Comparative aspects of zebrafish (*Danio rerio*) as a model for aging research

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**Abstract**

The zebrafish has emerged over the past decade as a major model system for the study of development due to its invertebrate-like advantages coupled with its vertebrate biology. These features also make it a potentially valuable organism for gerontological research. The main advantages of zebrafish include its economical husbandry, small yet accessible size, high reproductive capacity, genetic tractability, and a large and growing biological database. Although zebrafish life span is longer than rodents, it shares the feasibility of large-scale mutational analysis with the extremely short-lived invertebrate models. This review compares zebrafish with the more widely used model organisms used for aging research, including yeast, worms, flies, mice, and humans.

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The zebrafish has become a premiere model organism for the study of vertebrate development (Anderson and Ingham, 2003). Over the past few years, it has also been used to model a wide variety of disease processes (Rubinstein, 2003). Despite this enthusiasm in closely related fields, gerontology has been slow to embrace zebrafish as a model for the study of aging. For example, the number CRISP grant funding database hits for the keyword ‘zebrafish’ for all NIH institutes in 2002 was 32/2, while only five were found for grants funded by the National Institute on Aging. This review describes the utility of zebrafish as a biological model and analyzes its potential usefulness vis-a-vis the current models commonly used for aging research.

1. The origins of zebrafish as a biological model

The zebrafish, a hardy tropical fish commonly kept in home aquariums, has become a major model organism in biomedical research thanks primarily to the late George Streisinger (Grunwald and Eisen, 2002; Streisinger et al., 1981). He was one of a handful of scientists to first advocate studying neurobiology with a genetic approach using model organisms, which included Sydney Brenner’s use of *Caenorhabditis elegans* and Seymour Benzer’s studies on *Drosophila*. At the University of Oregon, Streisinger developed techniques to produce homozygous diploid zebrafish as well as methods to introduce mutations into the germ-line. These tools led to the identification of several mutations affecting embryonic development (Felsenfeld et al., 1990; Grunwald et al., 1988). Despite his progress, “Streisinger was constantly embattled to secure federal funding for his zebrafish project. His efforts endured only through the prescient and persistent intervention of a handful of scientists, who by chance were involved in the peer review and funding process at NIH (M. C. Capecchi, G. Lark and P. von Hippel, personal communications).” (Grunwald and Eisen, 2002).

The potential of zebrafish as a genetic model did not go unnoticed by *Drosophila* biologists, who had already performed large-scale mutational analysis of development in the fly (Nusslein-Volhard and Wieschaus, 1980). Christian Nüsslein-Volhard, winner of the Nobel prize for her work on *Drosophila* (Roush, 1995), began a similar mutagenesis screen for embryonic pattern mutations in zebrafish in Germany in 1993 (Mullins and Nüsslein-Volhard, 1993). Marc Fishman and Wolfgang Driever initiated a parallel screen in Boston (Fishman and Stainier, 1994). Over 4000 embryonic-lethal mutant phenotypes were recovered between the two efforts that are still being studied today. The Trans-NIH Zebrafish Initiative was begun soon afterwards to support zebrafish research, including the further development of the genetic map in order to facilitate...
the identification of the genes underlying the large number of mutant phenotypes. In 2000, the Sanger Centre initiated a project to sequence the zebrafish genome (www.sanger.ac.uk), which will be deposited in a centralized web-based database (www.ZFIN.org) that is maintained, along with a zebrafish stock center, at the University of Oregon.

