined by mutations with a paralytic phenotype and accumulations of acetylcholine, suggesting a defect in neurotransmitter release[7,8]. The binding of the mammalian homologue of *unc-18* (Munc-18) to syntaxin requires the N terminus of syntaxin whereas that of SNAP-25 involves a more C-terminal sequence. Our data suggest that Munc-18 is a previously unidentified essential component of the synaptic vesicle fusion protein complex.

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A recombinant protein containing the cytoplasmic domains of syntaxin A (also named HPC-1)[9,10] fused to glutathione *S*-transferase (GST) was immobilized on glutathione–agarose beads to form an affinity column. Detergent-treated rat brain homogenate was applied to this column, washed and eluted with a sodium chloridestep gradient, resulting in the purification of a single protein of M_r, 67,000 (Fig. 1*a*). The 67K protein (named Munc-18, see below) binds tightly to GST–syntaxin and elutes from it in an essentially pure form. Two control experiments were performed to ensure that the 67K protein bound to the affinity column by a specific interaction with syntaxin. First, affinity purifications were carried out on glutathione–agarose under a variety of control conditions. Glutathione–agarose beads containing no attached proteins, glutathione–agarose beads containing GST alone, a GST–neurexin fusion protein (GST-Neu I) or GST–syntaxin were incubated with total brain homogenate and washed extensively and their total protein content was analysed by SDS–polyacrylamide gel electrophoresis (PAGE). Endogenous rat brain GSTs were purified on all of the beads, but the 67K protein bound only to GST–syntaxin (Fig. 1*b*). Second, a recombinant protein in which the syntaxin A sequence was fused to a six-histidine tag instead of GST was used for a similar affinity purification. Again, the 67K protein was the major protein purified (data not shown). Together, these data demonstrate that the 67K protein binds specifically and tightly to syntaxin A and constitutes the single major protein in brain which interacts with this component of the synaptic vesicle fusion complex.

Purified 67K protein was subjected to amino-acid sequencing for identification. An N-terminal sequence and internal sequences from tryptic peptides were obtained and used to isolate cDNA clones from rat and bovine brain. The translated amino-acid sequences of the rat and bovine cDNAs predict synthesis of a 67.6K hydrophilic protein with no apparent transmembrane region (Fig. 2). The rat and bovine sequences are very similar, showing only a single amino-acid substitution over 594 residues (leucine to valine at position 291). In contrast to the conservation of the coding region, the untranslated regions of the rat and bovine cDNAs show little homology, suggesting

selective evolutionary pressure on the coding sequence of the protein (data not shown).

Sequence comparisons reveal that the 67K protein is highly homologous to the *unc-18* of *C. elegans*[7,8] and a related gene from *Drosophila*[11], and weakly homologous to the products of several yeast genes implicated in the secretory pathway, in particular *sec1* (refs 12, 13) (Fig. 2). The 67K protein exhibits an overall identity of 57% with the *unc-18* product, suggesting that it represents the mammalian *unc-18* homologue (referred to as Munc-18). Mutations in the *unc-18* gene in *C. elegans* are characterized by paralysis, accumulations of acetylcholine and resistance to the acetylcholinesterase inhibitor aldicarb. A similar phenotype is observed in *C. elegans* only with mutations in a small group of genes[8] that include the genes encoding synaptotagmin, a major synaptic vesicle Ca^{2+}-binding protein[14,15], and the putative vesicular acetylcholine transporter[16]. The accumulations of acetylcholine in these mutants suggests that their phenotype is caused by a block in neurotransmitter release, indicating that the *unc-18* gene product is essential for neurotransmission. The previously described weak homology between the products of the *unc-18* and the yeast *sec1* genes[17] can also be extended to the mammalian proteins that are slightly more homologous to *sec1* (23% overall identity) than is *unc-18* (21% identity) (Fig. 2). In addition, Munc-18 is weakly homologous to the yeast proteins slylp and slplp, which are related to seclp (ref. 13).

