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REVIEW

A call to fins! Zebrafish as a gerontological model

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Summary

Among the wide variety of model organisms commonly used for studies on aging, such as worms, flies and rodents, a wide research gap exists between the invertebrate and vertebrate model systems. In developmental biology, a similar gap has been filled by the zebrafish (*Danio rerio*). We propose that the zebrafish is uniquely suited to serve as a bridge model for gerontology. With high fecundity and economical husbandry requirements, large populations of zebrafish may be generated quickly and cheaply, facilitating large-scale approaches including demographic studies and mutagenesis screens. A variety of mutants identified in such screens have led to modelling of human disease, including cardiac disorders and cancer. While zebrafish longevity is at least 50% longer than in commonly used mouse strains, as an ectothermic fish species, its life span may be readily modulated by caloric intake, ambient temperature and reproductive activity. These features, coupled with a growing abundance of biological resources, including an ongoing genome sequencing project, make the zebrafish a compelling model organism for studies on aging.
Key words: aging; model; review; Zebrafish.

Introduction

Following an excellent and prescient review article on the use of the zebrafish model system to analyse embryonic development (Rossant & Hopkins, 1992), it became apparent that what was good and becoming great, for developmental biology, could also be fruitful for gerontology. In the 10 years since that publication, a plethora of data on vertebrate development has been generated using zebrafish (Driever *et al*., 1996; Haffter *et al*., 1996; Chen & Fishman, 2000; Grunwald & Eisen, 2002). In contrast, the use of zebrafish as a model for gerontology has lagged. In this review, we describe the versatility of zebrafish as a model organism and why we think the time has come to exploit this species as a model for aging research.

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Zebrafish biology

Zebrafish are a 2.5–5-cm-long freshwater tropical cyprinid fish native to India (Eisen, 1996). It has been a popular home aquarium fish for decades in part because of its active and 'playful' disposition and the ease with which it can be maintained. Initial studies performed by the late George Streisinger and colleagues at the University of Oregon were the driving force behind the emergence of the species as a major model organism for biological research (Streisinger *et al*., 1981). Main attractions for the use of zebrafish in developmental biology research included the transparency of the embryos and *ex utero* development, which greatly facilitate visual observation of developing anatomical structures (Eisen, 1996). A single female can produce several hundred eggs per week, although the onset of reproductive capacity is about 3–4 months, longer than that of the mouse. Thousands of fish can be quickly and simply produced, and large populations can be maintained at very low cost compared to rodents. Well-developed *in vitro* fertilization techniques also facilitate breeding programmes and mutant preservation. These invertebrate-like characteristics and a complex mammalian-like developmental script have led to an extensive characterization of zebrafish embryonic development (for example see Chen & Fishman, 2000). A wealth of data on cell determination and specialization during development, and the growing armamentarium of techniques such as *in vivo* imaging (Lawson & Weinstein, 2002), make the zebrafish an especially attractive model to study the interrelationship between differentiation and senescence. For example, investigation of the effects of aging on cell-cell communication may be quite feasible in zebrafish. Further information about zebrafish biology can be obtained at the Zebrafish Information Network (http://zfin.org/).

Mutagenesis

While the visual accessibility of zebrafish development has led to substantial descriptive information on cell fate and patterning (Schier, 2001), the true power of the zebrafish model for the analysis of complex biological processes, such as development and perhaps aging, lies in the ability to manipulate it genetically (Talbot & Hopkins, 2000). Streisinger conducted initial mutagenesis studies using pigmentation defects as a marker phenotype in the early 1980s (Streisinger *et al*., 1989; Grunwald &

