into grant applications, could serve as a way to collect supplemental funding from the researchers to whom stock centres bring the most direct benefit.

Networks represent the future of the worldwide animal model stock resource (BOX 4). We need to work together in larger conglomerates, avoiding duplication of activity, ensuring economies of scale, and cooperating in securing long-term funding platforms. If successful, these collective consciousness-raising efforts will serve as examples for the support of other model organisms. An expanded funding of stock centres will be necessary for the effective exploitation of the genetic and genomic information on the organisms they protect.

*Nadia Rosenthal is at the Mouse Biology Programme, European Molecular Biology Laboratory, via Ramarini 32, 00016 Monterotondo (Rome), Italy.*

*Michael Ashburner is at the Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK.*

*Correspondence to N.R.*
*e-mail: rosenthal@embl-monterotondo.it*

doi:10.1038/nrg891

1. Ashburner, M. Europe must grant crucial funds for biological research. *Nature* **402**, 12 (1999).
2. Wang, W. B., Leopold, R. A., Nelson, D. R. & Freeman, T. P. Cryopreservation of *Musca domestica* (Diptera: Muscidae) embryos. *Cryobiology* **41**, 153–166 (2000).
3. Leopold, R. A., Wang, W. B., Berkebile, D. R. & Freeman, T. P. Cryopreservation of embryos of the New World screworm *Cochliomyia hominovorax* (Diptera: Calliphoridae). *Ann. Entomol. Soc. Am.* **94**, 695–701 (2001).
4. Glenister, P. H. & Thornton, C. E. Cryoconservation — archiving for the future. *Mamm. Genome* **11**, 565–571 (2000).
5. Balling, R. ENU mutagenesis: analyzing gene function in mice. *Annu. Rev. Genomics Hum. Genet.* **2**, 463–492 (2001).
6. Nadeau, J. H. Modifier genes in mice and humans. *Nature Rev. Genet.* **2**, 165–174 (2001).
7. Balmain, A. Cancer as a complex genetic trait: tumor susceptibility in humans and mouse models. *Cell* **108**, 145–152 (2002).
8. Bucan, M. & Abel, T. The mouse: genetics meets behaviour. *Nature Rev. Genet.* **3**, 114–123 (2002).
9. Paigen, K. & Epping, J. T. A mouse phenome project. *Mamm. Genome* **11**, 715–717 (2000).
10. Eppig, J. T. Algorithms for mutant sorting: the need for phenotype vocabularies. *Mamm. Genome* **11**, 584–589 (2000).
11. Nadeau, J. H. *et al.* Sequence interpretation. Functional annotation of mouse genome sequences. *Science* **291**, 1251–1255 (2001).
12. Eppig, J. T. & Strivens, M. Finding a mouse: The International Mouse Strain Resource (IMSR). *Trends Genet.* **15**, 81–82 (1999).

Acknowledgements
The helpful comments of K. Matthews, S. Brown, N. Jenkins, J. Eppig and M. Hrabe de Angelis are gratefully acknowledged.

Online links

FURTHER INFORMATION
Gene Ontology: http://www.geneontolgy.org
Michael Ashburner's lab: http://www.gen.cam.ac.uk/dept/ashburner.html
Nadia Rosenthal's lab: http://www.embl-monterotondo.it
Access to this interactive links box is free online.

TIMELINE

# Headwaters of the zebrafish — emergence of a new model vertebrate

*David Jonah Grunwald and Judith S. Eisen*

The understanding of vertebrate development has advanced considerably in recent years, primarily due to the study of a few model organisms. The zebrafish, the newest of these models, has risen to prominence because both genetic and experimental embryological methods can be easily applied to this animal. The combination of approaches has proven powerful, yielding insights into the formation and function of individual tissues, organ systems and neural networks, and into human disease mechanisms. Here, we provide a personal perspective on the history of zebrafish research, from the assembly of the first genetic and embryological tools through to sequencing of the genome.

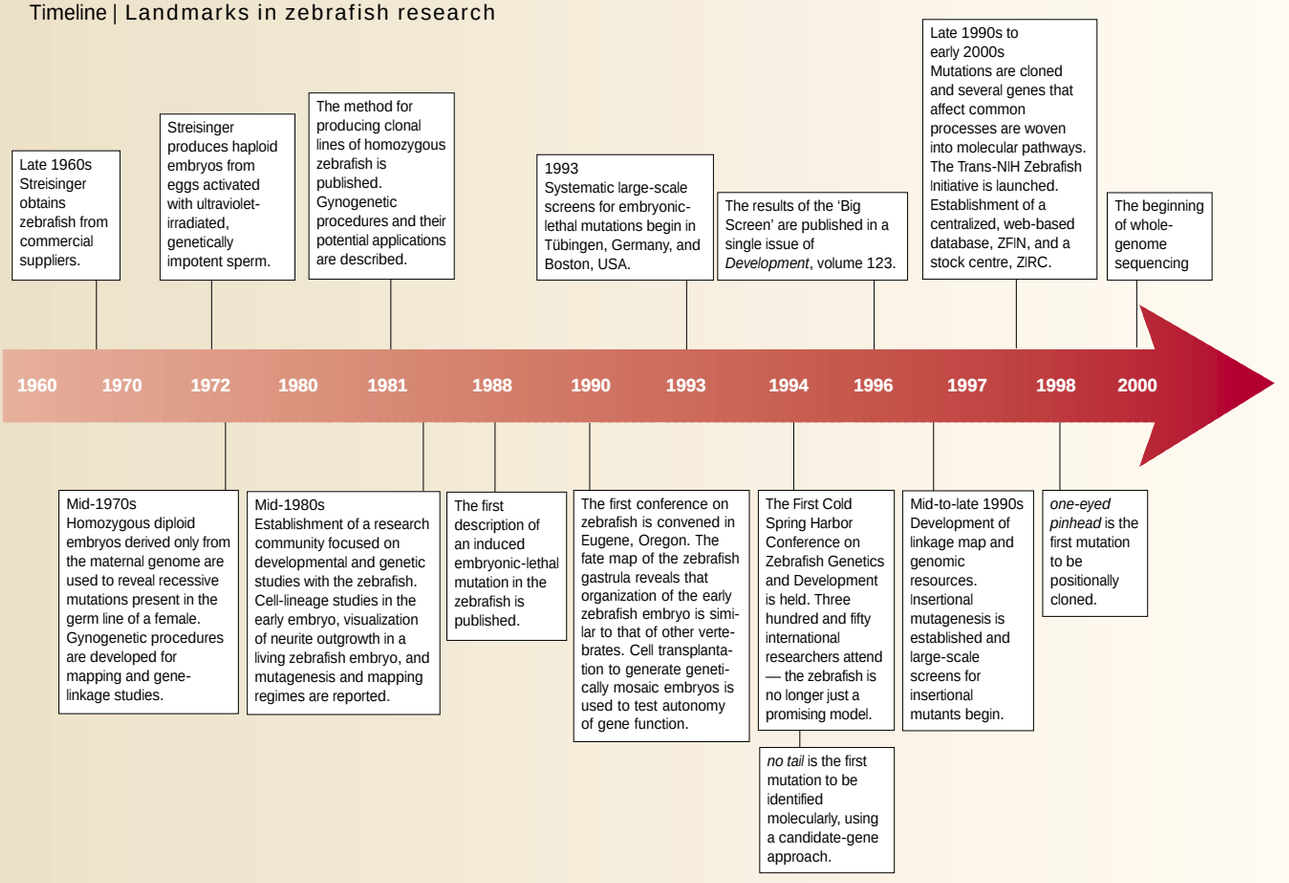
The zebrafish, a robust tropical fish that has long been a common feature in home aquariums, has recently attained a pre-eminent position in biomedical research. Zebrafish researchers have amassed something that was previously thought to be impossible in a vertebrate — a vast storehouse of mutations selected only on the basis of how they affect the living organism. Hundreds of mutations that perturb basic developmental processes have been described, including those that affect the establishment of the shape of the embryo, the generation of germ layers, complex organ systems and specific cell types, the organization of distinct brain regions and vascular architecture, and the establishment of defined neural circuits[1–4]. The mutants have provided models of human dysmorphologies[5,6], stimulating further efforts to achieve medical insights from the zebrafish into physiology, behaviour and chemical dependency[7–9]. Work with the zebrafish is also yielding insights into the relationship between single gene changes and structural adaptations that occur during the course of evolution, as well as the kinds of change in gene function that accompany the evolution of multigene families[10,11]. The significance of the insights anticipated from genetic work with the zebrafish has spurred an international public effort to complete the sequencing of its genome and its embracement by the biotechnology industry.

