Staring, a Novel E3 Ubiquitin-Protein Ligase That Targets Syntaxin 1 for Degradation*

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Syntaxin 1 is an essential component of the neurotransmitter release machinery, and regulation of syntaxin 1 expression levels is thought to contribute to the mechanism underlying learning and memory. However, the molecular events that control the degradation of syntaxin 1 remain undefined. Here we report the identification and characterization of a novel RING finger protein, Staring, that interacts with syntaxin 1. Staring is expressed throughout the brain, where it exists in both cytosolic and membrane-associated pools. Staring binds and recruits the brain-enriched E2 ubiquitin-conjugating enzyme UbcH8 to syntaxin 1 and facilitates the ubiquitination and protein degradation of syntaxin 1. These findings suggest that Staring is a novel E3 ubiquitin-protein ligase that targets syntaxin 1 for degradation by the ubiquitin-proteasome pathway.

Modulation of protein degradation is a major mechanism by which cells regulate the expression levels of specific proteins and consequently the cellular processes that these proteins participate in (1, 2). The ubiquitin-proteasome pathway plays a crucial role in the degradation of proteins involved in a variety of cellular processes, including differentiation, proliferation, and apoptosis. However, the role of the ubiquitin-proteasome pathway in the degradation of presynaptic proteins remains poorly characterized, despite the presence of ubiquitin at nerve terminals (3–5). In the ubiquitin-proteasome pathway, substrates are marked for degradation by covalent linkage to ubiquitin. The ubiquitinated substrate proteins are then recognized and degraded by the 26 S proteasome (1, 2, 6). Ubiquitination involves a highly specific enzyme cascade in which ubiquitin is first activated by an E1 ubiquitin-activating enzyme and then transferred to an E2 ubiquitin-conjugating enzyme and finally ligated to the substrate by an E3 ubiquitin-protein ligase (1, 7, 8). The E3 ubiquitin-protein ligase plays an essential role in determining the specificity of ubiquitination and subsequent protein degradation. Consistent with this role, it is estimated that an organism such as a human contains over 100 E3 ubiquitin ligases, in contrast to a single E1 ubiquitin-activating enzyme and about a dozen E2 ubiquitin-conjugating enzymes (9). Despite the importance of E3 ubiquitin-protein ligases in specific protein degradation and the estimated presence of more than 100 E3 ligases in the human genome, only a few E3 ligases have been characterized at the molecular level.

Syntaxin 1 is a neuronal membrane protein that was originally identified as a binding partner for synaptotagmin and the N-type calcium channel (10–12). It is well established that syntaxin 1 functions as a synaptic t-SNARE to mediate synaptic vesicle exocytosis at nerve terminals (13–15). Syntaxin 1 appears early during embryonic development (16, 17), and its expression level is dramatically up-regulated during synapse formation and brain maturation (16–19). Regulation of syntaxin 1 levels may contribute to the mechanism underlying learning and memory, since changes in syntaxin 1 levels have been found to correlate with long term potentiation and various learning and memory behaviors (20–22). Alteration in syntaxin 1 expression levels has been associated with several neurodegenerative diseases and psychiatric disorders, including schizophrenia, Alzheimer’s disease, and Creutzfeldt-Jakob disease (23–26).

Despite the importance of the regulation of syntaxin 1 levels in synaptic function and dysfunction, the molecular mechanisms underlying such regulation remain undefined. To identify proteins that regulate syntaxin 1, we carried out a search in rat brain for proteins that interact with syntaxin 1 using yeast two-hybrid screens. Here we report the isolation of a novel syntaxin 1-interacting protein, named Staring, that acts as an E3 ubiquitin-protein ligase to promote the ubiquitination and degradation of syntaxin 1 by the proteasome pathway.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Screen and cDNA Cloning—The bait plasmid, pPC97-Syntaxin 1, was constructed by subcloning the cytoplasmic domain (amino acids 5–270) of rat syntaxin 1B (27) into the pPC97 vector (28, 29). For the two-hybrid screen, the yeast strain CG-1945 (CLONTECH) was transformed sequentially with pPC97-Syntaxin 1 and a rat hippocampal/cortical two-hybrid cDNA library (29), using the lithium acetate method (30). Positive clones were selected on 3-amino-triazole (5 mM; Sigma)-containing medium lacking leucine, tryptophan, and histidine and verified with a filter assay for β-galactosidase activity.