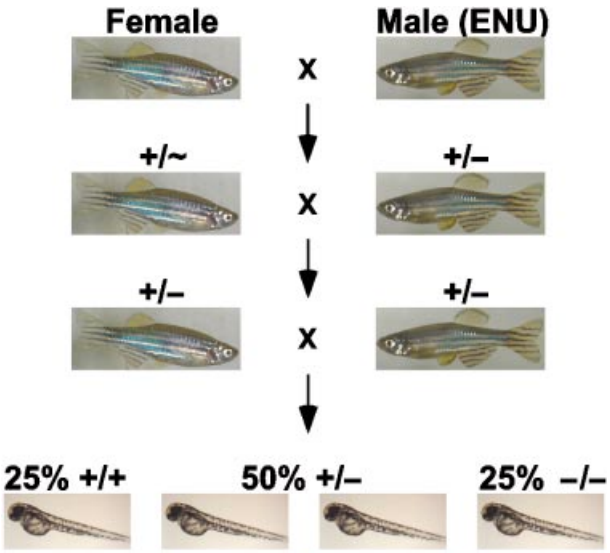

Fig. 1 Classic three-generation mutagenesis screen. Mutations in the germ cells of male fish are introduced by exposure to the chemical ethylnitrosourea (ENU). Crossing mutagenized males with wild-type females will produce heterozygous progeny harbouring various mutations. Successive sibling intercrosses produce offspring homozygous for mutant alleles at an expected incidence of 1 in 4.

Streisinger, 1992). The first large-scale mutagenesis screens of zebrafish to identify developmental mutations were conducted by Nusslein-Volhard and colleagues in Germany in the early 1990s (Haffter *et al*., 1996), based on her Nobel prize-winning studies on saturation mutagenesis screens in *Drosophila* (Nusslein-Volhard & Wieschaus, 1980), and by Fishman and colleagues in Boston (Stainier *et al*., 1996). Their approach was to mutagenize males with a chemical to induce point mutations, and then use a breeding strategy to produce individuals harbouring homozygous mutations. This classic three-generation screen (Fig. 1) has since been used to identify most of the mutants that have been published (Van Eeden *et al*., 1999). For the initial mutagenesis, male fish are treated with ethylnitrosourea (ENU), a chemical mutagen used to induce primarily point mutations. The mutagenized males are mated to normal females to produce 'founder' F1 fish, ideally each carrying a different mutation (designated by '~' and '–' in Fig. 1). Brother–sister matings of the F1 carriers is done to produce F2 families of fish heterozygous for the induced mutations. Random sibling matings of the F2 heterozygotes produce F3 homozygotes that are then screened for developmental abnormalities. A Mendelian frequency of one mutant in four F3 progeny indicates a recessive mutation carried by the parents.

What relevance could large-scale mutagenesis techniques using zebrafish have for gerontology? Mutant screens have generated a number of mutants with extended longevity in invertebrate models, especially *C. elegans* (Guarente & Kenyon, 2000; Murakami *et al*., 2000). Typically, ethyl methanesulphate was used to induce point mutations that resulted in null or reduced function alleles. Interestingly, only a few of the more than 40 or 50 longevity-extending mutations so far characterized were originally identified in screens for increased life span. In *Drosophila*, P-element insertional mutagenesis has been the predominant method to create mutants with extended longevity, either by directly screening for increased life span or by analysing a mutation in a known gene (Landis *et al*., 2001; Seong *et al*., 2001). The interpretation of the results from these invertebrate mutants may not be straightforward, however. For example, *age-1* mutants, which manifest extended longevity under standard conditions, exhibit a reduction in fitness when grown with wild-type worms under periodic dietary stress (Walker *et al*., 2000). If a general effect of longevity-extending mutations is reduced fitness, then their potential value for understanding the underlying mechanisms of aging may be limited. As a much longer-lived (see below) vertebrate, zebrafish may be a very useful species for this type of analysis.

In addition to chemical mutagenesis, insertional mutagenesis has proven feasible in zebrafish (Amsterdam *et al*., 1999). A primary advantage of insertional mutagenesis strategies, in which a retroviral vector randomly integrates into the genome to disrupt gene function, is the presence of a known sequence that allows direct cloning of the mutated gene, circumventing the laborious mapping techniques required to identify point mutations created by chemical mutagens (Golling *et al*., 2002). Two disadvantages to insertional mutagenesis are a lower mutation efficiency than that obtained with chemical mutagens, and the effort required to microinject the insertion vector into the embryo. Large-scale mutagenesis efforts have also been initiated using mice (Beckers & Hrabe De Angelis, 2002), although the costs and infrastructure required are much greater than that needed for zebrafish screens.

