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Synaptotagmin 11 interacts with components of the RNA-induced silencing complex RISC in clonal pancreatic β-cells

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ABSTRACT

Synaptotagmins are two C_2 domain-containing transmembrane proteins. The function of calcium-sensitive members in the regulation of post-Golgi traffic has been well established whereas little is known about the calcium-insensitive isoforms constituting half of the protein family. Novel binding partners of synaptotagmin 11 were identified in β-cells. A number of them had been assigned previously to ER/Golgi derived-vesicles or linked to RNA synthesis, translation and processing. Whereas the C2A domain interacted with the Q-SNARE Vti1a, the C2B domain of syt11 interacted with the SND1, Ago2 and FMRP, components of the RNA-induced silencing complex (RISC). Binding to SND was direct via its N-terminal tandem repeats. Our data indicate that syt11 may provide a link between gene regulation by microRNAs and membrane traffic.

Structured summary of protein interactions:
Syt11C2A physically interacts with **Vti1a** by pull down (View interaction)
Syt11C2B physically interacts with **SND1**, **PDIA6**, **Vti1b**, **Vti1a**, **Ago2** and **FMRP** by pull down (View interaction)
syt11C2B binds to **SND1** by filter binding (View interaction)
Syt11C2B physically interacts with **EIF3A**, **PDIA6**, **NPM1**, **EIF3B**, **NCL**, **RS3**, **RS3A**, **CBR1**, **ANP32B**, **LOC683961**, **SET**, **SND1**, **TBB2C**, **RS10** and **RS18** by pull down (View interaction)
SND1 physically interacts with **Ago2** by anti bait coip (View interaction)

1. Introduction

A number of transmembrane proteins are implicated in the transport, docking and fusion of vesicles during intracellular transport. Members of the synaptotagmin family (syts) have been shown to form calcium sensors in diverse trafficking pathways [1]. They share a common structure: a short intra luminal N-terminal domain, a single transmembrane domain and a cytoplasmic carboxy-terminal domain. In addition to this topology, synaptotagmins exhibit two C_2 domains in their cytoplasmic tail. Synaptotagmins are either Ca^{2+}-sensitive or -insensitive according to their capacity to bind the divalent ion and phospholipids via these C_2 domains, referred to as C_2A and C_2B. The C_2 domains may act as independent modules and their respective relevance and physiological targets are still not completely resolved. In addition to their role as Ca^{2+}-dependent trigger in membrane fusion, they may also participate in vesicle-membrane interactions [2].

The superfamily of synaptotagmins currently contains 17 isoforms in mammals which can be classified according to their biochemical properties or sequence alignments [1,3]. Syt1 to 3, 5 to 7, 9 and 10 are grouped in regard to their ability to bind phospholipids in a calcium-dependent manner suggesting an involvement of these isoforms in Ca^{2+}-regulated membrane fusion. On the contrary, syt4, syt8 and syt11 to 15 do not exhibit calcium-sensitivity [4–10]. The biochemical properties of syt16 and 17 are

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still unknown but sequence comparison suggests calcium-independent functions. Thus, function of several Ca^{2+}-sensitive isoforms have been characterized in detail in post-Golgi trafficking, whereas the localization and the biological role of most other isoforms are still poorly understood. In the attempt to characterize the role of synaptotagmins in the exocytosis of large dense core vesicles, we and others have been able to assign a role to syt1, 2, 7 and 9 using endocrine β-cells as a model system [11–16].

In contrast, the role and distribution of the Ca^{2+}-insensitive forms are still largely unknown although they constitute about a half of this protein family. In this setting, syt11 is of particular interest. Altered expression patterns have been associated with type 2 diabetes and with Parkinson's disease [17,18]. This protein appears as a syt4 paralog in vertebrates [3] and Ca^{2+}-sensitivity has been lost due to mutation of key amino acids representing an evolutionary adaptation [4]. Thus, knowledge about this isoform may contribute to elucidate the panel of functions of synaptotagmins beyond calcium sensing in membrane fusion. In the absence of any known or predictable cellular function, we decided to investigate its binding partners in the well-established model of insulin-secreting β-cells.

2. Materials and methods

2.1. Materials

The following commercial monoclonal antibodies were employed: anti-Vti1b and anti-Vti1a (Transduction Laboratories); anti-insulin (Sigma–Aldrich); anti- βCOP (Sigma–Aldrich), anti-AP1-AP2 (Sigma–Aldrich); (Transduction Laboratories); anti-GST (Sigma–Aldrich); anti-glucagon (Sigma–Aldrich). The following commercial polyclonal antibodies were used: guinea pig anti-SND1 (Progen), guinea pig anti-insulin (Sigma–Aldrich), rabbit anti-Ago2 (Cell Signalling) and rabbit polyclonal anti-FMRP (Abcam). The following secondary antibodies were employed: HRP-conjugated secondary antibodies (GE Healthcare). Several antibodies were kindly donated: the monoclonal anti-FMRP antibody 1C3 (Dr. Mazroui, Université Laval, Québec, Canada); a rabbit polyclonal anti-CaBP1/PDIA6 (Dr. Nguyen Van, Universität Göttingen, Germany), a monoclonal anti-Ago2 (851) (Dr. Hobman, University of Alberta, Edmonton, Canada), a monoclonal anti-EEA1 (Dr. Grunberg, Genève, Switzerland), a monoclonal anti-LAMP1 (Dr. Malosio, Milano), a rabbit polyclonal anti-syntaxin5, anti-syntaxin18 and anti-D12/Use1p (Dr. Hatsuzawa, Fukushima Medical University, Japan). The rabbit polyclonal anti SND1/p102 had been described previously [19].

