A Turing Model To Explain Heart Development

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The question of how the precise localization of the heart is achieved has been bothering me for some time. In 1926, Copenhaver showed that the heart field—that region of the mesoderm capable of responding to the inductive signal—is much larger than the formed heart will be. The extent of the inductive tissue is also fairly great. Fullilove (1970), working with the newt, showed that much of the pharyngeal endoderm is active. In the axolotl, we have shown that the midventral pharyngeal endoderm is most active, but that the lateral walls of the pharyngeal cavity are also active. The advancing sheets of lateral mesoderm are travelling over this inductive endoderm from at least stage 14 to stage 28. We have shown both that the endoderm retains inductive activity for this entire period, and that the mesoderm is capable of responding (at least *in vitro)* for most of it. Why is only the leading edge induced to form heart tissue? Perhaps there is significance in the fact that the heart is induced while the mesoderm is still separated into two advancing sheets. Thus, just the leading edges have to be specified to form heart, and we need some mechanism to prevent the tissue further back from doing so as well.

The second part to this puzzle is the experiments of Humphrey (1972) on the cardiac mutant. Cardiac hearts never start to beat (or do so only weakly in the conus region). Humphrey transplanted presumptive heart tissue from stage 28 cardiacs into wild-type hosts and found that the heart started to beat normally (at about stage 35), whereas wild-type heart tissue transplanted into cardiac hosts at the same stage failed to beat. He concluded that there was either a failure of induction in cardiac, or that the mutant produced an inhibitor of heart differentiation which was capable of preventing the initiation of beat in wild-type heart tissue transplanted into the mutant. Most of the subsequent literature on cardiac favored the inductive failure model, but we now know that +/+ heart-forming mesoderm explanted at stage 20 is fully capable of forming beating tissue, so why should it not do so in a cardiac enviroment unless there is inhibition? I do not find attractive the idea that only the mutant produces an inhibitor. It seems more likely that the inhibitor is also present in the wild-type to restrict in some way the extent of the heart, and that it is overproduced in the mutant.

In 1968, Jacobson and Duncan suggested that neural tissue was inhibitory, based on their experiments with the newt. We set out to determine whether this was also true in the axolotl, and tested some other tissues as well. All had relatively little effect, at least in our *in vitro* system. Then a possible solution came to me. Where is the inhibitor localized in systems where an inhibitor has been found, and there is precise spatial localization of structures? The best example that came to mind is the formation of the head (and foot) in Hydra (Bode and Bode, 1984). In this system, there are gradients of a head activator and a head inhibitor, and the high point of both is the head itself. The activator gradient peaks sharply at the head; the inhibitor gradient is somewhat shallower and extends further. This is exactly what is predicted for a Turing reaction-diffusion model (see, for example, Gierer and Meinhardt, 1972). In Gierer and Meinhardt's formulation, a shallow source gradient along a column of cells, or a small peak of source activity at one end, is sufficient to 'fire' the system and produce a sharp peak of activator (Fig. 1). What if the function of the endoderm is to induce the sources? Since the strongest inductive activity lies in the mid-ventral endoderm, with inductive activity decreasing somewhat up the sides of the pharyngeal cavity, the advancing mesodermal sheet will gradually be exposed to higher inductive activity. The leading

edge will receive the signal first, and a gradient will be set up within the mesoderm. The fact that a broad area of mesoderm is ultimately exposed to the inductive activity is unimportant. What is important is that the induction leads to a source gradient whose shape and extent are *not* the major factors determining the sharpness of the subsequent activator and inhibitor gradients.

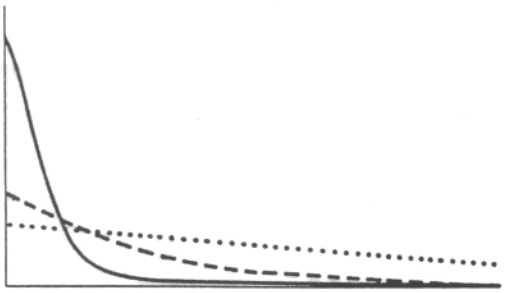

Fig. 1. Gradients of activator (—) and inhibitor (- - -) predicted by the equations of Gierer and Meinhardt (1972) assuming a shallow source gradient (·····).

How do we explain Humphrey's results for the cardiac mutant? Remember that the gradients, particularly of inhibitor, extend beyond the heart itself. When a wild-type heart is transplanted into a cardiac mutant, it is surrounded by mesoderm genetically programmed to produce a high level of inhibitor. Presumably this inhibitor diffuses into the transplanted tissue and prevents the activator from completing the sequence of events necessary to get a beating myocardium. Humphrey's reverse experiment (c/c heart into +/+ host) is a bit harder to explain. One may suggest that the high level of inhibitor in the cardiac tissue is bled off into the 'sink' of surrounding wild-type mesoderm. Why doesn't this happen when cardiac hearts are explanted? Actually, Kulikowski and Manasek (1978) reported that it does, though their observations have been contradicted by others. The explant result may depend on the medium used, and the fact that the 'sink' (in this case the culture medium) provides neither activator nor inhibitor. We believe, however, that Kulikowski and Manasek were wrong in concluding that there is no defect in cardiac cytodifferentiation, Lemanski's results (1973, 1976) clearly show a lack of organized sarcomeres, and in our model, the activator is what brings about that organization.

How do we test the model? I suppose, ultimately, by isolating both the activator and the inhibitor. Could it be that Davis and Lemanski's (1987) RNA is the activator? It certainly has some of the right properties: it corrects cardiac mutant hearts, but it does not seem to be the inducer (as they suggested) as it is without activity on uninduced stage 14 heart-forming mesoderm. It remains to be shown that the highest levels are in the heart itself, as would be required by our model. If cardiac overproduces the inhibitor, we could use cardiac hearts as a source of inhibitor to test on explanted wild-type hearts. If we find inhibitory activity, that activity can be characterized further.

If one really wants to go out on a limb, one might suggest that the activator/inhibitor system is first interpreted by the cells at the leading edge of the advancing mesodermal sheet as a signal to form endocardium. These cells then separate from the rest of the mesoderm, and the gradients in the remaining mesoderm readjust themselves (as they do following the amputation of a Hydra's head) so that they now signal the new leading edge to form myocardium. We don't get two endocardia because the first signalling has already sent the remaining mesoderm down a non-endocardial pathway.

The author would welcome comments, criticisms and suggestions, but please don't throw anything!

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