The vast majority of mutagenesis screens have focused on phenotypes displayed in embryonic or early stage larval fish. However, screening approaches continue to evolve. Zebrafish embryos hatch to become free-swimming larvae at about 2 days post-fertilization, and thus begin to display adult characteristics, such as sensory and motor responses. They are physically large enough to isolate specific tissues for experimental analysis, minimizing the disadvantages of pooled whole organism approaches commonly used for invertebrate analyses. Thus, screens for gerontologically relevant phenotypes, such as alterations in growth, or responses to oxidant or thermal stress should be highly feasible. Indeed, mutant screens related to age-related disease processes such as cancer have been initiated (Cheng & Moore, 1997; Cheng *et al.*, unpublished data).

A highly desirable objective would be to screen for mutants manifesting extended longevity. As a basis for such work, we have performed initial life span studies on outbred zebrafish and found the mean life span (from 17 months of age) to be about 42 months, with the longest living individuals surviving for 66 months (Gerhard *et al.*, 2002). Unfortunately, reproductive activity, such as number of eggs produced and frequency of mating, was not assessed in these group-housed populations containing a mix of males and females. The effects of reproductive effort on zebrafish longevity will be important to

determine since life span in other fish species may be impacted by reproduction (Reznick *et al*., 2001). Indeed, the 2- to 4-year life span anecdotally referred to for zebrafish (Higgs *et al*., 2002; Keller, 2002) may be the result of the need of developmental biologists to maximize reproductive output to provide embryos for studies on development and for mutagenesis. Caloric intake may also be a factor, since energy-rich diets are often used to increase reproductive capacity.

We also assessed the longevity of a relatively inbred strain that manifested 10–15% shorter mean and maximum life spans than the outbred zebrafish (Gerhard *et al.*, 2002), consistent with the effects of inbreeding depression. These results highlight a potential problem for the use of inbred lines in mutagenesis studies that select for longevity extending mutations. Mutations in genes that extend life span may merely restore a function that was impaired during inbreeding. Similarly, the artificial and potentially artefactual conditions of the laboratory environment likely cause a number of physiological adaptations. Longevous mutants may merely be better adapted to the laboratory environment and would be of limited general value in understanding the genetic basis of senescence. Such effects have been reported in comparisons of longevity among wild-derived stocks of mice (Miller *et al*., 2002) and *Drosophila* (Linnen *et al*., 2001) with commonly used inbred lines. Smaller body size and later onset of reproductive maturity were regarded as important differences in the longer lived wild-derived stocks in the murine study. The indeterminate and plastic nature of zebrafish growth make it a compelling model to extend and confirm such observations. In addition, since wild-type zebrafish stocks are readily available it should be possible to evaluate candidate loci identified in laboratory-derived strains by crossing the mutations into wild-type genetic backgrounds.

Since the life span of zebrafish is at least 50% longer than that of analagous mouse stocks, and longer than long-lived mouse mutants (Bartke *et al*., 2001), screening for longevity-extending mutations would extend beyond the typical 3- to 5-year grant funding cycle. However, our initial life span studies were performed with limited populations under one set of conditions. A number of variables may impact zebrafish longevity, including caloric intake, ambient temperature, housing density and reproductive activity. As mentioned above, anecdotal reports of shorter life spans may be due to differences in one or more of these variables. Thus, mutagenesis studies could be performed under conditions that result in a shorter longevity. While the life span we observed may be considered a major disadvantage of the model, it is still much less than other organisms that have been used in aging research, especially non-rodent mammals, and is far more rodent-like than the short-lived invertebrate model systems in which similar experimental approaches may be taken. In addition, the speed and economy with which large populations of zebrafish may be derived and maintained make possible large-scale demographic analyses (Vaupel *et al*., 1998), an advantage not shared by many vertebrate models.

Older zebrafish often manifest various degrees of spinal curvature (Fig. 2), as reported in other aging fish species (Comfort, 1961; Liu & Walford, 1969). In aging guppies, such curvature is thought to be due to muscle degeneration and may serve as a model for sarcopenia. Tens to hundreds of milligrams of skeletal muscle may be isolated from a single fish, providing an ample supply of tissue for study.

