Radim Žídek

"Wnt/β-catenin signalling in the development of the marine annelid *Platynereis dumerilii*"

(dissertation)

Abstract:

Wnt/β-catenin signalling is absolutely crucial for the early embryonic development of metazoan animals from the establishment of body axes, through the specification of germ layers and tissues to the development of organ systems. I used pharmacological manipulations of the Wnt/β-catenin pathway activity in the planktonic larvae of the marine polychaete annelid *Platynereis dumerilii*, the representative of the clade Spiralia, to investigate the role of Wnt/β-catenin signalling in the development and evolution of three hallmarks of Bilateria: the central nervous system, the body segmentation and the digestive tube.

Wnt proteins are produced in all three aforementioned systems in *Platynereis* where they trigger the Wnt/β-catenin pathway in neighbouring cells. I describe here, for the first time in *Platynereis*, a homologue of the endpoint transcription factor of the entire pathway, *Pdu-Tcf*, which is subjected to an alternative splicing and along with a Wnt target gene *Pdu-Axin* is expressed in tissues with the active Wnt signalling – in the brain ganglia, in the neuroectoderm along the ventral midline, in segments, in the posterior growth zone and in the gut.

Pharmacological manipulations suggest that Wnt/β-catenin signalling specifies neuronal progenitors in the ectoderm and promotes their proliferation, but it is not involved in the patterning of the nervous system in *Platynereis* as it does not significantly shift the boundaries of the expression domains of the neural-specific transcription factors. However, an analysis of their normal expression revealed a putative homology of the vertebrate and insect brain signalling centre, the isthmic organizer, with the ciliated posterior boundary of peristomium with the cryptic zero segment and suggested the existence of another in the anterior peristomium boundary. I thus propose that organizers of brain development are derived from the ciliated bands of an ancient planktonic bilaterian ancestor.

Wnt/β-catenin signalling positively regulates the segmentation gene *Pdu-Engrailed* on the intersegmental boundary, which confirms the current model of segmentation in *Platynereis* by the mechanism that is conserved between *Platynereis* and *Drosophila* and suggests a presence of a mutually exclusive, non-autonomous positive feedback loop between Wnt and Hedgehog signalling pathways. The over-activation of the Wnt/β-catenin pathway leads to a loss of chaetal sacs and of morphological, but not molecular boundaries between segments. The

Wnt/β-catenin signalling is active also in the posterior growth zone where it is probably involved in the formation of new segments.

The midgut development is delayed relative the anterior and posterior parts of the gut due to a high amount of yolk in the macromeres that inhibits cell division. It is reactivated much later in the nectochaete stage and entails the expression of neural-specific transcription factors *Pdu-Otx* and *Pdu-Nk2.1*. Wnt/β-catenin signalling positively regulates *Pdu-Cdx* in the hindgut and previously unrecognized domains in the ventral gut midline and the midgut/foregut boundary. The inhibition of Wnt/β-catenin pathway completely blocks the proliferation in the entire body of the larva and arrests the differentiation of the gut endoderm to a digestive epithelium. The typical expression of digestive enzymes diminishes from the midgut which instead retains the expression of *Pdu-Legumain* which is expressed here earlier, but is normally in this phase present already only in the hindgut. This state is not permanent and the differentiation continues once the inhibition is alleviated. I propose that the Wnt/β-catenin signalling specifies endodermal gut progenitors, promotes their proliferation and triggers steps that eventually lead to their differentiation.