2.2. Plasmids

cDNA encoding mouse syt11 was generated by RT-PCR from total brain RNA and inserted in a pGEM-T cloning vector as described in [6]. Truncated syt11 were obtained by PCR on pGEM-T-Syt11 using the following primers: syt11C_2A (amino acids 150–264), sense primer: CGCGGATCCTCGCCAGAGGAAGAG and antisense primer: CCGGAATTCTTACAATGGCACCATGAC; syt11 C_2B (amino acids 265–430), sense primer: CGCGGATCCGCTGGAGTGGACCCC and antisense primer: CCGGAATTCTTAGTACTCGCTCAG. PCR fragments were then digested with BamHI and EcoRI and inserted in the pGEX-2T vector (GE Healthcare) allowing the expression of a N-terminal GST fusion protein. Plasmids encoding the cytoplasmic portions of synaptotagmins fused to GST were as described [6]. The plasmid pGEX-4T-1-p100-SN(1-4), containing the four N-terminal SN-like domains of SND1 (amino acids 1–639), were kindly provided by Dr. O. Silvennoinen (University of Tampere, Finland) [20].

2.3. Cell culture, fluorescence microscopy and immunoprecipitation

INS-1E were cultured as previously described [21]. Islets were isolated from adult male Wistar rats (Iffa Credo, Lyon, France) using collagenase digestion and separation on a Ficoll density gradient [22]. For immunofluorescence studies, isolated cells from islets were obtained after dissociation with 0.025% trypsin/0.27 mM EDTA and then cultured in complete RPMI-1640 medium containing 5.6 mM glucose for 1 week. Islet cells were seeded on poly-L-lysine (Sigma–Aldrich) coated Lab-Tek® Chamber Slide™ System. After fixation with 2% paraformaldehyde for 20 min, cells were permeabilised for 5 min in 0.1% Triton X-100 and blocked in 2% BSA. Stained cells were mounted in Citifluor (Citifluor LTD, London, UK) and observed with the BioRad confocal microscope equipped with an argon-krypton laser.

Postnuclear supernatants (PNS) from INS-1E cells were prepared as described in [23]. PNS were then centrifuged at 100,000*g*, 2 h, 4 °C to obtain the membrane fraction. Triton X-100 extracts were obtained as described previously [23,24] except that sonication was omitted. Immunoprecipitation was performed on solubilized fractions by adding the relevant antibody and a 50% suspension of protein A-Sepharose beads CL-4B (GE Healthcare) for 2 h at 4 °C. Beads were washed several times, resuspended in Laemmli buffer and supernatants subsequently analyzed by SDS–PAGE and immunoblots as published [12]. To test specificity, anti-syt11 antibodies were preabsorbed prior to immunoblot with antigenic peptide (10–50 μM) for 1 h at room temperature.

2.4. Purification of recombinant proteins, pull-down experiments and direct binding assay

GST-Syt fusion proteins were expressed as described [24]. Recombinant proteins were either eluted directly or after overnight cleavage at 4 °C from the GST-tag by 4 U/ml of thrombin (GE Healthcare). The pGEX-4T1-p100-SN (1–4) expression vector was kindly donated by Dr. Valineva (University of Tampere, Finland). This construct was expressed in Rosetta host strain (Novagen) under the same conditions as for other GST-tagged proteins. Purified recombinant proteins were analyzed by Coomassie blue stained SDS–PAGE.

25 μg of GST fusion-proteins were immobilized onto gluthatione-Sepharose beads in buffer A (20 mM MOPS, 250 mM sucrose, 5 mM $MgCl_2$, pH 7). Beads were washed 4 times with buffer A/1 M NaCl and once without buffer A alone. Immobilized fusion proteins were subsequently treated for 10 min at 30 °C with micrococcal nuclease (Calbiochem) at a final concentration of 33 U/ml. Beads were washed again in Buffer A/0.01% Triton X-100. 500 μg of a freshly solubilized PNS was mixed with 25 μg of immobilized GST fusion proteins. After incubation at 4 °C for 2 h, beads were washed twice with buffer A/0.1% Triton X-100, twice with buffer A/300 mM KCl and finally with buffer A. Bound proteins were eluted by adding Laemmli's buffer. The eluted samples were resolved by SDS–PAGE followed by spectrometric mass analysis or by Western blot detection with the relevant antibodies.