The rise in prominence of research with the zebrafish has occurred only in the past decade (TIMELINE). The first international conference that focused on this organism was convened in 1990 (REF. 12). Sponsored by the US National Institutes of Health (NIH) and National Science Foundation (NSF), and hosted by the consortium of 'Oregon zebrafish laboratories', the gathering of ~40 scientists from the United States and Europe sought to appraise the potential of research with this organism. Here, we retrace the auspicious origins of the zebrafish field that were recognized at that meeting, and the subsequent innovations that transformed the zebrafish into a leading model organism. Our perspective is personal and therefore necessarily incomplete. We highlight the sequential contributions of individual scientists and reflect on the shifting cultural views in the scientific community that both propelled and retarded the ascent of this new model system.

## Fashioning a genetic system

The ability to carry out classical forward genetic analyses rendered the zebrafish unique among vertebrate model organisms and still continues to be largely responsible for its power as a tool for studying vertebrate biology. The idea of applying mutational analysis to study zebrafish embryonic development originated with George Streisinger (FIG. 1), who began working with the fish in the late 1960s. Streisinger had been among the principal contributors to the dawn of the modern era of molecular genetics. Having trained with Salvatore Luria and Max Delbrück, Streisinger was at the core of the historic phage group throughout the 1950s, working at Caltech, Cold Spring Harbor, and Cambridge, UK. Streisinger's phage work showed that the genetic code deduced *in vitro* coincided with the code *in vivo*, and

Timeline | Landmarks in zebrafish research




yielded insights into the molecular nature of induced mutations and the genetic structure of bacteriophage T4 (REFS 13–16). It was a heady time, focused on the primacy of the gene, its structure, and its capacity to encode dynamic molecular responses to a changing cellular environment and to endure across generations without becoming consumed or distorted. By the mid-1960s, the fundamental questions associated with the DNA–RNA–protein story were well on their way to a satisfying resolution. As Sydney Brenner wrote in his now-famous 1963 letter to Max Perutz, it was just a matter of elucidating "the chemical details of replication and transcription […]. The future of molecular biology [lay] in the extension of research to other fields of biology, notably development and the nervous system"[17]. The focus on the gene and the success of the mutational approach for understanding physiological regulation in bacteria[18] crystallized a commitment to the idea that both the components and the logic of increasingly complex systems could be deconstructed using mutation-based genetic analysis.

Then, as today, the workings of the nervous system and the basis of thinking and behaviour were regarded as the exceptional, almost mystical, problems still to be conquered by biologists. Brenner attacked this problem by bringing forth the new field of *Caenorhabditis elegans* genetic research. Seymour Benzer advocated harnessing the fruitfly to study nervous system function and behaviour. Streisinger aspired to unravel the genetic logic of neural development in a vertebrate. He began to tinker with a highly fecund tropical fish, the entire embryonic development of which — from egg to swimming larva — could unfold in a Petri dish.

Immersed as deeply as he had been in the wonders of phage, Streisinger was wedded to the idea that mutational analysis was needed in vertebrates. His goal from the beginning was to "study features of the organization and embryological development of the vertebrate nervous system through the use of mutant strains. [He was] particularly interested in the mechanisms leading to the formation of specific synaptic connections and in the nature of the signals that guide specific axons to particular target sites" (G. Streisinger, 1974 supplemental grant application to the National Science Foundation). The stumbling block, as he saw it, was the efficiency with which selected mutant phenotypes could be recovered in a diploid vertebrate. There were bound to be lots of genes in these higher organisms, and very few of them would be key to any single process under study. The trick was to identify interesting phenotypes associated with rare recessive mutations and to propagate those mutations efficiently in an unseen state — the heterozygous carrier. Streisinger was not alone in his conviction that obviating what Bill Dove called "the embarrassment of diploidy" was crucial for the success of the genetic approach. *C. elegans* profited from its hermaphroditic lifestyle in that single heterozygous carriers could produce both homozygous and heterozygous sibling progeny. Moreover, hermaphroditism freed the geneticist from limitations imposed by debilitating phenotypes that might have restricted mating in other organisms. *Drosophila* had a toolbox of genetic tricks that had been amassed over more than half a century, including marked and balancer chromo-

somes, which made it particularly easy to monitor the inheritance of newly found mutations. But, in vertebrates, tracking chromosome regions of interest was a great challenge, owing to the paucity of genetic markers. So, Streisinger focused initially on developing new tools that would allow him to recover recessive mutations efficiently from the germ line of zebrafish and to identify quickly the few gems of interest.

