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Morphogenesis in sea urchin embryos: linking cellular events to gene regulatory network states

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Abstract

Gastrulation in the sea urchin begins with ingression of the primary mesenchyme cells (PMCs) at the vegetal pole of the embryo. After entering the blastocoel the PMCs migrate, form a syncytium, and synthesize the skeleton of the embryo. Several hours after the PMCs ingress the vegetal plate buckles to initiate invagination of the archenteron. That morphogenetic process occurs in several steps. The non-skeletogenic cells produce the initial inbending of the vegetal plate. Endoderm cells then rearrange and extend the length of the gut across the blastocoel to a target near the animal pole. Finally, cells that will form part of the midgut and hindgut are added to complete gastrulation. Later, the stomodeum invaginates from the oral ectoderm and fuses with the foregut to complete the archenteron. In advance of, and during these morphogenetic events an increasingly complex gene regulatory network controls the specification and the cell biological events that conduct the gastrulation movements.

Keywords

sea urchin; gastrulation; morphogenesis; epithelial-mesenchyme transition; skeletogenesis; gene regulatory networks

1. Introduction

1.1 Sea Urchins are a well-established model for mechanistic studies of development including assembly of gene regulatory networks (GRNs)

Sea urchins have been an important model system for developmental biology for more than 130 years (Fig 1). Among the attributes that make them amenable for observation and experimentation are: 1) they are widely available in oceans around the world; 2) they produce millions of gametes in a single spawn; 3) eggs can be fertilized *in vitro* and develop synchronously; 4) embryos develop rapidly and are optically clear; and 5) they are robust to experimental manipulations such as egg bisections, cell ablation, cell dissociation, transplantation, and microinjection (Ettensohn et al., 2004). Even before the emergence of molecular developmental biology, researchers working with sea urchins made seminal contributions to our understanding of embryonic regulation (Driesch, 1891), the basis of heritability (Boveri, 1902; Boveri, 1918), intercellular induction (Horstadius, 1939), and cytokinesis (Rappaport, 1961), to name just a few. The extremely rich and varied history of experimental investigations in sea urchins established a foundation for more recent advances in the molecular dissection of fate specification and morphogenesis. In the last decade, sea urchins have been the favored model system for studies of gene regulatory networks

(Davidson, 2006). The sea urchin endomesoderm network is arguably the most complete GRN in any metazoan (Oliveri et al., 2008; Peter and Davidson, 2010).

An obvious goal for developmental studies is greater understanding of how embryogenesis is controlled. At the core of embryonic fate specification is transcriptional regulation. The sea urchin is especially useful for examining the GRNs that regulate embryonic specification, diversification and morphogenesis. A gene regulatory network describes the steps of cellular differentiation over time, with focus on the cis-regulatory connections between genes, particularly transcription factors and signaling molecules (Davidson, 2010). GRNs are modeled as logic maps containing a combination of predicted connections based on gene perturbation studies, and validated connections based on direct cis-regulatory analysis (Fig 2).

Sea urchins are an ideal system for building GRNs for several reasons. First, their development is rapid and relatively simple (Fig 1). In *Strongylocentrotus purpuratus* for example the fertilized egg develops into a swimming pluteus larva within a few days at 15°C and has differentiated ectoderm, mesoderm and endoderm tissues consisting of a total of about 14–15 different cell types. Second, many genes have been cloned and their expression profiles studied spatiotemporally and quantitatively by whole mount in situ hybridization (WMISH), quantitative PCR (qPCR), and more recently, nanostring technology (Geiss et al., 2008; Materna et al., 2010). Third, the embryos are easily injected with reagents that perturb development in a gene-specific way, such as synthetic mRNAs for over expression and dominant negative studies, and antisense morpholino oligonucleotides to inhibit translation. A series of perturbations with such reagents provided a basis for understanding markers associated with specific territories, then laid the framework that established the order of gene activation and repression (Davidson et al., 2002). In many cases the predicted connections between genes have been validated by cis-regulatory analysis that identifies and experimentally confirms functional binding sites in the enhancer region of the genes under investigation. This component of GRN analysis has typically been time-consuming, though recent advances offer approaches to greatly speed up the process (Nam et al., 2010). Improved bioinformatic tools have facilitated identification of putative enhancer regions by analyzing cis-regulatory regions in several species of urchins with conserved regulatory sequences. Additionally, sea urchin embryos are amenable to gene transfer through injected BAC constructs that contain the gene's regulatory region fused to GFP thus providing endogenous expression patterns. The regulatory sequences can then be deleted or mutated to confirm or identify functional enhancer sites. The Davidson lab at Caltech is host for the current and updated versions of the network (<http://sugp.caltech.edu/endomes>).

The network model is a systems-level view of the embryo, providing a major advance in examining aspects of development that were previously studied only at a phenomenological level. For example, the GRN has elucidated the logic of network architecture (Davidson, 2009), including how cells transition from initial to subsequent specification states (Oliveri et al., 2008; Peter and Davidson, 2010). It has been used to study developmental plasticity such as trans-differentiation and compensation (Ettensohn, 2009). It also provides a starting point for understanding the evolution of gene regulatory networks through comparison of networks in sea urchin and other echinoderms (Gao and Davidson, 2008; Hinman et al., 2009; Peter and Davidson, 2010). In this review, we highlight how the network is helping to identify the subroutines that control morphogenetic processes such as epithelial to mesenchymal transitions (EMT), cell migration, gastrulation and patterning.

1.2 Overview of sea urchin development

Initial patterning along the animal-vegetal axis is established prior to fertilization (Angerer and Angerer, 2003; Horstadius, 1973). The first two cleavages of the zygote are equal and parallel to the animal-vegetal axis. The 3rd cleavage is roughly equal and equatorial, separating four animal blastomeres from four vegetal blastomeres. At 4th cleavage the animal tier of cells divides meridionally, giving rise to a ring of 8 cells called mesomeres. By contrast, the four vegetal blastomeres divide asymmetrically. This produces four larger cells called macromeres, and four smaller and vegetally-situated cells, called micromeres (Fig. 1). At 5th cleavage the micromeres divide asymmetrically again to produce four small micromeres at the vegetal pole and four large micromeres just above them. At the 60-cell stage the embryo is composed of six tiers of cells whose fates have been mapped (Logan and McClay, 1997). The small micromeres are thought to give rise to the germ cells after dividing once during early development and migrating to reside in the coelomic pouches of the larva (Yajima and Wessel, 2011). Above the small micromeres are the large micromeres, which will become the skeletogenic cells and are often called primary mesenchyme cells (PMCs). The next tier of cells away from the vegetal pole are veg2 cells, which are descended from the macromeres, and give rise to the endoderm and non-skeletogenic mesoderm (NSM, including pigment cells, blastocoelar cells, muscle cells and coelomic pouch cells). Above the veg2 tier sits a tier of cells called veg1 that will give rise to endoderm and some ectoderm. The animal hemisphere contains the descendants of the mesomeres, which contribute to the dorsal and ventral (aboral and oral) ectoderm. The ectoderm is subdivided into a number of domains prior to the larval stage, including the apical plate domain with associated sensory cilia (where neurogenesis begins), the ciliated band separating the dorsal from the ventral ectoderm domains, and the mouth. The dorsal-ventral and left-right axes are established between early blastula stage and late gastrulation. They will not be addressed in this review, but we point readers to several recent papers that review these subjects (Duboc and Lepage, 2008; Lapraz et al., 2009). This chapter summarizes the sequence of events in the life of the micromere/PMC/skeletogenic cells and in the macromere progeny that form the archenteron.