Purified recombinant syt11C_2A and syt11C_2B (1 μg) were directly spotted onto PVDF membranes, which were subsequently washed in TBS (50 mM Tris, 190 mM NaCl, pH 8)-0.1% Tween and blocked in TBS-1% BSA prior to incubation in 10 μg of purified GST or GST-SN (N-terminal four staphylococcus nuclease domains of SND1 fused to GST). Membranes were washed with TBS-Tween and TBS, blocked in 5% milk TBS-Tween and incubated overnight with a guinea pig anti-SND1 antibody (1/2000). After three washes in TBS-Tween, blots were incubated for 1 h at room temperature with HRP-conjugated anti-guinea pig IgG secondary antibody. Binding of antibodies was visualized by chemiluminescence.

2.5. Mass spectrometry

Selected bands were excised from gels and digested with trypsin using standard protocols, the resulting peptide mixture being analyzed by tandem MS (MS/MS). Data were acquired on a MALDI Q-TOF Premier mass spectrometer used in MS/MS mode (Waters, Manchester, UK), with α-cyano-4-hydroxy-cinnamic acid (Sigma–Aldrich) used as a matrix (3.6 mg mL^{-1} solution in 50% acetonitrile in 0.1% aqueous trifluoroacetic acid). Mono-isotopic masses were corrected using the pseudomolecular ion of Glu-Fibrinopeptide as a lock mass (1570.6774 Da) [25]. Proteins were identified using the MS/MS search module from the online version of MASCOT software (http://www.matrixscience.com) against the non-redundant protein NCBI or Swissprot database. The following parameters were used: peptide charge +1, peptide tolerance ±0.03 Da, MS/MS tolerance ±0.1 Da and one missed cleavage allowed for trypsin.

3. Results

To obtain information about the role of syt11 in β-cells, we decided to search for protein–protein interactions. Synaptotagmins contain two independent cytosolic modules, C_2A and C_2B, which were fused separately to GST (Fig. 1A: GST-Syt11C_2A, amino acids 150–264; GST-Syt11C_2B, amino acids 265–430). Pull-down experiments were performed using these constructs as baits for binding partners in INS-1E cell extracts. No differences were observed between GST alone and GST-Syt11C_2A when used as bait and bound proteins were separated and visualized by SDS/PAGE. In contrast, using GST-Syt11C_2B as bait, 15 distinct specific bands in a molecular mass range from 17 to 160 kDa were detected in Coomassie blue-stained gels and further analyzed by mass spectrometry run onto a MALDI-Q-TOF used in MS/MS mode as given in Table 1. Among the peptides identified, certain are part of proteins known to be involved in the translation machinery such as eukaryotic translation initiation factor 3 subunits A and B or subunits of the 40S ribosomal complex (RS3A, RS3, RS10, RS13, RS18). Interestingly, the staphylococcal nuclease domain-containing protein 1, termed SND1 or p100 coactivator, was also identified as syt11-binding protein via N-terminal and C-terminal peptides. SND1 has been implicated in various cell functions in addition to its role as a transcriptional coactivator, namely in the secretion of lipid droplets in mammary epithelial cells and hepatocytes [19,26,27] and as part of the RISC complex by interacting with Argonaute 2 [28]. The protein is expressed in primary pancreatic α- and β-cells (data not shown). In addition we noticed the presence of the protein disulfide-isomerase PDIA6 (also termed CaBP1 or ERP5), an ER/ERGIC chaperone, of a carbonyl reductase ([NAPDH]1 or CBR1) and of several proteins known for their distribution in the nucleus, the cytosol, and endomembrane attachment.

To corroborate and extend these findings, pull-down experiments using the C_2A or the C_2B domain of syt11 were performed under the same conditions using cell extracts but analyzed by immunoblotting. The strong signal observed with the anti-SND1 antibody confirmed the interaction between GST-Syt11C_2B and SND1 already revealed by mass spectrometry (Fig. 1B and C). Similarly, PDIA6 interacted only with GST-Syt11C_2B but not with the C_2A domain of syt11. As synaptotagmins are involved in vesicular traffic we also examined potential interactions with SNARE proteins and tested first for SNARE proteins localized in the ER-Golgi region of the cell. The C_2B domain of syt11 exhibited clear interaction with the SNARE protein Vti1b whereas Vti1a interacted mainly with the C_2A domain (Fig. 1B and C) suggesting a potential role of syt11 in vicinity of the Golgi apparatus. However, interactions were not detected with the ER SNARE proteins D12/Use1p and syntaxin 18 or with the Golgi SNARE syntaxin 5 (Data not shown).

