Published in final edited form as:
Mol Reprod Dev. 2011 February ; 78(2): 68. doi:10.1002/mrd.21267.

Oxygen, pH, and oral-aboral axis specification in the sea urchin embryo

Alison E. Coluccio, **Taylor J. LaCasse**, and **James A. Coffman***
Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672 USA

Keywords

hypoxia; CO_2; acidification; nodal; teratogenesis

The oral-aboral axis of the sea urchin embryo is specified via the redox-regulated activation of *nodal* (Coffman et al., 2009, and references therein). Embryos cultured at high density under cover glass develop a radialized phenotype owing to inhibition of *nodal* expression (Coffman et al. 2004), an effect that we attributed to hypoxia. Recently Agca et al. (2009) reported that controlled hypoxia does inhibit *nodal* expression as well as ectoderm differentiation, but only when it is maintained until pluteus stage. However, we found that embryos developed under cover glass become radialized even when the cover glass is removed at early blastula stage (Supplemental Figure 1). We therefore asked whether other factors might contribute to this teratogenic effect.

Since embryos cultured under otherwise identical conditions develop normally, it is clear that the relevant effect of cover glass is to limit gas exchange, which in addition to hypoxia will promote acidification by preventing escape of CO_2. To determine which of these factors causes radialized development, embryos were developed to hatching blastula stage under normoxic (~10 ppm O_2) or hypoxic (≤1 ppm O_2) conditions in culture medium buffered to pH 8, 7.5, 7, or 6.5. While acidic pH was mildly radializing, hypoxia alone produced significant radialization, which was enhanced by acidification (Fig. 1 A, Supplemental Table 1 and Supplemental Fig. 2). Since the embryos were released from hypoxia at blastula stage, these results differ from those of Agca et al. (2009), for reasons that remain unclear. One difference that may be relevant is that we determined the level of hypoxia by measuring dissolved oxygen, whereas Agca et al. (2009) monitored atmospheric oxygen. In addition, we scored morphology at prism stage, whereas Agca et al. did so at pluteus stage, which may have allowed for recovery by regulative development. Finally, Agca et al. did not quantify the phenotypes they reported.

Ratiometric imaging was used to measure the effect of limiting gas exchange on intracellular H_2O_2 and pH (Fig. 1 B, Supplemental Fig. 3). Both mitochondrial and cytoplasmic H_2O_2 were reduced by this treatment, with a decrease in mitochondrial H_2O_2 comparable in magnitude to that produced by a mitochondrially-targeted catalase (Mt-Cat) shown previously to both inhibit *nodal* activation and entrain oral-aboral polarity (Coffman et al.

*Corresponding author: jcoffman@mdibl.org; phone: (207) 288-3605; fax: (207) 288-2130.

2009) (Fig. 1B). This decrease in H_2O_2, a major reactive oxygen species (ROS), contradicts the untested assumption of Agca et al. (2009) that hypoxia elevates ROS. As would be expected, limiting gas exchange also lowers intracellular pH, but less so than the decrease produced by pH buffering that did not induce radialization (Supplemental Fig. 3), suggesting that the relevant pH effects are extracellular.

We conclude that hypoxia is the major cause of the radialization caused by development under cover glass. This is probably attributable at least in part to decreased H_2O_2 levels, as the window of vulnerability corresponds to the initial H_2O_2-dependent activation phase of *nodal* expression (Supplemental Figure 1; Coffman et al., 2009). Extracellular acidification appears to play an ancillary role, perhaps weakening ligand-receptor interactions and thus by dampening the feedback amplification of *nodal* expression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: NIH R01- ES016722 and R25-ES016254

References

Agca C, Klein WH, Venuti JM. Reduced O_2 and elevated ROS in sea urchin embryos leads to defects in ectoderm differentiation. Dev Dyn. 2009; 238(7):1777–1787. [PubMed: 19517573]

Coffman JA, Coluccio A, Planchart A, Robertson AJ. Oral-aboral axis specification in the sea urchin embryo III. Role of mitochondrial redox signaling via H_2O_2. Dev Biol. 2009; 330:123–130. [PubMed: 19328778]

Coffman JA, McCarthy JJ, Dickey-Sims C, Robertson AJ. Oral-aboral axis specification in the sea urchin embryo II. Mitochondrial distribution and redox state contribute to establishing polarity in Strongylocentrotus purpuratus. Dev Biol. 2004; 273(1):160–171. [PubMed: 15302605]