*Why zebrafish?* Streisinger initially brought several species of tropical fishes into his laboratory, including medaka, which had an established history of genetic experimentation. In the absence of any apparent record of these preliminary investigations, we can only surmise his motivations for choosing to develop a genetic methodology with the zebrafish. Four factors seem to have contributed to his rationale. First, as the zebrafish bred prodigiously in the laboratory, it was well suited for standard genetic analyses. Adults could be maintained in breeding condition on a year-round basis and individual females would give rise to hundreds of progeny. Second, because of external fertilization in zebrafish, gametes could be harvested separately, and the conditions of fertilization and ploidy could be manipulated for the purpose of genetic analysis. Third, because all embryonic development proceeded in full view of the researcher, screening for specific developmental phenotypes or early vision-dependent behaviours was feasible. Last, Streisinger had a passion for tropical fish. As a teenager, he had worked with fish at the American Museum of Natural History in New York City, and later, as an accomplished phage geneticist, his family vacations would be punctuated with detours to local fish hobbyist stores (L. Streisinger, personal communication).

*New tools and methods.* As indicated by his early grant applications (with an initial grant from the NSF and later grants from the NIH), during the first ten years of work with the zebrafish, Streisinger's unwavering focus was to develop methods for rapidly uncovering recessive germ-line mutations. He wanted to free himself of the need to propagate each mutation through male and female heterozygous partners to produce homozygous offspring for screening. He reasoned that recessive phenotypes could be generated quickly by producing offspring derived solely from the maternal germ line (gynogenesis). His first accomplishment, which formed the basis of his 1973 grant application to the NSF, was to establish a highly efficient method for activating the development of eggs without genetic contribution from sperm, thereby producing haploid embryos. By the end of 1976, Streisinger and his associate Charline Walker had transformed this simple beginning into gynogenetic methods for producing wholly or partially homozygous diploid offspring[19]. To generate a diploid embryo that was homozygous at all loci, an egg was activated by ultraviolet light-irradiated (genetically impotent) sperm, its haploid set of maternal chromosomes was allowed to replicate and the initial segregation of chromosomes into daughter cells was prevented by suppression of the first mitotic cleavage of the zygote. To generate a gynogenote that was partially homozygous, the second meiotic division was inhibited in an activated egg, producing an embryo whose diploid genetic composition was wholly derived from sister chromatids (one half-tetrad). As genetic information between non-sister chromatids is recombined before the second meiotic division, this gynogenetic procedure yielded offspring that were heterogeneous in genotype: genes proximal to the centromere tended to be in homozygous condition on the sister chromatids and genes distal to the centromere tended to be in heterozygous condition. Each of the gynogenetic procedures met Streisinger's initial goal of recovering recessive phenotypes from maternal genomes in a single generation. In addition, these procedures could also be used to carry out genetic mapping and complementation analyses with extreme efficiency[20].

## Against all odds...

The work to develop the zebrafish as a model organism was an immense gamble. There was no history of genetic work with this organism. Moreover, in the 1970s, as Streisinger prepared the foundation for the zebrafish system, there was widespread scepticism as to whether his results could be translated into general principles. More than a century after the publication of Darwin's *On the Origin of Species by Means of Natural Selection*, there was little theoretical appreciation of the degree to which vastly diverged species would share the regulatory pathways that govern cell behaviour and embryonic development. Before the era of gene cloning, there were no data that

Figure 1 | **Principal architects of zebrafish developmental genetics.** From left to right, top row: George Streisinger (provided by the University of Oregon Archives), Charles Kimmel, Christiane Nüsslein-Volhard; bottom row: Marc Fishman, Wolfgang Driever and a pair of adult zebrafish.

addressed whether genetic programmes that regulate development were conserved. As a result, 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).

Streisinger's commitment to developing the tools for a new genetic system was nurtured both by where he had come from and where he was. The phage world was small and intimate, populated and mentored by physicists who had eschewed the potentially destructive applications of their research and redirected their research focus into biology with a special vigour. The early phage period was remarkable for the rate with which profound insights into basic biological mechanisms emerged. Changing research direction was accepted, and pursuing the breakthrough experiment was expected. In addition, confidence that a method of analysis was the key to asking questions was widespread. 'Hershey Heaven' — a term referring to the phage work of Al Hershey — was coined to describe the process of using a single, powerful analytical technique to probe several important questions. Streisinger found himself in unusual environments that were shaped by physicists-turned-biologists of exceptional moral character: first, as a postdoc with Delbrück at Caltech, and later, as a faculty member in the Institute of Molecular Biology founded by Aaron Novick at the University of Oregon. In both places, individuals were allowed or even expected to delve into their own interests, "people lived with mistakes" (G. Lark's description of Delbrück's policy at Caltech), and the community as a whole was willing to underwrite, financially and otherwise, the efforts of individual colleagues. (F. Stahl and P. von Hippel, personal communication. Despite the NIH policy to award funding to individual projects, the Institute of Molecular Biology, since its inception, had pooled all equipment, media facilities and administrative support into centralized facilities. It was through such devices that the Institute supplemented Streisinger's zebrafish research programme.) Commitment to answering a question was the only thing that mattered, and day-to-day progress was not measured closely. In this environment, the zebrafish work proceeded in a set of converted army barracks on the edge of the University of Oregon campus. Enveloped with corrugated metal, the barracks were cooled by a constant supply of water trickling down from the roof and heated with judiciously placed small electric heaters and fans, which occasionally would ignite small fires in the laboratory. Progress accrued incrementally: first, methods were established to produce haploid and diploid gynogenotes; second, a breeding programme was instituted to create strains that were devoid of confounding lethal mutations in their background; and third, methods were developed to induce new germ-line mutations. The first paper that described all the genetic procedures and announced the establishment of lethal mutation-free clonal lines was published in 1981 in *Nature*[19] and was heralded on the cover (FIG. 2). The accomplishment also received notice in several public venues, including a news commentary cartoon published in the Chicago Tribune and US television coverage. Subsequent work on mutagenesis was published in 1983 in *Genetics*[21,22].

"Changing research direction was accepted, and pursuing the breakthrough experiment was expected."

