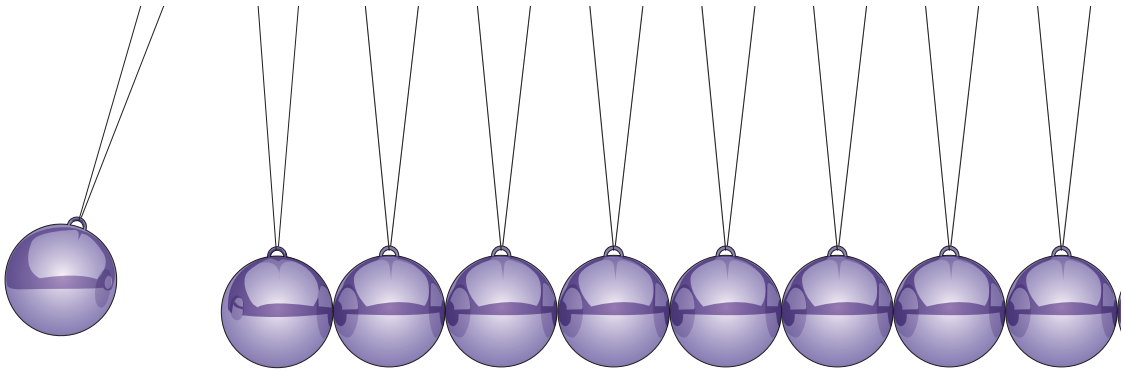

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CELL BIOLOGY OF THE NEURON

Balancing capture and release

> Strikingly, stationary *arl-8* mutant puncta showed a lower dissociation rate and a higher capture ability than their wild-type counterparts

Axonal transport is necessary for the formation of synapses, but it remains unclear how presynaptic protein transport and deposition are coordinated to ensure the correct patterning of synapses. A new study in *Caenorhabditis elegans* shows that the balance between the aggregation and dissociation of presynaptic proteins during their trafficking is a crucial determinant of presynaptic patterning.

In the presynaptic terminal, the active zone (AZ) contains specialist molecular machinery that allows synaptic vesicles (SVs) to undergo plasma membrane fusion. The delivery of SV and AZ proteins to the correct sites within the terminals relies on molecular motor-driven transport.

The authors studied *C. elegans* DA9 motor neurons, an *in vivo* model of presynaptic patterning. It had previously been shown that loss-of-function mutations in *arl-8*, which encodes a conserved ARF-like small G protein, caused the accumulation of presynaptic materials (including SV protein transport vesicles (STVs)) in proximal axons and a loss of presynapses in distal axon regions.

Here, through forward genetic screening, the authors identified two missense mutations in *jkk-1* (which encodes a homologue of mammalian mitogen-activated protein kinase kinase 7) that could suppress, to differing extents, SV protein mislocalization in *arl-8* mutant DA9 neurons. JKK-1 phosphorylates and activates c-Jun N-terminal kinase 1 (JNK-1), a homologue of mammalian JNK3 (also known as MAPK10), and the authors found that a null allele of *jnk-1* could also suppress the SV protein mutant phenotype, indicating that JKK-1-to-JNK-1 signalling regulates SV protein localization.

The abnormal STV accumulations in *arl-8* mutant DA9 neurons also contained AZ proteins, which could be co-transported with SV proteins in axons. A *jkk-1* null allele partially suppressed AZ protein mislocalization and reversed the synaptic transmission deficits, suggesting that the JNK pathway promotes presynaptic clustering, whereas ARL-8 suppresses it.

The authors examined the axonal transport of STVs in *arl-8* mutant and wild-type DA9 neurons by measuring, via time-lapse imaging, the movement of green fluorescent protein (GFP)-tagged RAB3, an SV protein. Stationary and motile GFP-tagged RAB3-positve puncta were detectable in neurons from both groups. Moreover, among the motile puncta, some coalesced with stationary puncta (a capture event), whereas others divided to generate stationary and motile puncta (a dissociation event).

Strikingly, stationary *arl-8* mutant puncta showed a lower dissociation rate and a higher capture ability than their wild-type counterparts, indicating that ARL-8 suppresses STV clustering during axonal transport by inhibiting STV coalescence and promoting movement events after STV capture. The *jkk-1* mutant normalized the dissociation rate in *arl-8* mutant neurons but did not rescue the capture defect, suggesting that the JNK pathway promotes presynaptic protein clustering by preventing STV dissociation.

Various AV proteins, including SYD-2, are required to recruit SV proteins to presynaptic terminals, and a loss-of-function mutation in *syd-2* had previously been shown to strongly suppress STV clustering in *arl-8* mutants. Here, stationary GFP-tagged RAB3 puncta showed a higher dissociation rate, and there was a higher number of movement events in *syd-2* mutants than in wild-type worms, indicating that SYD-2 promotes STV accumulation by promoting STV coalescence.

Finally, the authors identified a gain-of-function mutation in *unc-104* — which encodes a homologue of the mammalian molecular motor protein KIF1A — that suppressed the *arl-8* phenotype by reducing the capture probability of STV clusters. ARL-8, in its GTP-bound form, could directly bind to UNC-104, and disruption of this binding could induce an *arl-8* mutant-like phenotype, suggesting that UNC-104 is a downstream effector of ARL-8 and promotes STV motility.

This study reveals that presynaptic protein transport and assembly, and hence presynaptic patterning, are regulated by the balance between the coalescence and dissociation of STVs, and that various factors regulate one or both of these processes.

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ORIGINAL RESEARCH PAPER Wu, Y. E. *et al.* The balance between capture and dissociation of presynaptic proteins controls the spatial distribution of synapses. *Neuron* 30 May 2013 (doi:10.1016/j.neuron.2013.04.035)