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Fate Specification Along the Sea Urchin Embryo Animal-Vegetal Axis

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Introduction

Like those of a large majority of taxa, sea urchin embryos establish a spatial coordinate system for the initial body plan from one axis, the animal-vegetal (A-V), that is fixed during oogenesis by asymmetric deposition of maternal molecules (the embryologists' "determinants") and a second axis, dorsal-ventral (or, more descriptively, oral-aboral), that is specified sometime during the first few cleavage divisions (reviewed by Davidson, 1989). The ability of sea urchin embryos to establish these axes while continuously reorienting in culture suggests that neither axis is sensitive to the earth's gravitational field. In embryos of many sea urchin species, A-V polarity is evidenced by the unequal sizes of blastomeres of the 16-cell embryo, which consists of tiers of eight mesomeres, four macromeres, and four micromeres. Classical experimental micromanipulations of embryos (reviewed by Hörstadius, 1973), have established that the fates of micromeres are determined by inheritance of maternal molecules. In addition, the micromeres provide a vegetal focus of inductive influence that is critical in the normal embryo for appropriate specification of fates of overlying animal blastomeres, and that can induce vegetal differentiation (gut, secondary mesenchyme) in cells of more animal tiers when micromeres are transplanted to ectopic sites (Khaner and Wilt, 1991; Ransick and Davidson, 1993). Thus, specification of fates along the AV axis utilizes both major mechanisms familiar to developmental biologists—inheritance of maternally provided positional information, and communication among cells, presumably by ligand-receptor interactions.

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Studies with the Very Early Blastula Gene Set

The Very Early Blastula (VEB) gene set provides an entree to the mechanism of maternal determination of the AV developmental axis. Several years ago, we used subtractive cDNA screening methods to identify a set of mRNAs encoded by four different genes expressed transiently in the very early blastula (~150 cells), and absent from both the maternal pool of mRNAs and the message complement of later differentiated cells (Reynolds *et al.*, 1992). Surprisingly, the mRNAs identified by this simple temporal screen accumulate in the embryo with congruent spatial patterns and very similar temporal patterns. These genes, which we named the VEB genes, have two important features. First, the messages they encode accumulate asymmetrically along the AV axis, being present throughout the animal ~85% of the embryo but absent from the region around the vegetal pole. Second, the messages accumulate in embryonic blastomeres that are continuously separated beginning at the 2-cell stage. This latter property strongly suggests that activation of VEB gene expression, and consequently the asymmetry of accumulation, is regulated by a corresponding asymmetric distribution of maternal regulatory activities that corresponds to part of the molecular mechanism that establishes this axis. Our approach is to investigate the *cis*-acting elements in the regulatory apparatus of the VEB genes, and the *trans*-acting factors that drive their expression, to identify these critical molecular components.

Regulatory regions of the SpHE *and* SpAN *genes identify multiple positively acting factors that drive nonvegetal expression.* We have analyzed the regulatory regions of two of the VEB genes: *SpHE,* which encodes the hatching enzyme (Wei *et al.*, 1995), and *SpAN* (Kozlow-

ski *et al.*, 1996), which encodes an astacin-family protease that is likely to be involved in regulating the activities of cell signaling molecules (see below). These analyses include functional assays of expression of VEB promoter-driven transgenes microinjected into sea urchin eggs that are then allowed to develop into embryos, as well as standard *in vitro* assays of interaction of VEB promoter *cis* elements with proteins in nuclear extracts.

These studies have demonstrated that about 300 bp of sequence upstream of the transcription initiation site of *SpHE* and *SpAN* are each sufficient to drive expression of a β-galactosidase reporter in the correct nonvegetal pattern—*i.e.*, expression of the transgene is never observed in primary mesenchyme cells (PMCs) derived from the vegetal micromeres. Despite the congruence of expression of *SpAN* and *SpHE*, and the similar, compact structure of their regulatory regions, the repertoire of positively acting factors regulating these genes is rather different. On the basis of rate of accumulation of transcripts, the endogenous SpHE promoter is very strong. Reflecting this characteristic, it carries an apparent excess of positive regulatory sites (Wei *et al.*, 1995): The region within 300 bp of the transcription start site contains at least nine sites of protein binding which interact with six different factors. No one *cis* element appears to be critical for expression since replacement or deletion of individual sites decreases promoter activity no more than about twofold. Mutation of combinations of *cis* elements has little effect until three or four are inactivated. In contrast, the *SpAN* promoter contains fewer sites of protein-DNA interaction, two of which are essential for high transgene transcriptional activity (Kozlowski *et al.*, 1996).

