FEATURED ORGANISM OF ZEBRAFISH (DANIO RERIO)

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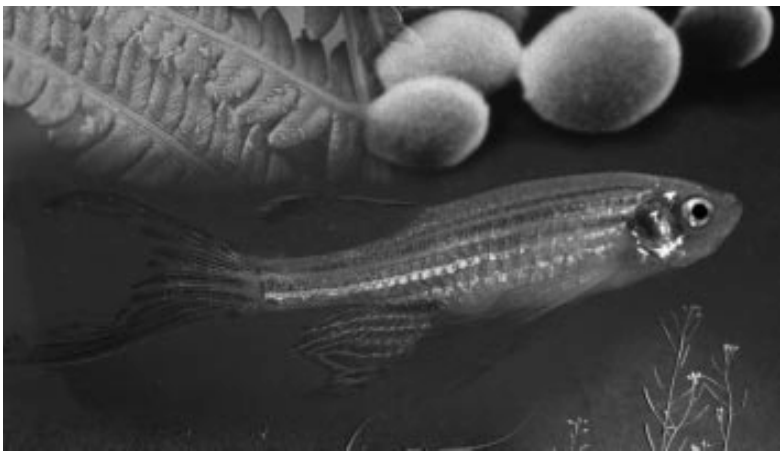
ABSTRACT

This study on zebrafish has long been a favourite model for the research of craniate development. Here I deliver an overview of the present state of information and resources of this fish. It is a slight, shoaling cyprinid, inborn to the floodplains of the Indian subcontinent, where it is originated in shallow, slow-flowing waters. Zebrafish are set spawners and egg scatterers, even though females are choosy about sites for oviposition and males protect territories everywhere such sites.

Keywords : model organism, mutation, zebrafish behaviour, genome.

I. INTRODUCTION

Zebrafish (Danio rerio) are slight, approximately 3 cm long. They are inherent to streams in India and are generally kept as pets. The males are slim and triggerman-shaped, with the black longitudinal streaks and generally a gold coloration on the middle and fins. Females are overweight when loaded with eggs and have little, if any, gold on their basements. Zebrafish are easy to increase, with a rapid generation period of 3 months, and the females can lay 100 of eggs at daily intervals. Fertilization is exterior, permitting easy access to kernels for opinion and manipulation. Rising embryos are effortlessly studied under a dividing microscope since they are translucent.



Zebrafish embryos grow fast in 2 - 4 days, with a beating heart and observable erythrocytes by 24 h. Additional benefit of cramming the zebrafish is that, in disparity

to other fish, which can be triploid or tetraploid (which makes the genetic analysis difficult), it preserves the diploid state. The zebrafish has been revealed to be a beneficial model for the growth of numerous complex tissues for instance the kidney, the olfactory system, and the visual system. In the study of hematopoiesis, zebrafish mutants have been detected to demonstrate amazingly alike phenotypes to those in human diseases.

II. TOOLS FOR STUDY

Notwithstanding the lack of a knowledge for gene knockout by the homologous recombination, zebrafish investigators have summative vast numbers of mutant lines. The majority of these were formed in a large-scale mutagenesis plan by means of the chemical mutagen, ethylnitrosourea (ENU), subsequently screening for progressive defects. ENU has the benefit of engendering slight mutations (associated with those instigated by irradiation), which often affect only one gene. These studies revealed mutants defective in the growth of many organ systems, for instance the retina (Malicki et al., 1996), the inner ear (Whitfield et al., 1996), and the cardiovascular system (Stainier et al., 1996). After comprehensive study of the mutants twisted from these experiments, it converted pure that saturation had not been grasped and the beginning of a second large-scale mutagenesis screen for zebrafish, the `Tubingen 2000' screen, was declared recently.

The screen will be accepted by Artemis Pharmaceuticals in association with labs at the Max-Planck-Institutes of Developmental Biology and Immune Biology, the University of Heidelberg, the Howard Hughes Medical Institute in Boston, and University College London (see this issue, pp. 232-234, for a meeting with Dr. Stefan Schulte-Merker of Artemis Pharmaceuticals on the mutagenesis screen). A zebrafish radiation hybrid (RH) panel was formed. This has been used as the basis for a map planned to aid the positional duplicating of the genes transformed in the strains from the mutagenesis screens (Geisler et al., 1999). The NIH Institute-wide Zebrafish Genome Initiative, overseen by the Trans-NIH Zebrafish Organizing Committee, was began in September 1998. A YAC public library has been formed, which contains of 17000 clones, and delivers approximately five-fold exposure of the zebrafish genome. There is correspondingly a P1 library, which entails of 4-5 genome equivalents, with clones taking a usual insert size of 115 kb. The creativity is also funding the formation of a 0.5-1.0 cM microsatellite map, mapping of the `Goodfellow' and `Ekker'

radioactivity hybrid panels, which are to be inhabited with approximately 10000 Expressed Sequence Tags (ESTs), and a meiotic map (Kelly et al., 2000), which is to comprise markers in public with the RH maps. To supply those making the maps with markers, there is similarly funding for the generation of approximately 100000 ESTs from oligo-fingerprinted cDNA libraries on behalf of various zebrafish embryo developing stages and dissimilar tissues.

