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Yeast synaptobrevin homologs are modified posttranslationally by the addition of palmitate

(palmitoylation/vesicle trafficking/SNARE/Snc proteins)

ANDRÉS COUVE*, VLADIMIR PROTOPOPOV*, AND JEFFREY E. GERST*†‡

*Department of Cell Biology and Anatomy, Mount Sinai School of Medicine, New York, NY 10029-6574; and †Department of Molecular Genetics and Virology, Weizmann Institute of Science, Rehovot 76100, Israel

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ABSTRACT **Yeast possess two homologs of the synaptobrevin family of vesicle-associated membrane proteins that function in membrane recognition and vesicle fusion. Yeast proteins Snc1 and Snc2 localize to secretory vesicles and are required for constitutive exocytosis. They also form a physical complex with a plasma membrane protein, Sec9, which is necessary for vesicle docking and fusion to occur *in vivo*. Formation of this molecular complex, as a prerequisite for vesicle fusion, appears to have been conserved evolutionarily. Here we demonstrate that Snc proteins undergo a single posttranslational modification with the addition of a palmitate moiety to Cys-95 in Snc1. Modification of Cys-95 (which is located proximal to the transmembrane domain) is rapid, occurs in the endoplasmic reticulum, and is long-lasting. Mutation of Cys-95 to Ser-95 blocks palmitoylation and appears to affect Snc protein stability. This provides evidence that synaptobrevin-like proteins are modified posttranslationally, and we predict that fatty acylation may be common to those found in higher eukaryotes.**

Recent studies have identified a number of membrane-localized receptors for components of the vesicle fusion machinery. These receptors, known generically as SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors), are thought to participate in the fusion of carrier vesicles with their target membranes by facilitating both vesicle docking and bilayer interaction (reviewed in refs. 1 and 2). In yeast, we have identified two proteins that appear to act as SNAREs on carrier vesicles (v-SNAREs) of the late secretory pathway. These proteins, Snc1 and Snc2 (3, 4), are homologs of neuronal proteins known as synaptobrevins, or vesicle-associated membrane proteins (VAMPs) (5, 6). A second homolog, cellubrevin, is constitutively expressed in other cell types (7). The synaptobrevins were first identified as components of synaptic vesicles (5, 6) and later as elements that participate in the binding of soluble *N*-ethylmaleimide-sensitive factor attachment proteins *in vitro* (8, 9). The yeast proteins were first identified as suppressors of cellular defects relating to the loss of function of the adenylyl cyclase-associated protein, or CAP (10, 11), which itself is an intriguing protein that appears to mediate diverse signaling pathways relating to cell proliferation, cytoskeletal regulation, and general growth control (12, 13).

Genetic studies demonstrate that Snc proteins are required for vesicle docking and fusion (4). Yeast lacking Snc protein expression accumulate secretory vesicles and fail to secrete normally. In addition, these cells show a variety of conditional-lethal phenotypes that result from the blockage of vesicle fusion. Like their neuronal counterparts, Snc proteins interact physically with SNARE proteins from the plasma membrane (target SNAREs) to mediate formation of a prefusion SNARE complex (14, 15). Specifically, these SNAREs include members of the Sec9/SNAP-25 (15, 16) and Sso/syntaxin (17–19) families of plasma membrane proteins. Snc proteins have been shown to interact tightly with Sec9 (14, 15), and genetic studies have revealed that the Sec9 function in cells is dependent on the presence of Snc protein (14). Thus far, evidence from both yeast and mammals implies that the mechanisms of membrane recognition and vesicle fusion are well conserved.

In an earlier study, we noted that the overexpression of Snc protein in yeast resulted in the appearance of two distinct forms of the protein when electrophoresed on polyacrylamide gels. One form of the protein has an apparent mobility of 17 kDa, while the presumed native form has a mobility of 18 kDa (4). This finding has led us to speculate that posttranslational modification may be involved in their processing. In this work we demonstrate that the proteins undergo a single posttranslational modification resulting in the addition of a palmitate moiety to a single cysteine residue located adjacent to the transmembrane domain. Lipid modification of Snc proteins is not required for their exocytic functions, but it does appear to affect protein stability.

