Mechanisms of Development 162 (2020) 103599

Contents lists available at ScienceDirect

Mechanisms of Development

journal homepage: www.elsevier.com/locate/mod

The gene regulatory control of sea urchin gastrulation

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ARTICLE INFO

Keywords:
Sea urchin
Echinoderm
Gastrulation
Gene regulatory network

ABSTRACT

The cell behaviors associated with gastrulation in sea urchins have been well described. More recently, considerable progress has been made in elucidating gene regulatory networks (GRNs) that underlie the specification of early embryonic territories in this experimental model. This review integrates information from these two avenues of work. I discuss the principal cell movements that take place during sea urchin gastrulation, with an emphasis on molecular effectors of the movements, and summarize our current understanding of the gene regulatory circuitry upstream of those effectors. A case is made that GRN biology can provide a causal explanation of gastrulation, although additional analysis is needed at several levels of biological organization in order to provide a deeper understanding of this complex morphogenetic process.

1. Introduction

The hallmark of gastrulation is coordinated cell movement. Like other morphogenetic processes, the cell movements of gastrulation can be analyzed at various levels of biological organization (Fig. 1). A prerequisite for understanding this process is a basic description of the cell and tissue movements that take place- which cells move and where do they move? With a description of the pattern of movements in hand, their mechanisms can then be analyzed in terms of 1) the mechanical forces that deform tissues, 2) the specific cell behaviors (e.g., changes in cell shape or motility) that generate mechanical force, 3) the molecules that drive the relevant cell behaviors, and/or 4) the genetically encoded programs that control the expression and activity of those molecules. A comprehensive understanding of gastrulation demands an integration of information across several levels of biological organization.

For decades, sea urchins have been a very valuable experimental model for the analysis of gastrulation. Historically, this was due to the ease with which early developmental processes could be observed in sea urchin embryos (which develop externally and are optically transparent) and to their apparently simple gastrulation movements. More recently, sea urchins have been a prominent model for systems level studies of gene regulatory mechanisms that underlie early embryogenesis, including the analysis of developmental gene regulatory networks (GRNs) (Peter and Davidson, 2015). GRNs are dynamic gene control systems that consist of transcription factors (TFs) (the products of regulatory genes) and the cis-regulatory elements to which those TFs bind. GRNs are typically represented as circuit diagrams that indicate the functional relationships among regulatory genes and the inputs of these genes into downstream effectors that mediate the cell type-specific properties or behaviors of the cells in which the network is operating. Cell type diversification during development can be explained as the appearance of distinct cell regulatory states (defined as the constellation of active TFs present in a cell at any given time), which arise through the progressive deployment of distinct GRNs in different lineages or territories of the embryo. GRNs have now been constructed for many territories of the early sea urchin embryo, although these vary in their level of detail (Su, 2009; Ben-Tabou de-Leon and Davidson, 2009; Peter and Davidson, 2015; Arnone et al., 2016; Martik et al., 2016; Shashikant et al., 2018).

The final readout of development is anatomy. Therefore, regulatory networks have much greater explanatory power if they can be linked to effector genes that control specific cellular behaviors that drive morphogenesis (Lyons et al., 2012; Ettensohn, 2013; Shashikant et al., 2018; Smith et al., 2018). The cell-level properties that regulate cell and tissue movements during gastrulation (e.g., cell motility, adhesion, shape, etc.) are controlled by transcriptional networks, as are other aspects of cell identity. This review discusses the major cell movements that take place during sea urchin gastrulation with an emphasis on the gene regulatory basis of those movements, as viewed through the lens of GRNs. More comprehensive reviews of various aspects of sea urchin gastrulation are available (Hardin, 1996; Kominami and Takata, 2004; McClay, 2004).

2. General features of sea urchin gastrulation

Gastrulation in echinoderms is representative of the process in deuterostomes more generally, but in a relatively (and deceptively) simple form that lacks certain evolutionary modifications characteristic

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https://doi.org/10.1016/j.mod.2020.103599
Received 2 December 2019; Received in revised form 21 February 2020; Accepted 24 February 2020
Available online 28 February 2020

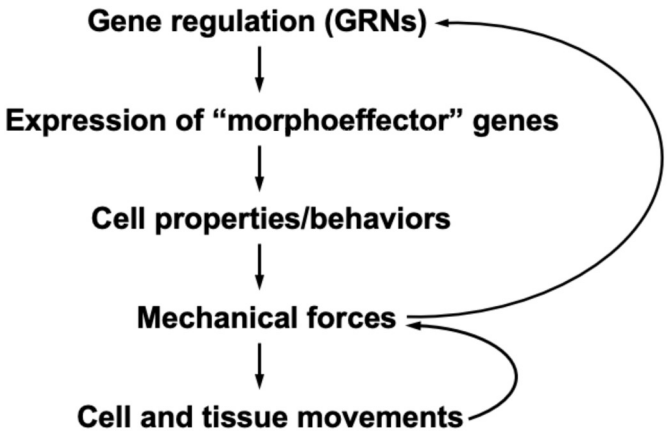

Fig. 1. The flow of biological information underlying morphogenetic processes, from gene regulatory mechanisms (which can be represented as GRNs) to overt cell and tissue movements. Feedback interactions also come into play, as cell and tissue deformations can produce mechanical stresses on neighboring cells and mechanical forces can modulate gene expression.

of vertebrates. The eggs of most echinoderms are small (100–200 μm in diameter) and have relatively little yolk. The zygote undergoes holoblastic cleavage, producing a spherical blastula with a monolayered epithelial wall. The embryo gastrulates by the internalization of prospective mesoderm and endoderm cells through a blastopore that subsequently forms the anus, while the mouth arises as a secondary opening later in development. The mechanisms of gastrulation have been studied most intensively in euechinoids, a group that includes most modern sea urchins and most species commonly used for research purposes. Comparisons with other echinoderms are extremely informative, however, as they reveal both evolutionary novelties and conserved features of gastrulation within the phylum.