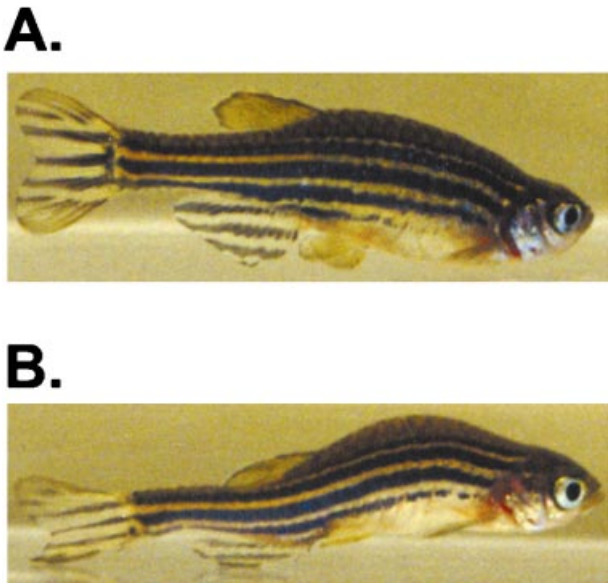

Fig. 2 Common senescent morphology in old zebrafish. (A) Old (52 months) zebrafish with fairly linear contour and subtle spinal curvature. (B) Old (52 months) zebrafish with moderate spinal curvature.

The feasibility of screening for extended longevity also depends not only upon length of life, but also the expected incidence of longevity-associated mutations. The presumably high numbers of mutation-carrying fish that would be required to identify mutants manifesting extended longevity would be beyond the capacity of most zebrafish facilities. Rather, it may be more effective to identify mutants with surrogate phenotypes, and then to determine whether the mutation has an effect upon life span, as has been the case for most of the *C. elegans* longevity mutants (Murakami *et al*., 2000), and several *Drosophila* strains (Clancy *et al*., 2001; Tatar *et al*., 2001). For example, several potentially relevant satiety and temperature mutants have already been described (Vogel, 2000b), although their impact upon life span has yet to be defined.

Genomics and genetics

A sufficient set of genomics resources is a necessary requirement for the identification of chemically induced mutations. The development of zebrafish genomics has been supported by a Trans-NIH Zebrafish Initiative (http://www.nih.gov/science/models/zebrafish/). Radiation hybrid and microsatellite maps have been assembled, and YAC, BAC and PAC libraries are available (Postlethwait & Talbot, 1997), and an abundance of ESTs have been cloned that has led to the generation of large-scale cDNA arrays for gene expression profiling (Herwig *et al*., 2001).

Sequencing of the zebrafish genome, estimated at 1700 Mb or about half the size of the human genome, was begun in 2001 and is expected to be available in draft form within the next 2 years (Vogel, 2000a). Ongoing are the creation of deletion mutants of the entire genome, and numerous mutagenesis screens (Wixon, 2000). The breadth and depth of these resources places the zebrafish in an elite group of model organisms that includes *C. elegans*, *Drosophila melanogaster* and *Mus musculus*. The sequencing of the *Fugu ripides* (Japanese pufferfish) genome provides a close evolutionary relative for comparative genomic studies as well (Venkatesh *et al*., 2000).

The zebrafish genome consists of 25 linkage groups corresponding to as many chromosomes (Postlethwait & Talbot, 1997). Most human genes examined thus far have orthologs in the zebrafish. Comparative analysis of the human and zebrafish genomes (Postlethwait *et al*., 2000) has also revealed large blocks of conserved synteny, indicating conservation prior to 450 million years ago, when zebrafish and human ancestors diverged. A genome-wide duplication event appears to have occurred more than 100 million years ago in the teleost lineage, well after humans and zebrafish split but prior to the divergence of zebrafish and pufferfish. Approximately 20% or more of the genes duplicated in this event may still be present, with many of the duplicates no longer redundant in function, having assumed subfunctions or evolved new roles. Thus, a mutation in one of two duplicated (but not identical) genes in zebrafish may cause a different phenotype than a mutation of the orthologous gene in mammals, where a single gene may subserve similar functions.