The multiplicity of positive factors driving VEB gene transcription argues against the simple hypothesis that a single spatially restricted, positive factor regulates the nonvegetal accumulation of VEB mRNAs. At least three mechanisms could achieve spatial regulation of the VEB genes: (1) the presence of a vegetal negative regulator sufficient to prevent expression; (2) exclusion of the majority of positive regulators from vegetal pole cytoplasm; and (3) repression of the VEB genes in vegetal cells by means of a unique chromatin structure. To test for a negative regulator operating in the vegetal region, we made a series of deletions across the −300 *SpHE* promoter. None of these led to ectopic expression in PMCs, indicating that if a negative mechanism operates, it must involve multiple, independently sufficient regulatory sites. A vegetal-specific repressive chromatin structure might be established in micromeres which, as a result of unequal cleavage at 16-cell stage, would inherit a lower quantity of any uniformly distributed positively acting factors that were stockpiled in the egg cytoplasm. Consistent with this hypothesis, micromeres have a distinctive bulk chromatin structure that is less accessible to nuclease digestion than is that of the larger blastomeres (Cognetti and Shaw, 1981). Furthermore, these small vegetal cells are believed to contain a special cytoplasmic domain, as reflected by a loss of the pigment granules that are tethered in the egg cortical cytoskeleton (Schroeder, 1980). Experiments are underway to discriminate among these possibilities by examining the DNA-binding proteins present in separated animal and vegetal blastomeres, as well as by using *in vivo* footprinting coupled with ligation-mediated PCR to examine actual occupancy of *cis* elements in animal and vegetal blastomeres.

SpAN *may function to convert maternal gene regulatory information asymmetrically arrayed along the AV axis into spatial regulation of cell-cell interactions.* Because the sea urchin embryo cellularizes rapidly, and because the fates of all but the most vegetal blastomeres (micromeres) are context-dependent, the maternal AV asymmetry must be translated into, or supplemented by, extensive cell-cell interactions. Interestingly, one of the VEB genes, *SpAN*, encodes a member of the astacin protease family. Other members of this family include *Drosophila tolloid* (Schimell *et al.*, 1991) and *tolloid related* (Nguyen *et al.*, 1994; Finelli *et al.*, 1995). These metalloendoproteases consist of an astacin protease module linked to domains expected to be involved in protein-protein interactions (complement C1r/s; EGF), implying that they may function as tethered proteases. *Tolloid* has been shown to interact genetically with *decapentaplegic* (Ferguson and Anderson, 1992a, b), a member of the TGFβ superfamily of signaling molecules, and both of these genes are essential for regulating the fates of cells along the dorsal-ventral axis in the early *Drosophila* embryo. The only vertebrate astacin protease identified to date, BMP-1, has recently been shown to be a collagen-processing enzyme (Kessler *et al.*, 1996). However, a member of the TFGβ superfamily, BMP4 (which is functionally homologous to *dpp;* Padgett *et al.*, 1993), has been strongly implicated in regulating mesodermal patterning along the DV axis of *Xenopus* embryos (reviewed by Hogan *et al.*, 1994). Because BMP4 is synthesized as a pro-protein, like other members of the TGF-β family, it presumably requires activation by proteolytic cleavage, and astacin proteases are potential candidates. We have therefore explored the effect of over- or misexpressing *Xenopus* BMP4 and sea urchin SpAN in early sea urchin embryos by injecting the corresponding mRNAs into unfertilized eggs. Both proteins cause similar and dramatic phenotypic effects, interpreted most simply as suppression of differentiation of vegetal structures. Misexpression in *Xenopus* embryos of both BMP4 and SpAN also perturbs the dorsal-ventral axis leading to ventralized phenotypes. We hypothesize that SpAN

operates in sea urchin embryos in a BMP4-like pathway which functions to limit the range of inductive influence emanating from the vegetal pole. In this way, the *SpAN* gene, through its regulation by maternal factors, could convert maternal spatial information into regulation of cell signaling along the AV-axis.

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