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NEURAL DEVELOPMENT

# No entry for AnkG

The scaffolding protein ankyrin G (AnkG; also known as ANK3) is restricted to the proximal part of axons, where it is required for the assembly of the axon initial segment (AIS) and for maintaining neuronal polarity. How this precise localization is controlled was not known, but now, Rasband and colleagues show that a cytoskeleton in the distal part of the axon forms a boundary that spatially restricts AnkG clustering and thereby AIS assembly. The authors first showed that in developing mouse brains, AnkG immunoreactivity in the proximal axon only appeared by the time the axons had reached (or almost reached) their final destination, indicating that axon specification precedes AnkG clustering. Interestingly, AnkG clustering always occurred at the same distance from the cell body, suggesting the existence of some sort of boundary that restricts the location of the AIS. Immunostaining experiments in cultured hippocampal neurons suggested that the axonal cytoskeletal proteins AnkB (also known as ANK2), αII-spectrin and βII-spectrin may act as such a boundary, as these proteins were localized in the distal axon and their staining patterns never overlapped with that of AnkG.

To investigate whether these three proteins indeed regulate the localized clustering of AnkG, the authors lowered the expression of AnkB, αII-spectrin or βII-spectrin in newly plated neurons using short hairpin RNAs. This prevented AnkG clustering in the AIS and instead led to its redistribution into the distal axon. Conversely, overexpression of AnkB resulted in a shortened AnkG-enriched region in the proximal axon. In addition, premature expression of AnkG in newly cultured neurons resulted in a longer AIS and a shorter region of AnkB immunoreactivity 3 days later. Thus, appropriate AnkG clustering depends on both the presence of AnkB, αII-spectrin and βII-spectrin and the correct temporal pattern of expression of all four proteins.

Experiments in mice lacking AnkB, mice lacking αII-spectrin and mice in which βII-spectrin was knocked out specifically in neurons confirmed the role of the cytoskeletal proteins in AnkG clustering *in vivo*. Together, these findings show that an exclusion mechanism provided by a cytoskeleton containing AnkB, αII-spectrin and βII-spectrin in the distal axon controls appropriate AnkG clustering and, therefore, the location of the AIS.

*Leonie Welberg*

**ORIGINAL RESEARCH PAPER** Galiano, M. R. *et al.* A distal axonal cytoskeleton forms an intra-axonal boundary that controls axon initial segment assembly. *Cell* **149**, 1125–1139 (2012)
**FURTHER READING** Rasband, M. N. The axon initial segment and the maintenance of neuronal polarity. *Nature Rev. Neurosci.* **11**, 552–562 (2010)