MATERIALS AND METHODS

DNA and Genetic Manipulations. Molecular cloning techniques were performed as described by Sambrook *et al.* (20). Site-directed mutagenesis was performed using a kit from Promega. DNA sequencing was performed using the dideoxynucleotide chain-termination method (21).

An oligonucleotide, 5′-TTACTAAAGCTAGCGACATCTTCATTT-3′, which contains an *Nhe* I site, was used to create a codon substitution in *SNC1* leading to the Cys-95 → Ser-95 mutation. This was performed by site-directed mutagenesis and was verified by DNA sequencing.

Standard techniques were used for the growth and genetic manipulation of yeast cells (22).

Phenotypic and Biochemical Assays. Assays for cell growth on rich medium and at 37°C were performed as described (12). The secretion of invertase was measured according to Goldstein and Lampen (23). Activity is expressed in units, where 1 unit equals 1 μmol of glucose released per min per 100 mg of dry weight of cells.

***In Vivo* Metabolic Labeling Experiments.** Yeast were labeled using either [^{3}H]palmitic acid (NET-043) or [^{35}S]-methionine/cysteine (NEG-072) (DuPont/NEN).

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Abbreviations: SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; v-SNARE, vesicle SNARE; VAMP, vesicle-associated membrane protein; ER, endoplasmic reticulum; IP, immunoprecipitation; HA, hemagglutinin antigen.

‡To whom reprint requests should be addressed.

Palmitoylation. Cells were resuspended at a concentration of 4–10 OD_{600} (optical density at 600 nm) units per ml in fresh medium containing [^{3}H]palmitic acid (0.25 mCi/ml; 1 Ci = 37 GBq). Cultures were grown at 30°C with constant shaking for 4 hr prior to harvesting. Cell lysates for use in immunoprecipitation (IP) experiments and immunoblots were prepared according to the procedure described by Couve and Gerst (14), except that 1% SDS was used instead of 1% Triton X-100 in the lysis buffer, and after extraction, the supernatants were boiled for 3 min prior to dilution with IP buffer. Either 10 μg of affinity-purified anti-hemagglutinin antigen (HA) antibody (12CA5) or 5 μl of rabbit anti-Snc polyclonal antibody (rabbit no. 20989) (4) was used for IP. For details on IP and protein detection see Couve and Gerst (14). Prior to autoradiography, gels were fixed overnight in 20% methanol/5% acetic acid prior to incubation with enhancer (EN^{3}HANCE; DuPont). Gels were subsequently dried and exposed (4–14 days) to x-ray film.

Pulse–chase experiments. For metabolic labeling experiments using [^{35}S]methionine/cysteine, cells were cultured in medium lacking methionine, but they were then grown for 1–2 min in fresh selective medium containing [^{35}S]methionine/cysteine (0.125 mCi/ml) at 30°C (pulse conditions). Between 4 and 8 OD_{600} units of cells per ml were incubated with labeled methionine. After the pulse, a 1-ml aliquot of the cells was taken for the zero time. This sample was washed once in 10 mM ice-cold NaN_3, aspirated, and placed in a dry ice/ethanol bath. Simultaneously, the chase was initiated by spinning down the remaining volume of cells and resuspending them in prewarmed medium containing 5 mM methionine and cysteine. At different times, 1-ml aliquots were removed and treated as above. The detection of labeled Snc protein was as described above.

