Roles of reticulons and REEPs in organisation of axonal endoplasmic reticlum in *Drosophila*

Belgin Yalçın[1], Niamh C. O'Sullivan[1], Martin Stofanko[1], Zi Han Kang, Annika Hartwich, Matt Thomas, Cahir J. O'Kane[2]
([1]equal contributions; [2]corresponding author)

Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK

The length of motor axons requires significant engineering for their formation and maintenance. Failures of this maintenance are seen in the Hereditary Spastic Paraplegias (HSPs), characterised by lower limb weakness, and degeneration of longer upper motor axons. To date over 50 causative Spastic Paraplegia Genes (SPGs) have been identified. Several encode endoplasmic reticulum membrane proteins, with intramembrane hairpin structures that curve and model ER membrane. Two of these protein families, reticulons and REEPs, are together required for formation of most tubular ER in yeast. To test whether they are also required for formation or maintenance of axonal ER, we have developed suitable markers in *Drosophila*, and analyzed mutants that lack members of these protein families.

Drosophila has two reticulon genes, one of which, *Rtnl1*, is widely expressed. YFP-tagged Rtnl1 localises strongly in axons and presynaptic terminals, in contrast to most conventional ER markers. *Rtnl1* knockdown reduces levels of smooth ER marker in longer distal motor axons, but not in shorter or proximal ones – analogous to HSP. This effect is also seen in labeled single axons, with no loss of ER continuity.

Drosophila has six REEP genes, two of which are widely and highly expressed. ReepA is orthologous to mammalian REEPs 1 to 4, and ReepB to mammalian REEP5 and REEP6. We can detect ReepB::GFP but not ReepA::GFP in axons and presynaptic terminals. When both ReepA and ReepB are removed, ER organisation is altered in epidermal cells but appears normal in axons. However, triple mutants that remove Rtnl1, ReepA and ReepB show major disruption of ER in motor axons.

We have therefore established *Drosophila* motor axons as a system for assaying the organisation of axonal ER, and the roles of spastic paraplegia genes in this process. This will allow additional and newly identified HSP genes to be tested for such roles, either singly or in combination with others.

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