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DEVELOPMENT

Reinforcing feedback

Photodisc

Imai *et al.* have identified a role for *Pinhead* gene transcription in specifying ventral fate in the sea squirt *Ciona intestinalis*. Furthermore, the genomic position of *Pinhead* — that is, adjacent to the bone morphogenic protein (Bmp) ligand antidorsalizing morphogenetic protein (*Admp*) — was found to be important for this specification, and this feature that looks likely to be conserved in animals.

Bmp signalling in dorsal–ventral specification is mediated by the spatially opposed expression of dorsal Admp and ventral Bmp2/4. Bmp2/4 locally induces ventral fate, whereas Admp migrates ventrally to induce ventral fate. To understand Bmp signalling further, the authors scanned the *C. intestinalis* genome for genes encoding cysteine-knot domains that are important in Bmp signalling. This scan identified the *Pinhead* gene, which is directly upstream of the *Admp* gene. During early dorsal–ventral axis specification, *Pinhead* was expressed ventrally and overlapped with *Bmp2/4* expression but not with *Admp*. By carrying out morpholino antisense knockdowns of these three genes, the authors then showed them to be essential for specifying ventral fate and for regulating each other. The authors also showed that Pinhead protein directly interacts with Admp *in vivo*, thus it might act as an antagonist by sequestering this protein.

To investigate the regulatory relationship between *Pinhead* and *Admp* further, Imai *et al.* cloned a region that included both adjacent genes and their putative regulatory regions, replacing *Pinhead* with an RFP reporter gene and *Admp* with a GFP reporter gene. Deletion of the *Admp* upstream region recapitulated endogenous expression of *Admp* and *Pinhead*. However, deletion of the *Pinhead* upstream region resulted in ectopic ventral GFP expression and decreased RFP expression, indicating that *Pinhead* transcription interferes with *Admp* transcription. Finer deletions identified an enhancer upstream of *Admp* that they termed 'G', which enhanced *Pinhead* expression, and an enhancer they termed 'A', which was adjacent to this and essential for *Admp* expression. A 'P' enhancer was also identified upstream of *Pinhead* that is controlled by Admp and Bmp2/4. A similar construct in Medaka recapitulated these results, indicating conservation of a *cis*-acting mechanism.

To examine the three-dimensional conformation of the *Pinhead* and *Admp* region, the authors carried out chromosome conformation capture (3C) on embryos that were modified to have inverse upregulation and downregulation of *Pinhead* and *Admp*. When *Pinhead* was upregulated, the authors observed a strong interaction between the *Pinhead* upstream promoter region and the A and G enhancers. Thus, when *Pinhead* is transcribed, the *Pinhead* upstream region contacts the G enhancer, which in turn sequesters the A enhancer, which is essential for *Admp* expression.

The authors' data thus indicate dual negative feedback between *Admp* and *Pinhead*. Admp migrates ventrally to activate *Pinhead*, and in turn *Pinhead* transcription sequesters the *Admp* enhancer to prevent its transcription in ventral regions. Pinhead protein interacts with Admp protein to reinforce its repression. The conservation of the genomic architecture of *Pinhead* and *Admp* from arthropods to vertebrates suggests that this dual negative feedback is conserved.

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ORIGINAL RESEARCH PAPER Imai K. S. *et al.* *Cis*-acting transcriptional repression establishes a sharp boundary in chordate embryos. *Science* **337**, 964–967 (2012)