Additional project in the Genome Creativity is to generate deletion mutants of the whole genome. This uses a tactic in which γ-irradiated sperm are used to impregnate wild-type eggs. DNA from gynogenetic haploid posterity of females fashioned from this fertilization is used as the pattern in multiplex PCR with introductions for any chosen locus. Females booming the required deletion are identified by reason of letdown to intensify that locus in a fraction of their progeny. The mutants twisted will be placed in the Zebrafish Stock Center at the University of Oregon, Eugene, which is also reinforced by the NIH initiative. Tools obtainable for functional analysis in zebrafish contain a selection of ectopic expression vectors (Hyatt and Ekker, 1999), with the option of having the gene of curiosity under the control of tissue specific or inducible promoters. One example, which allows embattled gene expression in the zebrafish, is called Gal4-UAS. This system includes two vectors, which are used to make detached transgenic zebrafish lines. The first carries the yeast Gal4 transcriptional activator, under the regulator of a given promoter; the second carries the gene of awareness fused to the Gal4 DNA-binding theme.

The zebrafish are then crossed to achieve spatial transcription of the chosen gene. Being transparent, zebrafish are idyllic for expression pattern nursing using lacZ or GFP fusion constructs. Another overexpression systems can use RNA or DNA injection and even huge clones for instance BACs have been used to release mutant phenotypes. This type of tactic is vital in classifying the gene in a selected mutant that is accountable for the pragmatic phenotype. A recent totaling to the battery of techniques for knocking out gene function in zebrafish is RNAi. This stratagem has been used in Caenorhabditis elegans, Drosophila and mouse and has now been exposed to work in zebrafish. If this can be shown to work dependably, it could open a stimulating new avenue for zebrafish genomics, in which a valueless phenotype can be realized for a chosen gene of interest. Additional alike tactic is to block gene appearance using morpholino antisense oligonucleotides. These modified oligos form steadier triple-helices with DNA than

usual oligonucleotides. Yet though, no in-depth learning of this method in zebrafish has been printed.

III. PRESENT STATUS OF GENOME KNOWLEDGE

The zebrafish genome is approximately 1700 Mb in size, as 25 chromosomes. The basis for all the maps of the genome contains of 25 linkage groups on which the 25 centromeres have been positioned. There are radiation hybrid (RH) and the meiotic maps of the genome onto which CA duplication markers, mutations and ESTs have been sited. There are presently 3000 microsatellites and over 1000 genes and ESTs on the meiotic map, and 3000 ESTs have been located on the `Goodfellow' RH panel, 1000 of which have also been planned on the `Ekker' RH panel. Comparison of the zebrafish gene map to a selection of mammalian genomes (Postlethwait et al., 1998) produced significant indication of synteny. A contrast of the zebrafish HOX gatherings to those of other vertebrates delivered suggestion for a genome duplication event, which is thought to have happened after the deviation of ray-finned and lobe-finned fishes but before the teleostan radiation. Additional contrast of the seven zebrafish HOX complexes with the 4 in Fugu confirmed gene loss as a foremost feature of the development of tetrapod and teleost fish HOX clusters.

IV. FUTURE AIMS

Professor Mark Fishman is the director of the Cardiovascular Research Center at the Massachusetts General Hospital. His group is relating a genomic approach to the study of the initial steps of growth of cardiovascular form and function in zebrafish. He feels that the key to the zebrafish is the genetic screen. He did the first large-scale screens in association with Wolfgang Driever (and in parallel with Professor Christiane Nusslein-Volhard's group) and they were unusually revealing. They were surprised by how modular the genetic disturbances were, i.e., that single gene mutations deleted or disconcerted single units of form (e.g., eliminating a chamber or a valve of the heart). This established the unitary logic of the organ assembly. The ensuing step will be to clone these genes, which will deliver the necessary handles on molecular trails for these units, for example the `ventricular pathway'. A 2nd contemplation in his work is that organs are working devices, and embryonic existence depends upon sure of them, such as the cardiovascular system, kicking in from day one.