Prey plasmids were then recovered and retransformed into yeast with pPC97-Syntaxin 1 or various control baits to confirm the specificity of the interaction. For cloning of full-length Staring, a partial Staring cDNA probe from the prey clone (clone 7) was used to screen a rat hippocampal cDNA library in AZAPII (Stratagene) according to the standard procedure (31). The cDNA inserts from positive Staring clones were sequenced multiple times on both strands using an Applied Biosystems 373A DNA sequencer.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF352815.

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‡ The abbreviations used are: E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin-protein ligase; GST, glutathione S-transferase; DMEM, Dulbecco’s modified Eagle’s medium; HA, hemagglutinin; SNARE, soluble NSF attachment protein receptors.
Expression Constructs—Conventional molecular biological techniques (3) were used to subclone DNA fragments encoding full-length and truncated forms of Staring into the following vectors: the pCP97 and pCP56 vectors for yeast two-hybrid interaction studies; the prokaryotic expression vectors pGEX-5X-2 (Amersham Biosciences) and pET28c (Novagen) for the production of GST- and His6-tagged fusion proteins; and the mammalian expression vectors pCDNA3.1(+) (Invitrogen) and pCHA (30) for transfection into HeLa cells. The expression construct pRK5-HA-UbcH5, pRK5-HA-UbcH7, and pRK5-HA-UbcH8 were obtained as generous gifts from Dr. Ted Dawson (32).

Antibodies—Four polyclonal anti-Staring antibodies, two in chicken (CS-N and CS-C) and two in rabbit (RS-N and RS-C), were generated against Staring N-terminal peptide MSGLSNKRAAGDGG and C-terminal peptide AAFGAHDFHRVYIS, respectively. The antibodies were affinity-purified using the immunogen peptide-coupled columns as described previously (30). Other antibodies used in this study include the following: anti-HA (3F10 (Roche Molecular Biochemicals) and HA.11 (Covance)), anti-Myc (9E10.3; Neosmarkers), anti-syntaxin 1 (HPC-1; Sigma), anti-actin (C4; Roche Molecular Biochemicals) and secondary antibodies coupled with horseradish peroxidase (Jackson Immunoresearch Laboratories, Inc.).

Northern and Western Blot Analyses—Northern blot analysis of Staring mRNA expression was performed on a rat multiple tissue Northern (MTNTM) blot and a human multiple tissue expression (MTE(TM)) arraying mRNA expression was performed on a rat multiple tissue Northern blot (31). For Western blot analysis, rat tissues were homogenized in 1% SDS and subjected to SDS-PAGE. The proteins were transferred onto nitrocellulose membranes and probed with anti-Staring and other antibodies. Antibody binding was detected by using the enhanced chemiluminescence method (Amersham Biosciences).

Subcellular Fractionations—Subcellular fractionations of rat brain into cytosolic fraction (100,000 × g supernatant) and membrane fraction (100,000 × g pellet) were performed as previously described (30). The membrane fractions were subjected to extraction studies as described (30), using 1.5 M NaCl or 4 M urea.

In Vitro Binding Assays—GST-Staring fusion proteins or GST control were immobilized on glutathione-agarose beads (Sigma) and incubated with rat brain homogenates as previously described (30, 33). After extensive washes, bound proteins were eluted by boiling in 2× Laemmli sample buffer and analyzed by SDS-PAGE and immunoblotting with appropriate antibodies.

Cell Transfections and Immunoprecipitations—HeLa or SH-SY5Y cells were transfected with indicated plasmids using LipofectAMINE (Invitrogen) as described by the manufacturer. Cell lysates were prepared and subjected to immunoprecipitation as described previously (35), using anti-HA antibody (3F10), anti-Myc (9E10.3), anti-Staring (RS-N), anti-syntaxin 1 antibody (HPC-1), or control IgG. The immunocomplexes were recovered by incubation with protein G- or protein A-Sepharose beads (Sigma). After extensive washes, the immunocomplexes were dissociated by boiling in the Laemmli sample buffer and analyzed by SDS-PAGE and immunoblotting.