Micromere/PMCs are a unique cell type in many ways and perhaps more is known about how this cell population works than is known about any other cell type in the embryo. Evolutionarily, micromere/PMCs are unusual in that they appear only in the euechinoids, the modern sea urchins and sand dollars. Other echinoderm larvae either lack calcium carbonate skeletons, or, in the case of Ophiuroid larvae, have a larval skeleton but do not have micromeres (Gao and Davidson, 2008; Yamashita, 1985). Because this cell type develops precociously, and is relatively easy to manipulate, much has been discovered about how it is specified, and how it contributes to the development of other cells in the embryo (see sections 2 and 3 below). PMCs produce the skeleton, which is a highly orchestrated patterning exercise. Production of the larval skeleton begins in late gastrulation and involves either 32 or 64 PMCs, depending on the species. Prior to making the skeleton the large micromeres are specified, and then 16 or 32 micromere progeny initiate morphogenesis. At that point they are called PMCs and they ingress via an epithelial-mesenchymal transition into the blastocoels, where they build the skeleton. They divide once more and do not divide again until feeding begins at a later time. The larval skeleton continues to grow as the larva increases in size and the adult rudiment prepares for metamorphosis (Fig 1). Following metamorphosis, the PMCs are likely lost, as there is no evidence that the PMCs are used for juvenile or adult structures. Indeed, it has been shown that the adult skeleton is produced by mesoderm and not by progeny of the PMCs (Yajima, 2007). This implies that the PMCs have a single function as skeletogenic cells of the larva.

The ectoderm, progeny of the mesomeres, later participates in gastrulation in a number of ways: ectoderm supplies positional information to the skeletogenic cells that enables PMCs

to produce a patterned skeleton; the oral ectoderm produces the stomodeum that later fuses with the archenteron; and the ectoderm later changes shape as the embryo transitions from a spherical to a prism shape (Fig 1) (section 3 below).

Following PMC ingression the vegetal plate thickens and the archenteron invaginates. That classic morphogenetic movement has been separated into two stages, primary invagination which involves inbending of the NSM cell sheet at the center of the vegetal plate, and secondary invagination during which the endoderm contributes to elongation of the gut tube (section 4 below describes these morphogenetic movements).

1.3 Morphogenesis in the context of GRNs

Prior to gastrulation, the embryo is already composed of distinct domains of cells, each controlled by specific regulatory states of the GRN. Patterning these GRN state differences along the animal-vegetal axis requires a combination of inherited maternal factors and signaling events between cells (Fig 2). Endomesoderm develops in the vegetal half the embryo. Initial activation of the endomesoderm GRN occurs at the 16-cell stage when beta-catenin, the mediator of canonical Wnt signaling, enters the nuclei of the micromeres (Logan et al., 1999; Wikramanayake et al., 1998). In the nucleus, beta-catenin complexes with the TCF/Lef transcription factor complex to up-regulate target genes. As soon as the large micromeres appear they can be isolated and in culture they become specified and will produce a skeleton showing that their specification is autonomous (Ettensohn, 2009; Okazaki, 1975). Beta-catenin in the nucleus of the micromeres leads to the expression of a crucial transcription factor, *pmar1*, which represses the *hesC* transcriptional repressor, to allow a micromere/PMC-specific program to unfold (Fig.3) (Revilla-i-Domingo et al., 2007; Sharma and Ettensohn, 2010) (see sections 2 and 3 below for details).

The micromeres also function as a crucial signaling center, inducing the differentiation of mesoderm and endoderm (Logan et al., 1999). There are at least three signals that emanate from the micromeres to pattern the endomesoderm. The first is an early signal that is sent from the micromeres during early cleavages (Ransick and Davidson, 1993). Its full molecular nature is yet to be understood but may involve the TGF-Beta family ligand Activin B (Sethi et al., 2009). Second, Wnt 8 is expressed in the micromeres where it acts cell autonomously, and also may send a signal to the neighboring cells to accelerate endomesoderm specification (Smith and Davidson, 2008; Wikramanayake et al., 2004). Third, a Delta signal is sent from the micromeres and received by the veg2 tiers of cells, which express the Notch receptor (Sherwood and McClay, 1997; Sherwood and McClay, 1999; Sweet et al., 2002). This signaling pathway induces components of the mesoderm. Many additional signals come into play as the endomesoderm continues to be specified and cell types diverge. When the embryo reaches the mesenchyme blastula stage it has specified about 14 different cell types, and these are programmed for differentiation and distinct roles in morphogenesis.

PMC ingression, migration of skeletogenic cells, patterning of the skeleton, and gastrulation movements constitute the morphogenetic movements necessary to shape the larva. Analogous and homologous morphogenetic events have been studied in great detail in many systems, and some general similarities can be drawn across cell types and phyla (Magie and Martindale, 2008; Micalizzi et al., 2010; Schock and Perrimon, 2002). The molecular components that are most often conserved are those involved in cell-cell adhesion and cytoskeletal dynamics, properties fundamental to all cell types. Despite similar morphogenetic patterns, the regulatory proteins coordinating the processes are divergent. In part this is because they often occur in differentiated tissues, already in an advanced state of specification. Since morphogenetic processes like EMT, migration and gastrulation are complex sequences of integrated cell and molecular changes, it is likely that subsets of

transcription factors run parts of these processes. By breaking morphogenetic processes down to smaller units, such as cell adhesion, polarity, shape, motility etc, one can determine the subroutines of the network that drive each. It is a challenge to understand how these processes, as individual units, function coordinately. In that context, each of these individual components of morphogenesis can be compared across cell types and species to provide a more meaningful assessment of similarities and differences. The sea urchin GRN provides an ideal platform for identifying and analyzing these subroutines.

