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Zebrafish: An integrative system for neurogenomics and neurosciences

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ABSTRACT

Rapid technological advances over the past decade have moved us closer to a high throughput molecular approach to neurobiology, where we see the merging of neurogenetics, genomics, physiology, imaging and pharmacology. This is the case more in zebrafish than in any other model organism commonly used. Recent improvements in the generation of transgenic zebrafish now allow genetic manipulation and live imaging of neuronal development and function in early embryonic, larval, and adult animals. The sequenced zebrafish genome and comparative genomics give unprecedented insights into genome evolution and its relation to genome structure and function. There is now information on embryonic and larval expression of over 12,000 genes and just under 1000 mutant phenotypes. We review the remarkable similarity of the zebrafish genetic blueprint for the nervous system to that of mammals and assess recent technological advances that make the zebrafish a model of choice for elucidating the development and function of neuronal circuitry, transgene-based neuroanatomy, and small molecule neuropharmacology.

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1. Introduction

Molecular neurobiological research in a model organism requires that its genome be fully sequenced and annotated. In addition, what will be needed is a comprehensive catalogue of the expression patterns of all protein coding genes, microRNAs and other non-coding RNAs, and their mutant phenotypes. This may sound, and in fact currently is, the stuff of dreams, but the recent development of high throughput technologies for DNA and RNA sequencing, transgenesis, and a number of other powerful tools

Abbreviations: TILLING, Targeting Induced Local Lesions In Genomes; KOMP, The knockout mouse project; IKMP, The international knockout mouse consortium; ZFIN, zebrafish information network; GRB, genomic regulatory block; WGD, whole genome duplication; CNE, conserved non-coding element; BAC, bacterial artificial chromosome; GFP, green fluorescent protein; UAS, upstream activating sequence; miRNA, micro RNA; 5HT, serotonin; GnRH, gonadotropin releasing hormone; KiSS, kisspeptin; LH, luteinizing hormone; POMC, proopiomelanocortin.

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poise the zebrafish for a prominent role in post-genome neurobiology (Table 1). The zebrafish genome is advancing towards a level of high quality finished sequence approaching that of the human and mouse genomes (King, 2009). Although the zebrafish is phylogenetically distant from humans, its development, including of the CNS, uses a genetic tool kit that is essentially conserved among all vertebrates (Canestro et al., 2007). In addition, zebrafish could allow the application of the tools that have made *Drosophila* and *C. elegans* such powerful genetic model systems to a model with a vertebrate nervous system (Burne et al., 2010). We will review the similarities in genetic makeup between humans and zebrafish and argue that in terms of genome architecture and gene regulation, zebrafish is a very good model not only for the investigation of nervous system development but also for maintenance, homeostasis, and physiology. Teleost genomes have undergone a whole genome duplication about 250 million years ago (Van de Peer et al., 2009) leading to many genes that have been retained in duplicate. However, this often turns out to be an advantage, at least with respect to forward genetics screens, because the resulting partial redundancy in gene function allows splitting of functions (de Martino et al., 2000; Navratilova et al., 2010). This can permit the teasing out of late functions of genes, whereas mutations in less genetically redundant species such as the mouse may result in early lethal phenotypes (Burgess and Granato, 2008).

Table 1
Advantages and disadvantages of zebrafish as a vertebrate neurobiological model.

