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Long- and short-range signals control the dynamic expression of an animal hemisphere-specific gene in *Xenopus*

Adnan Mir [a,b], Matthew Kofron [a], Janet Heasman [a], Melissa Mogle [a], Stephanie Lang [a], Bilge Birsoy [a], Chris Wylie [a,*]

[a] *Division of Developmental Biology, Cincinnati Children's Hospital Research Foundation, 3333 Burnet Ave, Cincinnati, OH 45229, USA*
[b] *Physician/Scientist Training Program, P. O. Box 670555, Cincinnati, OH 45267, USA*

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Abstract

Little is known of the control of gene expression in the animal hemisphere of the *Xenopus* embryo. Here we show that expression of *FoxI1e*, a gene essential for normal ectoderm formation, is expressed regionally within the animal hemisphere, in a highly dynamic fashion. In situ hybridization shows that *FoxI1e* is expressed in a wave-like fashion that is initiated on the dorsal side of the animal hemisphere, extends across to the ventral side by the mid-gastrula stage, and is then turned off in the dorsal ectoderm, the neural plate, at the neurula stage. It is confined to the inner layers of cells in the animal cap, and is expressed in a mosaic fashion throughout. We show that this dynamic pattern of expression is controlled by both short- and long-range signals. Notch signaling controls both the mosaic, and dorsal/ventral changes in expression, and is controlled, in turn, by Vg1 signaling from the vegetal mass. *FoxI1e* expression is also regulated by nodal signaling downstream of VegT. Canonical Wnt signaling contributes only to late changes in the *FoxI1e* expression pattern.

These results provide new insights into the roles of vegetally localized mRNAs in controlling zygotic genes expressed in the animal hemisphere by long-range signaling. They also provide novel insights into the role of Notch signaling at the earliest stages of vertebrate development.

Keywords: FoxI1e; Xenopus; Ectoderm; Animal gene expression

Introduction

The *Xenopus* blastula is conventionally divided into three regions, with respect to both cell fate and gene expression. Cells in the vegetal region give rise to the embryonic endoderm. They inherit the maternally encoded transcription factor VegT, which activates the synthesis of nodals. These, in turn, induce mesoderm in the adjacent marginal region. Cells in the animal region form the ectoderm. It is not known how this fate is initiated. In addition to forming the germ layers, the three blastula regions each become patterned to form the axes of the body, by the expression of dorsal and ventral genes.

This picture of gene expression in the blastula tends to be regarded as dynamic with respect to time, but static with respect to each region of the blastula; a linear progression within each region of transcriptional initiation, leading to specification to enter a particular lineage, or to exhibit a particular type of cell behavior. We show here that in fact, gene expression is highly dynamic within the animal region. The forkhead transcription factor *FoxI1e* is expressed in the animal hemisphere, starting at the blastula stage. It is required for the expression of genes in both the early neural and ectodermal lineages, and for the later differentiation of the epidermis (Mir et al., 2007). It is also required to repress mesoderm specification in the animal hemisphere (Suri et al., 2005). Here we show that its expression pattern changes rapidly in both time and space within the animal hemisphere. Expression is initiated dorsally at the blastula stage, then spreads to encompass the ventral region of the animal hemisphere by the mid-gastrula stage. At the early neurula stage, expression is lost from the dorsal ectoderm cells that form the neural plate. During this sequence, its expression domain also changes with respect to the layers of the animal cap. First, it is restricted to the inner cells of the animal cap at the blastula

* Corresponding author.
E-mail address: Christopher.Wylie@cchmc.org (C. Wylie).

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stage, and then to a layer of cells between the inner and outer layers of the animal cap at the gastrula stage. Furthermore, throughout this temporal and spatial progression, *FoxI1e* expression is always in a mosaic pattern, with positive cells interspersed with non-expressing cells.

These observations revealed a previously unsuspected regionalization of the animal hemisphere. The total expression domain of *FoxI1e* extends across the animal cap, excluding the outer cells at the blastula stage, and both inner and outer cells at the gastrula stage, and all the time is restricted to only some cells, but not others, in this expression domain. During the late blastula to mid-gastrula stages, the expression of *FoxI1e* moves across this expression domain in a dorsal to ventral direction. This pattern is completely different from the expression patterns of other animally localized transcripts previously reported, such as ectodermin (Dupont et al., 2005) and Xlim5 (Toyama et al., 1995; Houston and Wylie, 2003), which are not mosaic, nor dynamic with respect to the dorsal–ventral axis. We therefore set out to identify some of the factors that might control these spatial and temporal changes.

We found that both long- and short-range signals control this pattern of expression. First, mosaic expression is controlled by Notch signaling. Both gain- and loss-of-function experiments showed that Notch signaling represses *FoxI1e* expression, and that loss of Notch signaling causes expression of *FoxI1e* in all cells of its total expression domain, and eliminates both the temporal and spatial progression of expression within this domain. This raised the issue of how Notch signaling is controlled in the animal hemisphere. It could be intrinsic, or controlled at long-range from the other developing germ layer regions. We show that control of *FoxI1e* expression by Notch is, in turn, controlled by the TGF-β signal, Vg1, whose mRNA is maternally encoded, and inherited only by the vegetal cells of the embryo (Melton, 1987). We also find that the level of *FoxI1e*, but not its mosaic distribution, is controlled by nodal signaling downstream of VegT, a vegetally localized, maternally encoded transcription factor (Zhang and King, 1996).

