Sea Urchin Embryo: Specification of Cell Fate

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Introductory article

Article Contents

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Specification of cell fate in sea urchin embryos involves initial asymmetric distribution of maternal molecules that establish vegetal and nonvegetal domains of transcription activity. Subsequently, fates of most blastomeres are regulated by cell–cell interactions involving signalling ligands and cell surface receptors.

Introduction

Sea urchin larvae develop from bilaterally symmetric deuterostome embryos whose basic structure is closely related to that of vertebrates. Millions of synchronously developing sea urchin embryos can be obtained by mixing gametes from a single pair of parents. The species most commonly studied develop into free-swimming pluteus larvae in about 3 days at 15°C in a defined medium, sea water. The structure of the pluteus larva is relatively simple, consisting of only five major tissues and 14 known cell types. Development can be followed continuously in real time by microscopy, because the embryos are optically transparent and no histological preparation (e.g. fixing, sectioning and staining) is required. A wealth of information from experimental manipulations of the embryo, cell biology and molecular assays has established that pattern formation and cell differentiation at the beginning of development employ the two major mechanisms of specification of cell fate – the inheritance of maternal determinants and signalling among cells. Initially, an asymmetric distribution of transcription factors along the major axes (animal–vegetal (AV, corresponding approximately to anterior–posterior), oral–aboral (OA, roughly equivalent to dorsal/ventral and left right (LR)) establishes domains of specific gene expression. This endows cells in different regions of the embryo with the capacities to send and receive signals. These signals then lead to the expression of new sets of transcription factors which regulate genes that reinforce initial cell fate specifications and are required for production of specific proteins characterize the differentiated state of different cell types.

The sea urchin is an excellent system to study fundamental mechanisms underlying the development of vertebrate embryos for a number of reasons. It is evolutionarily related to these organisms as a basal deuterostome; it is relative simple; its genome has been sequenced; and the developmental functions of genes can by assessed because of the recent development of methods that permit experimental perturbations of gene expression. Together these features have led during the last 5 years to the identifications of several core gene regulatory networks that govern development of the three basic germ layers – ectoderm, endoderm and mesoderm.

Axes and Architecture

The first obvious morphological indicator of the AV axis is the unequal cleavage of blastomeres in the vegetal half of the eight-cell embryo, which gives rise to four small vegetal micromeres, and the macromeres above them (**Figure 1**). The OA axis is revealed morphologically around the time of gastrulation by the aggregation of clusters of micromere derivatives, the primary mesenchyme cells (PMCs), on either side of the embryo near its future oral side. Asymmetry along the LR axis is detectable morphologically only relatively late, by the formation of the adult rudiment on the left side of the oesophagus.

The temporally and spatially ordered cell divisions of cleavage create a reproducible arrangement of blastomeres aligned with respect to the AV, OA and LR axes. Lineage tracing experiments, in which individual blastomeres are labelled with dyes and differentiation of their progeny is followed, show that the fates of early blastomere tiers arrayed along the AV axis are predictable. Four vegetal micromeres of the 16-cell embryo give rise to four small micromere daughters that contribute to the coelomic rudiments and four large micromeres that form all skeletogenic mesenchyme (PMCs). At the 64-cell stage, the progeny of the macromeres, which are positioned just above the micromere lineage, give rise to eight cells of the veg_2 tier that produce all secondary mesenchyme derivatives and some endoderm. The eight cells in the next more animal tier (veg_1) contribute to both endoderm and ectoderm. The eight mesomeres of the 16-cell embryo's animal hemisphere divide to give the an_2 and an_1 tiers of eight cells each in the 32-cell embryo and provide the majority of ectoderm. Lineage tracing has shown that specification of the OA axis can be detected by lineage tracing experiments during cleavage stages, but this specification

does not become fixed , i.e., immune to experimental challenges, until after hatching blastula stage.

