available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/modo

MECHANISMS OF DEVELOPMENT

Respecification of ectoderm and altered Nodal expression in sea urchin embryos after cobalt and nickel treatment

Cavit Agca[a], *William H. Klein*[b], *Judith M. Venuti*[a,*]

[a]*Department of Cell Biology and Anatomy, Louisiana State University Health Sciences Center, 1901 Perdido Street, New Orleans, LA 70112, USA*

[b]*Department of Biochemistry and Molecular Biology, The University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030, USA*

ARTICLE INFO

Article history:
Received 7 October 2008
Received in revised form
18 January 2009
Accepted 20 January 2009
Available online 31 January 2009

Keywords:
Cobalt
Nickel
Nodal signaling
Oral ectoderm
Ectoderm
Oralization

ABSTRACT

In the sea urchin embryo, Nodal is the earliest known signal to play a role in the specification of the oral ectodermal territory. Nodal, a TGF-beta ligand, is first expressed in the presumptive oral ectoderm at ~7 H of development. Nodal overexpression produces a distinctive bell-shaped phenotype with expanded oral ectoderm, which resembles the oralized phenotype obtained as a result of nickel (Ni) treatment. To date, a detailed analysis of gene expression in Ni-treated embryos has not been undertaken. Because treatment with cobalt (Co) produces similar results to those seen with Ni treatment in other systems, we were interested in determining how Co influences sea urchin embryonic development. Here we report that Co also induces oralization of the ectoderm, and the effects of Ni and Co depend on functional Nodal signaling. Although both metals upregulate *nodal* gene expression, they do not initiate *nodal* transcription precociously. Analysis of the perturbation of Nodal receptor function suggests that Ni and Co contribute to *nodal* upregulation in the absence of *nodal* autoregulation, but cannot fully oralize the ectoderm in the absence of Nodal signaling.

1. Introduction

Nodal is a member of the activin subfamily of transforming growth factor-beta (TGF-beta) signaling molecules. In vertebrates, Nodal plays a highly conserved role in a number of developmental processes including mesoderm induction and patterning, axis formation and the establishment of LR asymmetry (Whitman, 2001; Hamada et al., 2002; Stainier, 2002). The *nodal* gene has not been identified in the protostomes, *Caenorhabditis elegans* and *Drosophila melanogaster*, but has been identified in sea urchins and urochordates, suggesting that Nodal signaling is unique to deuterstomes (Chea et al., 2005).

Nodal executes multiple functions during sea urchin development, it has been shown to be a key signal in organizing the embryonic dorsoventral (D/V) or oral–aboral (OA) axis (Duboc et al., 2004; Flowers et al., 2004), to play a role in the establishment of left-right asymmetry (Duboc et al., 2005) and in the determination of neuronal fates (Yaguchi et al., 2006, 2007). It has been reported that inhibition of Nodal signaling by morpholino antisense oligonucleotide (MASO) microinjection results in a failure in ectoderm specification (Duboc et al., 2004), in contrast, global overexpression of *nodal* causes oralization of the ectoderm (Duboc et al., 2004; Flowers et al., 2004).

The function of Nodal in early sea urchin development has been the subject of numerous investigations including two recent analyses of *nodal* transcriptional regulation. The *nodal* gene has two distinct cis-regulatory regions involved in the initiation and maintenance of transcription (Nam et al.,

* *Corresponding author*. Tel.: +1 504 599 0157; fax +1 504 568 4392.
E-mail addresses: jvenut@lsuhsc.edu, mrscs@cox.net (J.M. Venuti).
0925-4773/$ - see front matter
doi:10.1016/j.mod.2009.01.005

2007; Range et al., 2007). Once *nodal* transcription is initiated, *nodal* transcripts become amplified and are maintained through an autoregulatory feedback loop involving Smad factors, leading to the rapid accumulation of *nodal* transcripts (Nam et al., 2007). While the initiation of *nodal* transcription in the sea urchin has not been studied as extensively, there is evidence that redox signaling may be involved (Nam et al., 2007).

Classic experiments more than 60 years ago revealed that respiratory asymmetry influenced ectoderm specification (Pease, 1941, 1942a,b). Since changes in respiration can influence redox state, this suggests that respiratory asymmetry may influence *nodal* transcription and ectoderm specification. In further support for a role for redox and respiratory state the activity of the mitochondrial enzyme, cytochrome oxidase, was shown to be higher on the presumptive oral side of the embryo (Czihak,1963, 1971). More recently it was shown that mitochondria are asymmetrically distributed in the unfertilized sea urchin egg, that this maternal asymmetry is maintained in the zygote, and the side with the highest density of mitochondria tends to form the oral side (Coffman et al., 2004). If a respiratory asymmetry is induced by culturing the embryos in tight clusters, a redox gradient is established and the oral ectoderm forms on the most oxidative side (Coffman and Davidson, 2001). Thus, a more oxidative redox state, which is thought to be a consequence of asymmetric accumulation of mitochondria on the oral side, may play a role in the initiation of Nodal signaling.