2. Ingression of Primary Mesenchyme Cells

2.1 Initial Patterning of the PMCs primes them for later morphogenetic events

Following the initial activation of the micromere GRN an additional group of transcription factors are activated within 1 to 2 hours of the start of ingression (Tel, Erg, Hex, Tgif, FoxN2/3, Dri, FoxB, FoxO, Snail, Twist in Fig. 3) (Oliveri et al., 2002; Oliveri et al., 2003; Oliveri et al., 2008; Wu et al., 2007; Wu et al., 2008). All of these depend on an earlier group of transcription factors for their activation, though each transcription factor in the later group is activated by a unique combination of transcription factors and at a specific time (Oliveri et al., 2008; Rho and McClay, 2011). At a systems level, the *pmar1/hesC* double repression gate at the beginning of specification offers a way to rapidly activate the network (Davidson and Levine, 2008). Other components of the micromere GRN offer mechanisms to “lock down” the network and assure that it progresses forward (Davidson and Levine, 2008; Oliveri et al., 2008), and within the network there are devices that enable the GRN to be repaired following an earlier failure (Smith and Davidson, 2009).

The micromere GRN programs the skeletogenic cells for two functions: it prepares them for the process of ingression, and it prepares them for the events that follow ingression eventually resulting in production of the skeleton. Ingression is an epithelial-mesenchymal transition (EMT) and is the first in a series of morphogenetic changes by the PMC progeny of the large micromeres. Skeletogenesis then follows.

2.2 PMC ingression involves multiple, coordinated cell biological events

Prior to ingression during the late blastula stage the micromere progeny/presumptive PMCs and progeny of the veg2 cells flatten to form the vegetal plate (Fig 1). Within the vegetal plate the PMCs delaminate from their neighbors (small micromere and veg2 progeny) and crawl into the blastocoel, undergoing a classic EMT (Fig. 4). Once they have ingressed, the PMCs extend highly dynamic filopodia and migrate along the lining of the blastocoel forming a ring of cells below the equator. There they fuse and form bilateral clusters on the ventral side of the embryo within which spicule rudiments are produced (Okazaki, 1975). As this process occurs, the vegetal plate bends and the gut begins to elongate (see section 4 below).

In sea urchins, ingression is a movement of individual cells or small clusters of cells, distinguishing it from gastrulation in fly and frog, which is characterized by movements of sheets of cells. The first investigations to make detailed descriptions of PMCs ingression used TEM and SEM microscopy (Katow and Solursh, 1981; Fig. 4). The apical surface of the epithelium is covered with hyaline material, laminin, collagens, and echinonectin. The basal surface of this epithelium faces the blastocoel and is separated from it by a basal lamina that includes collagens and laminin. Each cell possesses a single cilium that is embedded, and protrudes through, the hyaline layer. Katow and Solursh (1981) demonstrated that the cilium of each PMC is lost prior to ingression, which frees the cells from the hyaline layer, while the small micromere progeny retain their cilia.

As PMCs ingress they must loosen connections to their neighbors, change shape, and breach the basal lamina to enter the blastocoel (Fig 4). The apical side of the epithelium faces the external environment and classic adherens junctions connect cells to one another. Katow and Solursh (1981) proposed that changes in cell adhesion must accompany ingression. Fink and McClay (1985) used an adhesion assay to demonstrate that as the cells ingress they lose affinity to the apical lamina and gain affinity to the basal lamina and blastocoelar matrix at the time of ingression. Hertzler and McClay (1999) showed that a sea urchin homolog of integrin is expressed on the basal surface of epithelial cells at the midblastula stage, which correlates with the timing of increased affinity to the laminin in the basal lamina. Once the PMCs ingressed and began migration, they had a lower affinity for laminin than for ectoderm, and this timing correlates with a loss of the integrin staining on the PMCs.

To get at the molecular basis of changes in adhesion during ingression Miller and McClay (1997a, 1997b) investigated the expression of cadherins and catenins ingression using antibodies (Fig. 4). Before ingression, cadherin and catenins are expressed in the apical adherens junctions. When the PMCs ingress, they lose the staining at the apical junctions, while their neighbors retain it. The proteins become internalized into several puncta in each PMC and are retained there only briefly; as soon as the PMCs begin to migrate the puncta are lost. This suggests that endocytosis is responsible for removing these proteins from the cell surface to free the PMCs. When ingression was inhibited the puncta were not seen. The authors interpreted these results to mean two things, first that changes in catenin localization could alter the intercellular connections between cadherins on apposing cell surfaces allowing the cells the freedom to move, and second that the internalization of cadherins is necessary for the PMCs to ingress.

Endocytosis removes the epithelial membrane components from PMCs as they transition to mesenchymal cells. A coincident exocytosis is also critical to ingression for inserting membrane proteins that will support the mesenchymal PMCs. Transcripts for genes that function in the PMCs often are made well in advance of ingression. For example, mRNA for the MSP130 glycoprotein is detected in the PMC lineage a few hours before ingression, but the glycoprotein is not detected on the cell membrane until the cells ingress (Anstrom et al., 1987)(Fig 4 G,H). Additionally, a sea urchin homolog of the neural glia CAM (NgCAM), is transcribed before ingression in the PMC lineage but staining with an antibody is seen only after the PMCs arrive in the blastocoel (Fig. 4H). When NgCAM is knocked down, PMCs still ingress but fail to carry out proper patterning and spicule formation, demonstrating that this protein functions after EMT (McClay and Peterson, unpublished results). Similarly, other proteins are seen in the PMC plasma membrane later, such as the VEGF receptor, which is necessary for migration and patterning of the PMCs once in the blastocoel; if it is knocked down, ingression still occurs (Duloquin et al., 2007).

Early in the ingression sequence PMCs undergo cell shape changes and become highly motile. The PMCs form a bottle cell shape and become elongated, with a stalk at the trailing edge still attached to the apical junction. They appear to pull at the adherens junction because when the cell finally deadheres the stalk quickly contracts. The motility of ingression is strongly dependent on actin, but microtubule involvement is minimal. Microtubule inhibitors applied before ingression do not interfere with ingression, while treating embryos with actin inhibitors during the same period prevents ingression (Anstrom, 1989; Anstrom, 1992). It is not understood how the PMCs breach the basement membrane, but it has been proposed that metalloproteases could be involved, since several metalloproteases are expressed at the right time and place (Quigley et al., 1993; Rise and Burke, 2002). However none, so far, have been directly associated with the movement through the basement membrane. It remains possible that invasion through the basement membrane is mechanical and does not require a protease.

2.3 Multiple transcription factors are necessary to execute PMC ingression

A current challenge is to understand how subnetworks of the micromere GRN drive specific cell biological processes. The mechanism by which ingression elements such as adhesion, cell shape and motility are controlled is beginning to emerge. *Lytechinus snail* is first expressed an hour before ingression (Wu and McClay, 2007). When *snail* is knocked down by morpholino, PMCs fail to ingress (Fig. 5). Importantly, *snail* is responsible for repression of cadherin expression and is also necessary for cadherin endocytosis from the cell membrane. This is probably not a protein-specific mechanism for endocytosis, but rather is representative of a massive membrane turnover during EMT (Wu et al., 2007). These experiments link a crucial EMT regulatory factor to a specific cell biological process necessary for EMT: membrane turnover and cell adhesion.