Ubiquitination Assays—HeLa cells were transfected with combinations of the following plasmids: pCHA-syntaxin 1, pCDNA3-Myc ubiquitin, pFLAG-Staring, and pFLAG-Staring3R, a C-terminal deletion mutant of Staring that lacks the RING finger motif. SH-SY5Y cells were transfected with pcDNA3-Myc ubiquitin in combination with pFLAG-Staring or pFLAG-Staring3R. Twenty-four hours after transfection, the cells were incubated for 8 h with 10 Ci of [35S]Met/Cys (1000 Ci/mmol) expressing Staring and syntaxin 1 were incubated for 8 h at 37°C with the proteasome inhibitor MG132 (20 μM; Calbiochem), the lysosomal protease inhibitor NH4Cl (50 mM) or chloroquine (100 μM; Sigma), or vehicle (MeSO; final concentration 0.1%). Cells were then lysed, and the protein concentrations of the lysates were determined by the BCA protein assay (Pierce). An equal amount of protein from each lysate was then analyzed by SDS-PAGE and immunoblotting.

RESULTS

Identification of Staring, a Novel Syntaxin 1-interacting RING Finger Protein—To identify syntaxin 1-interacting proteins, we screened a rat hippocampal/cortical cDNA library by yeast two-hybrid selection using the cytoplasmic domain of rat syntaxin 1B as bait. This screen led to the isolation of several clones encoding SNAP-25, a known syntaxin 1-interacting protein.
Novel E3 Ubiquitin Ligase Staring

As shown in Fig. 1, Staring contains six putative coiled-coil domains and a RING finger motif at the C terminus. The RING finger motif is a cysteine/histidine-rich (C₃H₄) structural domain that is found in a number of eukaryotic proteins, some of which have been implicated in vesicular transport (38). Emerging evidence indicates that the RING finger motif may function in protein ubiquitination as a key determinant of the E3 ubiquitin-protein ligase activity (39–41). The N-terminal region of rat Staring is 90% identical to human RBP95, an 838-amino acid protein recently identified from a yeast two-hybrid screen using retinoblastoma protein as bait (42). The expression of endogenous RBP95 protein has not yet been characterized; however, based on EST database searches, RBP95 seems to be a rare isoform derived from alternative splicing of the gene encoding the uncharacterized human ring finger protein 40 (RNF40, also called KIAA0661) (Fig. 2A).

Data base searches reveal the presence of Staring homologues as uncharacterized cDNAs or open reading frames obtained from genome projects in a number of organisms, including humans, mice, Drosophila, Caenorhabditis elegans, Arabidopsis, and yeast (Fig. 2A). Sequence comparison analysis indicates that RNF40 is the human orthologue of rat Staring. The human genome also contains a second Staring homologue, RNF20, which is encoded by a gene that is distinct from the RNF40 gene. In Drosophila, C. elegans, Arabidopsis, and yeast, there appears to be only one Staring homologue. Whereas the function of these Staring homologues is unknown, the deletion mutant of the yeast Staring homologue, Bre1p (also called YDL074c), was reported to be sensitive to multiple drugs, including brefeldin A and chlorpromazine (43, 44). Analysis of the deduced proteins from the Staring-homologue sequences reveals that these homologous proteins have a domain structure that is similar to that of Staring (Fig. 2A). Most notably, these Staring homologues contain a highly conserved RING finger motif at their C terminus (Fig. 2B). The conserved homology and conserved domain structure among Staring homologues from different species indicate that Staring is an evolutionarily conserved protein.

