

Effects of growth factors on the commitment of chick blastoderm

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The fate of the blastoderm in early chick embryos has been established by fate map studies. Cells of the epiblast will constitute the neural tube and the epithelial layer of the embryo, whereas the early mesoderm will contribute to the heart, the lateral mesoderm, the somites and the notochord (Garcia-Martinez *et al.*, 1993). The fate of the endoderm has not been determined so clearly, although it is clear that it will form the endodermal derivatives. A great variety of signaling molecules produced in the blastoderm contribute to the process of determination of the fate of these cells. Among them, there are several growth factors belonging to different families which are strongly produced in discrete regions of the embryo and affect the behaviour of blastodermal cells by activating gene pathways that finally lead to the production of different cell types.

Several signaling molecules have been tested and the results have led to important advances in the understanding of early morphogenesis in the chick. It is known that two groups of growth factors are important players in these early steps of patterning and specification in the chick embryo: the fibroblast growth factor family (FGFs) and the bone morphogenetic protein family (BMPs).

By delivering FGFs to ectopic places in the blastoderm one is able to change the fate of epithelial cells so that they become neural cells. Here, when heart specific markers were checked, the same growth factors show the ability to initiate and modulate early development of the heart.

Materials and Methods

Experimental procedures

Fertile chicken eggs were incubated until reached stage 3 (Hamburger and Hamilton, 1951). Embryos were cultured ventral side up on their vitelline membranes according to New (1955). Heparin acrylic beads were used as carriers for administering the candidate growth factors: recombinant human FGF-2, FGF-4 and FGF-8 proteins (R&D Systems; Minneapolis, Minnesota), and human recombinant BMP-2 and BMP-4 proteins (Genetics Institute; Cambridge, Massachusetts). Beads were implanted into the germinal cell crescent of the chick embryos.

Cell specific markers

Embryos were fixed and processed for whole mount *in situ* hybridization using several riboprobes, transcribed from: *Otx-2* cDNA (provided by A. Simeone), rostral regional marker (Bally-Cuif *et al.*, 1995); *cNkx-2.5* cDNA (provided by T.M. Schultheiss), which is expressed in the early cardiac crescent and in the differentiated

myocardium (Schultheiss *et al.*, 1995); *VMHC1* cDNA (provided by D. Bader), which is expressed in all differentiating cardiac myocytes, but its expression is later restricted to ventricular myocytes (Bisaha and Bader, 1991); *Shh* cDNA (provided by C. Tabin), which is expressed in the midline, node and notochord (Roberts *et al.*, 1995), and *paraxis* cDNA (provided by D. Susic and E. Olson), which is expressed in the somites and rostral segmental plate mesoderm (Garcia-Martinez *et al.*, 1997).

Results and Conclusions

Our results show that the growth factors used here induce the expression of different markers at the level of the cells located

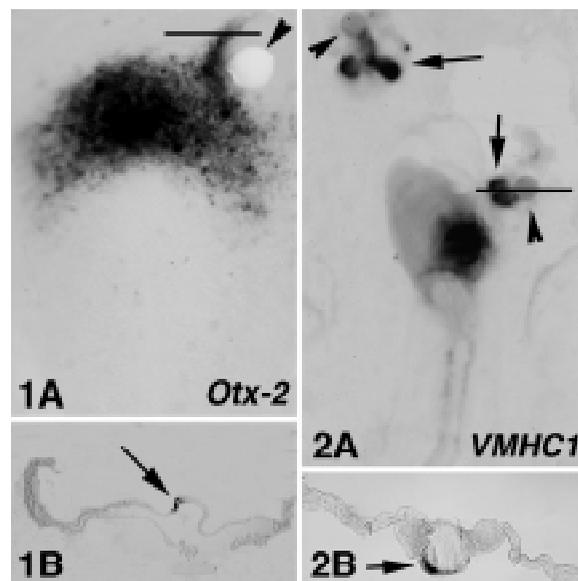


Fig. 1. Whole mount *in situ* hybridization with an antisense *Otx-2* riboprobe after implantation at stage 3 of a bead coated with FGF-4 (arrowhead). Note the expression in some cells located near the bead, in the epiblast layer (arrow), as confirmed by sectioning at this level (black line) in embryo at stage 6.

