

# Animal models of human disease: zebrafish swim into view

Graham J. Lieschke\* and Peter D. Currie†

**Abstract** | Despite the pre-eminence of the mouse in modelling human disease, several aspects of murine biology limit its routine use in large-scale genetic and therapeutic screening. Many researchers who are interested in an embryologically and genetically tractable disease model have now turned to zebrafish. Zebrafish biology allows ready access to all developmental stages, and the optical clarity of embryos and larvae allow real-time imaging of developing pathologies. Sophisticated mutagenesis and screening strategies on a large scale, and with an economy that is not possible in other vertebrate systems, have generated zebrafish models of a wide variety of human diseases. This Review surveys the achievements and potential of zebrafish for modelling human diseases and for drug discovery and development.

Biomedical research depends on the use of animal models to understand the pathogenesis of human disease at a cellular and molecular level and to provide systems for developing and testing new therapies. Mammalian models, such as the mouse, have been pre-eminent in modelling human diseases, primarily because of the striking homology between mammalian genomes and the many similarities in aspects spanning from anatomy to cell biology and physiology. Sophisticated transgenic approaches using dominantly acting disease-causing transgenes have allowed the creation of mouse models that accurately recapitulate the pathology of human diseases, a noteworthy example being the generation of cancer models through the tissue-specific expression of oncogenes. Furthermore, the generation of specific allelic modifications through gene targeting by homologous recombination has made the mouse the most widely used model of human disease.

However, a range of factors must be considered in addition to evolutionary proximity and anatomical similarity when selecting an animal disease model (TABLE 1). For example, larger mammals such as rats or sheep can have a physiologies and organ sizes that are more similar to humans, which are advantages when developing surgical therapeutic interventions. On the other hand, the surprising degree of functional conservation in basic cell-biological processes between mammals and invertebrates suggests that diseases that result from the disruption of these conserved cellular processes can be accurately modelled at a genetic and molecular level in flies and worms. In this regard, the large-scale 'forward-genetic'

mutagenic strategies that are available in invertebrate systems have been extraordinarily successful in determining gene functions, providing considerable insight into how orthologous human disease genes function in similar processes.

Despite these advantages, invertebrates lack many structures and organ systems that are involved in human disease pathogenesis, and their role in modelling human disease will therefore be limited. Conversely, although forward-genetic screens<sup>1–4</sup> and random-mutagenesis-based reverse genetics<sup>5,6</sup> are feasible in the mouse and are currently underway, they cannot be done on a scale that is possible in invertebrates because they require considerable staff and infrastructure support. Hence, such approaches in mice are limited to a few large projects, often operating as screening consortia. In this context, the zebrafish (*Danio rerio*) has come to attention recently as a genetically tractable vertebrate model system. As early as the 1930s, the zebrafish was being used as a classical developmental and embryological model. Early studies drew on the unique combination of the optical clarity of the embryos and larvae (allowing the *in vivo* visualization of cell-biological events) and embryological manipulability to make several important observations (reviewed in REF. 8). Through the 1980s, the development of zebrafish genetic techniques, such as 'cloning'<sup>9</sup>, mutagenesis<sup>10–13</sup>, transgenesis<sup>14</sup> and mapping approaches<sup>15</sup>, underpinned the use of zebrafish to apply invertebrate-style forward genetics to questions of vertebrate development. The identification of thousands of early developmental zebrafish mutants through genetic screens that were carried out in

\*Cancer and Haematology Division, Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria, 3050, Australia.

†Muscle Development Laboratory, The Victor Chang Cardiac Research Institute, 384 Victoria Street, Darlinghurst, Sydney, New South Wales 2010, Australia. Correspondence to G.J.L. or P.D.C.

e-mail: lieschke@wehi.edu.au; p.currie@victorchang.unsw.edu.au

doi:10.1038/nrg2091

Table 1 | Attributes of some key animals used to model human disease

Attribute of disease model	Model organism			
	Fly	Zebrafish	Mouse	Rat
<b>Practical issues</b>				
Husbandry infrastructure	\$	\$	\$\$\$	\$\$\$
Cost per animal per year	\$	\$	\$\$\$	\$\$\$
Characterized inbred strains	+	-	++++	+++
Outbred laboratory strains	+	+++	++	++
Anatomical similarity	-	+	++	++
Molecular or genetic similarity	+	++	+++	+++
Pathological similarity	-	++	+++	+++
Storage; for example, freezing sperm	No	Yes	Yes	Yes
<b>Molecular biology tools</b>				
Transgenesis*	++	++	++	++
Targeted gene modification*	+	-	++++	+
Transient <i>in vivo</i> assays*	++	++++	+	+
Allelic series from TILLING*	+++	++++	++	+
Feasibility of large-scale screens <sup>†</sup>	++++	+++	++	+
Affordability of large-scale screens <sup>†</sup>	++++	+++	+	-
Sequencing progress <sup>§</sup>	+++	++	+++	++
Annotation progress <sup>§</sup>	++	++	++++	++
<b>Cell-biology tools</b>				
Cell lines and tissue culture	++	+	++++	+
Antibody reagents	++	+	++++	++

\*Reverse-genetics approach; <sup>†</sup>forward-genetics approach; <sup>§</sup>genome sequence; -, not relevant, or not a strength; \$, \$\$, \$\$\$ and +, ++, +++, relative cost (\$) and strength (+) of the model in each category; +, ++, +++, outstanding strength of the model; TILLING, targeting induced local lesions in genomes.

the 1990s<sup>16–18</sup>, established the zebrafish as a mainstream model in developmental biology (BOX 1).