Plasmids. Vectors included pSE358, a *CEN4*-based plasmid that bears a *TRP1* marker; pAD4Δ, a 2-μm-based plasmid that bears a *LEU2* marker and the *ADH1* promoter; and pAD54, which encodes a peptide epitope from the HA of influenza virus downstream of *ADH1* in pAD4Δ (4). Described plasmids include pADH-SNC1, which expresses *SNC1* (3); pADH-LSNC1 (pADH-HASNC1), which expresses *HA–SNC1* (4, 14); and pTGAL-SNC1 (pGAL-TSNC1) (4) and pTGAL-LSNC1 (pTGAL-HASNC1) (14), which bear *SNC1* and *HA–SNC1* under the control of the *GAL10* promoter, respectively (14).

Plasmids created for this study include pALT-SNC1, which has *SNC1* cloned into the *Sal* I/*Sac* I sites of pALTER (Promega); pADH-HASNC1^{Ser95}, which carries *SNC1^{Ser95}* cloned into the *Sal* I/*Sac* I sites of pAD54; and pADH-SNC1^{Ser95}, which contains *SNC1^{Ser95}* cloned into pAD4Δ. All constructs were verified by restriction analysis.

Yeast Strains. *Saccharomyces cerevisiae* strains included JC1 (*Matα can1 his3 leu2 lys2 trp1 ura3 ade8*) (13), JG4 (*Mata can1 his3 leu2 trp1 ade8 snc1::URA3*) (3), JG6 (*Matα can1 his3 leu2 lys2 trp1 ura3 snc2::ADE8*) (4), and JG8 T15:85 (*Mata his3 leu2 trp1 snc1::URA3 snc2::ADE8* pGAL-TSNC1) (4).

Electron Microscopy and Immunogold Labeling. Yeast were harvested and fixed for thin-sectioning using standard procedures, similar to those described by Wright and Rine (24). Immunogold labeling was performed using protein A-gold particles (20 nm) (E-Y Laboratories). Further details about the fixation and immunogold labeling procedures will be provided upon request.

RESULTS

Overexpression of Snc Protein in Yeast Results in Multiple Forms. Snc proteins have an apparent electrophoretic mobility of 18 kDa on SDS/PAGE gels when expressed at native levels (ref. 4 and Fig. 1, lanes 1–3) or when mildly overexpressed from single-copy plasmids (Fig. 1, lane 4). In contrast, overexpression (≈10-fold) of Snc1 protein from a multicopy plasmid yields protein doublets having apparent mobilities of 17 and 18 kDa, in the case of the native protein (ref. 4 and Fig. 1, lane 6), and a doublet of 22 and 23 kDa in the case of an HA epitope-tagged form of Snc1 (HA–Snc1) (ref. 4 and Fig. 1, lanes 7 and 8). It would appear, then, that overexpression results in the accumulation of either immature or partially degraded forms of the protein. Moreover, smaller doublets of around 13–14 kDa, which lack the epitope tag, are often observed (Fig. 1, lanes 7 and 8). Although protein degradation could potentially account for all these forms, we have explored posttranslational modification as a possible mechanism for Snc protein maturation.