3. Morphogenesis of the primary mesenchyme

3.1. Overview

The onset of gastrulation in euechinoids is marked by the ingression of skeleton-forming cells known as primary mesenchyme cells (PMCs) (Fig. 2). PMCs are the progeny of the micromeres, four small blastomeres that arise at the vegetal pole of the 16-cell embryo through unequal cell division. Each of the four micromeres undergoes another unequal division at the fifth cleavage, producing one small daughter cell (small micromere) and one large daughter cell (large micromere, or LM). The four LMs are the founder cells of the PMC lineage. As the LMs divide further, their descendants become incorporated into the epithelial wall of the blastula in a torus-shaped region that surrounds the vegetal pole. At the onset of gastrulation, all LM descendants (now referred to as PMCs) migrate out of the wall of the embryo and enter the blastocoel in a striking example of ingression, a form of epithelial-mesenchymal transition (EMT). PMC EMT is accompanied by the onset of cell motility, first evident as vigorous pulsatory activity at the basal surfaces of the cells, and by changes in their adhesive properties, including a loss of adhesion to neighboring cells and the outer hyaline layer and a concomitant increase in adhesion to the basal lamina that lines the blastocoel (Amemiya, 1989; Fink and McClay, 1985; Gustafson and Wolpert, 1967). The adhesive properties of PMCs are modified by a burst of endocytosis that remodels the PMC surface and internalizes cell surface proteins, including G-cadherin, at the time of ingression (Miller and McClay, 1997; Wu et al., 2007; Wakayama et al., 2013).

After ingression, PMCs migrate away from the vegetal plate, moving along the blastocoel wall. During this early phase of their migration, most PMCs cells remain in the vegetal hemisphere and within the quadrant of the embryo from which they originally ingressed (Gustafson and Wolpert, 1967; Peterson and McClay, 2003). They move by means of filopodia, the dynamic behavior of which has been analyzed quantitatively in vivo (Malinda et al., 1995; Miller et al., 1995). PMC filopodia contact the thin basal lamina that covers the blastocoel wall and interact selectively with fibers that contain ECM3, the sea urchin ortholog of the vertebrate Frem2 protein (Hodor et al., 2000). As the PMCs migrate, their filopodia also contact neighboring PMCs. These contacts result in filopodial fusion and the formation a cable-like structure (the pseudopodial, or cytoplasmic, cable) that links the PMCs in a single, syncytial network (Okazaki, 1965; Hodor and Ettensohn, 1998). PMC fusion is mediated by a PMC-specific adhesion protein, KirrelL, which is required for filopodial contacts to induce membrane fusion (Ettensohn and Dey, 2017).

As PMCs migrate and fuse, they gradually arrange themselves in a characteristic, ring-like pattern along the blastocoel wall (the subequatorial PMC ring). Clusters of PMCs form at two sites along the ventrolateral aspects of the subequatorial ring. The formation of the embryonic skeleton begins at the mid-late gastrula stage, when one triradiate skeletal rudiment appears in each of the two ventrolateral PMC clusters. The formation of the PMC ring and the two skeletal rudiments is directed by ectoderm-derived cues that arise in a progressive fashion during gastrulation. The patterning of the ectoderm is beyond the scope of this review, but much is known about the signaling pathways that gradually compartmentalize the ectoderm during early embryogenesis (McClay, 2000; Angerer and Angerer, 2003; Su, 2009; McIntyre et al., 2014; Range, 2014). These processes convert the ectoderm into a mosaic of territories that secrete different signals. One critically important signal is VEGF3, which acts as a PMC guidance cue during early gastrulation and also regulates the expression of many biomineralization genes at skeletal growth zones on the ventral (oral) side of the embryo (Duloquin et al., 2007; Adomako-Ankomah and Ettensohn, 2013; Adomako-Ankomah and Ettensohn, 2014). At the late gastrula stage, after cell-cell fusion is complete, PMCs in the two ventrolateral clusters extend numerous filopodia toward the animal pole, and a syncytial strand of PMCs migrates from each cluster toward the pole. The guidance cues that direct this second phase of PMC migration have not been elucidated, but VEGF3 may play a role here as well (Adomako-Ankomah and Ettensohn, 2013).

The arms of the tri-radiate rudiments initially extend along the crystallographic "a-axis" of the growing biomineral. The directionality of this early growth is likely regulated by the local concentration of VEGF3 (Knapp et al., 2012) and the number of radii is controlled in part by IgTM, a PMC-specific, Ig domain-superfamily protein (Ettensohn and Dey, 2017). The three radii of each skeletal rudiment subsequently elongate and branch in a stereotypical pattern to produce the bilaterally symmetrical embryonic endoskeleton, each rudiment producing a half-skeleton that is the mirror image of its partner. The skeletal rods grow within a membrane-bound compartment inside the pseudopodial cables of the PMC syncytium through the secretion of amorphous calcium carbonate and associated proteins. These molecules are added to the surface of the existing biomineral, thereby causing the skeletal rods to grow in both length and girth (Wilt and Ettensohn, 2007).

3.2. The gene regulatory control of PMC morphogenesis

In sea urchins, zygotic transcription begins immediately after fertilization and the embryo has been compartmentalized into distinct territories of gene expression well before gastrulation begins. One of the earliest GRNs to be activated is the LM-PMC network. Our current understanding of the architecture of this GRN has been described in detail elsewhere and only an overview will be presented here (Oliveri et al., 2008; Shashikant et al., 2018; Khor et al., 2019). In brief, there are two major phases in the progressive deployment of the network: an early, cell-autonomous phase, which operates during cleavage and blastula stages, and a later, signal-dependent phase, which becomes dominant during gastrulation. The cell-autonomous activation of the