Thus, our results reveal that a protein encoded by the mammalian homologue of a *C. elegans* gene with an essential role in neurotransmission binds tightly to syntaxin, a component of the synaptic vesicle fusion machinery[1,6]. To characterize this interaction further, we investigated its possible modulation. Ca^{2+}, ATP-γS, or GTP-γS have no measurable effect on the binding of Munc-18 to GST–syntaxin (Fig. 3*a*). However, treatment of the brain homogenate with *N*-ethylmaleimide (NEM), a sulphhydryl-reactive reagent which interrupts membrane fusion events, inhibits binding, as does prior freezing of the brain homogenate. This result suggests that the binding of Munc-18 to syntaxin is not regulated in the same way as the binding of the complex between NSF and α/β/γ-SNAPs to syntaxin/SNAP-25/synaptobrevin[5], but that it is sensitive to perturbations in protein structure produced by NEM or freezing.

To investigate the stoichiometry of the Munc-18–syntaxin complex, glycerol gradient centrifugations were used. Syntaxin was cleaved from GST by thrombin and purified. Glycerol gradient centrifugations of purified syntaxin, purified Munc-18 and the Munc-18–syntaxin complex were performed. As judged by the values of their sedimentation constants, Munc-18 and the cytoplasmic domain of syntaxin migrate as monomers whereas the Munc-18–syntaxin complex has a sedimentation constant value corresponding to a 1:1 complex (Fig. 3*b*). In the experiment shown, the sample with the Munc-18–syntaxin complex contains an excess of syntaxin, resulting in the presence of monomeric syntaxin (Fig. 3*b*, closed arrows) and Munc-18–syntaxin complexes (open arrows). These results suggest that Munc-18 forms a stable complex with syntaxin, probably in a 1:1 ratio.

Syntaxin, SNAP-25 and synaptobrevin were identified as targets for clostridial neurotoxins[1–4] and as membrane receptors for the NSF–SNAP complex[5], suggesting that these proteins are part of the synaptic vesicle fusion machinery. Syntaxin, SNAP-25 and synaptobrevin interact directly with each other and/or with the NSF–SNAP complex and possibly also with

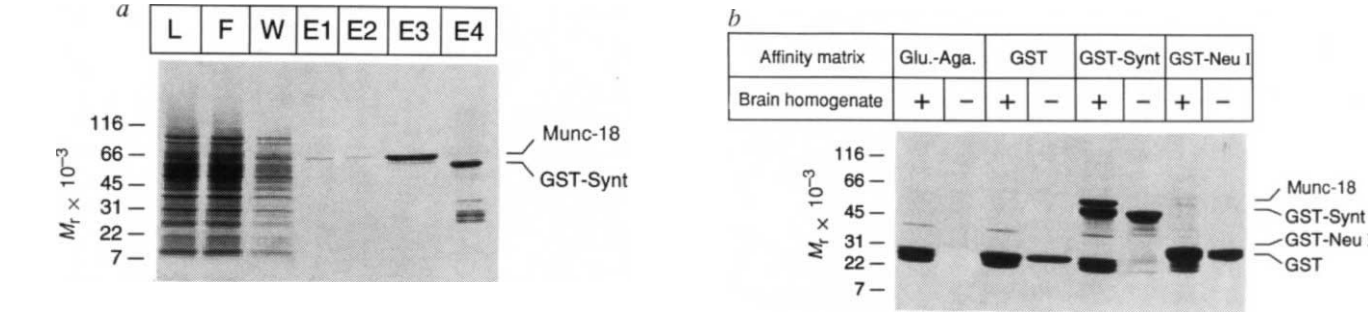

FIG. 1 Identification of a 67K brain protein that binds to syntaxin. *a*, Purification of a 67K brain protein (Munc-18) by affinity chromatography on immobilized syntaxin. Total rat brain homogenate was subjected to affinity chromatography on a GST–syntaxin fusion protein (GST-Synt) containing the cytoplasmic sequence of syntaxin A (HPC-1)[9,10] (residues 1–261) bound to glutathione–agarose. Fractions were analysed by SDS–PAGE and Coomassie blue staining: L, column loading material; F, flow-through; W, Ca^{2+} wash; E1, Mg^{2+}/EGTA eluent; E2, EDTA eluent in 0.1 M NaCl; E3, EDTA eluent in 1 M NaCl; E4, eluent with SDS–PAGE sample buffer. Migration positions of the 67K protein (Munc-18, see Fig. 2) and of GST-Synt are indicated on the right. The low M_r proteins eluted in sample buffer (E4) represent endogenous rat brain GSTs that bind to glutathione–agarose. *b*, Specifity of the binding of the 67K protein (Munc-18) to syntaxin. The picture shows a Coomassie blue-stained SDS–polyacrylamide gel of incubations of glutathione–agarose containing: no attached recombinant protein (labelled 'Glu.-Aga.'), recombinant GST, GST-Synt or GST–neurexin I fusion protein ('GST-Neu I'). Incubations were carried out in parallel with rat brain homogenate (left lanes) or control buffer (right lanes); positions of recombinant GST–fusion proteins are indicated on the right.

