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REVIEW

Animal–Vegetal Axis Patterning Mechanisms in the Early Sea Urchin Embryo

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We discuss recent progress in understanding how cell fates are specified along the animal–vegetal axis of the sea urchin embryo. This process is initiated by cell-autonomous, maternally directed, mechanisms that establish three unique gene-regulatory domains. These domains are defined by distinct sets of vegetalizing (β-catenin) and animalizing transcription factor (ATF) activities and their region of overlap in the macromeres, which specifies these cells as early mesendoderm. Subsequent signaling among cleavage-stage blastomeres further subdivides fates of macromere progeny to yield major embryonic tissues. Zygotically produced Wnt8 reinforces maternally regulated levels of nuclear β-catenin in vegetal derivatives to down regulate ATF activity and further promote mesendoderm fates. Signaling through the Notch receptor from the vegetal micromere lineages diverts adjacent mesendoderm to secondary mesenchyme fates. Continued Wnt signaling expands the vegetal domain of β-catenin's transcriptional regulatory activity and competes with animal signaling factors, including BMP2/4, to specify the endoderm–ectoderm border within veg_1 progeny. This model places new emphasis on the importance of the ratio of maternally regulated vegetal and animal transcription factor activities in initial specification events along the animal–vegetal axis. © 2000 Academic Press

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INTRODUCTION

An early critical step in the development of all animal embryos is the establishment of a three-dimensional coordinate system for patterning embryonic structures. Embryos may inherit this information in the form of asymmetric distributions of maternal determinants or morphogens that are established during oogenesis, or a polarity may be imposed by epigenetic mechanisms after fertilization. The presence of a morphologically detectable axis, and even of maternal molecular asymmetries, is not always accompanied by a demonstrable functional polarization of determinants (for review see Goldstein and Freeman, 1997). Embryos of diverse taxa rely to different extents on these two mechanisms. At the one extreme, the meroistic oogenesis of *Drosophila* establishes both anterior–posterior and dorsal–ventral axes before fertilization. At the other extreme, mouse oocytes lack any fixed polarity of developmental potential (Zernicka-Goetz, 1998). Embryos of the majority of different taxa utilize both mechanisms: The animal–vegetal (A-V) axis is constructed maternally, while the second axis is established after fertilization. The animal pole is assigned by the site of polar body extrusion, and, in yolky eggs (e.g., that of *Xenopus*), the yolk is concentrated at the vegetal pole. Within this latter group, different embryos vary in the extent to which fates of blastomeres arrayed along the A-V axis are primarily determined by inheritance of cytoplasmic determinants. In classic "mosaic" embryos, such as those of ascidians, fates of both animal and vegetal early blastomeres are fixed by this mechanism. In other embryos, maternal factors are sufficient to commit blastomeres at one pole, while patterning of the remainder of the embryo relies on inductive interactions (reviewed by Goldstein and Freeman, 1997).

The sea urchin embryo is one of the best studied of this last group, beginning with studies at the dawn of experimental embryology (Boveri, 1901a,b): When unfertilized eggs are bisected through the equator and the two halves are fertilized, the animal half gives rise to an incompletely differentiated epithelial ball, the *dauerblastula,* while the vegetal half can often produce a relatively normal pluteus larva with derivatives of endoderm, mesoderm, and ecto-

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derm (Hörstadius, 1939; Maruyama *et al.*, 1985). This difference demonstrates the presence of determinants of fate in the vegetal pole and the requirement for cell–cell interactions to complete fate specification of animal blastomeres. During cleavage, a maternally regulated, reproducible pattern of cell divisions partitions the egg cytoplasm among blastomeres that consequently have defined sizes and orientations relative to each other. Although, as discussed below, most blastomeres have the potential to assume a wide variety of fates, the geometric precision of cleavage restricts the range of cell–cell interactions that take place in the normal embryo, with the result that the fates of blastomeres at different positions along the A-V axis are reproducible and predictable. The fate map of the embryo at the 16- and 60-cell stages and the corresponding derivatives in the pluteus larva are diagrammed in Fig. 1. For a more detailed description, see Davidson *et al.* (1998). At the 16-cell stage, A-V polarity is morphologically evident: From animal to vegetal are arrayed tiers of eight mesomeres, four macromeres, and four micromeres. At the 32-cell stage, the mesomeres have divided equatorially to give two tiers of 8 cells each, named an_1 and an_2, while in the vegetal hemisphere the macromeres have divided meridionally to give one tier of 8 cells, and the micromeres have divided obliquely to give large and small daughters. At the 60-cell stage, the animal hemisphere consists of the daughters of an_1 and an_2, macromeres have given rise to two 8-cell tiers called veg_1 and veg_2, and the large micromeres have divided, while the small have not.

An extensive series of blastomere isolation and recombination experiments, both classical (reviewed by Hörstadius, 1973) and contemporary (reviewed by Davidson *et al.*, 1998), has shown that the only determined blastomeres are the larger progeny of the vegetal micromeres; when isolated in culture or placed in any experimental combination, they will differentiate as primary mesenchyme cells (PMCs). Fates of all other blastomeres remain plastic until the mesenchyme blastula–gastrula period. Their specification depends on a wave of inductive interactions initiated by signaling from the micromeres. Two experiments illustrate this mechanism: (1) When micromeres are transplanted to the animal pole, ectopic gastrulation is induced (Hörstadius, 1973; Ransick and Davidson, 1993) and (2) when micromeres are removed from the vegetal pole, gastrulation is greatly retarded and incomplete (Ransick and Davidson, 1995). Thus, the first border separating major tissue territories is between PMCs and mesendoderm and is established at the 16-cell stage by the separation of macromeres and micromeres. Borders between secondary mesenchyme (SMC) and endoderm and between endoderm and ectoderm are negotiated by inductive interactions among macromere progeny during the late cleavage-to-mesenchyme blastula period. For convenience, we will refer to the mechanisms that pattern vegetal blastomeres that specify mesenchyme and endoderm collectively as the "vegetal signaling mechanism" (VSM). This includes both maternally programmed cell-autonomous processes and initial signals sent between blastomeres during cleavage. At the same time, the animal blastomeres, although capable of remarkable developmental regulation, are maternally biased toward an ectoderm fate, represented by the *dauerblastula*, to which we will refer as "preectoderm."

In this review, we focus on maternal mechanisms that initiate differences in developmental potential along the A-V axis and downstream events that pattern major regions of the embryo. We discuss recent experiments that begin to define the molecular mechanisms establishing borders between PMC, SMC, endoderm, and ectoderm territories.

A MODEL FOR PATTERNING OF CELL FATES ALONG THE PRIMARY, A-V, AXIS

As a framework for discussing the mechanisms of cell fate specification in the sea urchin embryo, we begin with an updated model, which is summarized in Fig. 2. We then review the evidence supporting the individual components of this model. Its major features are as follows:

- Maternal mechanisms establish functionally distinct sets of gene regulatory activities in vegetal and animal domains.
- The VSM includes at least three temporally and spatially regulated components: a maternally regulated, cell-autonomous, wave of entry of β-catenin into nuclei that progresses from vegetal micromeres through the derivatives of veg_2; maternally initiated signaling through a Notch pathway; and zygotic, SpWnt8-mediated augmentation of nuclear β-catenin in cells in the vegetal hemisphere.
- The animal preectoderm domain is defined by a cohort of positive transcription factor (the animalizing transcription factor; ATF) activities that initially are present in macromeres and mesomeres, but are below functional levels in micromeres. In the absence of nuclear β–catenin, the ATFs conditionally specify an early preectoderm state and probably also are required for activation of downstream genes involved in differentiation of definitive ectoderm.
- During cleavage, the domains of nuclear β-catenin and of the ATFs overlap in the progeny of the macromeres. This initially provides the macromere lineages with a unique combination of gene-regulatory activities that conditionally specifies them as early mesendoderm. This definition excludes the micromeres, the founders of skeletogenic and coelomic mesoderm, that are maternally determined.
- In veg_2 and the lower progeny of veg_1, zygotic expression of Wnt8 in micromeres and veg_2 reinforces the maternally programmed wave of nuclear β-catenin, promoting the differentiation of both endoderm and secondary mesenchyme.
- Signaling from micromeres to vegetal progeny of macromeres by a Notch pathway diverts them to secondary mesenchyme fates.
- Zygotic activation of a BMP2/4 pathway in animal blastomeres promotes ectoderm differentiation and, acting

in opposition to the VSM, negotiates the ectoderm–endoderm border within the progeny of veg_1.

