Exocytosis mechanisms underlying insulin release and glucose uptake: conserved roles for Munc18c and syntaxin 4

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Departments of 1Pediatrics, 2Biochemistry and Molecular Biology, and 3Cellular and Integrative Physiology, 4Basic Diabetes Group of the Herman B. Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, Indiana

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Jewell JL, Oh E, Thurmond DC. Exocytosis mechanisms underlying insulin release and glucose uptake: conserved roles for munc18c and syntaxin 4. Am J Physiol Regul Integr Comp Physiol 298: R517–R531, 2010. First published January 6, 2010; doi:10.1152/ajpregu.00597.2009.—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 uses multiple Munc18-syntaxin isoform pairs, whereas insulin action in the peripheral tissues appears to use 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.

Sec1/Munc18 proteins; glucose homeostasis; diabetes; insulin resistance; soluble N-ethylmaleimide-sensitive factor attachment protein receptor 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 ~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 h 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 (for a review, see Ref. 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 (for reviews, see Refs. 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 that distinguish the specialization of events in each cell type (summarized in Table 2).

### Insulin Granule Exocytosis

Elevated postprandial glucose levels trigger a signaling cascade in the pancreatic islet beta cells to elicit insulin release (Fig. 2), depicted as an ~8-step process (for reviews, see Refs. 64 and 100). First, glucose entry into beta cells is facilitated via the plasma membrane-localized glucose transporter, GLUT2. GLUT2 has a relatively low affinity ($K_M \sim 30$ 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 ([$Ca^{2+}$]; [step 6]). The increase in [$Ca^{2+}$] 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 min following beta cell stimulation. Second-phase insulin secretion is less robust than the 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 use separate pools of insulin-containing granules. First-phase se-

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### Table 1. Expression of v- and t-SNARE isoforms in adipose, skeletal muscle, and pancreatic β-cells

<table>
<thead>
<tr>
<th>v-SNARE</th>
<th>Tissue (Localization)</th>
<th>Function</th>
<th>References</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>N/A</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>t-SNARE</th>
<th>Tissue (Localization)</th>
<th>Function</th>
<th>References</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>N/A</td>
<td>(137, 151, 173)</td>
</tr>
<tr>
<td>Syntaxin 3</td>
<td>Pancreatic β-cells, adipocyte (PM)</td>
<td>N/A</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, 152, 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>N/A</td>
<td>(90, 92)</td>
</tr>
<tr>
<td>Syntaxin 8</td>
<td>Adipocyte (endosome)</td>
<td>N/A</td>
<td>(92)</td>
</tr>
<tr>
<td>Syntaxin 10</td>
<td>Muscle</td>
<td>N/A</td>
<td>(127)</td>
</tr>
<tr>
<td>Syntaxin 12</td>
<td>Adipocyte</td>
<td>N/A</td>
<td>(92)</td>
</tr>
<tr>
<td>Syntaxin 16</td>
<td>Adipocyte (TGN)</td>
<td>GLUT4 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>

SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor protein; N/D, not determined; N/A, not applicable; PM, plasma membrane; TGN, trans-Golgi network; ER, endoplasmic reticulum.
cation appears to arise from plasma membrane-predocked 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 nonnutrient 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 uses 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 (for reviews, see Refs. 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 ($K_a \approx 4$ 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 α-subunits of the insulin receptor (IR) present on the surface of muscle cells and adipocytes (step 1), inducing tyrosine autophosphorylation within the intracellular IR, and increased kinase activity of the IRS-β 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 domain. Once bound, substrates are themselves tyrosine phosphorylated. Phosphorylated receptor substrates then serve as additional recruitment targets for specific proteins containing Src Homology 2 domains, including phosphatidylinositol kinase (PI3K) (step 3). Once recruited, PI3K is activated to catalyze the phosphorylation of phosphatidylinositols (steps 4 and 5) bisphosphate (PIP2) at the 3’ position, yielding phosphatidylinositol (steps 3, 4, and 5) trisphosphate (PIP3) (step 4). 3-Phosphoinositide-dependent kinase-1 (PDK-1) is able to recognize the 3’ site of PIP3 with its Pleckstrin homology (PH) 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 isofoms zeta and lambda (aPKC). As well defined as these initial signaling steps are, the identities of

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**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: 1st fast (spans 6-10 min); 2nd, slow and sustained (10 min–hours)</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 mixtures (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>
</tbody>
</table>