020068.s0003 ## Embryonic territories and tissue differentiation

020068.s0004 ### Cleavage

020068.p0005 The period of cleavage lasts for about 9 h after fertilization and includes about seven rounds of cell division, generating the 200–250 cells of the early blastula, which is a hollow, symmetric ball, one cell layer thick. Towards the end of cleavage, cell cycles slow and become asynchronous, and cells become visibly polarized with apical (outer) and basal (inner) surfaces. Each cell constructs a cilium for swimming on its apical surface after the embryo hatches from its fertilization membrane at about 18 h postfertilization.

020068.s0005 ### Morphogenesis of endoderm and mesenchyme

020068.p0006 This process begins at this point with the flattening of one side of the embryo to create the vegetal plate. Mesenchyme cells of various types begin an ordered series of epithelial–mesenchymal transitions and they delaminate from the embryo epithelium. First are the PMCs (primary mesenchyme cells) that ingress into the blastocoel from the central region of the vegetal plate and will secrete the skeletal calcite rods, called spicules. They are followed during blastula and early gastrula stages by SMCs (secondary mesenchyme cells) that include pigment and blastocoelar cells. While the function of the blastocoelar cells is poorly understood, recent work suggests that they are involved in development of the nervous system and in mediating adaptive immunity. Following PMC ingression, cells that will give rise to the endoderm begin to invaginate from the vegetal plate, initiating the process of gastrulation. When the archenteron or gut tube extends to the animal pole at the end of gastrulation, additional SMCs delaminate from the presumptive endodermal epithelium to produce muscle cells that encircle the oesophagus. About the same time, the daughters of the small micromeres migrate from the tip of the archenteron to the coelomic pouches. These cells are called coelomic mesenchyme and they express markers characteristic of germ cells in other systems. The interval between the beginning of mesenchyme cell ingressions and completion of gastrulation is approximately 1 day. By this time, the embryonic territories of ectoderm, endoderm and mesenchyme (pigment, muscle, blastocoelar, skeletogenic and putative germ) cell types have been specified. **See also**: Cleavage and Gastrulation in Sea Urchin <0001073>

020068.s0006 ### Morphogenesis of ectoderm

020068.p0007 During gastrulation, ectodermal regions become morphologically distinguishable since cells on the oral side are smaller and more tightly packed than those on the aboral side. A specific area on the oral side of the embryo and near the endoderm–ectoderm boundary is skeletogenic inducing centre because it sends signals that recruit the ingressed PMCs to form two clusters of cells that begin producing spicules. At the extreme animal pole, a distinct region of thick epithelium forms beginning before gastrulation and is termed the animal pole domain or neurogenic ectoderm. Between presumptive oral and aboral ectoderm, a ciliated band of cells is induced towards the end of gastrulation and a diffuse set of neurons begins to differentiate within this band. Thus, but late gastrula stage, the ectodermal embryonic territories include epidermal cells on the oral and aboral sides of the embryo, a band of cuboidal ciliated cells, the neurogenic ectoderm and a skeletal signalling centre.

020068.s0007 ### Coordination of morphogenetic events during gastrulation.

020068.p0008 During this critical period, many processes occur simultaneously. The embryonic gut differentiates into three parts (the foregut, stomach and intestine), the spicules elongate in a pattern dictated by ectodermal signals, the pigment cells invade the aboral ectoderm, the blastocoelar cells form a complex network throughout the blastocoel and around the gut, the aboral ectoderm expands in a thin sheet of squamous epidermal cells to produce the triangular, bilaterally symmetric pluteus, a mouth or stomodeum forms within the oral ectoderm and the nervous system, consisting of an apical ganglion above the mouth, oral ganglia and a diffuse network of cells begins to differentiate. This organism is equipped for swimming, feeding and differentiation of the adult rudiment, culminating after metamorphosis of the pentameral, radially symmetric adult.