Other key regulatory factors controlling PMC ingression are also involved in later steps of PMC specification. *Lytechinus twist* is expressed in PMCs right before ingression and persists for an hour or two after PMCs are in the blastocoel (Wu et al., 2008). When *twist* is knocked down ingression is severely delayed, and although PMCs eventually do ingress, spicules do not form (Fig. 5). PMCs lacking *twist* have reduced motility, which is necessary for completing ingression in normal development. The lack of spicule formation in *twist* morphants was shown to be a consequence of an inability of *twist* knockdown PMCs to fuse. Thus *twist* has an early role in proper timing of PMC ingression and a later role in PMC cellular behaviors.

Another transcription factor that has recently been studied is *foxN2/3*, and it too has multiple roles in EMT and later specification (Fig. 5) (Rho and McClay, 2011). Expression of *foxN2/3* begins about 1.5 hrs prior to ingression in the micromeres, and is quickly lost as PMCs undergo ingression. If *FoxN2/3* is knocked down, ingression is blocked or severely delayed. When *foxN2/3* morphant PMCs are transplanted into the blastocoel, they fail to organize properly in the ring, fail to fuse, and do not express markers for spiculogenesis. This demonstrates that a single transcription factor can have multiple roles in PMC specification. From these studies we can start to understand which genes are needed for earlier steps of ingression, such as *snail*, and which are needed also for later steps of ingression and PMC specification, such as *twist* and *foxN2/3*.

3. Skeletogenesis

3.1 PMCs engage in a stereotypic behavior prior to skeletal production

PMCs emerge on the vegetal floor of the blastocoel as a result of ingression (Fig. 1). In *Lytechinus* 32 cells ingress and immediately divide to produce approximately 64 PMCs, and in *Strongylocentrotus* 16 cells ingress then divide to produce 32 PMCs. The cell cycle then arrests for this population until after feeding begins at the larval stage. Before beginning skeletal synthesis PMCs engage in a series of movements. They migrate along the wall of the blastocoel toward the animal pole but stay roughly within longitudinal columns, i.e. they move upward then downward but with very little lateral movement (Peterson and McClay, 2003). During this time the PMCs extend thin filopodia in every direction (Fig. 6) (Miller et al., 1995), extensions that are hypothesized to provide an input of information to the PMCs for patterning (Malinda and Ettensohn, 1994; Malinda et al., 1995) (McClay, 1999). By midgastrula the PMCs form a ring around the blastocoel wall just inside the boundary between ectoderm and endoderm.

Just prior to beginning skeletal synthesis the PMCs fuse to form a syncytium (Hodor and Ettensohn, 1998). At the same time cells move within the ring to form two concentrated ventrolateral clusters of PMCs with the remaining PMCs scattered around the ring (Fig 7). The first signs of skeletal synthesis occur when a small tri-radiate skeletal element is seen in

each of the two ventrolateral clusters. Skeletal elongation occurs from the distal ends of the three elements with production of the calcium carbonate skeleton (Fig. 7) (Wilt, 2002). A small group of PMCs cluster at each of the tips of the growing skeleton where the most rapid growth occurs. Once the triradiate skeleton begins a small group of PMCs remains at the tip of each of the three lengthening spicule rods. These cells use the thin filopodia as sensors of positional information allowing the species-specific patterning of the skeleton to emerge (Fig. 7).

3.2 Patterning of the skeleton uses ectodermal cues and PMC genotypic responses

A major advance in understanding ectoderm specification came with the discovery that Nodal expression controls dorsal-ventral (aboral-oral) axis formation (Duboc et al., 2004). An as yet ill-understood set of signals activates expression of Nodal on the ventral side of the embryo and Nodal then feeds back to control its own synthesis. Nodal, in turn, activates BMP2/4 in the ventral half, the BMP ligand diffuses to the dorsal half of the embryo and activates a dorsal GRN there. Experiments with NiCl_2 were shown to radialize the ectoderm, and as a consequence radialize skeletal patterning (Fig. 7,8) (Hardin et al., 1992). When embryos were treated with NiCl_2 for about 3 hrs during cleavage they became ventralized (oralized; Fig. 8). Cell recombination experiments showed that NiCl_2 had no effect on the PMCs, but the PMCs required patterning information from the ectoderm (Fig. 8). It was later shown that Nodal in those embryos was expressed around the entire embryo causing oral organs such as the mouth to become circumferential (Duboc et al., 2004; Hardin et al., 1992). In the ventralized embryos skeletogenesis was initiated in multiple sites.

The PMCs are thought to pick up the patterning cues from the ectoderm using their thin filopodia (Miller et al., 1995). The evidence is indirect but convincing: Observation of thin filopodia shows no correlation between direction of cell movement and direction of filopodial extension, the cells most actively extending filopodia are the PMCs that are at the growing tips of the skeleton, and in the NiCl_2 - treated embryos, where all information apparently is ventral, filopodia grow to unusually long lengths (from an average of 25 μm to as many as 150 μm or more) (Fig. 7). This happens in embryos in which the PMCs were not exposed to NiCl_2 so it is in response to the altered state of the ectoderm, which was exposed to NiCl_2 .

Once PMCs receive patterning cues from the ectoderm they add to the skeleton using their own genotypic information (Fig 8). Further, each PMC is capable of producing any portion of the skeleton. These statements are supported by transplantation studies where single PMCs were transferred to different positions in the PMC ring. No matter where they were placed in the ring each PMC contributed to a normal skeleton (Armstrong et al., 1993; Armstrong and McClay, 1994). To demonstrate the genotypic nature of this position-specific patterning, several PMCs from a closely related species, *Tripneustes esculentus* (*Te*) were added to the blastocoel of *Lytechinus variagatus* (*Lv*) embryos. The added PMCs were labeled with fluorescence and joined into the syncytium. Locally, the *Te* PMCs produced a pattern that was correct for position in the embryo, correct for the genotypic pattern of the *Te* species, and genotypically distinct from the *Lv* skeletal pattern normally produced in that region (Armstrong et al., 1993; Armstrong and McClay, 1994; McClay et al., 1992). In a related experiment an entire complement of PMCs from *Te* was transferred and replaced endogenous PMCs in an *Lv* embryo. The skeleton was of the *Te* genotype, and it included elements in positions not occupied by normal *Lv* skeletons (Fig. 8). This experiment showed that the mechanism by which ectoderm patterned the skeleton was conserved in that *Tripneustes* cells were able to pattern a skeleton correctly. Of mechanistic importance, it also showed that the patterning was a consequence of signaling cues rather than an alternative possibility in which the ectoderm might lay down some sort of patterned substrate to be utilized passively by skeletogenesis. This is because the *Tripneustes* skeleton

occupied spatial positions not occupied by the *Lytechinus* skeleton, and therefore could not be due to an ectodermal production of some sort of extracellular matrix template (Armstrong and McClay, 1994). Those data force the conclusion that the ectodermal positional information supplied to the PMCs consists of cues, presumably signals, that provide spatial information to inform the PMCs of their position in the embryo.