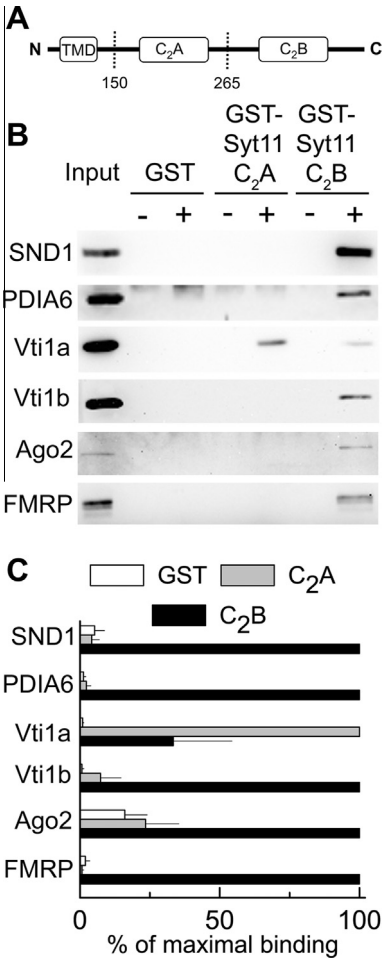

Fig. 1. Identification of syt11 binding partners by GST pull-down experiments. (A) Schematic representation of the GST-Syt11 constructs used. GST-Syt11C_2A corresponds to the GST protein fused to amino acids 150–264 of syt11 and GST-Syt11C_2B contains amino-acids 265–430. (B) Western-blots analysis of GST-pull-down experiments. Purified recombinant proteins were incubated with INS-1E post-nuclear supernatants and bound proteins were analyzed with appropriate antibodies: guinea pig polyclonal anti-SND1 (1/1000), anti-PDIA6 (1/1000), anti-Vti1a (1/2500), anti-Vti1b (1/500), anti-Ago2 (1/1000) and anti-FMRP (1/1000). Input represents 3% of the starting material. (C) Quantification of binding ($N = 3–5$).

Similarly, we did not find any evidence for interactions for a number of other proteins such as β-COP, a constituent of COPI vesicles, the clathrin adapters AP1 and AP2 as well as the endoplasmic reticulum chaperone BIP (data not shown). As syt11 interacts with SND1, which is part of the RISC complex, we also tested for the presence of fragile X mental retardation protein (FMRP) and the endonuclease Argonaute2 (Ago2), known binding partners of SND1 [28]. Indeed both were found in the specific eluate, but only in the case of syt11C_2B (Fig. 1B and C).

Unfortunately the distribution of syt11 along the secretory pathway could not been determined. Indeed, several commercial as well as antibodies generated in our or other laboratories were non-specific. Although they bound to recombinant syt11 and their binding was reduced or abolished by an excess of peptide, we did not observe any change in intensity after proven and substantial knock-down of syt 11 (data not shown).

To obtain further insight into the interaction with components of RISC, we next examined the interaction of endogenous SND1 and Ago2 by co-immunoprecipitation in INS-1E cells. For this purpose, SND1 was immunoprecipitated from INS-1E cells using a polyclonal guinea pig antibody and analyzed by Western-blot using

Table 1
Proteomic analysis of syt11C_2B binding partners.

	Name	NCBI Accession number	MW	MS/MS peptides	Mascot Score	Present on ERGIC, Golgi or derived vesicles
ANP32B	Acidic leucine-rich nuclear phosphoprotein 32 family member B	gi|18777770	31.2	2	85	
CBR1	Carbonyl reductase [NAPDH]1	gi|9506467	30.8	4	257	
EIF3A	Eukaryotic translation initiation factor 3 subunit 10 (theta)	gi|149040541	163.5	3	49	
	Eukaryotic translation initiation factor 3 subunit A	gi|261337190	163.5	3	49	
	ZH12	gi|95102034	150.4	3	49	
EIF3B	Eukaryotic translation initiation factor 3 subunit B	gi|72255511	91.2	3	99	
NCL	Nucleolin	gi|128844	77.1	3	141	a
NPM1	Nucleophosmin	gi|7242160	32.7	2	104	a, b
	Nucleolar protein B23.2	gi|203078	28.5	2	104	
PDIA6	Calcium binding protein/Protein disulfide-isomerase A6 precursor	gi|488838	47.1	4	99	a, c
RS3	40S Ribosomal protein S3	gi|57164151	26.6	3	130	a, b
RS3A	40S Ribosomal protein S3a	gi|8394221	30.1	1	58	b
RS10	40S Ribosomal protein S10-like	gi|62652203	19.6	1	43	a, b
RS13	40S Ribosomal protein S13-like	gi|109476367	17.8	1	44	
RS18	40S Ribosomal protein S18-like	gi|27658598	17.7	3	118	b
SET	SET Protein	gi|60499029	33.4	1	69	a
SND1	Staphylococcal nuclease domain-containing protein 1	gi|77404395	102.6	3	111	a, b
TBB2C	Tubulin beta 2C chain	gi|40018568	50.2	2	69	a

For each protein, the number of peptides leading to identification by MS/MS is indicated. Proteins in italics were identified on the basis of a single MS/MS and therefore cannot be considered as formally identified. Names, accession numbers (*Rattus norvegicus*), theoretical molecular weights and Mascot scores are reported (significance threshold was 24). a, b and c denote subcellular localization according to [27], [26] or [57], respectively.

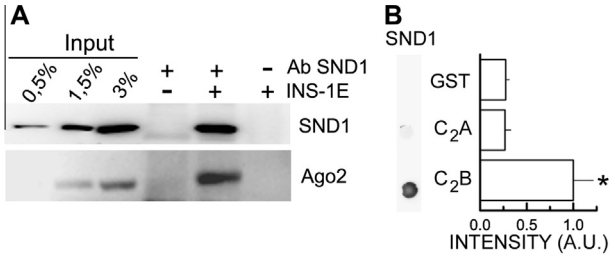

Fig. 2. Syt11 interacts directly with SND1. (A) Immunoprecipitation of SND1 were performed using a guinea pig anti-SND1 antibody. Ago2 was detected by Western blots, 0.5–3% of the initial INS-1E postnuclear supernatant was loaded on the gel as comparison. (B) Direct interaction between recombinant syt11C_2A or C_2B domains and N-terminal SND1 was investigated by dot-blot and quantifications are given ($N = 5$; $^*p < 0.05$, Bonferroni's).

