SPIM-FCS SHOWS THAT THE SECRETED SIGNALING PROTEIN WNT3 RESIDES IN PLASMA MEMBRANE LIPID DOMAINS IN VIVO

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Single Plane Illumination Microscopy (SPIM) combined with Fluorescence Correlation Spectroscopy (FCS) provides a tool that can measure spatiotemporal characteristics of molecular processes in 3D environments [1, 2]. SPIM-FCS has the characteristic advantages of light sheet microscopy of low photobleaching and phototoxicity compared to confocal FCS. However, SPIM-FCS is still diffraction limited. By combining the spatial with the dynamic information, however, one can obtain information even below the diffraction limit. This is possible since any structure, even if it is below the diffraction limit, influences molecular dynamics which can be observed above the diffraction limit. This relation between spatial organization and dynamics has been formulated as the so-called FCS diffusion laws [3, 4]. Here we apply the diffusion laws in SPIM-FCS to the investigation of a morphogen, Wnt3, whose localization within the plasma membrane of cells in live zebrafish is unknown. However, the information on the localization is essential in understanding Wnt3 secretion and its function in vivo.

Wnt3 is a signaling protein involved in development and disease (tetraamelia syndrome and oncogenesis). Recently, we have shown that Wnt3 regulates cerebellum development using zebrafish Wnt3 transgenics [5]. In particular, we demonstrated that the membrane-bound O-acyltransferase Porcupine is required for Wnt palmitoylation, secretion and biological activity. It was shown in vitro that for membrane localization in Wnt3-expressing cells Wnt3 undergoes two lipid modifications by palmitoylation at C77 and S209. Such posttranslational modifications have been linked with protein association with cholesterol-dependent lipid domains. This raises the question whether Wnt3 is targeted to these domains in live zebrafish.

Using SPIM-FCS we are able to show that Wnt3 localizes to cholesterol dependent domains in live zebrafish embryos and that this localization is disturbed when either the lipid modification of Wnt3 is inhibited by the porcupine inhibitor C59, or when the cholesterol concentration is reduced by methyl-β-cyclodextrin.

References