From the above it is clear that skeletal patterning is a collaboration between the ectoderm—which provides the spatial cues—and the skeletogenic mesoderm, which receives and processes that spatial information and in response produces the skeleton. Based on its position each PMC receives and processes complex spatial information and produces the correctly patterned piece of skeleton using the PMC genotypic information it has to pattern the growing crystal. Much remains to be learned about how this works. Two levels of information are needed to solve this patterning problem: there is a need to understand how the ectoderm is patterned and to discover the complexity of signals produced by the ectoderm, and it is necessary to learn how this information is processed by PMCs. It will be a major advance to learn how positional information is interpreted to produce the specific skeletal patterning decisions that are made as the skeleton grows. Much of this is unknown, but a few clues are beginning to emerge. The following section discusses those cues.

3.3 Programming PMCs for patterning

After ingressation the PMCs travel on the blastocoelar substrate as mesenchymal cells, join to form the syncytium, receive, process and respond to ectodermal information, and provide supersaturating concentrations of calcium carbonate at localized sites for skeletal growth, and produce spicule matrix proteins (Killian and Wilt, 2008; Livingston et al., 2006; Wilt, 2002; Wilt, 2005). Each of these tasks is a specialized function of the PMCs so it is not surprising that a number of molecules are transcribed uniquely by PMCs. The following examples illustrate a few of the expression patterns and reveal some of the molecular properties of these cell biological activities.

As mentioned above, a number of proteins that function after ingressation are transcribed and even translated well in advance of ingressation. An example is the MSP-130 protein that is transcribed and translated starting up to two hours pre-ingressation (Anstrom et al., 1987; Leaf et al., 1987). The MSP-130 protein initially is held in intracellular vesicles, likely the transgolgi network until ingressation begins (Fig. 4) (Wessel and McClay, 1985). At that time it is glycosylated and transported to the plasma membrane. Several monoclonal antibodies detect its appearance first in the intracellular vesicle, and then on the plasma membrane surface as ingressation continues (Wessel and McClay, 1985). Some skeletal matrix proteins are made well in advance of ingressation as well. SM50, for example, is an important skeletal matrix protein expressed initially as early as hatched blastula stage (Benson et al., 1987). The level of SM50 transcription continues to rise and increases to more than 10^3 molecules per cell at early mesenchyme blastula stage (the stage of ingressation). Also made in advance of ingressation are cell adhesion molecules, including integrins, that are involved in the post-ingressation cell movements (Hertzler and McClay, 1999).

At ingressation, or immediately thereafter, VEGF and FGF receptors (VEGFR and FGFR2) are expressed (Fig. 9) (Duloquin et al., 2007). These growth factor receptors are necessary for skeletogenesis since knockdown of either prevents skeleton formation (Duloquin et al., 2007; Rottinger et al., 2008). Shortly after the VEGFR and FGFR2 proteins are made in the PMCs, many of the PMCs in the early syncytial ring gather in two sites to form the ventrolateral clusters where skeletogenesis will initiate. These sites are immediately beneath the two bilateral sites in the ectoderm where both VEGF and FGF are produced (Duloquin et al., 2007) (Fig. 9). If either VEGF or FGF production is knocked down experimentally the ventrolateral clusters do not localize to the two sites. Thus at least two of the ectodermal

spatial cues are VEGF and FGF. It remains to be learned how the ectoderm gene regulatory network controls and localizes the expression of these two signals, and it also remains to be learned how these signals are used to initiate production of the triradial skeletons. That they are necessary is shown by additional experiments in which the receptors for those signals on the PMCs are necessary for the PMCs to respond and produce a skeleton (Duloquin et al., 2007).

Other clues are provided by PMCs' expression of a large number of genes associated with biomineralization (Livingston et al., 2006). As the time of skeletal deposition approaches PMCs secrete massive amounts of calcium salts combined with matrix proteins at the site of mineralization (Killian and Wilt, 2008). The PMCs at that site utilize the patterning information from the ectoderm and from their intrinsic patterning device to grow the mineral in the correct pattern. The PMCs insert a number of matrix proteins into the growing skeleton (Illies et al., 2002; Livingston et al., 2006). Also at the tip of the growing skeleton the PMCs express a changing mix of signals and transcription factors over time, and at least some of these changes must be utilized for skeletal patterning (Ettensohn, 2009).

4. Gastrulation

4.1 Invagination of the Archenteron: the cell types involved in invagination

Invagination of the archenteron is temporally separated from ingress of the PMCs (Fig 10). The PMCs complete ingress and shortly thereafter the invagination of the archenteron begins. As invagination is ongoing the PMCs form the ring structure that will surround the archenteron. Invagination can be simplified into two basic stages: primary (Fig. 10A), during which the initial inbending of the vegetal plate occurs, and secondary invagination (Fig. 10B), during which the tube of the archenteron elongates (Kominami and Takata, 2004).

Primary invagination is largely the responsibility of the NSM arising from the lower veg2 progeny at 8th cleavage (Fig 1). Secondary invagination involves the endodermal cells of the upper veg2 progeny and the veg1 progeny. Endoderm and NSM cell specification, as in the micromeres, begins early in cleavage (Fig 2). Those gene regulatory networks initially are combined as an endomesoderm GRN and then, when the NSM and endoderm cells segregate at, or shortly after 8th cleavage, the GRN subdivides into an endoderm and mesoderm state in the two cell types. In each subcircuit portions of the endomesoderm GRN are lost and other new transcription factors are gained to produce the two independent GRN states in the two cell types. The NSM progeny later subdivide further into pigment cells, blastocoelar cells, coelomic pouch cells and muscle cells, each with its own GRN state. The endoderm-fated GRNs also subdivide to produce foregut and midgut GRN states from the veg2 progeny, and midgut and the hindgut GRN states in cells that descend from Veg1 progeny. These groups of cells are programmed toward their several fates, and toward their distinct contributions to morphogenesis of the archenteron (Fig. 11).

4.2 Primary invagination

After the PMCs ingress the NSM cells (called SMCs or secondary mesenchyme cells in earlier papers) move in to fill the territory previously occupied by the PMCs at the vegetal pole. The lower portion of the embryo flattens to form a distinct vegetal plate as the NSM and endoderm cells become highly columnar. At the center of that vegetal plate the NSMs initiate primary invagination during which the cells change shape to form an inward bending dome of cells (Fig 10).