2. Zebrafish as a model for development

The zebrafish is a small (1–2 in.) fresh water tropical fish species native to India (Eisen, 1996). It is a nearly ideal model for systematic mutational analysis (Fishman, 2001). The embryos are transparent (Fig. 1), thus, they may readily screened for morphological abnormalities via gross and microscopic visual examination. An individual female can produce hundreds of eggs in each clutch, enabling huge numbers of progeny to be generated, facilitating the detection of rare mutations. The eggs are large enough to be easily manipulated and can be fertilized naturally or in vitro. Rapid ex utero development (Fig. 1) leads to free-swimming larvae with morphological and behavioral features of adults within 4–7 days. Organogenesis is easily visualized. For example, cardiac contractions begin during the second day after fertilization and all major organs recognizable by the fifth day. The generation time is 3–4 months and relatively modest amount of resources are required to maintain large numbers of fish (Eisen, 1996). They are also physically large enough to isolate significant amounts of specific tissues, especially skeletal muscle, yet are sufficiently small to allow for substantial 'economies of scale.'

Also vital for mutational analysis is the availability of genomic resources. Key for zebrafish biologists is the ongoing sequencing of its genome (Vogel, 2000a). The Sanger Institute initiated sequencing of the 1.7 Gb zebrafish genome in February 2001 using a two pronged approach, clone-based sequencing from BAC and PAC libraries, and whole genome shotgun sequencing from plasmids (http://www.sanger.ac.uk/Projects/D_rerio/). The clone-based approach is also being used to generate a physical map of the genome and will facilitate positional cloning of mutant genes. Further sequence information is being generated by the Washington University Zebrafish Genome Resources Project (http://zfish.wustl.edu/), which is developing cDNA libraries for EST sequencing and mapping genes and ESTs.

A number of other complementary genetic resources are available for zebrafish. The ability to generate transgenic zebrafish allows for overexpression studies as well as for the testing of candidate genes through complementation of mutants (Meng et al., 1999; Yan et al., 1998). While it is not yet possible to generate targeted knock-out mutations for evaluating null phenotypes of candidate genes, transgenic morpholino knock-down techniques allow for the transient suppression of gene expression during early development (Nasevicius and Ekker, 2000). As mentioned above, thousands of genetic mutants from large-scale mutagenesis experiments are also available for study. A key practical consideration is that zebrafish sperm can be frozen for future study.

A main component of the zebrafish armamentarium is the ability to perform high efficiency mutagenesis. Chemical mutagenesis protocols are well established (Knapik, 2000), in which single-base substitutions are induced that can result in loss-of-function, decreased-function, gain-of-function, and conditional (e.g. temperature sensitive) mutations. Mutagenesis is primarily accomplished using the DNA alkylating agent ethylNitrosourea (ENU), which has yielded average locus mutation rates of 1–3 mutations per 1000 haploid genomes (Mullins et al., 1994; Solnica-Krezel et al., 1994). Radiation (Walker, 1999) and insertional

![Fig. 1. Development in zebrafish. (A) Single cell embryo with evidence of early first cleavage. (B) 4-cell stage. (C) 8-cell stage. (D) Approximate 64–128 cell stage. (E) 3-h-old blastula stage embryo. (F) 24-h-old embryo curled inside chorion. (G) Free-swimming 48-h-old embryo following 'hatching' from chorion. (H) Adult male zebrafish at 8 months of age. (I) Old zebrafish at 52 months of age. (J) Following exposure to mitochondrial specific fluorescent dye MitoTracker Green (Molecular Probes, Eugene, OR), neuron body, axon, and putative satellite cell are vividly visualized. (K) Punctate mitochondrial staining in an epithelial-type cell, with nuclear sparing seen in some cells.](image-url)
mutagenesis (Amsterdam et al., 1999; Golling et al., 2002) techniques have also been developed.

Most induced mutations are recessive, thus, in mutant screens in a diploid organism, recessive mutations must be rendered homozygous to detect their phenotype. Screening mutation-bearing fish for relevant phenotypes is therefore done in individuals carrying homozygous recessive mutations. In the zebrafish, this is usually accomplished using a multi-generation back-cross paradigm, the approach used for the first major screen (Haffter et al., 1996; Stainier et al., 1996). Alternative techniques can be used to produce uniparental progeny, including a variety of gynogenetic (yielding embryos with only maternal genomes), and androgenetic (yielding embryos with only paternal genomes) uniparental techniques (Postlethwait and Talbot, 1997). Uniparental haploid screens utilize zebrafish strains devoid of recessive lethal mutations and exploit the ability of haploid embryos to survive for up to 4 days post-fertilization to detect subtle developmental phenotypes.