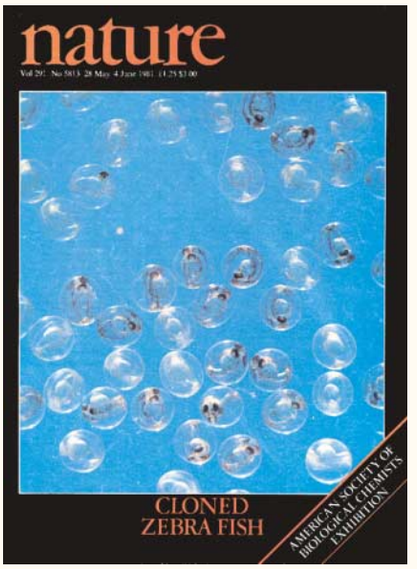



Figure 2 | **The debut publication.** Reproduction of the cover of the 1981 issue of *Nature* containing the landmark paper by Streisinger *et al.* that described genetic procedures for producing homozygous diploid clones of zebrafish. The photo depicts sibling homozygous diploid *golden* (clear) and wild-type (pigmented) zebrafish embryos.

### The first fruits

Although the tools for analysis were in place, little was understood of the embryology of the zebrafish and the question remained as to the general biological insights that could be derived from studying a fish. Influenced by contemporary studies on the role of cell lineage in embryogenesis, Streisinger harnessed the only genetic trait he had in hand — a recessive mutation that affected pigmentation — to analyse whether individual cells acquired restricted developmental fates at early stages of embryogenesis. As Beatrice Mintz and Anne McLaren had shown in the mouse, Streisinger found that early zebrafish blastomeres did not express a determinate cell lineage, but instead contributed on a stochastic basis to any specific differentiated tissue[23]. He collaborated with others to determine whether the nervous systems of genetically identical animals were less heterogeneous than those of outbred animals. He advocated the use of genetically determined stocks of zebrafish as sentinels for genotoxic (able to cause damage to DNA) agents in the environment[24,25]. Morphological and behavioural landmarks of visual development that could be assayed in embryos were described[26,27]. Finally, the lab began to screen on a regular basis for embryonic mutants with interesting phenotypes. Among the first mutants to be isolated was one that was later discovered to be deficient in a growth factor needed for axis determination, a second deficient in myofibril organization and a third in which a specific portion of its nervous system failed to form[28–30].

### Emergence of a research community

Meanwhile, the zebrafish was embraced at Oregon — first by Charles Kimmel (FIG. 1), and later by Monte Westerfield and Judith Eisen — as a model organism that was spectacularly amenable to the study of nervous system development in particular, and vertebrate embryology in general. Neurobiologists had never been fettered by concerns of "relevance". They found all nerve cells of potential interest, focusing principally on the suitability of a preparation for addressing a particular question. Kimmel quickly recognized the value of the zebrafish embryo itself. In the mid-1970s, well before Streisinger's work had come to fruition, Kimmel initiated a series of neuroanatomical studies of the zebrafish embryo that uncovered the segmental structure of the brain. By the time Streisinger's landmark paper arrived, Kimmel and colleagues had described more identifiable neurons in the

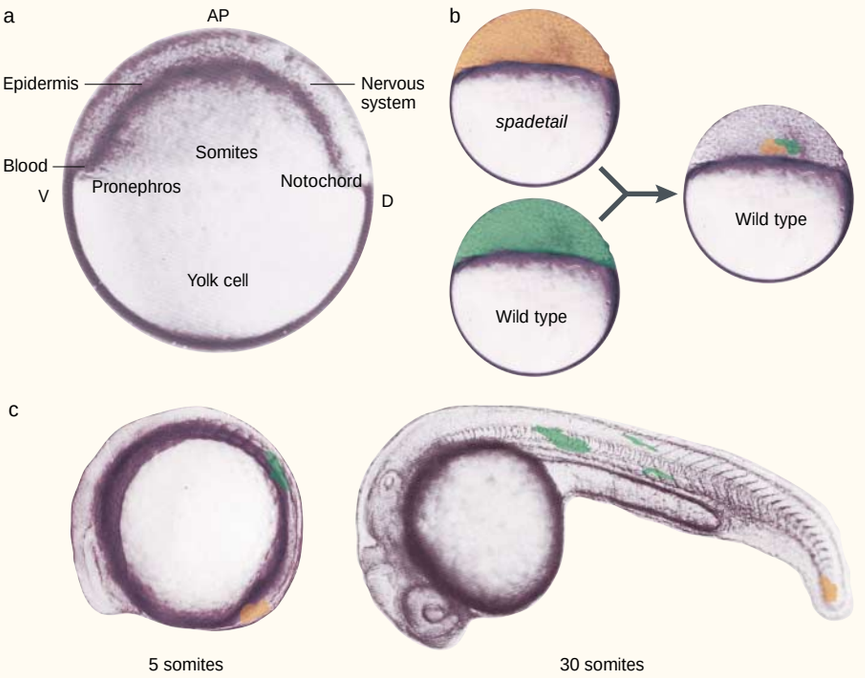



Figure 3 | **Determining cell autonomy of gene function. a** | Fate map of the early gastrula zebrafish embryo. The blastoderm of the early gastrula embryo is shaped like an inverted cup overlying the large yolk cell. Territories of future tissue fates are indicated on the picture of an early gastrula with respect to the dorsal (D) and ventral (V) midlines of the embryo and the animal pole (AP). Note that mesoderm fates arise from cells located near the outer margin of the blastoderm. **b,c** | Schematic illustration of how transplantation chimaeras were used to test cell autonomy of the *spadetail* mutation. Cells removed from the blastoderm margin of dye-labelled wild-type and *spadetail* mutant late-blastula-stage embryos were intermixed and implanted into a wild-type host embryo (**b**). Whereas transplanted wild-type cells (green) integrate into their host environment, participating in the normal formation of somitic muscle, the transplanted *spadetail* cells (orange) behave independently of their environment, failing to gastrulate normally and accumulating at the posterior of the embryo (**c**). Panel **a** is adapted from REF. 34. Panels **b,c** are adapted from REF. 37 © (1989) Macmillan Magazines Ltd.

zebrafish than had been recognized in any other vertebrate[31]. The morphology and arrangement of the brain neurons led Kimmel to suggest that they arose as part of a repeated developmental programme, and he began to probe the role of cell lineage in the production of these anatomically related, segmentally iterated brain neurons. Soon afterwards, Westerfield, Eisen and their colleagues described a stereotypic arrangement of distinct spinal-cord motor neurons in adult and embryonic zebrafish, and showed that outgrowth of the axons of these motor neurons could be visualized with astounding clarity in living embryos[32]. It was now clear that a detailed analysis of nervous-system organization, differentiation of specific cell types and establishment of neural circuits could be assayed in the zebrafish. If mutants that perturbed neural development could be generated, they would be recognized and harvested for all their worth. In a 1984 summary of his laboratory's principal objectives, Streisinger wrote that their primary goals were now to "investigate the genetic contribution of the zygote to early developmental decisions in the zebrafish and to identify specific developmental defects in photoreceptor development in fish homozygous for induced mutations" (G. Streisinger, unpublished laboratory documents).