In this model, construction of the A-V coordinate system begins with the maternal and cell-autonomous programming of animal and vegetal transcription domains. The region of overlap in the macromere daughters constitutes a conditionally specified early mesendoderm domain whose subsequent remodeling and subdivision into secondary mesenchyme, endoderm, and ectoderm requires signals from both animal and vegetal cells. This model considers only pathways for which at least some members have been identified in sea urchin embryos. Furthermore, we consider in detail only the first steps in the specification of major regions of the embryo—PMCs, SMCs, endoderm, and ectoderm—whose anlage are arranged in that order along the axis from vegetal to animal. We begin with the VSM that is required to specify mesoderm and endoderm.

THE VEGETAL SIGNALING MECHANISM IS TRIGGERED BY MATERNALLY REGULATED NUCLEAR β–CATENIN

The molecular pathway that constitutes the first step of the VSM recently has been identified, primarily in the laboratories of McClay (Logan *et al.*, 1999; Sherwood and McClay, 1999), Klein (Wikramanayake *et al.*, 1998), and Gache (Emily-Fenouil *et al.*, 1998) (see Fig. 2). The regulatory molecule is β-catenin, acting in its role as a transcription coactivator with sea urchin Lef1/Tcf1 (Huang *et al.*, 1999; Vonica *et al.*, 1999). Key components of this pathway are illustrated in Fig. 3. The VSM is initiated by entry of β-catenin into vegetal nuclei, which is first detectable at the 16-cell stage in the micromeres and gradually spreads through the presumptive vegetal plate (macromere derivatives) during cleavage (Logan *et al.*, 1999). Importantly, this appears to be a maternally controlled process that is independent of cell–cell interactions, both in micromeres and in the other vegetal blastomeres. This was demonstrated by the fact that it occurs in an appropriate fraction of cells when embryos are continuously dissociated into individual cells beginning at the 2-cell stage (Logan *et al.*, 1999). Furthermore, entry of β-catenin into nuclei of the veg_2 lineage does not appear to be dependent on signaling from adjacent micromeres, since it takes place in embryos from which micromeres have been removed immediately after fourth cleavage (Logan *et al.*, 1999). The nuclear concentration of β-catenin is graded, being highest in the most vegetal blastomeres, the small micromeres at the 32-cell stage. At the 60-cell stage, the β–catenin concentration in veg_2 nuclei is higher than in veg_1 nuclei and, after the next cleavage, it is sharply down regulated in veg_1. Because the concentration gradient initially established in vegetal blastomeres at the 16-and 32-cell stages is reciprocal to the difference in blastomere sizes, it does not simply reflect the potential reservoir of nuclear β-catenin. Thus, entry of β-catenin into nuclei must be regulated by upstream maternal components that are active in a vegetal-to-animal spatial and temporal gradient.

Nuclear β-catenin has critical functions in patterning vegetal tissues. First, it is necessary for micromeres to acquire signaling capacity, as well as for the subsequent differentiation of their larger daughters as PMCs. Injection of low levels of mRNA encoding a truncated sea urchin G-cadherin severely reduces the level of β-catenin in nuclei (Logan *et al.*, 1999), with the result that SMCs and endoderm fail to differentiate and the presumptive PMCs remain as incompletely differentiated epithelial cells in the wall of the animalized *dauerblastula* (Wikramanayake *et al.*, 1998; Li *et al.*, 1999; Logan *et al.*, 1999). Micromeres from an embryo treated in this manner cannot induce an archenteron when transplanted to the animal pole of a normal embryo (Logan *et al.*, 1999). Second, nuclear β-catenin is necessary for the overlying veg_2 progeny to develop competence to respond to micromere signaling. Embryos injected with cadherin mRNA cannot respond to induction when their micromeres are replaced with those from normal embryos and such chimeras consequently fail to differentiate vegetal structures (McClay, unpublished data). Conversely, embryos that have been injected with mRNA encoding a nonphosphorylatable, constitutively active form of β-catenin (Wikramanayake *et al.*, 1998) are severely vegetalized, differentiating expanded endoderm and secondary mesenchyme at the expense of ectoderm. These elegant experiments demonstrate that differentiation of vegetal tissues requires maternally regulated, cell-autonomous entry of β-catenin into vegetal nuclei both for initiation of micromere signaling and for reception of that signal by the overlying blastomeres.

The effects of altering expression of other members of the β-catenin signaling pathway are completely consistent with their demonstrated biochemical interactions (refer to Fig. 3). Tcf/Lef is a partner of β-catenin in transcriptional regulation (Clevers and van de Wetering, 1997). Introduction into sea urchin embryos of a dominant negative form of sea urchin or *Xenopus* Tcf/Lef that lacks the β-catenin binding domain produces animalized phenotypes (Huang *et al.*, 1999; Vonica *et al.*, 1999). Conversely, a constitutively active, β-catenin-independent form, consisting of the *Xenopus* Tcf-3 DNA binding domain linked to the strong transcription activation domain of VP16, vegetalizes embryos (Vonica *et al.*, 1999). For β-catenin to accumulate in nuclei, its phosphorylation by GSK3-β kinase and subsequent degradation must be prevented. Expression of dominant negative (kinase-dead) GSK3-β presumably blocks this turnover, leading to overaccumulation of β-catenin in nuclei and vegetalization (Emily-Fenouil *et al.*, 1998). Conversely, overexpression of GSK3-β causes severe animalization. Treatment of embryos with LiCl, an inhibitor of GSK3-β, expands the domain of nuclear β-catenin into the presumptive ectoderm territory (Logan *et al.*, 1999) and the embryos are vegetalized. dnTcf/Lef can completely reverse the vegetalizing effect of LiCl, consistent with the idea that most,