Recently, the same attributes that have propelled the rise of zebrafish in developmental biology research have also prompted the increased use of this organism as a model for several human diseases. Although there are obvious differences in the physiology of fish and humans (TABLE 2) that might affect the phenotypic outcome of diseases in zebrafish models, the zebrafish offers several advantages that make it an important complement to mouse models of disease. Many zebrafish models of monogenic human genetic disease have already been generated through forward-genetic screens<sup>19</sup>, allowing an enhanced understanding of the basic cell-biological processes that underlie the disease phenotype beyond that gained from existing models. Several zebrafish transgenic approaches have also been used in modelling acquired diseases (reviewed in REF. 20); for example, by marking cell types involved in infection and inflammation to facilitate the study of the processes that underlie disease progression. The ability to examine the onset and course of a pathological process *in vivo* and in real time is a particular strength of zebrafish models. Furthermore, zebrafish biology has established a new paradigm in therapeutics, that of whole-animal, high-throughput screening for small-molecule chemical modifiers of disease pathogenesis

**Shotgun and minimum tiling path sequencing**

Two approaches to whole-genome sequencing. Shotgun sequencing refers to the random acquisition of sequence. Minimum tiling path sequencing refers to the collection of sequence in an ordered, directed manner, such as the systematic sequencing of an entire BAC clone from one end to the other.

and severity, firmly establishing a role for zebrafish in the field of pharmaceutical drug discovery.

This Review discusses the recent progress in using zebrafish for modelling human disease. It surveys the methods that are available to zebrafish researchers to generate models of both genetic and acquired human diseases. Although not all types of disease and pathology are covered here, the following discussion and the examples in TABLE 3 outline a range of zebrafish models that highlight the breadth of their current and potential contributions to disease modelling. Finally, this Review examines the expanding area of zebrafish chemical genetics, which takes advantage of zebrafish disease models to identify new compounds with therapeutic potential.

**Zebrafish models of genetic disease**

The twin attributes underpinning the use of zebrafish as a model system are the ability to apply efficient invertebrate-style genetics to vertebrate-specific questions, and the optical clarity of embryos and larvae, which allow easy visualization of developmental processes. External fertilization, high fecundity, rapid development and high stocking densities mean that zebrafish are particularly genetically tractable. Furthermore, considerable genomic resources exist for zebrafish, including several high-density genetic maps for mapping induced mutations and the ongoing sequencing of the zebrafish genome. Both shotgun and minimum tiling path sequencing are being combined to generate a high-quality genome resource for zebrafish research (see [The Danio rerio Sequencing Project](#) web site), which has greatly facilitated the identification and characterization of disease-causing mutations.

**Forward-genetics approaches.** The main tool-of-trade for zebrafish biologists is a cadre of mutants — generated by random mutagenesis and selected on the basis of a common phenotype — that can be used to dissect a particular process of interest<sup>16,17</sup> (FIG. 1). To generate mutants, male fish are exposed to the mutagen ethylnitrosourea (ENU), which typically induces point mutations. Zebrafish are relatively resistant to ENU toxicity, allowing higher levels of mutagenesis and specific locus hit rates than can be achieved with other vertebrate models<sup>16,17,21,22</sup>. Random mutagenesis has also been successfully carried out in zebrafish using retroviral methods<sup>18,23</sup>. Although lower in efficiency than chemical mutagens, this method has the advantage of tagging each insertion event, greatly facilitating the identification of the mutated gene and circumventing the laborious positional cloning that is required with other forward-genetic approaches.

Zebrafish forward-genetic screens are facilitated by the transparency of embryos and larvae, attributes that increase the ease of phenotypic screening, allowing this to be done on a large scale without sophisticated infrastructure or equipment. The ease of phenotype assessment is an often overlooked facet of the design of forward-genetic screens, and it is here that the zebrafish excels as a model system. These features provide an advantage over other vertebrate genetic systems, including the mouse, in which aspects of organogenesis and disease pathology cannot be examined without interventions such as

surgery or post-mortem examination. By contrast, disease phenotypes in zebrafish can often be determined by simple optical microscopy that can be further assisted by transgenic approaches that drive the expression of fluorescent proteins in specific tissues and organs.

Utilising these attributes, large-scale forward-genetic screens<sup>16,17</sup> have recovered numerous zebrafish mutations in genes that are orthologous to those causing human congenital disease, and which result in clear phenotypic similarities (recently reviewed in REF. 19). These screens have collected recessive mutants with lethal developmental phenotypes that usually represent the most severe form of the corresponding human syndrome. In some instances, the mutant phenotypes have been sufficiently similar to the disease pathology in humans to allow the mutation to be identified by a candidate gene approach. In these instances, the identification of a zebrafish mutant as a model for a particular human disease has been relatively straightforward. For example, the cellular pathology of zebrafish muscle degeneration mutants presented superficial similarities to human muscular dystrophies. One of these mutants, *sapje*, carries a defect that impairs the zebrafish *dystrophin*

gene<sup>24</sup>, which is homologous to the human gene that is affected in **Duchenne muscular dystrophy**, the most common form of human muscular dystrophy. Similarly, photosensitive erythrocytes and altered porphyrin profiles led to the characterization of the anaemic mutants *yquem* and *dracula* as models of hepatoerythropoietic porphyria and erythropoietic protoporphyria, respectively<sup>25,26</sup>. Phenotypic similarities between *Tbx1* mutant mice and the zebrafish *van gogh* mutant also implicated *TBX1* in the human DiGeorge syndrome, identifying *van gogh* as a model of this disease<sup>27</sup>.

Once a zebrafish disease model is generated, the ease of embryo manipulation enables further investigation of the molecular and cellular basis of the disease. Time-lapse analysis of disease progression is often used to define the onset and nature of *in vivo* pathology at cellular resolution. Most analyses can be carried out in the context of the whole organism, allowing rapid assessment of the phenotype. Often, this has resulted in the formation of new hypotheses about disease-gene function, even when a well-studied mouse model exists for the same disease gene. In the case of muscular dystrophy, the zebrafish mutant *sapje* drew

**Candidate gene approach**

Characterizing mutants by focusing attention on individual genes that, based on some prior information, might plausibly underlie the mutant phenotype, as compared to a systematic positional cloning strategy.

**Hepatoerythropoietic porphyria**

A congenital syndrome that is characterized clinically by light-sensitive dermatitis and biochemically by high urinary uroporphyrin excretion, due to defects in the gene encoding uroporphyrinogen decarboxylase.

