Models have real value

Those of you who have kept tropical fish will almost certainly have had the Zebrafish (Danio rerio) in your menagerie. You may not know that this fish is now perhaps the most important tool in the investigation of normal development and in the identification of candidate genes that play a determining role in human malformation syndromes.

A deliberate programme of chemical mutagenesis has identified hundreds of mutations that affect vertebrate development affecting the form of the embryo, the generation of the germ layers, organogenesis, differentiation, the cytoarchitectonics of the brain and the vasculature (of which more later). In the Zebrafish, it is possible to explore the important area of how single gene changes may affect the structural adaptations that occur during evolution, as well as the kind of change in gene function that occurs in the evolution of multigene families (such as the homeoboxes).

What was the problem with other systems? It was really the difficulty with which selected mutant phenotypes could be recovered in a diploid vertebrate embryo. Since one of a very large number of genes might be the one involved in a developmental failure of phenotype, it was difficult to find a way to identify rare recessive mutations, and to propagate them in the heterozygous (carrier) state. In the favoured nematode model C. elegans, this could be done because its hemaphroditic lifestyle meant that single heterozygous carriers could produce both homozygous and heterozygous forms.

What’s good about the Zebrafish is that it breeds prodigiously (hundreds of offspring per female), and because fertilization is external, gametes can be harvested separately, allowing all kinds of genetic manipulation, including ploidy changes (see reference 1). As an example, to generate wholly or partially homozygous diploid offspring, an egg is activated (several methods are available) and its haploid set of chromosomes is allowed to replicate. The initial segregation of chromosomes into daughter cells is then prevented by suppression of the first mitotic cleavage of the zygote. To get partial homozygosity, the second meiotic division is inhibited in an activated egg, producing a diploid embryo whose genetic composition is derived entirely from sister chromatids (half tetrad). As recombination between non-sister chromatids occurs before the second meiotic division, this procedure yields a heterogenous set of offspring; genes near the centromere tend to be homozygous on the sister chromatids, and those distal will tend to be heterozygous. More simply, you can see through the embryos, which is a great help in watching things happen.

Two researchers—Kimmel in Oregon and Nusslein-Volhard in Tubingen, both with effective teams—are acknowledged to have led the work that has resulted in a clear appreciation of the uniformity of developmental processes in vertebrates by using the zebrafish. More than 4000 embryolethal mutant phenotypes have been recovered, and a number of candidate genes for human malformations have been identified in this way.

So to vascular development, a personal interest. I had long thought that while the form of the cardiovascular system was determined by the genes, the nature of vessel development (muscular rather than elastic artery, for example) was flow-dependent. Indeed, I and others had done a good deal of work that seemed to support the idea. Acardiac embryos have muscular rather than elastic aortas, the carotid arteries of anencephalics are muscular rather than elastic, and the common iliac artery in cases of the single umbilical artery syndrome is elastic on the side which will give rise to the umbilical artery—and has a high flow—while the other common iliac is muscular.

But work on the endothelium of the developing zebrafish suggests that at least part of the vessel is designated venous or arterial very early on—before the onset of the circulation. Notch is a large transmembrane receptor that has an important role in vascular signalling in vertebrates. Mice lacking notch ligands have abnormal vascular development and growth; Notch1 and Notch2 are expressed in endothelial cells, and the expression of Notch4 is confined to them. The expression of Notch—a major player in determining how cells make choices about the pathway of development they
will follow—at the time of somite formation, allows angioblasts to make this type of choice. In early development, Sonic hedgehog (Shh) produced by the notochord induces local mesoderm to produce vascular endothelial growth factor (vegf), and those angioblasts about to form the dorsal aorta are induced by Notch to produce arterial endothelial-related markers. EphrinB2, the product of Efnb2 and a member of a family of membrane-bound ligands, is expressed in arterial but not venous endothelial cells; Efnb4, which encodes the receptor for Efnb2, is found at much higher levels in veins than in arteries (there are a number of other markers that show this type of specificity in the chick, mouse, and frog). Activation of the Notch pathway also represses the venous cell fate, and in this regard the posterior cardinal vein cells represent the default situation.

This, of course, does not affect the later development of the vessel wall, but what is the significance of this early endothelial differentiation? It has been suggested that it is to enable other cells to join a developing artery or vein as they are signalled—after all, many arteries and veins develop in close proximity, and the pattern of a network might well be affected by this type of signalling.

We don’t know, but the Zebrafish will help. Zebrafish embryos in which vegf or Notch signalling is reduced have inappropriate connections between the dorsal aorta and the posterior cardinal vein, suggesting the two have failed to form as distinct entities. These can be observed as they develop. Further manipulations will no doubt follow.

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References