Spblimp1/krox: A Transcriptional Regulator with a Central Role in Endomesoderm Specification in Sea Urchin Embryos.

Spblimp1/krox: a sea urchin transcription factor

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Abstract

During cleavage stages, Spblimp1/krox is expressed in the large micromeres and veg2 descendents. During later blastula stages, it is expressed in the endoderm precursors of the veg1 ring of cells distal to the vegetal pole of the blastula. Later, its expression is restricted to the blastopore region and the posterior of the invaginating archenteron, and finally to the midgut and hindgut of the pluteus larva. The expression of Spblimp1/krox is thus dynamic, and involves several distinct spatial territories. A GFP-recombinant BAC was created to distinguish the expression pattern of the early form from that of the late form. The protein coding sequence of GFP was substituted for that of the second exon, 1b. This construct closely mimics Spblimp1/krox expression during early stages of sea urchin development. Using anti-sense morpholino (MASO) knockdown perturbations, we analyzed the downstream targets of Spblimp1/krox. We confirmed previously-published data stating that Spblimp1/krox autoregulates its own expression, and we found that it represses itself. This negative autoregulation is restricted to the mesodermal veg2 territory during the blastula stage, as shown by WMISH analysis of MASO-injected embryos.

Blimp1/Krox inputs expected from the perturbation analysis into other genes have been incorporated in our gene regulatory network.

Spblimp1/krox has two isoforms that are alternatively transcribed, 1a and 1b. Spblimp1/krox1a is expressed starting at gastrulation. Phylogenetic footprinting analysis reveals several conserved sequence patches several hundred bp long, in comparisons between Strongylocentrotus purpuratus and Lytechinus variegatus of the genomic region
surrounding the *blimp1/krox* locus. When included in an injected expression construct, one of these conserved patches recapitulates the expression of the 1a isoform during embryogenesis. This regulatory module of the *Spblimp1/krox* gene, which lies immediately upstream of the transcription initiation site for *Spblimp1/krox1a*, directs expression with great accuracy to the midgut and hindgut of the postgastrular embryo. Work on the regulation of the early 1b isoform will be reported elsewhere.
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Abstract

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Abstract

Results and Discussion

Genomic sequences surrounding the *blimp1/krox* gene

Identification by “Family Relations” analysis of conserved genomic sequence patches in the vicinity of the *Spblimp1/krox* gene

A DNA fragment which accurately generates the late expression pattern of the *blimp1/krox* gene

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Annotation of 900bp sequence immediately 5' of exon1a, including putative transcription factor binding sites

*cis*-Regulatory analysis of expression driven by module 43 and the network model for endomesoderm specification

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