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Staring is a Ubiquitously Expressed Protein That Exists in Both Cytosolic and Membrane-associated Pools—Northern blot analysis of Staring mRNA expression revealed the presence of a single Staring transcript of 5.1 kb (Fig. 3A). The Staring mRNA is relatively abundant in brain, testis, heart, liver, and kidney and expressed at low levels in lung, spleen, and skeletal muscle. Consistent with this result, analysis of human Staring mRNA expression using a multiple tissue expression array showed that Staring mRNA is ubiquitously expressed in various brain regions as well as all fetal and adult human tissues examined (data not shown). The broad tissue distribution of Staring mRNA expression suggests that Staring has a functional role important to many cell types, including neurons.

To characterize Staring at the protein level, we generated four polyclonal anti-Staring antibodies, two in chicken (CS-N and CS-C) and two in rabbit (RS-N and RS-C), against the N- and C-terminal 14-amino acid peptide of rat Staring, respectively. The antibodies (CS-N and RS-N) against the N terminus of Staring are expected to detect both Staring and RBP95 isoforms, whereas the antibodies (CS-C and RS-C) against the C terminus of Staring should only recognize the Staring isoform. Western blot analysis demonstrated that all four anti-Staring antibodies, but not their corresponding preimmune controls, recognized a single endogenous protein band of ~125 kDa (Fig. 3, B and C; data not shown), indicating that Staring is the predominant isoform expressed in rat. No 95-kDa protein band corresponding to RBP95 could be detected by either chicken (CS-N) or rabbit (RS-N) antibodies against the N terminus of Staring (Fig. 3B; data not shown), suggesting that RBP95 is a rare isoform that is either expressed at extremely low levels or not expressed at all. All four anti-Staring antibodies specifically react with recombinant Staring protein expressed in bacterial and mammalian cells (data not shown). Furthermore, preabsorption of these anti-Staring antibodies with recombinant Staring protein completely eliminated their immunoreactivity to recombinant as well as endogenous Staring protein (data not shown), confirming the specificity of these antibodies. In agreement with the result of Northern blot analysis (Fig. 3A), Western blot analysis using the anti-Staring antibodies revealed that Staring protein is ubiquitously expressed in all tissues tested, although the expression levels in heart and skeletal muscle are very low (Fig. 3B).

To examine the intracellular distribution of endogenous Staring, postnuclear supernatant of rat brain was separated into cytosol and membrane fractions and then subjected to Western blot analysis with anti-Staring antibodies (Fig. 3C). Staring immunoreactivity was detected in both cytosol and membrane fraction, although the relative amount of Staring in
the cytosol fraction was severalfold more than that in the membrane fraction. To investigate the nature of Staring as-
sociation with membranes, the membrane fraction was ex-
tracted with 1.5M NaCl or 4 M urea (Fig. 3).

Unlike the integral membrane protein syntaxin 1 that was resistant to
extraction by high salt and urea, a majority of Staring was
extracted by these treatments, suggesting that Staring is
peripherally associated with membranes via hydrophilic
interactions.

Staring Interacts with Syntaxin 1 in Vitro and in Vivo—
To determine whether the Staring-syntaxin 1 interaction detected
in yeast actually takes place in vitro, GST fusion proteins
containing various portions of Staring (Fig. 4A) were immobi-
lized on glutathione beads and used to bind endogenous syn-
taxin 1 from rat brain homogenate (Input). After extensive washes, bound
proteins were eluted and analyzed by SDS-PAGE and immunoblotting for syntaxin 1 (upper panel). GST-Staring fusion proteins were shown as
Ponceau S staining (lower panel).

C, co-immunoprecipitation of Staring with syntaxin 1 from transfected cells. Extracts from HeLa cells
co-transfected with pcDNA3-syntaxin 1 and pCHA-Staring were subjected to immunoprecipitation with an anti-HA antibody or control rat IgG.
Input, 30% of the HeLa extracts used for the immunoprecipitation. D, association of endogenous Staring with syntaxin 1 in rat brain. Detergent
extracts of rat brain synaptosomes (P2 fractions) were subjected to immunoprecipitation with anti-syntaxin 1 antibody or control mouse IgG.
Input, 30% of the brain extracts used for the immunoprecipitation. The immunoprecipitates in C and D were analyzed by immunoblotting with
anti-syntaxin 1 and anti-Staring (RS-C) antibodies. These co-immunoprecipitation experiments were replicated three times with similar results.