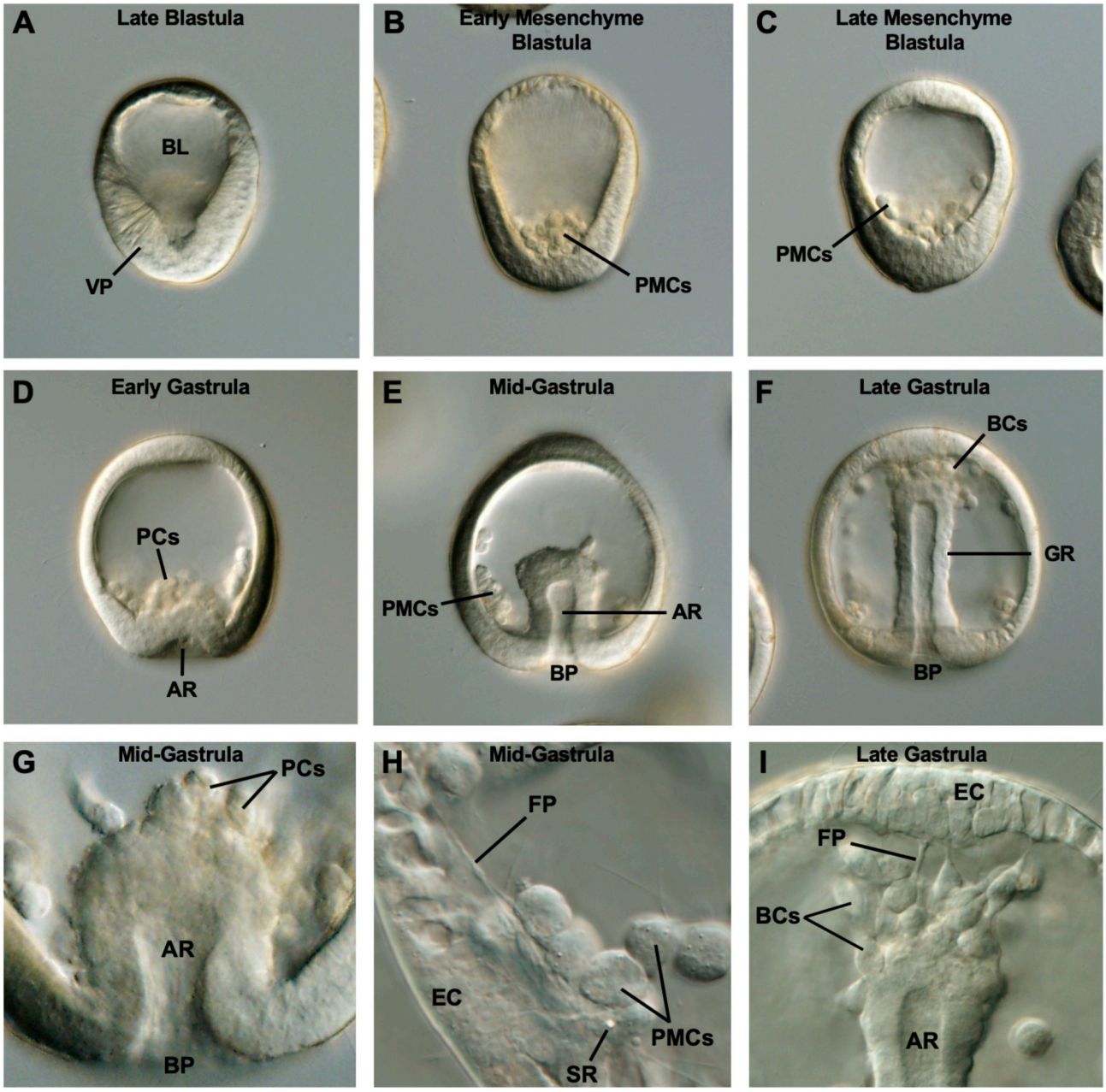

Fig. 2. Gastrulation in a model euechinoid sea urchin (*Lytechinus variegatus*). Gastrulation begins with PMC ingression and ends when the archenteron (AR) is fully extended. In this warm water species (23 °C), the entire process takes 8 h. All images are of living embryos viewed with differential interference contrast optics. (A–F) Low magnification views showing various stages of gastrulation. (G–I) High magnification views showing ingressed pigment cells (PCs) at the mid-gastrula stage (G); primary mesenchyme cell (PMC) filopodia (FP) interacting with the basal surface of the ectoderm (EC), also at the mid-gastrula stage, and a very small, birefringent skeletal rudiment (SR) within the PMC pseudopodial cable (H); and the tip of the gut rudiment during secondary invagination, showing ingressing blastocoelar cells (BCs) and filopodia extended by cells at the tip of archenteron interacting with ectoderm near the animal pole (I). AR- archenteron, BCs- blastocoelar cells, BL- blastocoel, BP- blastopore, EC- ectoderm, FP- filopodium, GR- gut rudiment, PCs- pigment cells, PMCs- primary mesenchyme cells, SR- skeletal rudiment, VP- vegetal plate.

network in the LM lineage is dependent on factors that are asymmetrically localized in the oocyte and partitioned to the micromere lineage (Logan et al., 1999; Weitzel et al., 2004; Peng and Wikramanayake, 2013). These maternal inputs lead to the expression of lineage-specific regulatory genes, including *pmar1/micro1*, *alx1* and *ets1* (Kurokawa et al., 1999; Oliveri et al., 2002; Nishimura et al., 2004; Ettensohn et al., 2003), which engage several downstream regulatory genes and ultimately activate a suite of several hundred effector genes that are expressed selectively by PMCs, many of which mediate biomineralization (Barsi et al., 2014; Rafiq et al., 2014) (Fig. 3).

The maternally entrained program is sufficient to drive PMC motility, cell fusion, and the expression of many biomineralization-related genes. The cell-autonomous nature of this program is revealed by the behavior of isolated micromeres cultured in seawater with no supplements. Under these conditions, micromere progeny activate their program of filopodial motility and undergo cell fusion, but do not deposit biomineral (Pucci-Minafra et al., 1968; Hagström and Lönning, 1969; Okazaki, 1975; Sano, 1977; Hodor and Ettensohn, 1998). Extensive skeletogenesis occurs in vitro, however, if the cells are exposed to serum at the equivalent of the mid-gastrula stage, when they ordinarily begin to secrete the skeleton (Okazaki, 1975; McCarthy and Spiegel, 1983; Knapp et al., 2012). These findings indicate that extrinsic