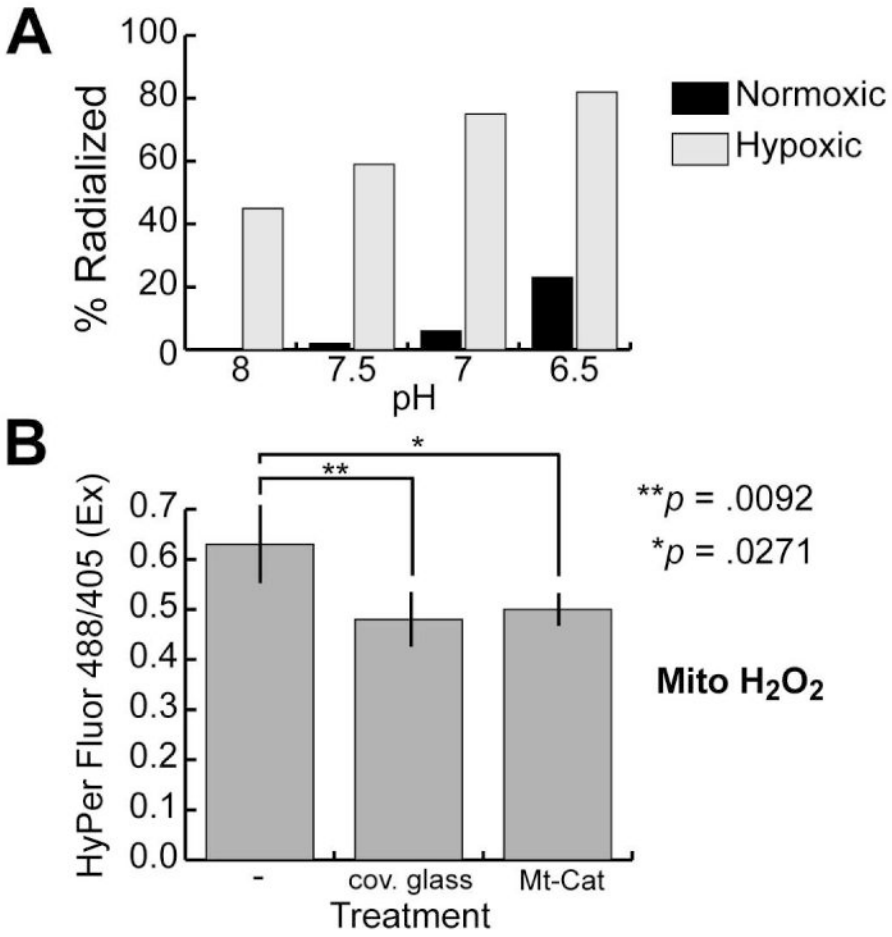

Figure 1.

Hypoxia induces radialized development and reduces mitochondrial H_2O_2 levels in sea urchin embryos. (A) Differential effects of controlled hypoxia and pH on development of oral-aboral polarity. Embryos were developed under the indicated conditions (see Supplemental Table 1 for details) until hatched blastula stage, then transferred to artificial seawater and developed to prism stage under normoxic conditions. At that point the embryos were scored for morphological radialization, defined as absence of ectodermal and endodermal asymmetry, and presence of multiple radially-arrayed spicules. The graph presents the combined results from the two experiments quantified in Supplemental Table 1. (B) Relative mitochondrial H_2O_2 levels in embryos subjected to the indicated treatments, measured using microinjected mRNA encoding HyPer-dMito (Evrogen), a mitochondrially-targeted yellow fluorescent protein derivative whose excitation maximum shifts from of 420 nm to 500 nm upon oxidation by H_2O_2. The indicated ratios were calculated from the average pixel intensities obtained from projected confocal fluorescence images of pre-hatching blastulae, obtained by exciting the embryos at 405 and 488 nm and collecting fluorescence emissions at 530 nm (three to six embryos imaged per treatment). The coverslip treatments were carried out as described in Coffman et al. (2004). Preparation and microinjection of mRNA and image analyses were performed as described in Coffman et al. (2009). Error bars depict the standard deviation; ANOVA followed by Dunnett's test was

used to determine the indicated significance values (JMP version 8.0; Cary, NC). A second experiment using HyPer-cyto mRNA (Evrogen) gave similar results (Supplemental Fig. 3).