To determine whether Staring associates with syntaxin 1 in vivo, we first performed co-immunoprecipitation experiments
using lysates of HeLa cells expressing exogenous syntaxin 1
and HA-tagged Staring. As shown in Fig. 4C, syntaxin 1 and
HA-Staring were co-immunoprecipitated by the anti-HA antibi-
ody, providing evidence for the association of these two pro-
teins in mammalian cells. By comparison, control IgG was
unable to precipitate either syntaxin 1 or Staring. We then
performed additional co-immunoprecipitation experiments to
examine the association of endogenous Staring and syntaxin 1
in rat brain synaptosomes (Fig. 4D). Anti-syntaxin 1 antibody,
but not the mouse IgG control, was able to co-immunoprecip-
itate syntaxin 1 and Staring from solubilized synaptosomes,
demonstrating the existence of endogenous Staring-syntaxin 1 complexes at nerve terminals. Under our experiment conditions, ~10% of total endogenous Staring was co-immunoprecipitated with syntaxin 1, indicating that only a fraction of Staring and syntaxin 1 co-exist in the Staring-syntaxin 1 complexes. These results are consistent with previous reports that syntaxin 1 interacts with more than a dozen proteins, including SNAP-25, nSec1/Munc-18, Munc-13, tomosyn, and syntaphilin (14, 45–48). Moreover, as suggested by its multidomain structure, Staring is likely to interact with additional proteins.

Staring Binds and Recruits the E2 Ubiquitin-conjugating Enzyme UbcH8 to Syntaxin 1—The presence of the RING finger motif in Staring raises the possibility that Staring may function as an E3 ubiquitin-protein ligase via interaction with a specific E2 ubiquitin-conjugating enzyme (41). As a first step to test this possibility, Myc-tagged Staring was transiently expressed in HeLa cells along with HA-tagged E2 enzyme UbcH5, UbcH7, or UbcH8, and the interaction of these E2 enzymes with Staring was examined by co-immunoprecipitation analysis (Fig. 5A). The results revealed that Staring specifically interacts with UbcH8, a brain-enriched E2 ubiquitin-conjugating enzyme (49). In contrast, no appreciable interaction could be detected between Staring and UbcH5 or UbcH7. These data indicate that UbcH8 is the cognate E2 ubiquitin-conjugating enzyme for Staring. To further determine whether Staring interacts with both UbcH8 and syntaxin 1 to form a ternary complex, co-immunoprecipitation experiments were performed using extracts of HeLa cells co-transfected with epitope-tagged Staring, syntaxin 1, and UbcH8 (Fig. 5B). We observed that syntaxin 1 and UbcH8 co-precipitate with Staring in a stable complex, suggesting that Staring has the ability to recruit its cognate E2 enzyme UbcH8 to syntaxin 1.

Staring Promotes the Ubiquitination of Syntaxin 1—Next, we sought to determine whether Staring acts as an E3 ubiquitin-protein ligase to ubiquitinate syntaxin 1 by using a well-established in vivo ubiquitination assay (50). HA-tagged syntaxin 1 was co-expressed in HeLa cells along with Myc-tagged ubiquitin in the absence or presence of exogenous Staring. Cell lysates were subjected to immunoprecipitation with an anti-HA antibody followed by immunoblotting with an anti-Myc antibody to detect ubiquitin-conjugated syntaxin 1 (Fig. 6). Increased levels of Myc-tagged ubiquitin were detected on syntaxin 1 in the presence of exogenous Staring, indicating that Staring promotes the ubiquitination of syntaxin 1. The Staring-mediated ubiquitination seems to be polyubiquitination, because the ubiquitinated syntaxin 1 was detected as a high molecular weight smear. In addition to the smear, several predominant ubiquitination bands were observed, which may represent predominant ubiquitinated syntaxin 1 species produced in vivo as the net result of the ubiquitination and deubiquitination reactions. Similar in vivo polyubiquitination patterns have previously been seen with other ubiquitinated proteins, such as synphilin-1 and CDCrel-1 (32, 51). The Myc-

![Fig. 5. Staring binds selectively to UbcH8 and forms a ternary complex with syntaxin 1 and UbcH8.](image)

![Fig. 6. Staring is a RING-type E3 ubiquitin-protein ligase that ubiquitinates syntaxin 1.](image)
immunoreactive band at ~70 kDa is probably a nonspecific band, because it also exists in the control lane that has no Myc-tagged ubiquitin.

