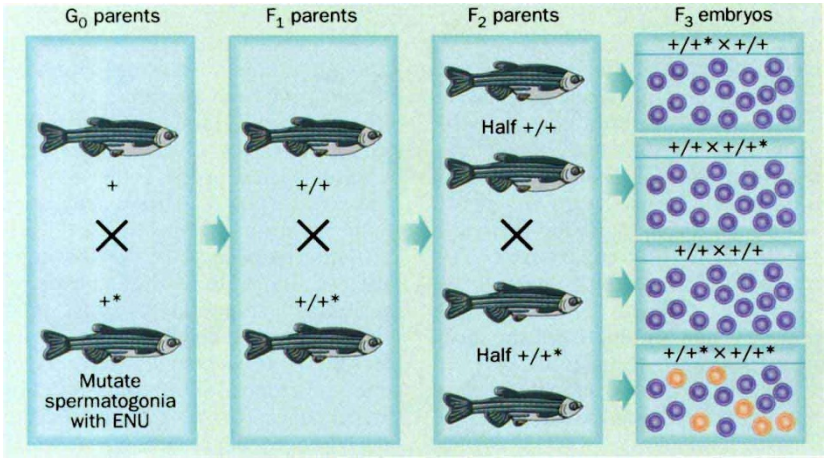

THE F_2 screen was developed by Nusslein-Volhard and Wieschaus to identify new genes in *Drosophila*, and it has now been used in zebrafish. Adult male fish are treated with ethyl nitrosourea (ENU), which generates point mutations in DNA. The chemical concentration and treatment regimes mean that, on average, just over one mutation is introduced per genome. Males are treated because mutations in spermatogonia — the stem cells of the male germ line located in the testis — can be conveniently introduced into the gene pool in large numbers from relatively few (40–50) fish. The resulting F_1 progeny are heterozygous for a unique set of mutagenized genes. F_2 families are then raised from single pairs of F_1 fish and, in any one family, 50 per cent of the siblings will be heterozygous for the same mutation. F_2 siblings are then crossed, and two siblings that are heterozygous for the same mutation will be crossed with each other, on average, in 25 per cent of cases. A quarter of the F_3 embryos created from these crosses will be homozygous for a particular mutation, and these are scored visually. □

So the zebrafish got the nod. It is one of the sad ironies of research that, having had the initial vision and done much of the hard work, Streisinger died in 1984 before he could bring his screens to fruition. The survival of the zebrafish as a vertebrate developmental system is largely due to Charles Kimmel and his colleagues in Oregon. They took up the mantle of Streisinger and put zebrafish developmental genetics on a firm footing, from which the Driever and Nusslein-Volhard groups took off.

In parallel, the two groups set out to saturate the zebrafish genome with mutations and to identify all of the major developmental-control genes. The scale of the enterprise was impressive — Nusslein-Volhard's *fischhaus* in Tübingen contained some 6,000 fish tanks, and Driever's group in Boston had almost as many. Following the initial F_2 screen (see box), complementation crosses were done to identify how many genetic loci had been affected. The screens were designed to reveal mutations that had a zygotic effect in a range of developmental events, such as establishment of the embryonic axes; patterning of the germ layers (ectoderm, mesoderm and endoderm); formation and morphogenesis of organs such as the heart, brain and sense organs; and generation and differentiation of neural crest. Mutants were identified by visual inspection of the largely transparent embryos and larvae at key stages during the first five days of their life.

More than 1,000 mutations were obtained in both screens. From the Boston screen, 577 mutations are reported in the special issue of *Development*, and complementation analysis has so far confirmed a total of 220 genes. The Tübingen screen reports 1,163 mutations, representing 369 genes. Many of the mutations give rise to fascinating phenotypes, affecting the development of various organ systems and the formation of the early embryonic axes. There is no doubt that these mutants represent an impressive outcome and an unparalleled resource for vertebrate developmental biologists.

Despite their obvious success, the screens did not achieve everything their originators might have initially hoped for — they were a learning process for all concerned. Because the groups only searched for obvious visible phenotypes, many potentially interesting mutations may have gone undetected or have been discarded prematurely. Also, it is not always clear what phenotype to expect from a mutation in a key gene. The original Nusslein-Volhard and Wieschaus screen in *Drosophila* indicated that failure to make important cell-fate choices often resulted in cell death. And extensive cell death was observed in many of the discarded mutants. So were interesting mutants lost amongst those that affected the less interesting housekeeping functions? It is clear from fly and worm genetics that the genes that are identified are defined by the type of screen performed.

An indication that the zebrafish screens did not reach saturation is the rate of generation of alleles for a particular gene. Although more than 10 alleles were found for some genes, only one allele could be found for many others. It is likely that, as in *Drosophila*, many of the main early control points are governed by maternally encoded information, and that a maternal-effect screen will be needed to examine some of the most interesting and elusive aspects of vertebrate embryogenesis. Other small-scale screens that have a particular developmental process in mind will also probably be successful in identifying new loci. One such ingenious screen, performed in Friedrich Bonhoeffer's laboratory, focused on the retinal–tectal connection in the formation of the visual system. They used dye-tracing from the retina to the tectum to uncover a number of hitherto unknown genes that alter axonal projections.

It is one thing to find the genes by mutation, but how can they be cloned? The answers to this persistent question were discussed at a meeting at Cold Spring Harbor (April 24–26 1996, "Zebrafish Development and Genetics"). A detailed genetic map complete with random markers spaced at approximately 3-centimorgan intervals is now available, and the resolution is likely to improve in the near future. The map has already been used to identify a cloned gene as the culprit for a specific mutation, and it should soon be used for the first time to positionally clone a gene. A yeast artificial chromosome (YAC) library is also available, which may be used to rescue the phenotype following injection into the fertilized egg. The gene of interest could then be identified by deletion analysis with the rescuing YAC. Somatic-cell hybrid panels and radiation hybrids are available, and there are definite regions of synteny (conservation of chromosomal structure) between zebrafish, pufferfish and human chromosomes. It shouldn't be long before we are able to clone this plethora of interesting genes — Streisinger would have been pleased. □

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