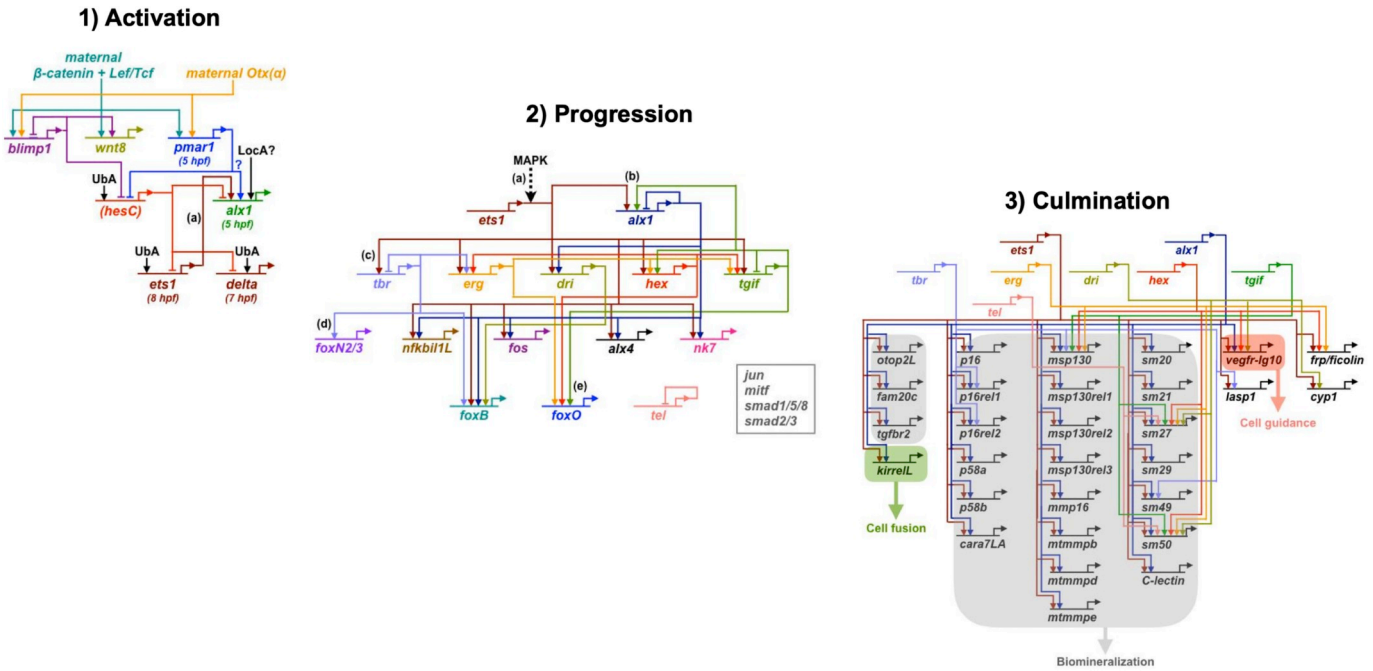

Fig. 3. A simplified view of the early (pre-gastrula) deployment of the PMC GRN, including maternal inputs that activate key regulatory genes (including *alx1* and *ets1*) selectively in the large micromere lineage ("Activation"), the engagement of downstream regulatory genes ("Progression"), and the deployment of effector genes that mediate directional cell migration, cell-cell fusion, and biomineralization ("Culmination"). For additional details see Oliveri et al., (2008); Shashikant et al., (2019), and Khor et al. (2019).

cues, which include VEGF3 and probably other signals, are required to promote biomineralization. Notably, one crucial outcome of the early phase of GRN deployment is the activation of genes that encode cell surface receptors, including VEGF, FGF, and TGF-β receptors, that render PMCs competent to respond to ectoderm-derived signals later in development (Page and Benson, 1992; Duloquin et al., 2007; Röttinger et al., 2008; Sun and Ettensohn, 2017).

PMC ingression, migration and fusion are under the control of several regulatory genes in the PMC GRN. Ets1 plays a key role, through a maternally-driven activation of the MAPK cascade and direct phosphorylation of Ets1 by ERK (Röttinger et al., 2004). In addition to Ets1, a surprisingly large number of lineage-enriched TFs have been implicated in PMC ingression. PMCs fail to ingress in Alx1 morphants, although some LM descendants (which are re-specified in the absence of *alx1* function) ingress later in gastrulation by Alx1-independent mechanisms (Ettensohn et al., 2007). A detailed analysis of the regulatory control of ingression in *Lytechinus variegatus* has implicated 15 different TFs in various sub-circuits that contribute to basal lamina disruption, cell shape change, motility, and/or de-adhesion (Saunders and McClay, 2014). In this species, *alx1* appears to regulate EMT through its positive regulation of *snail* and *twist*, possibly through the transcriptional and post-transcriptional down-regulation of cadherin (Wu and McClay, 2007; Wu et al., 2008). Notably, no other downstream effectors of ingression have been identified, including molecules that might activate PMC motility or filopodial activity. As noted above, after ingression the directionality of PMC migration is controlled by VEGF3 through its cognate receptor, VEGFR-10-Ig. An attractive (but unproven) hypothesis is that VEGF3 acts as a substrate-bound chemoattractant. Several positive regulatory inputs into *vegfr-10-Ig* have been established, including inputs from *alx1*, *ets1*, *hex*, and *dri*; those from *alx1* are direct (Oliveri et al., 2008; Rafiq et al., 2014; Khor et al., 2019). The regulatory control of *vegfr-10-Ig* and many other effector genes, including the cell fusion effector, *kirrelL*, involves coherent feedforward regulation by *ets1* and *alx1* (Oliveri et al., 2008; Rafiq et al., 2014; Khor et al., 2019).

The second, signal-dependent phase of GRN regulation is initiated soon after the PMCs begin to migrate, when they first come in close proximity with overlying ectodermal cells and are influenced by secreted signals. The shift to a signal-dependent mode is manifested by a striking change in spatial patterns of gene expression from an early pattern in which effector genes are expressed uniformly by all PMCs to a late pattern in which expression is restricted to localized domains within the PMC syncytium. These domains are invariably associated with sites of active skeletal growth (initially the ventrolateral PMC clusters, where the skeletal rudiments arise, and later the scheitel and tips of the arm rods). Local ectoderm-derived cues are required to maintain the expression of effector genes at sites of skeletal growth, whereas expression declines elsewhere. Although the PMCs become joined in a syncytium, mRNAs and proteins have limited mobility within the network and therefore non-uniform patterns of gene expression are maintained. As noted above, VEGF3 is an essential signal on the ventral side of the embyo, while a second, as yet unidentified, signal maintains the expression of effector genes in the scheitel region on the dorsal (aboral) side. Currently, the specific mechanisms by which these signals impinge on the PMC GRN are unknown.