General advantages
• Rapid generation time (2–3 months post-fertilization)
• Cost effective/high density stocking
• External development (free swimming larva at 4 dpf)
• Ethically relatively easy to justify (many experiments can be done in embryonic or larval animals)
Imaging/neuroanatomy
• Small size ideal for microscopy
• Non-invasive neuronal observation and manipulation due to transparency
• Live imaging in real time at cellular resolution
• Enquiries can involve thousands of larvae with automated image acquisition and processing
• Conservation of major nuclei/brain regions: arcuate nucleus, hippocampus, amygdala, locus coeruleus…)
• Small/compact neuronal network revealed at once by two photon or confocal imaging
• Live imaging with GECI (genetically encoded calcium indicator)
• Small adult brain size (5 mm long). Reduced number of sections for histological analysis and neurodegeneration
Genetic/genomic resources
• More than 12,000 annotated gene expression patterns (including miRNAs)
• Transgenesis using retroviral and transposon vectors (thousands of lines generated)
• Rapid mutagenesis (TILLING, ENU screens, insertional mutagenesis, zinc finger nucleases)
• Large collection of mutants
• Rapid gene knockdown using antisense morpholinos
Pharmacology/physiology
• Forward drug screens in 96 well format
• Conservation of pharmacological targets
• Conservation of classic fast neurotransmitters (glycine, GABA, glutamate, monoamines: dopamine, norepinephrin, epinephrin, serotonin, melatonin)
• Conservation of most neuropeptides (e.g. MCH2)
• Conservation of immediate early genes (e.g. cFos, Jun, krox 24, bdnf, homer)
Disadvantages
• No inbred strains
• Small size for tissue samples
• Evolutionarily distant from human
• Physiologically dissimilar
• No homologous recombination
• Some neuropeptides are not conserved (e.g. NPS)
• Some immediate early genes are not found (ARC)
• No electroencephalogram; no functional MRI
• No layered neocortex
• Some nuclei difficult to identify or not found yet (e.g. nucleus accumbens)
• Neuropathology/neurological disorders not well defined yet in zebrafish

1.1. Genetic manipulation of zebrafish

For molecular genetic neurobiology, a major requirement is the manipulability of the whole organism at multiple levels, be it forward genetic screens or reverse genetics, transgenesis, chemical screens, live non-invasive imaging, and/or genetic manipulation of neuronal circuits. The zebrafish model has, over the last decade, made remarkable progress on several of these fronts and has grown into a system that is used to investigate mechanisms of regeneration in the nervous system (Becker and Becker, 2008) and human disease development (Lieschke and Currie, 2007). Its many advantages include rapid development, fecundity, and modest (for a vertebrate) space requirements. Major organs are recognizable within 24 h, and embryos develop to a free swimming and hunting larva in 5 days. Clutches frequently reach over 100 synchronously developing embryos from a single mating pair. While zebrafish can be kept in regular aquaria at low stocking densities, there are now commercial holding systems for research laboratories in which zebrafish can be raised to sexual maturity in 2–3 months at a density of 10 individuals/liter at a water exchange rate of 3 times total volume/tank/hour. In addition, the zebrafish offers unique advantages in terms of accessibility, and optical transparency of its embryos. Melanization in larvae can be prevented by the addition of phenylthiourea (Elsalini and Rohr, 2003), and there are also mutant strains without melanophores or iridophores, such as the recently described casper strain (White et al., 2008).

Over the past decade, the zebrafish model received an enormous boost through the introduction of molecular technologies such as antisense oligonucleotides (morpholinos) for early knockdown of gene function (Nasevicius and Ekker, 2000), and the recovery of mutations in a gene of interest from mutagenized libraries of fish (Targeting Induced Local Lesions In Genomes (TILLING)) (Wienholds et al., 2003). In addition, there are now efficient strategies for genetic manipulation of zebrafish through transgenes (Amsterdam and Becker, 2005; Kikuta and Kawakami, 2009; Laplante et al., 2006; Scott et al., 2007). A more recent, and exciting, development are zinc finger nucleases for site directed mutagenesis (Doyon et al., 2008; Foley et al., 2009a; Foley et al., 2009b; Meng et al., 2008). However, this technology is still laborious and costly and it remains to be seen when it will become a routine tool in zebrafish laboratories.

A requirement for post-genome neurobiology is that large collections of gene expression patterns, as well as mutant and transgenic strains, are available. In addition to the advanced stage of assembly of the zebrafish genome there are approximately 12,000 annotated expression patterns allowing researchers to 'walk' across many given genomic regions of interest with patterns readily available at the click of the mouse. This includes expression patterns of micro RNAs, where zebrafish is arguably the vertebrate champion, as close to half of the 415 zebrafish miRNAs have documented expression patterns (Thatcher et al., 2008; Wienholds et al., 2005). The database of the ZebraFish Information Network (Sprague et al., 2008) (www.zfin.org) allows systematic searches for gene and miRNA expression patterns based on a variety of criteria. New expression patterns are continually added to the database from publications and also from several large-scale efforts with the goal to eventually include every gene in the fish genome (Thisse et al., 2004; Thisse and Thisse, 2008). Two large-scale chemical mutagenesis screens were initially performed in 1990s (Driever et al., 1996; Haffter et al., 1996), resulting in an entire issue of Development devoted to the description of mutants