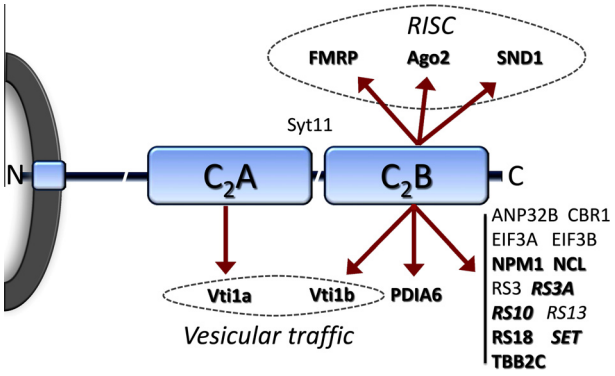

Fig. 3. Summary of observed interactions. Red arrows, interactions observed by pull-down and subsequent mass spectrometry and/or immunoblot; Proteins known to reside at the ERGIC or Golgi are given in bold (see also Table 1), proteins identified by one peptide only are given in italics.

rabbit polyclonal antibodies against SND1 or a monoclonal antibody against Ago2 (Fig. 2A). Clearly SND1 interacts with syt11 and with Ago2. We subsequently asked whether the interaction between SND1 and syt11 was of direct nature or required other scaffolding proteins. Full-length SND1 is poorly soluble and we therefore used a C-terminally truncated recombinant protein comprising only the N-terminal four staphylococcus nuclease (SN_{1-639}) domains fused to GST (GST-SN). As shown in Fig. 2B, GST-SN (SND1) interacted with GST-Syt11C_2B in a dot blot assay, but not with GST-Syt11C_2A. This designates the SN domains and the C_2B domains as minimal directly interacting modules(see Fig. 3).

4. Discussion

The calcium-sensitive synaptotagmins have been extensively characterized in post-Golgi trafficking [29]. Our study on a calcium-insensitive member, syt11, the vertebrate paralog of syt4 [30], revealed a defined link with the RNA-induced silencing complex RISC, known to be important in regulation of genes by microRNAs.

Obviously care has to be taken in regard to the specificity of protein–protein interactions. To avoid any potential interference by nucleic acids and electrostatic interactions, we used nuclease and high-salt treatment. Similarly, we used KCl instead of NaCl for washes. Moreover, a number of differential interactions were observed and most importantly, co-localization of binding partners including most of the ribosomal proteins identified here could be deduced from published proteomic data and expression of epitope-tagged proteins [31–33].

Protein interactions of syt11 identified here exhibited two striking features. First, a number of them had been assigned previously to Golgi derived-vesicles or to the ERGIC. Note that epitope-tagged syt11 localized to the Golgi and to vesicles upon transient expression in neuroendocrine PC12 cells [33]. Second, the majority of the interacting proteins is linked to RNA synthesis, translation and processing (Fig. 2). As to their localization, NCL, NPM1, RS10, RS18, RS3, RS3A, TBB2C, SET and SND1 had been found on ER-Golgi vesicles or Golgi membranes by proteomic analysis [31,32]. Strikingly, also FMRP, Ago2 and the Q-SNAREs Vti1a/Vti1b, which we subsequently identified as interacting proteins by immunoreactivity, reside on ER and on Golgi structures [32,34–36]. The differential binding observed here for the Q-SNAREs Vti1a and Vti1b underlines C_2-domain specificity in protein–protein interactions.

In contrast to a previous report [37], we did not detect any interaction with Parkin, a component of the E3 ubiquitin ligase complex present on the ER, the Golgi and derived vesicles [38] or with amyloid beta precursor protein binding protein 2 [39]. These interactions were described for full-length syt11 and may thus occur outside C_2 domains.

The preponderance of ribosomal or RNA binding proteins presents a striking feature (EIF3A, EIF3B, NPM1, NCL, RS10, RS13, RS18, RS3, RS3A, SND1). The involvement of synaptotagmin in the regulation of mRNA dynamics is not without precedence. The cytoplasmic RNA binding-protein SYNCRIP interacts with the C_2B domains of several syts including syt11, but not syt4 thus suggesting functional differences between the two paralogs [40]. The novel interaction of syt11 with the ancestral staphylococcal nuclease domain-containing 1 protein SND1 is of particular interest. This multifunctional regulator of gene expression has a broad expression profile [41], is enriched in secretory cells [27] and acts as transcriptional co-activator [42], in RNA editing [43], in the generation of RISC [28] and is linked to apoptosis [44]. SND1 is generally regarded as a nuclear and/or cytoplasmic protein and in line with a number of reports, [26,45,46] we observed only extranuclear expression in β-cells. The defined architecture of SND1 provides distinct binding sites and functions [47]. Whereas the C-terminal Tudor domain flanked by SN regions is involved in regulation of splicing [48], the N-terminal tandem repeats of SN-like domains are required to complex co-activators and transcription factors [42,49], to capture and cleave RNA substrates [50], to link Ago2 within the RISC complex [51] and, as shown here, to bind directly the C_2B domain of syt11. Thus tandem repeats of SN-like domains are capable to undergo multiple simultaneous interactions. As syt11 interacts with SND1 but also with Ago2 and FMRP, which are well characterized as RISC components [28,52], syt11 is linked to the RISC complex in β-cells. Recent data indicate that Ago2 is required for the compensatory increase in β-cell mass, as found during insulin resistance, and plays a role in the maintenance of the insulin secretory pathway [53,54].