Models for disease

Zebrafish have also now become a useful model organism to study disease (Dodd *et al*., 2000; Knapik, 2000). A number of organ systems have been studied, particularly the cardiac (Chen & Fishman, 2000) and haematopoietic systems (Amatruda & Zon, 1999), which has resulted in the identification of mutants closely paralleling several human diseases. For example, exploiting the transparency of zebrafish embryos to screen for the accumulation of fluorescent precursors in heme biosynthesis has led to the identification of mutant models for the human porphyria disorders (Wang *et al*., 1998). Mutations in genes causing cardiac (Xu *et al*., 2002) and sensory abnormalities (Ernest *et al*., 2000) also closely model several human genetic disorders. Genetic approaches using zebrafish have been proposed for aspects of age-related disorders, such as heart failure and stem cell therapies (Fishman, 2001). Several mutants with defects in genomic stability have also been identified (Cheng & Moore, 1997), which may result in further insight into the age-associated increase in cancer incidence.

Mice are still the premiere organism for disease modelling, primarily due to the ability to perform targeted gene disruption, i.e. gene knockouts, as well as transgenic overexpression. Overexpression in zebrafish can be achieved via tissue-specific or inducible promoters through microinjection of either RNA or DNA, including large segments of DNA such as BACs (Meng *et al*., 1999). Gene targeting by homologous recombination has not yet been accomplished in zebrafish, although embryonic cell cultures have been used to generate germ-line chimeras (Ma *et al*., 2001). However, 'knock down' techniques, in which antisense morpholino oligonucleotides are injected into embryos to partially block the translation of specific mRNAs, have become powerful tools for the dissection of gene function (Ekker, 2000; Nasevicius & Ekker, 2000).

Fish as models for aging

Fish represent the largest class of vertebrates, with more than 24 000 extant species (Patnaik *et al*., 1994), and have as members a majority of the longest living vertebrates currently known (Reznick *et al*., 2002). At least one member of this longevous group has been the focus of gerontological investigation (Black, 2002). A small number of other fish species have also been studied from a gerontological perspective (Liu & Walford, 1969; Markofsky & Perlmutter, 1972; Egami & Etoh, 1969; Schreibman *et al*., 1983; Woodhead, 1974; Satapathy & Patnaik, 1980; Beverton, 1987; Patnaik *et al*., 1994), although few studies specifically investigated the aging process throughout the life span (Woodhead, 1978). The experimental expediencies that attract developmental biologists have also been advocated for using fish as models for aging. These advantages include the ready availability of large cohorts, ectothermy that allows for modulation by external environmental changes, reasonably short life spans, and lower costs for breeding and maintenance relative to many mammalian species (Woodhead, 1978; Patnaik *et al*., 1994).

Oxidative stress

Oxidative stress has become a key paradigm in aging (Sohal & Weindruch, 1996). Little work has been done on oxidative stress during aging in any fish species. However, adaptive responses to toxicological and oxidative stressors in fish have been found to be similar, though not identical, to mammals (Winston, 1991; Kelly *et al*., 1998). Fish possess the major antioxidant enzymes, which generally occur at relatively higher levels than those of birds and mammals. Differences in oxidative stress have been proposed as a potential mechanism underlying the long life spans of certain species that live in extreme aquatic environments (Cailliet *et al*., 2001).

Zebrafish appear to be an excellent organism on which to investigate oxidative stress during aging. Studies on the effects of UV radiation on antioxidant status and survival, and on the use of transgenic zebrafish to serve as sentinels for oxidative stress, have been reported (Carvan *et al*., 2001; Black, 2002). We have initiated studies on oxidative stress by cloning several zebrafish antioxidant defence genes, including catalase (Gerhard *et al*., 2000). We have also measured levels of oxidative macromolecular damage in several tissues (Gerhard *et al*., unpublished data). The sequence of the zebrafish mitochondrial

genome is available in GenBank (Accession AC024175) and shares high sequence identity with mammalian mtDNAs.

The generation of reactive oxygen species (ROS) may be a primary pacemaker of aging (Merry, 2000). Most studies that have measured the production of ROS in the context of aging have used *in vitro* methods such as superoxide generation from submitochondrial particle fractions and hydrogen peroxide production from mitochondrial preparations (Farmer & Sohal, 1987; Sohal *et al*., 1994). Few *in vivo* studies have been performed to validate *in vitro* findings, in large part because such methods do not yet exist for higher organisms.