4. Gene regulatory mechanisms that underlie other aspects of sea urchin gastrulation

At present, primary mesenchyme morphogenesis is the gastrulation movement for which we have the most robust linkages between gene regulatory mechanisms and cell behaviors. The mechanisms of other gastrulation movements, most notably the key molecular effectors, remain poorly understood; consequently, there is a major conceptual gap between the early specification processes that establish cell identities and the cell movements associated with gastrulation. Nevertheless, there are prospects for closing some of those gaps and developing a more complete explanation of the genetic control of gastrulation in sea urchins.

4.1. Invagination of the gut rudiment

4.1.1. Overview of invagination in sea urchins and other echinoderms

The wall of the echinoderm blastula is a monolayered epithelial sheet. The vegetal plate, a thickened region of the blastula wall at the vegetal pole, appears at this stage (Fig. 2). In some echinoderms (e.g., euechinoids) the cells of the vegetal plate are highly elongated, making this region very conspicuous, while in others (e.g., cidaroids and sea stars) it is more difficult to discern. Invagination of the vegetal plate is a universal feature of echinoderm gastrulation. This process creates a circular blastopore (the future anus) and the gut rudiment, which is initially dome-shaped and composed entirely of prospective mesoderm. The lumen of the gut rudiment (the archenteron) is continuous with the external environment via the blastopore. The archenteron elongates dramatically during gastrulation, and cells from the surrounding prospective endoderm are gradually recruited into the invaginating region. In most echinoderms, the archenteron extends more or less through the center of the blastocoel, but in some species (e.g., *S. purpuratus*) it is positioned closer to the ventral wall of the blastocoel. Eventually, the tip of the gut rudiment makes contact with the ectoderm at a site where the mouth will form. In echinoderms with a relatively small blastocoel (including euechinoids) the gut rudiment extends completely across the blastocoel and the mouth forms very near the animal pole, while in species with a large blastocoel (e.g., cidaroids and asteroids) the archenteron extends only about halfway across the blastocoel and the mouth forms near the equator. The final phase of the extension of the gut rudiment involves the contractile activity of filopodia extended by cells at the tip of the archenteron (Dan and Okazaki, 1956; Gustafson and Kinnander, 1956; Hardin, 1988; Hardin and McClay, 1990). Involution (i.e., recruitment of cells that surround the blastopore into the invaginating region) occurs during sea urchin gastrulation (Ettensohn, 1984; Burke et al., 1991; Logan and McClay, 1997; Piston et al., 1998; Ettensohn, 1999) but is much less extensive than the involution movements associated with vertebrate gastrulation. Considerable involution, including all of the prospective hindgut endoderm, also occurs after the conclusion of what is typically considered gastrulation (reviewed by Kominami and Takata, 2004).

Traditionally, invagination of the gut has been divided into “primary” and “secondary” invagination, the former referring to the initial phase of gut rudiment extension and the latter to the stage of elongation mediated by filopodial contraction. This distinction was initially made based partly on the finding that there is a sharp increase in the rate of gut rudiment elongation during the final phase of invagination in some sea urchin species (Gustafson and Kinnander, 1956). It has since been shown, however, that in some sea urchins the archenteron elongates at a relatively constant rate (Kominami and Masui, 1996; Takata and Kominami, 2004). The terms “primary” and “secondary” invagination still have some utility in distinguishing the end-phase of archenteron extension in most euechinoids, mediated by filopodial contraction, from mechanisms that operate earlier in gastrulation.

4.1.2. Cellular and molecular mechanisms of invagination

The mechanisms that drive the initial invagination of the vegetal plate remain enigmatic. The relevant mechanical forces are generated locally, as isolated vegetal plates have the capacity to invaginate (Moore and Burt, 1939; Ettensohn, 1984). These local mechanical forces are sufficient to overcome a positive hydrostatic pressure in the blastocoel, which resists the inpocketing. Invagination occurs in the presence of drugs that depolymerize microtubules or inhibit DNA synthesis, demonstrating that cell division is not required (Ettensohn, 1984; Stephens et al., 1986). PMC ingression, which closely precedes invagination in euechinoids, is also not essential for the inpocketing of the vegetal plate, which occurs even when PMC specification and ingression are inhibited (Langelan and Whiteley, 1985; Kurokawa et al., 1999; Ettensohn et al., 2003). In some echinoderms (e.g., cidaroid sea urchins and sea stars), invagination begins several hours before any mesodermal cells ingress from the vegetal plate, providing further evidence that ingression and invagination are not coupled.

It was originally proposed that invagination is initiated by the rounding of cells in the vegetal plate (Gustafson and Wolpert, 1967), but quantitative analysis of cell shape changes indicates this is unlikely to be the mechanism (Ettensohn, 1984). Subsequently, three hypotheses have been advanced to explain the inpocketing of the vegetal plate: 1) tractoring of the apical surfaces of cells along the hyaline layer (Burke et al., 1991), localized secretion of ECM (Lane et al., 1993), and apical constriction (Nakajima and Burke, 1996). Each proposed mechanism places different constraints on the mechanical properties of the epithelial cell layer and ECM (Davidson et al., 1995). The apical constriction hypothesis is widely accepted in textbooks, partly because bottle-shaped cells appear in the archenteron wall concomitant with early invagination and appear to assist in the initial inpocketing of the vegetal plate (Nakajima and Burke, 1996; Kimberly and Hardin, 1998). These cells are almost certainly prospective pigment cells, (Takata and Kominami, 2004), which in many euechinoids undergo ingression early in gastrulation (see below). Other echinoderms, however, invaginate in the absence of pigment cells or early-ingressing mesenchyme cells of any type. Therefore, bottle cells may be an unusual feature of invagination in euechinoids and not a characteristic of gastrulation in echinoderms more generally.

