Of mice and frogs

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'It may eventually be possible to obtain as complete an account of the developmental mechanics of the mammalian embryo as Spemann and his school have provided in the Amphibia, but it is probable that operations on the mammalian embryo will always be attended with considerable difficulty...owing to its transparency, toughness and stickiness...[which] is probably the most annoying characteristic of all, since it results in any fragment of tissue... intended for a graft, adhering to the operating knives with such tenacity that it frequently becomes entirely macerated during attempts made to free it'.
C.H. Waddington, 1934

With this series of experiments, Waddington[1] was attempting to extend the famous demonstration by Spemann and Mangold[2] that one particular region of the newt embryo, the dorsal lip of the blastopore, causes a duplication of the body axis and the formation of a double embryo when transplanted. They demonstrated that the transplanted tissue profoundly influences the development of other cells by instructing them to change their fate from epidermal to neural and by generating a well-organized axis. Appropriately, they called this region the 'organizer'. In her recent paper in *Development*, Beddington[3] demonstrates the location of the mouse organizer.

What is the organizer? Several criteria can be used to define it: its position in the embryo, the tissues to which it will contribute, the genes it expresses, and its functional properties. Are all these features conserved among different vertebrate classes?

The patterns of cell movements during gastrulation suggest that the primitive streak of the amniote embryo and the blastopore of amphibians are equivalent, since both are the site at which ingression occurs to form the mesoderm. Therefore, the tip of the primitive streak (called Hensen's node in the chick and simply 'node' in the mouse) occupies a position equivalent to that of the dorsal lip of the blastopore (Fig. 1). Cells in the organizer contribute to the same tissues in the different species (Table 1), despite some quantitative differences. Several genes are expressed exclusively in the organizer region (Table 1). Of these, only the homeobox-containing gene *goosecoid* has been cloned in all three species. Interestingly, *goosecoid* mRNA from both frog and chick can mimic some of the properties of the organizer when injected into the ventral side of a *Xenopus* embryo[4,5].

On the basis of their position in the embryo, the fate of the cells and their patterns of gene expression, the dorsal lip, Hensen's node and the mouse node seem to be homologous regions. However, the original definition of the organizer was based mainly on its ability to induce a second embryonic axis. By 1932, Waddington[6] had already obtained double embryos by grafting Hensen's between duck embryos. As Spemann and Mangold had done with the newt, he showed that the ectopic nervous system is host-derived (and therefore induced). Therefore, Waddington assigned similar properties to the node and the dorsal lip of the blastopore. Soon after, he began work on the mammalian embryo, transplanting pieces of rabbit primitive streak into chick or rabbit embryos[1,7]. The occasional development of a secondary axis led him to the conclusion that it is 'probable that the embryonic development of mammals is influenced by factors similar to those which have become familiar in Amphibia and birds'. However, because of a lack of suitable markers, he was unable to distinguish host

TABLE 1. Comparison of properties of the organizer in frog, chick and mouse

	Organizer region		
	Frog	**Chick**	**Mouse**
Developmental stage	**Stage 10–11**	**Stage 3–4**	**6.75–7 d**
Fate	a	b	c
Notochord	+	+	+
Head mesoderm	+		
Somites	+/–	+	+/–
Endoderm	+	+	+
Neural tube	+/–	+	+
Gene expression			
forkhead family	Yes[d]		Yes[e]
goosecoid	Yes[f]	Yes[g]	Yes[h]
noggin	Yes[i]		
nodal			Yes[j]
Function			
Induction of a second axis	Yes[k]	Yes[l]	Yes[m]
Synthesis of retinoic acid	Yes[n]	Yes[o]	Yes[p]
Duplication of digits		Yes[q]	Yes[p]

[a]Refs 10, 11, [b]Ref. 12, [c]Refs 3, 13–15, [d]Refs 16–18, [e]Ref. 21, [f]Ref. 19, [g]Ref. 5, [h]Ref. 9, [i]Ref. 20, [j]Ref. 22, [k]Ref. 2, [l]Ref. 6, [m]Ref. 3, [n]Ref. 23, [o]Ref. 24, [p]Ref. 25, [q]Ref. 26.
In the different species organizer cells contribute to tissues to a different extent: +, major contribution; +/– minor contribution.