Dichlorodihydrofluorescein diacetate (DCF), a fluorescein derivative that fluoresces upon reaction with ROS, has been used to detect the generation of reactive oxygen intermediates inside living cells in culture (Miranda *et al*., 1999; Leach *et al*., 2001). While not specific for particular ROS (or for reactive nitrogen species), it is sensitive for the detection of ROS activity from a number of sources. We have detected adequate fluorescent signal following exposure of zebrafish larvae for 1 h to 30 μM DCF added to the water, with no noticeable effects on survival or morphology (Fig. 3). This approach may also be applicable to several lines of adult fish manifesting pigmentation defects and increased optical clarity. The ability to repeatedly measure the level of ROS generation in a living vertebrate over time may have multiple applications in gerontology, especially in screening for ROS mutants.

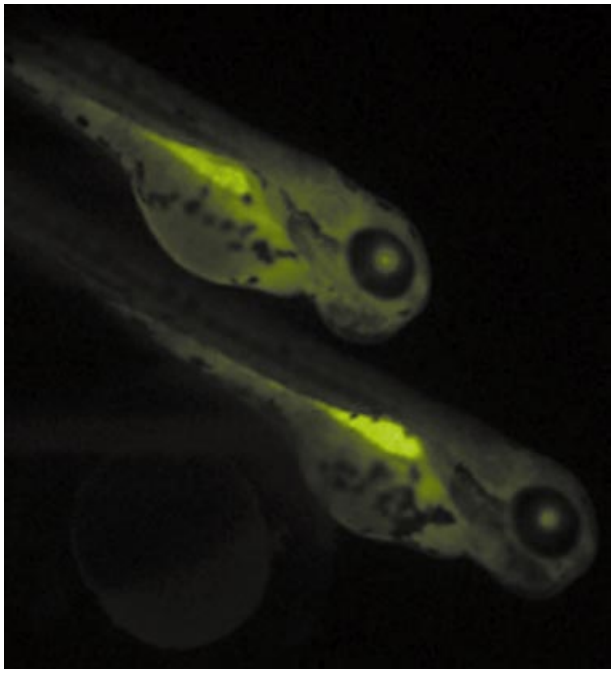

Fig. 3 Dichlorodihydrofluorescein diacetate (DCF)-treated embryos (200×). Embryos at several stages of development were incubated with 30 μM of DCF for 1 h then examined by fluorescence microscopy. In embryos at 2–3 days of age, the gastrointestinal tract was strongly fluorescent but other tissues also exhibited relatively strong fluorescence, including the retina and other structures of the head region. No effect through adulthood could be detected in 2–3-day-old embryos exposed for 1 h to 30 μM DCF.

Caloric restriction

Another general advantage of a fish species as a model system is the ability to control caloric intake with reasonable accuracy. However, the only reported laboratory-based study on the impact of caloric restriction on aging in fish was conducted with the guppy (*Lebistes*), whose maximum life span is about 5 years (Comfort, 1963). A 50% reduction in food intake maintained only for the first 600 days of life increased maximum life span by approximately 15%. Surprisingly, the mortality rate curves suggested that rather than altering the rate of aging, caloric restriction appeared to prevent a specific or perhaps singular cause of early mortality, such as an infection. Confirmation of this work in a similar species, such as the zebrafish, under specific pathogen-free conditions with a more suitable caloric restriction protocol is warranted.

We have performed short-term studies in zebrafish using a caloric restriction regimen akin to those used in rodent and primate studies (Pugh *et al*., 1999). We have designed specific diet formulations to ensure that calorically restricted and *ad libitum* fed fish consume equivalent amounts of essential nutrients yet decreased calories, primarily carbohydrates. Gene expression in tail muscle from restricted fish was profiled using a small-scale zebrafish cDNA array. Several mitochondrial genes were found to be down-regulated (Gerhard *et al*., unpublished data), similar to gene expression profiling results reported for skeletal muscle of rhesus monkeys subjected to caloric restriction (Kayo *et al*., 2001).

Temperature modulation

In ectothermic organisms, life span is generally inversely proportional to ambient temperature, although both phenomenological and mechanistic studies have been performed almost exclusively in invertebrates (Sohal & Allen, 1986). In general, oxidative damage to lipids and proteins and ROS generation are increased at higher temperatures, although exceptions are apparent (Farmer & Sohal, 1987). Even the relationship between metabolic rate and temperature is not simple or direct (Mcarthur & Sohal, 1982). In these models, potentially important variables have not been accounted for, including caloric intake, metabolic rate and activity measurements, all feasible to measure in zebrafish. Indeed, the potential role of caloric restriction as the primary mediator of the longevity extending effects of temperature modulation has not been adequately assessed, though zebrafish would be an ideal model for such studies. As an ectotherm, zebrafish are likely to manifest differences in stress physiology relative to mammals, although little data are yet available on their responses to specific stressors.

