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Gene regulatory control in the sea urchin aboral ectoderm: Spatial initiation, signaling inputs, and cell fate lockdown

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ABSTRACT

The regulation of oral–aboral ectoderm specification in the sea urchin embryo has been extensively studied in recent years. The oral–aboral polarity is initially imposed downstream of a redox gradient induced by asymmetric maternal distribution of mitochondria. Two TGF-β signaling pathways, Nodal and BMP, are then respectively utilized in the generation of oral and aboral regulatory states. However, a causal understanding of the regulation of aboral ectoderm specification has been lacking. In this work control of aboral ectoderm regulatory state specification was revealed by combining detailed regulatory gene expression studies, perturbation and *cis*-regulatory analyses. Our analysis illuminates a dynamic system where different factors dominate at different developmental times. We found that the initial activation of aboral genes depends directly on the redox sensitive transcription factor, hypoxia inducible factor 1α (HIF-1α). Two BMP ligands, BMP2/4 and BMP5/8, then significantly enhance aboral regulatory gene transcription. Ultimately, encoded feedback wiring lockdown the aboral ectoderm regulatory state. Our study elucidates the different regulatory mechanisms that sequentially dominate the spatial localization of aboral regulatory states.

Introduction

Cell fate specification and differentiation are controlled by complex regulatory networks encoded in the genome (Davidson, 2006, 2010). The architecture of gene regulatory networks (GRNs) determines their information processing properties, and defines the temporal order of specification events (Ben-Tabou de-Leon and Davidson, 2007). Thus the structure-function relations of GRNs as they progress through developmental time provide systems level understanding of the progressive molecular control of developmental processes. The construction of experimentally based models of gene regulatory networks requires advanced experimental tools and methodologies.

The sea urchin embryo presents key experimental and theoretical advantages for the study of developmental GRNs: a relatively simple developmental program that has been used to address fundamental questions in development (Horstadius, 1939), easy gene transfer technology coupled with quantitative perturbation analysis of gene expression and a novel high throughput method for testing the expression level of more than a hundred *cis*-regulatory reporters in one experiment (Nam et al., 2010; Nam and Davidson, 2012). Predictive mathematical models were developed based on the knowledge gained from sea urchin regulatory studies, simulating the dynamics of gene regulatory circuits (Bolouri and Davidson, 2003; Ben-Tabou de-Leon and Davidson, 2009, 2010; Ben-Tabou de-Leon, 2010) and more recently, computing how the logic functions utilized by GRNs give rise to unique spatial regulatory states (Peter et al., 2012). These experimental and theoretical methodologies enabled the construction of one of the most elaborate models of developmental GRNs, a model of the control system governing specification of endomesoderm in the sea urchin embryo (Oliveri et al., 2008; Peter and Davidson, 2009, 2011). It has the capacity to explain the molecular control of various developmental phenomena, such as spatial and temporal patterning of gene expression (Smith and Davidson, 2008; Peter and Davidson, 2011), the endoderm–mesoderm cell fate decision (Ben-Tabou de Leon and Davidson, 2010; Peter and Davidson, 2011), and the activation of sets of differentiation and structural genes in a specific cell lineage (Oliveri et al., 2008; Smith and Davidson, 2009). The recent computational model mentioned above, demonstrates that the endomesoderm GRN in fact includes sufficient information to compute predicatively almost all known regulatory gene expression in space and time, in this portion of the embryo up to gastrulation (Peter et al., 2012). Experimentally based models of

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the GRNs governing specification of the various fate and regulatory state domains of the sea urchin embryo ectoderm are now being constructed and carry the promise to illuminate the regulatory control of ectodermal patterning (Su et al., 2009; Li et al., 2012).

In its external developmental morphology, the sea urchin embryo ectoderm consists of four prominent territories: the oral ectoderm, within which the mouth forms through fusion of the foregut and the ectoderm; the aboral ectoderm, which differentiates into squamous epithelium; the ciliary band, positioned at the border between oral and aboral ectodermal domains; and the apical neurogenic domain (Fig. 1A left panel). The neurons of the sea urchin larva are mostly localized within the apical domain, in the ciliary band, and around the mouth (Burke et al., 2006). Oral/aboral diversification along the secondary embryonic axis of the sea urchin embryo forms as the result of an asymmetric redox gradient that derives originally from uneven maternal mitochondrial distribution (Coffman and Davidson, 2001; Coffman et al., 2004, 2009). At the high end of this gradient, the future oral side of the embryo, the *nodal* gene is activated where its *cis*-regulatory control system responds to a redox sensitive transcription factor (Coffman and Davidson, 2001; Coffman et al., 2004, 2009; Nam et al., 2007; Range et al., 2007; Range and Lepage, 2011). Nodal signaling then controls the specification and differentiation of the oral ectoderm domain (Duboc et al., 2004). Targets of Nodal signaling within the oral ectoderm include genes encoding both the ligand BMP2/4 and its antagonist Chordin (Duboc et al., 2004, 2010). The BMP ligand diffuses to the aboral ectoderm, to which its signaling activity is confined, due to inhibition of BMP reception by Chordin within the oral ectoderm (Duboc et al., 2004; Chen et al., 2011).