METHODS. Using standard procedures[24,25], the cytoplasmic domain of syntaxin A was cloned by the polymerase chain reaction from rat brain cDNA (oligonucleotide sequences: CGCGAATTCCCGCGAGCATGAAGGACCGAAC and GCGAAGCTTATGCCTTGCTCTGGTACTTGAC), transferred into the bacterial expression vector pGEX-KG (ref. 26), sequenced, expressed and purified on glutathione–agarose[27]. Other GST–protein used (Figs 3 and 4) were obtained similarly[28]. To prepare total solubilized brain homogenate, frozen rat brains were homogenized in 4 mM HEPES/NaOH pH 7.4, 0.1 g l^{-1} phenylmethylsulphonyl fluoride (PMSF), extracted for 4 h at 4 °C after addition of an equal volume of 4 mM HEPES/NaOH pH 7.4, 0.1 g l^{-1} PMSF, 0.2 M NaCl, 2% NP-40, 2 mM EDTA and centrifuged (100,000*g* for 30 min at 4 °C; yield: 1 g protein from 10 rats in 50 ml). For affinity chromatography (*a*), rat brain homogenate was precleared by incubation with 2 ml glutathione–agarose (6 h at 4 °C) and centrifugation (800*g* for 2 min). After addition of 3.5 mM $CaCl_2$ and $MgCl_2$, the homogenate was applied to a 1-ml glutathione–agarose column containing 6 mg GST–Synt and pre-equilibrated with buffer A (50 mM Tris/HCl pH 8.0, 0.1 M NaCl, 2.5 mM $MgCl_2$) containing 0.5% NP-40 and 2.5 mM $CaCl_2$. The column was washed with 20 ml buffer A containing 0.5% CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulphonate) and 2.5 mM $CaCl_2$ ('W' in *a*) and sequentially eluted with 10 ml of: buffer A containing 0.5% CHAPS and 5 mM EGTA (E1); 50 mM Tris/HCl pH 8.0, 0.1 M NaCl, 0.5% CHAPS and 5.0 mM EDTA (E2); 1 M NaCl, 0.5% CHAPS and 5 mM EDTA (E3); and 1.5 ml SDS–PAGE sample buffer (E4) (yield: 0.8 mg Munc-18 per g protein). For the batch affinity chromatography procedure (*b*), 50 μl glutathione–agarose without attached proteins or containing 0.5 nmol of the indicated GST–proteins were incubated overnight at 4 °C with 2 ml total brain homogenate (40 mg protein) in buffer B (4 mM HEPES/NaOH pH 7.4, 0.1 M NaCl, 1% NP-40, 1 mM EDTA, 3.5 mM $CaCl_2$, 3.5 mM $MgCl_2$) or in buffer B only. The glutathione–agarose was recovered by centrifugation (800*g* for 2 min), washed six times with 1.2 ml buffer A, resuspended in 0.18 ml SDS–PAGE sample buffer, and 40 μl was analysed by SDS–PAGE. Numbers on the left of the figures indicate positions of M_r markers.