3. The origins of zebrafish as a gerontological model

Several small tropical fish have been the subject of previous gerontological studies, whose investigators have cited a number of advantages for studying aging including the availability of large cohorts of offspring from single matings, the ectothermic nature of fish (which facilitates environmental modulation by external changes), and their reasonably short life span relative to many mammalian species (Woodhead, 1978). Others have highlighted the low costs for breeding and maintenance, the ability to manipulate life span by both temperature reduction and food restriction, and the large variety of species available as potential models (Patnaik et al., 1994).

These advantages hold for many of the more than 24,000 extant fish species, why then the zebrafish? One reason is the large and increasing number of investigators who use the model. A critical mass of scientists who generate a wide range of potentially useful reagents and resources for aging studies should not be underestimated. The current surge in zebrafish biology was apparent even in the early 1990s prior to the initiation of the first large-scale screen (Rossant and Hopkins, 1992), after which zebrafish leapt into the mainstream of biological research (Chen and Fishman, 2000; Driever et al., 1996; Haffter et al., 1996). Around this time, a life span study was initiated in the author’s laboratory with the expectation that the anecdotal reports of zebrafish longevity would be 2–4 years, an estimate still referred to and reported (Higgs et al., 2002). The median life span of outbred zebrafish (from the age of 17 months) was found to be about 42 months, with the longest living individuals surviving for 66 months (Gerhard et al., 2002).

The longevity of a relatively inbred zebrafish strain was also assessed and manifested a 10–15% shorter median and maximum life spans than the outbred zebrafish, consistent with the effects of inbreeding depression. This initial study did not assess potential effects of gender and reproductive activity, which could affect life span (Reznick et al., 2001). The life span of outbred zebrafish is thus at least 50% longer than that of commonly used mouse strains, and is slightly longer than long-lived mouse mutants (Bartke et al., 2001).

A maximum life span of at least 5 years is a major disadvantage relative to the extremely short-lived invertebrate organisms, and to rodents. However, the key utility of the zebrafish as a model is that it may be genetically manipulated like invertebrates, yet it offers a life span that is more similar to, and as a vertebrate perhaps more relevant to, mammals. The derivation of large populations can be achieved quickly and cheaply making possible large-scale demographic analyses (Vaupel et al., 1998), an advantage not shared by many vertebrate models.

Zebrafish also appear to develop various degrees of spinal curvature with age, as reported with aging in other fish species (Comfort, 1961; Liu and Walford, 1969). Muscle degeneration appears to underlie the curvature and may thus serve as a model for sarcopenia (Gerhard et al., 2002). Tens to hundreds of milligrams of skeletal muscle may be isolated from a single fish, providing an ample supply of tissue for study. The pathologies zebrafish incur, such as various tumors, are morphologically similar to those found in mammals (Beckwith et al., 2000), yet the costs for such analyses are much less due to the smaller size of zebrafish.

The next phase of zebrafish gerontology should include a more detailed examination of life span and pathology, in parallel with exploitation of some of the available techniques described below. This should avoid the situation that existed for C. elegans, where the detailed phenotype of aging (Herndon et al., 2002) was not characterized for many years after the application of powerful genetic approaches began to yield significant results.

4. Zebrafish as a unicellular model of aging

Although a metazoan, zebrafish spend about 30–40 min as a readily obtainable, accessible, and manipulable single cell embryo (Fig. 1). Its adult size also enables the accesssion of specific tissues for in vitro studies. Zebrafish may thus serve as a useful model for studies related to two well characterized unicellular models of aging, budding yeast and the cellular senescence of cultured cells.