Recent work shows that the oral ectoderm contains an unexpectedly complex, bilaterally arranged set of spatial regulatory state subdomains, (Li et al., 2012) and the same is true of the aboral ectoderm despite its seemingly uniform morphology (Chen et al., 2011). Prior to gastrulation the aboral ectoderm specifically expresses a set of regulatory genes encoding the transcription factors *Tbx*2/3, Irxa, Dlx, Msx, Hmx and Hox7 (Su et al., 2009; Chen et al., 2011). Although these genes turn on within a narrow time window (Fig. 1B (Materna et al., 2010)), their spatial expression is differential, defining regulatory state sub-regions within the aboral ectoderm (Chen et al., 2011). The dynamic changes in spatial subdomain expression of these genes between early and late blastula stages are shown diagrammatically in Fig. 1C (Chen et al., 2011). Previous analyses revealed that the reception of BMP2/4 enhances expression of the aboral ectoderm regulatory genes (Duboc et al., 2004; Su et al., 2009; Saudemont et al., 2011). However, the effect of BMP2/4 knockdown was less severe than the effect of BMP receptor knock-down (Yaguchi et al., 2010), and this suggests that there are additional BMP ligands operating at this time. Furthermore the aboral *tbx*2/3 gene is expressed before the phosphorylation of the BMP mediator, SMAD1/5/8, is detectable (Chen et al., 2011), indicating the presence of an early activator of aboral ectoderm genes not related to BMP signaling. Thus, a redox-dependent aboral ectoderm transcription factor was predicted to initiate the expression of *tbx*2/3 (Su et al., 2009). While intricate positive regulatory interactions were suspected to exist among the aboral ectoderm transcription factors (Su et al., 2009; Saudemont et al., 2011), prior to the present work temporal resolution of observations indicating such interactions was lacking, particularly for early time points.

Here we study the dynamic regulatory control of the aboral ectoderm specification by combining gene expression studies, perturbation analysis, and *cis*-regulatory analyses. We show that the redox sensitive transcription factor HIF1α (Hypoxia Inducible Factor 1α) is directly activating the early expression of aboral ectoderm regulatory genes, possibly mediating the primordial redox gradient. Both BMP2/4 and BMP5/8 then contribute to the magnitude of expression of the aboral ectoderm regulatory genes, and their inputs were verified for key genes at the *cis*-regulatory level. As typical for specification GRNs, the system later graduates to the use of encoded cross-regulatory feedback interactions, which thereafter internally control its transcriptional functions.

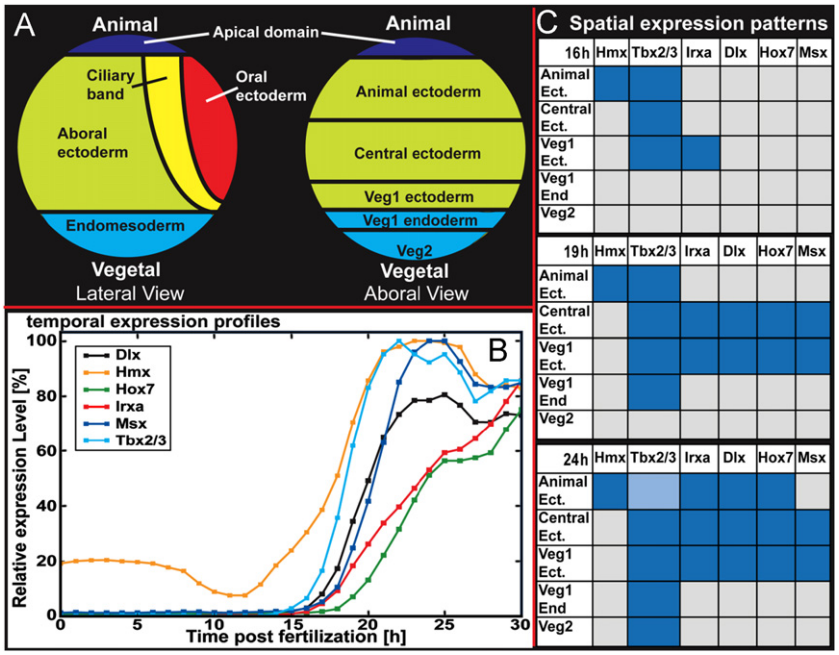

Fig. 1. Spatio-temporal patterning of the sea urchin aboral ectoderm. A. Diagram of sea urchin embryogenesis marking the different embryonic territories and ectodermal sub-domains. Left—lateral view, right—aboral view. B. Temporal expression profiles of the aboral ectoderm regulatory genes (based on Materna et al. (2010)). C. Spatial expression patterns of aboral ectoderm transcription factors at different time points at early development. Dark blue marks high expression level in this region, light blue marks low expression in this region, and gray marks regions where the gene is not detectable in WMISH (based on Chen et al. (2011)).