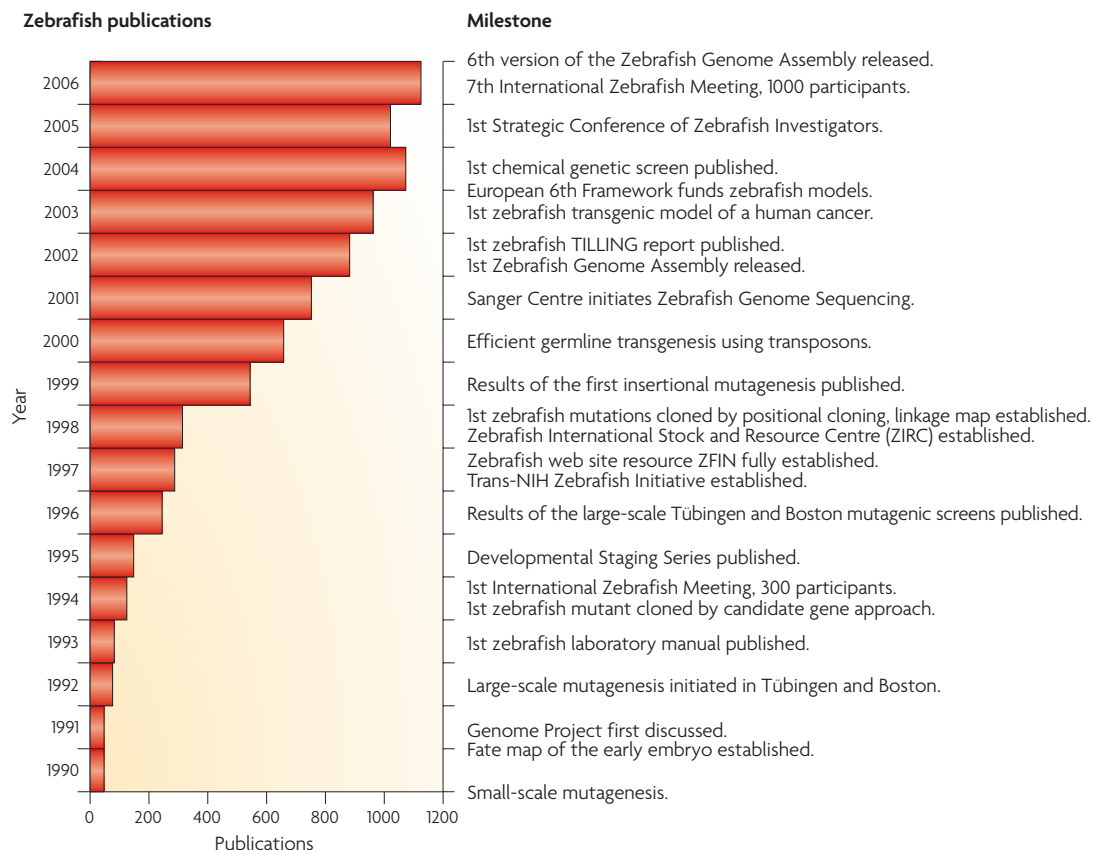
**Erythropoietic protoporphyria**

A congenital syndrome that results from overproduction of protoporphyrin due to a defect in the haem-synthesis enzyme ferrochelatase. It is characterized by light-sensitive dermatitis, mild anaemia and occasionally liver dysfunction and neuropathy.

**DiGeorge syndrome**

A syndrome that combines craniofacial, aortic, cardiac, thymic and auditory developmental defects, many of which are attributable to haploinsufficiency of the *TBX1* gene.

**Box 1 | A historical perspective of zebrafish genetic research**



The past decade and a half has seen a rapid uptake in the use of zebrafish as a model. Building on landmark embryological studies of the previous decade, the large-scale mutagenic programmes of the mid-1990s and the sequencing of the zebrafish genome by the **Sanger Centre** have fuelled a 40-fold rise in the number of zebrafish publications listed in PubMed. The number of mouse publications has risen 2.5-fold in the same period. TILLING, targeting induced local lesions in genomes.