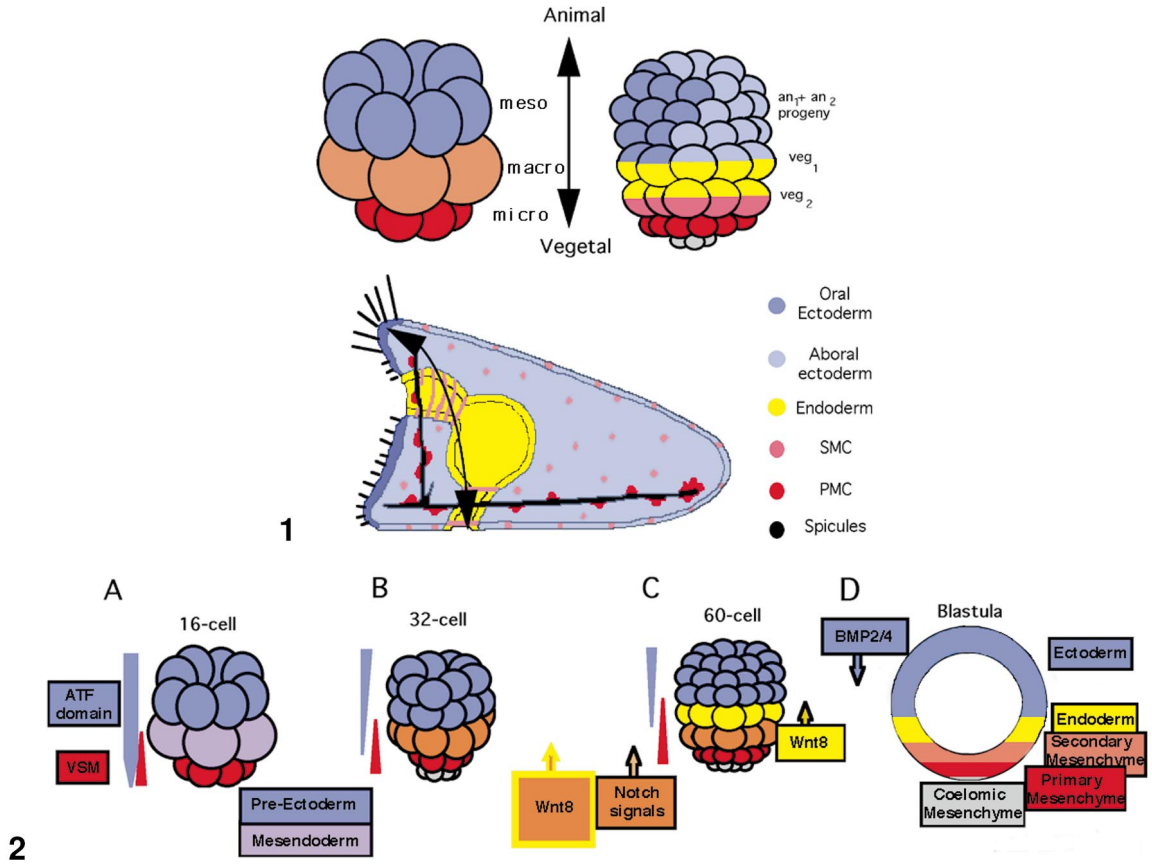


FIG. 1. Fate map of the sea urchin embryo. Lineages of major regions of the sea urchin embryo are illustrated at the 16-cell, 60-cell, and pluteus stages. At the 16-cell stage, A-V polarity is demonstrated by unequal blastomere sizes: 4 vegetal micromeres, 4 macromeres, and 8 mesomeres. At the 60-cell stage, there are 8 vegetal micromere granddaughters that will give rise to the skeletogenic PMC lineage and 4 vegetal small micromere daughters that contribute to the coelomic rudiments. The macromeres have produced two tiers, each of 8 blastomeres, called veg_1 (more animal tier) and veg_2 (more vegetal tier). The mesomeres have divided to give two tiers of 8 cells each at the 32-cell stage, termed an_1 and an_2; these divide again to produce 32 an_1 + an_2 progeny in the 60-cell embryo. Major tissues of the pluteus larva include oral and aboral ectodermal epithelia separated by the ciliary band; endoderm differentiated as fore-, mid-, and hindgut separated by myoepithelial sphincters; secondary mesenchyme derivatives including pigment cells, blastocoelar cells, esophageal muscle fibers, and coelomic rudiments; and a supporting skeleton synthesized by the primary mesenchyme cells.

FIG. 2. A model for patterning along the A-V axis. (A) At the 16-cell stage maternal mechanisms establish distinct combinations of vegetal β-catenin and animal transcription factor activities in micromeres, macromeres, and mesomeres. The animal transcription factors specify mesomeres as preectoderm (blue). β-Catenin is required in micromeres (red) for both their signaling function and their differentiation. The combination of β-catenin and the animal transcription factors begins to specify macromeres as mesendoderm (purple). (B) Between the 16- and the 32-cell stages, micromeres signal overlying macromeres via a Notch pathway and also begin to express zygotic SpWnt8. β-Catenin enters nuclei of macromere progeny cell autonomously. (C) At the 60-cell stage veg_1 and veg_2 tiers separate. The action of the vegetal signaling mechanism progressively down regulates zygotic expression of animal transcription factors in the veg_2 and the more vegetal veg_1 progeny. In the early blastula, internalization of Notch is observed in presumptive secondary mesenchyme, while continued signaling by Wnt8 is required for differentiation of endoderm and secondary mesenchyme. (D) At a later blastula stage, continued SpWnt8 signaling completes endoderm specification, antagonizing the function of zygotic SpBMP2/4 in the pre-ectoderm.

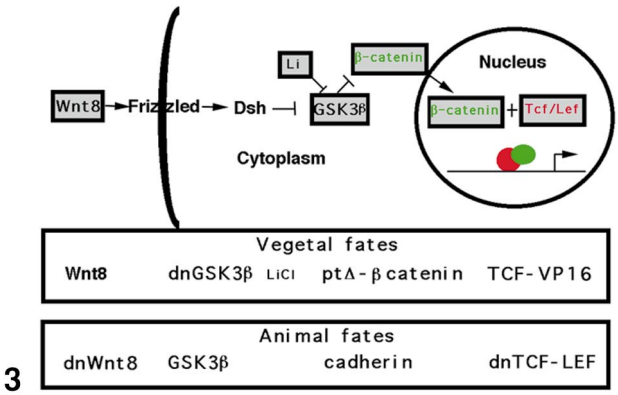
if not all, of LiCl's effects are mediated through Tcf/Lef-responsive promoters (Vonica *et al.*, 1999). Finally, the sensitive period for the teratogenic effect of LiCl is during early cleavage (Hörstadius, 1973) and the period of sensitivity to increased Tcf/Lef activity is before the 60-cell stage. The latter has been shown by introducing a glucocorticoid receptor–*Xenopus*-Tcf-3 fusion protein and treating embryos at various times during cleavage with the ligand, dexamethasone. Dex relieves the cytoplasmic tethering of the fusion protein, allowing timed entry of this activating factor into blastomere nuclei (Vonica *et al.*, 1999) and causing vegetalization if administered before the 60-cell stage. Thus, not only are the different developmental effects of these components of the β-catenin pathway consistent

with their known interactions, but also embryos are sensitive to perturbations of this pathway at an appropriate time. Because the window of sensitivity to either dnGSK3-β or LiCl is very early, these treatments probably affect predominantly the maternal, cell autonomous mechanism regulating nuclear entry of β-catenin.

THE MATERNALLY INITIATED ANIMAL TRANSCRIPTION FACTOR DOMAIN

Mesomeres that constitute the animal hemisphere are remarkable in their ability to alter fate in response to inductive signals from more vegetal blastomeres. Although mesomeres have little, if any, inductive capacity, they are not completely naïve, uncommitted cells. Isolated animal halves of eggs or eight-cell embryos form dauerblastulae consisting of a morphologically polarized epithelium with a thickened region of cuboidal cells bearing long stereocilia that are near the animal pole and a more squamous region that superficially resembles the epithelia of the aboral and perioral regions (Wikramanayake *et al.*, 1995; Wikramanayake and Klein, 1997). This morphology indicates the developmental potential of presumptive ectoderm in the absence of vegetal signals. Further differentiation of ectoderm into squamous oral, cuboidal neurogenic ciliary band, and squamous aboral regions, which are arrayed in that order along the oral–aboral axis, requires vegetal signaling (Livingston and Wilt, 1990a,b; Wikramanayake and Klein, 1997). Patterning along this second axis is outside the scope of this review; for further recent discussion, see Davidson *et al.* (1998) and Ettensohn and Sweet (1999).

Discovery of the ATF domain began with the independent identification in *Paracentrotus lividus* (LePage *et al.*, 1992a,b) and *Strongylocentrotus purpuratus* (Reynolds *et al.*, 1992) of several genes that are activated during early cleavage in a spatially regulated pattern that reveals A-V polarity: The messages accumulate in animal but not in vegetal blastomeres. The best studied of these genes are those encoding the hatching enzyme (*HE, SpHE*) and an astacin protease related to Tolloid and BMP1 [*P. lividus, BP10* (Lepage *et al.*, 1992); *S. purpuratus, SpAN* (Reynolds *et al.*, 1992)]. Because mRNA levels peak at the very early blastula stage, we named these the VEB genes. Most important are the facts that (1) the genes are transcribed at least by 8-cell stage and thus are among the first strictly zygotic genes activated in the embryo; (2) at the 16-cell stage transcripts of all four *S. purpuratus* VEB genes accumulate in animal macromeres and mesomeres, but are not detectable in micromeres (Reynolds *et al.*, 1992; Nasir *et al.*, 1995); and (3) the VEB genes are activated cell autonomously in embryos that are continuously separated into individual blastomeres beginning at 2-cell stage (Reynolds *et al.*, 1992; Ghiglione *et al.*, 1993). These facts indicate that initial activation of the VEB genes is under maternal control.