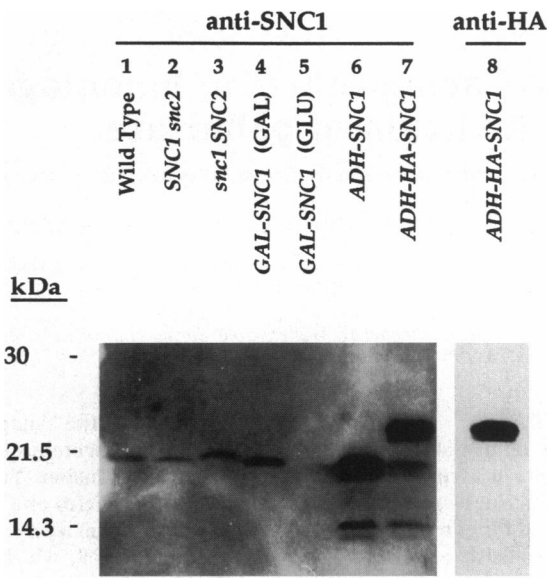

FIG. 1. Western blot detection of Snc protein expression. Snc proteins were detected by Western analysis, using either anti-Snc antibodies (4) or affinity-purified anti-HA monoclonal antibodies. Protein samples (50 μg per lane) were prepared from yeast expressing Snc proteins from their genomic loci or from episomal plasmids. Samples were processed as described in *Materials and Methods* and are as follows: lane 1, wild-type cells (JC1); lane 2, *snc2* cells (JG6); lane 3, *snc1* cells (JG4); lane 4, *snc1 snc2* pTGAL-SNC1 cells (JG8) grown on galactose-containing medium; lane 5, *snc1 snc2* pTGAL-SNC1 cells (JG8) grown on glucose-containing medium; lane 6, *snc1 snc2* cells constitutively expressing *SNC1* from a multicopy plasmid; lane 7, *snc1 snc2* cells constitutively expressing *HA–SNC1* from a multicopy plasmid; lane 8, same as lane 7. Lanes 1–7 were detected with an anti-Snc antiserum (1:1000 dilution), whereas lane 8 was detected with anti-HA antibodies (1:5000 dilution).

We have explored three modifications: protein phosphorylation, glycosylation, and acylation. Labeling experiments performed *in vivo* with [^{32}P]orthophosphoric acid showed no incorporation of labeled phosphate into Snc protein (data not shown). Likewise, we were unable to precipitate Snc protein from cell lysates using immobilized concanavalin A or to show any change in the electrophoretic mobility of Snc protein after treatment with endoglycosidase H (data not shown). Thus, Snc proteins are not likely substrates for either protein phosphorylation or glycosylation.

Yeast Snc Proteins Are Palmitoylated. We next determined whether Snc proteins are lipid-modified. A single conserved Cys residue (Cys-95), found proximal to the transmembrane domain, was the sole candidate for fatty acylation in Snc1. To test whether Cys-95 is acylated, both native and HA-tagged Snc1 proteins were immunoprecipitated from lysates prepared from yeast grown on medium containing [^{3}H]palmitic acid (see *Materials and Methods*). Autoradiography of the immunoprecipitates demonstrated that a single band, corresponding to the higher molecular mass form of Snc protein (e.g., 18 or 23 kDa),

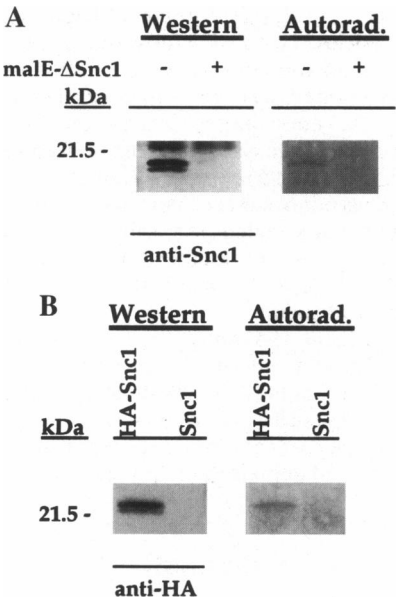

FIG. 2. Palmitoylation of yeast Snc proteins. *snc* null cells expressing *SNC1* or *HA–SNC1* were labeled with [^{3}H]palmitic acid, as described (see *Materials and Methods*). Cell lysates were subjected to IP with anti-Snc or anti-HA antibodies. Protein detection was performed using immunoblots (Western), while radiolabeling of the proteins was detected in separate gels that were fixed and dried for autoradiography (Autorad.). (*A*) Native Snc1 protein was immunoprecipitated with anti-Snc antibodies and was detected in immunoblots or was electrophoresed and exposed to film (7 days). IP was performed in either the presence (+) or absence (−) of added exogenous malE–ΔSnc1 fusion protein (50 μg) (4). (*B*) Lysates from cells expressing either HA–Snc1 or native Snc1 were subjected to IP with anti-HA antibodies. Immunoprecipitates were immunoblotted or exposed to film as described for *A*.

