Universität Basel

Research Project

Integrative analysis of gene regulatory networks with essential functions in limb bud development

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Project title Integrative analysis of gene regulatory networks with essential functions in limb bud development
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During vertebrate embryonic development, organ and tissue morphogenesis are orchestrated by interactions of gene regulatory networks (GRNs) and signaling systems that regulate the proliferation, survival, specification and differentiation of progenitor cell populations. Vertebrate limb bud organogenesis provides an excellent model to study the interactions that coordinate patterning and proliferative expansion of limb bud mesenchymal progenitors (LMPs) with their commitment to the chondrogenic lineage, which will give rise to the limb skeletal elements. One major asset of the limb bud model is that alterations of patterning and/or outgrowth manifest themselves in easily scorable skeletal phenotypes that correlate in general well with the underlying molecular alterations. Therefore, we use mouse limb buds to address questions of fundamental relevance to organogenesis. In particular, we study the GRNs and signaling systems that coordinately control limb bud development by combining genome editing, genetics and experimental embryology with transcriptional profiling, genome-wide chromatin immunoprecipitation analyses (ChIP-seq) and protein biochemistry. The first part of our research aims to comprehensively analyse the GRNs regulated by key transcription factors during the onset of limb bud development. These GRNs set-up the initial antero-posterior and proximo-distal polarities and restrict SHH to the posterior mesenchyme. This are the main aims: 1. TBX3 interactions with HAND2 in pre-patterning the early limb bud mesenchyme. We will analyse the TBX3 specific and shared HAND2/TBX3 transcriptional targets and GRNs in early mouse limb buds. One main focus will be the analysis of HAND2/TBX3 interactions establishing the posterior Gli3 expression boundary that hallmarks the initial AP polarisation of the limb bud mesenchyme. 2. Direct regulation of Hand2 expression by PBX1/2 and analysis of the target genes co-regulated by PBX and HAND2 in early limb buds. We will analyse the PBX1/2 interacting CRMs in the Hand2 cis-regulatory landscape to gain insight into their functions in the spatial-temporal regulation of Hand2 expression. In addition, we will identify the transcriptional targets and GRNs co-regulated by PBX and HAND2 and analyse the function of key targets in the onset of limb bud development.3. Toward a comprehensive understanding of the molecular interactions and regulatory circuits that govern limb bud pre-patterning. We will determine if HAND2 forms complexes with TBX3, PBX1/2 and/or transcriptional regulators in early limb buds. In addition, we will use simulations of the HAND2, TBX3 and/or PBX controlled GRNs to gain insights into the underlying regulatory logic, which should inform further analysis.The second part of our research aims to gain functional insights into the self-regulatory signaling system defined by the SHH/GREM1/AER-FGF feedback loop. The BMP antagonist Grem1 is a key node in this system that controls distal progression of limb bud outgrowth. Our ongoing analysis has pro-

vided evidence for the underlying robustness of this signaling system. Therefore, we aim to uncover the molecular basis and regulatory logic underlying the phenotypic robustness of the signaling system.4. Probing the robustness of the self-regulatory limb bud signaling system. We have generated several Grem1 alleles with reduced expression levels, which provides an unique opportunity to gain insight into the potential robustness of the signaling systems controlling limb skeletal development. We will profile the limb bud transcriptome of Grem1 alleles with stable (=robust) and variable (=labile) pentadactyly and loss of specific digits. The transcriptional signatures will identify genes/pathways for further analysis of their potential involvement in providing the signaling system with robustness.5. Functions of BMP antagonist SMOC1 in the limb bud signaling system. We have identified Smoc1 as an additional BMP antagonist in the signaling system. Therefore, we will assess the requirement of Smoc1 for the signaling interactions that control limb bud organogenesis and assess its role in robustness by compensating reduced Grem1 levels up to a certain threshold.The proposed research takes a holistic approach and should provide fundamental insights into the transcriptional and signaling interactions that ensure robust and stereotypic development of vertebrate limbs, but still permitted tinkering with these interactions during evolutionary diversification. The proposed analysis of the GRNs and signaling systems that control limb bud development will provide insights into how embryonic progenitors integrate signaling inputs into a dynamic, but at the same time robust transcriptional response.

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