## "the fish is a frog… is a chicken… is a mouse"

As the first embryonic mutants emerged, Streisinger and Kimmel began to plan collaborative screening efforts on the basis of their shared interests in the patterning and differentiation of the nervous system. Then, with unexpected suddenness, efforts to test the promise of a mutational analysis of zebrafish development were thwarted by the death of Streisinger in August 1984. By this time, Christiane Nüsslein-Volhard (FIG. 1) and other European *Drosophila* geneticists had recognized the potential of the zebrafish, but beyond the boundaries of the Oregon laboratories the project was largely viewed in the United States as Streisinger's personal quest. Consequently, US medical research funding institutions were reluctant to invest in its continuation.

In a remarkably uncommon move in US academic institutions, where the independence of each research group is defended with resolve, the groups in Oregon worked in concert to maintain and expand Streisinger's programme of genetic research with the zebrafish. They established an informal course on zebrafish husbandry, genetics and embryonic anatomy, hosting several visiting scientists and sending representatives to other labs. Westerfield devised a primer on methods that was widely distributed[33]. Kimmel — an insightful character who was known among his Oregon colleagues for both his attention to detail and his homespun music and artistry — quietly laid out a road map to focus attention on the important biological questions that could be asked in the zebrafish.

Intrigued by the striking organization of the identified neurons of the zebrafish brain, in 1982 Kimmel embarked on an ambitious programme, which was to continue for more than ten years, to illuminate the developmental steps that led to the origin and organization of distinct tissue types in the zebrafish embryo. Kimmel's work defined a crucial stage, just at the onset of gastrulation, when the prospective organization of the entire embryonic body plan emerged. This extended series of studies had two powerful effects on zebrafish research. First, it provided both the conceptual framework and the tools needed to explore the regulation of cell fate in the zebrafish embryo. Second, it placed the zebrafish in the context of vertebrate developmental biology. Kimmel's description of the zebrafish fate map[34] (FIG. 3a) came on the heels of analogous studies in other vertebrate model systems at a time when many were looking for common principles to unify the understanding of metazoan development in general and vertebrate development in particular. One illustration of this quest was the burgeoning effort in the mid-1980s to discover and understand the functions of Hox genes in vertebrates. In his highly influential summary of the zebrafish lineage work, Kimmel announced "the fish is a frog… is a chicken… is a mouse"[35].

Kimmel and his colleagues at Oregon mounted a tour-de-force effort to show the depth and range of developmental questions that could be addressed with the few

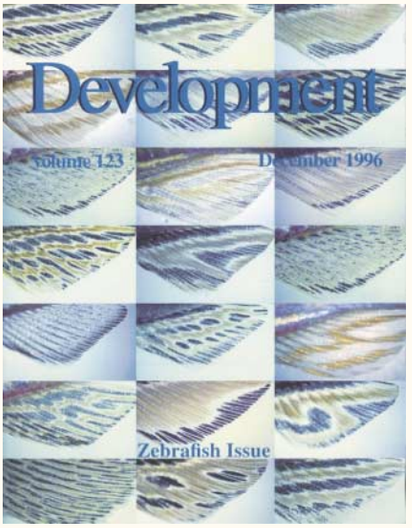



Figure 4 | **Results of the the 'Big Screen' are announced.** Reproduction of the cover of the issue of *Development*, volume 123. This issue included 37 papers that reported results from the first large-scale screens for developmental mutants in the zebrafish. Approximately 4,000 embryonic-lethal mutants were recovered and characterized.

zebrafish embryonic mutants that were in hand. Most notable was work published in 1989 and 1990 on the *spadetail* mutant, recently shown to be defective in a regulatory T-box transcription factor[36]. Kimmel showed that *spadetail* governed the morphogenetic behaviour of embryonic cells during gastrulation, and therefore it was crucial to the formation of the vertebrate body plan[37]. Then, in a highly influential experiment, Kimmel's colleagues, Robert Ho and Don Kane, showed that chimeric embryos, which had been generated using very simple cell-transplantation methods, could be used to identify the specific cells that required *spadetail* function[38] (FIG. 3b,c). The cell-autonomy analyses that they introduced can be carried out with an ease and precision that is unrivalled in any other model organism used at present, and have become *de rigeur* in the zebrafish field.

The work done in Oregon in the 1980s highlighted the synergy that grew out of blending classical embryological and genetic approaches in the zebrafish to ask a range of developmental and neurobiological questions. Concurrently, two profound advances in the Geist of developmental biology readied world science to embrace the zebrafish. Foremost, the work of Nüsslein-Volhard and Eric Wieschaus revolutionized the study of all biology, by showing that collections of fly mutant phenotypes could be woven into broader contexts, elucidating the regulatory logic and cell behaviours that are involved in executing a developmental programme. These ideas were reinforced by the spectacular success of research with *C. elegans*. Moreover, the link between phenotype and the action of individual genes had been bridged in *Drosophila* by molecular biology, and many felt confident that it was only a hurdle of technology that separated mutant phenotypes in zebrafish from an understanding of their molecular nature.

Second, a new appreciation of the consequences of evolution slowly emerged with the demonstration that genetic and cell-biological pathways were highly conserved among all existing life forms. This idea was illustrated by many kinds of experiment. Yeast cell-cycle mutants could be rescued with human genes. Homologous genes that had been identified initially only by sequence similarity had analogous expression patterns, as revealed by RNA *in situ* hybridization. A zebrafish embryonic mutant, *no tail*, and the historic mouse developmental mutant *Brachyury* not only resembled each other phenotypically, but also were subsequently discovered to have defects in orthologous genes[39]. The neurobiologists had been right all along. It did not matter which animal you chose — fundamental processes were fundamentally conserved.