The forces underpinning primary invagination must occur autonomously within the vegetal plate since vegetal plates isolated by microsurgery prior to gastrulation initiate invagination

normally (Ettensohn, 1984). Prior to gastrulation NSM cells are localized as a ring of about twenty to thirty cells at the center of the vegetal plate, encircling the cluster of small micromere progeny. Laser ablation of even one quarter of the NSM ring severely delays the onset of gastrulation (Kimberly and Hardin, 1998). Just prior to invagination, the NSM cells acquire a distinct wedge-like appearance and some of these are also referred to as bottle cells (Fig. 10A) (Nakajima and Burke, 1996). This shape change is thought to be induced by an actin-mediated constriction at the apical end of the cell and likely aids in the bending of the vegetal plate.

The idea that an active narrowing of cellular apices would drive a cellular sheet to bend has existed in the field of morphogenesis for over one hundred years (Sawyer et al., 2010). However, apical constriction alone may be insufficient to drive this process in sea urchins since treatment with cytochalasin D, an inhibitor of F-actin assembly, fails to block primary gastrulation (Lane et al., 1993), suggesting that cytoskeletal changes are not a major component of primary invagination. That work suggests that physical mechanisms are the drivers of the inbending (Davidson et al., 1995). The bending bilayer model (Lane et al., 1993), for example, proposes that secretion of a hygroscopic (water-absorbing) material into the extracellular matrix between the apical surface of the epithelium and the hyaline layer, will swell and provide a stiffening force to buckle the vegetal plate. Mechanical testing of the blastula wall demonstrated that the hyaline layer is able to provide adequate stiffness to support this model (Davidson et al., 1999). Thus, the extracellular matrix, unlike the cytoskeleton, is a possible contributor to primary invagination.

Although bottle cell formation is one of the first events described to occur at the onset of primary invagination, their absence does not ultimately impede gastrulation. Serial ablation of bottle cells significantly delays invagination within that region, but embryos inevitably form archenterons (Kimberly and Hardin, 1998). The NSM cell type(s) that produce the bottle cells remains controversial. In the tropical sea urchin, *Echinometra methaei*, the pigment cells were determined to be the bottle cells since this species of urchin produces pigment granules prior to gastrulation enabling them to be traced during the invagination process (Takata and Kominami, 2004). However, this has not yet been confirmed in other species of sea urchin, and is complicated because the pigment cells begin to ingress just as the primary inbending of the archenteron begins, and when mesenchyme cells ingress they display a bottle cell appearance for a short time. Since bottle cells are a temporary phenomenon, lack specific markers and are a subtle, temporary change in a small population of cells, their role in initiation of invagination remains an enigma. Specific markers for each NSM cell type exist, however markers for these molecules have not been adapted for live cell analyses, which is needed to correctly assess the dynamics of bottle cell formation.

Primary invagination at the molecular level, appears to involve non-canonical Wnt signaling via the planar cell polarity (PCP) pathway (Fig. 12)(Croce et al., 2006). This hypothesis is based on the observation that the frizzled receptor, Fz5/8, is expressed in the NSM prior to the onset of gastrulation and is a required for gastrulation and blastopore formation. Experimentally, if Fz5/8 expression is knocked down, primary invagination fails to occur, but that knockdown can be rescued by expression of activated RhoA, a known component of PCP signaling. It is not known how the PCP signaling connects to mechanisms of primary invagination, and, the Wnt ligand involved in this signaling is not known. RhoA, a small, monomeric GTPase, downstream of Frizzled/PCP signaling, appears to be required in the initiation of primary invagination since constitutively active RhoA induces a precocious invagination as early as the prehatched blastula stage and dnRhoA blocks primary invagination (Fig. 12) (Beane et al., 2006). Of interest, constitutively active RhoA produces a single early invagination in the embryo (even at high concentrations) and after a stall in

development, these embryos proceed through gastrulation at the same time as controls. There is no evidence that RhoA contributes to later components of gastrulation.

FGF signaling also plays a role in primary invagination (Rottinger et al., 2008). FgfA morphants exhibit a severe delay in invagination. The archenteron that eventually forms is not tripartite and does not connect with the stomodeum indicating a role in both primary invagination and gut regionalization. Inhibition of FGFR1 (and not FGFR2) represses Fz5/8 expression in the vegetal plate and also delays gastrulation. A role for calcium signaling has also been indicated since treatment with the calcium channel inhibitor verapamil blocks invagination of the vegetal plate and the formation of bottle cells (Nakajima and Burke, 1996).

4.2 Extension of the archenteron

Once primary invagination is complete, the archenteron rests as a shallow, dome-capped lumen, hardly changing in length for a short period of time. As secondary invagination proceeds, the lumen of the archenteron will deepen and change shape becoming more cylindrical with a flat top (Fig 10B). The diameter of the archenteron changes drastically during this time. It is reduced from about thirty cells in circumference to around eight (Ettensohn, 1985). Narrowing of the archenteron occurs simultaneously with elongation in a type of cell movement called convergent extension (CE). Elongation proceeds, not by the addition or formation of new cells, but by the intercalation of existing endodermal cells which slide in between one another lengthening while narrowing the gut tube (Hardin, 1989; Hardin and Cheng, 1986). However, after the archenteron reaches its full length, additional cells from the Veg1 lineage provide a late addition to the archenteron (Fig. 14).

RhoA does not regulate the CE movements of sea urchin gastrulation as one might expect based on the evolutionarily conserved role of RhoA in CE movements (Beane et al., 2006). Beta-catenin is severely decreased in endodermal cells as they rearrange lengthwise (Miller and McClay, 1997). It has been proposed that this loss of beta-catenin may impart a change in associated adherens junctions enabling these cells to loosen the epithelium to enable cells to slide past one another in the epithelial cell sheet. Details and mechanisms underlying the lengthening of the archenteron remain to be discovered.

During the lag in elongation that follows primary invagination, the NSM at the tip of the archenteron begin extension of long, thin filopodia (Fig 13). These filopodia extend and withdraw, sometimes reaching distant sites along the wall of the blastocoel where they attach for 5–10 min before collapsing. As gastrulation continues these filopodial extensions eventually reach the animal pole (Miller et al., 1995). The filopodia scan the inner blastocoel in a continual process of extension and retraction until a target site of attachment is found (Hardin and McClay, 1990). The molecular identity of the target has not yet been identified, but the attachment site appears to cause a change in the filopodial behavior of the NSM cells because if it is unavailable the NSM cells continue to extend filopodia for many hours past the time they normally stop this activity, and that cessation normally occurs coincident with NSM binding to the target. The target is not at the site where the stomodeum will appear but is not far from that location. Once the target site is located, the filopodia pull the archenteron to its final length and align the archenteron for mouth formation. This pulling accounts for about 1/3 of the total extension of the archenteron. Laser ablation of the NSM cells that extend the filopodia arrests gut elongation at 2/3 its final length, confirming that thick filopodia are critical for full-length elongation (Hardin, 1988).