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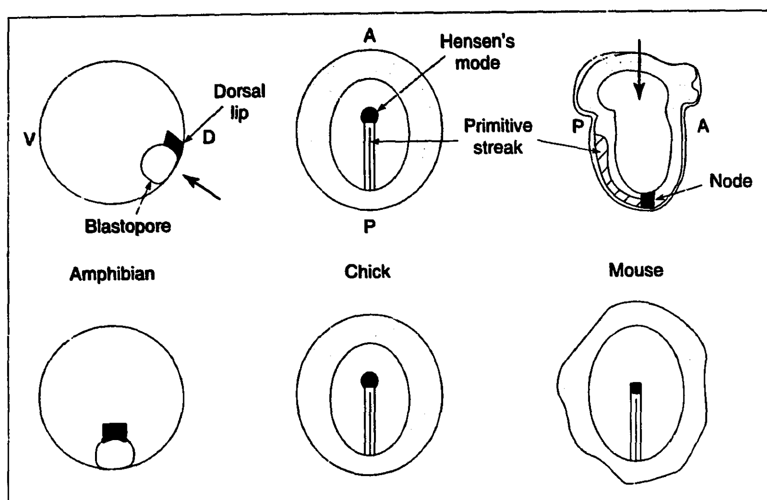

FIGURE 1. Homologous structures in amphibian, chick and mouse embryos during gastrulation. Cells ingress through the blastopore in the frog, and through the primitive streak in chick and mouse. Mesoderm forming at the dorsal lip of the blastopore or the tip of the primitive streak will be axial–dorsal in character; for example, the notochord. The filled area represents the organizer region; the stippled area, extraembryonic tissues. Top: Diagrams showing the embryos as they are usually represented. Bottom: Diagrams showing the same embryos taking a viewpoint (arrow in upper row) that places the organizer in the centre. A, anterior; D, dorsal; P, posterior; V, ventral.

from donor tissue with any confidence, and therefore could not distinguish induction from self-differentiation of the graft.

Because of the obvious technical difficulties with mammalian embryos, studies have turned to a different strategy, as begun by Waddington: combining inducing and responding tissues from different organisms (Table 2). We now know that frog embryos can respond to signals emanating from the chick node[8]. In a recent attempt to demonstrate organizer activity in the mouse, Blum and colleagues[9] showed that an apical fragment of the mouse embryo, which includes the region that expresses *goosecoid* (the node), can induce a second axis in frog embryos. These two studies show that similar signals and mechanisms may indeed be involved in setting up the organization of the body axis in different vertebrates. As to the exact location of the organizing centre in mammals, Beddington's studies[3] have now clearly assigned it to the node. She studied two aspects of the organizer: the fate of its cells and its ability to induce a second axis. Labelling with the carbocyanine dye DiI was used to follow the descendants of ventral node cells, revealing that in mouse, as in chick and frog, they contribute to the notochord. To investigate whether the mouse node has organizer properties when transplanted, donor tissue labelled with DiI or derived from transgenic mice expressing the *lacZ* gene was used to distinguish between graft and host tissue; this showed that, provided the transplanted cells differentiate into notochord, the graft induces a second axis containing host-derived neural tissue.

Thus, although the superficial appearance of embryos of different vertebrate classes seems to differ considerably, there are topographically and functionally homologous regions in frogs, chicks and mice: the dorsal lip, Hensen's node and the 'node'. Beddington's study has, at long last, made the mouse a respectable organism for manipulative embryology (albeit only for the privileged few who share her skills), paving the way for new approaches to the study of mouse development that combine molecular techniques, classical genetics and embryology.

TABLE 2. Cross-species transplantations of the organizer region

	Graft		
	Amphibian	**Bird**	**Mammal**
Host			
Amphibian	Yes[a]	Yes[b]	Yes[c]
Bird		Yes[d]	Yes[e]
Mammal		Yes[f]	Yes[g]

[a]Ref. 2, [b]Ref. 8, [c]Ref. 9, [d]Refs 6, 27, [e]Refs 1, 7, [f]Ref. 1, [g]Refs 3, 7.

