

Title: Genetic and Physical Regulation of Ventricular Development and Reissner Fiber Formation in Zebrafish

ABSTRACT

The vertebrate brain develops around fluid-filled cavities whose growth and patterning depend on epithelial secretion, membrane organisation, and mechanical forces transmitted through cerebrospinal fluid (CSF). Within this environment, the Reissner fiber (RF) is an evolutionarily conserved extracellular structure found in vertebrates with horizontal body posture. RF is assembled largely from the glycoprotein SCO-spondin (Sspo), which is secreted by the subcommissural organ (SCO; anterior RF) and floor plate (posterior RF). RF acts in proprioception during straightening of the body axis and its maintenance, as well as in ventricular homeostasis. In this thesis, I used zebrafish to test how molecular regulation and physical constraints jointly shaped ventricular development and contributed to the initiation of the anterior RF.

I addressed three related questions. First, I examined how the voltage-gated potassium channel Kv2.1, which consists of the electrically active subunit Kcnb1 and the modulatory subunit Kcng4b, influenced Sspo secretion and anterior RF assembly. Mutations in Kv2.1 subunits produced strong, opposing anterior RF phenotypes. These phenotypes coincide with changes in RF-associated transcript levels, Sspo localization and secretion, and differences in SCO apical architecture. They are consistent with an effect of Kv2.1 on membrane potential and Sspo secretion.

Second, I tested whether membrane composition contributed to these defects. Manipulating cholesterol availability disrupted the conditions required for Sspo secretion and anterior RF initiation. These findings support a model in which cholesterol-dependent membrane organisation shaped signalling competence and polarised secretion in ventricular epithelia.

Third, I tested whether gravity-dependent mechanics influenced anterior RF formation. Sustained changes in embryo orientation during a defined developmental window reduced anterior RF and were accompanied by altered ventricular morphology and transcriptional responses in mechanosensory genes expressed in CSF-contacting neurons (CSF-cNs).

Overall, my results supported the view that RF formation depends on SCO secretion, membrane potential, and body position during early brain morphogenesis.