SINGLE MOLECULE TRANSLATION IMAGING: QUANTIFYING PROTEIN SYNTHESIS SPATIALLY AND TEMPORALLY IN GROWING AXONS

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ABSTRACT: During neurodevelopment retinal ganglion cells (RGCs) have to project their axons from the eye to the back of the brain. The outmost tip of a growing axon, the growth cone, navigates to its remote target, the optical cortex, by sensing the local concentrations of extracellular cues, such as Netrin-1, and responds to such guidance cues by turning, advancing, or pausing in a timely manner. Previous studies [1] showed that this complex task is achieved by the growth cone mostly autonomous via local protein synthesis. Specifically, β-actin is a major candidate protein to mediate the sensation of guidance cues to structural responses as it is a key structural component of growth cones and its mRNA has been localized in axons.

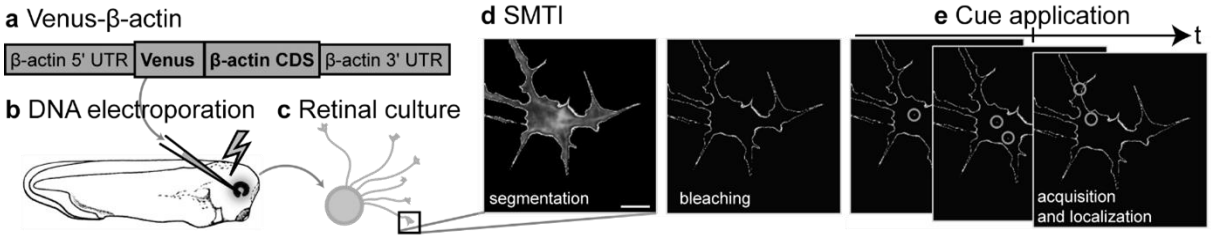

Figure 1: SMTI in *xenopus'* retinal ganglion cell axons to study the effect of the cue Netrin-1.

To gain access to a direct readout of local protein synthesis, we developed *single molecule translation imaging* [2]. Hereby, the coding sequence of the fast-folding and fast-bleaching fluorescent protein Venus is incorporated into the DNA coding sequence of the protein of interest, β-actin, and the thus generated constructed injected into the RGC cell body. In a procedure similar to FRAP, the fluorescent actin-mesh of already synthesised protein is photobleached and under more moderate illumination intensities the translation of individual molecules is observed as sub-second long photon-bursts to yield data-sets similar to those generated by PALM. From the localisation data, we reconstruct density maps of local protein expression and quantify the rate kinetics of local synthesis in dependence of guidance cues.
Particularly, we discovered that Netrin-1 triggers a burst of β-actin synthesis at multiple non-repetitive sites, particularly in the axon-tip periphery. The response is remarkably rapid starting within 20 seconds of cue application.

REFERENCES

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