Table 2 | **Comparative zebrafish biology for modelling human disease**

Characteristics	Key similarities to humans	Key differences and unknowns
<b>General biology</b>		
Genome structure	Diploid; essentially contains the full vertebrate repertoire of genes	Gene duplication resulting from ancestral whole-genome duplication, resulting in subfunctionalization and neofunctionalization
Anatomy	Vertebrate body plan	Aquatic adaptations include streamlined body plan and different locomotor strategies
Diet and metabolism	Omnivorous	Poikilothermic, grows optimally at 28.5°C
Growth	Growth is determinate (that is, proceeds to a limited maximum adult size); exhibits a saltatory growth pattern (for example, in fin regeneration)	Significant capacity for regeneration of many tissues and organs, for example, heart, fin, retina
Lifespan	Juvenile and adult phases of growth around the point of reproductive maturity; evidence for age-related physiological changes, for example, in cognitive function	Lifespan of 3–5 years; generation time of 3 months
<b>Systematic biology</b>		
Embryology	Stages and processes of cleavage, early patterning, gastrulation, somitogenesis, organogenesis are all represented	Very rapid; non-placental, occurs <i>ex vivo</i> ; influence of maternal transcripts; involves hatching
Skeletal system	Complex ossified skeleton comprising cartilage and bone	Lack long bone, cancellous bone, and bone marrow; joints are not weight-bearing
Muscle	Axial and appendicular muscle groups; skeletal, cardiac and smooth muscle cell types; fast and slow skeletal muscle fibres	Fast- and slow-twitch muscle are topographically separate; tail-driven locomotion depends on alternating contraction of myotomal muscle; appendicular muscle bulk is proportionately small
Nervous system and behaviour	Representative anatomy: fore-, mid- and hind-brain, including diencephalon, telencephalon and cerebellum; peripheral nervous system with motor and sensory components; enteric and autonomic nervous systems; specialized sensory organs: eye, olfactory system and ear; exhibit 'higher' behaviours and integrated neural function: memory, conditioned responses and social behaviours (for example, schooling)	Telencephalon has only a rudimentary cortex; fish-specific sensory organs, such as the lateral line; fish behaviours and cognitive function are abstracted or simplified compared with human behaviour
Haematopoietic and lymphoid/immune systems	Multiple haematopoietic cell types: erythrocytes, myeloid cells (neutrophils, eosinophils, monocytes and macrophages), T- and B-lymphocytes; coagulation cascade for haemostasis; innate and adaptive humoral and cellular immunity	Erythrocytes are nucleated; possess thrombocytes rather than platelets; kidney interstitium is the haematopoietic site; details of humoral regulation of haematopoiesis are largely unknown; could have evolved fish-specific immune system components (for example, a family of immune receptors)
Cardiovascular system	Multi-chamber heart with an atrium and ventricle; circulation within arteries and veins; separate lymphatic circulation	Has left–right distinctions in cardiac anatomy, but does not have separate left–right circulations, that is, the heart has only two chambers; so far no evidence for secondary heart field derivatives; lymph nodes have not been described
Respiratory system	Cellular gas exchange; oxygenation is dependent on circulation and haemoglobin carriage	Respiration occurs in gills, not lungs; no pulmonary circulation; possess an endoderm-derived swim bladder (functioning as a variable buoyancy device), which corresponds embryologically but not functionally to the lungs
Gastrointestinal system	Major organs: liver, exocrine and endocrine pancreas, gall bladder; zonal specializations along the length of the absorptive alimentary tract; immune cells in lamina propria	Lack an acidified digestive organ; have an intestinal bulb rather than stomach; intestinal Paneth cell not present
Renal and urinary systems	Glomerular anatomy and function	Filtration occurs in anterior and posterior kidneys; mesonephric rather than metanephric adult kidney; no bladder or prostate gland
Reproductive system	Molecular and embryological biology of germ-cell development; cellular anatomy of germ-cell organs, the testis and ovary	No sex chromosomes; mechanism of sex determination is uncertain; fertilization is <i>ex vivo</i> (that is, no uterus or the related internal female reproductive organs); oocytes are surrounded by a chorion, not the zona pellucida, which must be penetrated by sperm; non-lactating; no breast equivalent
Endocrine system	Most endocrine systems represented, for example, hypothalamic/hypophyseal axis (glucocorticoids, growth hormone, thyroid hormone, prolactin), parathyroid hormone, insulin and renin	Differences in anatomical distribution of glands, for example, discrete parathyroid glands do not seem to be present; prolactin has a primary role in osmoregulation
Skin and appendages	Ectodermal derivative; pigmentation pattern is due to neural-crest-derived pigment cells including melanocytes	Have structures unique to fish that are specialized for the aquatic environment (for example, elasmoid scales, mucous cells); lack mammalian appendages (for example, hair follicles, sebaceous glands); possess two additional pigment cell types: xanthophores and iridophores

Table 3a | Zebrafish disease models

Disease, pathological process or exposure	Example of zebrafish model	Phenotype and/or studies of disease pathogenesis	References
<b>Congenital and hereditary disease</b>			
Birth defects, paediatric syndromes	Random mutants from ENU and insertional mutagenesis	Several thousand mutants with early phenotypes affecting developmental processes and organogenesis	16,17,23
Disease-susceptibility traits	ENU-mutation in <i>adenomatous polyposis coli</i> ( <i>apc</i> ) gene	<i>apc</i> <sup>-/-</sup> fish develop intestinal polyps	34
	Ataxia telangiectasia morphant	Increased radiation sensitivity	106
<b>Carcinogenesis</b>			
Drug carcinogenicity testing	Chemical carcinogen exposure	Assorted tumours including sarcoma, seminoma	57
Cellular hyperproliferation	Genetic screen for hyperproliferation mutants	<i>bmyb</i> <sup>-/-</sup> ENU mutant with hyperproliferation cell phenotype; a subsequent chemical screen identified a specific suppressor	61,77,105
Oncogenesis	Panel of insertion mutants in ribosomal protein gene loci	Unexpected increased incidence of tumours suggests a new mechanism of oncogenesis	107
Genomic instability	Mutants resulting from forward-genetic screens	Increased incidence of spontaneous tumours or tumour susceptibility	59–61
Leukaemogenesis	Tg( <i>rag2:Myc</i> ) zebrafish	Lethal acute lymphoblastic leukaemia; conditional variant using Cre-lox technology	63,53
	Tg( <i>rag2:bcl-2</i> ) zebrafish	Lymphocytosis, transgene conferred steroid- and irradiation-resistance	64
Melanoma oncogenesis	Tg( <i>mitfa:BRAF</i> ) zebrafish	Malignant melanoma	67
Cooperative tumorigenesis	<i>p53</i> <sup>-/-</sup> ENU mutant zebrafish crossed with tumour-prone zebrafish strains	Accelerated tumorigenesis	45,67
	Interbreeding of transgenic zebrafish expressing leukaemogenic genes	Accelerated leukaemogenesis	66
<b>Infection</b>			
Gram-positive spp.	Infect embryos with <i>Bacillus subtilis</i>	Observe leukocyte behaviour	82
Mycobacterium spp.	Infect embryos or adults with <i>Mycobacterium marinum</i>	Assess vulnerability, organism virulence, contribution of adaptive cellular immunity, transcriptome response	81,93,96
Gram-negative spp.	Infect embryos with <i>Escherichia coli</i> , <i>Salmonella arizonae</i>	Observe leukocyte behaviour	82,93
<b>Inflammation and wound healing</b>			
'Sterile' wounding	Transect embryonic tail, or wound fin	Observe, quantitate and modify leukocyte behaviour	83,90–92
Regeneration	Fin transection	Observe and quantitate regrowth	73,108,109
	Removal of cardiac ventricular muscle	Cardiac muscle regeneration	110,111
<b>Immunological disease</b>			
Immune suppression	Immune suppression by irradiation	Myelosuppression; suppresses immune allograft rejection	112,113
	Immune suppression due to T-cell dysfunction	Heightened susceptibility to <i>M. marinum</i> infection	96

ENU, ethylnitrosurea; Tg, transgenic model.

attention to disruption at the myotendinous junction as a possible site of pathogenesis for the disease<sup>24</sup>.