020068.s0008 # The Cell and Molecular Biology of Early Development

020068.s0009 ## Initial maternal asymmetries

020068.p0009 There is positional information asymmetrically distributed along the AV axis of the egg. When fertilized, the animal half of a bisected egg gives rise to a hollow ball of poorly differentiated ectoderm, whereas blastomeres in the vegetal hemisphere often produce a reasonably well-differentiated pluteus larva. Thus, some maternally supplied gene products are spatially localized to the vegetal halves of eggs and animal halves require additional information from vegetal blastomeres to differentiate appropriately.

020068.p0010 The major mechanism underlying creation of animal and vegetal domains has been traced to the nuclear function of β-catenin, a transcriptional activator that is the primary effector of the canonical Wnt signalling pathway. As discussed in more detail below, at 16-cell stage, β-catenin enters the nuclei of most vegetal cells, the micromeres, where it activates the PMC-specific gene regulatory network and the production of at least two signalling mole-

cules required for the development of the adjacent endomesodermal territory. Nuclear entry of β-catenin is thought to be cell autonomous, because it happens in the absence of signals from other cells of the embryo. Because of this, the process that drives nuclearization of β-catenin must be maternally regulated. From work in other systems, it is known that immediately upstream of β-catenin nuclearization is dishevelled. Recent studies show that immediately after fertilization, this molecule concentrates in the vegetal cortex of the sea urchin egg. This is the earliest known regulatory asymmetry along the AV axis of the sea urchin embryo, although what causes its translocation to the vegetal cortex is not understood. The finding that β-catenin can enter nuclei in individual cells of dissociated embryos does not eliminate the possibility that disheveled is activated in the vegetal cortex through an autocrine wnt signal acting through its receptor, frizzled, at the vegetal pole of the egg and/or early embryo. Such a process has recently been shown to occur through Wnt11 in dorsal axis specification of the *Xenopus* embryo. Here, the production of coreceptors and extracellular matrix proteoglycans is also required to mediate the maternal wnt signal. If a similar process operates in sea urchin embryos, it may explain why there is a 5.5 h lag between fertilization and the first detectable β-catenin in micromere nuclei at the 16-cell stage.

020068.p0011 The micromeres are thought to be maternally determined because they always differentiate as skeletogenic (primary) mesenchyme when they are cultured separately or tested in any combination with other blastomeres. These cells are also the first to contain β-catenin in their nuclei. The facts that disheveled is localized to the region of the egg that gives rise to micromeres and is required for β-catenin's transcriptional function fits very nicely with this idea.

020068.p0012 Nuclear β-catenin is required not only for the development of micromere progeny but also for the rest of the embryo. It is transiently maintained in micromere descendants and in overlying vegetal derivatives where it initiates a cascade of events that include both activation of signalling pathways and production of transcription factors. These are required for specification of endodermal and mesenchymal cells. Also required is the downregulation of regulatory activities that promote ectoderm differentiation. At least the initial phases of nuclear entry of β-catenin into vegetal blastomeres is cell autonomous and therefore thought to be supported primarily by maternally supplied gene products.

020068.s0010 Developmental regulatory activities that pattern the early embryo

020068.s0011 The endomesoderm gene regulatory network

020068.p0013 The transcriptional regulatory function of β-catenin is required in the progenitors of endoderm and mesenchyme derived from the macromeres as well as in the micromere lineage. β-Catenin are present in nuclei of macromere progeny between 5th and 9th cleavages. This is essential because neither tissue in embryos express excess cadherin which sequesters β-catenin in the cytoplasm. Cadherin can also block endomesoderm development if it is present in the macromeres, but not in the micromeres or vice versa. This indicates that micromeres must send and macromere progeny must receive signals that are nuclear β-catenin-dependent. The fact that cadherin can completely block endomesodermal development served as the basis to select mRNAs encoding transcription factors (~ 50) and signalling molecules, that are required for this process in normal embryos. The mRNAs expressed at the right time and place were then arranged into potential gene regulatory networks (GRNs). This was because new technology allowed interference of the production of specific gene products. Gene-specific 'knock downs' were achieved by injecting specific modified nucleotides, called morpholinos, that block either RNA splicing or translation. This is analogous to a null mutation. The effect of loss of each protein was monitored by measuring the expression levels (mRNA concentrations) of all the other candidate genes, or a compilation of the epistatic relationships among regulatory genes. Because of the rapid development of the sea urchin embryo and the ease of perturbing gene function, this approach led rapidly to the construction of the endomesoderm gene regulatory network (GRN), currently the largest and most detailed network described in any embryo.