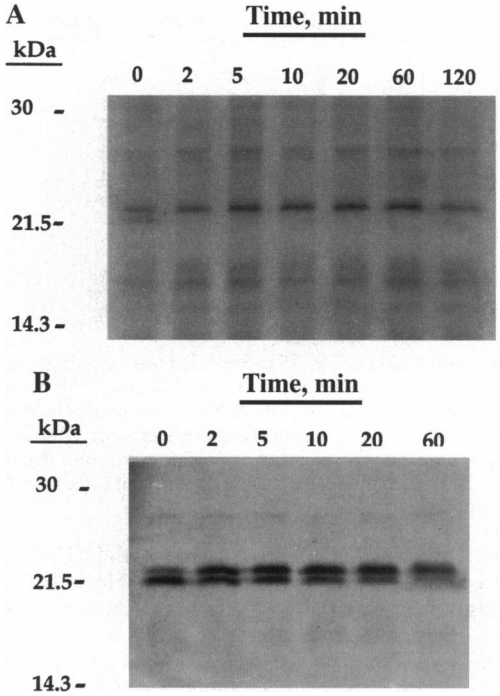

FIG. 3. Pulse–chase labeling of HA–Snc1 protein. *snc* null cells expressing HA–Snc1 were pulsed with EXPRE^{35}S^{35}S for 1 min. Cells were then washed and treated with unlabeled methionine and cysteine (chase) for 0–120 min. HA–Snc1 protein was immunoprecipitated, electrophoresed, and detected in immunoblots using anti-HA antibodies (not shown) or was autoradiographed by exposure to x-ray film. (*A*) Autoradiogram of immunoprecipitated HA–Snc1 protein from cells bearing the single-copy plasmid (pTGAL-HASNC1). Exposure to film was for 10 days. (*B*) Autoradiogram of immunoprecipitated HA–Snc1 protein from cells bearing the multicopy plasmid (pADH-HASNC1). Exposure to film was for 5 days.

could be detected (Fig. 2). In contrast, the lower bands of 17 kDa and 22 kDa, as detected by Western blotting, were not seen in the autoradiograms. The labeling of the higher molecular mass bands was found to be specific, as the radiolabeled 18-kDa band was eliminated when excess malE–ΔSnc1 fusion protein (4) was added to the lysates prior to immunoprecipitation (Fig. 2*A*), and the labeled 23-kDa band could not be detected in immunoprecipitates made from cells overexpressing only the native protein (Fig. 2*B*). Competition experiments using excess HA peptide also blocked the appearance of the of 23-kDa band both in Western blots (17) and in the autoradiograms (data not shown). These results indicate that only the higher molecular mass form of Snc1 protein has been modified by palmitate addition. Similar labeling results have been obtained from cells expressing Snc2 protein (data not shown).

Pulse–Chase Analysis of Snc Protein Modification. To determine both the kinetics of palmitate addition and the stability of the unpalmitoylated form of the protein, we performed pulse–chase analysis using [^{35}S]methionine/cysteine (EXPRE^{35}S^{35}S) to label Snc1 protein. Cells pulsed briefly with EXPRE^{35}S^{35}S show that newly synthesized, labeled HA–Snc1 protein has an apparent mobility of 22 kDa and that one modification (e.g., palmitate addition) occurs rapidly and completely within 2 min after addition of the chase in cells expressing *HA–SNC1* from a single-copy plasmid (Fig. 3*A*). The final 23-kDa form of the protein is stable for as long as 60 min and appears to be the mature Snc protein.

In contrast, only a fraction of Snc1 protein is modified in cells overexpressing (≈10-fold) protein from multicopy plasmids (Fig. 3*B*). Typically, around 40–50% of Snc protein accumulates as the unpalmitoylated form in these cells. Moreover, no further modification is observed after the 2-min chase. Stability of the palmitoylated protein is similar to that shown in Fig. 3*A*; however, the unpalmitoylated protein is lost at a faster rate. A densitometric comparison between the 5-min and 20-min time points reveals that while the intensity of the upper band is unchanged (≤3%), nearly 60% of that of the lower band is lost (data not shown). Overall, the intensities of the upper and lower bands declined by 53% and 73%, respectively, by 60 min. However, the ratio between the palmitoylated and unpalmitoylated forms of the proteins changes from 1.3:1 at the early time points (2 and 5 min) to 3:1 at the later time points (20 and 60 min).

