Transcription Regulation in Development and Disease

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Opening Remark

We use molecular- and biochemical-cell-systems as well as transgenic mice and gene targeting approaches to investigate how differential gene expression patterns are established and maintained during mammalian development and under disease conditions. We address this question through investigating the biology of SOD1 and two transcription factors Runx1 and Runx3 (Fig. 1). The RUNX transcription factors are master regulator of lineage specific gene expression in developmental pathways. SOD1 and *RUNX1* reside on human chromosome 21 and are possibly involve in ALS and in Down syndrome leukemia whereas *RUNX3* reside on chromosome 1 at a region known to be involved in several human diseases. Below is a spotlight account summarizing our approach to the issue "how transcription regulators are regulated".

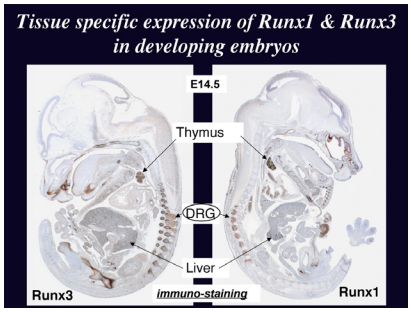


Fig. 1 *Runx1 and Runx3 are expressed in common organs but in different cell types. Immunohistochemistry with anti Runx1 and anti Runx3 abs shows that both genes are expressed in embryonic thymus, DRG, liver and developing skeletal elements. Runx1 is also expressed in the nasal epithelium.*

Long Range Enhancer-Promoter communications regulate Tissue Specific expression of Runx1 and Runx3

The mammalian RUNX transcription factors (TF) are master regulators of gene expression in several important developmental pathways and when mutated are involved in human diseases. Even though the RUNX TF recognize the same DNA-motif and regulate their target genes through interaction with common transcriptional co-activators or co-repressors, the functional overlaps between them are minor, and each RUNX has a distinct subset of biological functions (Fig. 1). This paradigm is underscored by the findings that each of the corresponding RUNX knockout mice displays a unique subset of phenotypic abnormalities that correlate with cell-autonomous functions of RUNX as master regulators of cell lineage specifications. We have previously shown that this lack of functional redundancy results from a tightly regulated spatio/temporal expression

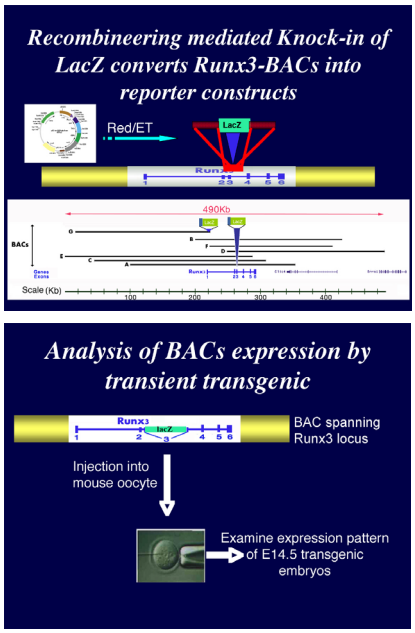


Fig. 2 *Recombineering mediated knock-in of LacZ converts Runx3-BACs into reporter constructs.* ***a.*** *Upper part: Insertion of LacZ using the Red/ET recombineering system of GENEBRIDGES. Lower part: collection of BACs (marked A-G) spanning 490 kb of Runx3 genomic locus each modified by in-frame insertion of LacZ into exon #3.* ***b.*** *Each LacZ expressing BAC was microinjected into fertilized ova and transient-transgenics analyzed at E14.5 for LacZ expression pattern.*

mediated by an intricate transcriptional control mechanism. For example, both Runx1 and Runx3 genes are expressed in developing dorsal root ganglia, but in different classes of sensory neurons similarly, both are expressed in mature T cells, but at different stages during T cell development.

How this tissue specific expression of *RUNX* in a distinct subset of cell types and at defined time windows during development is attained? How these transcriptional regulators are regulated?

We employed a series of overlapping BACs spanning large genomic regions (i.e. hundreds Kb) of *Runx3* and *Runx1* loci to identify and characterize the long-range regulatory elements mediating tissue and stage specific expression of *Runx1 and* Runx3 during development and in adults. Using recombineering each BACs was modified to include in-frame reporter (LacZ) and then employed to produce transient transgenic mice (Figs. 2a & 2b).

This approach culminated in generating a series of transgenic mouse strains each expressing a different Runx1 *or* Runx3 reporter BAC (Fig. 2a & 2b). Analysis enabled us to identify several subsets of conserved non-coding elements, residing hundred Kb upstream of the basic promoter/transcriptional start site, that confer stage and cell-type specific expression of Runx1 and Runx3 during embryogenesis and postnatally (Figs. 3a and 3b).

Significantly, we have identified the specific dorsal root ganglia (DRG) enhancers mediating expression of Runx1 and Runx3 in DRG TrkA nociceptive and TrkC proprioceptive neurons, respectively (Fig. 3a and Figs. 4a and 4b).