Polarized, local cell rearrangements occur within the wall of the archenteron as the invagination deepens. These cell rearrangements have the effect of simultaneously lengthening and narrowing the gut rudiment as invagination proceeds (Ettensohn, 1985; Hardin, 1989; Martik and McClay, 2017). Precisely when these rearrangements begin has not been established, but they accompany much of the elongation of the gut rudiment. They are not simply a passive response to filopodial tension, as evidenced by the ability of the gut rudiment to elongate extensively in exogastulae, although some passive cell rearrangement may occur normally late in gastrulation (Hardin and Weliky, 2019). In other organisms, similar oriented cell rearrangements are associated with polarized cell protrusive activity and signaling through the planar cell polarity (PCP) pathway (Huebner and Wallingford, 2018). Whether these processes contribute to the active epithelial cell rearrangements that occur during sea urchin gastrulation is not yet known. Three components of the PCP pathway, Frizzled 5/8 (Croce et al., 2006), RhoA (Beane et al., 2006), and Jun kinase (Long et al., 2015), have been implicated in invagination in sea urchins, particularly in the initial inpocketing of the vegetal plate. The primary function of these molecules may be to regulate cell rearrangements, if these begin early in gastrulation.

The role of filopodial contraction in gastrulation in euechinoid sea urchins is well-established. This mechanism plays a less prominent role, however, in the elongation of the gut rudiment in other echinoderms. In *Eucidaris tribuloides*, a cidaroid sea urchin, the gut rudiment extends only about halfway across an expansive blastocoel and filopodia are never observed directed toward the animal pole (Hardin, 1989). In this species, and probably also in sea stars, which gastrulate in a similar manner, filopodia have little or no role in the extension of the archenteron along the AV axis. They likely pull the tip of the archenteron laterally and ventrally toward the future mouth opening, however, and this could be considered the equivalent of secondary invagination in these animals.

Surprisingly, in euechinoids, the identity of the cells at the tip of the archenteron that engage in filopodial contraction remains unclear. It seems likely that many, perhaps all, of these cells are prospective blastocoelar cells, which normally undergo ingression late in gastrulation. The small micromere descendants (prospective germ cells), however, are also located at the tip of the gut rudiment and extend filopodia late in gastrulation (Campanale et al., 2014). These cells are surrounded by future coelomic pouch cells (Materna et al., 2013b), which are also positioned appropriately to participate in gastrulation, although it is not known whether they extend filopodia.

4.1.3. The gene regulatory control of invagination

The gene regulatory processes that specify the endoderm and mesoderm have been studied extensively in euechinoid sea urchins, and a detailed, GRN-based model of the specification of these territories during pre-gastrula development has been developed (for a recent and comprehensive review, see Peter and Davidson, 2015). Several different signaling pathways, including the Notch and Wnt pathways, play important roles in the specification of presumptive endomesoderm and in its subsequent compartmentalization into mesodermal and endodermal domains (Sherwood and McClay, 1997; Sherwood and McClay, 1999; Sweet et al., 1999; McClay et al., 2000; Sweet et al., 2002; Croce et al., 2006; Röttinger et al., 2006; Range et al., 2008: Croce and McClay, 2010; Croce et al., 2011; Sethi et al., 2012; Materna and Davidson, 2012; Cui et al., 2014). In addition, Nodal signaling, acting orthogonal to the animal-vegetal (AV) axis of the egg, further subdivides the non-skeletogenic mesoderm and endoderm along the dorsal-ventral (DV) axis (Duboc et al., 2010; Materna et al., 2013a). As a consequence of these early embryonic patterning events, the vegetal plate, which contains the cells that will execute gastrulation movements, is compartmentalized into at least five distinct domains at the onset of invagination (Fig. 4). The regionalization of the vegetal plate at the start of gastrulation is similar, but somewhat simpler in sea stars, which lack pigment cells (McCauley et al., 2010).

It is worth noting that the compartmentalization of the vegetal plate shown in Fig. 4 represents a snapshot in time. Before and during gastrulation, the regulatory states of cells in the vegetal region are highly dynamic. Prior to gastrulation, several endomesodermal regulatory genes are initially expressed in relatively broad domains that progressively sharpen, and many exhibit an "expanding torus" of expression that moves outward from the vegetal pole prior to gastrulation. Some further regionalization of the mesoderm and endoderm along both the AV and DV axes occurs during gastrulation, although a more extensive compartmentalization of the gut occurs later in embryogenesis (Tu et al., 2006; Materna et al., 2013a; Annunziata et al., 2014; Annunziata et al., 2019). This dynamic pattern of cell regulatory states complicates the picture, as any of the transient regulatory states observed in the vegetal plate and gut rudiment before or during gastrulation might drive cell behaviors that are important for gastrulation. It seems clear that the onset of filopodial motility by cells at the archenteron tip requires molecular changes in these cells after gastrulation begins, but this change in behavior could be controlled at the post-transcriptional level. Without clear evidence to the contrary, it is possible that the principal gene regulatory processes that drive gastrulation have been completed prior to the onset of invagination.

Because the force-generating mechanisms and immediate molecular effectors of invagination remain mostly unknown, it is not currently possible to establish direct linkages between this morphogenetic process and the GRNs that control endodermal and mesodermal specification. There are, however, candidates for regulatory genes that might play relatively direct roles in invagination. *Brachyury* (*bra*), a T-box gene that has been implicated in gastrulation movements in other organisms (Gentsch et al., 2017), becomes progressively restricted in its expression to the posterior, veg1-derived endoderm where it is expressed transiently by cells as they move over the blastopore lip (Croce et al., 2001; Gross and McClay, 2001; Rast et al., 2002; Peter and Davidson, 2011). Later, *bra* is expressed in the prospective stomodeum,

