*Invited Review*

**Exocytosis Mechanisms Underlying Insulin Release and Glucose Uptake: Conserved Roles for Munc18c and Syntaxin 4**

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Abstract

Type 2 diabetes has been coined “a two hit disease” as it involves specific defects of glucose-stimulated insulin secretion from the pancreatic beta cells in addition to defects in peripheral tissue insulin action required for glucose uptake. Both of these processes, insulin secretion and glucose uptake, are mediated by SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) protein core complexes composed of Syntaxin, SNAP-23/25, and VAMP proteins. The SNARE core complex is regulated by the Sec1/Munc18 (SM) family of proteins, which selectively bind to their cognate Syntaxin isoforms with high affinity. The process of insulin secretion utilizes multiple Munc18-Syntaxin isoform pairs, whereas insulin action in the peripheral tissues appears to utilize only the Munc18c-Syntaxin 4 pair. Importantly, recent reports have linked obesity and Type 2 diabetes in humans with changes in protein levels and single nucleotide polymorphisms (SNPs) of Munc18 and Syntaxin isoforms relevant to these exocytotic processes, although the molecular mechanisms underlying the observed phenotypes remain incomplete (5, 104, 144). Given the conservation of these proteins in two seemingly disparate processes, and the need to design and implement novel and more effective clinical interventions, it will be vitally important to delineate the mechanisms governing these conserved SNARE-mediated exocytosis events. Thus, we provide here an up-to-date historical review of advancements in defining the roles and molecular mechanisms of Munc18-Syntaxin complexes in the pathophysiology of Type 2 Diabetes.

Keywords: SM proteins, glucose homeostasis, diabetes, insulin resistance, SNARE proteins, glucose-stimulated insulin secretion
COORDINATION OF WHOLE BODY GLUCOSE HOMEOSTASIS

Circulating blood glucose levels are tightly regulated in mammals and are maintained at about 5 mM (~80-100 mg/dL). Following intake of high-carbohydrate food, blood glucose levels rise to approximately 8 mM (~120-140 mg/dL), which under normal homeostatic circumstances induces the pancreas to secrete insulin from the beta cells within the Islets of Langerhans. This release of insulin subsequently signals to the liver to reduce glucose output while simultaneously inducing clearance of excess glucose from the blood by the skeletal muscle and adipose tissue. Normally, this process restores the blood glucose levels to 5 mM within 2 hours after the meal. However during pathological progression from a normal to a clinically-defined Type 2 diabetic phenotype, there are clear and progressive aberrations in both insulin secretion as well as glucose uptake/clearance mechanisms. Given that a significant number of proteins required for insulin secretion and glucose clearance are identical, alterations in their abundance and/or function would materially impact both mechanisms and increase susceptibility to aberrant glucose homeostasis.

SNARE-MEDIATED EXOCYTOSIS

The SNARE core complex is composed of three proteins in a heterotrimeric 1:1:1 ratio: 1) Syntaxin, a 35 kDa protein containing an N-terminal regulatory domain, a C-terminal SNARE domain, and a far C-terminal transmembrane domain anchoring it to the plasma membrane (PM); 2) SNAP23 or SNAP25, two complementary isoforms that lack transmembrane domain anchors but localize to the PM due to palmitoylated cysteine residues; and 3) VAMP2 (also known as synaptobrevin), an 18 kDa protein containing a far C-terminal transmembrane domain anchoring
it to the vesicle membrane. As depicted in Fig. 1, the vesicle (v-) SNARE VAMP2 pairs with the two target PM (t-) SNAREs Syntaxin and SNAP23/25 forming the SNARE core complex. Together, the three SNARE proteins produce a stable bundle of the four α-helices, with one α-helix from VAMP, one from Syntaxin, and the remaining two from SNAP23/25 [reviewed in (52)]. This complex is remarkably SDS-resistant once formed. To date, 6 v-SNARE isoforms and 13 t-SNARE isoforms have been identified in cell types relevant to insulin secretion and insulin action (Table 1). Interestingly, the underlying vesicle/granule exocytosis events of insulin secretion and glucose uptake share numerous commonalities with those of neuronal synaptic vesicle exocytosis (Table 2). Glucose-stimulated insulin secretion (GSIS) from the pancreatic beta cell and glucose uptake in the muscle and adipose are both mediated by the same SNARE protein isoforms: Syntaxin 4, SNAP23 and VAMP2 [reviewed in (29, 131)]. Additionally, beta cells use t-SNARE isoforms Syntaxin 1 and SNAP25 to mediate the first-phase of insulin secretion (27). However, key differences exist which distinguish the specialization of events in each cell type (summarized in Table 2).

INSULIN GRANULE EXOCYTOSIS

Elevated post-prandial glucose levels trigger a signaling cascade in the pancreatic islet beta cells to elicit insulin release (Fig. 2), depicted as an ~8-step process [reviewed in (64, 100)]. First, glucose entry into beta cells is facilitated via the plasma membrane-localized glucose transporter, GLUT2. GLUT2 has a relatively low affinity (K_M ~30 mM) for glucose, is constitutively present within the plasma membrane, and does not require SNARE proteins for translocation and membrane localization. Following GLUT2-facilitated uptake, glucose is phosphorylated by glucokinase to generate glucose-6-phosphate, which is subsequently
metabolized via mitochondrial oxidative phosphorylation to effect an increase in intracellular ATP:ADP ratio (step 2). Elevated beta cell ATP levels induce closure of ATP-dependent potassium channels (step 3), resulting in cell depolarization (step 4) and an influx of calcium ions (Ca^{2+}) through voltage-dependent calcium channels (step 5) to yield a net increase in the intracellular Ca^{2+} concentration ([Ca^{2+}]_i; step 6). The increase in [Ca^{2+}]_i signals SNARE complex formation (step 7) to facilitate insulin release from the granules (step 8), although the precise mechanism(s) by which Ca^{2+} triggers granule fusion remains somewhat unresolved.

Insulin secretion occurs in pulsatile fashion in sync with Ca^{2+} influxes during two major phases, and is termed ‘biphasic’. First-phase insulin secretion occurs within 5-10 minutes following beta cell stimulation. Second-phase is less robust than first-phase, but can be sustained for several hours if elevated blood-glucose levels persist (21, 40, 45, 46). These two phases of secretion are thought to utilize separate pools of insulin-containing granules. First-phase secretion appears to arise from plasma membrane pre-docked granules, termed the ‘readily releasable pool’ (RRP), while second-phase secretion is believed to involve release from a granule pool deeper within the cell, the ‘storage-granule pool,’ which presumably replenishes the RRP (4, 99). In addition, KCl and other non-nutrient secretagogues can induce a first-phase type release, while only fuel-type secretagogues, such as glucose, can produce a sustained second-phase insulin release (36). First and second-phase release events also differ in their requisite SNARE protein isoforms. First-phase insulin release utilizes Syntaxin 1A, Syntaxin 4, SNAP25 or SNAP23, and the v-SNARE VAMP2, whereas second-phase secretion is managed by Syntaxin 4, SNAP25 or SNAP23, and VAMP2, but specifically not Syntaxin 1A (Table 3).
GLUT4 VESICLE TRANSLOCATION