4.1. Yeast

In the budding yeast, Saccharomyces cerevisiae, aging has been studied in two main experimental paradigms, replicative and chronological life span (Jazwinski, 2002; Sinclair, 2002). Replicative life span is measured as the number of daughter cells that bud from a mother cell. On
an average, a mother cell will give rise to about 20 daughter cells prior to losing the ability to proliferate. The mechanism by which this occurs may be due to the accumulation of extrachromosomal rDNA circles, which may cause senescence by titrating essential transcription and replication factors. The SIR2 gene mediates silencing of rDNA and represses the formation of extrachromosomal rDNA circles. SIR2 is a histone deacetylase that requires NAD as a cofactor, suggesting a link to cellular metabolism (Imai et al., 2000). Overexpression of the SIR2 gene increases rDNA silencing and can extend replicative life span (Tissenbaum and Guarente, 2001).

The potential role of rDNA or SIR 2 in zebrafish aging has not been reported. The moderately repetitive 5S rDNA sequences have been characterized in zebrafish cell lines (Phillips and Reed, 2000) and several zebrafish histone deacetylase homologs have been identified (www.TIGR.org). Two single cell phases of zebrafish may be useful for such studies. First, similar to other metazoans, specific tissues may be harvested from zebrafish, including those that consist primarily of post-mitotic or proliferating tissues (availability of cultured cell lines discussed below). In addition, the fertilized oocyte may serve as a vehicle to track molecular changes occurring in early embryonic cell divisions that could have a subsequent influence on aging in the adult organism.

Chronological aging in yeast is measured in a stationary phase, when glucose becomes limiting following fast logarithmic growth, in which the cells stop dividing but can survive for several months. In this final phase, they are resistant to heat and oxidative stress and have lower metabolic rates (Sinclair, 2002). Oxidative stress may play a role in determining life span of the stationary phase, since yeast lacking superoxide dismutase have a greatly reduced chronological life span (Longo et al., 1999). A genetic screen for oxidative stress resistant mutants with extended chronological life span identified SCH9, which encodes a protein kinase with homology to C. elegans AKT-1 and AKT-2 part of the IGF-1/DAF-2 insulin-like signaling pathway, and, CYR1, which encodes adenylate cyclase (Fabrizio et al., 2001).

The potential role of oxidative stress in zebrafish aging has begun with the cloning of the mitochondrial genome and antioxidant defense genes, including catalase (Gerhard et al., 2000). Aging skeletal muscle also appears to manifest abnormal mitochondrial morphology (Gerhard et al., 2002). Potentially important technical advantages of zebrafish for studies on oxidative stress, are the ability to accurately measure variables such as caloric intake, metabolic rate, and physical activity, in addition to the use of non-toxic compounds as indicators of reaction oxygen species (Gerhard and Cheng, 2002). The primary drawback for such studies is that the effects of selected mutations or environmental manipulations upon life span cannot be ascertained quickly. Nevertheless, large numbers of embryos can be generated easily and cheaply and subject to a wide variety of experimental manipulations.

4.2. Cellular senescence

Cellular senescence occurs in cells capable of dividing (e.g. fibroblasts, endothelial cells, etc). Similar to yeast replicative aging, as cell division occurs in vitro, eventually the ability to replicate is lost and a wide variety of related biochemical changes occur (Cristofalo, 1997). It has been proposed that cellular senescence is critical for suppressing tumorigenesis in eukaryotes and might cause or contribute to the aging of tissues that proliferate (Campisi, 2003). Cellular senescence appears to be caused by the progressive shortening of telomeres, which occurs with each cell division (Bodnar et al., 1998). Shortened telomeres may be recognized as damaged DNA by cells, which then triggers an irreversible arrest of cell division and thus senescence. The enzyme telomerase can repair such shortening, but telomerase is not expressed in most human cells. In addition, other stimuli may induce cellular senescence such as oxidative stress and certain oncogenes.

Species differences exist in the levels of telomerase expression in post-embryonic tissues (Campisi, 2001). In species with relatively short telomeres and low levels of telomerase expression, such as humans, telomere shortening likely causes cellular senescence. In other species, such as mice, telomeres are longer and telomerase expression is higher. In these species, cellular senescence may occur through non-telomeric pathways.

