Functional roles of the dual Ca^{2+} sensors in the nematode C. elegans

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Neurotransmitter release at the C. elegans neuromuscular junction is governed by a dual Ca^{2+} sensor system composed of SNT-1 and SNT-3, which are functional analogs of synaptotagmin-1 and -7 (Syt1/Syt7) in mammalian central synapses. In this study, we investigated how SNT-1 and SNT-3 mediate fast and slow neurotransmitter release through their potential interactions with the SNARE complex and their polybasic motifs. AlphaFold 3 models of SNT-1–SNARE and SNT-3–SNARE complexes accurately recapitulated the canonical Syt1 C2B–SNARE primary interface (Zhou et al., *2015, Nature*) and precisely identified conserved binding residues within the C2B domains, as well as in SNAP-25 and Syntaxin, highlighting the evolutionary conservation of this interaction. Electrophysiological analyses using targeted mutagenesis demonstrated that both SNT-1 and SNT-3 require C2B–SNARE interactions and polybasic motifs within their C2 domains to drive evoked fast and slow neurotransmitter release. Notably, SNT-1 and SNT-3 exhibited differential dependence on distinct regions of the C2B–SNARE interface and their respective polybasic motifs, suggesting that Ca^{2+}-triggered fast and slow release operate via distinct mechanistic strategies. Furthermore, we found that SNT-1 mediates spontaneous neurotransmitter release through multiple pathways, involving not only the primary C2B–SNARE interface but also additional putative SNARE-binding interactions. Together, our findings uncover both conserved and divergent mechanisms for synaptic exocytosis regulated by the dual Ca^{2+} sensors in *C. elegans*.