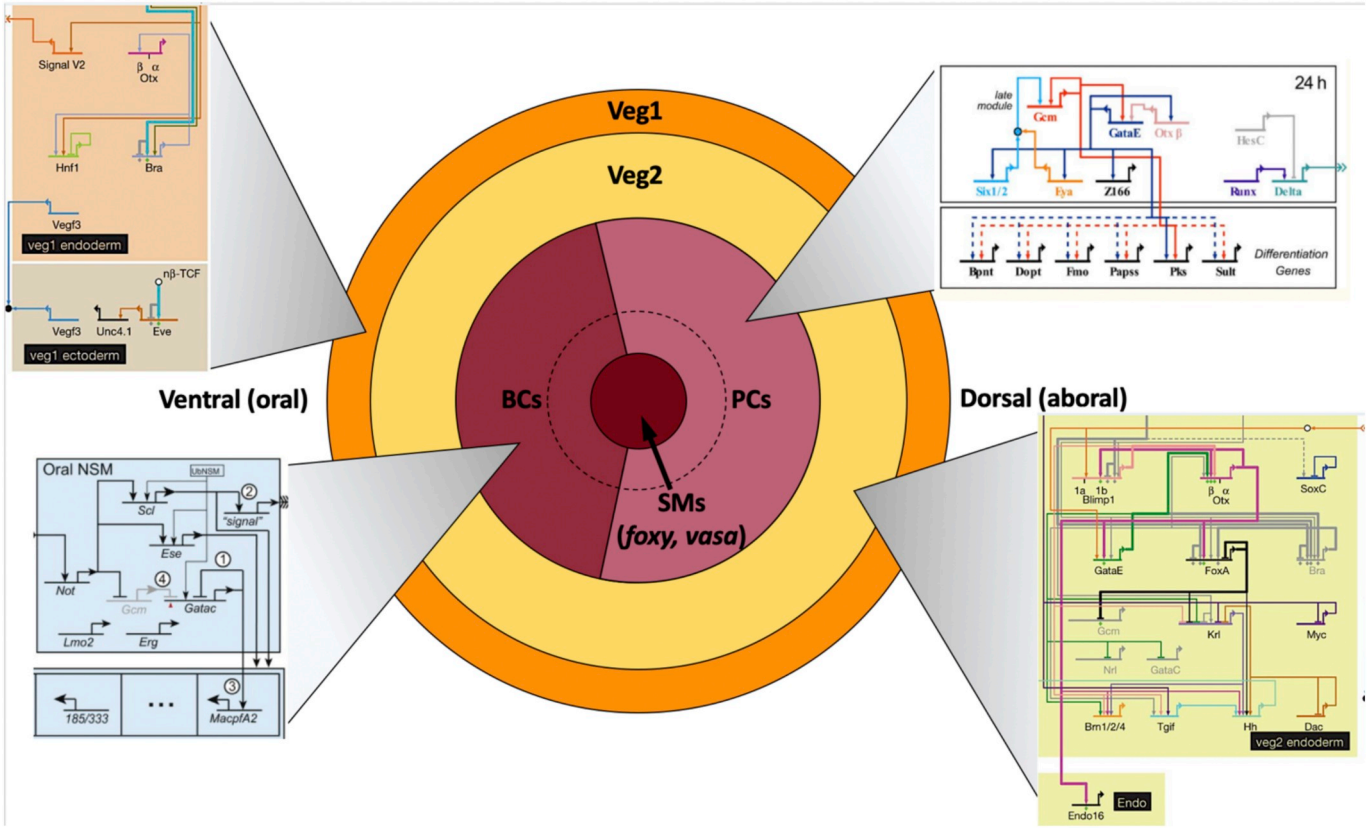

Fig. 4. Regulatory domains within the vegetal plate at the onset of invagination (*Strongylocentrotus purpuratus*, 24 h post-fertilization). By this stage, PMCs have ingressed from the vegetal plate, which is now composed of prospective non-skeletogenic mesoderm (shown in pink and red) and prospective endoderm (shown in yellow and orange). Portions of GRNs deployed in the prospective pigment cells (PCs), prospective blastocoelar cells (BCs), Veg1-derived endoderm, and Veg2-derived endoderm, are shown. The small micromere (SM) territory, which consists of prospective germ cells, is marked by Vasa and *foxy* expression (Voronina et al., 2008; Materna et al., 2013b). The dotted line surrounding the SM territory indicates the approximate location of prospective coelomic pouch cells, which are specified via an expansion of *foxy* expression after the onset of gastrulation (Materna et al., 2013b). GRN models are adapted from Peter and Davidson (2011), Materna et al. (2013a), and Solek et al. (2013) and additional details can be found in those references.

another embryonic territory that undergoes invagination. Perturbation of *bra* function using a dominant negative construct (Gross and McClay, 2001) or an antisense morphlino (Rast et al., 2002) interferes with invagination of the vegetal plate, although the effects are somewhat variable and concentration-dependent. Whether the entire process is affected or only later stages of invagination, and to what extent the phenotype can be attributed to a delay in gastrulation, are questions that have not been fully addressed. Several effector genes that are targets of *bra* have been identified using a subtractive hybridization strategy (Rast et al., 2002), but this is an area that would benefit from further analysis using current methodologies.

Like *bra*, *foxA* is initially expressed dynamically in the vegetal plate and becomes progressively restricted to the endoderm, but to a different compartment- the anterior, or veg2-derived endoderm (Oliveri et al., 2006; Ben-Tabou de-Leon and Davidson, 2010; Peter and Davidson, 2011). Intriguingly, also like *bra*, *foxa* is expressed in the stomodeal invagination later in development. Morpholino-based knockdown of *foxa* results in a dose-dependent block or delay in gut invagination and an inhibition of mouth formation Oliveri et al., 2006). The segregation of cell fates in the vegetal plate is perturbed, which could contribute to the effects of *foxa* knockdown on vegetal plate morphogenesis. Genes related to *foxa* have been implicated in cell movements in various metazoans (reviewed by Ben-Tabou de-Leon, 2011), but in sea urchins the specific cell behaviors and key molecular effectors regulated by *foxa* have not been elucidated.