020068.p0014 Understanding the structure of the regulatory network illuminates how development of vegetal tissues work. First, there are only a few regulatory steps between nuclear β-catenin activity and expression of genes that define terminal differentiation of different cell types. For example, in the micromere-PMC lineage, the first step is derepression of pmar1, the gene at the top of the regulatory hierarchy, the second is activation of a set of transcription factors some of which are direct activators of differentiation genes while others are one step removed. In macromere-endomesoderm lineages, signals sent from micromeres + β-catenin-dependent production of transcription factors also led to expression of differentiation genes with only 1 or 2 additional steps. This architecture explains why the highly differentiated 3-day pluteus larva can be made after only ~ 10 rounds of cell division and only 6 after the initial entry of β-catenin into micromere nuclei. Second, and more importantly, some transcription factors are cross-regulatory. This property makes their production independent of earlier events (such as nuclearization of β-catenin) and their function constitutes a key driving force forward to the next regulatory step in the GRN.

020068.p0015 An important initial regulatory component of the endomesoderm gene regulatory network is the regulated removal of transcriptional regulators that function in differentiation of cell types derived from more animal regions of the egg. This set of factors includes the animalizing

transcription factors (ATFs), which are uniformally distributed throughout the egg and early embryo. In the absence of nuclear β-catenin (cadherin-injected), ATFs accumulate in all nuclei, whereas in normal embryos, the ATF-positive domain gradually retracts towards the animal hemisphere of the embryo in parallel with the advancing wave of nuclear β-catenin. Studies on one of these SoxB1 illustrate the developmental significance of this process. Most importantly, this factor antagonizes nuclear β-catenin function; when SoxB1 is eliminated with a morpholino, the transcriptional activity of β-catenin rises many fold. Thus, for the nuclear β-catenin GRN to operate, SoxB1 concentrations must be downregulated in vegetal blastomeres. This is accomplished through asymmetric partitioning of SoxB1 mRNA and protein at the asymmetric 4th cleavage, by β-catenin-dependent repression of *SoxB1* transcription as well as β-catenin-dependent degradation of SoxB1 protein in vegetal cells. This last mechanism was discovered by introducing mRNA encoding SoxB1 protein linked to green fluorescent protein and watching its distribution in developing live embryos.

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Ectodermal gene regulatory networks

020068.p0016 Our understanding of the molecular regulation of ectoderm differentiation is less advanced, but some critical genes that encode transcription factors and signalling molecules have been identified. The initial patterning along the oral–aboral axis involves a combination of unknown signals from vegetal cells and an asymmetry in redox potential that is present in the embryo shortly after fertilization. This combination activates expression of the transforming growth factor β (TGF-β), nodal in presumptive oral ectoderm. Nodal signaling then activates production of goosecoid (a transcription factor) in the oral ectoderm and bone morphogenetic proteins 2/4 (BMP2/4, a signalling molecule) required for aboral ectoderm. The transcription factor, orthopedia, is required for skeletogenesis and is expressed at the right time in the skeletogenic inducing centre. In the animal pole domain the transcription factor NK2.1 regulates production of long cilia characteristic of cells in this region, but regulatory proteins required for specification of neurectoderm have not yet been identified. Studies are in progress in a number of laboratories to uncover the GRNs underlying development of each of these different ectodermal domains.

