Realizing the potential of zebrafish as a model for human disease

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Barut, Bruce A., and Leonard I. Zon. Realizing the potential of zebrafish as a model for human disease. Physiol Genomics 2: 49–51, 2000.—The value of the zebrafish (Danio rerio) as a model for human disease has been substantiated by a number of recently published papers. Several zebrafish mutants with “human” diseases have been found, spanning a variety of human pathologies. These successful studies utilizing the zebrafish have been made possible by the development of key reagents such as YAC, PAC, and BAC libraries, as well as radiation hybrid panels. With the further establishment of new tools and access to the newly generated resources, the zebrafish is poised to serve as a novel model for human disease.

positional cloning; human disease models; mutagenesis screens; hematopoiesis

THE ZEBRAFISH (Danio rerio) was originally envisioned as a model to bridge the gap between fly/worm and mouse/human for understanding embryonic development. As a vertebrate, the zebrafish has the advantage of external development with optically transparent embryos. Furthermore, the zebrafish has tremendous potential as a genetic model due to its relatively small size, which allows for the maintenance of large numbers of animals, relatively short generation time (2–3 mo), and production of over 200 embryos per female per week. In addition to its developmental advantages, recent studies have indicated that the zebrafish has a great potential to serve as a model for human disease.

Early events in zebrafish studies ensured that a variety of human disease conditions are primed to be studied utilizing the zebrafish model. The initial characterization by Streisinger and co-workers (7, 15, 16) of the zebrafish provided a strong foundation for the ease of genetic studies. The combination of easy mutagenesis and powerful phenotypic screens of the earliest developmental stages resulted in the undertaking of large-scale screens. Phenotypic analysis of embryonic development in animals obtained from two mutagenic screens of the zebrafish genome isolated mutations that affect virtually all major organ systems [an entire issue of Development (volume 123, 1996) was dedicated to zebrafish].

The zebrafish is particularly amenable to the study of hematopoiesis (1). Two different genetic approaches were successfully utilized to demonstrate a link between mutations in the zebrafish and human blood disorders. Hematopoiesis mutants number 50, comprising 26 distinct complementation groups that affect the erythroid lineage (13). The phenotype of these hematopoietic mutants span the stages of differentiation and segregate into five classes: bloodless, blocked progenitor proliferation, blocked progenitor differentiation, hypochromia, and photosensitive blood (1). Many of these distinct phenotypes resemble anemias or thalassemias and thus serve as potential models with which to study defects in differentiation or hemoglobin production [iron metabolism, heme synthesis, globin expression].

Using a positional cloning approach, Brownlie and colleagues (3) isolated the gene sauternes (sau). This zebrafish mutant has a microcytic, hypochromic anemia, with the embryos having severely reduced hemoglobin levels and displaying immature circulating erythrocytes. Sau proved to be due to a defect in the erythroid δ-aminolevulinate synthase (ALAS-2) gene, a critical enzyme that regulates the first step in heme biosynthesis in embryonic red cells. In humans, mutations in ALAS-2 cause X-linked congenital sideroblastic anemia with many characteristics that resemble the sau phenotype. The sau zebrafish mutant is the first animal model of this human disease.

A second human hematopoietic “disease” gene was identified in zebrafish using the candidate gene approach. Wang and co-workers (17) characterized a photosensitive porphyria zebrafish mutant, yqeen (yq). Biochemical analyses of the yq photosensitive erythrocytes demonstrated an accumulation of porphyrins in the cells, suggesting a uroporphyrinogen decarboxylase (UROD) deficiency in the mutant. A missense UROD mutation was found in yq mutant DNA. The phenotype of yq mutants resembles that of human hepatoerythropoietic porphyria (HEP). The authors concluded that the homozygous mutant zebrafish could be considered the first genetic animal model of HEP. These two zebrafish phenotypes (sau and yq) demonstrate similarity to two human blood disorders, a sideroblastic anemia and a porphyria, and represent the first zebrafish...
models of human disease. These initial successful studies demonstrate the advantages of zebrafish to study the molecular events of hematopoiesis and illustrate the ease of whole embryo in situ hybridization and mutant rescue in the zebrafish system.

A variety of mutations in other organ systems provide a rich source for the study of genetic components of human disease. The screens produced over forty cardiovascular mutants with defects that affect early development of the heart, vasculature, and blood (14). In addition, mutations exist in most other organ systems including the central nervous system, the eye, limb development, and the kidney (4). These mutations potentially serve as models for a multitude of human diseases. For example, the zebrafish cardiac mutant, griddock, has a defect that resembles the human condition of coarctation of the aorta (18), and in the kidney mutants there are animals with cystic kidneys that may represent polycystic kidney disease of humans (5).

The value of the zebrafish system as a model to effectively study human disease has improved with the availability of new genetic tools. Positional cloning approaches in the zebrafish have been made possible by the development of large insert genomic yeast artificial chromosome (YAC), P1-derived artificial chromosome (PAC), and bacterial artificial chromosome (BAC) libraries (2). In addition to the sau gene, positional cloning was successful in the isolation of the one-eyed pinhead mutation, which disrupts an epidermal growth factor (EGF)-signaling pathway in zebrafish and phenotypically resembles the human condition holoprosencephaly (19). The advent of somatic cell and radiation hybrid panels facilitates the assignment of genes of interest to particular linkage groups (6, 9). Increased resolution of the zebrafish map allows for fine-scale mapping. Of greatest utility in establishing the usefulness of the zebrafish for understanding human disease has been the discovery that for many chromosomal loci, there is an obvious synten between the fish and the human (12). This conservation of genes facilitates both positional cloning and candidate mapping, with researchers having the ability to “ping-pong” between the human and zebrafish genomes (20).

A number of studies have demonstrated that the zebrafish genome serves as an easily accessible source for the isolation of homologs of human genes, and the fish can help in the functional analysis of these genes. The work of Karlovich et al. (8), Lai et al. (10), and Leimer et al. (11) provide examples demonstrating the potential function of the zebrafish as a model for human disease using this methodology. Karlovich and colleagues (8) have isolated the huntington’s disease gene homolog in zebrafish and are investigating the potential role for this gene in early vertebrate development. Lai et al. (10) have cloned two zebrafish homologs of human enzymes involved in steroidogenesis, which should facilitate study of human disease associated with steroid imbalance. The recent studies of Leimer and colleagues (11) in utilizing the zebrafish to clone genes known to cause familial Alzheimer’s disease have yielded surprisingly comparative activities and mutations between human and fish amyloid associated proteins, further supporting the relevance of the zebrafish model to study a variety of human pathological conditions.

A second set of tools to further the value of the zebrafish/human disease model will be the resulting mutants from additional screens. Screens targeted to generate and isolate mutants in specific lineages or with late onset phenotype will achieve a higher level of saturation of detectable genes and expand the scope of zebrafish models of human disease states (4).

The rapid progression of mapping and identification of gene function in zebrafish demonstrates the value of this organism in the field of physiological genomics. With continued development of genomic tools for the zebrafish system, there is a greater likelihood of the identification of the function of newly discovered genes and determination of relevance to human disease.

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