The interaction of RISC components with the transmembrane protein syt11 indicates the association of RISC with organelles. A link has been reported between RISC and endosomes or multivesicular bodies (MVB) [55,56] promoting their recruitment or loading at a strategic site relevant for membrane traffic, recycling, export or viral entry. Intriguingly data mining (see Table 1) and our observations suggest that respective components such as SND1 and Ago2 are attached already to membranes earlier in the secretory pathway. Moreover, recent work has shown that RISC is linked the ER membrane and ERGIC/Golgi may be implicated in recycling of RISC components in plant and in animal cells [57,58]. In conclusion, our observations indicate a novel interaction between RISC and a transmembrane protein implicated in intracellular traffic and suggest an unprecedented function for a member of the synaptotagmin family.

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References

[1] Südhof, T.C. (2013) A molecular machine for neurotransmitter release: synaptotagmin and beyond. Nat. Med. 19, 1227–1231.
[2] Sorensen, J.B. (2009) Conflicting views on the membrane fusion machinery and the fusion pore. Annu. Rev. Cell Dev. Biol. 25, 513–537.
[3] Craxton, M. (2010) A manual collection of Syt, Esyt, Rph3a, Rph3al, Doc2, and Dblc2 genes from 46 metazoan genomes–an open access resource for neuroscience and evolutionary biology. BMC Genomics 11, 37.
[4] von Poser, C., Ichtchenko, K., Shao, X., Rizo, J. and Südhof, T.C. (1997) The evolutionary pressure to inactivate. A subclass of synaptotagmins with an amino acid substitution that abolishes Ca^{2+} binding. J. Biol. Chem. 272, 14314–14319.
[5] Cao, P., Maximov, A. and Südhof, T.C. (2011) Activity-dependent IGF-1 exocytosis is controlled by the Ca^{2+}-sensor synaptotagmin-10. Cell 145, 300–311.
[6] Monterrat, C., Boal, F., Grise, F., Hemar, A. and Lang, J. (2006) Synaptotagmin 8 is expressed both as a calcium-insensitive soluble and membrane protein in neurons, neuroendocrine and endocrine cells. Biochim. Biophys. Acta Mol. Cell Res. 1763, 73–81.
[7] Maximov, A., Shin, O.H., Liu, X. and Südhof, T.C. (2007) Synaptotagmin-12, a synaptic vesicle phosphoprotein that modulates spontaneous neurotransmitter release. J. Cell Biol. 176, 113–124.
[8] von Poser, C. and Südhof, T.C. (2001) Synaptotagmin 13: structure and expression of a novel synaptotagmin. Eur. J. Cell Biol. 80, 41–47.
[9] Fukuda, M. (2003) Molecular cloning, expression, and characterization of a novel class of synaptotagmin (Syt XIV) conserved from Drosophila to humans. J. Biochem. 133, 641–649.
[10] Fukuda, M. (2003) Molecular cloning and characterization of human, rat, and mouse synaptotagmin XV. Biochem. Biophys. Res. Commun. 306, 64–71.
[11] Gauthier, B.R., Duhamel, D.L., Iezzi, M., Theander, S., Saltel, F., Fukuda, M., Wehrle-Haller, B. and Wollheim, C.B. (2008) Synaptotagmin VII splice variants alpha, beta, and delta are expressed in pancreatic beta-cells and regulate insulin exocytosis. FASEB J. 22, 194–206.
[12] Grise, F., Taib, N., Monterrat, C., Lagree, V. and Lang, J. (2007) Distinct roles of the C_2A and the C_2B domain of the vesicular Ca^{2+} sensor synaptotagmin 9 in endocrine beta-cells. Biochem. J. 403, 483–492.
[13] Gut, A., Kiraly, C.E., Fukuda, M., Mikoshiba, K., Wollheim, C.B. and Lang, J. (2001) Expression and localisation of synaptotagmin isoforms in endocrine beta-cells: their function in insulin exocytosis. J. Cell Sci. 114, 1709–1716.