Occasionally, zebrafish mutants have phenotypic similarities to human diseases for which the molecular bases are unknown. For example, *retsina* is an anaemic mutant with binucleate erythrocytes that are highly reminiscent of those found in the marrow of patients with congenital dyserythropoietic anaemia type 2, for which a disease locus has not yet been found. This suggests that Erythroid band 3 (the protein that is altered in *retsina* mutants), or other proteins in the same complex, might be disrupted in this congenital disease<sup>28</sup>.

There is an important difference between this phenotype-driven approach for generating disease models and reverse-genetic strategies such as gene knockout. That is, when a zebrafish phenotype is associated with a mutation in the orthologue of a known human disease gene, other mutants with similar phenotypes immediately present themselves as potential candidate genes for that disease. This is a particularly compelling hypothesis when a zebrafish mutant phenotype has pathological characteristics that are highly suggestive of a specific human disorder, even when the mutation maps away from known disease loci. For example, several putative polycystic kidney disease (PKD)

#### Congenital dyserythropoietic anaemia type 2

An hereditary anaemia characterized by binucleate marrow erythroid precursors, ineffective erythropoiesis, and acidified-serum-sensitive red cells (a positive Ham test). Although it maps to 20q11.2, the genetic basis is unknown.

Table 3b | Zebrafish disease models

Disease, pathological process or exposure	Example of zebrafish model	Phenotype and/or studies of disease pathogenesis	References
<b>Metabolic disease</b>			
Iron-storage disorder	ENU-induced mutation in <i>ferroportin</i>	Discovery of <i>ferroportin</i> basolateral iron transporter, later implicated in Type IV haemochromatosis	114,115
Porphyria (exemplifying inborn errors of metabolism)	ENU-induced mutations affecting various haem-synthesis enzymes	Light-sensitive anaemia and haemolysis	25,26
<b>Endocrine disease</b>			
Hypothyroidism	Expose embryos to anti-thyroid drugs	Biochemical hypothyroidism with developmental effects	116–118
Growth hormone excess	Transgenic overexpression or direct administration	Increase in muscle bulk	119
<b>Nutritional disease</b>			
Fasting and starvation	Restrict food	Affects fin growth	120
Vitamin deficiency	Antagonize vitamin K by warfarin administration	Anti-coagulation	121
<b>Psychological and behavioural abnormalities</b>			
Addiction	Genetic screen for altered cocaine sensitivity	ENU mutants with cocaine insensitivity	39
Social behaviour	Computer-assisted quantification of schooling and chasing behaviour	With tools to quantify behaviour, perturbations can be detected more easily and objectively	35
Mating behaviour	Natural variation in behaviour	Correlate reproductive success with territorial behaviour	122
Cognitive function	Video recording of locomotor activity	Age-related decline in defined cognitive responses, accelerated by genotoxic stress and attenuated by cholinergic upregulation	40
<b>Toxicity and poisoning</b>			
Teratogenicity screening	Transgenic zebrafish with scorable target transgene	Assay mutagenicity of chemicals	123
Teratogenicity mechanisms	Thalidomide treatment of embryos and selected morphants	Antiangiogenic effect is mediated by C2-ceramide and sphingosine-1-phosphate pathway	124
Exposure to environmental chemicals	Arsenate and perchlorate exposure	Disruptive effects on thyroid histology	125

ENU, ethylnitrosurea.

**Polycystic kidney disease**  
A genetically heterogeneous group of disorders that are characterized by multiple renal cysts, associated with liver cysts and cerebral aneurysms.

**Hermansky–Pudlak syndrome**  
A genetically heterogeneous syndrome that combines albinism, a bleeding diathesis and lysosomal storage defects with characteristic pigmented reticuloendothelial cells.

**Familial dilated cardiomyopathy**  
A spectrum of familial cardiomyopathies, a subset of which have been associated with mutations of the *TTN* gene.

loci were identified from a panel of zebrafish mutants with cystic kidney phenotypes, but only one carried a lesion in an orthologue of a known human PKD gene<sup>29</sup>. A more speculative example is provided by the *fade out* mutant, which shows retinal degeneration that is reminiscent of Hermansky–Pudlak syndrome, but which maps away from any of the known disease loci<sup>30</sup>. Given these examples of strong phenotypic parallelism between human diseases and zebrafish mutants, despite the involvement of apparently different genes, zebrafish mutants should be regarded as pointing to potential candidate disease genes, rather than dismissing them as quirks of fish physiology.

Zebrafish mutants can also provide functional evidence for connecting a particular locus with a human disease phenotype, as demonstrated by the implication of titin (*TTN*) in familial dilated cardiomyopathy. Linkage of the large *TTN* locus with the human disorder had already been established<sup>31</sup>. However, the finding that *ttn* is aberrantly spliced in the zebrafish *pickwick* mutant<sup>32</sup> (which has a dysfunctional, poorly contractile heart) was important in making the connection between titin mutations and the disease<sup>33</sup>.

Humans who are heterozygous for dysfunctional alleles can show a predisposition to disease, delayed disease onset, a prodromal disease phase or sensitivity to a disease precipitant. These states can be modelled by zebrafish that are heterozygous for mutant alleles. For example, like their human and murine counterparts, zebrafish that are heterozygous for loss-of-function mutations in *apc* (the adenomatous polyposis coli orthologue) develop intestinal neoplasias and are susceptible to intestinal carcinogens<sup>34</sup>. However, in both zebrafish and mice, the number of examples of haploinsufficiency-related disease loci is small compared with humans, and diseases that are prevalent in dominant human forms are not uncommonly modelled by recessive alleles in mouse and zebrafish models. Whether this phenomenon results from biological differences between these species, the nature of the mutations that have been recovered or a bias in the phenotypic analysis remains to be determined.