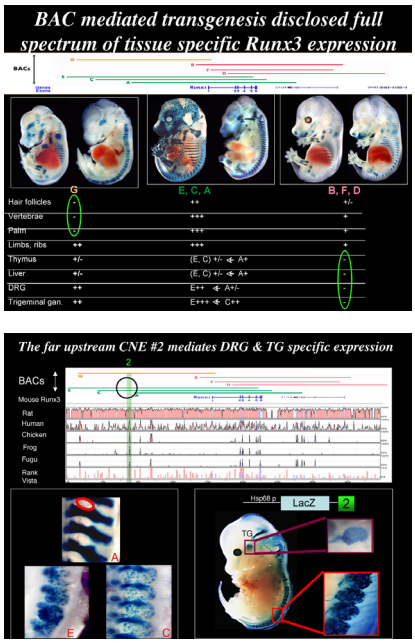
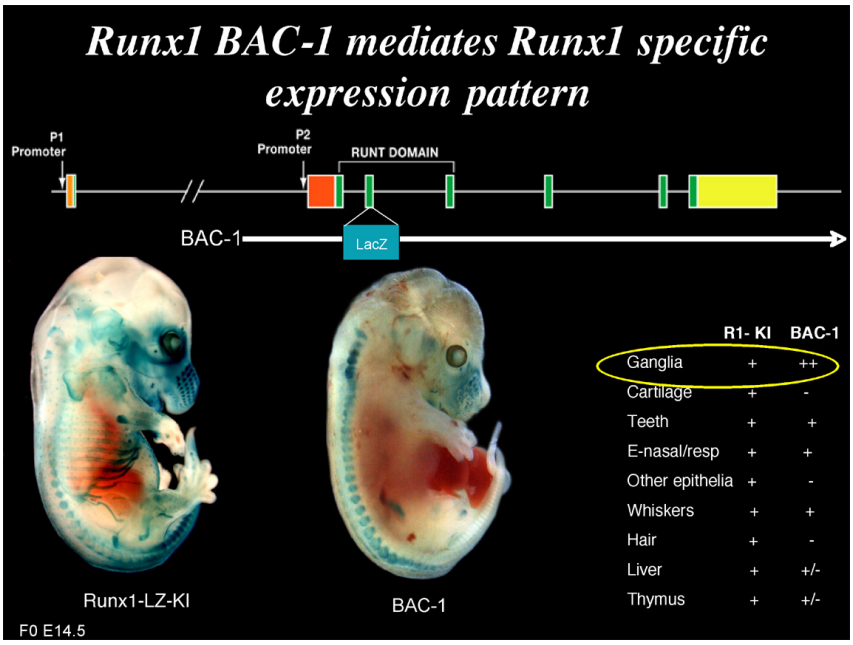


Fig. 3 *BAC mediated transgenesis disclosed full spectrum of tissue specific Runx3 expression.* ***a.*** *Analysis of LacZ staining of overlapping BAC array identified the regions (designated in the table below) encompassing tissue specific regulatory elements.* ***b.*** *Circled in the upper scheme is a conserved non-coding element (CNE#2) located in BACs overlapping region that mediated DRG specific LacZ expression. CNE#2 was isolated and linked to a LacZ reporter gene driven by the constitutive hsp68 promoter (lower right panel). Microinjection of CNE#2-driven reporter plasmid mediated DRG and trigeminal ganglia (TG) specific expression.*



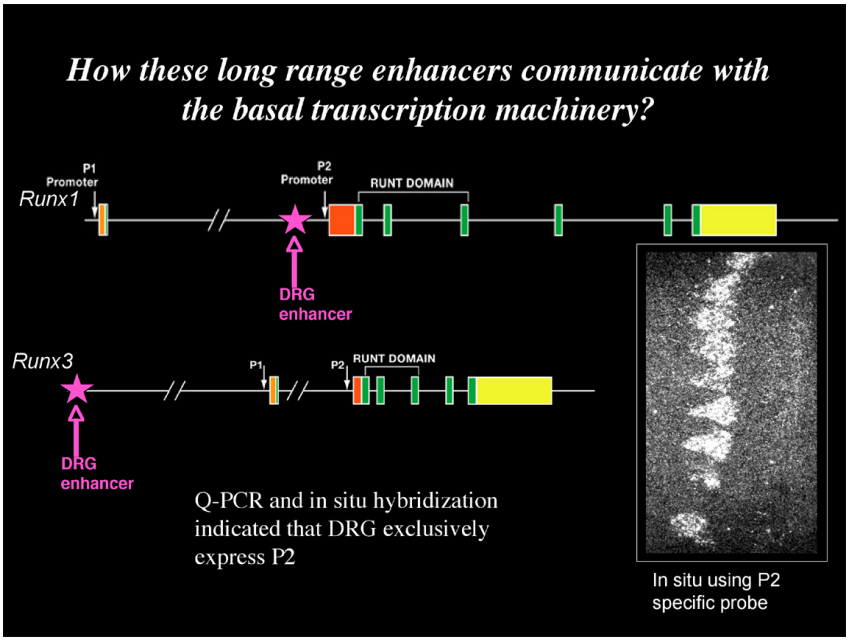


Fig. 4 *Runx1 BAC-1 mediates Runx1 specific expression.* ***a.*** *BAC-1 of Runx1 spans 50 kb upstream of the P2 promoter and 50 kb downstream of the gene 3'-end. Transient transgenics of LacZ knock-in BAC displayed Runx1 specific expression listed in the table. Note the prominent expression in the DRG.* ***b.*** *Schemes depicting the genomic location of DRG specific enhancer of Runx1 and Runx3. A CNEs of BAC-1 located upstream of P2 promoter was identified, isolated and showed to confer DRG specific expression in transient transgenics. In situ hybridization (right panel) indicated Runx1-P2 specific expression in DRG.*

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