Approximately 80% of homeostatic glucose clearance is handled by skeletal muscle, with the remainder by adipose and other insulin responsive tissues. Skeletal muscle glucose clearance involves transduction of the extracellular insulin signal into intracellular signaling events to induce translocation of intracellular GLUT4 vesicles to the surface of muscle cell t-tubule and sarcolemmal membranes. In this process, SNARE-regulated GLUT4 vesicle fusion is the most distal event [reviewed in (14, 50, 155)]. Under basal conditions, GLUT4 protein is localized to intracellular vesicles within cells, and unlike GLUT2, GLUT4 has a higher affinity for glucose ($K_M \sim 4$ mM), thus providing a steep gradient for rapid glucose clearance. Insulin signaling to evoke GLUT4-mediated glucose clearance entails at least 11 steps, as modeled in Fig. 3. The process ensues with insulin binding to extracellular $\alpha$-subunits of the insulin receptor (IR) present on the surface of muscle cells and adipocytes (step 1), inducing tyrosine autophosphorylation within the IR protein and increased kinase activity of the IR $\beta$-subunits (step 2). Tyrosine autophosphorylation of the IR fosters recruitment of IR substrates, including the canonical Insulin Receptor Substrate (IRS), typically through a phosphotyrosine binding (PTB) domain. Once bound, substrates are themselves tyrosine-phosphorylated. Phosphorylated receptor substrates then serve as additional recruitment targets for specific proteins containing Src Homology 2 (SH2) domains, including phosphatidylinositol kinase (PI3-Kinase) (step 3). Once recruited, PI3-Kinase is activated to catalyze the phosphorylation of phosphatidylinositol (4, 5) bisphosphate (PIP2) at the 3’ position, yielding phosphatidylinositol (3, 4, 5) trisphosphate (PIP3) (step 4). 3-Phosphoinositide-dependent kinase-1 (PDK-1) is able to recognize the 3’ position of PIP3 with its PH (Pleckstrin homology) domain and is recruited to the plasma membrane where it is activated (step 5). At the plasma membrane, PDK-1 phosphorylates and
activates AKT/PKB (also known as protein kinase B, step 6) along with atypical PKC isoforms zeta and lambda (aPKC). As well-defined as these initial signaling steps are, the identities of downstream substrates/targets of these serine/threonine kinases which result in GLUT4 vesicle translocation remain unclear, although some recent progress has been made: Akt signals downstream (step 7) to AS160 (a Rab GTPase-activating protein) (112), and AS160 signals downstream to multiple Rab targets (step 8) (72). Rab proteins have been described in GLUT4-containing vesicles (step 9), and are presumed to facilitate their trafficking to/docking at the plasma membrane. However, a gap exists beyond this step to link to the SNARE complex at the plasma membrane: t-SNARE isoforms Syntaxin 4 and SNAP23 with the v-SNARE VAMP2 are known to be required for GLUT4 vesicles to fuse with the plasma membrane (step 10) to facilitate glucose uptake into the skeletal muscle cell (step 11).

Independent of insulin, exercise and muscle contraction have also been shown to increase glucose uptake into skeletal muscle, although far less is known regarding the requirements and mechanisms of SNARE proteins in this process. It is clear that exercise increases the translocation of GLUT4 and VAMP2 to the plasma membrane of human and rat skeletal muscle (61, 105). VAMP3 was also initially implicated in contraction-stimulated GLUT4 translocation, however subsequent studies of skeletal muscle from the VAMP3 (-/-) mice failed to support this role (169). With the plethora of knockout mouse models of SNARE proteins now available, it is anticipated that the requirements and roles for these proteins in exercise-stimulated glucose uptake will be soon forthcoming.
DIFFERENTIAL SNARE ISOFORM FUNCTION IN EXOCYTOSIS EVENTS OF GLUCOSE HOMEOSTASIS

Insulin secreting islet beta cells and insulin-responsive muscle and adipose cells contain multiple isoforms of each of the SNARE proteins required for the distal exocytosis events occurring at the plasma membrane (Table 1).

The Syntaxin Family

The pancreatic beta cell expresses plasma membrane-localized Syntaxin isoforms 1A, 2, 3 and 4, though additional non-plasma membrane Syntaxin isoforms are also expressed, albeit their function in exocytosis remains untested (Table 1). To date, only isoforms 1A and 4 are clearly known as required for insulin exocytosis (85, 122), primarily from data obtained using knockout mouse models. Mice with Syntaxin 1A deficiency show selectively impaired insulin release during the first phase, while islets from Syntaxin 4 heterozygous (-/+ ) knockout mice display defects in both first and second phases of GSIS. Furthermore, islets isolated from transgenic mice over-expressing Syntaxin 4 in the islet secrete approximately 30% more insulin in both phases, implying a positive role for Syntaxin 4 in both first- and second-phase insulin secretion (122). Oddly, Syntaxin 1 over-expressing transgenic mice show insulin resistance with impaired insulin secretion (63). Although the molecular basis for this phenotype is unclear, it has been hypothesized that stoichiometry of particular SNARE proteins in cells is crucial for optimal function in exocytosis.

In contrast to pancreatic beta cells and neuronal cells, neither muscle nor adipose tissues—two of the primary insulin-responsive tissues—express Syntaxin 1, but instead appear to rely upon Syntaxin 4 (51, 137, 151). Syntaxin 4 (-/+ ) mice exhibit a blunted insulin-stimulated
GLUT4 translocation and decreased glucose uptake into skeletal muscle (168). Consistent with this, transgenic mice over-expressing Syntaxin 4 in skeletal muscle tissue show a two-fold increase in GLUT4 translocation into the sarcolemmal and t-tubule membranes of hindlimb muscle (120). Syntaxin 4 is thus the only syntaxin isoform currently known to be required for insulin-stimulated GLUT4 vesicle translocation. Recently, GLUT4 was found to be expressed in the hypothalamus, suggesting that Syntaxin 4 has a role in the brain as well (9). Syntaxin isoforms 2, 3, 5, 6, 7, 8 and 12 are reportedly expressed in adipocytes but not as critical participants in insulin-stimulated GLUT4 vesicle exocytosis (Table 1).

**SNAP25, SNAP23 and SNAP29**

SNAP25, the principal neuronal isoform, is present in the beta cell but absent from muscle and adipose cells (108). SNAP23 is also expressed in beta cells, and is capable of compensating for an absence of SNAP25 (107). However, skeletal muscle and adipose express and utilize only SNAP23 (2, 57, 95). SNAP29 is also widely expressed (124), though differs from SNAP23 and SNAP25 in that it binds to intracellularly-localized syntaxin isoforms in addition to the plasma membrane-bound syntaxins (48). SNAP29 has not yet been reported to function in insulin exocytosis or GLUT4 vesicle exocytosis.

**The VAMP family**

There are seven currently identified VAMP isoforms, all of which are attached by a C-terminal transmembrane domain to vesicle/granule membranes, including insulin granules, synaptic vesicles, GLUT4-containing vesicles, or ER-Golgi compartments (17). Islet beta cells express VAMP2/synaptobrevin, VAMP3/cellubrevin and VAMP8, with VAMP2 as the
predominate isoform required for GSIS (77, 96, 106). Remarkably, 3T3L1 adipocytes express all VAMP isoforms except for VAMP1 (160), but only VAMP2, VAMP3, and VAMP7 have been directly linked to GLUT4 vesicle exocytosis (69, 169). In skeletal muscle, VAMP2, 3, 5 and 7 co-immunoprecipitate with GLUT4 vesicles, and all but VAMP3 translocate to the plasma membrane with GLUT4 in response to contraction (105). In L6 myoblasts, VAMP7/TI-VAMP is expressed and required for both insulin stimulated and osmotic shock triggered-GLUT4 vesicle translocation (67).