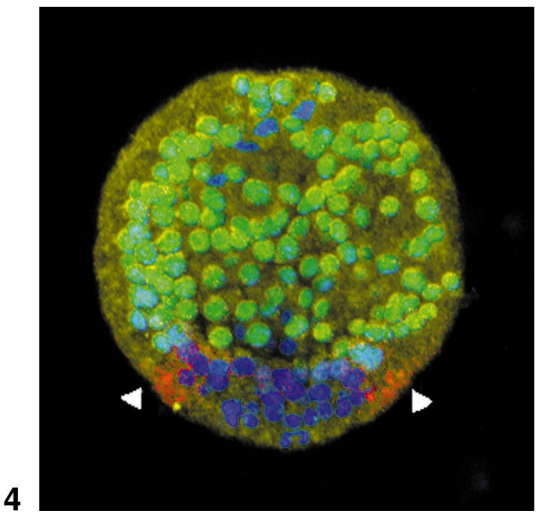


FIG. 3. The Wnt signaling pathway (top). Only the central components of this pathway are shown. The effects of experimental alterations in activities of the components highlighted in boxes have been tested. These effects are listed below, grouped according to whether they promote animal or vegetal fates.

FIG. 4. Molecular markers demonstrate patterning of major regions of the embryo at the mesenchyme blastula stage. Shown is a partial confocal stack of a mesenchyme blastula that has been triply stained: The vegetal plate is indicated by blue DAPI staining of nuclei at the vegetal pole (bottom). Presumptive veg_1 endoderm is identified by continued accumulation of apical Notch (red; anti-Notch antibody kindly provided by D. R. McClay). Preectoderm is identified by blue/green nuclear staining with DAPI and an antibody specific for the SpSoxB1 ATF (Kenny *et al.*, 1999) and the absence of Notch. (Blue staining in the animal hemisphere is due to mitotic figures that stain with DAPI but have released SpSoxB1 protein to the cytoplasm.) The arrowheads indicate the presumptive endoderm–ectoderm boundary.

Analysis of the regulatory regions of *SpHE* and *SpAN* has been carried out in order to identify the *cis*-acting elements that mediate this maternal spatial regulation (Kozlowski *et al.*, 1996; Wei *et al.*, 1995) and the corresponding *trans*-acting factors (Kenny *et al.*, 1999; Wei *et al.*, 1999a,b).

High-resolution dissection of these regions by functional tests *in vivo* of the effects of deletions and replacements, coupled with *in vitro* analyses of DNA binding, catalogued a large number of different *cis* elements in each promoter, collectively interacting with at least 10 different factors. Most surprisingly, all identified *cis* elements confer positive transcription activity and several different individual elements, and combinations of elements, of the *SpHE* promoter all sponsor transgene expression that was excluded from vegetal derivatives, i.e., PMCs. Therefore the VEB genes were proposed to be regulated by a set of multiple activating transcription factors whose activities are spatially regulated by a maternal mechanism(s) (Wei *et al.*, 1997). A variety of potential regulatory mechanisms exists, such as mRNA localization, differential mRNA or protein turnover, posttranslational modification, etc. Sea urchin embryos have a demonstrated capacity to prelocalize both mRNAs (Di Carlo *et al.*, 1996; Montana *et al.*, 1996; Vlahou *et al.*, 1996) and proteins (Romancino *et al.*, 1998; Romancino and Di Carlo, 1999) during oogenesis and have also been demonstrated to selectively exclude about one-quarter of different maternal transcripts from micromeres or to rapidly degrade them in micromeres after the fourth cleavage (Rodgers and Gross, 1978; Ernst *et al.*, 1980).

Our laboratory has recently cloned two of the animal transcription factors. SpEts4 is a positive activator of *SpHE* (Wei *et al.*, 1999b), and the cognate SpEts4 *cis* element, in combination with the *SpHE* basal promoter, is sufficient to drive expression of a reporter transgene that is correctly excluded from PMCs (Wei *et al.*, 1999a). SpSoxB1 is an essential positive regulator of *SpAN* promoter activity that appears to lack inherent transcription activation function, but has DNA bending activity that likely serves an architectural role (Kenny *et al.*, 1999; unpublished observations). Zygotic roles of both SpEts4 and SpSoxB1 in spatial regulation of gene activity are indicated by the fact that both zygotic mRNAs accumulate in a pattern very similar to the "nonvegetal" expression pattern of the VEB genes that they regulate. Consistent with maternal function, both *SpEts4* and *SpSoxB1* mRNAs are present in unfertilized eggs. However, somewhat surprisingly, both messages are *uniformly distributed* in eggs and early cleavage-stage embryos, implying that the maternal mechanism for spatial regulation of the activity of these factors operates downstream of mRNA production and storage in the egg. The critical task for understanding the initial polarity of their function, then, is to define the mechanism(s) that first restricts their activity to macromeres and mesomeres.

The distribution of SpSoxB1 protein during cleavage suggests a major candidate mechanism. This factor accumulates uniformly in nuclei of two-, four-, and eight-cell embryos, reflecting the uniform mRNA distribution and suggesting the lack of early translational or posttranslational regulation. The SpSoxB1 protein distribution changes abruptly at the fourth cleavage, when levels become significantly lower in micromere nuclei than in those of the larger mesomeres and macromeres (Kenny *et al.*, 1999). Since *SpSoxB1* mRNA equilibrates to the cytoplasm during mitosis (our unpublished observations), it follows that the asymmetric fourth cleavage provides a smaller reservoir of the essential SpSoxB1 factor in micromeres. This process probably is a major contributor to the lack of SpSoxB1 function in micromeres and PMCs, although it is probably not the only mechanism. Operation of an additional mechanism(s) is suggested by the fact that the relative level of SpSoxB1 *cis* element binding activity is severalfold lower in micromere whole-cell extracts, on a per-microgram-protein basis (Kenny *et al.*, 1999), a difference that cannot be attributed to asymmetric cleavage. Amplification of this initial asymmetry could be achieved if the various ATFs regulate their own and/or each others' expression, as is suggested by the zygotic patterns of SpEts4 and SpSoxB1 mRNA accumulation.

Studies on the VEB genes have identified the ATF domain as a maternally produced, zygotically sustained cohort of positive transcription factor activities. We propose that, in parallel to specification of mesendoderm by β-catenin, this cohort of factors conditionally specifies the general preectoderm state, represented by the epithelial differentiation of *dauerblastulae*.

THE MATERNALLY REGULATED VSM AND ATFs CREATE THREE DISTINCT TRANSCRIPTION REGULATORY DOMAINS AT THE 16-CELL STAGE

Although cell lineage analyses demonstrate that, within the macromere progeny, the SMC–endoderm and endoderm–ectoderm borders are not established until shortly after the 120-cell stage (early blastula) and 400-cell mesenchyme blastula stages, respectively, there is evidence that, at the 16-cell stage, macromeres are already specified as a general mesendoderm domain. For example, *SpKrox1* transcripts appear only in macromeres at the 16-cell stage (Wang *et al.*, 1996). The *SpKrox1* message continues to accumulate until mesenchyme blastula stage and remains confined to the vegetal plate. Subsequently, during gastrulation, expression is down regulated in that region of presumptive endoderm that has invaginated, suggesting that SpKrox1 functions in specifying mesendoderm, rather than in terminal differentiation of SMCs or endoderm. This argues that an immediate function of the maternally constructed, 16-cell-stage, mesendoderm domain is the localized zygotic activation of downstream genes encoding transcription regulatory proteins, such as SpKrox1. We propose that nuclear β-catenin and the ATFs are major components of this *unique gene-regulatory domain*.