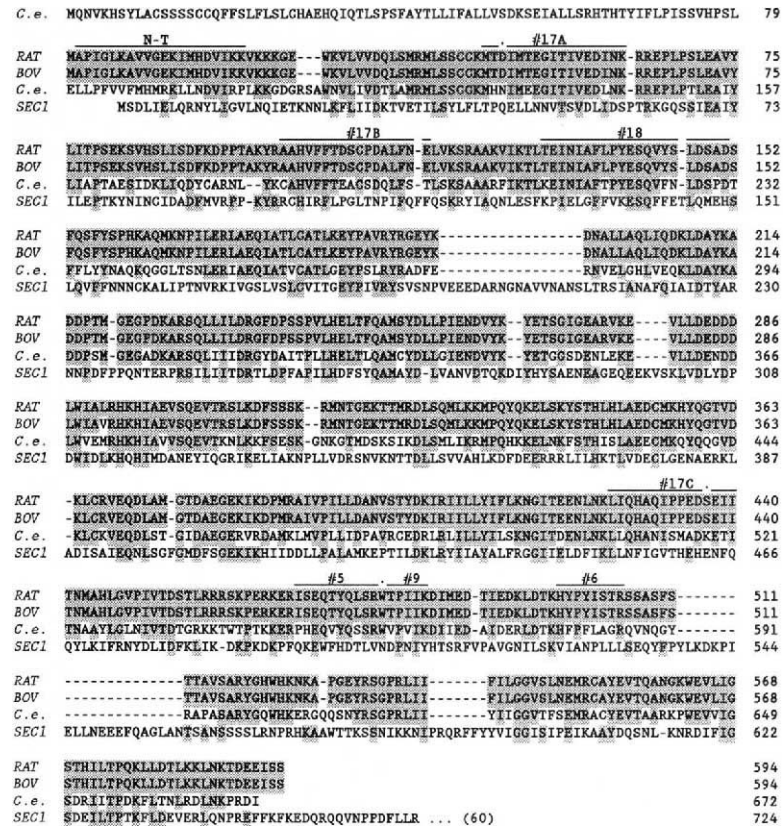

FIG. 2 Structures of rat and bovine 67K proteins (Munc-18) determined by cDNA cloning: homologies to *C. elegans unc-18* and yeast *sec1* gene products. The amino-acid sequences of the rat and bovine 67K proteins as deduced from cDNA sequences (top two lines) are aligned with the sequences of the *C. elegans unc-18* gene product[8] and of yeast sec1p[12]. Sequences are shown in single-letter amino-acid code and numbered on the right. Residues identical to the rat sequence are shaded, revealing an overall identity of 57% of the rat 67K protein with the *unc-18* gene product and identifying it as mammalian *unc-18* homologue (Munc-18). Sec1p is 23% identical to rat and bovine Munc-18 and 21% identical to the *unc-18* product[17]. Locations of amino-acid sequences determined from the purified protein are indicated by lines above the sequence (N-T, N-terminal sequence; #5, 6, 9, 17A, 17B, 17C and 18, sequences of tryptic peptides).

METHODS. Amino-acid sequencing of the purified 67K protein (Fig. 1) was performed as described[29]. Full-length cDNA clones were isolated from rat and bovine brain cDNA libraries using oligonucleotides predicted by the amino-acid sequences and fully sequenced[24,25]. In addition to the alignment shown here, a weak homology of Munc-18 to S1y1p and Slp1p, similar to the homologies of these proteins to Sec1p[13], was detected (not shown). The nucleotide sequences of the cDNA clones reported here are deposited in Genbank. The accession number of rat *munc-18* is L26087 and that of bovine *munc-18* is L26088.