Although single-gene disorders constitute a serious human disease load, the diseases that have the greatest impact on human populations, such as addiction, infectious disease susceptibility, heart disease and cancer, seem to have complex polygenic modes of inheritance.

An advantage of modelling disease in zebrafish is the opportunity to do genetic suppressor and enhancer screens in the background of known disease-causing genes. Such screens are currently underway, and this approach provides one way to track down genes that influence disease susceptibility.

Zebrafish also provide the opportunity to study genetic influences on complex, integrated neurological functions that affect behaviour. Several pharmacological agents that alter synaptic transmission and neural membrane stability in humans show analogous activities in zebrafish, suggesting the existence of similar neural networks in these organisms. Although cognitive function can be quantitated in mice, this is difficult to do on a scale that would be necessary for a genetic screen. Zebrafish behaviours are readily quantitated in bulk (for example, chasing or swimming behaviours<sup>35–39</sup>), for both larvae and adults (for example, by using a conditioned place preference test<sup>40</sup>). Superficially, these behaviours seem remote from complex human behaviours, and part of the challenge lies in abstracting human behaviour or deconstructing it to its components, so that analogies with fish behaviour can be drawn. One pointer to the potential contribution of zebrafish models to understanding the pathogenesis of behavioural disorders comes from the area of addiction: zebrafish exhibit strain-dependent variations in alcohol susceptibility<sup>41</sup> and specific mutants have been isolated with varying cocaine sensitivity, as monitored by a conditioned place-preference test<sup>39</sup>.

**Reverse-genetics approaches.** Reverse genetics involves studying the phenotypic consequences of perturbing the function of a gene of interest. Mouse geneticists have excelled in this regard, with gene targeting by homologous recombination making the mouse a powerful model for studying genetic diseases. However, zebrafish mutants in orthologues of selected human disease loci can be generated by a reverse-genetic approach termed TILLING (targeting induced local lesions in genomes)<sup>42</sup> (FIG. 1). Some of the first zebrafish mutants to be generated using this approach<sup>43,44</sup> have mutations in genes involved in human disease: for example, *rag1* (*recombination activating gene 1*)<sup>21</sup>, which is a lymphocyte V(D)J recombinase associated with human severe combined immunodeficiency, and the tumour suppressor genes *p53* (REF. 45) and *apc*<sup>34,46</sup>. TILLING offers several advantages over gene targeting by homologous recombination. First, provided with a sufficiently large library to screen, an allelic series of random mutations can be collected<sup>21</sup>; such series have been extremely informative about gene function in invertebrate genetic models. An allelic series enables better genotype–phenotype correlations to be made, which can provide insights into the molecular pathogenesis of disease. Importantly, from a population perspective, human congenital diseases are also typically generated by an allelic series of point mutations, which are more accurately modelled by a set of point mutations introduced by chemical mutagenesis than by a single model that is made using standard homologous recombination, which often leaves a large and complex genomic footprint.

Furthermore, missense and gain-of-function mutations can also be generated using TILLING, and these can be extremely informative in assaying gene function.

Retroviral mutagenesis in zebrafish, which was initially developed as a tagged insertional mutagenesis strategy for forward genetics, has also been developed commercially for the reverse-genetic identification of specific gene disruptions. Here, genetically mapped insertions in a given orthologue of a specific disease gene can be ordered ‘off-the-shelf’ by researchers (see the **Znomics** web site).

In embryonic and larval zebrafish, reverse-genetic analyses are also facilitated by assays of gene function using transient rather than stable misexpression, which is technically easier than in mice as injection into the female pronucleus is not required. Microinjection of early embryos with either mRNA<sup>47</sup> or antisense morpholino oligonucleotides<sup>48</sup> results in transient gene overexpression or knockdown, respectively. Sophisticated compartmentalization and caging techniques allow tissue-specific and temporal-specific expression<sup>49</sup>. These techniques have been extremely useful for studying gene function during early zebrafish development. In the context of disease modelling, they provide a relatively quick approach for examining potential genetic interactions that affect disease phenotype severity or penetrance, and for evaluating the therapeutic effect of removing or adding selected gene products in the context of disease-related phenotypes. For example, the transient alteration of uridine-rich small nuclear ribonucleoprotein particle (U snRP) levels has helped to elucidate the cellular processes that underlie the neurodegenerative disorder spinal muscular atrophy<sup>50</sup>. In addition, the rescue of the *sauternes* mutant by overexpressing the haem biosynthesis pathway enzyme  $\delta$ -aminolevulinic synthase was presented as the first example of gene therapy for the analogous human disease, congenital sideroblastic anaemia<sup>51</sup>.

### Transgenic approaches

The absence of a comparable technique to gene targeting by homologous recombination in mouse embryonic stem (ES) cells prevents the construction of zebrafish disease models using engineered endogenous loci. This form of genetic engineering is still not possible in zebrafish, despite the generation of zebrafish cell lines with similar properties to ES cells<sup>52</sup> and the demonstration that Cre recombinase can function in zebrafish embryos<sup>53</sup>. However, non-homologous engineering of the zebrafish genome has been feasible for many years, and the efficiency of germline transgenesis has recently been markedly improved using several transposon-mediated systems<sup>54,55</sup>. Transposon-mediated transgenesis by simple injection of constructs into fertilized oocytes can result in a 50–80% efficiency of germline transgenesis without the need to target the female pronucleus. This is far more rapid and efficient than the generation of transient and stable transgenic mice. This advantage, along with the optical transparency of the zebrafish embryo, is resulting in an ever-expanding panel of transgenic zebrafish expressing fluorescent proteins in various cell types, organs and anatomical patterns (reviewed in REF. 20). These lines facilitate

#### Prodromal disease phase

A phase of a disease in which a specific early symptom prefigures the full development of a disease, or a symptom indicates that a disease attack is imminent.

#### Conditioned place preference test

A psychological test to determine whether the regular association of a particular stimulus with a particular location leads to an alteration in behaviour that favours the location, regardless of whether the stimulus is present.