The zebrafish is quite feasible and energetic without a functioning cardiovascular system because it subsists by diffusion from the water during the first few days. This is

in distinction to the mouse, in which subordinate deterioration starts proximately upon close of cardiac function. Thus, for the first time, it is conceivable to analyze the genes that initiative function in the embryo. Consequently, he can now understand a way to answer questions like: `What makes the first heartbeat?'; How does contraction drive cellular growth?'; and `Does blood flow cause or modify vessel formation?'. He also realizes parallel questions being replied with respect to the onset of function for renal, gut and additional organ systems. The phenotypes of many of these mutations deliver eerily precise phenotypic models of complex human diseases, for which we have insufficient candidate genes.

Incidentally, he feels that the zebrafish will be the redemption of physiology and the way to search the human genome project for the real gold. Professor Fishman's view is that the foremost value of mapping and sequencing of the genome is that it will speed up mutation cloning. Nevertheless, he notes that zebrafish genome contrasts with other species will stipulate good initial points for study of controlling elements, and vision into how their deviation might cause large-scale physical changes throughout evolution. He forestalls that new screen will be useful to the understanding of organ creation and physiology, evolutionary biology, and other areas. Behaviour, culture, and memory are previously the focus of some early phase studies and suppresser, and garnish screens will help refine pathway imageries. Furthermore, the comparatively ready penetrance of many chemicals into the worthwhile embryo makes them goals for chemical screens, related to drug discovery and toxicology. Dr. Stephen Wilson is a Wellcome Senior Research Fellow and Reader at the Department of Anatomy and Developmental Biology at University College London. His group is reviewing the mechanisms underlying the modeling of cells and tissues in the embryonic zebrafish, with a focus on the embryonic growth of the forebrain.

He feels that genome sequencing is a chief priority for the communal for various reasons, but perhaps mainly to ease the fast cloning of genes pretentious by mutations inaccessible in a wide diversity of genetic screens, using an applicant gene approach. He sees full-length cDNA sequencing as a vital equivalent project to genome sequencing to smooth the affinity of coding orders within the genome. This will be vital to aid in the copying of mutations and for functional analysis of the genome, as a extensive diversity of mis-expression plans now exist in zebrafish, delivered that one has access to full-length coding order. Generation of the new libraries, from wild-type and the transgenic lines, is likely to be indispensable for this project. As further cDNA

resources become available, for instance Unigene sets, then the generation of tremendously well characterized arrayed libraries will be probable. He also feels that zebrafish are maybe the most appropriate craniate model system in which to achieve large-scale look profiling analysis of genes by in situ hybridization. By means of whole embryos at a diversity of growing stages, it is likely to determine sites and times of expression of 1000s of genes quickly, correctly, and economically throughout embryonic and larval growth.

This type of study has been started in several groups, but Stephen's feeling is that it wants to be long-drawn-out and funded on a larger scale. From these large-scale mien profiling projects, large numbers of genes with highly specific expression designs will be inaccessible. In his view it will be vital to found selected transgenic GFP lines that label specific cell populaces in the fish by using supervisory sequences that control the expression of these genes. Such lines will be vital for complete phenotypic analysis, for full screening procedures and for the separation of cell type-specific RNA populations that may be understated in more general libraries. Stephen points out that if antisense morpholinos go out to dependably inhibit gene function, then immediately full-length cDNA sequences are gained, large-scale `knock-out' analysis becomes likely.

This would positively not replace true genetic tactics but may be an initial first pointer of gene function. He also notes that prevailing screens have inaccessible only a fraction of the mutant lines that can be gotten and so additional focused screens are vital. These should comprise screens by morphology, by gene and protein expression, by behaviour, physiology and as many other norms as investigators can found. Also, Stephen proclaims that more knowledge of anatomy, physiology, cell biology and behaviour of developing and adult fish, prearranged and obtainable on the web via ZFIN, will be indispensable to fully make sense of gene function analysis studies.

V. CONCLUSIONS

Zebrafish seem to be mostly a floodplain species, inhabiting shallow millponds and ditches or the sluggish reaches of streams. They are a plentiful species and are amongst the least fish species in the meetings in which they happen. Their diet, based on gut satisfied analysis, contains mostly of insects and zooplankton, among inanimate material. These outcomes specify that they feed through the water column, steady with observations of their upright distribution, and the conclusion that they incline to be limited to the shallow margins of waterbodies. The `Featured Organism' study purposes

to present a summary of an organism, mostly for those working on other systems. It delivers related info on the organism itself and on genomics lessons presently in progress. These sections are an individual critical analysis of the existing studies of the organism. The `Future Aims' section is planned to be of attention to readers who work on the selected organism and those who study other systems, and the feelings expressed therein are those of the named contributors.

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