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How Kiss-and-Run Can Make Us Sick: *SOX4* Puts a Break on the Pore

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Insulin is released by regulated exocytosis of secretory granules. At the heart of this process is a tight complex of three SNARE proteins (SNAP25 and syntaxin in the plasma membrane and synaptobrevin in the vesicle membrane) that forms during exocytosis (1). The complex pulls the two opposing membranes close enough to enable formation of a narrow fusion pore (~1.5 nm), a proteolipidic structure that behaves somewhat similar to an ion channel (2). Owing to its small size, the pore essentially acts as a molecular sieve that allows passage of small molecules but prevents release of the larger peptide hormones (3,4) (Fig. 1*A*). Once formed, the fusion pore can then either expand irreversibly ("full fusion," required for insulin secretion) or revert to the closed state ("kiss-and-run") (5). The sieve effect is of particular interest in β-cells as insulin granules contain many peptides, nucleotides, and amino acids that are cosecreted with insulin and have their own signaling functions (6). Purines, in particular ATP, stimulate both insulin secretion and β-cell survival via P2X and P2Y receptors (7), and the resulting autocrine and paracrine feedback may be involved in synchronizing pulsatile insulin release (8). Glutamate stimulates and GABA inhibits glucagon release from neighboring α-cells (9). However, fusion pore dynamics are not well understood mechanistically, although in β-cells there is some evidence for a role of dynamin and regulation by cAMP (10,11).

In this issue of *Diabetes*, Collins et al. (12) provide strong evidence that in humans the transcription factor *SOX4* regulates the expression of STXBP6/amisyn and that this protein in turn affects insulin secretion negatively by an effect exerted on the fusion pore. Mice expressing a mutant *SOX4* have reduced glucose-dependent insulin secretion, which is surprising given that both intracellular Ca^{2+} signaling and depolarization-evoked exocytosis are normal (12,13). The breakthrough of the current work by Collins et al. is the finding that release of ATP from individual granules is markedly slowed in the *SOX4* mutant mice, suggesting that insulin remains trapped inside the granules because the fusion pore does not expand beyond ~1 nm. By comparing the gene expression profiles of *SOX4* mutant and wild-type mice, Collins et al. then zoom in on the protein STXBP6/amisyn as the most likely *SOX4* target mediating these effects. Indeed, overexpression of the protein reduces the amount of ATP released during single exocytosis events, confirming an earlier observation that it might affect the fusion pore (14). Moreover, *SOX4* expression correlates well with that of *STXBP6* in a large collection of human donor islets. Combined, these data nicely make the case that *SOX4* drives expression of *STXBP6*/amisyn, which in turn restricts fusion pore expansion and therefore leads to reduced insulin secretion.

So how can we envision amisyn to affect fusion pore behavior and insulin release? Amisyn is a 24-kDa protein that consists of an N-terminal pleckstrin homology (PH) domain and a COOH-terminal R-SNARE–like coiled-coil domain (15). The latter has similarities with synaptobrevin and allows amisyn to bind to syntaxin 1. One possibility is therefore that the protein acts as competitive inhibitor of SNARE complex formation, similar to the related STXBP5/tomosyn (16). SNARE complexes are limited by the local availability of the three cognate SNARE components, and amisyn could via its SNARE domain compete with synaptobrevin for binding to endogenous syntaxin and SNAP25. As neither amisyn nor tomosyn have a transmembrane domain, the resulting alternative SNARE complexes will be futile, which could decrease fusion efficiency and lock the fusion pores in a narrow state (Fig. 1*B*). However, amisyn only affects fusion pore expansion, whereas tomosyn 1 prevents exocytosis altogether (16,17). The obvious difference is that tomosyn has bulky WD40 repeats, whereas amisyn contains a predicted lipid-binding PH domain that could act as soft membrane anchor. Thus, when amisyn replaces synaptobrevin as "pseudo-SNARE" the force exerted on the membrane might be just enough to open the fusion pore but not enough to drive pore expansion (Fig. 1*C*). This scenario is indirectly supported by the finding that flexible linkers inserted between synaptobrevin's SNARE motif and