#### Caging

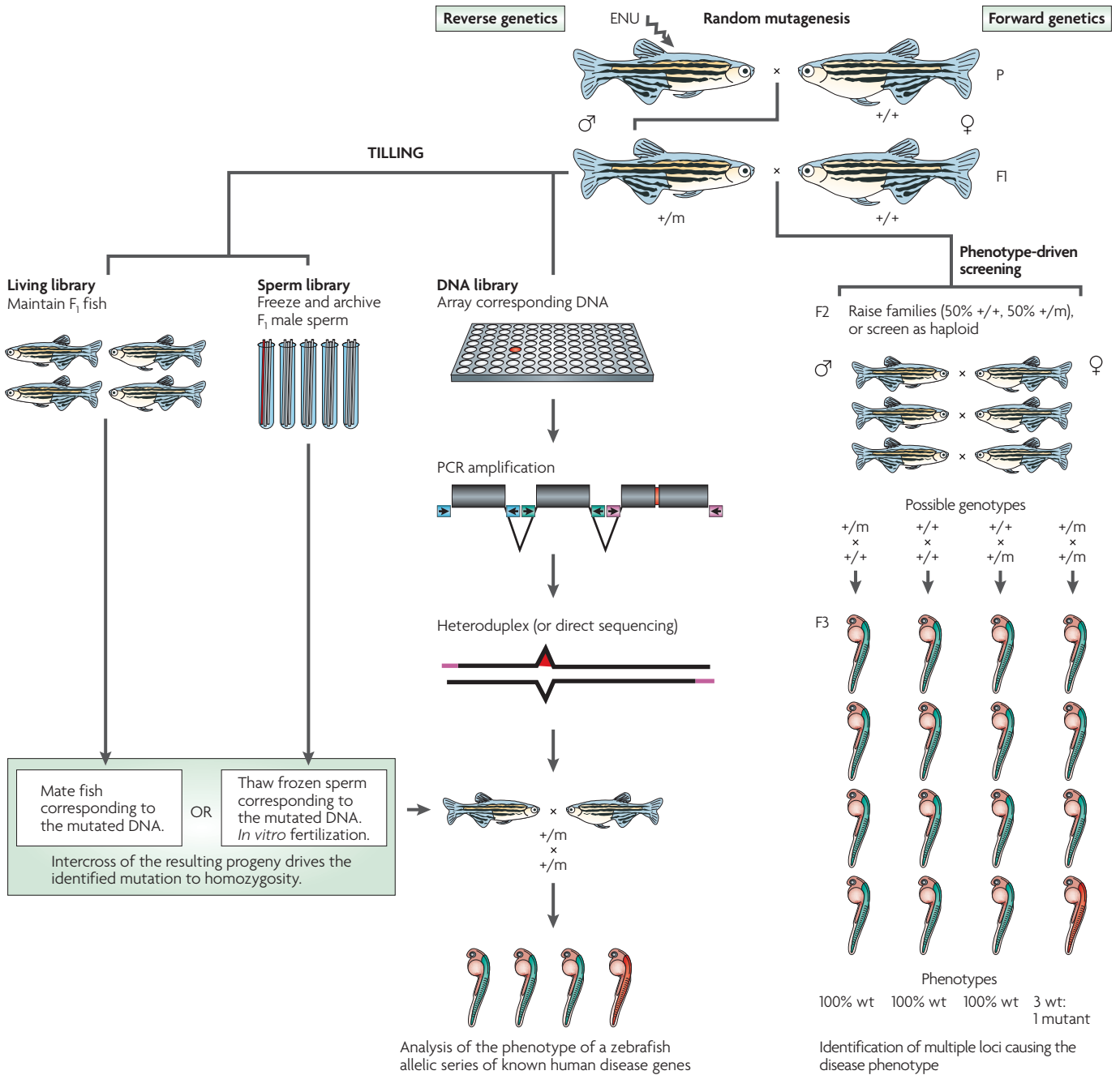
An experimental approach that delivers reagents in an inactive ‘caged’ form that can later be activated by a chemical, physical or genetic ‘uncaging’ event.

#### Spinal muscular atrophy

A collection of syndromes caused by mutations in the *SMN1* gene, characterized by spinal motor neuron degeneration causing muscle weakness and wasting.

#### Congenital sideroblastic anaemia

A congenital anaemia that is morphologically characterized by marrow red cell precursors with aggregates of non-haem iron around their nuclei, which is detectable by Prussian blue staining. The condition results from mutations in the gene encoding the haeme-synthesis enzyme  $\delta$ -aminolevulinic synthetase.



**Figure 1 | Generating zebrafish disease models.** Adult male zebrafish that have been mutagenized by immersion in water containing ethylnitrosourea (ENU) are outcrossed to generate the F<sub>1</sub> founder population, which has a high density of mutations. A forward-genetic methodology, shown here as a classical diploid F<sub>3</sub> schema<sup>16,17</sup>, drives the induced recessive mutations to homozygosity through two generations of intercrosses, resulting in one-quarter of the F<sub>2</sub> family sibling intercrosses producing mutant progeny in one-quarter of the F<sub>3</sub> embryos. Screening can be facilitated using transgenes that express GFP tissue-specifically. Mutations are selected by phenotype analysis and disease models are identified by their relatedness to human pathologies. Alternatively, males of the F<sub>1</sub> founder population can be used to create linked DNA and sperm libraries. These libraries can be used to target the screening of individual loci by TILLING (targeting induced local lesions in genomes), a reverse-genetic process<sup>21,43,44,46</sup>. This method uses PCR to amplify exons of a specific disease gene of interest from individual or pooled DNA from the F<sub>1</sub> DNA library. The fish corresponding to the DNA library can either be a living library<sup>21,43</sup>, or can be more conveniently stored as a library of frozen sperm<sup>44</sup>. Multiple mutations are detected within amplicons by various heteroduplex-detecting methods<sup>43,44</sup> or direct sequencing<sup>21,44</sup>. Once a mutation is detected within a given amplicon, the pedigree carrying the mutation is recovered by breeding directly from the corresponding fish in a living library or by thawing the corresponding frozen sperm to use *in vitro* fertilization. The resulting fish are intercrossed to generate heterozygous and homozygous mutants for the disease gene. With a large enough library, multiple different ENU-induced mutations will be identified, resulting in an allelic series. m, mutant allele; wt, wild type.

efficient genetic screening and allow pathological processes to be followed in real time by microscopy. Transgenesis also enables the overexpression of dominantly acting disease genes within specific tissues using different tissue-specific promoters and this approach has been used to generate several zebrafish cancer models (see below).