Our results imply that the addition of palmitate is rapid and, thus, is likely to occur in the early part of the secretory pathway—i.e., in the endoplasmic reticulum (ER). To test this, we performed pulse–chase labeling in cells bearing a temperature-sensitive mutation in *SEC18* at both permissive (25°C) and nonpermissive (37°C) temperatures. Cells bearing this mutation are unable to engage in the transport of proteins from the ER to the Golgi at 37°C (25). Thus, the modification of newly synthesized protein is not expected to occur beyond the ER under these conditions. In labeling experiments using *sec18* cells shifted briefly (30 min) to 37°C, we found no significant difference in the rate of Snc protein modification or in the total amount of labeled protein (data not shown), with respect to wild-type cells labeled under similar conditions. This implies that the palmitoylation of Snc proteins occurs prior to their transport to the Golgi.

Replacement of Cys-95 by Ser Abolishes Palmitoylation. To determine whether the sole Cys residue in Snc protein is the site of acylation, we substituted Ser for Cys-95 by site-directed mutagenesis. When expressed in wild-type yeast, a mutant Snc1 protein (HA–Snc1^{Ser95}) ran as a single band with an apparent mobility of 22 kDa, in contrast to HA–Snc1 (Fig. 4).

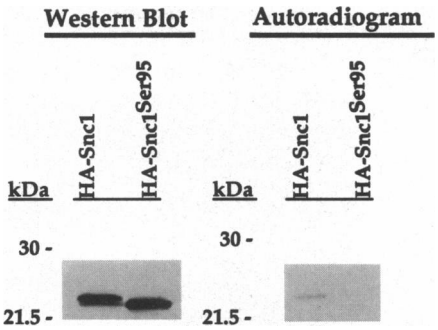

FIG. 4. Alteration of Cys-95 to Ser-95 abolishes palmitoylation. *snc* null cells expressing either *HA–SNC1* or *HA–SNC1*^{Ser95} were labeled with [^{3}H]palmitic acid, as described. Yeast cell lysates were subjected to protein detection in immunoblots (Western) or IP with anti-HA antibodies. Immunoprecipitates were electrophoresed, dried, and exposed to x-ray film for 8 days (Autoradiogram). Results from two similar experiments are shown.

Moreover, labeling experiments *in vivo* with [^{3}H]palmitic acid revealed that the mutant protein was unable to be palmitoylated (Fig. 4). Thus, Cys-95 is the site of acylation in Snc1 and Cys-94 is the likely site in Snc2.

We next determined whether the loss of palmitoylation affects the growth and exocytic functions of Snc proteins. The loss of Snc function in yeast results in a variety of conditional-lethal phenotypes; such cells are temperature sensitive, unable to grow on nutrient-rich growth medium (yeast extract/peptone/dextrose), are morphologically abnormal, and are defective for normal secretion (4). We tested the function of HA–Snc1^{Ser95} by expression in *snc* null cells. Such cells bear a galactose-inducible *SNC1* gene and in the absence of galactose demonstrate the null phenotypes (4). Cells shifted from galactose- to glucose-containing medium remained temperature resistant, were able to grow on yeast extract/peptone/dextrose, showed no morphological abnormalities when examined by light microscopy, and were able to secrete invertase at levels similar to those seen with cells expressing HA–Snc1. The amounts of secreted and nonsecreted invertase in a representative experiment were 92.8 and 22.7 units, respectively, for glucose-derepressed cells expressing HA–Snc1 and 81.9 units and 22.6 units for cells expressing HA–Snc1^{Ser95}. Thus, the loss of Snc palmitoylation does not affect the general growth characteristics of yeast and does not interfere with the secretory processes.

