Signal Integration by Gene Regulatory Landscapes during Mouse Limb Bud Organogenesis – Robustness and Evolutionary Diversification

We are interested in understanding the molecular mechanisms underlying the regulatory circuits that govern vertebrate organogenesis. To this aim we study how gene regulatory landscapes integrate multiple signaling inputs into robust but highly dynamic transcriptional outputs. We are taking advantage of our in-depth knowledge of the self-regulatory signaling systems that control vertebral limb bud organogenesis. In particular, we are identifying the functionally relevant cis-trans-regulatory interactions that control the establishment and propagation of this self-regulatory signaling system during initiation and progression of mouse limb bud organogenesis. Often, gene expression is regulated by multiple cis-regulatory modules (CRMs) that are scattered in large genomic landscapes, but how these landscapes integrate transcriptional inputs is still largely unknown. Therefore, we profile chromatin architecture, epigenetic marks, interaction of transcriptional regulators with CRMs and transcriptionomes to identify the relevant CRMs, which are functionally analysed by CRISSP/Cas9 genome editing (Fig. 1). Experimental analysis is combined with bioinformatics and in silico simulations to reveal gene regulatory networks (GRNs) and interaction kinetics. A key node in the self-regulatory limb bud signaling system is the BMP antagonist Grem1, whose expression is controlled by a large genomic landscape integrating inputs from all major signaling pathways. Within the Grem1 landscape, we have identified the region required for limb bud expression, which encodes three CRMs regulated by key transcriptional regulators in limb buds. Currently, we are studying these CRMs that appear to regulate Grem1 expression in a cooperative manner, possibly as part of a super enhancer. As modern tetrapods limbs display amazing evolutionary diversity, a second fascinating aspect of your research is insights into how this robust and self-regulatory signaling system evolved and diversified. Recently, we showed that the HAND2 transcription factor is essential to this process and polarization of the nascent mesenchyme. C-Myc in combination with genetic analysis revealed the gene regulatory logic by which the HAND2-target GRN establishes proximal, anterior and posterior compartments and activates SHH expression in early limb buds (Fig. 2). Recently, we have shown that HAND2 controls similar GRNs during early heart development. Our studies begin to reveal the similarities in the HAND2-target GRNs controlling both limb and heart organogenesis, which points to a possible origin of these two structures. Finally, we have shown that SMAD4, which is part of the transcriptional complexes mediating response to canonical BMP signaling, is essential for normal limb bud development. We have used C-Myc and comparative transcriptome analysis to identify the genes and GRNs that are regulated by SMAD4 chaotic circuitry. Our analysis provides insight into the functionally relevant cis-regulatory dynamics of SMAD4-mediated BMP signal transduction during limb bud organogenesis and chondrogenic differentiation. With respect to the latter, we are performing an in depth analysis of the functions of the BMP antagonists Grem1 and Noggin and SMAD4-mediated BMP/TGFβ signal transduction during chondrogenesis and digit ray formation. Understanding BMP functions in the switch from proliferating mesenchymal to differentiating chondrogenic progenitors is directly relevant to development-inspired engineering of cartilage (joint SFP project with I. Martin).

Selected Publications


