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New regulatory circuit controlling spatial and temporal gene expression in the sea urchin embryo oral ectoderm GRN

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ABSTRACT

The sea urchin oral ectoderm gene regulatory network (GRN) model has increased in complexity as additional genes are added to it, revealing its multiple spatial regulatory state domains. The formation of the oral ectoderm begins with an oral–aboral redox gradient, which is interpreted by the *cis*-regulatory system of the *nodal* gene to cause its expression on the oral side of the embryo. Nodal signaling drives cohorts of regulatory genes within the oral ectoderm and its derived subdomains. Activation of these genes occurs sequentially, spanning the entire blastula stage. During this process the stomodeal subdomain emerges inside of the oral ectoderm, and bilateral subdomains defining the lateral portions of the future ciliary band emerge adjacent to the central oral ectoderm. Here we examine two regulatory genes encoding repressors, *sip1* and *ets4*, which selectively prevent transcription of oral ectoderm genes until their expression is cleared from the oral ectoderm as an indirect consequence of Nodal signaling. We show that the timing of transcriptional de-repression of *sip1* and *ets4* targets which occurs upon their clearance explains the dynamics of oral ectoderm gene expression. In addition two other repressors, the direct Nodal target *not*, and the feed forward Nodal target *goosecoid*, repress expression of regulatory genes in the central animal oral ectoderm thereby confining their expression to the lateral domains of the animal ectoderm. These results have permitted construction of an enhanced animal ectoderm GRN model highlighting the repressive interactions providing precise temporal and spatial control of regulatory gene expression.

Introduction

This work was undertaken as an effort to generate a realistic and relatively complete GRN model that would encompass the genomic regulatory code for the sea urchin (*Strongylocentrotus purpuratus*) embryo oral ectoderm. Both additional genes and additional spatial regulatory state domains have recently been added to the initial draft GRN model for oral ectoderm specification (Li et al., 2012; Su et al., 2009), and we continue that process here. The oral ectoderm GRN is activated initially in cells that both express and receive Nodal signals (Bolouri and Davidson, 2010; Duboc et al., 2004; Nam et al., 2007). These cells are located on the oral side of the cleavage stage embryo in consequence of *nodal cis*-regulatory response to a redox gradient set up very early in development by a primordial asymmetric distribution of mitochondria (Coffman et al., 2004, 2009; Coffman and Davidson, 2001; Nam et al., 2007; Range et al., 2007). Sea urchin embryos become radialized and lose oral–aboral polarity when Nodal signaling is blocked by morpholino anti-sense oligos, or by Nodal pathway inhibitors (Duboc et al., 2004; Saudemont et al., 2010). The Nodal receptor has been identified as the Alk4 receptor kinase, which activates the Smad signal transduction pathway (Yaguchi et al., 2007). However, many regulatory genes that apparently respond to Nodal signaling in the oral ectoderm do so indirectly. For example, we recently found that an immediate Nodal signaling target, the homeobox gene *not*, plays an essential role in establishing oral–aboral polarity (Li et al., 2012; Materna et al., 2012).

Specification of the ectoderm is progressive and dynamic. Regulatory genes are activated, affecting one another's spatial domain of expression often by repression, and the result is an increase in the spatial complexity of the regulatory state patterns. Thus various new subdomains emerge during the blastula stage (Li et al., 2012). Early cell lineage tracing experiments showed that the oral ectoderm is parsed into veg1 and animal oral ectoderm, which was supported by subsequent gene expression analysis. Inside the animal oral ectoderm, the subject of the present work, a stomodeal subdomain forms at the late mesenchyme blastula stage; while outside, ciliary band (CB) genes are expressed bilaterally. Furthermore, the regulatory genes of the animal oral ectoderm and future stomodeum are activated only sequentially, over the period between the early