The significance of Streisinger's work to establish the framework for genetic manipulation of a vertebrate had been immediately grasped by several of the leading *Drosophila* developmental geneticists, who were already convinced of the insights to be gleaned from a concerted mutational analysis of development. Nüsslein-Volhard (personal communication) and Jose Campos-Ortega (personal communication) each recall excited discussion of Streisinger's 1981 paper in journal clubs that took place soon after its publication. After Streisinger's death, Nüsslein-Volhard set a small aquarium of zebrafish on a windowsill and pondered how to accomplish a study of the magnitude that she deemed necessary (C. Nüsslein-Volhard, personal communication). Perhaps more than anyone else, she realized that all of the ~120 *Drosophila* mutant genes that had come out of her work with Wieschaus and Gerd Jurgens were proving to be of compelling interest. As Campos-Ortega recalls (personal communication), by the late 1980s, "*Drosophila* researchers had obtained a thorough description of embryonic development from the points of view of embryology, genetics and molecular biology. The synthesis of biological disciplines had [almost] been achieved, only cell biology was missing. A new way of working out biological problems, using a multidisciplinary approach, had been firmly established in everyone's minds thanks to the work in *Drosophila*". The zebrafish system was intriguing because it offered the potential of good embryology and good genetics with the prospect that cellular resolution could be achieved in a way that would yield insights into vertebrate development in particular, and cell biology more generally.

"It did not matter which animal you chose — fundamental processes were fundamentally conserved."

### The 'Big Screen'

By the late 1980s, a handful of prominent *Drosophila* developmental geneticists had established research programmes with the zebrafish, although the revolutionary impact of these programmes could scarcely have been foreseen. As noted by Nüsslein-Volhard (personal communication), "At the time, the fields of vertebrate and fly biology were miles, miles apart […] fly people would speak of genes [only], frog people of factors […] Nobody yet believed in what was later felt to be the incredible conservation of molecular mechanisms."

Nüsslein-Volhard's plan was by far the most ambitious. She would recapitulate the *Drosophila* screen for embryonic pattern mutations in a vertebrate at the Max Planck Institute in Tübingen, Germany. At the same time, Marc Fishman recruited her gifted student, Wolfgang Driever, to Massachusetts General Hospital to establish a parallel effort (FIG. 1). Initially, aspects of husbandry that would be required for a large-scale genetic analysis of embryonic development needed to be conquered. New procedures for mutagenesis were developed and a standard multi-generational breeding protocol was instituted to allow recovery of recessive mutations[40,41]. A core of brilliant young postdoctoral associates and students was assembled. The 'Big Screen' for embryonic mutants was begun in 1993 and brought to a conclusion exactly two years later. Between Tübingen and Boston, ~4,000 embryonic-lethal mutant phenotypes were recovered. To be of ultimate value to the general research community, the mutants needed to be described, sorted into complementation

groups and preserved in a way that would make them accessible to others. A decision was made that proved historic: description of the mutant phenotypes would not trickle out as tantalizing anecdotal individual elements — instead, they would be published together once the genetic and preliminary phenotypic characterizations of the entire group had been completed. This effort took another year and culminated in the publication of 37 papers gathered together in a single issue (volume 123) of the journal *Development*, devoted entirely to the zebrafish mutants (FIG. 4).

The impact of the 'Big Screen' has been assessed in many reviews. The zebrafish had been catapulted to the forefront of developmental biology research. The model for genetic analysis of development and physiology that had been established in *Drosophila* had been extended fruitfully to new vertebrate problems. Several mutants that affect common processes helped to identify interacting genetic pathways[3,4,42,43]. Hypomorphic mutations had a special role for modelling human disease states[6]. In an immediate sense, the 'Big Screen' provided researchers with an array of mutants that were relevant to many aspects of vertebrate embryonic development. It also provided impetus for the establishment of numerous mutational analyses focused on specific developmental programmes in the zebrafish[44] and made it clear that such methods could be applied in the mouse[45]. The reverberating effects of the effort are still being felt.

## Classical genetics to the molecular era

Interest in the mutant phenotypes immediately focused attention on the molecular identification of the defects. Years earlier, John Postlethwait anticipated the need for molecular landmarks scattered across the zebrafish genome and initiated work to create a linkage map. The early mapping work, carried out largely by a cadre of undergraduate students at the University of Oregon, relied on the particular strengths of the zebrafish genetic system and introduced two methodologies that are now commonplace in the mapping of zebrafish mutations. First, construction of the linkage map depended wholly on Streisinger's gynogenesis methods, as segregation of polymorphic DNA markers was assessed among sibling haploid progeny[46]. Even today, analysis of marker segregation among half-tetrad gynogenotes is used to assign new mutations rapidly to linkage groups[47]. Second, a technique called 'bulk segregant analysis' was introduced as an initial mapping device for new mutants[48].

> "…the emergence of the zebrafish as a prominent biological tool required open-mindedness and tenacity by researchers with extraordinary vision."

In this procedure, marker segregation is analysed in pooled groups of wild-type or mutant offspring that are produced from a single pair of heterozygotes. For a polymorphic locus that is not linked to a mutation, all alleles will be found in both mutant and wild-type pools, whereas for a linked marker, only one allele will be present in the pool of mutant offspring. So, using bulk segregant analysis and a small set of DNA markers that are distributed across the map, only a very limited initial analysis is required to assign a new mutation to a chromosomal location. Furthermore, using standard methods of gene mapping, the vast numbers of progeny that can be recovered from heterozygous mating partners means that thousands of meioses can be scored readily, and therefore mutations can be mapped with a fine-scale precision not approached in other vertebrate models.

With the tools for forward genetic analysis tested, and the pathway towards positional cloning established, it was clear that the development of supportive resources would transform the zebrafish into a widely embraced model. Under the guidance of Harold Varmus, then Director of the NIH, and prompted by the persistent advocacy of Len Zon, Marc Fishman and Nancy Hopkins, the NIH chose to transcend its traditional institutional boundaries and invest significantly in the development of genomic resources for the zebrafish. This decision reflected a new confidence of the NIH in the relevance of simple model organisms to medical research — a view that is substantiated, for example, by the successful use of the zebrafish as a bioassay for the function of human genes involved in establishing left–right asymmetries[49]. As a result of the Trans-NIH Zebrafish Initiative, the zebrafish map was quickly consolidated and became densely populated with anonymous molecular markers, as well as newly discovered genes and expressed sequence tags. Mutations were positionally cloned. Syntenic relationships between the zebrafish and mammalian species were used to identify candidate genes for the zebrafish mutations. Independently, the Hopkins laboratory developed tools for insertional mutagenesis and undertook a massive effort to cover much of the zebrafish genome with insertional mutations that could be rapidly cloned[50,51]. Antisense methods that had been mistrusted by molecular biologists for decades were proved by Stephen Ekker and his colleagues to be highly informative for studying gene function in the zebrafish[52]. In 2000, the Sanger Centre initiated a project to sequence the zebrafish genome. Most importantly, to facilitate the rapid exchange of genomic data and genetic resources, a centralized web-based database (see online link to ZFIN) and a zebrafish stock centre (Zebrafish International Resource Center) were established by Monte Westerfield.