These data reveal multiple levels of control of early zygotic genes in *Xenopus*. First, long-range signals from the vegetal cells of the embryo, as well as short-range signals through the Notch pathway, combine to control both amount and position of expression of animal-specific genes during the blastula and gastrula stages. Second, none of these signals controls the "total expression domain" of *FoxI1e*, which remains confined to the group of cells that normally express it during its changing temporal expression at the blastula and gastrula stages. Instead, they control the temporal and spatial sequence of expression within this domain.

Materials and methods

Oocytes and embryos

Oocytes were generated for host transfer experiments by manual defolliculation of surgically removed ovary as previously described (Heasman et al., 1991). Culture and injections were carried out in L15-based oocyte culture medium (OCM). The length of the culture period between injection and host transfer varied by experiment. All mRNA injections were cultured overnight. VegT-depleted embryos were generated using a morpholino oligo [5′-CCCGACAGCAGTTTCTCATTCCACG-3′], and cultured for 3 days after injection. Vg1 depletions were carried out using the oligo Vg1c as previously described (Birsoy et al., 2006), with 4 days of culture after injection. *Xotch* was depleted using AS14MP [5′-GGAAGGGCTCAGCGCTAC-3′], with 3 days of culture. For rescue experiments, mRNA was injected on the last day of culture, before progesterone treatment, or at the 2-cell stage after fertilization. Eggs were collected in high-salt solution, fertilized in vitro with isolated testis, and cultured in 0.1× MMR. Staging was according to Nieuwkoop and Faber (1967). Dissections were performed at stage 9 or stage 10 on agarose-coated dishes in 1× MMR and then cultured in OCM. β-Catenin-depleted embryos were generated using a morpholino as previously described (Heasman et al., 2000). Synthetic mRNAs encoding β-catenin, BMP4, cmBMP7, β-galactosidase, NICD, Su(H)-DBM, and Vg1 were generated using Ambion mMessage mMachine kits.

Real-time RT–PCR

Total RNA was extracted as previously described (Zhang et al., 1998). Unless otherwise indicated, input was 2 whole embryos, 3 marginal zones, or 5 vegetal masses per sample. cDNA was synthesized using oligo dT primers, and semi-quantitative real-time RT–PCR was carried out using the LightCycler system as described (Kofron et al., 2001). *Ornithine decarboxylase* (*ODC*) was used as a loading control, and all values were normalized to *ODC* levels. In all cases, water-only and reverse transcriptase-negative controls failed to produce specific products. Each experiment was repeated a minimum of three times in independent experiments to verify reproducibility of results. Primer sequences were: *Siamois*: U: 5′-CTGTCCTACAAGAGACTCTG-3′, D: 5′-TGTTGACTGCAGACTGTTGA-3′. *Xnr3*: U: 5′-CTTCTGCACTAGATTCTG-3′, D: 5′-CAGCTTCTGGCCAAGACT-3′. *FoxI1e*: U-5′-GCACCTGCTGTGGTTCATAA-3′, D-5′-CACCACTG-TAGTGCGTCAGAA-3′. *Xotch*: U: 5′-AGTAACCCGTGCAAAAATGG-3′, D: 5′-AGCTTCCGGTAAATCCAGGT-3′. *ESR-1*: U: 5′-TGGCAAAACTGGAA-CAGGAT-3′, D: 5′-TGGGATACAACAGGGAGCTT-3′.

Whole-mount in situ hybridization, membrane staining, and Red-Gal staining

In situ hybridizations were carried out as described by Harland (1991), with a probe concentration for *FoxI1e* of 5 μg/ml, and using BMB Purple (Roche) as the alkaline phosphatase substrate. For membrane staining, embryos were stained for *FoxI1e*, sectioned, and stained with Alexa-488-conjugated Wheat Germ Agglutinin (Molecular Probes) at 0.01 μg/μl in PBS+0.1% Tween-20 for 30 min. For lineage labeling, embryos injected with 50 pg of nuclear β-galactosidase mRNA in the A1 blastomere were fixed for 1 h at room temperature in MEMFA, stained with Red-Gal (Research Organics) for 20 min at 37 °C, and fixed for another hour at room temperature before in situ hybridization for *FoxI1e*.

Results

FoxI1e is expressed in a dorsal to ventral wave, in a subset of cells in the animal cap

In previous work, we found that *FoxI1e* expression was mosaic in the early embryo (Mir et al., 2007). This could be due either to an asynchronous onset of expression, leading to a more stable homogeneous expression pattern, or it could be that gene expression in the animal hemisphere is not uniform, but controlled in more complex ways than previously thought. To distinguish between these possibilities, we carried out in situ hybridization on embryos from the late blastula stage (stage 9) to the mid-gastrula stage (stage 11). We found that expression in all embryos began in a localized manner above the equator on