The Sec1/Munc18 (SM) protein family

In the early 1990’s, the yeast Sec1p protein was implicated as a regulator of SNARE assembly and exocytosis function, through its ability to directly interact with Syntaxin. Homologues were subsequently identified in C. elegans (unc18), D. melanogaster (Rop), and mammals [reviewed in (103)]. Collectively, proteins of this type are referred to as ‘SM’ proteins, for Sec1/Munc18. The mammalian members of this family, Munc18 proteins, are ~66-68 kDa soluble proteins with no apparent transmembrane domain, yet are frequently found at the plasma membrane through direct interaction with their cognate Syntaxins (43, 132). Plasma membrane-associated SM proteins present in mammalian cells include Munc18a, Munc18b and Munc18c (also referred to as -1, -2 and -3, respectively); non-plasma membrane-associated mammalian SM proteins are mVps45 and mSly1. Endogenous Munc18a (referred to as Munc18-1 hereafter) and Munc18b bind to the Syntaxin isoforms 1-3, whereas Munc18c binds with high-affinity solely to Syntaxin 4 (Table 4).

The regions/residues within the SM proteins that are responsible for syntaxin partnering specificity are still undetermined, remarkable given the high degree of similarity that exists
amongst Munc18 isoforms (Munc18b and Munc18c show 62% and 51% amino acid identity, respectively, to Munc18-1). Of the many proteins known to bind directly to syntaxins in both insulin-secreting and insulin-responsive cell types, Munc18 proteins bind with highest affinity (54). Munc18-Syntaxin complexes are found principally at the plasma membrane. However, the Munc18 proteins are soluble and equally abundant in the cytosolic compartment as they are at the membrane, though cytosolic Munc18 proteins are not associated with syntaxins (30, 132, 134). Munc18 proteins are presumed to localize to the plasma membrane by association with membrane-localized proteins, such that increased expression of Syntaxin 4 selectively attracts Munc18c to the plasma membrane (132). The purpose or function of soluble Munc18 within the cytosolic cellular compartment is currently unknown. Islet beta cells express all three isoforms, while adipocytes and skeletal muscle express only Munc18b and Munc18c (130, 158). Depletion studies using RNAi or genetic ablation of either Munc18-1 or Munc18c typically show loss of exocytic function, indicative of their conserved functional importance in SNARE-mediated exocytosis events (82, 83, 147).

**MUNC18 AND SNARE PROTEIN MOUSE MODELS: ALTERATIONS OF GLUCOSE HOMEOSTASIS**

Reduced protein and/or mRNA levels of Syntaxin 1A, Syntaxin 4 and/or Munc18c have been reported in islets and skeletal muscle of diabetic and obese human patients (5, 89). Similarly, rodent models of obesity and diabetes, including the GK rat, Zucker rat, ob/ob and streptozotocin-induced diabetes mouse models exhibit significantly lower levels of these same SNARE isoforms (35, 59, 78, 170). In corroborating fashion, numerous knockout and transgenic mouse models selectively targeted for SNARE and Munc18 proteins have defects in glucose
homeostasis (Table 3). As nearly all of the classic whole-body SNARE and Munc18 protein homozygous knockout mice die either in embryogenesis or at birth, the majority of the current understanding arises from studies utilizing haploinsufficient mouse models. Overall, data generally support the concept that Type 2 Diabetes is a polygenic disease, likely emanating from haploinsufficiencies. Since these are in vivo models of altered glucose homeostasis, effects upon whole body homeostasis as well as the tissue-specific effects underlying the whole body phenotypes are discussed together below.

**Syntaxin mouse models:** Shown in Table 3, mouse models of Syntaxin 1A protein over-expression (beta cell specific transgenic), Syntaxin 4 protein over-expression (pancreas, skeletal muscle and adipose specific transgenic), Syntaxin 1A and/or Syntaxin 1B deficient and Syntaxin 4 haploinsufficient have been generated, and many characterized for glucose homeostatic control.

**Syntaxin 1:** Consistent with clonal cell studies and Syntaxin 1A ablation/interference, islets isolated from classic whole-body Syntaxin 1A knockout mice display impaired first-phase insulin release associated with a decrease in pre-docked granules as determined by total internal reflection fluorescence microscopy (TIRFM) and electron microscopy (EM), and show normal expression levels of Munc18-1 and Munc18b (33, 85). Surprisingly, mice with beta-cell specific over-expression of Syntaxin 1A display fasting hyperglycemia, hypoinsulinemia, and impaired glucose tolerance (63). One possible explanation for this disparate phenotype may be reduced Munc18-1 levels in the Syntaxin 1A over-expressing islets, but the cause of the paucity of Munc18-1 is unclear. Additionally, non-tissue specific knock-in/knockout mice engineered with two mutations in Syntaxin 1B (L165A/E166A) presumed to confer an ‘open’ state Syntaxin 1B
molecule, on a Syntaxin 1A knockout background have been generated (37), though effects upon insulin secretion have not yet been reported.

**Syntaxin 4:** Syntaxin 4 homozygous (-/-) null mice die early in embryogenesis, apparently due to a requirement for Syntaxin 4 in the fusion of the GLUT8-containing vesicle with the plasma membrane in the mouse blastocyst (162). However, Syntaxin 4 heterozygous (+/-) knockout mice are viable and exhibit insulin resistance and impaired insulin secretion (122, 168). This insulin resistance is largely due to significantly reduced skeletal muscle glucose uptake and GLUT4 translocation, while insulin secretion deficit is attributed to decreased first- and second-phase insulin release (122). Notably, in addition to the expected 50% decrease in Syntaxin 4 protein in the Syntaxin 4 (-/-) mouse tissues, Munc18c protein levels were decreased in parallel, while no other protein levels were altered (168). In consistent fashion, Syntaxin 4 over-expressing transgenic mice show a parallel upregulation of endogenous Munc18c protein abundance in the three tissues over-expressing the Syntaxin 4 transgene: adipose, skeletal muscle and pancreas (120). Syntaxin 4 transgenic mice show increased insulin sensitivity, which is likely linked to their increased GLUT4 translocation and enriched GLUT4 deposition in the sarcolemmal and t-tubule membranes of skeletal muscle (120). Islets isolated from Syntaxin 4 transgenic mice also exhibit 30% greater GSIS during both phases (122). These data corroborate studies which correlate reduced Syntaxin 4 and Munc18c protein levels with aberrant insulin action in human and mouse skeletal muscle (5, 170). Collectively, these findings raise the possibility that strategies which increase Syntaxin 4 protein levels may coordinately protect against the development of insulin secretion and insulin resistance defects.

Unlike skeletal muscle and islet cells, primary adipocytes do not exhibit significant changes to glucose uptake, either in the Syntaxin 4 over-expressing transgenic mice or the
Syntaxin 4 (+/-) knockout mice (120, 168). Although this seemingly contrasts with the earlier
3T3-L1 adipocyte studies implicating Syntaxin 4 in glucose uptake, it is important to note that
those early studies utilized a dominant-negative mutant form of Syntaxin 4 to interfere with
endogenous VAMP2 trafficking and ablate insulin-stimulated GLUT4 translocation (87); an
approach which does not necessarily reflect requirement for Syntaxin 4. As such, whether or not
there is an absolute requirement for Syntaxin 4 in GLUT4 vesicle translocation in adipocytes,
or—assuming Syntaxin 4 is required—the minimum “threshold” Syntaxin 4 required, remain
questions open for investigation.