Thus, micromeres, macromeres, and mesomeres already constitute distinct gene regulatory domains at the 16-cell stage, through the action of cell-autonomous, maternal mechanisms that polarize the functions of β-catenin and the ATFs. This suggests that the *ratio of ATF to β-catenin activity is an important initial factor in regulating blas-*

tomere fates along the A-V axis. Support for this hypothesis is found in the effects of elevating or reducing β-catenin activity in embryos, as discussed above. Our laboratory has carried out similar gain-of-function and loss-of-function assays with SpSoxB1: Ectopic expression by mRNA injection at the 1-cell stage progressively reduces the domain of mesendoderm and, at the higher doses, causes the embryo to convert to an epithelial sphere resembling early ectoderm (unpublished data). This phenotype is very similar to that produced by blocking the VSM by cadherin mRNA injection (Wikramanayake *et al.*, 1998; Logan *et al.*, 1999). Remarkably, SpSoxB1 misexpression even prevents differentiation of PMCs, and micromere progeny remain as epithelial cells in the wall of the expanded "blastula." In contrast, dominant negative interference by expression of a repressor variant, consisting of the SpSoxB1 DNA binding domain linked to the engrailed repression domain, causes most cells to express the PMC-specific 6e10 epitope (unpublished data). These results suggest that even in the maternally determined micromeres, ectopic expression of the ATFs can antagonize the maternal determinants of the VSM.

SUBDIVISION OF MESENDODERM FATES—SMCs AND ENDODERM

A continued vegetal-to-animal cascade of cell–cell interactions would involve signaling by presumptive SMCs to drive overlying mesendoderm to an endoderm fate. Current data suggest that this is not the case. Instead, it appears that Notch- and Wnt-mediated signaling from micromeres to macromeres and/or their daughters specifies SMC fate, while lack of Notch signaling leads to differentiation of mesendoderm as endoderm (Sherwood and McClay, 1999).

In *L. variegatus*, LvNotch protein undergoes striking regional changes in its subcellular distribution during early development. Through cleavage, LvNotch is uniformly distributed on cell surfaces throughout the embryo. At the early blastula stage, just before the SMC–endoderm border is established, it is sharply down regulated in presumptive SMCs in the center of the vegetal plate (Sherwood and McClay, 1997). Down regulation is accompanied by internalization of LvNotch in cytoplasmic vesicles (Sherwood and McClay, 1999). Slightly later, at mid- to late mesenchyme blastula stage, LvNotch expression is sharply up regulated on the apical surfaces of presumptive endoderm surrounding the future SMCs (red staining; Fig. 4) (Sherwood and McClay, 1997). Notch internalization in vesicles has been associated with signaling in other systems (Henderson *et al.*, 1994; Klueg *et al.*, 1998) and suggested to reflect either activation or subsequent down regulation of the pathway. The latter is more likely in this case because Notch down regulation is not observed until SMCs are specified after the 120-cell stage and after the micromere signal(s) is sent, which occurs primarily between the 16- and the 32-cell stages (Ransick and Davidson, 1995). Regardless of the precise mechanisms, Notch internalization in presumptive SMCs and apical Notch accumulation in presumptive endoderm cells are precisely correlated with their subsequent differentiation, as illustrated by the experiments discussed below.

Sweet *et al.* (1999) have provided evidence that the Notch-dependent specification of SMCs requires signals from micromeres: When micromeres are removed from normal embryos, Notch is not down regulated in presumptive SMCs and their differentiation is markedly inhibited. The same result is obtained when normal micromeres are replaced with micromeres expressing cadherin (D. R. McClay, personal communication), suggesting that the Notch stimulatory signal depends on nuclear β-catenin. In both these cases, apical Notch accumulates throughout the vegetal plate and presumptive mesenchyme cells differentiate as endoderm. Conversely, when micromeres are placed at the animal pole, they induce internalization of Notch and ectopic formation of SMCs, although at reduced levels compared to normal embryos (Sweet *et al.*, 1999; some types of SMCs, especially muscle, show less sensitivity to such experimental perturbations). Also supporting the idea that micromeres supply the signals that activate the Notch pathway, McClay and co-workers (personal communication) have found that the size of the region of Notch down regulation and the number of SMCs can be simultaneously and progressively recovered in micromereless embryos by reimplanting increasing (one to four) numbers of micromeres. Although other possibilities exist, all these data suggest that micromere signaling regulates Notch receptor activation in overlying cells. An attractive hypothesis is that micromeres secrete a ligand related to Delta or Serrate, as a result of the activity of the maternal nuclear β-catenin-dependent pathway.

These effects of micromeres on specification can be replicated by manipulation of signaling through the Notch pathway. Injection of mRNA encoding a dominant negative variant of Notch, dnLvNotch, that lacks the majority of the intracellular domain, decreases the number of differentiated SMCs, but a normal gut differentiates (Sherwood and McClay, 1999). In these embryos, as in those lacking micromeres (Sweet *et al.*, 1999), apical Notch is observed throughout the vegetal plate. Conversely, injection of mRNA encoding the constitutively active, intracellular domain of LvNotch causes overproduction of SMCs by respecification of adjacent presumptive endoderm cells that also have high levels of nuclear β-catenin resulting from both maternal cell-autonomous and zygotic Wnt-mediated signals. This respecification is accompanied by rapid internalization of LvNotch in the converting endoderm (Sherwood and McClay, 1999). Thus, under a variety of experimental perturbations, there is a strong correlation between commitment to SMC fate and Notch internalization, and both require micromere (and veg_2) nuclear β-catenin.

ZYGOTIC WNT8 SIGNALING AUGMENTS MATERNAL NUCLEAR β-CATENIN

Wikramanayake and Klein (personal communication) have discovered that *SpWnt8* mRNA is expressed during cleavage stages in a temporal and spatial wave that begins in the micromeres at the late 16-cell stage and eventually extends to veg_1 blastomeres. Expression of mRNA encoding a dominant negative SpWnt8 variant blocks differentiation of endoderm and secondary mesenchyme in intact embryos. Overexpression of SpWnt8 in animal hemispheres separated at the 8-cell stage causes much of this presumptive ectoderm to form multiple ectopic archentera. Ectopic secondary mesenchyme is not detectable, perhaps because the putative micromere-generated Notch ligand is not present or because animal cells convert more easily to endoderm than to the more vegetal SMC fates. Since the Wnt pathway augments β-catenin stability through Dsh and GSK3-β (Fig. 3), these results strongly suggest that SpWnt8-mediated reinforcement of nuclear β-catenin levels is required to complete specification of mesendoderm. This requirement also is suggested by the fact that, for embryos to express normal levels of the early vegetal plate marker, Endo16, micromeres must be present throughout the interval between the 16- and the 60-cell stages (Ransick and Davidson, 1995).

We propose that the VSM down regulates zygotic expression of the ATFs in the mesendoderm domain during cleavage. This function is suggested by two observations. First, Ghiglione *et al.* (1993) showed that the domain of expression of the *P. lividus* hatching enzyme gene (*HE*), a target of ATFs, can be dramatically displaced toward the animal pole when the β-catenin pathway is stimulated with LiCl, an observation we have confirmed for *SpHE* (unpublished results). Similar animal displacement of the *HE* transcription domain results when dnGSK3-β is introduced into the embryo (Emily-Fenouil *et al.*, 1998). Thus, LiCl, an inhibitor of GSK3-β, and dnGSK3-β mimic the effect of overactivation of zygotic vegetal SpWnt8 signaling. This leads to increases in nuclear β-catenin levels that ultimately down regulate the ATF factors that drive the VEB genes and probably other ectoderm-specific genes. Second, the SpSoxB1 ATF protein is abundant in nuclei of macromeres and mesomeres, but gradually disappears from presumptive SMCs and endoderm until, at the mesenchyme blastula stage, the vegetal border of SpSoxB1-positive cells corresponds to the ectoderm/endoderm boundary (Fig. 4, arrowheads). The ATF genes could be down regulated directly by β-catenin or by downstream components of the VSM such as the Notch pathway.

In summary, current data are most consistent with the idea that zygotic SpWnt8 signaling provides an essential increment in nuclear β-catenin levels required for completion of mesendoderm specification, while Notch signaling diverts the more vegetal mesendoderm progeny to secondary mesenchyme fate. The combined action of these pathways leads to activation of genes required for endoderm and mesoderm differentiation and down regulation of the ATF genes in vegetal macromere progeny (Fig. 2).