Nonpalmitoylated Snc1 Protein Targets to Secretory Vesicles. Previously, we (4) demonstrated that Snc proteins localize to post-Golgi carrier vesicles, using immunogold labeling of thin-sectioned cells. Moreover, we were able to detect the presence of both the 22-kDa and 23-kDa forms of HA–Snc1 in secretory vesicles purified from a late *sec* mutant (4). Thus, both palmitoylated and unpalmitoylated Snc proteins may reside on secretory vesicles. However, it is unclear whether palmitoylation is necessary for the efficient sorting of Snc proteins to vesicles. To test this possibility, we examined the intracellular localization of HA–Snc1^{Ser95}. Immunogold labeling of thin-sectioned *sec6* cells that express HA–Snc1^{Ser95} showed that the nonpalmitoylated protein associates with vesicles that accumulate when cells are shifted to nonpermissive temperatures (Fig. 5). Moreover, under conditions when vesiculation does not occur (i.e., permissive temperatures), the mutant protein is associated primarily with the plasma membrane. These results are identical to those described for HA–Snc1 (4). Thus, replacement of Cys-95 by Ser does not alter protein localization.

As noted, mutant HA–Snc1^{Ser95}, like HA–Snc1, is found only on vesicles and not on the plasma membrane during vesiculation. This implies that both forms probably recycle back to secretory vesicles (4). In contrast, HA-tagged VAMP2 and certain VAMP2–Snc1 chimeras do not show this relocalization pattern and remain membrane-associated (V.P. and J.E.G., unpublished results).

DISCUSSION

Protein acylation is an important modification that occurs on a number of proteins having varied functions (reviewed in ref. 26). The palmitoylation of nonintegral membrane proteins, including the α subunits of guanine nucleotide-binding proteins (27), p21 ras (28), and others is thought to enhance both membrane association and protein function. This enhancement of function is likely a consequence of protein localization, whereby the palmitoylated protein interacts more efficiently with either its regulatory or target molecules. However, the palmitoylation of integral membrane proteins, such as the β-adrenergic receptor (29), has also been described. Alteration of the acylation site(s) has been shown to down-regulate the function of some membrane proteins and enhance their degradation. Moreover, a requirement for palmitoyl-CoA has been also described for an *in vitro* Golgi transport assay (30), although the nature of this requirement remains obscure.

In this study we demonstrate that members of the synaptobrevin family are substrates for palmitoyltransferase activity. Our results show that the modification of Snc protein is rapid, occurs in the ER and on all protein expressed under native conditions, and is stable. We suggest, then, that the palmitoylation of Snc proteins occurs in the manner described for other proteins and does not necessitate a distinct mechanism. We also suggest that our original observation of the protein doublets was due to the intracellular accumulation of the unpalmitoylated form of Snc protein. This is likely to be an artifact of protein overexpression and may be due, in part, to the depletion of available palmitoyl-CoA, the saturation of the palmitoyltransferase activity by excess substrate, or both.

The full role of palmitoylation in Snc protein function remains unknown. Since the nonpalmitoylated form localizes to secretory vesicles and appears to confer normal secretion,

