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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