A novel role of presynaptic periactive zone proteins in extracellular vesicle trafficking

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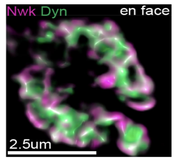
Introduction

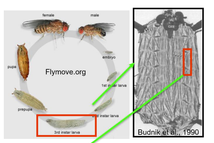
- Extracellular vesicles (EVs) are small, endosomally-derived, membrane bound vesicles that can transport protein, lipid, and nucleic acid cargoes between cells (Colombo et al 2014, Mulchany et al., 2014; French et al., 2017; van Niel et al 2018)
- Trafficking of EVs within the nervous system is important for cell-cell communication (Fauré et al 2006, Korkut et al., 2009, Koles et al 2012, Morel et al 2013, Korkut et al 2013, Fröhlich et al, 2014, Budnik et al 2016, Pastuzyn et al 2018, Ashley et al 2018) and EVs may also spread toxic proteins between cells in disease (Coleman & Hill, 2015)
- We have identified a novel role for presynaptic periactive zone (PAZ) membrane remodeling proteins in regulating the traffic of EV cargoes at the *Drosophila* NMJ, genetically separable from their well-established functions in synaptic vesicle recycling and synaptic growth
- This includes the FBAR SH3 protein Nwk, which is known for its role in membrane trafficking (Rodal et al., 2008; Becalska et al., 2013; Kelley et al., 2014)
- We hypothesize that PAZ membrane remodeling machinery plays a novel role in protecting EV cargoes from a degradative fate, therefore allowing for their release

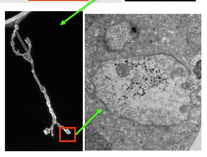
Drosophila NMJ

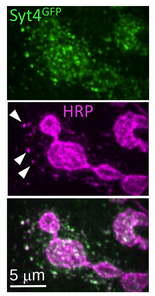


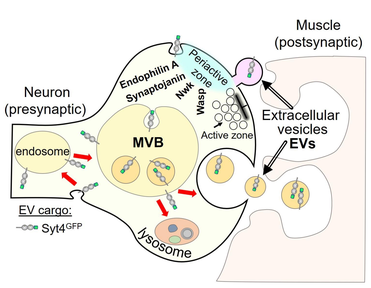
surround sites of SV release (peri-active zones)

Using the *Drosophila* NMJ as a model to study the regulation of EV cargoes

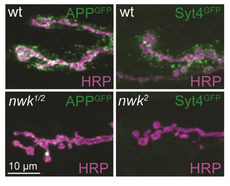


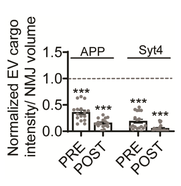




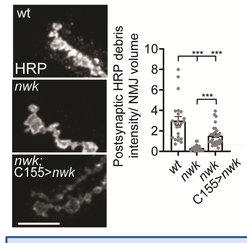


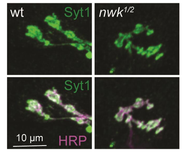
Neuronal Nwk specifically regulates levels of EV cargoes at the NMJ

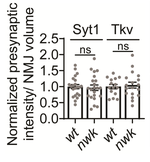


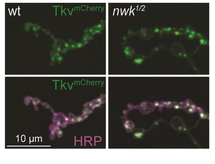


- *nwk* mutants exhibit a significant reduction in pre- and postsynaptic levels of EV cargoes Amyloid Precursor Protein (APP) and Synaptotagmin-4 (Syt4) at the NMJ
- Loss of *nwk* also leads to a significant reduction in postsynaptic HRP debris, which can be partially rescued through neuronal expression of *nwk*
- Levels of canonical endocytic, non-EV cargoes (Tkv and Syt1) are similar between control and *nwk* NMJs