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The role of signalling among cells in embryo development

020068.p0017 While much research devoted to the creation of GRNs has focused on the regulatory relationships among transcription factors, the role of signalling among cells is also critical for cell-fate specification in this embryo. By removing blastomeres or transplanting them to ectopic locations, it has been shown that essential signals are sent from vegetal blastomeres to overlying cells, as well as laterally among cells within each tier. The existence of such an elaborate set of signals endows this embryo with its great regulative power, i.e., the ability of all but the most vegetal cells to transfate when experimentally challenged. For example, fate mapping by dye injection establishes that a secondary axis, the oral–aboral axis, is in place before four-cell stage in *Strongylocentrotus purpuratus* embryos. However, normal quadruplets arise when each of the four blastomeres is separated and cultured in isolation. Or when primary mesenchyme cells are removed, secondary mesenchyme cells change fate to replace them. And when portions of the archenteron are surgically removed, cells that could otherwise become ectoderm instead convert into an endodermal pathway. Cells derived from animal blastomeres fated to become endoderm can differentiate into nearly all cell types of the embryo if supplied with signals from micromeres. In fact, surprisingly normal embryos can develop from a combination of micromeres and the animal-most eight blastomeres from a 60-cell stage embryo. Thus, cells are continuously talking to each other to ensure that tissues critical to survival of the embryo are present.

Critical signals

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Signals from the micromeres

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020068.p0018 Nuclear β-catenin activates at least three signals in the micromeres. The first is undefined and begins to be sent to overlying lineage of macromeres during 16–60-cell stages and facilitates formation of an endomesodermal state. Two subsequent signals, Wnt8 and Delta, promote differentiation of endoderm and secondary mesenchyme, respectively. Wnt8 may affect endoderm differentiation either by reinforcing the pathway that stabilizes β-catenin leading to its nuclearization or by activating genes required for gastrulation cell movements, or perhaps both. Delta signals through the receptor, Notch, which is required for pigment and blastocoelar cell specification.

Signals from macromere progeny

020068.s0016

020068.p0019 Delta also is produced in macromere descendants and its signalling through Notch among cells in this territory is required for additional blastocoelar and muscle cells. An unknown signal from these cells is required to activate nodal in the oral ectoderm.

Signals from mesomere progeny

020068.s0017

020068.p0020 As mentioned above, nodal signalling in the oral ectoderm of the early blastula stage embryo is required for specification of cells in this territory, in part by suppressing differentiation of aboral ectoderm and the ciliated band. It is required for other signals like BMP, which are necessary to establish the aboral ectoderm. Once the oral and aboral territories are specified, signals between them set up the ciliated band.

020068.p0021 All of these signals ultimately derive from nuclear β-catenin. Without this factor and the resulting signals, cells of the embryo acquire a primitive ectoderm state and form a hyperciliated blastula. Such embryos are very similar, if not identical, to those derived solely from the animal tier of four blastomeres of an eight-cell embryo, which never receives vegetal signals. Half of the cells in these embryos consist of a poorly differentiated, unpolarized epidermal-like epithelium while the other half has neural properties and represents an expansion of the neural territory in the absence of vegetal signals. Thus, the β-catenin-dependent GRN and associated signalling interactions among blastomeres serve to produce new gene products required for endomesoderm and for ectodermal oral–aboral polarity and to repress the innate neurogenic capacity of most cells in the embryo except those at the animal pole. What endows this special region with immunity to oral and aboral specification signals is not understood.

020068.s0018 ## The transition from conditional specification to determination is controlled by localized repression of gene expression

020068.p0022 Specification of cell fates occurs when differences in gene expression can be detected in different cells. In sea urchin embryos, specification of most cells occurs well before they are determined. Their fates are plastic and may be altered experimentally, as, for example, by rearranging positions of blastomeres. When experimental perturbations can no longer alter their fate, cells are said to be determined. During the period of developmental plasticity, cells are said to be conditionally specified, they express a broader range of gene products. An important concept is that the transition from conditional specification to determination requires the progressive silencing of regulatory genes in different embryonic territories. An example of this phenomenon is the production of the transcriptional repressor, goosecoid, in the oral ectoderm which represses genes activated by a closely related, but ubiquitous transcription factor, Otx, which are required for aboral ectoderm differentiation. Thus, transcriptional repression is a key upstream step in cell-fate determination.