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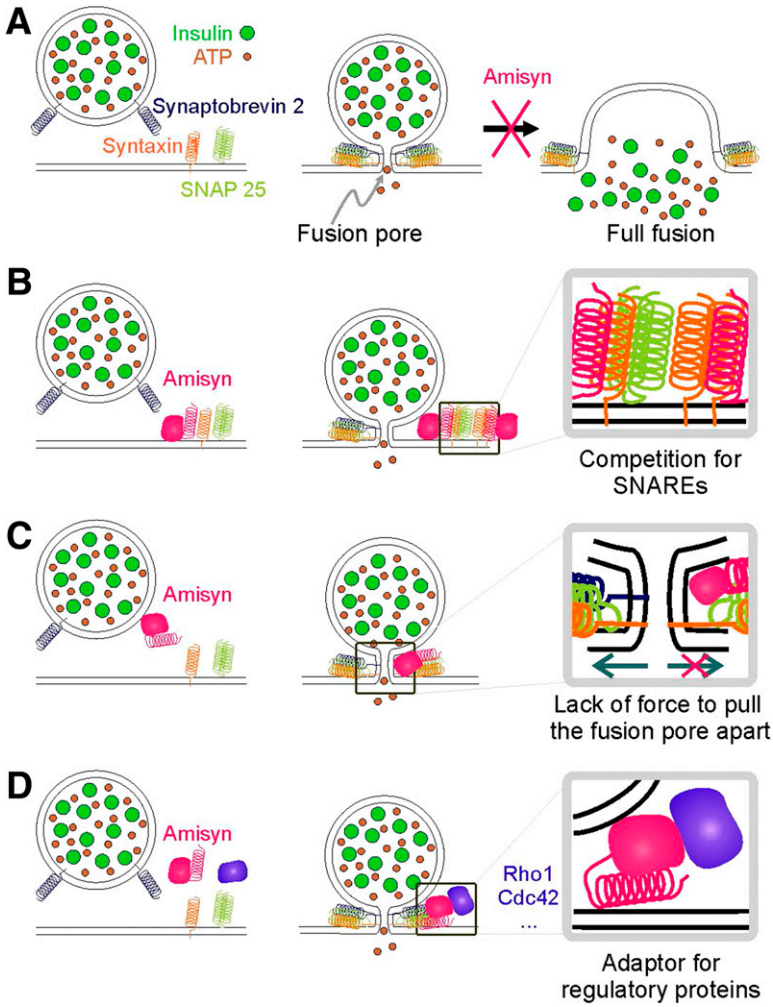

Figure 1—Amisyn prevents fusion pore expansion. *A*: Model of secretory granule exocytosis. Formation of the SNARE complex forces membrane fusion, which begins with the opening of a narrow fusion pore that allows passage of small molecules (middle). Subsequent pore dilation leads to insulin release (right). *B–D*: Hypothetical scenarios by which STXBP6/amisyn could prevent expansion of the fusion pore; see text for details.

transmembrane domain slow pore expansion (18). A third possibility is that amisyn acts as an adaptor for recruitment of regulatory proteins to the release site (Fig. 1*D*). PH domains are increasingly recognized as protein–protein interaction platforms (19), and the strong similarity of amisyn's PH domain with the exocyst component Sec3/Exoc1 suggests that it could act as coincidence detector by simultaneously binding phospholipids and small GTPases at the release site (20).

Although the concept of the fusion pore has been recognized for nearly three decades (21), Collins et al. (12) provide the first direct evidence for its role in human disease. Given the complexity of the exo- and endocytic machinery, additional proteins and signaling pathways are likely involved and may shift the balance of cargo release from insulin granules. Targeting these pathways may ultimately lead to novel treatments for diabetes, and with the development of high-resolution imaging techniques to study the fusion pore behavior, we are bound to learn more about syntaxin's friends tomosyn and amisyn ("tomo" meaning friend in Japanese and "ami" in French). Finally, the study highlights once again the power of combining genome-wide expression analysis with detailed functional analysis at the cellular level.

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