LONG RANGE SIGNALLING PROCESS IN EMBRYONIC DEVELOPMENT

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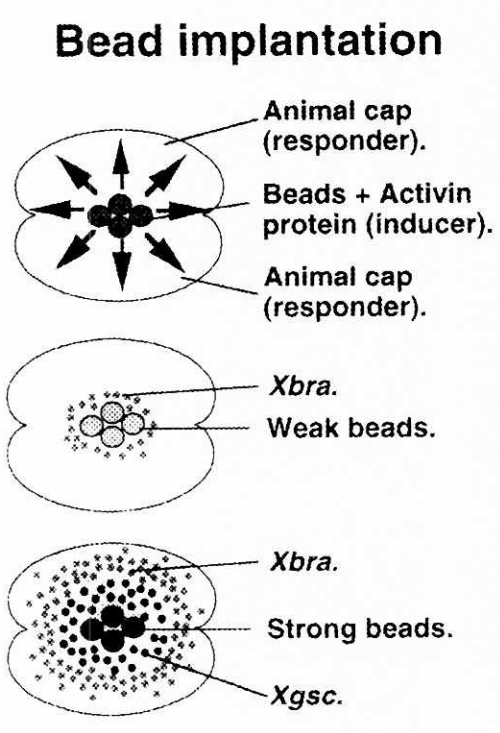
Long range signalling includes situations in which a cell or a group of cells emit secreted signalling molecules (usually proteins) that influence other cells located several cell diameters away from the source. Such processes are believed to operate during early amphibian development, as in mesoderm (Nieuwkoop) induction and neural (Spemann) induction. They are very likely also to be involved in neural induction in the chick and in chick limb morphogenesis. In these cases, it is believed that the secreted signalling molecule forms a concentration gradient to which cells in the path of that gradient make different kinds of responses. Thus, this is a developmental mechanism of considerable potential importance because a single signalling event may generate many different cell types and differentiation pathways. Furthermore, a general understanding of how long range signalling between cells takes place could have considerable therapeutic benefits in those cases where it could be useful to alter the effective range of signalling molecules.

In this laboratory, we devised a new experimental approach to the analysis of cell response to a morphogen gradient. We use cells injected with mRNA or chromatographic beads to which purified protein is bound as a signal source and place these sources next to a field of responsive but uncommitted cells derived from the animal cap of a *Xenopus* blastula. In this system we assay the response of cells to different concentrations of our secreted morphogen (the TGFb molecule activin) by in situ hybridization with gene-specific probes. We have found that cells respond to low concentrations of activin by transcribing the gene *Xbrachyury* (*Xbra*), and to higher concentrations by transcribing the gene *Xgoosecoid* (*Xgsc*). Cells respond to an increasing concentration gradient of activin by first expressing *Xbra* and subsequently *Xgsc.* A ripple of *Xbra* expression moves centripetally from the source of activin signalling as the concentration of activin increases with time. The ripple of *Xbra* low response is followed immediately by *Xgsc* high response in all those cells where the activin concentration is believed to have exceeded a threshold value. Fig. 1 illustrates the way in which this experimental system works. The key features of this system are the following. First, the whole process from the start of signalling to detectable gene response takes place within 2 - 3 hours at 23°C. Second, there is no cell movement and very little cell division; as a result, we have an essentially static lawn of cells through which an increasing concentration gradient of morphogen passes, activating different genes as the concentration increases. The key advantage of this particular experimental system is that the same cells are seen to have at least three choices of response, namely no change of fate (ectoderm), or low response (*Xbra*) or high response (*Xgsc*). Several other genes recently tested fall into the class of low or high response genes (Gurdon *et al.*, 1996).



This experimental system is being pursued in several directions. First, there is evidence (S. Dyson *et al.*, submitted) that activin is a necessary signalling molecule in early *Xenopus* development. Second, McDowell *et al.*, (in preparation) have evidence that activin is itself required for a long range distance response, suggesting that this signalling process takes place by diffusion of activin rather than by a local action of activin causing release of another kind of signalling molecule. Third, we have recently identified two very early response genes, that is genes which seem to respond directly to activin. One of these, namely *Eomesodermin*, is a new T-domain gene that is expressed 1 - 2 hours before *Xbra* and that is able to activate all other mesoderm-specific genes when overexpressed (Ryan *et al.*, submitted). We have also identified another new T-domain gene, namely *Antipodean*; this gene is also activated zygotically at the same time as *Eomesodermin*, and can activate several early mesodermal genes. Furthermore, there is a substantial maternal content of *Antipodean* transcripts which are strongly localized to the vegetal end of *Xenopus* oocytes, suggesting a role in very early events (Stennard *et al.*, submitted). Apart from a general interest in these new DNA-binding transcription factors, we are especially interested in the identification of the promoters of these genes, in order to find proteins that interact with these promoters. This will help us to work backwards from gene transcription resulting from activin signalling to the activin signalling process itself. The aim is to understand how different concentrations of the same morphogen, in this case activin, can cause activation of different immediate response genes.

As a longer term objective, we plan to use our experience gained with the above activin signalling system to analyze the mechanism of another signalling process, referred to as the community effect. Currently, several known secreted signalling factors are being tested for their role in the community effect (Carnac *et al.*, in preparation).

References

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