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TECHNICAL TIPS

Efficient induction and preparation of fusion proteins from recombinant phage λgt11 clones

After the required recombinant phage λgt11 clone is detected and plaque-purified, it is often necessary to obtain preparative amounts of the recombinant protein, which is specified by fusion of the cloned sequence to the carboxyl terminus of β-galactosidase in this expression system. The conventional method[1] for preparing fusion proteins from these clones involves production of phage lysogens of *E. coli* strain Y1089 and induction of *lacZ*-directed expression of the fusion protein using isopropyl-β-D-thiogalactopyranoside (IPTG). This method has two limitations: it is time consuming, and phage lysogeny occurs at a low frequency. We have previously described[2] a method for preparing fusion proteins from L agar plates of *E. coli* Y1090 infected with a high concentration of recombinant λgt11 phages (up to 5×10^6 p.f.u. per 150×15 mm plate). More recently, Runge[3] has described a method for preparing fusion proteins from liquid cultures of *E. coli* Y1090 infected with λgt11 clones. Here, we present some improvements to our plate-induction method.

First, the concentration of IPTG used can be reduced to 5 mM (Fig. 1). Second, the induction of fusion protein expression can be repeated three or four times by adding IPTG-containing medium. For any single induction of the ERP72 protein, the maximum effect was seen after 3–5 h. ERP72 fusion protein could be induced and eluted from the plate as many as five times[4] (Fig. 2). It was not necessary to scrape off the top agar to extract the fusion protein. While the liquid culture method allows the recovery of only 0.2–1% of total protein[5,6], this method generally yields ~5–10% of expressed protein in solution: most lysed cells are trapped in the agarose and the expressed proteins are recovered in a small volume of inducing solution, resulting in a higher final concentration of protein. More than 200 μg of fusion protein can be obtained from one plate. Supernatants from each induction are saved and analysed by western blotting.

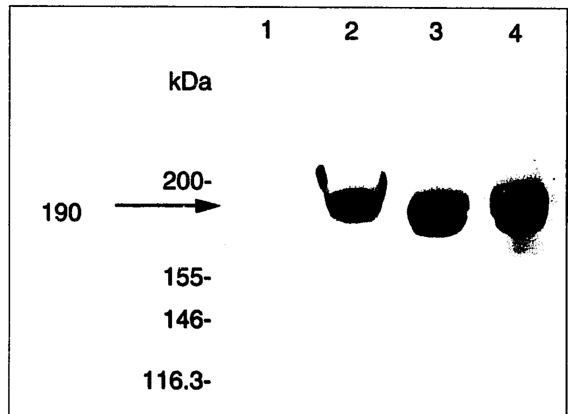

FIGURE 1. Induction of the 190 kDa ERP72 fusion protein with various concentrations of IPTG (lane 1, no IPTG; lane 2, 2.5 mM; lane 3, 5 mM; lane 4, 10 mM). Fusion protein was detected by western blot analysis with anti-ERP72 antibody.

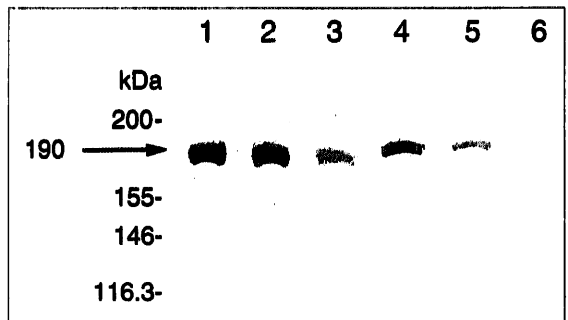

FIGURE 2. Repeated induction and elution of the 190 kDa ERP72 fusion protein from agar plates. *E. coli* Y1090 cells were infected and plated by the standard procedure[1]. Plates were incubated for 3 h at 42°C, and 5 ml of 5 mM IPTG, 10 mM $MgSO_4$, $0.5 \times$ L broth added to each plate. Induction was carried out at 37°C for 3 h (lane 1) and the supernatant recovered. Induction and elution were repeated five times with one-hour intervals between inductions (lanes 2–6). Fusion protein recovered after each induction was analysed by western blotting.

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