Nothing yet has been published on zebrafish cellular senescence or on related aspects of telomeres or telomerase. The zebrafish telomere repeat factor sequence has been identified (Genbank AY099522) and telomerase activity has been reported in other fish species. Permanent lymphoid cell lines have been readily obtained from peripheral blood cells of channel catfish (Ictalurus punctatus), which increase telomerase activity upon immortalization (Barker et al., 2000). Cell lines derived from pufferfish (Fugu) species have also been found to possess high, perhaps indefinite proliferative potential, and high telomerase activity (Bradford et al., 1997). High telomerase activities have been found in multiple tissues in the rainbow trout, attributed to the indeterminate growth in many fish species (Klapper et al., 1998). If indeed linked to proliferative activity, high somatic telomerase activity may then be expected in all species with indeterminate growth. At least several fibroblast-like zebrafish cell lines are publicly available (www.ATCC.org) that were derived from embryos or adult fins, as well as an epithelial line derived from liver. The determination of telomere length and relationship to various aspects of aging, such as the occurrence of neoplasia and cellular senescence in zebrafish cells may be of particular interest.

Screens for zebrafish mutations that affect cell proliferation and differentiation are already underway (Cheng and...
extend life span in another RNAi screen (Dillin et al., 2002). The expression of several mitochondrial genes was also found to affect transcripts were found to affect more than 5600 genes, a number of mitochondrial related bacterially expressed double-stranded RNA. In a screen of corresponding gene in vivo occurs through consumption of the (dsRNA), from which decreased expression of the corresponding gene in bacteria expressing double-stranded RNA. Worms (C. elegans) have identified key genetic pathways regulating cell proliferation and apoptosis. Findings from zebrafish could determine whether the function of these genes is conserved, or whether they are only relevant to invertebrates. Also, zebrafish provide a model to directly test whether events occurring in embryonic development impact function in adult animals.

5. Worms: debate for fish?

The self-fertilizing hermaphrodite nematode C. elegans has become the primary genetic model system for gerontology (Finch and Ruvkun, 2001) due to its short and reproducible longevity (almost 3 weeks at 20 °C), inexpensive maintenance, completely sequenced genome, complete cell fate map, and the identification of a number of genes and pathways that increase its life span (Johnson, 2003; Tissenbaum and Guarente, 2002). Mutations in several components of the daf-2/insulin-like signaling pathway cause an extension of life span (Guarente and Kenyon, 2000; Murakami et al., 2000). Other genes that increase C. elegans life span include those underlying the eat mutants, which have manifest abnormalities in feeding and may operate through caloric restriction, and the clk (biological timing abnormality) mutations, that cause slowing of many physiological processes (Hekimi et al., 2001). Other classes of mutations have also been found (Johnson et al., 2000).

Recently reported large-scale systematic RNA interference (RNAi) screens to identify genes that extend life span when suppressed attest to the power of C. elegans as a genetic model (Dillin et al., 2002; Lee et al., 2003). Worms can be grown on bacteria expressing double-stranded RNA (dsRNA), from which decreased expression of the corresponding gene in vivo occurs through consumption of the bacterially expressed double-stranded RNA. In a screen of more than 5600 genes, a number of mitochondrial related transcripts were found to affect C. elegans life span (Lee et al., 2003). These genes appear to act downstream of, or parallel to, the daf-2/insulin-like signaling pathway. Suppression of several mitochondrial genes was also found to extend life span in another RNAi screen (Dillin et al., 2002). Exposure to the RNAi was shown to be required only during development and not throughout the life span, suggesting that a reconfiguration of mitochondrial function early on in life can extend longevity.

The RNAi of mitochondrial genes had other effects as well (Dillin et al., 2002). Body size was reduced, though probably not through a reduction in cell number, and pharyngeal pumping for food consumption was decreased. Lower levels of ATP and oxygen consumption, and marked abnormalities in mitochondrial structure and morphology were noted (Lee et al., 2003). Life span extension was also accompanied by resistance to heat shock and hydrogen peroxide treatment.