Munc18 mouse models:

SM proteins in yeast, flies, and worms have been universally characterized as positive
and essential regulators of exocytosis events. Moreover, the impairment phenotypes of Munc18-
1 (+/-) knockout (142, 147) and Munc18c (+/-) knockout (82, 83) mouse models indeed support
a positive required role of these SM proteins in exocytosis events in vivo (Munc18-1 and
Munc18c homozygous knockouts are lethal). In terms of glucose homeostasis, the Munc18c
heterozygous knockout mouse model exhibits glucose intolerance due to peripheral insulin
resistance coupled with deficient GSIS (82, 83). Insulin-stimulated GLUT4 vesicle translocation
in the Munc18c (+/-) hindlimb skeletal muscle was dramatically abolished, indicating that
Munc18c deficit is likely responsible for the peripheral insulin resistance (82). In terms of the
GSIS impairment, RNAi-mediated depletion of Munc18c from isolated islets or clonal beta cells
in culture thoroughly recapitulate the defective GSIS seen in the Munc18c (-/+ ) knockout islets
(83). In contrast to previously discussed studies of SM proteins as positive factors in exocytosis,
a line of MEF-derived adipocytes from a second Munc18c (-/-) mouse model showed increased
GLUT4 presence in plasma membrane subcellular fractions (56). However, since this model consists of derived rather than primary cells or tissues, direct comparisons cannot be made. With regards to the role of Munc18-1 in glucose homeostasis, the Munc18-1 (-/+), have yet to be assessed for defects. However, intriguing data gained by RNAi-mediated depletion of Munc18-1 from clonal beta cells suggest they would likely have defects in GSIS that would contribute to glucose intolerance (139).

A transgenic mouse model of Munc18c protein over-expression in adipose, skeletal muscle and pancreas, akin to the Syntaxin 4 over-expression transgenic mouse model (Table 3), exhibits peripheral insulin resistance resulting from impaired insulin-stimulated glucose uptake and GLUT4 vesicle exocytosis, and decreased GSIS (121). This in vivo phenotype fully recapitulated data from Munc18c protein over-expression in 3T3-L1 adipocytes, skeletal muscle and islet beta cells (60, 84, 126, 132). Notably, Munc18c transgenic mice and Munc18c (+/-) knockout mice have normal Syntaxin 4 protein abundance, suggesting that Syntaxin 4 expression directs Munc18c expression, but not vice versa (82, 121).

The negative effect of SM protein over-expression has been recapitulated in Drosophila (42). Consensus interpretation of these data postulates that the overly abundant SM proteins bind to and sequester cognate endogenous Syntaxins, preventing interaction with the v-SNARE proteins, and in doing so impair exocytosis. However, Munc18-1 over-expression in neuronal cells in culture rather enhances exocytosis (141), and over-expression of Munc18-1 in clonal beta cells has no effect upon insulin secretion [(139); Oh and Thurmond, unpublished results]. With regards to whether SM proteins have a clear positive or negative role, these disparate results may arise from differential protein abundance and stoichiometry, since over-expression studies rarely control for protein expression level on a per cell basis. Alternatively, the differences may be
related to the different types of exocytosis reactions in which Munc18-1 and Munc18c reportedly participate, with Munc18-1 functioning in rapid vesicle release (synaptic neurotransmission and first-phase insulin release) and Munc18c serving in sustained second phase insulin release and GLUT4 vesicle exocytosis events (Table 2).

**VAMP and SNAP mouse models:**

Of the two v-SNARE isoforms found in insulin-secreting and insulin-responsive cell types, only VAMP3 (-/-) knockout mice have been characterized for glucose homeostasis. Though VAMP3 was initially implicated in GLUT4 vesicle translocation in 3T3-L1 adipocytes (87, 125, 150) using dominant-negative and toxin cleavage approaches, VAMP3 null mice show normal insulin and glucose tolerance (169). Insulin-stimulated glucose uptake into adipocytes is normal in these mice, suggesting that VAMP3 is dispensable for GLUT4 translocation, perhaps due to compensation from VAMP2. VAMP2 (+/-) knockout mice thrive while the null mice die immediately after birth (114). Furthermore, calcium-triggered synaptic vesicle exocytosis in neurons of VAMP2 haploinsufficient mice is significantly impaired, and given the numerous overlaps between this neuronal process and that of first-phase insulin release from the beta cell, it is anticipated that VAMP2 would be required for glucose homeostasis *in vivo*, provided it remains the only other v-SNARE protein in these tissue types. VAMP8 (-/-) knockout mice survive and have apparent defects in pancreatic acinar cell zymogen granule content and platelet secretion (98, 152), although have yet to be characterized for glucose homeostasis.

Although SNAP25 homozygous (-/-) knockout mice fail to thrive beyond birth, studies conducted using embryonic and fetal tissue have been demonstrated a lack of evoked synaptic vesicle exocytosis (154). While SNAP25 heterozygous (+/-) knockout mice do thrive, no
characterizations of potential effects upon whole body glucose homeostasis or insulin release from isolated islets are reported to date. No SNAP23 knockout mice are reported in the current literature.

MUNC18-SYNTAXIN COMPLEXES: MOLECULAR MECHANISMS

**Protein-protein interaction studies—*in vitro:***

The functional *in vivo* models and cell-based studies of SM and Syntaxin proteins indicate SM-Syntaxin coupling is the key to understanding exocytosis events, and has direct pertinence to many diseases. By 1999, the first structures of Munc18-1 bound to Syntaxin 1A were revealed, solved using crystallographic and NMR approaches (8, 25, 74), where Munc18-1 was seen to hold Syntaxin in a ‘closed’ conformation in a 1:1 stoichiometric complex, and interpreted as a conformation precluding Syntaxin participation in SNARE core complex assembly (modeled in Fig. 4A). Studies of SNARE proteins in cell and tissue lysates support this model, along with *in vitro* ultracentrifugation studies showing the exclusion of Munc18-1/nSec1 protein from the SNARE core complex (32). More recently, the Munc18c isoform has been co-crystallized with the N-terminal 19 residue peptide of Syntaxin 4, indicating the importance of this particular site for protein-protein interaction (49). While it was initially argued that this interaction fostered a new conformation (modeled in Fig. 4B), subsequent studies in adipocytes and beta cells cast doubt on its relative importance in relevant exocytosis events, as the same peptide failed to confer binding to Munc18c and is ineffective as a competitive inhibitor (22, 54).
A third binding mode has recently been proposed, whereby the SM protein associates with the four alpha-helical bundle comprised by the SNARE core complex (modeled as Fig. 4C). Support for this new binding mode comes from in vitro reconstitution assays using recombinant Munc18-1, Syntaxin 1A, SNAP-25 and VAMP2 proteins (115). This model relies upon the hypothesis that Munc18-1 functions principally as a positive and necessary factor to promote exocytosis, consistent with the mouse model data. The latest mechanistic hypothesis incorporates the SNARE binding protein Complexin (38, 70). Complexins (CPXs), also named synaphtins, were originally described in neuronal cells to play an essential role in Ca\textsuperscript{2+}-dependent neurotransmitter release (71, 97, 138). In vitro, complexins bind to assembled heterotrimeric SNARE complexes (16). However the question of whether they promote or inhibit SNARE-regulated exocytosis is unresolved due to conflicting in vivo data (11). Complexins do not appear to bind individual SNARE proteins, but a recent study shows that CPX1 can bind to Syntaxin/SNAP25 binary complexes (157). Furthermore, it has been reported that Munc18-1 and CPX1 can bind simultaneously to the SNARE complex (24). Complexin expression in beta cells was noted prior to the emergence of the current mechanistic model (1), and as such its linkage to the insulin exocytosis mechanism has not yet been determined.

Notably, detection of Munc18-SNARE complex association, as depicted in Figs. 4B and 4C, requires low-stringency assay conditions, where little to no detergent is included (<0.1% Triton X-100). In contrast, older titration studies performed under higher stringency conditions demonstrated the ability of Munc18-1 to displace SNAP25 and VAMP2 from Syntaxin 1 (43, 93, 167). Similarly, Munc18c reduces the binding of SNAP23 to Syntaxin 4 in a concentration-dependent manner when evaluated in 1% Triton X-100 solubilized cell lysates (2), yet under low-stringency detergent conditions Munc18c binding to Syntaxin 4-bound SNARE core...
complexes was detected (65). While the benefit of low-stringency buffers has enabled the ability to detect the otherwise elusive transient docking complex, co-immunoprecipitation data obtained from cell or tissue lysates currently supports only Model A (Figure 4A). Interestingly, a novel immunofluorescent approach conducted in cells supports the concept of an intermediate transition complex of Syntaxin 1 bound simultaneously to Munc18-1 and SNAP25, and that the addition of VAMP2 subsequently displaced Munc18-1 (174). Biochemical validation of the formation of this and the macromolecular complexes in Models B and C by endogenous proteins in these cell types awaits further investigation.