020068.s0019 ## Summary Model

020068.p0023 The most critical molecular asymmetry is the establishment of distinct vegetal and nonvegetal domains of transcriptional activity. The vegetal domain is set up by the maternally regulated, cell-autonomous, influx into nuclei of a transcription regulatory protein, β-catenin, that begins in micromeres at 16-cell stage and progressively extends to more animal blastomeres during cleavage. This endows micromeres with inductive capacity, thus creating an embryonic organizing centre. Nuclear β-catenin also is required for overlying macromere progeny to become competent to receive micromere signals. This initial maternal asymmetry is required both to initiate and to mediate a series of inductive interactions that is relayed between adjacent tiers of vegetal blastomeres during cleavage, presumably by sequentially activating production of cell-type-specific transcriptional activators and repressors as well as ligands and receptors in interacting tiers. The vegetal signalling cascade is opposed by multiple positively acting transcription regulators expressed in the region of the embryo. Immediate functions of these regulators are to specify this region as early, conditionally specified ectoderm and to limit the range of vegetal signalling mechanisms. Around mesenchyme blastula stage, the opposing activities of the vegetal cascade and the animalizing domain reach equilibrium in the region of veg_1 progeny near the embryo equator and establish the endoderm–ectoderm border. The creation of this equilibrium state allows the developmental capacities of cells in each embryonic territory to become fixed during the last round or two of cell divisions before the larval form is completed.

Further Reading

Angerer LM and Angerer RC (2000) Animal–vegetal patterning mechanisms in the early sea urchin embryo. *Developmental Biology* **213**: (1) 1–12.

Cameron RA and Davidson EH (1991) Cell type specification during sea urchin development. *Trends in Genetics* **7**: 212–218.

Davidson EH (1999) A view from the genome: spatial control of transcription in sea urchin development. *Current Opinion in Genetics and Development* **9**: 530–541.

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

Ettensohn CA and Sweet HC (2000) Patterning the early sea urchin embryo. *Current Topics in Developmental Biology* (in press) . AU:2

Kenny AP, Kozlowski D, Olekyn DW, Angerer LM and Angerer RC (1999) SpSoxB1, a maternally encoded transcription factor asymmetrically distributed among early sea urchin blastomeres. *Development* **126**: 5473–5483.

Logan CY, Miller JR, Ferkowicz MJ and McClay DR (1999) Nuclear beta-catenin is required to specify vegetal cell fates in the sea urchin embryo. *Development* **126**: 345–357.

Lowe CJ and Wray GA (1997) Radical alterations in the roles of homeobox genes during echinoderm evolution. *Nature* **389**: 718–721.

Wessel GM and Wikramanayake A (1999) How to grow a gut: ontogeny of the endoderm in the sea urchin embryo. *BioEssays* **21**: 459–471.

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Glossary:

CDNA#DNA copy of an individual mRNA.
Cell autonomous#Function that requires no interaction with other cells.
Determinant#Molecule, usually RNA or protein, controlling the developmental fate of a cell.
Immunocytochemistry#Method for detecting location of proteins in embryos by microscopy, using fluorescently labelled or enzyme-linked antibodies.
Maternal determination#Regulation of cell fate by inheritance of molecules (determinants) stored in the egg cytoplasm during oogenesis.
Spicules#Calcite skeletal rods secreted by the primary mesenchyme cells.
Transcription factor#Nuclear protein that either activates or suppresses RNA synthesis.