It has been proposed that activation of a redox-dependent transcription factor initiates *nodal* transcription. This is supported by the identification of a bZIP binding site in the *nodal* gene regulatory region (Nam et al., 2007; Range et al., 2007), and bZIP transcription factors are known to be redox sensitive (Amoutzias et al., 2006). Further evidence for redox signaling stems from demonstration of a requirement for P38 MAPK (Mitogen-activated protein kinase) upstream of Nodal signaling (Bradham and McClay, 2006). P38 is known to be activated by redox signaling and to activate bZIP transcription factors (Clerk et al., 1998; Inoue et al., 2005).

Nickel (Ni) treatment produces the same phenotype as Nodal overexpression (Duboc et al., 2004; Flowers et al., 2004), but it is not clear how Ni influences ectoderm specification. Ni treatment has been shown to generate reactive oxygen species (ROS) in tissue culture cells leading to oxidative stress (Kawanishi et al., 2002; Cavallo et al., 2003; Pourahmad et al., 2003). Ni treatment can also stabilize hypoxia inducible factors (Namiki et al., 1995; Salnikow et al., 2000; Maxwell and Salnikow, 2004) in human cell lines. Cobalt (Co) has similar effects to Ni in human cell lines including the stabilization of hypoxia inducible factors (Namiki et al., 1995; Chandel et al., 1998; Salnikow et al., 2000; Maxwell and Salnikow, 2004), and generation of ROS (Chandel et al., 1998; Salnikow et al., 2000; Pourahmad et al., 2003). The ability of Ni and Co to generate ROS and to stabilize the hypoxia inducible factors suggests that Ni and Co might similarly affect ectoderm specification by influencing redox signaling and/or the respiratory asymmetry.

Here, we compare the effects of Ni and Co treatments on the specification of the oral ectoderm by a high-resolution analysis of their effects on *nodal* expression at early stages of development, and by analysis of their effects on the expression of other oral and aboral ectoderm-specific markers. We show that the effects of Ni and Co on oral ectoderm specification are through Nodal signaling and that these metals share common features: they depend on Nodal signaling to oralize the ectoderm; they upregulate *nodal* transcription at the same developmental time points; they do not initiate *nodal* transcription precociously, but they both can influence *nodal* transcription in the absence of a functional *nodal* autoregulatory feedback loop.

2. Results

2.1. *Morphological effects of Co treatment*

To determine whether Co might have a similar effect as Ni on ectoderm specification, sea urchin embryos were treated continuously after fertilization with different concentrations of $CoCl_2$ ranging from 5 to 750 μM and examined for morphological effects at the pluteus stage (Pl). As the concentration of Co increased, the effects on embryonic development increased in a graded manner. At the lowest concentration tested (Fig. 1B) embryos look morphologically similar to controls (Fig. 1A). Morphological effects of Co treatment were first detected at 25 μM Co (Fig. 1C) as the embryos lose their pointed apex. As the Co concentration increases, embryos are progressively shorter along their OA axis and the aboral ectoderm appears reduced compared to that of control embryos suggestive of a change in the allocation of ectodermal fates. However, development of other lineages such as the endoderm and the skeletogenic mesenchyme does not appear to be adversely affected at moderate concentrations (50–100 μM) of Co (Fig. 1D and E). Severe alterations in morphology were observed at concentrations of 200 and 500 μM Co (Fig. 1F and G). At these concentrations embryos appear rounded but unlike Ni-treated embryos, Co-treated embryos do not form a bell-shape, are not radialized and their gut is oriented towards the oral side as in control embryos. Bilateral skeletal rods were observed at low to moderate concentrations of Co (Fig. 1A–D, lower panels), however, at the highest Co concentrations tested, primary mesenchyme cells (PMCs) were disorganized and spicule growth was inhibited (Fig. 1F–H, lower panels). The effect of Co on spicule growth is distinct from that observed in Ni-treated embryos, where PMCs are radially arrayed and multiple triradiate spicules are observed (Hardin et al., 1992). At the highest concentrations of Co tested, an epithelial thickening develops on the oral side in the region of the presumptive oral hood and the archenteron is reduced in size compared to that of control embryos (Fig. 1G). At 750 μM Co (Fig. 1H), as in higher concentrations of Ni (500 μM), the endoderm fails to invaginate (Flowers et al., 2004). All these results suggest that both metals may affect OA axis specification, although Co treatment has a milder effect.

2.2. *Co treatment expands the oral ectoderm at the expense of aboral ectoderm*

Co-treated embryos exhibit reduced aboral ectoderm compared to controls, but the Co phenotype is distinct