Ultrastructural analyses of Munc18-1 in complex with Syntaxin 1A (74), and Munc18c complexed with the N-terminal peptide of Syntaxin 4 (but not the entire soluble region of Syntaxin 4 protein) (49), have yielded tremendous insight into the potential function(s) of these complexes. Syntaxin 4 contains four cytosolic alpha helices; from the N-terminus the domains are: Ha, Hb, Hc, and H3, followed by a transmembrane domain, as determined by its homology to Syntaxin 1. The C-terminal H3 domain is the canonical SNARE protein motif which participates in the SNARE core complex (25), and based upon homology, the bundled coils of Syntaxin 4 are predicted to fit into the cleft of an arch/crescent-shaped Munc18c (Fig. 5A). The Hc and H3 domains are connected by a flexible linker region believed to catalyze syntaxin’s transition from a ‘closed’ to an ‘open’ conformation, with the open form being active for engagement in the SNARE core complex. Consistent with this, residues within the 118-194 region constituting the Hc and linker domains of Syntaxin 4 are critical Munc18c interaction contacts (54), with the N-terminal peptide of Syntaxin 4 fitting into a small pocket nearer the top of the overall Munc18c “crescent” shape (49). Interestingly, mutations in Munc18c which significantly impair binding to Syntaxin 4 are situated at the apex of the crescent (22, 134, 135),
distanced from the arched cleft, and in close proximity to the binding sites for Doc2β and the Syntaxin 4 N-terminal peptide (Fig. 5B). The significance of Doc2β is discussed in a subsequent section below.

**Protein-protein interaction studies in cells and tissues—impact of post-translational modifications:**

Munc18-Syntaxin complexes are regulated through post-translational modifications, including serine/threonine phosphorylation, tyrosine phosphorylation, O-linked glycosylation, and nitrosylation of either or both proteins. Serine phosphorylation of Munc18-1 by protein kinase C and/or threonine phosphorylation by Cdk5 (cyclin-dependent kinase 5) disrupts Munc18-Syntaxin interaction and promotes secretory granule exocytosis (3, 7, 28, 31, 118). Munc18c has been shown recently to be phosphorylated by protein kinase C, causing dissociation from Syntaxin 4 and increasing SNARE complex assembly in pancreatic acinar cells (20). Syntaxin 1A can also be serine phosphorylated by the death-associated protein kinase (DAPK), which significantly decreases its interaction with Munc18-1 (136). The catalytic subunit of the serine/threonine phosphatase protein 2B (PP2B) has been shown to directly interact with Munc18c in human umbilical vein endothelial cells (HUVECs) (80).

While serine/threonine phosphorylation of Munc18 or Syntaxin isoforms in insulin-secreting or insulin-responsive cell types has yet to be demonstrated, both proteins can undergo tyrosine phosphorylation in a stimulus-dependent manner. In clonal MIN6 beta cells, Munc18c becomes tyrosine phosphorylated at residue Tyr219, and as a result dissociates from Syntaxin 4 (54, 84). In 3T3-L1 adipocytes, Munc18c residue Tyr521 was modified in response to either insulin or PDGF stimulations (113, 145). Interestingly, Tyr521 resides in a disordered region,
though prediction mapping places it in close proximity to Tyr219 (Fig. 5B), suggesting that this vicinity of Munc18c may be an important site for stimulus-induced conformational changes. In addition, Syntaxin 4 becomes phosphorylated at residues Tyr115 and Tyr251 in insulin-stimulated 3T3-L1 adipocytes (113). Remarkably, Syntaxin 4 does not appear to undergo tyrosine phosphorylation in clonal beta cells (84), perhaps indicating a form of mechanistic “bifurcation.” Still further, Munc18c can be modified by O-linked glycosylation in 3T3-L1 adipocytes under insulin-resistant conditions with glucosamine, concurrent with impaired insulin-stimulated GLUT4 translocation (15) and deficient Munc18c localization to the plasma membrane (79).

**Impact of additional binding partners on SM-Syntaxin interactions:**

While *in vitro* studies are invaluable for detailing kinetic and binding site information, cellular studies have elucidated a major role for post-translational modifications in how the SM-syntaxin complexes associate and dissociate. Moreover, SM-syntaxin complex accessory proteins which are relevant to insulin secretion and insulin action have been identified, as discussed below.

**Doc2β:** As a soluble 45 kDa double C2 domain-containing protein, Doc2β is expressed in adipocytes and islet beta cells, and exerts positive effects upon GLUT4 vesicle and insulin granule exocytosis events, respectively (34, 54, 58, 75). In islet beta cells Doc2β protein over-expression increases GSIS by ~40%, and the siRNA-mediated depletion of Doc2β attenuates insulin release (58, 75), with similar effects observed in 3T3-L1 adipocytes (34). Doc2β can mediate similar outcomes in synaptic vesicle exocytosis through association of its first C2
binding domain (C2A) with Munc18-1 (146). In islet beta cells, the second C2 domain (C2B) mediates its association with residues 173-255 of Munc18c, including the regulatory Y219 phosphorylation site (58). Tyrosine phosphorylation of Munc18c decreases Munc18c-Syntaxin 4 interactions with a concomitant 2-fold increase in Munc18c-Doc2β binding (54). Doc2β effectively competes with Syntaxin 4 for Munc18c binding, and its endogenous association with Munc18c is required for GSIS. In contrast, it has been suggested that Doc2β instead exerts its effects through interaction with Syntaxin 4 in a calcium-dependent manner (75). Although calcium was present in both beta cell studies, methodological differences such as use of low stringency binding conditions, use of calcium-chelators, and use of a transmembrane-containing insoluble syntaxin protein may have permitted detection of Syntaxin 4 association with Doc2β. Thus, while there is full agreement that Doc2β plays a positive role in Syntaxin 4-mediated exocytosis, details of the underlying mechanism must await further examination.

**Munc13-1:** Munc13-1 is a soluble 200 kDa protein expressed in pancreatic islet beta cells (89, 116), but not in adipocytes or skeletal muscle. Munc13-1 is composed of one C1 and two C2 domains which mediate phorbol ester and diacylglycerol binding and phospholipid-dependent Ca^{2+} binding, respectively. Munc13-1 can pair directly with Munc18-1, Doc2β or Syntaxin 1A (6, 146). Munc13-1 over-expression amplifies insulin secretion and is proposed to function in granule priming (116). Reduced expression of Munc13-1 is observed in islets isolated from diabetic humans or Zucker fa/fa rats (89, 116), consistent with glucose intolerance and the deficient insulin release characteristics of Munc13-1 (+/-) knockout mice (62), and is therefore believed to have a required role in insulin exocytosis.
**WNK1:** A unique member of the serine/threonine kinase family, WNK1 (With No K (lysine)) has been linked to the inherited hypertension syndrome Pseudohypoaldosteronism II (161, 163). WNK1 is a soluble 230 kDa kinase expressed in 3T3-L1 adipocytes and islet beta cells (55, 66), and is a Munc18c binding protein (81). WNK1 and Munc18c associate via direct interaction of the N-terminal 172 residues of Munc18c (distinct from residues bound by Doc2β) and the kinase domain of WNK1, and competitive inhibition of this complex impairs Syntaxin 4-mediated insulin granule exocytosis (81). Also, WNK1-Munc18c complexes are found localized to the plasma membrane, though complexes are also found in the cytosol, a unique feature amongst Munc18c binding proteins to date. Two other unique features of this complex exist: 1) despite the requirement for the kinase domain of WNK1, its intrinsic kinase activity is apparently dispensable for interaction (i.e. Munc18c does not serve as a WNK1 substrate); 2) siRNA-mediated WNK1 depletion does not impact insulin secretion from clonal beta cells nor glucose uptake into adipocytes (55), complicating specific designation of its role in exocytosis events pertinent to glucose homeostasis.

