PERSPECTIVES: GENOMICS

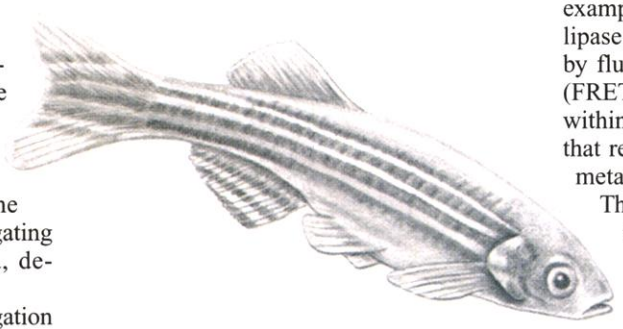
# Zebrafish—the Canonical Vertebrate

Mark C. Fishman

The zebrafish is a dream system for scientists riding the next wave of genome-wide exploration. This photogenic creature is the first vertebrate that has proven tractable to the type of large-scale genetic screening used so successfully in fruit flies and worms. Mutations induced by chemicals, radiation, or viral insertion cause visible changes (phenotypes) that can be readily observed in this vertebrate. The lessons we learn from zebrafish will prove invaluable for those now contemplating the awesome task of interpreting similar random mutagenesis screens in the mouse. Thanks to its transparency, the zebrafish embryo facilitates analysis of mutations because changes in its phenotype can be tracked at the level of the individual cell in the living animal. The similarity of developmental programs among all vertebrates means that the zebrafish is a great model for investigating human development (or vice versa, depending on your specio-centricity).

With a genetic screen, the investigation is launched by spotting the phenotype induced by the random mutation. Thus, one discovers the function of a protein before deciphering the sequence of its gene. Many important zebrafish genes have been cloned by first identifying a mutation and then using a technique called positional walking to obtain the complete gene sequence. This process will accelerate as the complete zebrafish genome sequence becomes available over the next couple of years. Zebrafish genetics is a powerful system with which to "annotate" the human genome (that is, to assign functions to all of the proteins encoded by the genome). Perhaps even more important, the breadth of the genetic screen offers the opportunity to assign an overall logic to the assembly of complex body plans during development (*1*).

Many of the signaling pathways discovered by genetic screening in the fruit fly *Drosophila* are conserved in vertebrates, but their molecular components are not transferred to vertebrate structures in a predictable way. For example, the partitioning of mesoderm into metameric somites during vertebrate development borrows elements from signaling pathways that dictate segmentation in fruit flies, but with distinctive differences (*2*). Certain lipids control germ cell migration during fruit fly development, and, in the developing zebrafish, the lysosphingolipids control the migration of precursor cells that eventually form the heart (*3*). Zebrafish genetics may fill in unanticipated missing components of known signaling pathways. For example, development of the frog or chick body plan reflects the axially restricted activity of proteins that comprise the Wnt, fibroblast growth factor, Hedgehog, and transforming growth factor (TGF-β) signaling pathways. The first mutant genes identified in zebrafish could be assigned to these pathways because the mutations induced dramatic alterations, such as dorsalization or ventralization, in body plan. The first zebrafish gene cloned entirely by positional means, *one-eyed pinhead* (*oep*), proved to be a previously unrecognized critical permissive cofactor for the TGF-β protein Nodal, an important developmental signaling molecule (*4*). The discovery of this gene helped to explain features of the Nodal pathway in germ layer formation, left-right asymmetry in brain and mesoderm, and ventral midline formation.



Some vertebrate features have evolved since the last shared chordate ancestor. For example, unlike the presumptive ancestral primitive chordate, the vertebrate cardiovascular system is lined with endothelial cells, and blood flow is driven unidirectionally by a chambered heart that includes a thick-walled ventricle. Distinctive signaling pathways that direct the assembly and structure of the ventricle—including its size (*5*), wall thickness, and position downstream of the atrium—have been characterized. For each of these organotypic decisions, a new gene, or a new function for a known gene, has been identified by positional cloning in zebrafish. The zebrafish has proved powerful in deciphering uniquely vertebrate decisions because mutations in its genes remove individual organ elements or functions while leaving the remainder of development intact. A link between axial determinants and organ form is emerging—this has been most clearly shown for development of the cranial skeleton, which depends on the intersection of dorsoventral, mediolateral, and anteroposterior signaling pathways (*6*).

