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A conserved gene regulatory network subcircuit drives different developmental fates in the vegetal pole of highly divergent echinoderm embryos ☆

Brenna S. McCauley, Erin P. Weideman, Veronica F. Hinman *

Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213, USA

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ABSTRACT

Comparisons of orthologous developmental gene regulatory networks (GRNs) from different organisms explain how transcriptional regulation can, or cannot, change over time to cause morphological evolution and stasis. Here, we examine a subset of the GRN connections in the central vegetal pole mesoderm of the late sea star blastula and compare them to the GRN for the same embryonic territory of sea urchins. In modern sea urchins, this territory gives rise to skeletogenic mesoderm; in sea stars, it develops into other mesodermal derivatives. Orthologs of many transcription factors that function in the sea urchin skeletogenic mesoderm are co-expressed in the sea star vegetal pole, although this territory does not form a larval skeleton. Systematic perturbation of *erg*, *hex*, *tbr*, and *tgif* gene function was used to construct a snapshot of the sea star mesoderm GRN. A comparison of this network to the sea urchin skeletogenic mesoderm GRN revealed a conserved, recursively wired subcircuit operating in both organisms. We propose that, while these territories have evolved different functions in sea urchins and sea stars, this subcircuit is part of an ancestral GRN governing echinoderm vegetal pole mesoderm development. The positive regulatory feedback between these transcription factors may explain the conservation of this subcircuit.

Introduction

The gene regulatory network (GRN) for early specification of the sea urchin endomesoderm is extremely well resolved and provides one of the most insightful accounts of the mechanisms of development in any animal system. This network explains the development of the vegetal domain of the sea urchin from its specification by maternal factors through the final differentiation of cells as endoderm or one of several types of mesoderm (Davidson, 2006; Davidson et al., 2002b; Oliveri et al., 2008; Smith and Davidson, 2008). A comparison of this GRN to the orthologous endomesoderm network in the sea star, *Asterina miniata*, revealed that some of the regulatory connections are conserved between these divergent echinoderms (Hinman and Davidson, 2007; Hinman et al., 2003a). Presumably, these interactions have been retained from a very ancient ancestor that existed some 500 million years ago (Wada and Satoh, 1994). Other connections, however, have diverged in the time since these organisms last shared a common ancestor. The conserved regulatory interactions found in these two GRNs exhibit a high degree of positive feedback between the transcription factors involved. Such feedback is thought to stabilize expression of target genes and may function as a mechanism to "lock down" early specification events (Davidson, 2006). These observations led to speculation that positive feedback within GRN subcircuits may be refractory to evolutionary change and thus might provide a molecular mechanism for the constraint that results in a "phylotypic" developmental plan (Davidson and Erwin, 2006). Hints of positive feedback regulatory loops are also found within other conserved developmental programs (Davidson, 2006; Olson, 2006), but there are currently very few direct comparisons of GRN architecture. Therefore, the hypothesis that recursive wiring is causative of evolutionary constraint remains speculative.

In this study, the comparison of endomesoderm GRN architecture in sea urchins and sea stars is expanded with a focus on mesoderm development. Unlike the endoderm, which forms morphologically similar larval digestive tracts in these echinoderms, mesodermal cell types have evolved in the time since sea urchins and sea stars diverged. Sea urchins have at least two mesodermal cell types, pigment cells and micromere-derived skeletogenic mesoderm, that are absent in the young/larval sea star. Indeed, these cell types are not present in the larvae of other groups of echinoderms, and are likely an evolutionary novelty of modern sea urchins (euechinoids). Thus, the comparison of mesoderm regulatory networks in sea urchins and sea stars may prove insightful about how novel cell lineages arise during evolution.

Endomesoderm is derived from the vegetal pole of echinoderm embryos. In sea stars, cell divisions are equal, and mesoderm and endoderm segregate by the late blastula stage (Fig. 1). Fate mapping shows that mesodermal precursors are located at the central vegetal pole, and endoderm is found in a ring surrounding them (Kuraishi and Osanai, 1992). During gastrulation, mesoderm is internalized as part

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* Corresponding author. Department of Biological Sciences, 634 Mellon Institute, Carnegie Mellon University, 4400 5th Ave., Pittsburgh, PA 15213, USA. Fax: +1 412 268 7129.
E-mail address: veronica@cmu.edu (V.F. Hinman).

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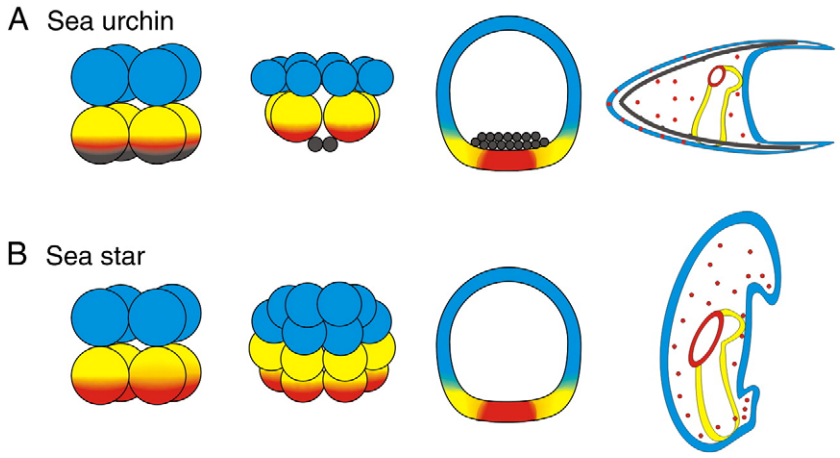