## Conclusions

Thirty years after George Streisinger began his solo voyage to develop a new avenue of genetic research with a vertebrate, nearly 800 scientists gathered at the Fifth International Conference on Zebrafish Development and Genetics (in June 2002) to discuss with animation and exuberance their latest findings on the genetic basis of zebrafish development, physiology and behaviour. In retracing the origins of the zebrafish field, we ask whether elements that were crucial to its success can be used to foster similar leaps in the future. Certainly, the emergence of the zebrafish as a prominent biological tool required open-mindedness and tenacity by researchers with extraordinary vision. In addition, it required a considerable investment of time and an environment that was permissive to the necessarily slow maturation of new ideas and technologies. It is incredible to realize in today's entrepreneurial scientific environment that Streisinger worked for more than nine years on the project before its debut publication. His decision to wait until he had developed a robust network of genetic tools, much like the decision by Nüsslein-Volhard and Driever to publish their mutant strains as a comprehensive set, had an immense impact on the future of the field. In both cases, unveiling work at a relatively mature stage broadcast a clear outline of the potential trajectory of the field and thereby attracted many newcomers. Without investment in such long-term ventures, a luxury provided to Streisinger by his unique environment, we might not today harbour the conviction that genetic studies with a little tropical fish will reveal treasures of understanding for the basis of human diseases.

*David Jonah Grunwald is at the Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, Utah 84112, USA.*

*Judith S. Eisen is at the Institute of Neuroscience, University of Oregon, Eugene, Oregon 97403, USA.*