Fig. 2. Whole mount *in situ* hybridization with an antisense *VMHC1* riboprobe after implantation at stage 3 of two separate beads coated with FGF-4 (arrowheads). Note the expression in some cells located near the bead in the interface between the epiblast and hypoblast of the germ cell crescent (arrow), as confirmed by sectioning at this level (black line).

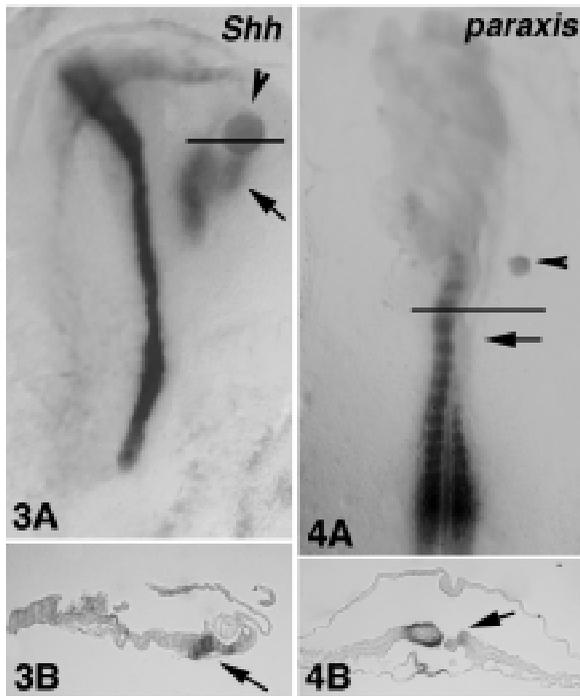


Fig. 3. Whole mount *in situ* hybridization with an antisense *Shh* riboprobe after implantation at stage 3 of bead coated with FGF-4 (arrowhead). Note the expression in some cells located near the bead in the interface between the epiblast and hypoblast of the germ cell crescent (arrow), as confirmed by sectioning at this level (black line).

Fig. 4. Whole mount *in situ* hybridization with an antisense *paraxis* riboprobe after implantation at stage 3 of bead coated with BMP-2 (arrowhead). Note the inhibition of paraxial mesoderm, resulting in fewer somites on the same side as the implanted bead (arrow), as confirmed by sectioning at this level (black line).

around the implanted soaked beads. The ectopic application of members of the fibroblast growth factor (FGF) family changes the specification of the ectodermal cells from epithelial to neural (Alvarez *et al.*, 1998), but its effect after short periods of time has not been checked. Here we show that as early as four hours after the application, epiblast cells can respond to the source of FGF by inducing the neural marker *Otx-2* in some cells near the bead (Fig. 1). Only posterior neural tissue is produced when the beads are applied at HH4 and the embryos analyzed 24 h later, when the main organization of the neural tube is already completed (Alvarez *et al.*, 1998; Streit *et al.*, 1998). Here we have shown that FGF is also able to induce anterior neural tissue and therefore fulfills the requirements to be considered complete neural inducers.

Mesodermal markers were also studied after administration of several growth factors. After FGF-2 and FGF-4 administration, the expression of cardiac specific genes was induced around the bead:

cNkx-2.5 and *VMHC1* (Fig. 2). We can hypothesize that these growth factors may participate in cardiogenesis, initiating and modulating this morphogenetic process. Also FGF-2, FGF-4 and FGF-8 induced *Shh* expression in non-medial tissues of the embryo (Fig. 3), reflecting their role as proteins implicated in the organization of axial structures in uncommitted cranial cells. On the other hand, members of BMPs showed an inhibition of the mesoderm differentiation at the level of the paraxial mesoderm (Fig. 4), decreasing the number of somites on the same side as the implanted beads.

These findings demonstrate that several members of FGFs and BMPs are involved in early steps of morphogenetic processes. The same protein can regulate, by inducing or by inhibiting, different morphogenetic processes in different layers of the blastoderm. Thus, inductive and suppressive signals may regulate the expression pattern of several genes, implicated in determination processes.

Acknowledgments

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