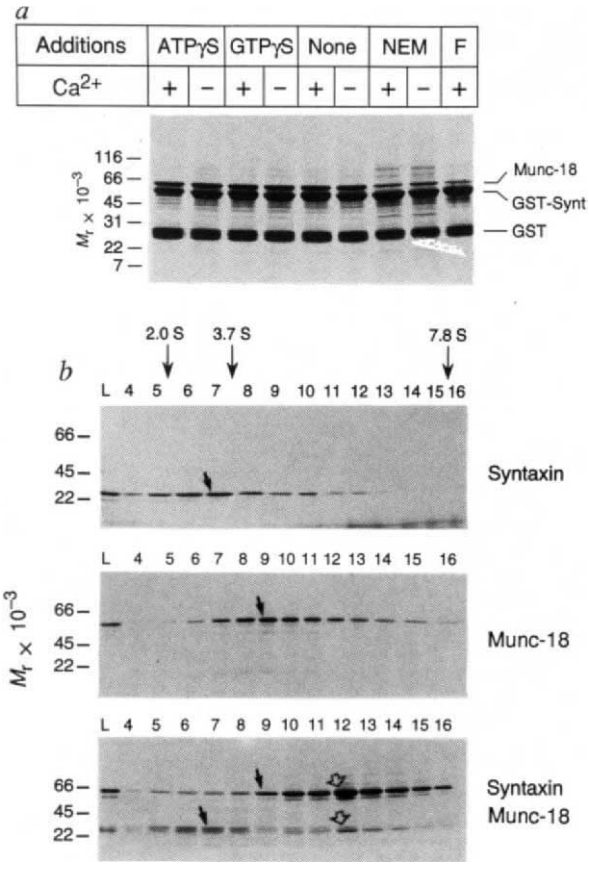

FIG. 3 Nature of the Munc-18–syntaxin complex. *a*, Dependence of Munc-18 binding to syntaxin on Ca^{2+}, ATP-γS, GTP-γS, NEM and prior freezing of the brain homogenate. GST–syntaxin attached to glutathione–agarose was incubated with total rat brain homogenate in the presence or absence of Ca^{2+} (3.5 mM $CaCl_2$ or 5 mM EGTA) with 50 μM ATP-γS ('ATPγS'), 50 μM GTP-γS ('GTPγS'), or no further additions ('None'), or after pretreatment of the brain homogenate with 2 mM NEM for 2 h at 4 °C followed by the addition of 20 mM dithiothreitol ('NEM'), or after freezing the extracts at −20 °C for 24 h before use ('F'). Proteins bound to glutathione–agarose after extensive washing were analysed by SDS–PAGE and Coomassie blue staining. Positions of Munc-18, GST-Synt and endogenous brain GSTs purified on the glutathione–agarose are indicated on the right. *b*, Size analysis of the cytoplasmic domain of syntaxin, Munc-18 and the Munc-18–syntaxin complex by glycerol density gradient centrifugation. Fractions were analysed by SDS–PAGE and silver staining and are identified by numbers above each panel. Protein sizes are estimated[30] by comparison with known protein standards analysed on parallel gradients (2.0S, equine myoglobin (17.5K); 3.7S, chicken ovalbumin (44K); 7.8S, bovine γ-globulin (158K)). Closed arrows point to peak positions of uncomplexed syntaxin (2.8S, 31K) and Munc-18 (4.5S, 62K). Open arrows point to peak position of the Munc-18–syntaxin complex (5.9S, 94K).

METHODS. Experiments testing the effects of different treatments on Munc-18 binding to syntaxin were carried out as for Fig. 1*b* with additional buffer components or manipulations as stated. For the glycerol gradient centrifugations, GST–syntaxin either alone or complexed with Munc-18 (see Fig. 1) were cleaved with thrombin and purified[27]. Partially purified Munc-18 was obtained as described in Fig. 1 legend. Samples (0.3 ml) adjusted to a glycerol concentration of 1% (w/v) were layered onto 11 ml of a 2–20% (w/v) linear glycerol gradient containing 4 mM HEPES/NaOH pH 7.4, 0.1 M NaCl, 0.5% NP-40, 2.5 mM $CaCl_2$ and 2.5 mM $MgCl_2$, and overlaid with 0.5 ml of 4 mM HEPES/NaOH pH 7.4. After centrifugation (15 h at 21,000*g* at 4 °C), 30 fractions were collected from the gradients and analysed by SDS–PAGE and silver staining[28].

synaptotagmin[18,19]. Munc-18 binding to syntaxin may have been missed previously because freezing inhibits this interaction (Fig. 3*a*). Stoichiometric binding of SNAP-25, synaptotagmin and synaptobrevin to syntaxin was not observed on the Coomassie blue-stained gels in our experiments (Figs 1 and 3). However, such binding could be detected when more sensitive immunoblotting techniques were applied (Fig. 4*a*). For these experiments, recombinant fusion proteins of syntaxin, SNAP-25 and synaptobrevin with GST were used to bind proteins from total rat brain homogenates, demonstrating reciprocal but substoichiometric interactions between syntaxin, SNAP-25, synaptotagmin and synaptobrevin. These interactions were not modulated by Ca^{2+}, ATP-γS or GTP-γS, suggesting that they represent direct constitutive binding reactions (data not shown).

