required for this early ese and gcm expression but to resolve and pattern a population of cells with an initial mixed identity. By the time of delamination of the primary mesenchymal cells, segregation of ese and gcm expression and DV patterning of the NSM occurs. Ventral NSM cells retain expression of ese and lose expression of gcm, while dorsal NSM lose expression of ese and retain expression of gcm. Perturbation and overexpression experiments indicate that Ese is necessary and sufficient to promote expression of blastocoelar cells lineage specific transcription factors such as gataC, prox1 and scl on the ventral population of NSM as well as to restrict gcm expression to the dorsal population of NSM precursors. Thus, we identify Ese as an key component of the gene regulatory network responsible of blastocoelar cell specification.

41 SoxC functions in neural precursor cells in sea urchin embryo neurogenesis

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Previously we showed that Six3 was necessary for development of all nerves in the sea urchin embryo (Wei et al., 2009). The expression of Six3 begins during cleavage stages broadly throughout the embryo and then is confined to patches of contiguous cells; but it is not expressed in individual neural precursors. This suggests that Six3 is involved in establishing neuroectoderm territories but is not directly involved in initiating neural differentiation. To look for Six3-dependent genes that might execute Six3's function in neurogenesis, we carried out a microarray-based screen at the mesenchyme blastula stage when expression of proneural factor orthologs begins to appear in individual cells. Among the set of Six3-dependent transcription factors expressed in individual cells, SoxC has a unique function in neural precursor cells. By in situ hybridization, we found that SoxC is expressed in individual cells in neuroectoderm. More importantly, when SoxC was knocked down with morpholino oligos, development of all neurons was greatly reduced as is also the case in Six3 morphants. However, in a double in situ with Synaptagmin B, which is a marker for almost all neurons, no cells expressed both. This observation suggested that SoxC-expressing cells could be in an intermediate state and other factors mediate their further differenciation. We carried out a RNA-Seq screen for potential candidates. Among the affected genes, we tested Z167 and Brn1/2/4 and found that they both are partially co-expressed with SoxC and partially co-expressed with Synaptagmin B or Tph, a marker for Serotonin cells. Therefore, in sea urchin embryo, neurogenesis is a multistep process and SoxC functions in neural precursors, which is a conserved function observed in other systems.

42 Suppression of nodal expression in prospective dorsal cells of the early sea urchin embryo by the Hbox12 homeodomain regulator

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Dorsal/Ventral (DV) axis formation in the sea urchin embryo depends upon the expression of nodal on the ventral side, which behaves as a DV organizing centre. However, only fuzzy clues are known as to the early symmetry-breaking steps that lead to the positioning of such an organizer. An extremely interesting candidate for this role is the hbox12 homeobox-containing gene. In Paracentrotus lividus , hbox12 expression is antecedent and complementary with respect to that of nodal , being confined in prospective dorsal cells. We show that ectopic expression of Hbox12 provokes DV abnormalities and attenuates nodal as well as nodal -dependent gene transcription. By blastomere transplantation, we also establish that DV defects arise from hbox12 misexpression in the animal hemisphere. To impair Hbox12 function we expressed ubiquitously a truncated form of the protein, encoding for the homeodomain. Such a perturbation disrupts DV axis formation by allowing ectopic expression of nodal across the embryo. Moreover, clonal loss-of-function imposed by either blastomere transplantation or gene transfer assays highlights that Hbox12 action in prospective dorsal cells is necessary for DV polarization. Remarkably,

the localized knock-down of nodal restores DV polarity of embryos lacking hbox12 function. Finally, we show that hbox12 is involved in the dorsal-specific inactivation of the p38 MAPK, which is known to be required for nodal expression. Altogether, our results indicate that Hbox12 prevents the ectopic activation of nodal transcription within the future dorsal side of the early sea urchin embryo.

43 Evolution of ectoderm-mesoderm communication during skeletal patterning in echinoid larvae

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The echinoid larval endoskeleton is a classic model for studies of cell differentiation and morphogenesis. It is also a model for the evolution of embryonic patterning, since specific parts of the skeleton can vary between species. Two of the most variable elements are the recurrent and posterior connecting rods. The developmental basis of this variation is not well understood. By comparing the sea urchin Lytechinus variegatus , which forms a small recurrent rod and no posterior connecting rod, with the sand dollar Mellita quinquiesperforata , which forms both, we found that the difference in their skeletal patterning is already obvious in the arrangement of primary mesenchyme cells (PMCs) at gastrula stages. Mellita embryos possess an antero-dorsal chain of PMCs between the tips of the longitudinal strands, which emanate from the ventro-lateral clusters. These PMCs contribute to the recurrent rod and the anastomosed posterior connecting rod. No antero-dorsal chain exists in Lytechinus , but at prism stages it is from an analogous location that the recurrent rods form from a branching event at the tip of each longitudinal strand. Because the position of the PMCs is dictated by ectodermal patterning in sea urchins, we investigated the role of ectodermal patterning in Mellita PMC arrangement. We found that Nodal signaling controls the position of the antero-dorsal chain in the oral/aboral axis. Ectodermal signals that control the spatiotemporal pattern of underlying PMCs have diverged between these two echinoids. These data provide the framework for studies in both species that address the details of these two patterning systems at the molecular level.

44 Experimental approach to divergence in test organization between euechinoid and cidaroid sea urchins

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The two extant crown groups of echinoids are the subclasses Cidaroidea and Euechinoidea, which diverged around P/T boundary and differs strikingly in several aspects of skeletal morphogenesis. Such divergence thus represents a compelling opportunity for understanding the evolutionary mechanisms of specific body plan innovations in echinoderms. Our initial focus is on the difference of their test pattering, a major phylogenetic character as revealing from their development and fossil records. Embryos from Strongylocentrotus purpuratus (Euechinoid) and Eucidaris tribuloides (Cidaroid), were cultured in the lab to the stage six weeks after metamorphosis, and samples were collected at different time points. The whole animal and extracted skeletal elements were scanned under SEM and micro-CT to know how their tests are built differently in these two species by addition of successive rows of body plates at the start of adult skeletogenesis. The difference on their test patterning was first visible from one week after metamorphosis with compound plates in Sp vs simple plates in Et from the ambulacral region while no discernible difference from the interambulacral region. WMISH was done on genes from the gene regulatory network underlying the development of the skeletogenic lineage of Echinoderm to find what accounts for the clade–specific differences in the spatial deployment of this GRN on the ambulacral region in Sp and Et. Gene regulatory analysis and synthetic experimental evolution will be