**80K-H:** The 80K-H protein (80 kDa) was originally identified as a PKCζ binding partner and is widely expressed, especially at the plasma membrane of insulin-sensitive 3T3L1 adipocytes and L6 myotubes (47). 80K-H has been implicated in vesicle trafficking events via a close relationship to the protein VASAP-60 (13), and in GLUT4 vesicle transport through its ability to interact with both Munc18c and PKCζ in an insulin-dependent manner (47, 119). The requirement of 80K-H in glucose uptake in vivo and/or primary cells and its putative role as a signaling link between PKCζ and Munc18c await further investigation.
**Rab3A:** Rab proteins are a large family of small GTPases responsible for the regulation of many membrane trafficking events and are thought to participate in insulin exocytosis as well as GLUT4 vesicle exocytosis through interaction with Munc18-Syntaxin complexes. However, despite efforts over the past decade, only Rab3A, a Munc18-1 binding protein, has been identified to date (39), with no Munc18c binding candidate to modulate insulin action as of yet. Rab3 null mice exhibit glucose intolerance coupled to ablated first-phase insulin release but without insulin resistance (164), consistent with its role as solely a Munc18-1 and not a Munc18c binding factor.

**Synip:** Synip (syntaxin-interacting protein) is a 62 kDa protein that was initially discovered as a novel Syntaxin 4-binding protein and has been implicated in the control of glucose transport and GLUT4 vesicle translocation in 3T3-L1 adipocytes (73). It binds only to the Syntaxin 4 isoform in an insulin-sensitive manner. This mechanism appears to function through Synip phosphorylation at Ser99 in response to activation of Akt2, and subsequent dissociation from Syntaxin 4 to promote GLUT4 vesicle exocytosis (86, 165), although this is a disputed finding (111). Synip expression in βHC-9 clonal beta cells has also been reported and a role for it implicated in Syntaxin 4-mediated insulin exocytosis using an over-expression paradigm (109). Synip knockout mice or RNAi-mediated knockdown data will be required to determine if Synip is necessary during Syntaxin4-based exocytosis events relevant to glucose homeostasis.

**Tomosyn and Cab45b:** Tomosyn proteins are syntaxin binding factors, with 7 different isoforms expressed from two genes, Tomosyn-1 and Tomosyn-2 (41). b-Tomosyn-1 (b=big), a cytosolic
protein, was identified as a Syntaxin 4-binding partner in 3T3-L1 adipocytes, and its over-expression inhibited GLUT4 translocation to the plasma membrane (159). Similarly, in beta cells depletion of an analogous Syntaxin 1A binding isoform of Tomosyn-1 was found to decrease stimulated exocytosis (18). In contrast, depletion of a related m-tomosyn-1 (m=medium) isoform (which also binds to Syntaxin 1A) in clonal beta cells was shown to increase insulin release while over-expression was inhibitory, suggesting that it functions as a negative regulator of insulin exocytosis (172). Although tomosyn-1 knockout mice exist and have enhanced synaptic transmission (110), the mice are not yet characterized for effects upon glucose homeostasis, and it remains unclear which isoforms are ablated.

Cab45b was recently identified as a soluble 42 kDa calcium binding protein associated with Munc18b in pancreatic islet beta cells (173). In clonal INS-1E beta cells, antibody-mediated interference of endogenous Cab45b or RNAi-knockdown of Munc18b expression reduced depolarization-evoked membrane capacitance, implicating potential roles for each protein in insulin exocytosis.

**Novel roles for Munc18 and Syntaxin proteins in granule mobilization and pool refilling**

New evidence suggests that non-traditional roles exist for SM and syntaxin proteins in exocytosis. Both Munc18-1 and Munc18c (+/-) knockout mouse models reveal the necessity of these proteins in granule localization to the plasma membrane (83, 141). Munc18-1-depleted clonal beta cells also exhibit defective docking of insulin granules to the plasma membrane (139). Although this might suggest that the soluble fraction of SM proteins somehow directs granule mobilization through the cytoskeletal matrix, pilot *in vitro* F-actin binding studies argue against Munc18c protein as a direct binding factor of F-actin (Kalwat and Thurmond, unpublished)
results). In contrast, Syntaxin 4 was recently shown to indeed bind directly to F-actin, through an N-terminal ‘spectrin-like’ domain that is relevant to insulin exocytosis in clonal beta cells (53). In contrast, Syntaxin 1A failed to directly associate with F-actin, although does co-immunoprecipitate with F-actin in beta cell lysates and dissociate transiently in response to glucose stimulation (133). Syntaxin 4 can also associate with α-fodrin, an F-actin binding factor, in primary rat adipocytes (68). Given that Syntaxin 4 and Munc18c in particular, are required for the mobilization phase of insulin exocytosis, and are also responsible for the relatively long-range trafficking of GLUT4 vesicles in adipocytes and myocytes, future exploration regarding their interactions with cytoskeletal elements should prove very exciting. However, it is important to note that stimulus-induced actin remodeling appears to be different for exocytosis in beta cells than it is in adipocytes and myocytes. Current data suggest that disruption of the actin cytoskeleton using agents such as Latrunculin in beta cells potentiates insulin exocytosis, but in adipocytes inhibits GLUT4 vesicle exocytosis (12, 88, 133, 140, 143).

PERSPECTIVES AND SIGNIFICANCE

Munc18c and Syntaxin 4 are clearly common links in the known mechanisms of insulin granule exocytosis and GLUT4 vesicle translocation, and yet the molecular details of their actions in these processes remain incomplete. With regards to Munc18c, significant progress has been made in its characterization as a positive effector of both processes, as well as the recent identifications of novel binding factors that implicate it in both Syntaxin 4-dependent (vesicle docking/fusion at the plasma membrane) and Syntaxin 4-independent (granule mobilization/localization) mechanisms. The possibility that Munc18c functions in a Syntaxin 4-independent role in facilitating insulin granule delivery is particularly important, given that
granule recruitment to the readily releasable pool of a β-cell is a rate-limiting component of insulin release. Pharmacological targeting of Munc18c function, directly or indirectly, via a binding partner implicated in that mechanism, could presumably exert profound effects upon the capacity of the β-cell to sustain insulin release beyond the first few minutes. The ability to sustain insulin release in a regulated biphasic manner, as opposed to the constitutive release triggered by current popular oral medications which cause hypoglycemia and hasten beta cell failure, would be of tremendous advantage as it would allow for restoration of glucose homeostasis with lower resting insulin levels and hence less risk of hypoglycemic episodes.

In addition to gaining more insight into how Munc18c-Syntaxin 4 complexes are regulated, it will be particularly important to determine how and why Syntaxin 4 and Munc18c protein levels decrease under conditions of obesity and Type 2 diabetes in humans and rodent models. Interestingly, knockout and transgenic mouse model studies suggest that Munc18c expression is controlled by Syntaxin 4. Thus, future studies aimed towards gaining the ability to control Syntaxin 4 expression could be advantageous to improving whole body glucose homeostasis.
Acknowledgements

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REFERENCES


Fig. 1. SNARE core complex formation. Upon stimulation, Syntaxin (orange) adopts an ‘open’ conformation exposing its H3 domain to form the SNARE core complex with VAMP2 (red) and SNAP23/25 (green).