Since the RING finger motif is thought to be essential for the enzymatic activity of a RING-type E3 ubiquitin-protein ligase (40, 41), we examined the effect of deletion of the Staring RING finger motif on the in vivo ubiquitination of syntaxin 1. A mutant form of Staring (StaringΔR) that lacks the RING finger motif was generated and analyzed for its ability to ubiquitinate syntaxin 1 using the same in vivo ubiquitination assay as described above (Fig. 6). The results revealed that deletion of the RING finger motif abolished the ability of Staring to ubiquitinate syntaxin 1, providing further evidence supporting the role of Staring as a RING-type E3 ubiquitin-protein ligase.

Staring Targets Syntaxin 1 for Degradation by the Proteasome Pathway—Polyubiquitination of a cellular protein generally leads to the degradation of the protein by the proteasome pathway (52). To determine whether Staring-mediated ubiquitination of syntaxin 1 promotes its degradation, we performed pulse-chase experiments to compare the turnover rate of syntaxin 1 protein in the absence and presence of exogenous Staring. As shown in Fig. 7, the half-life of syntaxin 1 was ~16.7 h in the absence of Staring. By comparison, the half-life of syntaxin 1 was reduced to 6.1 h in the presence of Staring, indicating that Staring has the ability to regulate the degradation of syntaxin 1. Consistent with this result, measurement of the syntaxin 1 protein level by Western blot analysis revealed that the steady-state expression level of syntaxin 1 was significantly decreased in the presence of Staring as compared with the syntaxin 1 level in the absence of Staring (Fig. 7C). Furthermore, the Staring-induced degradation of syntaxin 1 was blocked by MG132, a potent inhibitor of proteasome function (53), but not by E64, an inhibitor of lysosomal cysteine proteases. Together, these results suggest that Staring promotes the degradation of syntaxin 1 by the proteasome pathway.

Staring Regulates the Ubiquitination and Degradation of Endogenous Syntaxin 1 in SH-SY5Y Cells—We next investigated whether Staring is able to regulate the ubiquitination and degradation of endogenous syntaxin 1. For these studies, we used human neuroblastoma SH-SY5Y cells, a well-characterized dopaminergic cell line that expresses endogenous syntaxin 1 and exhibits SNARE-mediated neurotransmitter release (54, 55). Overexpression of Staring in SH-SY5Y cells significantly enhanced the ubiquitination of endogenous syntaxin 1, as indicated by the appearance of a high molecular weight smear containing Myc-ubiquitin-modified syntaxin 1 (Fig. 8A). The effect of overexpressing Staring on syntaxin 1 ubiquitination was abolished by the deletion of Staring RING finger motif, demonstrating that Staring promotes the ubiquitination of endogenous syntaxin 1 in a RING finger-dependent manner. In addition, overexpression of Staring significantly accelerated the turnover of endogenous syntaxin 1, as measured by the pulse-chase analyses in the absence and presence of exogenous Staring (Fig. 8B). The half-life of endogenous syntaxin 1 was reduced from 19.6 to 8.6 h by overexpression of Staring. The increased turnover rate of syntaxin 1 was accompanied by a decrease in the steady-state expression level of syntaxin 1 in the presence of exogenous Staring (Fig. 8C). Moreover, the Staring-induced degradation of endogenous syntaxin 1 was blocked by the proteasome inhibitor MG132 but not by the lysosomal protease inhibitor E-64, NH4Cl, or chloroquine (Fig. 8C). Taken together, these data provide strong support for a role of Staring in regulating the degradation of endogenous syntaxin 1 via the ubiquitin-proteasome pathway.