Integrative physiology and homeostasis are by definition best studied in the intact animal. The transparency of the zebrafish embryo permits direct assessment of cardiovascular pacemaking, rhythm, and contractility, which are proving tractable to single-gene analysis. Physiological imaging can highlight functional targets. For example, enzymatic cleavage of phospholipase $A_2$ in the gut can be tracked in vivo by fluorescence resonance energy transfer (FRET) between two fluorescent moieties within the substrate. In this way, mutations that result in physiological defects in lipid metabolism have been defined (*7*).

The zebrafish adds to its genetic tractability by being remarkably permeable to small molecules added to the water, an asset for those studying the interactions of genes and environment. When the effects of a small molecule are similar to those of a particular mutation, the component targeted by the small molecule can be identified. Such chemical screening holds promise for preclinical drug discovery and testing as well as for toxicological evaluation in the normal animal and in specific mutants. Stereotypical behaviors of zebrafish embryos can be modified by alcohol and cocaine, suggesting that the developing embryo may enable molecular pathways of drug abuse and addiction to be unraveled.

Patterning, pathfinding, and connectivity in the central nervous system can all be dissected through mutations in zebrafish genes. Zebrafish transparency facilitates analysis of single neuron activity during the execution of normal behaviors. Zebrafish embryos manifest stereotypical behaviors. For example, they are able to visually track the movement of vertical bars or of elements in a computer-animated visual environment (which presumably enables them to remain fixed while the river flows around them), and they exhibit flight from an apparent threat (*8*). To date, most mutations affecting these behaviors are in genes that control neuronal connections in the eye or brain. Zebrafish embryos also ex-

ILLUSTRATION: KATHARINE SUTLIFF

The author is at the Massachusetts General Hospital and Harvard Medical School, Cardiovascular Division, Boston, MA 02114, USA. E-mail: mcfishman@partners.org

hibit even more complex behaviors, including choice of fish with which to shoal, circadian rhythmicity of locomotion, and right-eye dominance in the decision to bite.

Most human genes studied so far have orthologs in the zebrafish. In fact, large regions of human and zebrafish chromosomes show synteny (conservation of gene order) that has been conserved for more than 420 million years (the approximate date of the last common ancestor). However, within these large gene blocks, the order of genes may be transposed or inverted (*9*). This conserved synteny has promoted our understanding of genome evolution, including the relative contributions of chromosomal translocations and inversions. For example, there is a genome duplication at the base of the teleost radiation about 100 million years ago, and about 20% of these duplicated genes are believed to have been retained in the zebrafish. Interestingly, where evaluated, the duplicated genes are not redundant in function, but rather subdivide the function of the ancestral gene. Thus, mutations in one of the duplicated genes may give rise to a more discrete phenotype than mutation of the ortholog in a species, such as the mouse, where gene duplications have not been found. In addition to identifying new genes, the complete zebrafish genome sequence will reveal noncoding sequences shared through evolution, thereby pinpointing important candidate regulatory elements.

The zebrafish will undoubtedly be key to understanding human disease. First, mutations in orthologous zebrafish genes have provided models for several human genetic disorders. For example, mutations in genes encoding enzymes of the heme biosynthetic pathway cause the porphyrias. These diseases are characterized by a constellation of hematological, hepatic, and skin abnormalities. Mutations in orthologous zebrafish genes cause similar phenotypes, dramatically illuminated by fluorescent porphyrins visible in the transparent embryo. The otic vesicle defects in the *mariner* zebrafish mutant are caused by mutations in the myosin VIIA gene; this is the gene disrupted in human Usher 1B deafness syndrome (*10*). Second, the conservation of body plan among all vertebrates means that the phenotype of a zebrafish mutation may resemble a human disease in search of a molecular explanation. This applies both to congenital and adult-onset human diseases. For example, in the *gridlock* zebrafish mutant, the aortic branch point is blocked, which suggests that mutations in the *gridlock* gene (*11*) or other components of its signaling pathway may contribute to congenital malformations of the aortic arch in the human fetus. Even characteristic combinations of organ systems affected in certain genetic disorders, such as craniofacial and pigmentation defects, are mimicked by single-gene defects in zebrafish. Complex diseases in the adult, such as heart failure, often have a clear genetic predisposition but are refractory to standard gene mapping techniques. Dozens of mutations in zebrafish yield a phenotype characterized by inefficient contraction of cardiac muscle; identifying the mutated genes may yield candidates involved in human heart failure. Third, zebrafish mutations that result in loss of stem cell populations, for example, those of gut or blood, or that perturb regeneration of injured fins, will be valuable for designing drugs for use in regenerative medicine.