ESTABLISHMENT OF THE ENDODERM–ECTODERM BORDER

Although the macromere lineages constitute the initial mesendoderm domain, the most animal region of veg_1 contributes to the ectoderm**.** Lineage analysis shows that the ectoderm–endoderm border is not specified until the blastula stage (Logan and McClay, 1997). Coincidentally, the initial maternally regulated wave of nuclear β-catenin is followed by its reappearance specifically in the veg_1 progeny that give rise to endoderm (Logan *et al.*, 1999). This increase is likely to be mediated by zygotic SpWnt8 signaling from adjacent veg_2 progeny and/or among veg_1 derivatives, since the vegetal-to-animal wave of SpWnt8 mRNA accumulation persists through the mesenchyme blastula stage and treatments that increase β-catenin in embryo nuclei shift the endoderm–ectoderm border toward the animal pole.

A sea urchin homologue of BMP2/4 appears to work in opposition to vegetal signaling at the endoderm–ectoderm border (Angerer *et al.*, 1999). Injection of this mRNA at the one-cell stage in increasing doses radializes embryos and progressively expands the animal region of epithelial cells, while suppressing the domain of vegetal derivatives. At the higher doses, endoderm is reduced to a small vesicle which, however, shows patterned expression of foregut and hindgut markers. This latter observation suggests that SpBMP2/4 overexpression resets the endoderm–ectoderm boundary, rather than simply eliminating the more animal endoderm derivatives. *SpBMP2/4* mRNA also has been shown to have strong ventralizing activity in *Xenopus* embryos, as does the *Xenopus* homologue (Dale, personal communication). Injection of mRNA encoding *Xenopus* Noggin into sea urchin embryos appears to antagonize endogenous SpBMP2/4 because it produces a largely reciprocal phenotype, in which ectoderm is reduced and vegetal structures are expanded. These results suggest that SpBMP2/4 functions in normal embryos to antagonize SpWnt8 signaling and to help regulate the position of the endoderm–ectoderm border. The similarity of phenotypes produced by SpSoxB1 and SpBMP2/4 overexpression suggests that BMP2/4 could be a downstream target of ATF activities, although our preliminary experiments suggest that these are not sufficient. An appealing counterpart mechanism, analogous to the pathway in *Xenopus* dorsal specification (e.g., Fagotto *et al.*, 1997; Laurent *et al.*, 1997; reviewed recently by Moon and Kimelman, 1998), is that an ultimate downstream consequence of stimulating a Wnt signaling pathway is production by vegetal blastomeres of a BMP antagonist such as Noggin or Chordin, although homologues of these proteins have not yet been identified in sea urchin embryos.

NEGATIVE SIGNALING PREVENTS BLASTOMERES FROM ADOPTING THE FATES OF THEIR VEGETAL NEIGHBORS

In the preceding discussion we have emphasized positive transcription-regulatory and cell-signaling activities that direct blastomeres toward their normal fates. However, it is clear that negative regulatory signals also are important for maintaining initial specifications before these cells are finally determined (reviewed by Brandhorst and Klein, 1992). In this process, the progeny of more vegetal blastomeres send signals to cells derived from more pluripotent animal blastomeres that prevent them from adopting the more vegetal fate of the signaling cells. For example, PMCs are replaced in embryos from which micromeres or PMCs have been removed, by fate conversion of presumptive SMCs (reviewed by Ettensohn, 1992). Thus, in normal embryos, PMCs prevent SMCs from following the PMC program. Similarly, when a core of presumptive SMCs is removed from the vegetal plate, adjacent presumptive endoderm internalizes apical Notch and converts to SMC fates (Sherwood and McClay, 1997) and when most endoderm is removed, even at the late gastrula stage, it is replaced by conversion of presumptive ectoderm (McClay and Logan, 1996). It is reasonable to expect that similar repressive feedback mechanisms are involved at even earlier stages in the refinement and subdivision of the preectoderm and mesendoderm.

NEW ANSWERS AND SPECULATIONS

The initial patterning steps of sea urchin embryos have been discussed in several recent reviews (Davidson *et al.*, 1998; Ettensohn and Sweet, 1999). In updating his original model (Davidson, 1989), Davidson has emphasized the maternal specification of a vegetal signaling center in the micromeres that initiates an inductive cascade in which each tier of early blastomeres signals its animal neighbors. Sweet and Ettensohn (1999) modified this general model to emphasize the importance of maternally specified, cell-autonomous processes in patterning, an idea that had been previously discussed by Livingston and Wilt (1990b). Here we have reviewed evidence that further supports and emphasizes the role of spatially regulated maternal activities. In particular, we propose the following: (1) Maternally specified events already establish three unique gene-regulatory domains in the 16-cell embryo, corresponding to micromeres, macromeres, and mesomeres. Each of these territories currently is defined by a distinct set of vegetalizing (β-catenin) and animalizing (ATF) activities. Macromeres already constitute a separate transcriptional domain by virtue of their maternally controlled acquisition of both animal and vegetal factors, which is independent of any inductive signaling. Similarly, mesomeres already are maternally biased by the ATFs toward an animal preectoderm fate. Thus, while Davidson envisioned early transcription territories that were created by inductive activation of preexisting maternal transcription factors, we now know that broader territories are established even earlier by purely maternal mechanisms. (2) Major factors that function in patterning mesendoderm have been identified: Zygotic SpWnt8 reinforces maternally regulated levels of β-catenin, promoting mesendoderm fates and Notch signaling diverts macromere progeny to secondary mesenchyme fates. (3) During cleavage the VSM down regulates the domain of zygotic production of at least some ATF factors, progressively clearing them from the mesendoderm domain. In competition with animal factors, including BMP2/4, this specifies the endoderm–ectoderm border within veg_1 progeny (reviewed in Angerer and Angerer, 1999).

NEW QUESTIONS

(1) What is the cell-autonomous maternal mechanism that regulates entry of β-catenin into vegetal nuclei of early cleavage-stage blastomeres? Cell autonomy suggests that the maternal mechanism acts downstream of a Wnt receptor, while the effects of dominant negative GSK3-β and LiCl imply that it operates upstream of GSK3-β kinase activity. Thus, initial steps in the VSM might involve a phosphorylation or dephosphorylation event that is triggered by fertilization and regulates the activity of a Dsh homologue or that of GSK3-β. A recently identified kinase activity that inhibits GSK3-β kinase offers a prototype that functions downstream of several different signaling pathways (reviewed in Vanhaesebroeck *et al.*, 1997). Interestingly, initial nuclearization of β-catenin in the *Xenopus* Nieuwkoop center may also be cell autonomous since it appears to be independent of the Wnt receptor (Frizzled) (review, Moon and Kimelman, 1998). It does, however, depend on the localization of Dsh to the future dorsal side after fertilization (Miller *et al.*, 1999).

(2) Although nuclear β-catenin is essential for induction of vegetal derivatives in normal embryos, it has not been detected in animal cells that are induced to form ectopic archentera by transplanted micromeres (Logan *et al.*, 1999). Nevertheless, a role for β-catenin in these cells is suggested by the ability of SpWnt8 to induce gut formation in embryos derived from isolated eight-cell animal halves (A. H. Wikramanayake and W. H. Klein, personal communication). The role of the Wnt pathway also can be further tested by examining the ability of transplanted micromeres to form archentera in recipient embryos in which β-catenin function has been blocked by cadherin or the dominant negative Lef/Tcf. If β-catenin is required, then the fact that the levels induced are well below those observable in vegetal blastomeres would require that some inherent maternal polarity in downstream biochemistry makes animal cells more sensitive to β-catenin, as has been suggested previously (Davidson *et al.*, 1998). This idea is not easily reconciled with the fact that maternally regulated ATFs,

several of which are able to divert vegetal cells to ectoderm fates, are expressed in animal, but not in the most vegetal nuclei. One potential, speculative, resolution of this paradox could be that, in addition to β-catenin, a separate maternal molecular polarity extends from the vegetal pole and is mutually antagonistic with β-catenin. For example, this mechanism might activate downstream genes responsible for the well-documented *negative* regulation discussed above, which prevents blastomeres from adopting the fates of their more vegetal neighbors. If this negative influence does not extend into the animal region, then high levels of β-catenin would not be required to counteract it.