*Correspondence to D.J.G.*
*e-mail: grunwald@genetics.utah.edu*

doi:10.1038/nrg892

1. Driever, W. *et al.* A genetic screen for mutations affecting embryogenesis in zebrafish. *Development* **123**, 37–46 (1996).
2. Haffter, P. *et al.* The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio*. *Development* **123**, 1–36 (1996).
3. Stainier, D. Y. A glimpse into the molecular entrails of endoderm formation. *Genes Dev.* **16**, 893–907 (2002).
4. Thisse, C. & Zon, L. I. Organogenesis — heart and blood formation from the zebrafish point of view. *Science* **295**, 457–462 (2002).
5. Weinstein, B. M., Stemple, D. L., Driever, W. & Fishman, M. C. Gridlock, a localized heritable vascular patterning defect in the zebrafish. *Nature Med.* **1**, 1143–1147 (1995).
6. Zhong, T. P., Rosenberg, M., Mohideen, M. A., Weinstein, B. & Fishman, M. C. *gridlock*, an HLH gene required for assembly of the aorta in zebrafish. *Science* **287**, 1820–1824 (2000).
7. Baier, H. Zebrafish on the move: towards a behavior–genetic analysis of vertebrate vision. *Curr. Opin. Neurobiol.* **10**, 451–455 (2000).
8. Darland, T. & Dowling, J. E. Behavioral screening for cocaine sensitivity in mutagenized zebrafish. *Proc. Natl Acad. Sci. USA* **98**, 11691–11696 (2001).
9. Warren, K. S., Wu, J. C., Pinet, F. & Fishman, M. C. The genetic basis of cardiac function: dissection by zebrafish (*Danio rerio*) screens. *Phil. Trans. R. Soc. Lond. B* **355**, 939–944 (2000).
10. Force, A. *et al.* Preservation of duplicate genes by complementary, degenerative mutations. *Genetics* **151**, 1531–1545 (1999).
11. Kimmel, C. B., Miller, C. T. & Keynes, R. J. Neural crest patterning and the evolution of the jaw. *J. Anat.* **199**, 105–120 (2001).
12. Barinaga, M. Zebrafish: swimming into the development mainstream. *Science* **250**, 34–35 (1990).
13. Okada, Y. *et al.* Molecular basis of a mutational hot spot in the lysozyme gene of bacteriophage T4. *Nature* **236**, 338–341 (1972).
14. Streisinger, G., Edgar, R. S. & Denhardt, G. H. Chromosome structure in phage T4. I. Circularity of the linkage map. *Proc. Natl Acad. Sci. USA* **51**, 775–779 (1964).
15. Streisinger, G. *et al.* Frameshift mutations and the genetic code. *Cold Spring Harbor Symp. Quant. Biol.* **31**, 77–84 (1966).
16. Tsugita, A. *et al.* Frameshift mutations resulting in the changes of the same amino acid residue (140) in T4 bacteriophage lysozyme and *in vivo* codons for Trp, Tyr, Met, Val, and Ile. *J. Mol. Biol.* **41**, 349–364 (1969).
17. Brenner, S. in *The Nematode* Caenorhabditis elegans (ed. Wood, W. B.) ix–xiii (Cold Spring Harbor Laboratory Press, New York, 1988).
18. Jacob, F. & Monod, J. Genetic regulatory mechanisms in the synthesis of proteins. *J. Mol. Biol.* **3**, 318–356 (1961).
19. Streisinger, G., Walker, C., Dower, N., Knauber, D. & Singer, F. Production of clones of homozygous diploid zebra fish (*Brachydanio rerio*). *Nature* **291**, 293–296 (1981).
20. Streisinger, G., Singer, F., Walker, C., Knauber, D. & Dower, N. Segregation analyses and gene–centromere distances in zebrafish. *Genetics* **112**, 311–319 (1986).
21. Chakrabarti, S., Streisinger, G., Singer, F. & Walker, C. Frequency of γ-ray-induced specific locus and recessive lethal mutations in mature germ cells of the zebrafish. *Genetics* **103**, 109–124 (1983).
22. Walker, C. & Streisinger, G. Induction of mutations by γ-rays in pregonial germ cells of zebrafish. *Genetics* **103**, 125–136 (1983).
23. Streisinger, G., Coale, F., Taggart, C., Walker, C. & Grunwald, D. J. Clonal origins of cells in the pigmented retina of the zebrafish eye. *Dev. Biol.* **131**, 60–69 (1989).
24. Streisinger, G. Attainment of minimal biological variability and measurements of genotoxicity: production of homozygous diploid zebra fish. *Natl Cancer Inst. Monogr.* **65**, 53–58 (1984).
25. Streisinger, G. & Levinthal, C. Report to the Planning Committee to build a new Marine Resources Center at Woods Hole Marine Biological Laboratory (1976).
**Their report emphasized "the use of isogenic marine forms for the testing of teratogenicity". They argued that "Successful achievements in biomedical research have always depended in a critical way on the selection of appropriate model animal systems in which to study phenomena of concern… It is important to test whether agents give rise to teratogenic effects with linear dose response. In order for dose response curves to be interpretable, isogenic strains need to be used".**
26. Branchek, T. A. *Functional and Structural Development of Photoreceptors in Zebrafish*. Ph.D. thesis, University of Oregon, Eugene, Oregon (1981).
27. Clark, D. T. *Visual Responses in Developing Zebrafish* (Brachydanio rerio). Ph.D. thesis, University of Oregon, Eugene, Oregon (1981).
28. Felsenfeld, A. L., Walker, C., Westerfield, M., Kimmel, C. & Streisinger, G. Mutations affecting skeletal muscle myofibril structure in the zebrafish. *Development* **108**, 443–459 (1990).
29. Grunwald, D. J., Kimmel, C. B., Westerfield, M., Walker, C. & Streisinger, G. A neural degeneration mutation that spares primary neurons in the zebrafish. *Dev. Biol.* **126**, 115–128 (1988).
30. Hatta, K., Kimmel, C. B., Ho, R. K. & Walker, C. The cyclops mutation blocks specification of the floor plate of the zebrafish central nervous system. *Nature* **350**, 339–341 (1991).
31. Kimmel, C. B. Reticulospinal and vestibulospinal neurons in the young larva of a teleost fish, *Brachydanio rerio*. *Prog. Brain Res.* **57**, 1–23 (1982).
32. Eisen, J. S., Myers, P. Z. & Westerfield, M. Pathway selection by growth cones of identified motoneurones in live zebra fish embryos. *Nature* **320**, 269–271 (1986).
33. Westerfield, M. *The Zebrafish Book* 4th Edn (University of Oregon Press, Eugene, Oregon, 2000).
34. Kimmel, C. B., Warga, R. M. & Schilling, T. F. Origin and organization of the zebrafish fate map. *Development* **108**, 581–594 (1990).
35. Kimmel, C. B. Genetics and early development of zebrafish. *Trends Genet.* **5**, 283–288 (1989).
36. Griffin, K. J., Amacher, S. L., Kimmel, C. B. & Kimelman, D. Molecular identification of spadetail: regulation of zebrafish trunk and tail mesoderm formation by T-box genes. *Development* **125**, 3379–3388 (1998).
37. Kimmel, C. B., Kane, D. A., Walker, C., Warga, R. M. & Rothman, M. B. A mutation that changes cell movement and cell fate in the zebrafish embryo. *Nature* **337**, 358–362 (1989).
38. Ho, R. K. & Kane, D. A. Cell-autonomous action of zebrafish *spt-1* mutation in specific mesodermal precursors. *Nature* **348**, 728–730 (1990).
39. Schulte-Merker, S., van Eeden, F. J., Halpern, M. E., Kimmel, C. B. & Nüsslein-Volhard, C. *no tail* (*ntl*) is the zebrafish homologue of the mouse T (*Brachyury*) gene. *Development* **120**, 1009–1015 (1994).
40. Driever, W., Stemple, D., Schier, A. & Solnica-Krezel, L. Zebrafish: genetic tools for studying vertebrate development. *Trends Genet.* **10**, 152–159 (1994).
41. Mullins, M. C., Hammerschmidt, M., Haffter, P. & Nüsslein-Volhard, C. Large-scale mutagenesis in the zebrafish: in search of genes controlling development in a vertebrate. *Curr. Biol.* **4**, 189–202 (1994).
42. Hammerschmidt, M. *et al. dino* and *mercedes*, two genes regulating dorsal development in the zebrafish embryo. *Development* **123**, 95–102 (1996).
43. Mullins, M. C. *et al.* Genes establishing dorsoventral pattern formation in the zebrafish embryo: the ventral specifying genes. *Development* **123**, 81–93 (1996).
44. Patton, E. E. & Zon, L. I. The art and design of genetic screens: zebrafish. *Nature Rev. Genet.* **2**, 956–966 (2001).
45. Anderson, K. V. Finding the genes that direct mammalian development : ENU mutagenesis in the mouse. *Trends Genet.* **16**, 99–102 (2000).
46. Postlethwait, J. H. *et al.* A genetic linkage map for the zebrafish. *Science* **264**, 699–703 (1994).
47. Johnson, S. L. *et al.* Centromere-linkage analysis and consolidation of the zebrafish genetic map. *Genetics* **142**, 1277–1288 (1996).
48. Postlethwait, J. H. & Talbot, W. S. Zebrafish genomics: from mutants to genes. *Trends Genet.* **13**, 183–190 (1997).
49. Bamford, R. N. *et al.* Loss-of-function mutations in the EGF-CFC gene *CFC1* are associated with human left–right laterality defects. *Nature Genet.* **26**, 365–369 (2000).
50. Amsterdam, A. *et al.* A large-scale insertional mutagenesis screen in zebrafish. *Genes Dev.* **13**, 2713–2724 (1999).
51. Talbot, W. S. & Hopkins, N. Zebrafish mutations and functional analysis of the vertebrate genome. *Genes Dev.* **14**, 755–762 (2000).
52. Nasevicius, A. & Ekker, S. C. Effective targeted gene 'knockdown' in zebrafish. *Nature Genet.* **26**, 216–220 (2000).

## Acknowledgements

Many colleagues have been extremely generous with their time, contributing to this project by supplying us with written and verbal accounts of their perspectives on the origin of the zebrafish field. In particular, we thank T. Alber, J. Campos-Ortega, M. Capecchi, C. Kimmel, K. G. Lark, C. Nüsslein-Volhard, F. Stahl, L. Streisinger, P. von Hippel, C. Walker and E. Wilson for their insights and patience, and the University of Oregon Division of Archives for its assistance. D.J.G. and J.S.E. are funded by grants from the National Institutes of Health.

## Online links

DATABASES

**The following terms in this article are linked online to:**

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink
*Brachyury*
ZFIN: http://zfin.org
*no tail* | *spadetail*

**Access to this interactive links box is free online.**