Keywords: Forward genetics; Drug screening; Zebrafish

Many of the critical pathways that govern vertebrate development are highly conserved between humans and zebrafish (Danio rerio). The zebrafish genome shares a high degree of sequence similarity to that of humans. Approximately 70% of genes associated with diseases in humans have functional homologs in the zebrafish [1]. In addition, zebrafish as an experimental model offers many advantages including their ability to produce large numbers of eggs (a single cross can generate 200–300 embryos), thereby developing outside the body, are transparent making them amenable to follow during organogenesis. Development is rapid, with major organs primordial forming by 24 hours after fertilization. Compared to other vertebrate models, zebrafish are easy and inexpensive to raise and maintain. [2]. George Streisinger, a founding father of zebrafish research was one of the first to work with zebrafish in the late 1960s [3]. He began to study embryonic development particularly that of the nervous system by employing mutant strains [4-6]. Realizing the importance of the zebrafish model, Grunwald and Eisen used this developmental model to study the segmental structures of the brain and characterized neurons in the zebrafish that had not been reported in other vertebrate models [7]. Christiane Nüsslein-Volhard, a fruit fly geneticist at the University of Tübingen, who identified 120 developmentally important genes in Drosophila melanogaster [8], recognized the usefulness of zebrafish as a vertebrate model, and established collaboration with Marc Fishman at the Massachusetts General Hospital [9] to study these developmentally important genes in zebrafish.

Forward Genetics

In Forward Genetics heritable mutagenic lesions are created with the use of chemical (ENU: Nethyl- N-nitrosourea) [9] or insertional (retroviruses or transposons) [10,11] mutagenesis approaches. Mutagenic lesions are screened for particular phenotypes and the causative genes responsible for a given phenotype are identified through positional cloning and/or through the candidate gene approach [12,13]. The success of the "Big forward genetic screen" Nüsslein-Volhard et al. [9] commonly referred as "Tübingen/Boston screens" created a significant impact on the use of zebrafish as a promising system to model disease and development. The results were published in the entire volume of the Journal Development [14]. A major drawback of this approach is, with the large size of the zebrafish genome the identification of mutant genes can be time consuming and laborious [15].

Reverse Genetics

Reverse Genetics involves the selection of a target gene and creation of mutants of the selected gene and investigation of the associated phenotypes to uncover function of the gene in question. Many reverse genetic approaches have been developed recently [16]. These include the use of antisense morpholino (MO) oligonucleotide mediated gene knockdown technology [17,18], Targeting induced local lesions in genome (TILLING) [16,19], Zinc Finger Nucleases (ZFN) [20,21], Transcription Activator-Like Effector Nucleases (TALENs) [22] Tol2 mediated Transgenesis [23,24], GAL4-UAS System [25], To2-mediated Gal4/UAS [26], Cre/Lox system [27] and a tamoxifen-inducible Cre/lox method [28]. Among them TALENs, ZFNs and Tol2 mediated transgenesis methods are becoming successful in defining the functional roles of target genes. Both GAL4/UAS and Cre/Lox methods are less efficient due to the limited understanding of tissue/cell specific promoters and there is no guarantee that they will work as expected. Further development in these technologies would facilitate their better application in zebrafish research.

Modeling Vertebrate Development

Both forward and reverse genetic approaches have been employed in zebrafish to define the role of genes involved in the development of vertebrate organs, tissues and cells. A recent key word search of PubMed revealed 8596 publications on the use of zebrafish in vertebrate development. Some of the examples include development of the cardiovascular system [29], the endoderm [30], motor neurons [31,32] and craniofacial structures [33]. The insights gained from zebrafish are directly applicable to humans since molecular mechanisms that regulate vertebrate development are highly conserved between the two species.

Modeling Disease

With the exception of few organs namely the lungs, prostrate and mammary gland, most of the tissues and organs present in humans are found in the zebrafish. The cloning of mutated genes screened for specific phenotypes have revealed similarity in humans and thus serves as models for human disease and to study underlying mechanisms. The first human disease model in zebrafish to be defined in this was the sauternes (sau) mutant responsible for a blood disorder involving a specific defect in hemoglobin production. The mutated gene responsible for the blood disorder was ALAS-2. Many other mutants showing phenotypic similarity to human diseases have been screened and later identified to have human homologues. These include hematological disorders [34,35], neurological disorders [36], cardiovascular diseases [37], muscle disease [38] and cancers [39,40]. Detailed reviews on modeling human diseases in zebrafish have been published [1,7,41,42].

Drug Screening

High throughput chemical screening in zebrafish is a very promising tool since it can be undertaken by simply placing zebrafish embryos in 96 well plates, adding chemicals to the water and then looking for...
the suppression of a given phenotype. The efficacy and toxicity of new compounds can be simultaneously assessed by employing this method [43]. The applicability and advantages of using zebrafish embryos over other models in drug screening have been reviewed in detail [44-46]. Large scale chemical screening using zebrafish have been conducted recently with the aim of identifying novel biological and therapeutic compounds. Important compounds discovered in this way are being tested with the intent of identifying potential lead components and initial validation as potential therapeutics.

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