Temperature reduction has previously been studied in the annual fish, *Cynolebias bellottii,* which resulted in close to a doubling of mean and maximum life spans with modest (6 °C) reductions in tank temperatures (Liu & Walford, 1966). Interestingly, fish kept at the higher temperature for the first

8 months of life, then switched to the lower temperature, had life spans even longer than fish kept continuously at the lower temperature, which in turn lived longer than fish kept continuously at the higher temperature (Liu & Walford, 1975). These studies do not appear to have been replicated in any other vertebrate species.

Insulin-like pathways

Studies conducted over the past several years primarily in *C. elegans* indicate that insulin-like signalling plays a key role in determining life span (Murakami *et al*., 2000). Mutations in *daf-2*, *age-1* and other genes in *C. elegans* that are components in an insulin signalling pathway significantly increase longevity by determining whether the organisms enter a diapause-like metabolic state (Guarente & Kenyon, 2000). Mutations in the *Drosophila* orthologs of the insulin receptor (Tatar *et al*., 2001) and insulin receptor substrate (Clancy *et al*., 2001) also cause a modest increase in life span. In mammals, studies in the long-lived Ames and Snell dwarf mice (Bartke *et al*., 2001) implicate a role for the insulin-like growth factors in aging. Some information on these pathways is already available in zebrafish. IGF receptors with characteristics of the mammalian type I IGF receptor have been described in zebrafish cells (Maures *et al*., 2002). The two major signal transduction pathways, MAPK and PI3 kinase, are activated by IGF-I (Pozios *et al*., 2001). Based on data from several fish species, including zebrafish, the major components of the IGF signalling system appear to be structurally and functionally similar to those in mammals, although several key differences are apparent. Zebrafish possess two structurally distinct IGF-I receptor genes that display different expression patterns, and thus may play different roles in development, growth and aging. In addition, zebrafish appear to grow throughout the life span (Gerhard *et al*., 2002), similar to other fish species. The IGF signalling system has been suggested to play an important role in this growth pattern (Maures *et al*., 2002). Screening for growth-related phenotypes should be feasible in mutagenesis studies.

Prospects for the future

As would be the case for any new model organism, a substantial database of basic gerontological information for zebrafish is needed. Although we have conducted initial studies, longevity data on several outbred and inbred strains, performed by several investigators, accounting for several key variables, such as calories, temperature, reproductive capacity, and housing density would be a good start. Careful documentation of pathologies during aging, easily and economically accomplished in zebrafish, would also be invaluable for the interpretation of age-related phenotypes. Mutagenesis screens for mutants bearing gerontologically relevant phenotypes, such as alterations in levels of oxidative damage or disruption of insulin pathways, could proceed in parallel. By the time mutants are identified and initially characterized, initial longevity studies could be well underway. Mutant lines could then be crossed to various genetic backgrounds and expanded to establish populations for longevity assessment. One practical reason for investigating aging in zebrafish is that a major investment has already been made in basic zebrafish biology that has been driven by developmental biology research. The availability of reagents such as clones, antibodies and cell lines, experimental procedures such as transgenesis, and expertise of numerous investigators world-wide using zebrafish provides a deep set of resources upon which to base future gerontological studies.

Conclusion

By virtue of a burgeoning wave of biological resources accumulating for the zebrafish, driven primarily by the ability to perform large-scale mutagenesis studies, zebrafish have become a major model system for biomedical research. Few mutants with gerontological phenotypes have been identified, though much promise lies ahead for a genetic approach. The zebrafish appears to be a promising model to study several aspects of aging, such as the relationship of differentiation and senescence, the mechanisms by which caloric restriction and temperature reduction modulate life span, and large-scale demographic analyses. Despite a longer life span than mice, numerous invertebrate-like advantages, especially the ease and low cost of deriving and keeping large populations, make the zebrafish uniquely suited for a variety of gerontological studies.

Acknowledgments

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