Limited only by the imagination of the investigator and by the precision of the phenotype, the zebrafish is turning out to be a species for all genomic seasons.

**References**


1. C. Nüsslein-Volhard, E. Wieschaus, *Nature* **287**, 795 (1980).
2. S. A. Holley, R. Geisler, C. Nüsslein-Volhard, *Genes Dev.* **14**, 1678 (2000).
3. E. Kupperman, S. An, N. Osborne, S. Waldron, D. Y. R. Stainier, *Nature* **406**, 192 (2000).
4. J. Zhang, W. S. Talbot, A. F. Schier, *Cell* **92**, 241 (1998).
5. W. Rottbauer *et al.*, *Dev. Cell* **1**, 265 (2001).
6. C. B. Kimmel, C. T. Miller, C. B. Moens, *Dev. Biol.* **233**, 239 (2001).
7. S. A. Farber *et al.*, *Science* **292**, 1385 (2001).
8. H. Baier, *Curr. Opin. Neurobiol.* **10**, 451 (2000).
9. J. H. Postlethwait *et al.*, *Genome Res.* **10**, 1890 (2000).
10. S. Ernest *et al.*, *Hum. Mol. Genet.* **9**, 2189 (2000).
11. T. P. Zhong, M. Rosenberg, M.-A. P. K. Mohideen, B. Weinstein, M. C. Fishman, *Science* **287**, 1820 (2000).


**NOTA BENE: PROTEIN FUNCTION**

# Clockedout—an Archetype of a Functionless Protein

Proteomics is set to revolutionize our knowledge of protein function. But is the assumption that all proteins are functional really valid? A recent study indicates that at least one protein does not do anything useful—for all practical purposes, it merely comes along for the ride. Skumway *et al.* have studied the function of Missingear (Mr), initially believed to be a clock protein in *Drosophila*. Mr was always coexpressed with Tic, Toc, and Buzzer. However, no change in clock dynamics could be discerned at any level of expression of Mr, leading to the speculation that the protein had some other function. After an extensive functional, genetic, and structural survey, Skumway *et al.* now conclude (*1*) that Mr does not do anything. They have tentatively renamed the protein as clockedout.

Clockedout has no distinctive structural motif but appears to have borrowed elements from several other protein families. Enzymatic pockets are turned inward, and binding sites are tagged with odd lipids and saccharides so that the protein gives the appearance of a molecular junk pile. All ubiquitination sites are inactivated, as if the protein had made a deal with these enzymes to take five and leave it be.

How does the cellular economy deal with a deadbeat like clockedout? If evolutionary niches—even molecular ones—can be thought of as "jobs," then clockedout has hit upon an ideal strategy: If one's niche is doing nothing, how can a competitor do more than nothing? It is not clear whether clockedout is unemployed or whether it is like one of the mythical cubicle dwellers who serve no function but still get paid. The latter may in fact serve a function, in that they allow headcount to be reduced without any loss of overall productivity (the "bait and switch" strategy).

Those looking for the true null of background signal in functional assays will find clockedout invaluable as a control. It binds to no known chemical or other protein, turns over no substrate, and undergoes neither import nor export. Whether clockedout is fully optimized to do nothing or, like some designed enzymes, simply does everything badly is still open because of its limited homology with the protein incompetent. Perhaps its true function lies in a symbiotic relationship with the recently evolved *Homo sapiens* ssp. *sleepless*, that is, the molecular biologist.

Homologs are being found across the evolutionary spectrum (*2*), including *nodoff* in the dormouse, *kickback* in *Xenopus*, *wormedoutofit* in *Caenorhabditis elegans*, *flatbread* in *Saccharomyces*, *float* in zebrafish, *DORMANT* in *Arabidopsis*, and *lazy* in the rat. Studies in the human are still in progress, but tentative evidence for the gene *layabout* is being gathered—slowly.

—CHIP READER

**References and Notes**


1. H. S. Skumway *et al.*, *J. Overinterpret. Res.* **2**, 449 (2001).
2. H. S. Skumway *et al.*, in perpetration.

3. The author is currently between industrial positions. He can be reached through Phil Szuromi, a Supervisory Senior Editor at *Science*.