Are similar large-scale genetic approaches practical using zebrafish? Chemical mutagenesis methods are a major strength of the zebrafish model and are being widely exploited for a number of experimental purposes. However, several issues complicate the use of life span as a phenotype for screening. A maximum life span of more than 5 years exceeds conventional funding intervals. Although this has not prevented aging studies on species with even longer life spans, it is a major practical constraint. The appropriate control group for comparison of mutant longevity also needs to be well defined. Further, baseline data on aging in commonly used zebrafish strains is thus needed, including the effects of caloric intake, temperature, housing density, reproductive effort, and physical activity on life span.

In addition, to achieve saturation mutagenesis, thousands of fish must be generated. This number is feasible when screening for developmental mutations because phenotypes are assessed only during the first few days of life and the fish are therefore quite small, minimizing space requirements, and fish without phenotypes can be euthanized freeing space quickly. Using longevity as a phenotype requires maintaining mutation-bearing fish for long periods as adults, dramatically increasing space and husbandry requirements. Enough fish carrying each mutation must be maintained in order to obtain sufficient statistical power to discern potential increases in longevity.

Several approaches may be taken to address these disadvantages. Mutation-bearing cohorts that exhibit shortened median longevities can be continually replaced with cohorts carrying new mutations, a sort of ‘rolling’ mutagenesis strategy to maximize use of space. However, this strategy will miss mutations that extend maximum longevity though decreasing median longevity. Another possibility is to separate the measurement of longevity from the mutagenesis by screening for surrogate phenotypes. Indeed, most of the first 40 or 50 longevity extending mutations identified in C. elegans were mutants initially identified through screening for developmental abnormalities (Murakami et al., 2000). Characteristics of long-lived worm mutants include resistance to heat and oxidative stress (Johnson et al., 2000), as well as the various changes noted in RNAi treatment with mitochondrial genes described above. Screening for these sorts of phenotypes at a young age could identify a small group for subsequent longevity studies.

Zebrafish may be a particularly useful model to screen for such surrogate phenotypes. Zebrafish are quite small for the first several months of life, so large numbers of mutant fish may be generated and subject to phenotypic screening. Generation of haploid progeny may also be used for screening to decrease space and time requirements. Non-toxic fluorescent compounds may be used to visualize mitochondrial morphology and function (Fig. 1), and various physiological measurements may be performed on individual fish such as oxygen consumption, caloric intake, and physical activity.
Mutants with potentially relevant phenotypes have already been described, including a temperature preference mutant and two caloric intake mutants (Vogel, 2000b).

Initial RNAi approaches in zebrafish have not been promising (Zhao et al., 2001), but ‘knock-down’ morpholino oligonucleotide techniques are well developed (Nasevicius and Ekker, 2000). The reduced expression is only effective for the first 4–5 days of development, although during this time, essentially all major organs assume adult morphology. Whether a reduction in the expression of a single gene during the first few days of life could extend the life span of an organism that may live more than 1800 days is not known. However, the finding that the RNAi of mitochondrial genes was required only during development and not throughout life (Dillin et al., 2002) suggests that this may be possible. Indeed, the easy and direct accessibility to early development may make zebrafish an extremely useful model to determine how interventions early in development affect subsequent longevity.

Expression profiling has also been reported in aging C. elegans (Lund et al., 2002). However, the small size and non-compartmentalized vascular system of C. elegans make the isolation of specific cells or tissues for use in microarray studies difficult. Thus, whole organism homogenates have been used to obtain RNA for microarray studies, an option not scientifically acceptable for vertebrate systems. For studies difficult. Thus, whole organism homogenates have been used to obtain RNA for microarray studies, an option not scientifically acceptable for vertebrate systems. For C. elegans, alternative approaches using mutants, transgenics, tissue culture, and computational strategies have been used to interpret whole organism data (Reinke, 2002). An advantage of zebrafish for such studies is the ability to obtain significant amounts of several tissues, especially skeletal muscle, and the availability of large-scale microarrays, both cDNA and oligonucleotide (Lo et al., 2003; Stickney et al., 2002), so that reliance upon cross-species hybridization is not necessary.