(3) Experiments in which micromeres or their progeny are removed at successive cleavage divisions indicate that the micromere signals that specify endoderm and SMCs are sent primarily at the 16- and 32-cell stages, i.e., well *before* these lineages separate after the 120-cell stage. How is the downstream effect of Notch signaling inherited by only the more vegetal components of the mesendoderm, the vegetal derivatives of veg_2 blastomeres?

(4) What are the molecular and cellular mechanisms that establish the ATF domain and then modulate it to that of definitive ectoderm? To date we have cloned only two of these factors, but analysis of only two target promoters suggests that there are many more. Are the activities of all of the ATFs primarily regulated in the same way, and is asymmetric cleavage a sufficient mechanism to explain their reduced function in micromeres? Asymmetric cleavage cannot explain subsequent retraction of SpSoxB1 expression to the endoderm–ectoderm border. Will all ATFs show a similar modulation or, for example, might their zygotic expression be more or less sensitive to the proposed repression by the VSM? Differential repression could lead to different combinations of ATF factors in animal and vegetal macromere derivatives, which could be an additional factor in their patterning.

(5) What are the immediate target genes of the ATFs and β-catenin? Although dominant negative interference and misexpression experiments clearly establish that these factors play important developmental roles, to date in the cases of only two factors (SpEts4 and SpSoxB1) have downstream target genes of these maternal regulators been identified, i.e., the hatching enzyme gene (*SpHE, HE*) and the tolloid-related gene, *SpAN*. In contrast, very detailed analyses of the regulatory elements of genes encoding terminal differentiation products of major cell types have identified a large number of *trans*-acting factors (reviewed in Coffman and Davidson, 1992; Maxson and Tan, 1994; Kirchhamer *et al.*, 1996). The sea urchin embryo presents a remarkably tractable and powerful system for analysis of gene regulatory networks in early embryogenesis (Arnone and Davidson, 1997; Yuh *et al.*, 1998). Further analysis should be able to link upstream and downstream *cis–trans* interactions, providing a map of the transition from maternal to zygotic control of patterning of cell fates along the A-V axis.

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REFERENCES

Angerer, L. M., and Angerer, R. C. (1999). Regulative development of the sea urchin embryo: Signaling cascades and morphogen gradients. *Semin. Cell Dev.* **10,** 327–339.

Angerer, L. M., Oleksyn, D. W., Logan, C. Y., McClay, D. R., Dale, L., and Angerer, R. C. (1999). A BMP pathway regulates cell fate allocation along the sea urchin animal–vegetal embryonic axis. *Development,* in press.

Arnone, M., and Davidson, E. H. (1997). The hardwiring of development: Organization and function of genomic regulatory systems. *Development* **124,** 4649–4659.

Boveri, T. (1901a). Die Polarität von Oocyte, Ei und Larve des *Strongylocentrotus lividus. Zool. Jb. Abt. Anat. Ont.* **14,** 630–653.

Boveri, T. (1901b). Über die Polarität des Seeigeleies. *Verh. Phys.-Med. Ges. Wurzburg* **34,** 145–176.

Brandhorst, B. P., and Klein, W. H. (1992). Territorial specification and control of gene expression in the sea urchin embryo. *Semin. Dev. Biol.* **3,** 175–186.

Clevers, H., and van de Wetering, M. (1997). TCF/LEF factors earn their wings. *Trends Genet.* **13,** 485–489.

Coffman, J. A., and Davidson, E. H. (1992). Expression of spatially regulated genes in the sea urchin embryo. *Curr. Opin. Genet. Dev.* **2,** 260–268.

Davidson, E. H. (1989). Lineage-specific gene expression and the regulative capacities of the sea urchin embryo: A proposed mechanism. *Development* **105,** 421–445.

Davidson, E. H., Cameron, R. A., and Ransick, A. (1998). Specification of cell fate in the sea urchin embryo: Summary and some proposed mechanisms. *Development* **125,** 3269–3290.

Di Carlo, M., Romancino, D. P., Ortolani, G., Montana, G., and Giudice, G. (1996). "BEP" RNAs and proteins are situated in the animal side of sea urchin unfertilized egg, which can be recognized by female pronuclear localization. *Biochem. Biophys. Res. Commun.* **229,** 511–517.

Emily-Fenouil, F., Ghiglione, C., Lhomond, G., Lepage, T., and Gache, C. (1998). GSK3beta/shaggy mediates patterning along the animal–vegetal axis of the sea urchin embryo. *Development* **125,** 2489–2498.

Ernst, S. G., Hough-Evans, B. R., Britten, R. J., and Davidson, E. H. (1980). Limited complexity of the RNA in micromeres of sixteen-cell sea urchin embryos. *Dev. Biol.* **79,** 119–127.

Ettensohn, C. A. (1992). Cell interactions and mesodermal cell fates in the sea urchin embryo. *Development* **Suppl.,** 43–51.

Ettensohn, C. A., and Sweet, H. C. (1999). Patterning the early sea urchin embryo. *Curr. Top. Dev. Biol.*, in press.

Fagotto, F., Guger, K., and Gumbiner, B. M. (1997). Induction of the primary dorsalizing center in Xenopus by the Wnt/GSK/beta-catenin signaling pathway, but not by Vg1, activin or noggin. *Development* **124,** 453–460.

Fan, M. J., and Sokol, S. Y. (1997). A role for Siamois in Spemann organizer formation. *Development* **124,** 2581–2589.

Ghiglione, C., Lhomond, G., Lepage, T., and Gache, C. (1993). Cell-autonomous expression and position-dependent repression by Li^+ of two zygotic genes during sea urchin early development. *EMBO J.* **12,** 87–96.

Goldstein, B., and Freeman, G. (1997). Axis specification in animal development. *BioEssays* **19,** 105–116.

Henderson, S. T., Gao, D., Lambie, E. J., and Kimble, J. (1994). *lag-2* may encode a signaling ligand for the GLP-1 and LIN-12 receptors of *C. elegans. Development* **120,** 2913–2924.

Hörstadius. (1939). The mechanics of sea urchin development, studied by operative methods. *Biol. Rev. Cambridge Philos. Soc.* **14,** 132–179.

Hörstadius, S. (1973). "Experimental Embryology of Echinoderms." Clarendon, Oxford.

Huang, L., Li, X., El-Hodiri, H. M., Wikramanayake, A. H., and Klein, W. H. (1999). Involvement of Tcf/Lef in establishing cell types along the animal–vegetal axis of sea urchins. *Dev. Genes Evol.*, in press.

Kenny, A. P., Kozlowski, D. J., Oleksyn, D. W., Angerer, L. M., and Angerer, R. C. (1999). SpSoxB1, a maternally encoded transcription factor asymmetrically distributed among early sea urchin blastomeres. Submitted for publication.

Kirchhamer, C. V., Yuh, C. H., and Davidson, E. H. (1996). Modular *cis*-regulatory organization of developmentally expressed genes: Two genes transcribed territorially in the sea urchin embryo, and additional examples. *Proc. Natl. Acad. Sci. USA* **93,** 9322–9328.

Klueg, K. M., Parody, T. R., and Muskavitch, M. A. (1998). Complex proteolytic processing acts on Delta, a transmembrane ligand for Notch, during *Drosophila* development. *Mol. Biol. Cell* **9,** 1709–1723.

Kozlowski, D. J., Gagnon, M. L., Marchant, J. K., Reynolds, S. D., Angerer, L. M., and Angerer, R. C. (1996). Characterization of a *SpAN* promoter sufficient to mediate correct spatial regulation along the animal–vegetal axis of the sea urchin embryo. *Dev. Biol.* **176,** 95–107.

Laurent, M. N., Blitz, I. L., Hashimoto, C., Rothbacher, U., and Cho, K. W. (1997). The *Xenopus* homeobox gene twin mediates Wnt induction of goosecoid in establishment of Spemann's organizer. *Development* **124,** 4905–4916.