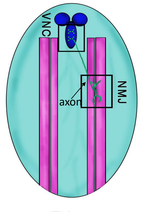


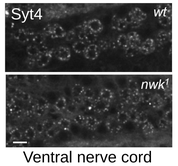


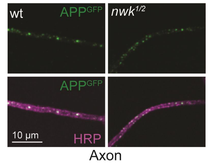


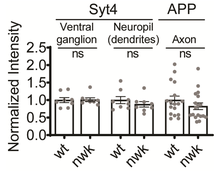


Nwk locally regulates EV cargo levels at the NMJ



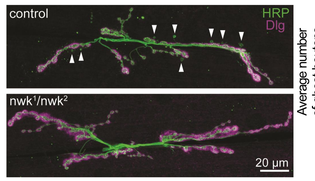


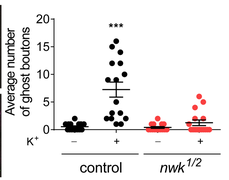


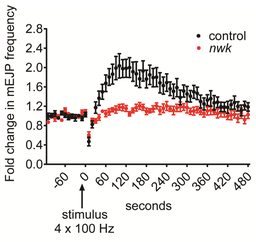


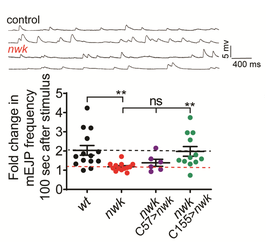
EV cargoes are locally decreased at the motorneuron axon terminals in *nwk* mutants. Cargo levels are similar between control and *nwk* mutants in the cell bodies, dendrites, and axons of the motorneurons.

nwk mutants exhibit loss of EV cargo function

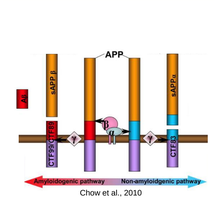


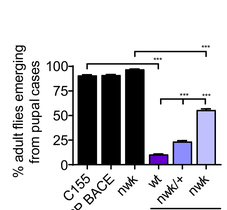


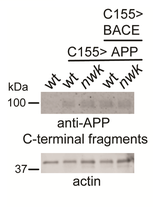


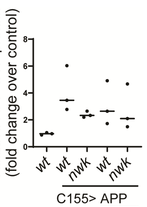


- Spaced stimulation increases the formation of ghost boutons (nascent boutons that lack active zones and postsynaptic proteins) (Ataman et al., 2008) and *syt4* mutants fail to form ghost boutons upon stimulation (Korkut 2013, Piccioli et al., 2014, Harris et al 2016).
- High frequency stimulation induces an increase in mini EJP frequency, which is blocked in *syt4* mutants (Yoshihara et al., 2005, Barber et al 2009, Korkut 2013, Cho et al 2015).
- *nwk* mutants exhibit a failure to form ghost boutons with spaced stimulation, and a failure to increase mini frequency upon stimulation, indicating a loss of EV cargo Syt4 function at NMJ axon terminals in *nwk* mutants.





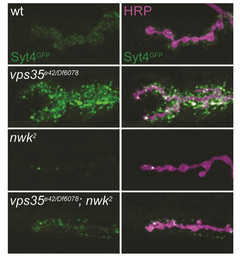


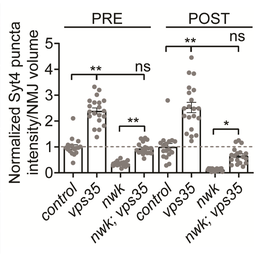


- Overexpression of APP and its amyloidogenic cleavage enzyme beta secretase (BACE) in the nervous system leads to a reduction in eclosion of adult flies from pupal cases
- Loss of *nwk* suppressed the eclosion defects of flies overexpressing APP and BACE in the nervous system in a dose-dependent manner, suggesting that *nwk* mutants suppress the pathological effects of APP in the nervous system
- Loss of *nwk* does not impact levels of neuronally expressed APP, or APP and BACE, in adult fly brains

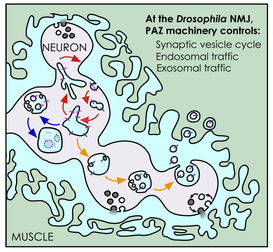
Loss of retromer restores EV cargo levels at *nwk* mutant NMJs

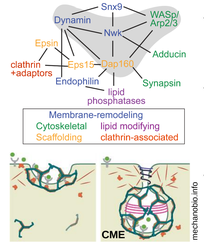
- Vps35 is a component of the retromer complex which has important roles in recycling endosomal cargoes back to the plasma membrane (Wang et al., 2014)
- *vps35* mutants have increased levels of pre- and postsynaptic EV cargoes which accumulate in a Rab11-positive compartment. This suggests that Vps35 functions to retrieve EV cargoes from a recycling route in order to remove them from an EV-permissive pathway (Walsh et al., bioRxiv 645713).
- Loss of *vps35* restores EV cargo levels at *nwk* NMJs, suggesting Nwk and Vps35 exert opposing actions on EV cargo proteins