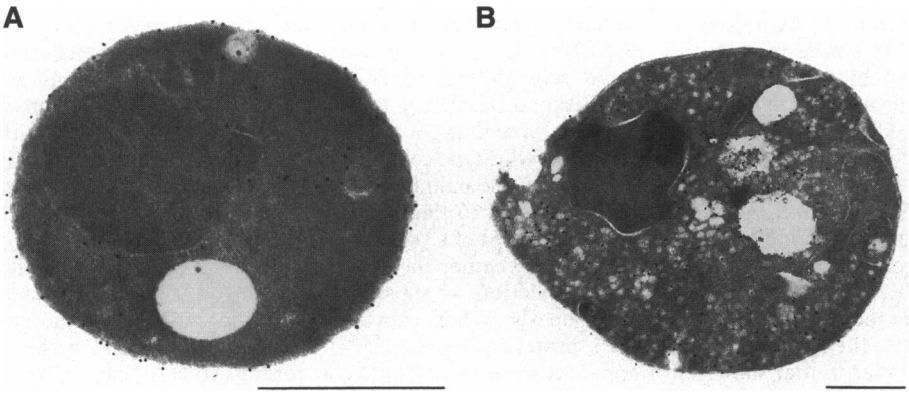

FIG. 5. Localization of HA–Snc1^{Ser95} in *sec6* cells. *sec6* cells expressing HA–Snc1^{Ser95} were grown at 25°C prior to shifting a portion of the cells to 37°C for 1 hr (nonpermissive conditions). Cells maintained at 25°C and temperature-shifted cells were both processed for thin-section microscopy and were colabeled with anti-HA antibodies and protein A-gold particles (20 nm). (*A*) Representative cell maintained at 25°C. (*B*) Representative cell that was temperature-shifted. (Bars = 1 μm.)

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Yeast  Snc2   088  DLKMRMCLFLVVIILLVVIIVPIVVHFT.
Yeast  Snc1   089  DLKMKMCLALVIIILLVVIIVPIAVHFSR.

Rat    VAMP2  092  NLKMMIILGVICAIILIIIIV----YFST.
Human  Syb2   092  NLKMMIILGVICAIILIIIIV----YFSS.
Rat    VAMP1  094  NCKMMIMLGAICAIIVVVIVI----YIFT.
Human  Syb1   094  NCKMMIMLGAICAIIVVVIVI----YFFT.
Rat    Ceb1   079  NCKMWAIGISVLVIIVIIIIV----WCVS.
Tor    VAMP1  096  NCKMMIMLGGIGAIIVIVIII----YFFT.
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FIG. 6. Cys residues in Snc proteins and their homologs. Amino acid sequences corresponding to the carboxyl-terminal regions of various synaptobrevin-like proteins are shown. Cys residues are underlined and in boldfaced type. The lines above the sequences correspond to the transmembrane regions. Amino acid position numbers are given on the left. Syb1 and 2, synaptobrevins 1 and 2; Ceb1, cellubrevin 1; Tor, *Torpedo*.

it is unlikely that palmitoylation is absolutely required for exocytic functions. Indeed, our data suggest palmitoylation may be relevant to Snc protein stability, as the palmitoylated form of the protein persists longer than the unpalmitoylated form. It is likely, then, that palmitoylation renders these proteins more resistant to degradation, as has been shown for other membrane proteins.

We have examined other members of the synaptobrevin family and note that many of these proteins bear Cys residues proximal to, or buried in, their transmembrane regions. In particular, rat and *Torpedo* VAMP1, human synaptobrevin 1, and cellubrevin each contain a Cys residue proximal to the transmembrane domain, similar to that found in Snc proteins (Fig. 6). Some members of this family even contain multiple Cys residues in this region. We raise the possibility, then, that the acylation of synaptobrevin-like proteins is conserved evolutionarily. Moreover, this modification may be distinct to v-SNAREs functioning at the exocytic level, as other proteins of similar structure, such as Bet1 (31), Bos1 (32), and Sly2/Sec22 (33), that are implicated in vesicle trafficking between the ER and the Golgi all lack Cys residues in this region. More work will be required to determine the precise role of palmitoylation both in Snc protein function and, perhaps, in other v-SNAREs on the secretory pathway.

A.C. and J.E.G. contributed equally to the data collection. We are grateful to Drs. Randy Schekman and Peter Novick for antibodies and helpful advice. J.E.G. was the recipient of a Hirschl Career Scientist Award and an American Cancer Society Junior Faculty Research Award (no. 453) and currently is the recipient of an Allon Fellowship and is the incumbent of the Henry Kaplan Career Development Chair for Cancer Research.

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