Until recently, the morphological phenotype of aging worms had not been characterized (Herndon et al., 2002). A major phenotype in old worms appears to be muscle degeneration, akin to sarcopenia in mammals and other species. Muscle degeneration also appears to be a major senescent change in zebrafish (Gerhard et al., 2002).

6. The ‘vertebrate Drosophila’ versus the invertebrate, Drosophila

Nusslein-Volhard’s Nobel Prize-winning work with Drosophila was the precedent for pursuing large-scale mutagenesis of zebrafish development. In gerontology, Drosophila has also been well studied for many decades (Helfand and Rogina, 2003). Selective breeding based upon the onset of reproduction has shown that naturally occurring genetic variants can cause large differences in life span (Arking, 2001). Long-lived Drosophila lines are also more resistant to stress, similar to long-lived C elegans mutants. Selection for specific traits such as onset of reproduction has not yet been reported in zebrafish.

Large-scale mutational analysis has also been used to identify genes that extend Drosophila life span. As was the case for C. elegans, most of the mutations reported thus far were not identified based upon screening for longevity. In contrast to C. elegans, mutations in most components of the insulin signaling pathway do not extend longevity. Nevertheless, certain insulin receptor mutant alleles do extend life (Tatar et al., 2001) as do mutations in the insulin receptor substrate chico in females (Clancy et al., 2001). The insulin pathway in Drosophila regulates cell, organ, and total body size and affects metabolism (Garofalo, 2002).

Zebrafish may be a particularly useful species to extend studies on the role of insulin pathways upon aging, given the indeterminate nature of growth in fish. The major proteins involved in insulin signaling appear to be structurally and functionally similar to those found in mammals. Zebrafish possess two structurally distinct IGF-I receptor genes that display different expression patterns (Pozios et al., 2001). IGF signaling system has been suggested to play a key role in the indeterminate growth of fish species (Maures et al., 2002). Screening for growth related phenotypes may therefore be a feasible surrogate phenotype for mutations affecting the IGF pathway.

Other genes have been identified that extend longevity in Drosophila. Mutation in the Methuselah gene extends life span by about one-third and increases resistance to stress (Lin et al., 1998). The Indy (I’m not dead yet) gene also extends longevity and is homologous to a dicarboxylate transporter, suggesting a role in intermediary metabolism and perhaps operating through a mechanism related to caloric restriction (Rogina et al., 2000). Zebrafish may be subject to caloric restriction regimens similar to those used in rodent and primate studies (Pugh et al., 1999).

Overexpression of the antioxidant defense genes, including Mn superoxide dismutase (Sun et al., 2002), Cu/Zn superoxide dismutase with (Sohal et al., 1995) or without catalase overexpression (Sun and Tower, 1999) has also been reported to increase Drosophila life span, although some controversy exists (Orr and Sohal, 2003). Transgenic overexpression is easily accomplished in zebrafish (Udvadia and Limney, 2003) and several antioxidant defense genes have been cloned. Importantly, effects on specific pathologies may be determined using the zebrafish model.

7. Zebrafish versus lab mice—50 times smaller yet live 50% longer

The life spans of many commonly used strains of laboratory mice do not extend much beyond 2–3 years. However, several mutations that result in a dwarf phenotype, i.e. the Ames dwarf (Prop1 df/df) and Snell dwarf (Pit1 df/dw) mice can increase life span by over 50% (Bartke et al., 2001). The growth abnormalities are due to multiple hormonal
deficiencies, including the growth hormone/insulin-like growth factor-1 pathway, suggesting potential common regulatory aspects of life span determination across a wide range of species. Mice selectively bred for post-natal growth rate also had a wide range of life spans (Miller et al., 2000).