To determine whether interactions of syntaxin with different proteins are mediated by distinct sequences, two deletion mutants of GST–syntaxin were constructed and used for affinity purification of Munc-18 and SNAP-25. Deletion of residues 7–76 of the cytoplasmic domains of syntaxin completely abolishes Munc-18 binding but has no effect of the binding of SNAP-25 (Fig. 4*b*). Conversely, deletion of residues 116–261 abolishes binding of both Munc-18 and SNAP-25. These data suggest that Munc-18 binds to syntaxin at an N-terminal site that is distinct from the more C-terminal binding site for SNAP-25.

Our data demonstrate that syntaxin forms a tight stoichiometric complex with a 67K protein in brain which by amino-acid sequencing and cDNA cloning was identified as the mammalian homologue of the *C. elegans unc-18* gene, therefore referred to as Munc-18. Because syntaxin, together with SNAP-25 and synaptobrevin, is part of the synaptic vesicle fusion complex[1–6], Munc-18 probably represents a novel component of this complex. Figure 4*c* depicts a model of the protein–protein interactions operating in this complex in which Munc-18 is envisaged as playing a central role because of its stable interaction with syntaxin, although the exact roles of Munc-18 and other components of the complex in the fusion reaction are unknown. As *unc-18* seems to be essential for neurotransmission in *C. elegans*, Munc-18 is probably an essential component of the fusion complex. In addition to *unc-18*, Munc-18 is weakly homologous to yeast *sec1*, mutations in which inhibit exocytosis[20]. *sec1* interacts with a family of genes named *SSo1* and *SSo2* whose products are related to syntaxin[21] and, according to our data, are likely to interact physically with Sec1p. Multiple syntaxin isoforms are present in yeast and mammals which differ primarily in the N-terminal sequences and may act in different cellular fusion reactions[22,23]. As Munc-18 binds to the N-terminus of syntaxin, it seems likely that mammalian cells will also contain a series of different Munc-18 isoforms, each of which may interact only with a specific isoform of syntaxin. Thus, Munc-18 and its isoforms could have a function in determining the specificity of intracellular fusion reactions. □