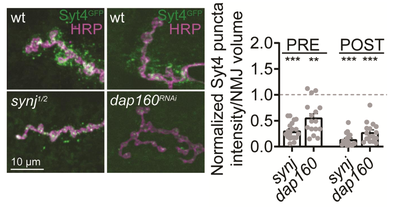
Nwk is a component of the periactive zone membrane remodeling machinery



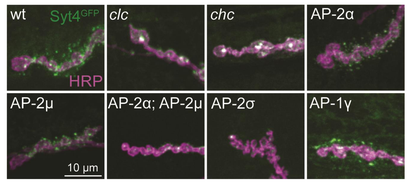


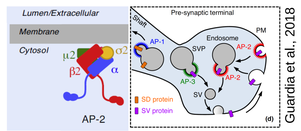
- Nwk functions with a network of periactive zone machinery with canonical roles in clathrin mediated endocytosis (CME)
- The periactive zone (PAZ) is the presynaptic region surrounding the active zone where PAZ membrane remodeling proteins are localized
- PAZ proteins have diverse functions in neurons, including the regulation of synaptic vesicle recycling and endosomal growth factor receptor trafficking

Directed screen identifies subset of PAZ proteins required for EV cargo trafficking



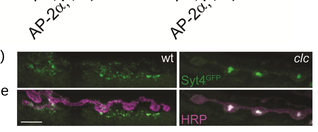
Loss of Synaptojanin and neuronal knockdown of Dap160 leads to a decrease pre- and postsynaptically in EV cargo levels, like loss of Nwk, suggesting a role for PAZ machinery molecules in regulating EV cargo traffic



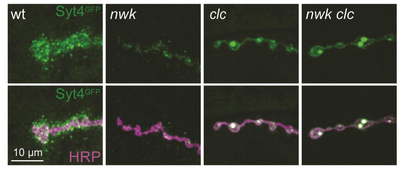


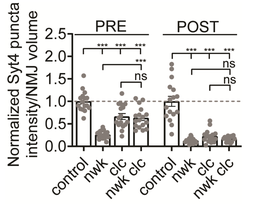
Clathrin and the AP-2 adaptor complex (α, β, σ, and μ subunits) function in CME to regulate synaptic vesicle cycling and receptor endocytosis in neurons

Guardia et al., 2018



- Clathrin mutants (*clc*, *chc*) exhibit a reduction in postsynaptic EV cargo levels, with no change in presynaptic levels but a change in presynaptic localization, showing presynaptic accumulations of Syt4 that are also HRP positive
- AP-2α and AP-2μ have mild effects on EV traffic, while their simultaneous loss, or loss of AP-2σ leads to a significant decrease in EV cargo levels both pre- and postsynaptically, like loss of *nwk*.
- Suggests that the regulation of EV cargoes at synapses is a clathrin and AP-2 adaptor complex dependent process
- Are these presynaptic accumulations in clathrin mutants sensitive to loss of *nwk*?





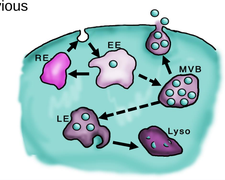
nwk clc flies phenocopy *clc* mutant levels and localization of EV cargo Syt4, suggesting cargoes are sequestered within a compartment that is inaccessible to the mechanism downregulating EV cargoes in *nwk* mutants

Conclusions

- We have found a novel role for the presynaptic PAZ machinery in regulating EV cargo traffic
  - Genetically separable from its well-established role in synaptic vesicle recycling: AP-2α mutants have a strong SV cycling defect with mild effects on EV traffic, while *nwk* mutants exhibit a milder SV cycling defect with a strong defect in EV traffic
- PAZ proteins specifically regulate EV cargoes at synapses and do not regulate canonical endocytic cargoes
- The physiological function of Syt4 and the pathological function of APP is diminished in *nwk* mutants and likely in other PAZ mutants such as shibire, making it important to reconsider previous mutant phenotypes of these genes
- Nwk acts at a similar point in the endosomal system to Vps35, likely at the level of recycling endosomes

Future Directions

- What do EV cargoes go in *nwk* flies? Are they being degraded?
- What impact does neuronal stimulation have on EV cargoes in PAZ mutants?
- Is the involvement of PAZ proteins in EV regulation conserved in mammalian cells?



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