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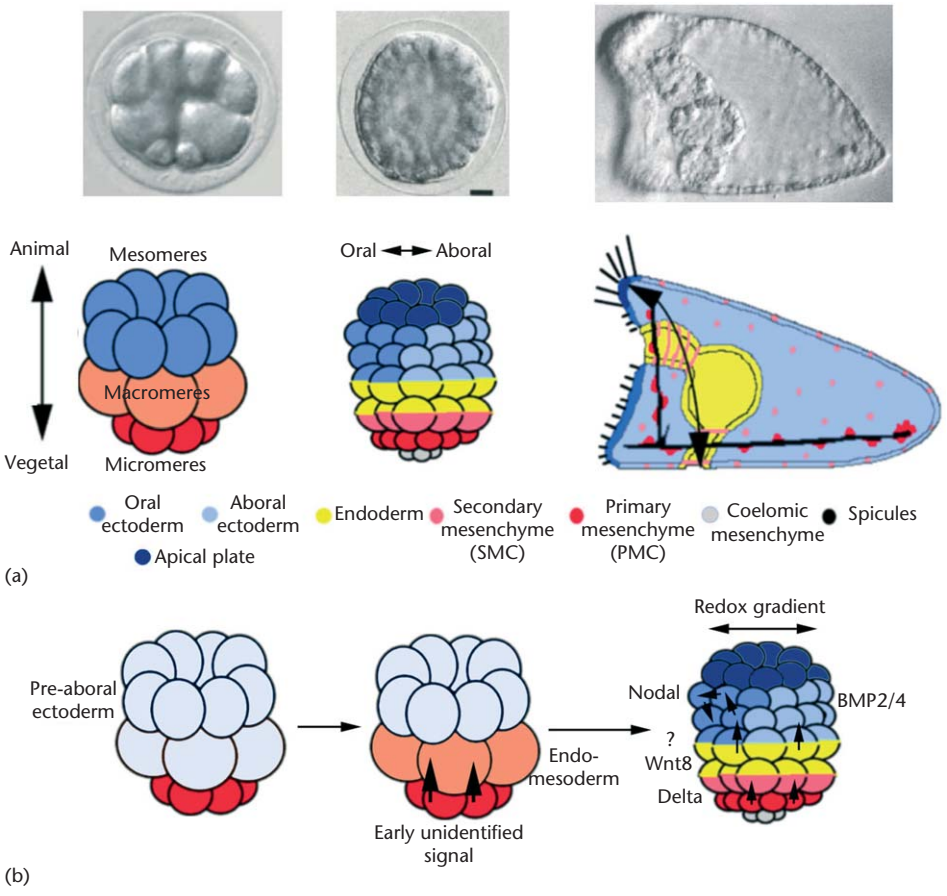

AU:3 **Figure 1** Model for early patterning steps in sea urchin development. Ectoderm, blue; endoderm, yellow; mesenchyme, red. At the 16-cell stage (a), mesomeres and micromeres give rise only to ectoderm and mesenchyme, respectively. In contrast, macromeres give rise to cells of all three germ layers and are coloured grey to signify this combination (blue, yellow and red). The mesomere and macromere nuclei are blue, indicating that they initially contain transcription factors specifying a pre-ectoderm state, while those in micromere nuclei are red, signifying a vegetal transcriptional domain whose most important known factor is β-catenin. At the 60-cell stage (b), macromere progeny include veg_1, coloured green because they will give rise at early blastula stage (c) to ectoderm (blue) and endoderm (yellow), while veg_2 are coloured orange because they will give rise to endoderm (yellow) and SMCs (red). Veg_1 nuclei are green to signify that they contain transcription factors specifying both ectoderm and endoderm. During blastula stages (c, d), endoderm and SMCs segregate in veg_2 progeny as a result of signalling from micromeres (arrowhead) to overlying SMC precursors expressing the Notch receptor (*). Among veg_1 progeny, ectoderm and endoderm segregate as a result of BMP2/4 signalling from animal blastomeres (blue arrow) and vegetal β-catenin-dependent signals (yellow arrow).

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