[14] Iezzi, M., Kouri, G., Fukuda, M. and Wollheim, C.B. (2004) Synaptotagmin V and IX isoforms control Ca^{2+} -dependent insulin exocytosis. J. Cell Sci. 117, 3119–3127.
[15] Lang, J., Fukuda, M., Zhang, H., Mikoshiba, K. and Wollheim, C.B. (1997) The first C_2 domain of synaptotagmin is required for exocytosis of insulin from pancreatic beta-cells: action of synaptotagmin at low micromolar calcium. EMBO J. 16, 5837–5846.
[16] Monterrat, C., Grise, F., Benassy, M.N., Hemar, A. and Lang, J. (2007) The calcium-sensing protein synaptotagmin 7 is expressed on different endosomal compartments in endocrine, neuroendocrine cells or neurons but not on large dense core vesicles. Histochem. Cell Biol. 127, 625–632.
[17] Andersson, S.A. et al. (2012) Reduced insulin secretion correlates with decreased expression of exocytotic genes in pancreatic islets from patients with type 2 diabetes. Mol. Cell. Endocrinol. 364, 36–45.
[18] Pihlstrom, L. et al. (2013) Supportive evidence for 11 loci from genome-wide association studies in Parkinson's disease. Neurobiol. Aging 34 (1708), e7–e13.
[19] Palacios, L., Ochoa, B., Gomez-Lechon, M.J., Castell, J.V. and Fresnedo, O. (2006) Overexpression of SND p102, a rat homologue of p100 coactivator, promotes the secretion of lipoprotein phospholipids in primary hepatocytes. Biochim. Biophys. Acta 1761, 698–708.
[20] Yang, J., Valineva, T., Hong, J., Bu, T., Yao, Z., Jensen, O.N., Frilander, M.J. and Silvennoinen, O. (2007) Transcriptional co-activator protein p100 interacts with snRNP proteins and facilitates the assembly of the spliceosome. Nucleic Acids Res. 35, 4485–4494.
[21] Roger, B. et al. (2011) Adenylyl cyclase 8 is central to glucagon-like peptide 1 signalling and effects of chronically elevated glucose in rat and human pancreatic beta cells. Diabetologia 54, 390–402.
[22] Shibata, A., Ludvigsen Jr., C.W., Naber, S.P., McDaniel, M.L. and Lacy, P.E. (1976) Standardization fo a digestion-filtration method for isolation of pancreatic islets. Diabetes 25, 667–672.
[23] Boal, F., Le Pevelen, S., Cziepluch, C., Scotti, P. and Lang, J. (2007) Cysteine-string protein isoform beta (Cspbeta) is targeted to the trans-Golgi network as a non-palmitoylated CSP in clonal beta-cells. Biochim. Biophys. Acta Mol. Cell Res. 1773, 109–119.
[24] Boal, F., Laguerre, M., Milochau, A., Lang, J. and Scotti, P.A. (2011) A charged prominence in the linker domain of the cysteine-string protein Cspalpha mediates its regulated interaction with the calcium sensor synaptotagmin 9 during exocytosis. FASEB J. 25, 132–143.
[25] Liu, J., Valencia-Sanchez, M.A., Hannon, G.J. and Parker, R. (2005) MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. Nat. Cell Biol. 7, 719–723.
[26] Broadhurst, M.K., Lee, R.S., Hawkins, S. and Wheeler, T.T. (2005) The p100 EBNA-2 coactivator: a highly conserved protein found in a range of exocrine and endocrine cells and tissues in cattle. Biochim. Biophys. Acta 1681, 126–133.
[27] Keenan, T.W., Winter, S., Rackwitz, H.R. and Heid, H.W. (2000) Nuclear coactivator protein p100 is present in endoplasmic reticulum and lipid droplets of milk secreting cells. Biochim. Biophys. Acta 1523, 84–90.
[28] Caudy, A.A. et al. (2003) A micrococcal nuclease homologue in RNAi effector complexes. Nature 425, 411–414.
[29] Pang, Z.P. and Südhof, T.C. (2010) Cell biology of Ca^{2+}-triggered exocytosis. Curr. Opin. Cell Biol. 22, 496–505.
[30] Craxton, M. (2004) Synaptotagmin gene content of the sequenced genomes. BMC Genomics 5, 43.