Fig. 2. Glucose-stimulated insulin secretion from the islet beta cell. An 8-step model: (1) Glucose enters the cell through the constitutively active PM localized GLUT2 transporter and gets metabolized, which in turn (2) increases the ATP:ADP ratio resulting in (3) the closure of the ATP dependent potassium channels (K$_{ATP}$). The closure of the K$_{ATP}$ channels leads to (4) plasma membrane (PM) depolarization, (5) opening of the voltage-dependent calcium channels (VDCC), causing calcium influx into the cell (6). As a result, intracellular calcium [Ca$^{2+}$]$_i$ levels rise, and through a largely uncharacterized series of events, the SNARE proteins mediate (7) vesicle fusion to facilitate (8) insulin release.

Fig. 3. Glucose uptake via insulin-stimulated GLUT4 translocation and fusion in muscle and adipose tissues. An 11-step model: (1) extracellular insulin binds to the α-subunit of the insulin receptor (IR), triggering autophosphorylation and activation of the β-subunit kinase activity. (2) This induces recruitment of IRS-1, and (3) IRS-1 recruits PI3-K. (4) PI3-K phosphorylates PIP2 to yield PIP3. (5) PIP3 recruits PDK1 to the PM, where it (6) phosphorylates and activates AKT. (7) AKT phosphorylates AS160, and (8) AS160 targets multiple Rabs present on GLUT4 containing vesicles (9), although the precise mechanisms beyond this remain unclear. (10) Vesicle fusion occurs via the SNARE proteins resulting in GLUT4 integration into the PM to facilitate (11) glucose uptake.

Fig. 4. Proposed models of Munc18c-Syntaxin 4 interaction. (A) Unstimulated state model of Munc18 holding Syntaxin in a ‘closed’ conformation which in turn inhibits Syntaxin from being able to participate in the SNARE tertiary complex, thus inhibiting exocytosis. (B) A new model in the field derived from in vitro data; in stimulated state Munc18 remains bound to ‘open’ Syntaxin at the N-terminus allowing Syntaxin to participate in the SNARE core complex. (C) Munc18 interacts with the SNARE core complexes through the four α-helical bundle.

Fig. 5. Modeling of Munc18c-Syntaxin 4 interaction. (A) Depicting full length Munc18c (green) interacting with Syntaxin 4 (orange). The peptide termed 18c/pep3 (residues 460-483) is illustrated in pink and arginine 240 shown in yellow; both have been shown to play important roles in Munc18c-Syntaxin 4 interaction (134, 135). (B) Upper panel—Munc18c Tyr219 sits in the Doc2β binding region on Munc18c (blue) juxtaposed to the binding cleft for the N-terminus of Syntaxin 4 (red). Lower panel—enlarged view of the boxed region, illustrating the close proximity between Tyr219 and Tyr521, though Tyr521 is not present in the crystal structure due to its location within a disorder region (drawn into the model with white dashed line).
Table 1. Expression of v- and t-SNARE isoforms in adipose, skeletal muscle and pancreatic β-cells

<table>
<thead>
<tr>
<th><strong>v-SNARE</strong></th>
<th><strong>Tissue (localization)</strong></th>
<th><strong>Function</strong></th>
<th><strong>References</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>VAMP2/Synaptobrevin</td>
<td>Pancreatic β-cells, adipocyte, muscle (vesicles)</td>
<td>Exocytosis of insulin granules (β-cells), GLUT4 vesicles at the PM</td>
<td>(87, 96, 149, 160)</td>
</tr>
<tr>
<td>VAMP3/Cellubrevin</td>
<td>Pancreatic β-cells, adipocyte, muscle (vesicles)</td>
<td>Exocytosis of insulin granules (β-cells), GLUT4 vesicles at the PM</td>
<td>(125, 149, 158, 160)</td>
</tr>
<tr>
<td>VAMP4</td>
<td>Adipocyte, muscle (TGN)</td>
<td>ND</td>
<td>(123, 160)</td>
</tr>
<tr>
<td>VAMP5/myobrevin</td>
<td>Adipocyte, muscle (PM)</td>
<td>Myogenesis</td>
<td>(160, 171)</td>
</tr>
<tr>
<td>VAMP7/TI-VAMP</td>
<td>Adipocyte (PM, endosome)</td>
<td>Osmotic shock-induced GLUT4 translocation</td>
<td>(160)</td>
</tr>
<tr>
<td>VAMP8/Endobrevin</td>
<td>Pancreatic beta cells, adipocyte (endosome)</td>
<td>GLUT4 endocytosis, insulin secretion (β-cells)</td>
<td>(77, 160)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>t-SNARE</strong></th>
<th><strong>Tissue (localization)</strong></th>
<th><strong>Function</strong></th>
<th><strong>References</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Syntaxin 1A</td>
<td>Pancreatic β-cells (PM)</td>
<td>First phase insulin secretion in pancreatic β-cells</td>
<td>(76, 85, 91)</td>
</tr>
<tr>
<td>Syntaxin 2/Epimorphin</td>
<td>Pancreatic β-cells, adipocyte (PM)</td>
<td>ND</td>
<td>(137, 151, 173)</td>
</tr>
<tr>
<td>Syntaxin 3</td>
<td>Pancreatic β-cells, adipocyte (PM)</td>
<td>ND</td>
<td>(137, 156, 158)</td>
</tr>
<tr>
<td>Syntaxin 4</td>
<td>Pancreatic β-cells, adipocyte, muscle (PM)</td>
<td>Both phases of insulin secretion (β-cells), GLUT4 translocation</td>
<td>(87, 120, 122, 151)</td>
</tr>
<tr>
<td>Syntaxin 5</td>
<td>Adipocyte (TGN)</td>
<td>GLUT4 endocytosis in adipocyte</td>
<td>(156)</td>
</tr>
<tr>
<td>Syntaxin 6</td>
<td>Adipocyte, muscle (TGN)</td>
<td>Putative involvement in GLUT4 endocytosis (adipocytes)</td>
<td>(92, 117, 133)</td>
</tr>
<tr>
<td>Syntaxin 7</td>
<td>Pancreatic β-cells, adipocyte (endosome)</td>
<td>ND</td>
<td>(90, 92)</td>
</tr>
<tr>
<td>Syntaxin 8</td>
<td>Adipocyte (endosome)</td>
<td>ND</td>
<td>(92)</td>
</tr>
<tr>
<td>Syntaxin 10</td>
<td>Muscle</td>
<td>ND</td>
<td>(127)</td>
</tr>
<tr>
<td>Syntaxin 12</td>
<td>Adipocyte</td>
<td>ND</td>
<td>(92)</td>
</tr>
<tr>
<td>Syntaxin 16</td>
<td>Adipocyte (TGN)</td>
<td>GLU4 intracellular trafficking</td>
<td>(117)</td>
</tr>
<tr>
<td>SNAP23</td>
<td>Pancreatic β-cells, adipocyte, muscle (PM)</td>
<td>Exocytosis of insulin granules (β-cells), GLUT4 vesicles at the PM</td>
<td>(94, 153)</td>
</tr>
<tr>
<td>SNAP25</td>
<td>Pancreatic β-cells (PM)</td>
<td>Exocytosis of insulin granules (β-cells)</td>
<td>(108)</td>
</tr>
</tbody>
</table>

ND, not determined; N/A, not applicable; PM, plasma membrane; TGN, trans golgi network; ER, endoplasmic reticulum
Table 2. Comparison of exocytosis events of insulin granules, GLUT4 vesicles and synaptic vesicles