4.3. Archenteron completion and formation of the stomodeum

Archenteron formation proceeds even after secondary invagination is complete (Fig 14). When the invagination first reaches the animal pole target the endoderm cells are highly stretched along the long axis of the archenteron. That stretching is resolved through the involution of additional endodermal cells (mostly from the original Veg1 lineage) at the base of the archenteron (Fig. 14) (Martins et al., 1998; Piston et al., 1998). This late recruitment of cells continues to contribute to the archenteron well into the prism stage. Turgor pressure, or the osmotic pressure generated by the blastocoel, has been implicated as a potential mechanism for this process (Kominami and Takata, 2004), though it has not been ruled out that these cells autonomously move into the archenteron. When gastrulation ends the next phase of gut morphogenesis begins.

Stomodeum formation and connection of the stomodeum to the archenteron occur relatively late, several hours after the gut reaches its full length. The first insights into the sub-circuit that regulates stomodeum formation have recently been elucidated. The ventral/oral ectoderm of the sea urchin is a signaling center that uses two major signals, Nodal and BMP2/4, to establish the ventral-dorsal (oral-aboral) axis (Duboc et al., 2010). A global perturbation approach based on morpholino knockdown data and quantification via qPCR and Nanostring was used to build provisional oral ectoderm GRNs (Su et al., 2009). These papers also include a comprehensive examination of all known oral ectoderm genes and expand the suite of genes involved in oral ectoderm specification. As a consequence there now are a number of candidate transcription factors known to be upstream of stomodeum formation, in the same way transcription factors of the endoderm and NSM GRNs must be upstream of the morphogenetic events of invagination. The transcription factor *Goosecoid*, activated by Nodal, is a key upstream regulator of the known stomodeal genes *foxA* and *brachyury*. *goosecoid* morphants lack mouths and down-regulate the expression of *foxA* and *brachyury*. Because *goosecoid* is a transcriptional repressor, it is probable that its regulation of *foxA* and *brachyury* is through a double repression mechanism whereby *goosecoid* represses an unknown repressor of both genes. Once activated, *foxA* and *brachyury* constitute a positive feed-forward loop. The continual expression of either gene requires the presence of the other.

As the larval stage approaches archenteron subdivides to form a tripartite gut consisting of an esophagus, stomach, and hindgut (intestine) as determined both morphologically and with regionalized expression of endodermal markers (Wessel and McClay, 1985). Although an advanced GRN exists for the early specification of endomesoderm (Peter and Davidson, 2010), later patterning and differentiation events are not well explored. A small, three gene network—*Cdx*, *Lox*, and *Endo16*—has been assembled to begin to examine the patterning of the hindgut (Fig. 11) (Cole et al., 2009). Briefly, *lox* and *endo16* are coexpressed in the hindgut until *Lox* repression restricts *endo16* anteriorly to the midgut. The midgut is then defined by *endo16* and the hindgut by *lox*. Expression of *cdx* then begins in the posterior region of *Lox* expression. *Lox* may have a role in the activation of *cdx*, but once activated, *Cdx* represses *lox* and restricts its expression to the anterior hindgut where it forms a morphological constriction at the midgut-hindgut boundary. The connection between these network states and control of the morphogenetic events of gastrulation is just beginning to be explored but promises to reveal new insights about the mechanisms that join the stomodeum and the archenteron to form the complete gut.

Once the pluteus stage is reached the larva begins to feed, and it grows over the next weeks to months until metamorphosis. Within the larva the rudiment of the adult grows and becomes the adult at metamorphosis. Much less is known about these stages in the life history of the organism.

Mechanistically, in the sea urchin the focus has been on understanding how gastrulation works. As has been seen from the above, much has been learned, but at the same time, though gastrulation in the sea urchin is among the best understood of all embryos, much remains to be discovered about how this remarkable set of movements works and is controlled.

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Figure 1.

Sequence of development of sea urchin embryos. This diagram shows several stages in the development of the embryo up to the pluteus larval stage. Mesomeres, macromeres and micromeres originate at 4th cleavage, which includes an asymmetric cleavage in the vegetal half of the embryo. The micromeres (red) are fated to produce the larval skeleton. The macromeres are fated to produce mesoderm (shades of red), endoderm (yellow), and a small amount of ectoderm (blue). The animal half of the embryo produces ectoderm and neural tissues. The PMCs ingress at the mesenchyme blastula stage, and shortly thereafter the archenteron begins its invagination. As the skeleton grows the embryo changes shape, first to a prism shape and then to the pluteus larval shape.

Figure 2.

Endomesoderm gene regulatory network (GRN). Each node represents a gene with its enhancer region above an arrow to indicate activation. Inputs into the enhancer include arrows to indicate an activating input, or repression, indicated by a bar input. The background of each field indicates the subnetwork operating in the micromeres (pink), NSM (purple), and endoderm (yellow and orange). Time roughly is displayed with the first events occurring at the top of the diagram, and later events occurring toward the bottom. This graphic model is from the Davidson Lab website <http://sugp.caltech.edu/endomes/> and the graphic is updated periodically as new information becomes available.

Figure 3. Micromere GRN. This subnetwork represents specification of the skeletogenic lineage. Following initial specification at the top provided by maternal inputs, a series of transcription factors are activated sequentially. About 1 to 1.5 hrs before ingress the bottom two rows of transcription factors (Tel, Erg, Hex, Tgif, FoxN2/3, Dri, FoxB, FoxO) are activated and will control both ingress and events of PMC differentiation. Some of the genes expressing differentiation proteins are shown at the bottom of the graphic. Modified from the Davidson website.

Figure 4.

Ingression of PMCs. (A) Diagram modified from Katow and Solursh, (1981), based on an ultrastructural analysis of ingression. The yellow cell is the PMC as seen during ingression. (B,C) Ingression as visualized from a time-lapse sequence. The (B) sequence shows fluorescent PMCs as they assume the teardrop shape and ingress into the blastocoel. The same sequence is shown in (C) with added DIC images to show where the PMCs are located during the ingression process. (D) Two images of ingression in which the plasma membranes of the embryo are labeled with expressed cadherin-GFP. Note that the ingressed PMCs have lost the cadherin from their surface. (E) an image of PMCs expressing a PMC marker (green), that have internalized cadherin (red). (F) An enlarged image of an embryo

stained as in E to show the intracellular vesicles containing cadherin (red). (G,H) Two images taken 30 min apart. In (G) an antibody directed against glycosylated MSP-130 is first seen in intracellular vesicles as ingression begins (green). Thirty minutes later MSP-130 is expressed on the cell surface of all the PMCs (green) and is joined by NgCAM (red), a protein that is inserted into the PMC membrane after ingression is completed. (Miller and McClay, 1997a; Peterson and McClay, unpublished images).

Figure 5.