[31] Chen, X., Simon, E.S., Xiang, Y., Kachman, M., Andrews, P.C. and Wang, Y. (2010) Quantitative proteomics analysis of cell cycle-regulated golgi disassembly and reassembly. J. Biol. Chem. 285, 7197–7207.
[32] Foster, L.J., de Hoog, C.L., Zhang, Y., Xie, X., Mootha, V.K. and Mann, M. (2006) A mammalian organelle map by protein correlation profiling. Cell 125, 187–199.
[33] Fukuda, M. and Mikoshiba, K. (2001) Characterization of KIAA1427 protein as an atypical synaptotagmin (Syt XIII). Biochem. J. 354, 249–257.
[34] Cikaluk, D.E., Tahbaz, N., Hendricks, L.C., DiMattia, G.E., Hansen, D., Pilgrim, D. and Hobman, T.C. (1999) GERp95, a membrane-associated protein that belongs to a family of proteins involved in stem cell differentiation. Mol. Biol. Cell 10, 3357–3372.
[35] Fischer von Mollard, G. and Stevens, T.H. (1998) A human homolog can functionally replace the yeast vesicle-associated SNARE Vti1p in two vesicle transport pathways. J. Biol. Chem. 273, 2624–2630.
[36] Siddiqi, S.A., Siddiqi, S., Mahan, J., Peggs, K., Gorelick, F.S. and Mansbach 2nd, C.M. (2006) The identification of a novel endoplasmic reticulum to Golgi SNARE complex used by the prechylomicron transport vesicle. J. Biol. Chem. 281, 20974–20982.
[37] Huynh, D.P., Scoles, D.R., Nguyen, D. and Pulst, S.M. (2003) The autosomal recessive juvenile Parkinson disease gene product, parkin, interacts with and ubiquitinates synaptotagmin XI. Hum. Mol. Genet. 12, 2587–2597.
[38] Mouatt-Prigent, A., Muriel, M.P., Gu, W.J., El Hachimi, K.H., Lucking, C.B., Brice, A. and Hirsch, E.C. (2004) Ultrastructural localization of parkin in the rat brainstem, thalamus and basal ganglia. J. Neural Transm. 111, 1209–1218.
[39] Rual, J.F. et al. (2005) Towards a proteome-scale map of the human protein-protein interaction network. Nature 437, 1173–1178.
[40] Mizutani, A., Fukuda, M., Ibata, K., Shiraishi, Y. and Mikoshiba, K. (2000) SYNCRIP, a cytoplasmic counterpart of heterogeneous nuclear ribonucleoprotein R, interacts with ubiquitous synaptotagmin isoforms. J. Biol. Chem. 275, 9823–9831.
[41] Fashe, T., Saarikettu, J., Isomäki, P., Yang, J. and Silvennoinen, O. (2013) Expression analysis of Tudor-SN protein in mouse tissues. Tissue Cell 45, 21–31.
[42] Yang, J., Aittomaki, S., Pesu, M., Carter, K., Saarinen, J., Kalkkinen, N., Kieff, E. and Silvennoinen, O. (2002) Identification of p100 as a coactivator for STAT6 that bridges STAT6 with RNA polymerase II. EMBO J. 21, 4950–4958.
[43] Yang, W., Chendrimada, T.P., Wang, Q., Higuchi, M., Seeburg, P.H., Shiekhattar, R. and Nishikura, K. (2006) Modulation of microRNA processing and expression through RNA editing by ADAR deaminases. Nat. Struct. Mol. Biol. 13, 13–21.
[44] Sundström, J.F. et al. (2009) Tudor staphylococcal nuclease is an evolutionarily conserved component of the programmed cell death degradome. Nat. Cell Biol. 11, 1347–1354.
[45] Scadden, A.D. (2007) Inosine-containing dsRNA binds a stress-granule-like complex and downregulates gene expression in trans. Mol. Cell 28, 491–500.
[46] Garcia-Arcos, I., Rueda, Y., Gonzalez-Kother, P., Palacios, L., Ochoa, B. and Fresnedo, O. (2010) Association of SND1 protein to low density lipid droplets in liver steatosis. J. Physiol. Biochem. 66, 73–83.
[47] Shaw, N. et al. (2007) The multifunctional human p100 protein 'hooks' methylated ligands. Nat. Struct. Mol. Biol. 14, 779–784.
[48] Friberg, A., Corsini, L., Mourao, A. and Sattler, M. (2009) Structure and ligand binding of the extended Tudor domain of D. melanogaster Tudor-SN. J. Mol. Biol. 387, 921–934.
[49] Valineva, T., Yang, J., Palovuori, R. and Silvennoinen, O. (2005) The transcriptional co-activator protein p100 recruits histone acetyltransferase activity to STAT6 and mediates interaction between the CREB-binding protein and STAT6. J. Biol. Chem. 280, 14989–14996.
[50] Li, C.L., Yang, W.Z., Chen, Y.P. and Yuan, H.S. (2008) Structural and functional insights into human Tudor-SN, a key component linking RNA interference and editing. Nucleic Acids Res. 36, 3579–3589.
[51] Paukku, K., Kalkkinen, N., Silvennoinen, O., Kontula, K.K. and Lehtonen, J.Y. (2008) P100 increases AT1R expression through interaction with AT1R 3'-UTR. Nucleic Acids Res. 36, 4474–4487.
[52] Jin, P. et al. (2004) Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. Nat. Neurosci. 7, 113–117.
[53] Tattikota, S.G. et al. (2014) Argonaute2 mediates compensatory expansion of the pancreatic β-cell. Cell Metab. 19, 122–134.
[54] Tattikota, S.G. et al. (2013) Argonaute2 regulates the pancreatic beta-cell secretome. Mol. Cell Proteomics 12, 1214–1225.
[55] Gibbings, D.J., Ciaudo, C., Erhardt, M. and Voinnet, O. (2009) Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. Nat. Cell Biol. 11, 1143–1149.
[56] Lee, Y.S. et al. (2009) Silencing by small RNAs is linked to endosomal trafficking. Nat. Cell Biol. 11, 1150–1156.
[57] Li, S. et al. (2013) MicroRNAs inhibit the translation of target mRNAs on the endoplasmic reticulum in Arabidopsis. Cell 153, 562–574.
[58] Stalder, L. et al. (2013) The rough endoplasmatic reticulum is a central nucleation site of siRNA-mediated RNA silencing. EMBO J. 32, 1115–1127.