**DISCUSSION**

Whereas it is becoming increasingly clear that the ubiquitin-proteasome proteolytic pathway is involved in synaptic function and neurodegeneration (56–58), very little is known about neuronal protein substrates that are targeted by this pathway. On the other hand, alterations in the expression levels of presynaptic proteins have been linked to synaptic plasticity and neurodegeneration, yet it is not understood whether and how the ubiquitin-proteasome proteolytic pathway regulates the degradation of these proteins at nerve terminals. In this study, we have identified and characterized Staring, a novel RING finger protein that interacts with syntaxin 1, an essential component of neurotransmitter release machinery. Our data suggest that Staring may function as an E3 ubiquitin-protein
ligase to promote the ubiquitination and degradation of syntaxin 1 by the proteasome pathway.

Traditionally, it is thought that the lysosomal pathway is used for the degradation of transmembrane proteins like syntaxin 1, whereas the ubiquitin-proteasome pathway is involved in the degradation of cytosolic and nuclear proteins. However, several recent papers reported the involvement of the ubiquitin-proteasome pathway in the degradation of transmembrane proteins, such as GABA_A receptor, Netrin-1 receptor DCC (deleted in colorectal cancer), amiloride-sensitive epithelial Na^+ channels, and the Epstein-Barr virus latent membrane protein 1 (LMP1) (59–63). In addition, our recent work has demonstrated that the degradation of synaptic vesicle membrane protein synaptophysin is regulated by the E3 ubiquitin-protein ligase Siah in a proteasome-dependent manner (35). The evidence presented here suggests that the ubiquitin-proteasome pathway also regulates the degradation of the plasma membrane t-SNARE syntaxin 1. These findings implicate an important role for the ubiquitin-proteasome pathway in the degradation of both cytosolic and membrane proteins at nerve terminals. Since in neuron the lysosome is localized in the cell body instead of the nerve terminal, degradation by the ubiquitin-proteasome pathway may provide a more efficient means for the regulation of presynaptic protein levels locally at the nerve terminal.

Staring is a novel member of a growing family of RING finger proteins that function as E3 ubiquitin-protein ligases. The RING type E3 ligases consist of two classes: single-subunit RING E3 ligases and multisubunit RING E3 ligases. A single subunit RING E3 ligase contains the substrate-binding domain and the RING finger motif in the same molecule. In contrast, a multisubunit RING E3 ligase is a multiprotein complex that contains the RING finger protein as one subunit and a separate protein for substrate recognition (41, 64). Our data indicate that Staring is a single-subunit RING E3 ligase that uses its coiled-coil domain H3 to bind substrate syntaxin 1. In addition to the H3 domain, Staring also contains five other coiled-coil domains, suggesting that Staring may bind and ubiquitinate additional substrates.

Among the molecular components of the ubiquitin-proteasome pathway, the E3 ubiquitin-protein ligase is perhaps the most important player, because it binds specific substrates and determines their ubiquitination and subsequent degradation. Furthermore, regulation of the E3-mediated substrate recognition and ubiquitination is a major mechanism for modulation of specific protein degradation (52, 64). Aberrations in the E3 ubiquitin-protein ligase activities have been implicated in the pathogenesis of several human diseases (64). For example, mutations or exon deletions in the gene encoding the E3 ubiquitin-protein ligase parkin are a major cause of autosomal recessive juvenile parkinsonism (58, 65, 66). Interestingly, parkin is widely expressed in many tissues, yet autosomal recessive juvenile parkinsonism is characterized by a selective degeneration of nigral neurons, suggesting that accumulation of a neuron-specific substrate(s) may be involved in the pathogenesis (65, 66). Similar to parkin, Staring is also ubiquitously expressed. We have found that Staring selectively interacts with the brain-enriched E2 ubiquitin-conjugating enzyme UbcH8 and promotes the ubiquitination and degradation of the neuron-specific protein syntaxin 1. Since altered syntaxin 1 levels have been associated with several neurodegenerative diseases, it would be tempting to speculate that Staring-mediated syntaxin 1 degradation contributes to the pathophysiology of these diseases. Further studies of the molecular mechanism that controls the degradation of presynaptic proteins will not only enhance our understanding of neurodegeneration but will also advance our knowledge of synaptic plasticity such as learning and memory.

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