Genes involved in control of ingression. In each case either a green dyed control micromere or a red-dyed morpholino-injected micromere was transferred to the vegetal pole of a control or morpholino-injected embryo. (A,G) A *Snail*-morpholino injected micromere (red) failed to ingress in a control embryo. (B,H) A control green micromere ingressed in an embryo that had been injected with a *Snail* morpholino. (C,I) Micromeres containing *Twist* morpholino (red) fail to ingress in a control (green) embryo. (D,J) Control micromeres ingress in an embryo containing *Twist* morpholino. (E,K) Control PMCs produce the skeleton in an unlabeled host embryo. (F,L) An unlabeled host embryo produces a skeleton but PMCs containing *FoxN2/3* morpholino do not participate in the skeleton production. (Wu et al., 2007; Wu et al., 2008; Rho and McClay, 2011).

Figure 6.

PMCs actively extending thin filopodia. Four frames taken approximately 10 min apart show the dynamics of thin filopodial extension and withdrawal. The four cells to the right in each frame are PMCs. During this time period a Non-skeletogenic cell wanders into the frame. Over time thin filopodia extend and shorten. The filopodia that shorten usually form a kink as they begin to shorten. (From Miller et al., 1995)

Figure 7.

PMC patterning involves filopodial interactions with the ectoderm. (A) an antibody to both PMCs and NSM (at tip of archenteron) shows that both cells extend many thin filopodia. (B) The PMCs at the beginning of skeletogenesis stained with an antibody to MSP-130. The 64 PMCs form a ring around the archenteron and on the two sides a cluster of PMCs harbors the early triradial skeleton. Thin filopodia are abundant at the growing tips of the skeleton. (C) An embryo producing a skeleton. Half the embryo was injected with a dye. (D) A single thin filopodium extends about 60 – 70 μm . (E) An embryo fertilized at the same time as the embryo in (B), but grown for three hours in dilute NiCl_2 sufficient to radialize the embryo. The filopodia produced by the PMCs wrap around the wall of the blastocoel with some up to 150 μm in length. (F) An embryo in which the red half was injected with a truncated cadherin which prevented specification of that half of the embryo. The PMCs migrate but tend to avoid contact with the un-specified, and therefore uninformative half embryo. (Armstrong, et al., 1993; Armstrong and McClay, 1994).

Figure 8.

PMCS use ectoderm for patterning information. On the left half the blue embryo was treated with NiCl₂ and after the treatment PMCs were swapped between blue and red embryos. NiCl₂-treated PMCs produce a normal skeleton if placed in a control red embryo (top skeleton). Control red PMCs produce a radialized skeleton if placed in a NiCl₂-treated embryo, demonstrating that the NiCl₂ effect is on the ectoderm, and the PMCs receive information for patterning from that ectoderm. On the right half the red embryos are *Lytechinus variegatus* and the blue embryos are *Tripneustes esculentus*. The top half shows the skeletons produced in control embryos of each species. On the bottom PMCs are transferred between species, and again the correct skeleton is produced in each case. Of importance, the *T. esculentus* skeleton includes elements not found on *L. variegatus* skeletons, and the ectodermal information necessary for those elements could only be delivered as positional cues. (Harden, et al., 1992; Armstrong and McClay, 1994).

Figure 9.

VEGF and FGF are two ectodermal signals that contribute to PMC patterning. (A) VEGF is produced by the ectoderm just lateral to the two ventrolateral clusters, and in (E) The VEGF receptor is produced by the PMCs that receive the VEGF signal. (B) NiCl_2 -treatment radializes expression of VEGF, and correspondingly in (F) the VEGFR-expressing PMCs do not form ventrolateral clusters as the do in control embryos in (E). (C) Ectoderm produces FGF just opposite the ventrolateral clusters. Cells in (G) express FGF receptor and form ventrolateral clusters immediately beneath the cells expressing the FGF. (D) A control embryo produces a ring of cells and is initiating skeletogenesis (bright triradiate skeleton at bottom of embryo). If FGF is knocked down, as in (H) the PMCs do not form ventrolateral clusters. (I) Control larva and (J) a larva in which VEGF signaling was eliminated. (K) Control larva, and (L) a larva in which FGF signaling was eliminated.

Figure 10.

Sea Urchin Gastrulation. (A) Primary invagination. Micromeres (red) have already ingressed into the blastocoel. At the beginning of primary invagination, cells in the vegetal plate take on a bottle cell shape (orange) helping to buckle the epithelium. (B) Secondary invagination. After the archenteron buckles the gut lengthens. Secondary mesenchyme cells at the tip of the archenteron (orange) extend filopodia and search the inside of the blastocoel. They make contact the roof of the blastocoel and stretch the archenteron to its full length. Adapted from Kominami and Takata 2004

Figure 11.

A subnetwork that helps drive restriction of hindgut differentiation state. Adapted from Cole et al. 2009.

Figure 12.

Frizzled 5/8 and RhoA are necessary for gastrulation in *Lytechinus variegatus*. (A). Comparison of control embryos at late gastrula and prism stages (left, top and bottom, respectively) and embryos injected with mRNA coding for a dominant negative form of Frizzled5/8 (FzTM1, right). Inhibiting Frizzled 5/8 leads to failure to form the gut. (B) RhoA is downstream of Frizzled5/8. If embryos are co-injected with dnFrizzled5/8 (FzTM1) and mRNA for a constitutively active form of RhoA, normal development is rescued in 28% of the cases. (C). RhoA promotes gut formation. If a dominant negative form of RhoA is injected into embryos, no gut forms (even at later stages, data not shown). If a constitutively active form of RhoA is injected a precocious archenteron forms at the hatched blastula stage,

at the appropriate location. (A) and (B) Adapted from Croce et al. 2006; and (C) from Beane et al. 2006).

Figure 13.

Filopodia are not necessary for lengthening the gut up to two-thirds its final length. Ablation of filopodia on NSM cells delays archenteron lengthening. Time-lapse of an ablation experiment at the onset of archenteron elongation. (A) gastrula with archenteron at 1/3 its full length, right before the ablation. A prominent secondary mesenchyme cell is marked by an arrow. (B) immediately following the ablation; remnants of mesenchyme cells can still be seen (arrow). (C) 2h and 17min after ablation. Significant elongation has occurred. There is a normal aggregation of primary mesenchyme cells into ventrolateral clusters (large arrow). Remnants of severed filopodia ablated after the first series of laser pulses are still visible (small arrows). Adapted from Hardin 1988.

Figure 14.

Veg 1 cells are added to the archenteron late in gastrulation. A lineage tracer labels progeny of a single Veg 1 cell (the upper daughter of the macromere division at sixth cleavage). Those Veg 1 cells reach the blastopore in panel (C) when the gut is just beginning to elongate. In (D) the gut has almost completely elongated yet the Veg 1 cells are still near the blastopore. In (E) after the gut reaches the animal pole, the Veg 1 cells enter the archenteron some of the progeny reach as far as the midgut of the archenteron.