Meeting report

Fish are rising

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A report on the second European conference on zebrafish genetics and development, University College, London, 19-22 April 2001.

Several major factors that are bringing the zebrafish to the fore as a genomic resource and genetic model system were showcased at this meeting. The small, cheap, tropical, freshwater zebrafish is an important vertebrate developmental model organism that is widely used for random mutagenesis projects and is well suited to embryological manipulation because of its externally developing transparent embryos. More recently, new techniques including insertional mutagenesis, imaging and ablation of cells in live embryos, using easy-to-make transgenics carrying green fluorescent protein derivatives under a host of promoters, and the GAL4-UAS system of targeted expression have been added to the zebrafish repertoire, revealing its sheer elegance as a model organism. Some people would say that the mouse has the advantage of reverse genetics (gene knockout technology), but the advent of translation-blocking morpholino oligonucleotides in the zebrafish (and *Xenopus*) communities provides similar results for a fraction of the cost and time of mouse gene knockouts without positional effects.

The zebrafish genome sequence

If this all sounds too good to be true, then there is more: the zebrafish genome will be fully sequenced by way of an annotated physical map by 2003. Funding from the Wellcome Trust is allowing the Sanger Centre (represented at the meeting by Jane Rogers) to shotgun-sequence the genome, with daily release of data to the Sanger's own FTP site and repositories of raw sequence traces at the European Bioinformatics Institute (Hinxton, UK) and the National Center for Biotechnology Information (National Institutes of Health, Bethesda, USA). Coverage of the genome is predicted to reach threefold by December 2001, and the Sanger Centre is already providing a high-stringency search engine for the zebrafish community to use in accessing the sequence. This will be followed by increased efforts to sequence a minimal overlapping set of bacterial artificial chromosomes (BACs) covering the whole genome (being generated by Robert Geisler, Max Planck Institute for Developmental Biology, Tübingen, Germany and Ron Plasterk, Hubrecht Laboratory, Utrecht, The Netherlands). High-throughput restriction fingerprinting of BAC libraries is under way to generate tenfold coverage of the 25 chromosomes of the diploid zebrafish genome, equivalent to 2,600 cM or 1.7 gigabases. Contigs generated using overlapping restriction profiles are being anchored onto the zebrafish radiation-hybrid and genetic maps using BAC end sequences. The mapping and sequencing project is patently of tremendous significance to the zebrafish community. Together with the incomplete genome sequence of another model fish species, *Fugu rubripes*, in the public domain, and with the mouse and human sequence, it will finally allow better identification of conserved intergenic enhancers and control regions.

From genes to phenotypes by multiple approaches

Two ways to get from your gene of interest to a loss-of-function phenotype were presented, with the new morpholino technique leading the charge. Morpholinos are antisense oligonucleotides with altered backbone chemistry made by Gene Tools LLC [http://Gene-Tools.com] that can be directed to the AUG region of a transcript and block translation (see *Genome Biology* **2**(5):reviews1015). Delivery *in vivo* for zebrafish is achieved by injection into the early embryo between 1-cell and 16-cell stages. Many groups showed specific, consistent and reproducible phenotypes, with only minor caveats. Morpholinos directed against the *extradenticle*-related *Pbx* homeobox genes were used by Andrew Waskiewycz (Cecilia Moens' group, Fred Hutchinson Cancer Research Center, Seattle, USA), singly, in combination and

even in a mutant background to produce a dramatic phenotypic series involving several family members. It is worth taking into account his warnings, however: a mild neural phenotype is seen even in negative controls that contained a mismatch to the target sequence, and his western blot controls showed that only a 95% reduction in protein level had been achieved with the sequence-matched morpholinos. Other speakers pointed out that very high levels of morpholino have to be injected in order to block translation from maternally deposited messages, which may be present in a relatively inaccessible compartment or conformation.

The other reverse genetic method presented was resequencing, which involves sequencing your gene of interest from frozen testes of thousands of F1 males generated by random mutagenesis in the search for mutations in that gene. This was the work of Ron Plasterk, with the collaboration of Artemis Pharmaceuticals (Köln, Germany), a forward-genetics company that is looking for disease genes using animal models. Resequencing has been used by Plasterk and colleagues to establish an allelic series of the gene *rag1*, which encodes recombination activating protein; the alleles have been taken through fertilization and subsequent breeding to homozygosity of the mutations. Finally, totipotent cell lines and homologous recombination *in vitro* may be on the horizon...watch this space.

Multi-colored fish and chips

Mapping of zebrafish mutations using a gene-chip array (with the familiar Cy3 and Cy5 red and green dyes) of single-nucleotide polymorphisms (SNPs) was detailed by Will Talbot's group (Stanford University, USA and Protogene Inc., Menlo Park, USA). Together, they are setting up panels of SNPs across the genome and eventually across individual regions or chromosomes. These can be hybridized with differently labeled mutant and wild-type DNA. Tell-tale red and green spots emerged from a sea of yellow when they tested a prototype panel of 100 SNPs on previously mapped loci, showing the viability of the approach. Currently, zebrafish mutations are mapped using a panel of 239 CA-repeat-length polymorphisms; this is PCR- and gel-intensive, but a chip-based approach will really speed things up.

Overall the meeting was a tremendous success, so that topping it in Paris in 2003 will be a tall order. As is often the case at zebrafish meetings, there was some bias towards early developmental studies; future meetings might hope to address this by including talks on organ formation, differentiation and disease models. This aside, the buzz about the success of morpholinos in gene 'knockdown' studies must have inspired many of those attending to greater efforts and new approaches.