Lepage, T., Ghiglione, C., and Gache, C. (1992a). Spatial and temporal expression pattern during sea urchin embryogenesis of a gene coding for a protease homologous to the human protein BMP-1 and to the product of the *Drosophila* dorsal–ventral patterning gene tolloid. *Development* **114,** 147–163.

Lepage, T., Sardet, C., and Gache, C. (1992b). Spatial expression of the hatching enzyme gene in the sea urchin embryo. *Dev. Biol.* **150,** 23–32.

Li, X., Wikramanayake, A. H., and Klein, W. H. (1999). Requirement of SpOtx in cell fate decisions in the sea urchin embryo and possible role as a mediator of beta-catenin signaling. *Dev. Biol.* **212,** 425–439.

Livingston, B. T., and Wilt, F. H. (1990a). Range and stability of cell fate determination in isolated sea urchin blastomeres. *Development* **108,** 403–410.

Livingston, B. T., and Wilt, F. H. (1990b). Determination of cell fate in sea urchin embryos. *BioEssays* **12,** 115–119.

Logan, C. Y., and McClay, D. R. (1997). The allocation of early blastomeres to the ectoderm and endoderm is variable in the sea urchin embryo. *Development* **124,** 2213–2223.

Logan, C. Y., Miller, J. R., Ferkowicz, M. J., and McClay, D. R. (1999). Nuclear beta-catenin is required to specify vegetal cell fates in the sea urchin embryo. *Development* **126,** 345–357.

Maruyama, Y. K., Nakaseko, Y., and Yagi, S. (1985). Localization of cytoplasmic determinants responsible for primary mesenchyme formation and gastrulation in the unfertilized egg of the sea urchin *Hemicentrotus pulcherrimus. J. Exp. Zool.* **236,** 155–163.

Maxson, R., and Tan, H. (1994). Promoter analysis meets pattern formation: Transcriptional regulatory genes in sea urchin embryogenesis. *Curr. Opin. Genet. Dev.* **4,** 678–684.

McClay, D. R., and Logan, C. Y. (1996). Regulative capacity of the archenteron during gastrulation in the sea urchin. *Development* **122,** 607–616.

Miller, J. R., Rowning, B. A., Larabell, C. A., Yang-Snyder, J. A., Bates, R. L., and Moon, R. T. (1999). Establishment of the dorsal–ventral axis in *Xenopus* embryos coincides with the dorsal enrichment of dishevelled that is dependent on cortical rotation. *J. Cell Biol.* **146,** 427–437.

Montana, G., Romancino, D. P., and di Carlo, M. D. (1996). Cloning, expression, and localization of a new member of a *Paracentrotus lividus* cell surface multigene family. *Mol. Reprod. Dev.* **44,** 36–43.

Moon, R., and Kimelman, D. K. (1998). From cortical rotation to organizer gene expression: Toward a molecular explanation of axis specification in *Xenopus. BioEssays* **20,** 536–545.

Nasir, A., Reynolds, S. D., Angerer, L. M., and Angerer, R. C. (1995) VEB4: Early zygotic mRNA expressed asymmetrically along the animal–vegetal axis of the sea urchin embryo. *Dev. Growth Differ.* **37,** 57–68.

Ransick, A., and Davidson, E. H. (1993). A complete second gut induced by transplanted micromeres in the sea urchin embryo. *Science* **259,** 1134–1138.

Ransick, A., and Davidson, E. H. (1995). Micromeres are required for normal vegetal plate specification in sea urchin embryos. *Development* **121,** 3215–3222.

Reynolds, S. D., Angerer, L. M., Palis, J., Nasir, A., and Angerer, R. C. (1992). Early mRNAs, spatially restricted along the animal–vegetal axis of sea urchin embryos, include one encoding a protein related to tolloid and BMP-1. *Development* **114,** 769–786.

Rodgers, W. H., and Gross, P. R. (1978). Inhomogeneous distribution of egg RNA sequences in the early embryo. *Cell* **14,** 279–288.

Romancino, D. P., and Di Carlo, M. (1999). Asymmetrical localization and segregation of *Paracentrotus lividus* BEP4 maternal protein. *Mech. Dev.* **87,** 3–9.

Romancino, D. P., Montana, G., and Di Carlo, M. (1998). Involvement of the cytoskeleton in localization of *Paracentrotus lividus* maternal BEP mRNAs and proteins. *Exp. Cell Res.* **238,** 101–109.

Sherwood, D. R., and McClay, D. R. (1997). Identification and localization of a sea urchin Notch homologue: Insights into vegetal plate regionalization and Notch receptor regulation. *Development* **124,** 3363–3374.

Sherwood, D. R., and McClay, D. R. (1999). LvNotch signaling mediates secondary mesenchyme specification in the sea urchin embryo. *Development* **126,** 1703–1713.

Sweet, H., Hodor, P. G., and Ettensohn, C. A. (1999). The role of micromeres in Notch signaling and mesoderm specification in the sea urchin embryo. *Development,* in press.

Vanhaesebroeck, B., Leevers, S. J., Panayotou, G., and Waterfield, M. D. (1997). Phosphoinositide 3-kinases: A conserved family of signal transducers. *Trends Biochem. Sci.* **22,** 267–272.

Vlahou, A., Gonzalez-Rimbau, M., and Flytzanis, C. N. (1996). Maternal mRNA encoding the orphan steroid receptor SpCOUP-TF is localized in sea urchin eggs. *Development* **122,** 521–526.

Vonica, A., Weng, W., Gumbiner, B. M., and Venuti, J. M. (2000). TCF is the nuclear effector of the beta-catenin signal that patterns the sea urchin animal-vegetal axis. *Dev. Biol.* **217,** 230–243.

Wang, W., Wikramanayake, A. H., Gonzalez-Rimbau, M., Vlahou, A., Flytzanis, C. N., and Klein, W. H. (1996). Very early and transient vegetal-plate expression of SpKrox1, a Kruppel/Krox gene from *Strongylocentrotus purpuratus*. *Mech. Dev.* **60,** 185–195.

Wei, Z., Angerer, L. M., and Angerer, R. C. (1997). Multiple positive *cis* elements regulate the asymmetric expression of the *SpHE* gene along the sea urchin embryo animal–vegetal axis. *Dev. Biol.* **187,** 71–78.

Wei, Z., Angerer, L. M., and Angerer, R. C. (1999a). Spatially regulated SpEts4 transcription factor activity along the sea urchin embryo animal–vegetal axis. *Development* **126,** 1729–1737.

Wei, Z., Angerer, L. M., Gagnon, M. L., and Angerer, R. C. (1995). Characterization of the *SpHE* promoter that is spatially regulated along the animal–vegetal axis of the sea urchin embryo. *Dev. Biol.* **171,** 195–211.

Wei, Z., Angerer, R. C., and Angerer, L. M. (1999b). Identification of a new sea urchin ets protein, SpEts4, by yeast one-hybrid screening with the hatching enzyme promoter. *Mol. Cell Biol.* **19,** 1271–1278.

Wikramanayake, A. H., Brandhorst, B. P., and Klein, W. H. (1995). Autonomous and non-autonomous differentiation of ectoderm in different sea urchin species. *Development* **121,** 1497–1505.

Wikramanayake, A. H., Huang, L., and Klein, W. H. (1998). Beta-catenin is essential for patterning the maternally specified animal–vegetal axis in the sea urchin embryo. *Proc. Natl. Acad. Sci. USA* **95,** 9343–9348.

Wikramanayake, A. H., and Klein, W. H. (1997). Multiple signaling events specify ectoderm and pattern the oral–aboral axis in the sea urchin embryo. *Development* **124,** 13–20.

Yuh, C. H., Bolouri, H., and Davidson, E. H. (1998). Genomic *cis*-regulatory logic: Experimental and computational analysis of a sea urchin gene. *Science* **279,** 1896–1902.

Zernicka-Goetz, M. (1998). Fertile offspring derived from mammalian eggs lacking either animal or vegetal poles. *Development* **125,** 4803–4808.

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