ABSTRACT

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Grant Title: ***The roles of glycans in the intra-axonal compartmentalization of neuron***

1. Objectives

Molecules carrying BP102 antigen, a marker of *Drosophila* central nervous system, are localized specifically in the proximal axon segment of isolated primary cultured neurons (1). Specific localization on proximal axon segments requires dynamin-dependent endocytosis. However, the mechanism underlying the preferential trafficking of molecules to the proximal axon segment is largely unknown. To elucidate it, we examined (i) which kind of molecules is localized on proximal axon segments and (ii) the ultrastructural structures at the intra-axonal boundary, and then (iii) screened the key molecules for segmentation by using *Drosophila* mutants.

2. Methods

The cells, including postmitotic neurons before axonogenesis, were obtained from *Drosophila* embryos at stages 9-11 and cultured on glass bottom dishes for 24h (1,2). Then we performed immunostaining, lectin-staining and FluoroNanogold labelling of fixed primary cultured cells. A confocal microscope was used for the detection of the optical images. EM images were taken on the ClairScope ASEM (atmospheric scanning electron microscopy) system (3,4).

3. Results

The major *O*-glycan, T antigen, localized in the proximal axon segments of *Drosophila* neuron. Ultrastructural analysis by ASEM showed that microtubule bundles cross one another at the intra-axonal boundary, and that T antigens accumulate near the boundary and formed circular pattern before the boundary. We then screened and identified spectraplakin, a crosslinker protein between F-actin and microtubules, as a key molecule for the proximal localization of T and BP102 antigens. Spectraplakin was distributed in the proximal axon segment like T antigen. Null mutation of spectraplakin inhibited preferential localization of T and BP102 antigens. Moreover, we demonstrated that the F-actin binding domain of spectraplakin is required for this special trafficking. Therefore, our results suggest a novel trafficking pathway; (i) spectraplakin bridges between microtubules and F-actin near the intra-axonal boundary, (ii) changes the trafficking direction of *O*-glycan-carrying vesicles and (iii) makes them move to the proximal plasma membrane (5).

Reference

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