4.2. Movements of non-skeletogenic mesoderm

In euechinoid sea urchins, two populations of non-skeletogenic, migratory cells ingress into the blastocoel during gastrulation; pigment cells and blastocoelar cells. Both cell types are derived from the veg2 blastomeres of the early embryo and both rely on Notch signaling for their specification (Sherwood and McClay, 1999; Sweet et al., 1999; McClay et al., 2000; Sweet et al., 2002). The progenitors of these two cell types have acquired distinct regulatory states by the onset of gastrulation and occupy two distinct compartments of the vegetal plate; prospective pigment cells and blastocoelar cells are located in the dorsal (aboral) and ventral (oral) regions of the vegetal plate, respectively (Ruffins and Ettensohn, 1996).

Pigment cells are the first non-skeletogenic mesenchyme cells to ingress (Fig. 2). Typically, ingression takes place soon after invagination begins, although there is some variability in timing among species Gibson and Burke, 1985; Takata and Kominami, 2004). After ingression, pigment cells re-insert themselves selectively into the dorsal ectoderm in response to cues from ectoderm-derived ephrin (Krupke et al., 2016). At post-gastrula stages, pigment cells become concentrated primarily at the apex of the body and along the ciliary band. Blastocoelar cells ingress later in gastrulation and give rise to cells that have been described as fibroblastic or immunocyte-like. They extend filopodia which join the cells in an extensive network concentrated around the gut and skeletal rods (Tamboline and Burke, 1992; Katow et al., 2004). At least some blastocoelar cells fuse (Hodor and Ettensohn, 1998) resulting in the formation of large, multinucleate cells; it therefore seems likely that the extensive filopodial network formed by blastocoelar cells is syncytial, although direct evidence is lacking. Blastocoelar cells may represent a heterogeneous population of cells, as there have been numerous reports of possible additional subpopulations of non-skeletogenic mesenchymal cells at various stages of gastrulation (Shoguchi et al., 2002; Kominami and Takata, 2003; Ohguro et al., 2011; Takata and Kominami, 2011).

Provisional GRNs have been constructed for both these cell lineages (Fig. 4). With respect to pigment cell specification, attention has centered on glial cells missing (*gcm*), a regulatory gene directly responsive to Delta signaling, which plays a key role in this process (Ransick et al., 2002; Ransick and Davidson, 2006; Ransick and Davidson, 2012; Oulhen and Wessel, 2016). Many pigment cell-specific mRNAs have been identified, including those that encode enzymes involved in pigment biosynthesis (Calestani et al., 2003; Barsi et al., 2015). *Gcm* is a direct driver of at least some of these biosynthetic genes (Calestani and Rogers, 2010). Several regulatory genes that control PMC ingression (e.g., *alx1*, *ets1*, and *snail*) are not expressed by presumptive pigment cells, suggesting that the upstream inputs that control EMT in these two cell types are distinct.

Presumptive blastocoelar cells express a characteristic suite of transcription factors at the early gastrula stage, including *scl*, *gatac*, and three ETS family members- *ets1*, *erg*, and *ese* (Rizzo et al., 2006; Duboc et al., 2010; Flynn et al., 2011; Solek et al., 2013). Effector genes that control the ingression, migration, or fusion of blastocoelar cells have not been identified; therefore, direct linkages between the upstream regulatory circuitry and the behaviors of these cells during gastrulation are not currently possible. Notably, several cytoskeletal genes (e.g., *α-actinin*, *arp1*, *arp3*, *cdc42*, *cofilin*, *talin*, and *p21-arc*) are selectively expressed by both PMCs and blastocoelar cells (Rafiq et al., 2012), suggesting that similar programs of cell motility might be deployed in these two cell types, both of which undergo EMT and migrate exclusively by means of filopodia.

5. Conclusions and future prospects

The experimental accessiblity of the sea urchin embryo, coupled with several decades of concerted effort on the part of cell and developmental biologists, have led to a highly detailed picture of the cell behaviors that accompany gastrulation. Nevertheless, important questions remain unanswered, most notably with respect to the mechanical basis of epithelial movements that take place during gastrulation; i.e., the initial inpocketing of the vegetal plate and the oriented rearrangement of cells in the wall of the archenteron. One useful approach might be to exploit the diversity of echinoderms to reveal conserved aspects of these processes- for example, it would be informative to analyze mechanisms of early invagination in sea stars, an experimental model not complicated by mesenchyme cell ingression. In general, the movements of cells as individuals during gastrulation are better understood than epithelial movements. Gene products that direct the migration of PMCs and pigment cells have recently been revealed, as have components of the molecular machinery of PMC fusion. Two important issues that remain unresolved, however, are the molecular changes that trigger mesoderm cell motility and the molecular basis of target site recognition by filopodia during secondary invagination.

There is also an increasingly detailed picture of the transcriptional networks that are deployed in specific territories of the early embryo. Indeed, remarkable progress has been made in this area over the past decade. Of course, some gene regulatory programs are better understood than others. Even the PMC GRN, arguably one of the most comprehensive in any developing embryo, lacks certain important details, including the architecture of genetic sub-circuits downstream of several late regulatory genes.

Given these various gaps in our knowledge, it is perhaps not surprising that relatively few direct connections have been made between the transcriptional programs of mesoderm and endoderm cells and the movements of these cells during gastrulation. This should not be taken as a message of gloom; rather, that this important area is ripe for further work. One example of a successful linkage is related to the *vegfr-10-Ig* gene. The upstream regulatory control of *vegfr-10-Ig* is largely understood. The activation of this gene during the cell autonomous phase of PMC specification renders these cells competent to respond to local regions of VEGF3 production within the ectoderm, regions that are specified by a complex sequence of earlier cell-cell interactions that have also been largely elucidated. A second example involves the activation of the *kirrelL* gene, another output of the cell-autonomous program of PMC specification and a mediator of PMC fusion. Currently, the most important challenge is to pinpoint additional, pivotal effectors of cell and tissue movements during gastrulation; it should then be

rather straightforward to link the genes that encode such effectors to the relevant upstream transcriptional circuitry. Eventually, these studies may reveal “morphogenetic cassettes”- modular genetic circuits that control cell behaviors deployed repeatedly during gastrulation in different contexts or cell types, such as EMT, filopodial motility, and cell-cell fusion.

Acknowledgements

The preparation of this manuscript was supported by the National Science Foundation (IOS-1354973, to C.A.E.).

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