



**Karolinska
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Institutionen för Cell- och Molekylärbiologi

Morphogen Interpretation in the Developing Nervous System

AKADEMISK AVHANDLING

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ABSTRACT

Development of the central nervous system relies on the generation of specialized cell types in a tightly controlled spatial and temporal order from neural progenitor cells. Morphogen molecules, secreted by defined sources, spatially organize neural progenitors by inducing discrete expression patterns of cell fate determinant genes in a concentration-dependent manner. The combinatorial expression of these patterning genes defines distinct progenitor domains from which specific neuronal subtypes are generated. This thesis deals with one of the big challenges in developmental sciences, which is to understand how these inductive gradients are translated into precise transcriptional outputs.

Sonic Hedgehog (Shh) is a morphogen essential for the generation of ventral neuronal subtypes. In **Paper I**, we have identified the *cis*-regulatory modules (CRM) of neural Shh-target genes, which we use as tools to elucidate the mechanisms imposed by Gli proteins, the bifunctional transcriptional mediators of Shh gradient. We find that Gli activators have a non-instructive role in long-range patterning and in synergy with SoxB1 proteins activate Shh target genes in a largely concentration independent manner. Instead, Gli repressors are interpreted at transcriptional levels into precise spatial gene patterns in combination with regional homeodomain co-repressors. Moreover, the local interpretation of Shh displays lower CRM context sensitivity and requires Gli activators to accumulate to a threshold level sufficient to counteract Gli repressors. Thus our data propose a novel mechanism for transcriptional interpretation of Shh gradient.

Paper II studies a feedback circuit between Shh and its downstream homeodomain targets that establishes the non-graded regulation of Shh signaling activity. We show that by regulating *Gli3* expression, Nkx2 proteins amplify and Pax6 antagonizes Shh signaling. The amplified Shh response is important for specification of the two most ventral cell fates: the floor plate (FP) and V3. However, the spatial separation of the two domains appears to be achieved by the acquisition of neurogenic potential over time in the p3 domain, rather than by different Shh concentrations. These data establish that the non-graded, intrinsic changes in responding cells operate in parallel with graded mechanisms and are required for correct interpretation of Shh signaling.

Morphogens are pleiotropic signals that regulate development of various tissues, but how they induce tissue-specific responses remains unresolved. **Paper III** explores the tissue-specific interpretation of Shh, Bone Morphogenic Proteins (BMP) and Retinoic Acid (RA) signaling and shows that direct transcriptional integration of these pathways with SoxB1 proteins at the CRM level is required for activation of neural targets. We further show that the genome-wide collocation of binding sites for SoxB1 and morphogen-mediatory transcription factors in CRMs can faithfully predict the neural-specific gene activity. Moreover, misexpression of SoxB1 proteins in the limb bud confers mesodermal cells with the potential to activate neural-specific target genes upon activation of Shh, BMP or RA signaling. Accordingly, our data offers a fairly simple conceptual explanation for morphogen-mediated transcriptional regulation of neural-specific target genes during embryogenesis.