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Gene expression in the endoderm during sea urchin development

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Understanding how the embryonic germ layers become competent to form their characteristic tissue types is a problem of fundamental importance to developmental biology. Knowledge of how the endodermal layer is first determined and then differentiates has only recently begun to accumulate. In sea urchins, several different signals have been implicated in endoderm formation, beginning as early as the fourth cleavage division and continuing until just prior to invagination of the endoderm. Recent experiments in sea urchin embryos have shown that the activity of glycogen synthase kinase 3-β and entry of β-catenin into the nucleus during cleavage stages is required for mesoderm and endoderm formation (Emily-Fenouil *et al.*, 1998; Logan *et al.*, 1999), implicating the Wnt signalling pathway in this process. Overexpression of β-catenin leads to an exaggeration of endoderm and mesoderm in the embryo at the expense of ectoderm (Wikramanayake *et al.*, 1998). Since this signal is required for both mesoderm and endoderm, some other signal must be present to differentiate between these two germ layers. Micromeres formed by the fourth cleavage division have the ability to induce endoderm (Ransick & Davidson, 1995). This induction can occur independently of the entry of β-catenin into the nucleus of the cells induced to form endoderm (Logan *et al.*, 1999), indicating micromere induction acts through a different signalling pathway. Final determination of endoderm also requires cell interactions through the late mesenchyme blastula stage, since cells from embryos dissociated prior to that stage fail to develop into endoderm autonomously (Chen & Wessel, 1996). A sea urchin member of the hedgehog family of signalling molecules has been reported to be expressed in the vegetal plate, indicating it also may play a role in endoderm formation.

The expression of genes that reflect the differentiated state of the endoderm requires that the cis-regulatory region of these genes can integrate the information from a variety of signals, both positive and negative. The output of this process is the transcription of the genes in the right place and at the right time. We have isolated a sea urchin gene encoding a member of the winged helix family of transcription factors, *Spfkh1*, that is expressed in the endoderm immediately following final specification (Luke *et al.*, 1997). This gene provides an excellent tool to investigate how a gene integrates information from several signalling pathways that ultimately regulate spatial and temporal gene expression in the endoderm. We have data suggesting the lef/tcf sites in the cis-regulatory region of the *Spfkh1* gene are occupied at the 64-cell stage by a complex of proteins that includes β-catenin. At later stages β-catenin is no longer present in the complex that binds this site. We propose that activation of the Wnt signalling pathway during cleavage results in transport of the lef/β-catenin complex into the nucleus of mesoderm and endoderm cells. One potential target of the lef/β-catenin complex is the *Spfkh1* gene.

Binding of this complex makes *Spfkh1* competent to be activated in endoderm. Additional input from other signal transduction systems involved in endoderm specification leads to binding of other factors required for *Spfkh1* expression in endoderm. Transcription begins at the mesenchyme blastula stage, when factors not present earlier bind to the gene with the aid of the previously bound factors. One potential factor is SpOtx, a sea urchin member of the orthodenticle family of transcription factors. This factor has been shown to act downstream of β-catenin signalling, but the mechanism is unclear (Li *et al.*, 1999). We have identified a potential SpOtx binding site in the *Spfkh1* cis-regulatory region, and this site is bound by nuclear proteins isolated from sea urchin embryos. Binding first occurs at low levels at hatched blastula and is maintained through late gastrula. A GATA binding site is also bound at hatched blastula, but the binding is transient, disappearing by late gastrula.

There is also a cubitus interruptus (ci) binding site within the 5′ coding region of the *Spfkh1* gene. ci is a member of the Gli family of transcription factors, and is downstream of the hedgehog signalling pathway (Chen et. al., 1999). ci can act as either an activator or a repressor of transcription, depending on the form generated by proteolytic processing. We see a complex pattern of proteins binding to the *Spfkh1* binding site, with a unique band appearing at late mesenchyme blastula/early gastrula.

Our results are consistent with the view that sequential signals that determine endoderm cell fate result in

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sequential binding of factors to the cis-regulatory region of endoderm-specific genes. Final specification of endoderm coincides with the assembly of a complex of factors that is capable of initiating transcription (Fig. 1).

References

Chen, S.W. & Wessel, G.M. (1996). *Dev. Biol.* **175**, 57–65.
Chen, C.H., von Kessler, D.P., Park, W., Wang, B., Ma, Y. & Beachy, P.A. (1999). *Cell* **98**, 305–16.
Emily-Fenouil, F., Ghiglione, C., Lhomond, G., Lepage, T. & Gache, C. (1998). *Development* **126**, 2489–98.
Logan, C.Y., Miller, J.R., Ferkowicz, M.J. & McClay, D.R. (1999). *Development* **126**, 345–57.
Luke, N.H., Killian, C.E. & Livingston, B.T. (1997). *Dev. Growth Differ.* **39**, 285–94.
Ransick, A. & Davidson, E.H. (1995). *Development* **1212**, 3215–22.
Wikramanayake, A. H., Huang, L. & Klein, W.H. (1998). *Proc. Natl. Acad. Sci. USA* **95**, 9343–8.

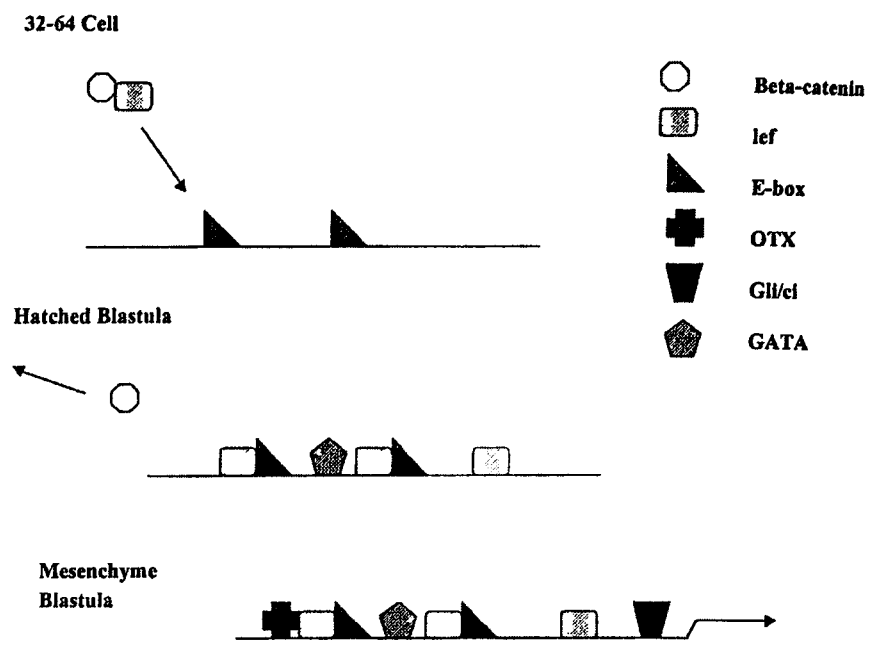


Figure 1 Factors involved in the specification of endoderm during sea urchin development.

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