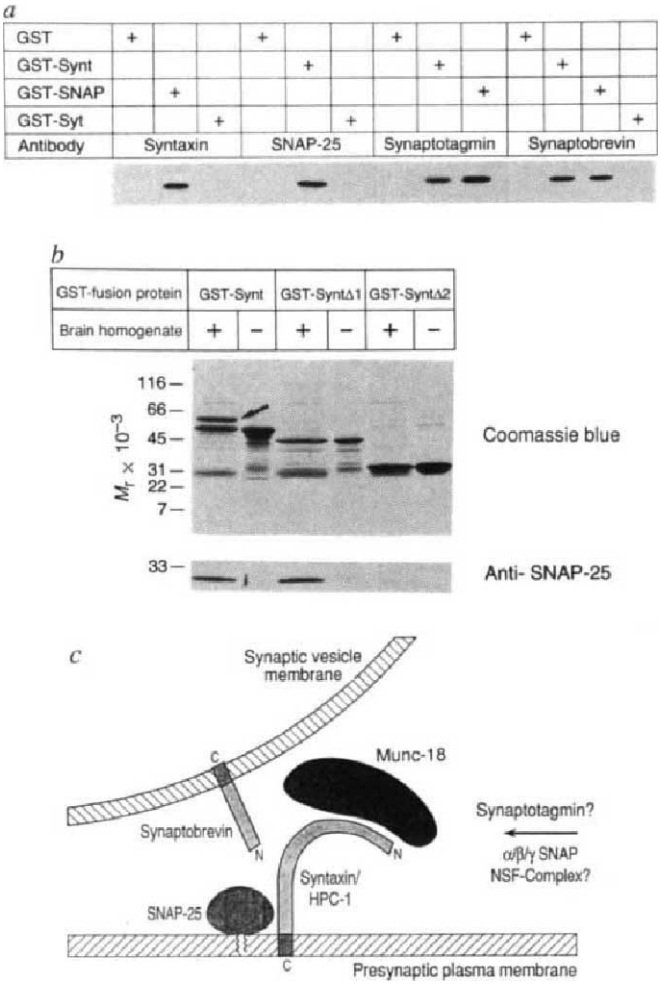

FIG. 4 Relation of the Munc-18–syntaxin complex to syntaxin binding of SNAP-25, synaptotagmin and synaptobrevin. *a*, Complex formation of syntaxin, SNAP-25, synaptotagmin and synaptobrevin. Total rat brain homogenates were incubated with the indicated GST–fusion proteins (GST alone, GST–syntaxin, GST–SNAP containing full-length SNAP-25, and GST-Syt, containing cytoplasmic domains of rat synaptotagmin I starting at residue 140; ref. 15). Proteins bound after extensive washing were analysed by SDS–PAGE and immunoblotting using the indicated antibodies. *b*, Sequence requirements of syntaxin for binding Munc-18 and SNAP-25. GST–fusion proteins containing the cytoplasmic domain of syntaxin (GST-Synt; residues 1–261), or deletions of residues 7–76 (GST-SyntΔ1), or of the C-terminal part of the syntaxin cytoplasmic domain (GST-SyntΔ2; residues 1–115) were attached to glutathione–agarose and incubated with rat brain homogenate or control buffer. Proteins bound to the agarose were analysed by SDS–PAGE followed by Coomassie blue staining (top) or immunoblotting with an antibody to SNAP-25 (bottom). Arrow points to position of Munc-18. *c*, Model of the interactions of proteins of the synaptic vesicle fusion complex. The interaction of syntaxin with SNAP-25, synaptobrevin and Munc-18 shown could be either simultaneous or sequential. Syntaxin also interacts with the NSF–SNAP complex[5] and with synaptotagmin[10], but the timing of these interactions and their stoichiometry are unclear. METHODS. Affinity purifications of proteins from brain homogenates using GST–fusion proteins were performed as for Fig. 1. Samples were analysed by SDS–PAGE followed by immunoblotting with the indicated antibodies described previously[28,29], and by Coomassie blue staining. Numbers on the left of the gels indicate positions of M_r markers.

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GTP binding and hydrolysis by the signal recognition particle during initiation of protein translocation

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THE signal recognition particle (SRP) consists of one RNA and six protein subunits[1,2]. The N-terminal domain of the 54K subunit contains a putative GTP-binding site, whereas the C-terminal domain binds signal sequences and SRP RNA[3–7]. Binding of SRP to the signal sequence as it emerges from the ribosome creates a cytosolic targeting complex containing the nascent polypeptide chain, the translating ribosome, and SRP[8]. This complex is directed to the endoplasmic reticulum membrane as a result of its interaction with the SRP receptor[9–11], a membrane protein composed of two subunits, SRα and SRβ, each of which also contains a GTP-binding domain[12,13]. In the presence of GTP, SRP receptor binding to SRP causes the latter to dissociate from both the signal sequence and the ribosome[13,14]. GTP is then hydrolysed so that SRP can be released from the SRP receptor and returned to the cytosol[15]. Here we show that the 54K subunit (M_r 54,000) of SRP (SRP54) is a GTP-binding protein stabilized in a nucleotide-free state by signal sequences, and that the SRP receptor both increases the affinity of SRP54 for GTP and activates its GTPase. We propose that nucleotide-mediated conformational changes in SRP54 regulate the release of signal sequences and the docking of ribosomes at the endoplasmic reticulum.

To analyse the role of GTP in protein targeting to the endoplasmic reticulum, (ER), we pursued the observation that the interaction of SRP with its receptor induces GTP hydrolysis[16]. Purified SRP had no detectable GTPase activity and the purified SRP receptor hydrolysed GTP only poorly, but SRP and its receptor together hydrolysed GTP about ten times faster than the receptor alone (Fig. 1).

To determine which components of SRP interact with the SRP receptor to increase GTP hydrolysis, SRP was dissociated into its subunits under non-denaturing conditions. The dissociated proteins can be purified and reconstituted with SRP RNA to regenerate fully functional SRP[17]. Surprisingly, a partially reconstituted SRP containing only the 19K and 54K subunits and SRP RNA was almost as active as native SRP in the presence of SRP receptor (Fig. 1), but was inactive without it (not shown). Moreover, omission of SRP19, which stabilizes the binding of SRP54 to SRP RNA[18], reduced activity only slightly (Fig. 1). SRP RNA and SRP54, however, were both essential (Fig. 1), indicating that the complex of SRP54 and SRP RNA is both necessary and sufficient to elicit GTP hydrolysis in conjunction with the SRP receptor. All subsequent analysis was carried out with this 'minimal' SRP [SRP(54/RNA)].