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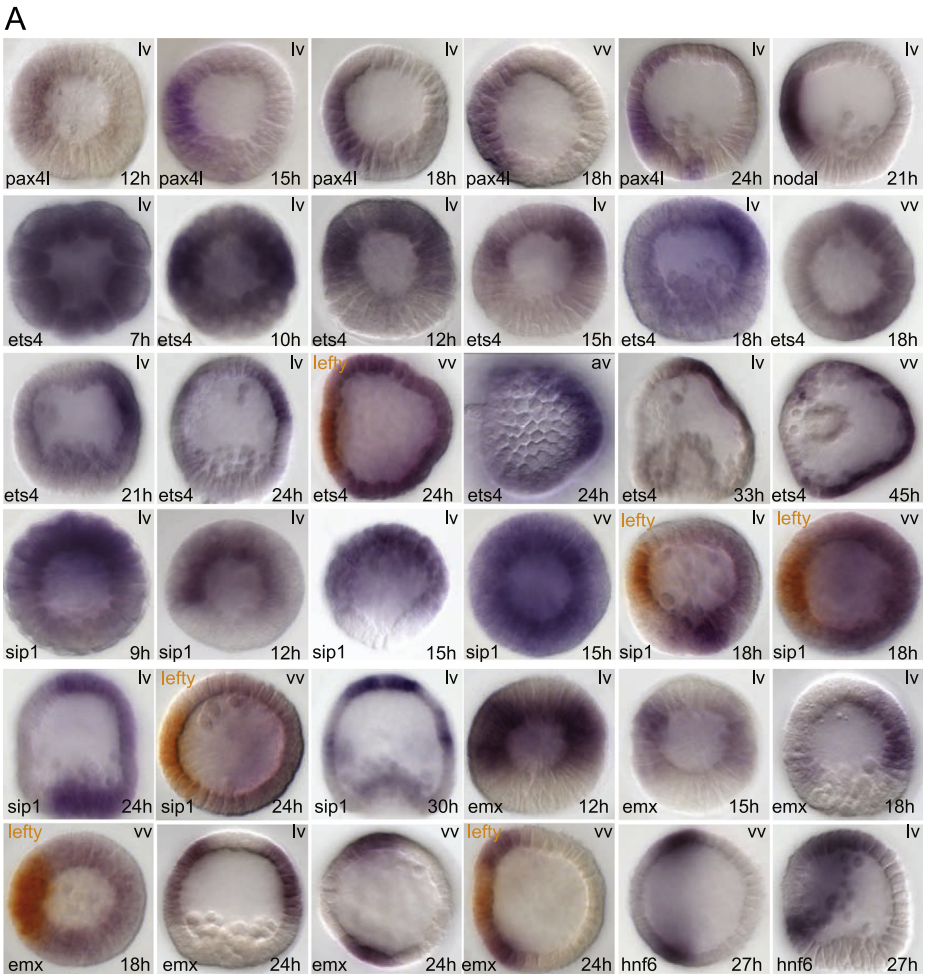
blastula (~9 h) and mesenchyme blastula (>22 h) stages. Considering that in this species at 15° the time typically elapsing between activation of an upstream regulatory gene and the activation of its immediate downstream target gene is ~3 h (Bolouri and Davidson, 2003; Peter et al., 2012), the dynamics of progressive gene activation during oral ectoderm specification cannot simply be due to a single



C

	ets4			sip1			emx			nodal			not			pax4l			gsc			foxg			bra		
hours post fertilization	12	18	24	12	18	24	12	18	24	12	18	24	12	18	24	12	18	24	12	18	24	12	18	24	12	18	24
apical				■	■	■																					
animal oral	■			■			■			■	■	■		■	■		■	■		■	■		■	■			
animal lateral	■	■	■	■	■	■	■	■	■																		
animal aboral	■	■	■	■	■	■	■	■																			
stomodeal																											■
veg1 oral										■	■	■		■	■		■	■									

Fig. 1. Dynamic spatial gene expression patterns and territories of the oral ectoderm. (A) Expression of *pax4l, ets4, sip1 and emx. pax4l* transcripts are localized in the oral ectoderm during the blastula stage, similar to *nodal*. Initially *ets4, sip1, and emx* transcripts cover both oral and aboral ectoderm. Oral expression of these genes fades at mid-blastula and becomes complementary to the central oral ectoderm (marked by *lefty* expression in the double WMISH) after18 h. (B) Diagrams illustrating ectodermal gene expression domains shown in lateral view. Developmental stages are the early blastula stage (12 h), late blastula stage (18 h), and mesenchyme blastula stage (24 h). For simplicity, endomesodermal domains are not indicated. Ectodermal domains are color-coded and labeled on the left; domain-specific genes are shown in Table S1. Apical—apical plate; Ec—ectoderm. lv—lateral view; vv—vegetal view; av—apical view. All embryos in lateral or vegetal views were shown with the oral ectoderm facing left. (C) Expression matrix for ectodermal genes during the blastula stage. Three time points were included representing the early blastula stage (12 h), late blastula stage (18 h), and mesenchyme blastula stage (24 h). A graphic presentation of the expression patterns is shown in Table S1.