Fig. 1. Schematic of sea star and sea urchin development through the early larva. Sea urchin embryonic development is depicted in A and sea star development in B. During early cleavage, sea urchin and sea star embryos are similarly organized: vegetal blastomeres give rise to endomesoderm (yellow and red), while the animal blastomeres become ectoderm (blue). The 4th embryonic cleavage is unequal in sea urchins, dividing the vegetal half of the embryo in macromeres (endomesoderm) and micromeres (shown in grey), which give rise to the larval skeleton. Cleavage is equal in sea stars and no micromeres form. The blastula of sea urchins and sea stars are organized similarly, with mesodermal progenitors (red) in the central vegetal pole, surrounded by presumptive endoderm (yellow); the remainder of the embryo will become ectoderm. Prior to gastrulation, the skeletogenic mesoderm has ingressed in sea urchins, while sea star blastulae contain no mesenchyme. By the larval stage, sea stars and sea urchins have formed a gut tube (archenteron) and have mesodermally derived coelom, blastocoelar cells, and muscle. Sea urchin larvae also are pigmented and have a skeleton (shown in grey).

of the archenteron, and only after gastrulation is nearly complete does any mesenchyme migrate into the blastocoel as blastocoelar cells (Byrne and Barker, 1991; Kuraishi and Osanai, 1992). A large population of mesoderm remains at the tip of the archenteron and forms the coelomic pouches, which will eventually give rise to the adult rudiment (Hyman, 1955). Larval circumesophageal muscle is also derived from the coelomic mesoderm. This mode of development is thought to be basal among echinoderms.

In contrast, in modern sea urchins, an unequal 4th cleavage divides the vegetal half of the embryo into polar micromeres and overlying macromeres (Fig. 1). The large micromere daughters give rise to the skeletogenic mesoderm, ingress into the blastocoel prior to gastrulation, and later form the larval skeleton. Starting at late cleavage stages, the micromeres produce the Delta ligand, inducing the overlying macromere descendents to become other mesodermal cell types (Sherwood and McClay, 1999; Sweet et al., 2002; Sweet et al., 1999) including coelom, circumesophageal muscle, blastocoelar cells, and pigment cells. The remaining macromere descendents become the larval endoderm (reviewed in Davidson et al., 1998). From a phenomenological viewpoint, it therefore appears as if the entire micromere/skeletogenic mesoderm lineage is a novelty of euechinoids.

Thus, in both sea stars and sea urchins, endomesoderm precursors form at the vegetal pole of the embryo; subsequent segregation places the mesoderm at the central vegetal pole, surrounded by endoderm. Although much is known about mesoderm development and differentiation in sea urchins, the molecular details of sea star mesoderm formation have only begun to be resolved. A handful of transcription factors are known to be expressed in the mesoderm progenitors at the central vegetal pole of *A. miniata* blastulae, namely *ets1/2*, *gatac*, *otx*, and *tbr* (Hinman and Davidson, 2007; Hinman et al., 2003a; Hinman et al., 2003b). Orthologs of these genes participate in the formation of both skeletogenic and non-skeletogenic mesoderm in sea urchins (Chuang et al., 1996; Davidson et al., 2002a; Davidson et al., 2002b; Fuchikami et al., 2002; Kurokawa et al., 1999; Oliveri et al., 2002). Additionally, in sea urchins, *hesc* has an early function in repressing micromere cell fate by blocking the expression of key transcription factors in this territory (Oliveri et al., 2008; Revilla-i-Domingo et al., 2007).

To understand the basal mode of mesoderm development in echinoderms, we analyzed the regulatory interactions between five sea star transcription factors orthologous to genes with known function in the development of the sea urchin skeletogenic mesoderm. Surprisingly, although the vegetal pole domains in these organisms have very different developmental fates, both utilize a conserved, recursively wired subcircuit downstream of initial specification events. This suggests that a conserved subcircuit can drive distinct developmental outcomes in divergent organisms.

Methods

Isolation of sea star genes from a mid-gastrula cDNA library

Heterospecific probes were prepared by PCR amplifying approximately 0.7-1 kb regions of *hex*, *erg*, *tgif*, and *foxn2/3* from *Strongylocentrotus purpuratus* cDNA. Primer sequences are available upon request. PCR products were radiolabeled and hybridized to an *A. miniata* late gastrula cDNA library as described (Hinman and Davidson, 2007). 5′ RACE was performed on a gastrula stage RACE library to extend gene sequences to include the start codon using the GeneRacer system (Invitrogen; Carlsbad, California). Primer sequences are available upon request. Final cDNA sequences were deposited in Genbank (Accession Numbers: *AmHex* GU251972, *AmErgS* GU251974, *AmErgL* GU251975, *AmTgif* GU251973, *AmFoxn2/3a* GU251977, *AmFoxn2/3b* GU251978).

Analysis of gene expression patterns by whole mount in situ hybridization (WMISH)

Spatial gene expression patterns were determined by WMISH as described (Hinman et al., 2003b), except the color reaction contained 10% dimethylformamide to reduce background. Embryos were photographed with DIC optics on a Leica DMI4000B at 200× magnification using the Leica Application Suite software (Leica; Wetzlar, Germany).

Gene perturbation experiments

Gene expression was blocked by injecting zygotes with translation-blocking morpholino antisense oligonucleotides (MASOs; Gene Tools LLC; Philomath, OR). Injections were performed as described (Hinman et al., 2003a), with the addition of 0.5 mg/mL rhodamine green dye in the injection solution. The AmTbr MASO was described previously (Hinman and Davidson, 2007); sequences for other MASOs available upon request. Sibling embryos were injected with the Gene Tools standard control MASO to ensure gene-specific results. In some experiments, MASOs were injected into a single blastomere of the two cell embryo to generate an internal control. The plane of first cleavage