To determine whether SRP54, SRα or SRβ catalyses the GTP hydrolysis, we monitored nucleotide binding to the proteins by ultraviolet crosslinking[19,20]. This approach allowed us to detect the relatively low-affinity binding of these proteins to GTP and to measure GTP binding to each of the three GTP-binding proteins in the reaction independently. When SRP receptor was incubated with [α-^{32}P]GTP and crosslinked using ultraviolet radiation, both SRα and SRβ were labelled (Fig. 2*a*, lane 1). Similarly, when SRP(54/RNA) was used, SRP54 was labelled (Fig. 2*a*, lane 2). SRP54 was crosslinked to the same extent when SRP RNA was omitted (not shown), and was the only SRP protein labelled when intact SRP was crosslinked, indicating that the labelling reaction was specific for GTP-binding proteins (not shown). SRP54, SRα and SRβ must therefore be GTP-binding proteins, as predicted from their amino-acid sequences.

When SRP(54/RNA) and SRP receptor were mixed to stimulate GTP hydrolysis (Fig. 1, reaction 7), GTP crosslinking to SRP54 was dramatically stimulated (Fig. 2*a*, lane 3), but there was no significant change in crosslinking to either SRα or SRβ.

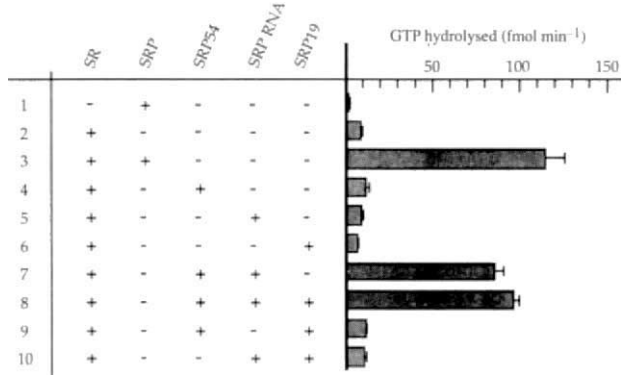

FIG. 1 Stimulated GTPase activity of SRP and partially reconstituted SRPs. GTP hydrolysis rates are the average of three independent experiments; the standard deviation of the measurements is indicated. The reaction was linear with time over the period analysed. tRNA could not replace SRP RNA in this reaction. In the presence of the SRP receptor (SR), all partially reconstituted SRPs that contained both the SRP RNA and SRP54 were about equally active; that is, the additional presence of SRP68/72 and/or SRP9/14 had no effect on the reaction. In the absence of SR, purified SRP proteins, SRP RNA and all partially reconstituted SRPs were inactive.
METHODS. SRP and SRP receptor were purified as described[28,29] as were the individual SRP components[18]. Partially reconstituted SRPs were formed by mixing components at a concentration of 500 nM each in 300 mM potassium acetate, 5 mM $Mg(OOCCH_3)_2$, 25 mM HEPES pH 7.5, 0.01% Nikkol detergent, (octaethyleneglycol mono-*n*-dodecyl ether; Nikko Chemical, Tokyo), 1 mM dithiothreitol (DTT). After mixing, reactions were incubated for 10 min on ice, 10 min at 37 °C and then kept on ice until the GTPase reaction. GTPase reactions (20 µl) contained 20 nM SR and/or either 20 nM SRP or 20 nM partially reconstituted SRPs in GTP hydrolysis buffer containing 50 mM $KOOCCH_3$, 50 nM triethanolamine, pH 7.5, 2.5 mM $Mg(OOCCH_3)_2$, 0.5% Nikkol detergent, 1 mM DTT. GTP 1 µM included 0.5 mCi ml^{-1} [γ-^{32}P]GTP (ICN). Reactions were incubated at 25 °C for 20 min and assayed by charcoal adsorption followed by Cerenkov counting.