<table>
<thead>
<tr>
<th>Insulin secretion</th>
<th>GLUT4 vesicle translocation</th>
<th>Neurotransmitter release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large secretory granules (&gt;100 nm radius)</td>
<td>Large secretory granules (&gt;100 nm radius)</td>
<td>Small synaptic vesicles (&lt;25 nm radius)</td>
</tr>
<tr>
<td>Recycling via Golgi complex</td>
<td>Recycling via Golgi complex</td>
<td>Local recycling</td>
</tr>
<tr>
<td>Two secretion phases: 1&lt;sup&gt;st&lt;/sup&gt; fast (spans 6-10 min); 2&lt;sup&gt;nd&lt;/sup&gt;, slow and sustained (10 min-hrs)</td>
<td>Slow (5-15 min) and sustained</td>
<td>Fast, short-lasting secretion (0.1-6 ms)</td>
</tr>
<tr>
<td>Small number of predocked granules</td>
<td>Few predocked vesicles</td>
<td>Large number of predocked vesicles</td>
</tr>
<tr>
<td>Exocytosis targeted to large plasma membrane section</td>
<td>Exocytosis targeted to large plasma membrane section</td>
<td>Exocytosis restricted to synaptic active zone</td>
</tr>
<tr>
<td>Complex secretory mixes (e.g., multiple peptides, catecholamines, nucleotides)</td>
<td>Cargo proteins to integrate into the plasma membrane</td>
<td>Release of one or two low-molecular-weight compounds</td>
</tr>
<tr>
<td>SNARE isoforms: Syntaxins 1 and 4, SNAP23 and SNAP25, VAMP2 and VAMP3</td>
<td>SNARE isoforms: Syntaxin 4, SNAP23, VAMP2</td>
<td>SNARE isoforms: Syntaxin 1, SNAP25, VAMP2</td>
</tr>
<tr>
<td>SM isoforms: Munc18-1, Munc18c</td>
<td>SM isoform: Munc18c</td>
<td>SM isoform: Munc18-1</td>
</tr>
<tr>
<td>Latrunculin potentiates secretion</td>
<td>Latrunculin inhibits GLUT4 translocation</td>
<td>Latrunculin potentiates secretion</td>
</tr>
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Table 3. Genetically-engineered mouse models of SNARE protein ablation/over-expression for studies of glucose homeostasis in vivo.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Aberration</th>
<th>Phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syntaxin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syntaxin 1A (-/-)</td>
<td>No Syntaxin 1A expression</td>
<td>Fewer docked granules during only first phase of insulin secretion</td>
<td>(85)</td>
</tr>
<tr>
<td>Syntaxin 1B (-/-, open)</td>
<td>Syntaxin 1B LE expression only, no endogenous</td>
<td>ND</td>
<td>(37)</td>
</tr>
<tr>
<td>Syntaxin 1A (-/-), Syntaxin 1B (-/-, LE)</td>
<td>No Syntaxin 1A or 1B, LE mutant expression</td>
<td>ND</td>
<td>(37)</td>
</tr>
<tr>
<td>Syntaxin 1A Tg</td>
<td>Beta cell specific Syntaxin 1A over-expression, decreased Munc18-1 expression</td>
<td>Fasting hyperglycemia, impaired glucose tolerance and insulin exocytosis</td>
<td>(63)</td>
</tr>
<tr>
<td>Syntaxin 4 (+/-)</td>
<td>Reduced Syntaxin 4 and Munc18c expression, null lethal</td>
<td>Insulin resistant; reduced GLUT4 translocation; defective insulin secretion</td>
<td>(122, 168)</td>
</tr>
<tr>
<td>Syntaxin 4 Tg</td>
<td>Increased Syn4 and Munc18c expression in pancreas, skeletal muscle and adipose tissues only</td>
<td>Insulin sensitive; enhanced GLUT4 translocation and insulin secretion</td>
<td>(120, 122)</td>
</tr>
<tr>
<td>Munc18c</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Munc18-1 (-/-, +/+)</td>
<td>Reduced expression of Munc18-1, null lethal</td>
<td>ND</td>
<td>(147, 148)</td>
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<tr>
<td>Munc18-1 Tg</td>
<td>Over-expression of Munc18-1 in neuron</td>
<td>ND</td>
<td>(142)</td>
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<tr>
<td>Munc18c Tg</td>
<td>Over-expression of Munc18c</td>
<td>Insulin resistant; impaired insulin secretion</td>
<td>(121)</td>
</tr>
<tr>
<td>Munc18c (-/-)</td>
<td>No Munc18c expression</td>
<td>Enhanced GLUT4 uptake in MEF-derived adipocytes</td>
<td>(56)</td>
</tr>
<tr>
<td>Munc18c (+/-)</td>
<td>Reduced Munc18c expression, null lethal by E7.5</td>
<td>Insulin resistant; impaired insulin secretion; reduced GLUT4 translocation in skeletal muscle</td>
<td>(82, 83)</td>
</tr>
<tr>
<td>VAMP</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>VAMP2 (-/-)</td>
<td>No VAMP2 expression, lethal</td>
<td>ND</td>
<td>(114)</td>
</tr>
<tr>
<td>VAMP3 (-/-)</td>
<td>No VAMP3 expression</td>
<td>Normal insulin and glucose tolerance, and normal glucose uptake</td>
<td>(169)</td>
</tr>
<tr>
<td>VAMP8 (-/-)</td>
<td>No VAMP8 expression</td>
<td>ND</td>
<td>(98, 152)</td>
</tr>
<tr>
<td>SNAP25 (-/-, +/-)</td>
<td>Reduced SNAP25 expression, null lethal at birth</td>
<td>ND</td>
<td>(154)</td>
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ND, not determined; Tg, transgenic; (-/-), homozygous; (+/-) heterozygous
Table 4. SM-Syntaxin binding specificities

<table>
<thead>
<tr>
<th>SM proteins</th>
<th>Syntaxin partner</th>
<th>Function</th>
<th>Reference</th>
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<tbody>
<tr>
<td>M. musculus</td>
<td>Syntaxin 1, 2, 3</td>
<td>Synaptic vesicle exocytosis</td>
<td>(43)</td>
</tr>
<tr>
<td>Munc18a/-1</td>
<td>Syntaxin 1, 2, 3</td>
<td>Apical membrane trafficking</td>
<td>(101, 102)</td>
</tr>
<tr>
<td>Munc18b/-2</td>
<td>Syntaxin 4</td>
<td>GLUT4 vesicle exocytosis, insulin granule secretion</td>
<td>(84, 126, 129, 132, 134)</td>
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<tr>
<td>Munc18c/-3</td>
<td>Syntaxin 5, 18</td>
<td>ER to golgi transport</td>
<td>(23, 166)</td>
</tr>
<tr>
<td>mSly1</td>
<td>Syntaxin 16/Tlg2p</td>
<td>TGN transport</td>
<td>(26, 128)</td>
</tr>
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<td>mVPS45</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>H. Sapiens</td>
<td>Syntaxin 3</td>
<td>Primary neutrophil granule fusion</td>
<td>(10)</td>
</tr>
<tr>
<td>Munc18a/-2</td>
<td>Syntaxin 4</td>
<td>Insulin action in human skeletal muscle</td>
<td>(5)</td>
</tr>
<tr>
<td>Munc18c/-3</td>
<td>Syntaxin 1A</td>
<td>Neuronal disorder (migraine predisposition)</td>
<td>(19)</td>
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<tr>
<td>ND</td>
<td>Syntaxin 7,8</td>
<td>Heterotypic fusion of late endosome</td>
<td>(44)</td>
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<tr>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND, not determined
Fig. 1

extracellular

intracellular

VAMP2

SNAP23/25

H3

Vesicle

‘open’ syntaxin

Hc

Hb

Ha

PMP

e xtracellular

intracellular

PM
Fig. 4
Fig. 5

A) 

B) Disordered region containing Y521