**SM, Sec1/Munc18; GLUT4, glucose transporter 4.**
Table 3. Genetically engineered mouse models of SNARE protein ablation/overexpression 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></td>
<td></td>
</tr>
<tr>
<td>Syntaxin 1A (−/−), Syntaxin 1B (−/−, LE)</td>
<td>No syntaxin 1A or 1B, LE mutant expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syntaxin 1A Tg</td>
<td>Beta cell-specific syntaxin 1A overexpression, 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 syntaxin 4 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>Munc18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Munc18-1 (−/−, −/+)</td>
<td>Reduced expression of Munc18-1, null lethal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Munc18-1 Tg</td>
<td>Overexpression of Munc18-1 in neuron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Munc18c Tg</td>
<td>Overexpression of Munc18c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Munc18c (−/−)</td>
<td>No Munc18c expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Munc18c (−/+)</td>
<td>Reduced Munc18c expression, null lethal by E7.5</td>
<td></td>
<td></td>
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<tr>
<td>VAMP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAMP2 (−/−)</td>
<td>No VAMP2 expression, lethal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAMP3 (−/−)</td>
<td>No VAMP3 expression</td>
<td></td>
<td></td>
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<tr>
<td>VAMP8 (−/−)</td>
<td>No VAMP8 expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNAP25 (−/−, −/+)</td>
<td>Reduced SNAP25 expression, null lethal at birth</td>
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</tbody>
</table>
| ND, not determined; Tg, transgenic; (−/−), homozygous; (−/+), heterozygous.

Genetically engineered mouse models of SNARE protein ablation/overexpression for studies of glucose homeostasis in vivo.

![Diagram of glucose uptake via insulin-stimulated GLUT4 translocation and fusion in muscle and adipose tissues.](image)

**Fig. 3.** Glucose uptake via insulin-stimulated GLUT4 translocation and fusion in muscle and adipose tissues. An 11-step model: step 1: extracellular insulin binds to the α-subunit of the insulin receptor (IR), triggering autophosphorylation and activation of the β-subunit kinase activity; step 2: this induces recruitment of IRS-1, and (step 3) IRS-1 recruits PI3K. Step 4: PI3K phosphorylates PIP2 to yield PIP3. Step 5: PIP3 recruits PDK1 to the PM, where it (step 6) phosphorylates and activates AKT. Step 7: AKT phosphorylates AS160, and (step 8) AS160 targets multiple Rabas present on GLUT4-containing vesicles (step 9), although the precise mechanisms beyond this remain unclear. Step 10: vesicle fusion occurs via the SNARE proteins, resulting in GLUT4 integration into the PM to facilitate (step 11) glucose uptake.

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).
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).

**Syntaxin family.** The pancreatic beta cell expresses plasma membrane-localized syntaxin isoforms 1A, 2, 3, and 4, though additional nonplasma 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 to be 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 overexpressing syntaxin 4 in the islet secrete 30% more insulin in both phases, implying a positive role for syntaxin 4 (Table 4). Oddly, syntaxin 1-overexpressing 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 overexpressing syntaxin 4 in skeletal muscle tissue show a twofold 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 tissue express and use only SNAP23 (2, 57, 95). SNAP29 is also widely expressed (124), although it 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.

**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 predominant 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 coimmunoprecipitate 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).