Zebrafish may be a valuable species to investigate the possible relationships between early growth rates and longevity. Wide variation in growth rates is apparent in outbred zebrafish populations, suggesting the presence of naturally occurring polymorphisms on which selection could easily be performed. Growth may also be impacted by environmental conditions. Data from a South American annual fish indicate that decreased ambient temperature resulted in both faster growth rates and increased life span (Liu and Walford, 1966). No follow-up studies have been reported on this observation.

Mouse and zebrafish both offer conventional transgenic overexpression approaches, although the costs and technical difficulties associated with generating transgenic zebrafish are much lower. The generation of germ-line founder transgenic fish by microinjection of DNA into zebrafish embryos is similar to that obtained in mice (Udvadia and Linney, 2003). The injected DNA usually integrates as a concatemer in both species as well. Several alternative transgenic techniques are available for zebrafish including retrovirus infection (Linney et al., 1999) and the use of nuclear localization signals (Collas and Alestrom, 1998).

The mouse has become the prime vertebrate model system to disrupt gene function through homologous recombination using embryonic stem cells. In zebrafish, gene expression may be reduced for the first several days of embryonic development through microinjection of modified antisense oligonucleotides called morpholinos (Nasevicius and Ekker, 2000). Unfortunately the ‘knock-down’ of gene expression is only transient. True ‘knock-out’ techniques have not yet been reported but germ-line chimeras have been generated from embryo cell cultures (Ma et al., 2001) and zebrafish have been cloned by substitution of the embryonic nucleus with a nucleus from a cultured embryonic fibroblast (Lee et al., 2002).

Accessibility to the fish equivalents of most major mammalian organs is possible in zebrafish. Post-mitotic tissues, including skeletal muscle, brain, heart, and eye may thus be easily harvested for study. Replicating tissues may also be obtained, and zebrafish develop a wide variety of neoplasms with morphological similarity to mammalian tumors (Beckwith et al., 2000). Pathological analysis is also more cost-effective in zebrafish due to the ability to place an entire adult fish on a single microscope slide.

8. Zebrafish, a new angle on human disease

Despite nearly a half a billion years of evolutionary divergence, zebrafish possess many anatomical and functional features that are similar to humans. This has led investigators to use mutagenesis screens for phenotypes related to human disease as well as disease therapeutics (Nasevicius and Ekker, 2001; Rubinstein, 2003). Shin and Fishman (2002) have presented zebrafish as a ‘modular medical model.’ They argue that zebrafish orthologs have been identified for most human genes, and large regions of conserved chromosomal synteny are present between zebrafish and mammals. There are also certain aspects of zebrafish mutagenesis screens that facilitate the identification of certain aspects of specific human diseases. For example, complex phenotypes may be sought, paralleling many human disorders, and the mutations induced by chemical mutagenesis often produce hypomorphic mutations rather than the null mutations in mouse knockouts. A number of clinically important age-related human disorders may be modeled in zebrafish including heart failure, cardiac ischemia, cancer, diabetes, osteoporosis, Parkinson’s disease, Alzheimer’s disease, hearing loss, and macular degeneration. Most of the work done so far has been to identify mutations that affect anatomically important cell types related to these various disorders. Little work has been done on screening for age-related phenotypes at older ages.

Another potential use that zebrafish may have in the study of age-related human disease, is the ability to perform high throughput screening for compounds affecting a specific biochemical or morphological phenotype (Moon et al., 2002). Zebrafish embryos are permeable to a wide variety of small molecules that may be dissolved directly in the water. The high reproductive capacity, economy, and small size all facilitate the use of zebrafish for such screening.

9. Summary

The zebrafish is a prime organism for the study of embryonic development that is now being used to model a number of physiological and pathological processes. It is anticipated that the experimental attributes that have led to its widespread use in many diverse disciplines will also make it a fruitful gerontological model in the near future.

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References