**Sec1/Munc18 protein family.** In the early 1990s, the yeast Sec1 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 Caenorhabditis elegans (unc18), Drosophila melanogaster (Rop), and mammals (Munc18) (for a review, see Ref. 103). Collectively, proteins of this type are referred to as SM proteins, for Sec1/Munc18 (SM). The mammalian members of this family, Munc18 proteins, are ~66- to 68-kDa soluble proteins with no apparent transmembrane domain, yet they 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); nonplasma 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 among 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-synaptin 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 Goto-Kakizaki 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 overexpression (beta cell transgenic), syntaxin 4 protein overexpression (pancreas, skeletal muscle, and adipose specific transgenic), syntaxin 1A and/or syntaxin 1B deficient, and syntaxin 4 haploinsufficient mice 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 predocked granules, as determined by total internal reflection fluorescence microscopy and electron microscopy, and show normal expression levels of Munc18-1 and Munc18b (33, 85). Surprisingly, mice with beta-cell-specific overexpression 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-overexpressing islets, but the cause of the paucity of Munc18-1 is unclear. Additionally, nontissue 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-overexpressing transgenic mice show a parallel upregulation of endogenous Munc18c protein abundance in the three tissues overexpressing 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 that 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 that 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-overexpressing 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 used a dominant-negative mutant form of syntaxin 4 to interfere with endogenous VAMP2 trafficking and ablate insulin-stimulated GLUT4 translocation (87), an approach that 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, because this model consists of derived rather than primary cells or tissues, direct comparisons cannot be made. With regard 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 overexpression in adipose, skeletal muscle, and pancreas, akin to the syntaxin 4 overexpression 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 overexpression 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 overexpression 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 overexpression in neuronal cells in culture rather enhances exocytosis (141), and overexpression of Munc18-1 in clonal beta cells had no effect upon insulin secretion (139). With regard to whether SM proteins have a clear positive or negative role, these disparate results may arise from differential protein abundance and stoichiometry, since overexpression 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. Although 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 because of 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 they 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 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 SNAP25 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 cocrystallized with the N-terminal 19 residue peptide of syntaxin 4, indicating the importance of this particular site for protein-protein interaction (49). Although 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 comprising 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 synaphins, were originally described in neuronal cells to play an essential role in Ca^{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 Fig. 4, B and C, 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). Although the benefit of low-stringency buffers has enabled the ability to detect the otherwise elusive transient docking complex, coimmunoprecipitation data obtained from cell or tissue lysates currently support only model A (Fig. 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 on the basis of 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
PKC and/or threonine phosphorylation by Cdk5 (cyclin-dependent or both proteins). Serine phosphorylation of Munc18-1 by PKC, 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 has been shown recently to be phosphorylated by PKC, causing dissociation from syntaxin 4 and increasing SNARE complex assembly in pancreatic acinar cells (20).

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 (75). Although calcium was present in both islet beta cells, Doc2B protein overexpression increases GSIS by ~40%, and the siRNA-mediated depletion of Doc2B attenuates insulin release (58, 75), with similar effects observed in 3T3-L1 adipocytes (34). Doc2B can mediate similar outcomes in synaptic vesicle exocytosis through association of its first C2 domain with the N terminus of syntaxin 4 (red). Lower panel-extended 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).

While in vitro studies are invaluable for detailing kinetic and binding site information, cellular studies have elucidated a major role for posttranslational 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.

**Doc2B.** As a soluble 45-kDa double-C2 domain-containing protein, Doc2B is expressed in adipocytes and islet beta cells, and it exerts positive effects upon GLUT4 vesicle and insulin granule exocytosis events, respectively (34, 54, 58, 75). In islet beta cells, Doc2B protein overexpression increases GSIS by ~40%, and the siRNA-mediated depletion of Doc2B attenuates insulin release (58, 75), with similar effects observed in 3T3-L1 adipocytes (34). Doc2B 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 twofold increase in Munc18c-Doc2B binding (54). Doc2B 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 Doc2B 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

![Image](http://ajpregu.physiology.org/content/298/3/525/F5.large.jpg)
use of a transmembrane-containing insoluble syntaxin protein may have permitted detection of syntaxin 4 association with Doc2β. Thus, although 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 that mediate phorbol ester and diacylglycerol binding and phospholipid-dependent Ca²⁺ binding, respectively. Munc13-1 can pair directly with Munc18-1, Doc2β, or syntaxin 1A (6, 146). Munc13-1 overexpression 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 pseudohypaldosteronism 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, although complexes are also found in the cytosol, a unique feature among 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) and 2) siRNA-mediated WNK1 depletion does not impact insulin secretion from clonal beta cells or 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 3T3-L1 adipocytes and L6 myotubes (47). 80K-H has been implicated in vesicle trafficking events via a close relationship to the protein VASAP-60 (13), as well as 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 awaited 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 is implicated in syntaxin 4-mediated insulin exocytosis using an overexpression paradigm (109). Data on Synip knockout or RNAi-mediated knockout mice will be required to determine whether Synip is necessary during syntaxin 4-based exocytosis events relevant to glucose homeostasis.

**TOMOSYN.** Tomosyn proteins are syntaxin binding factors, with 7 different isoforms expressed from two genes, tomosyn-1 and tomosyn-2 (41). b-Tomosyn-1 (b stands for big), a cytosolic protein, was identified as a syntaxin 4-binding partner in 3T3-L1 adipocytes, and its overexpression 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 stands for medium) isoform (which also binds to syntaxin 1A) in clonal beta cells was shown to increase insulin release while overexpression 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.** 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 nontraditional 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 (M. Kalwat and D. C. 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 it does coimmunoprecipitate 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
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 regard 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 complexes are regulated, it will be 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.

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MUNC18C-SYNTAXIN 4 COMPLEXES MAINTAIN GLUCOSE HOMEOSTASIS


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