### Zebrafish models of acquired disease

Although genetic approaches have underpinned the initial growth of zebrafish as a model of single-gene hereditary disorders and monogenic adult disease, an exciting emerging area is zebrafish modelling of acquired disease. These diseases, which constitute the bulk of the human disease load, have complex aetiologies, varied patterns of onset and diverse clinical manifestations, reflecting the complex genetic contribution to their pathogenesis. Although there are excellent mammalian models of many acquired diseases, these are generally not as readily manipulated in large-scale genetic or chemical screens as compared with zebrafish models. The need for zebrafish models is also driven by the fact that, although simplified *in vitro* cell-based assays have been useful for dissecting the components of complex physiological processes and pathological mechanisms, they have not provided a tractable integrated model of *in vivo* disease pathogenesis. To highlight how acquired diseases are now being studied in zebrafish, we focus below on two of the most developed models; in addition, TABLE 3 provides a systematic overview with selected examples.

**Carcinogenesis.** The use of zebrafish for carcinogenicity testing of chemicals and in toxicology long predates their use as a genetic model<sup>62</sup>. Zebrafish develop malignant tumours spontaneously and in response to mutagens<sup>56</sup> and carcinogens<sup>57,58</sup>, a propensity that is increased on genetically unstable genomic backgrounds<sup>59–61</sup> or on loss of tumour suppressor function (for example, *p53*)<sup>45</sup>. These spontaneous tumours are generally not of the types that are common in humans, and include a range of epithelial, liver and peripheral nerve sheath tumours, as well as seminomas and sarcomas. However, several common human tumour types have been modelled in zebrafish using transgenesis, confirming that the molecular mechanisms that underpin mammalian tumorigenesis also apply in zebrafish.

Perhaps the best-studied example of induced tumorigenesis in zebrafish is lymphoid leukaemia. In zebrafish, this cancer can result from overexpression of a classical dominant oncogene, Tg(*rag2:mouse-c-Myc*)<sup>53,63</sup>, a pro-survival molecule, Tg(*rag2:zebrafish-bcl2*)<sup>64</sup>, a classical fusion oncogene, Tg(*xenopus-E1 $\alpha$ :human-ETV6-RUNX1* synonym *TEL-AML1*)<sup>65</sup> and an activated signalling pathway, Tg(*rag2:human-NOTCH1*)<sup>66</sup>. These effector transgenes are collectively derived from mice, zebrafish and humans, attesting to the high degree of functional interspecies conservation in these oncogenic pathways. The long latency and incomplete penetrance of leukaemia occurrence in these transgenic fish suggests that, as in mammals, these oncogenes require cooperative mutations to cause flagrant leukaemia. Genetic modifier screens, which are feasible in zebrafish,

would be well suited to detecting these mutations. Indeed, interbreeding several of these transgenic lines has resulted in cooperative leukaemogenesis (which has a shorter disease latency)<sup>66</sup>, providing genetic evidence in support of this approach.

Melanoma has also been modelled in transgenic zebrafish that overexpress in neural crest cells an activated human *BRAF* oncogene, carrying the RAS-pathway-activating mutation that is the most prevalent in human melanoma<sup>67</sup>. These transgenic zebrafish develop pigmented naevi and, when *p53* is also inactivated, melanomas. In addition, tumour–host interactions have been studied in zebrafish through xenografting cells from a human melanoma cell line into early zebrafish embryos<sup>68</sup>. The secretion of specific signalling molecules by the xenograft induced embryonic patterning defects, and expression analysis and functional studies identified a role for specific signalling pathways in melanoma tumorigenesis. Xenografts of colorectal and pancreatic cancer cell lines into zebrafish have also been described<sup>69</sup>. Finally, the study of a zebrafish pigmentation mutant (*golden*) has provided new insight into the genetic basis of racial pigmentation differences<sup>70</sup>. Given that skin pigmentation type is a strong determinant of melanoma risk in humans<sup>71</sup>, it will be interesting to investigate the extent to which variation at the *golden* locus is a determinant of ultraviolet-light-related melanoma susceptibility in zebrafish.

The optical transparency of zebrafish and the availability of lines with fluorescently marked cells provide attractive opportunities to understand mechanisms of tumorigenesis. Inducing malignancy in transgenic fish carrying oncogenes with fluorescent tags, or in fish with appropriate fluorescently marked cell types, results in fluorescent tumours<sup>53,63,64</sup>, allowing recognition of tumour onset, location and the estimation of tumour bulk. These approaches can be used to design efficient and practical mutational screens for mutations that affect different aspects of tumourigenesis. Similarly, the host response to tumour development could be studied *in vivo*; for example, by exploiting the fluorescent vasculature and lymphatic vessels of Tg(*fli1:GFP*) transgenic zebrafish<sup>72</sup>, an approach already applied to the study of regeneration and wound healing<sup>73</sup>.

Forward-genetic screens have identified mutants with cancer predisposition phenotypes. One imaginatively designed screen used a mosaic eye assay to identify potential genomic instability mutants. This approach resulted in the identification of 12 mutants, some of which showed genetic interactions as transheterozygotes<sup>59</sup>. Many of these mutants — which have not yet been cloned — exhibit increased rates of spontaneous tumour development as heterozygotes. Another screen, which was designed to detect hyperproliferation through the analysis of phosphohistone H3 expression, recovered eight cell-proliferation mutants; two of these have been described in detail and exhibit cancer susceptibility in carcinogenesis assays<sup>60,61</sup>.

It remains to be determined whether all aspects of human tumour biology are replicated in zebrafish. In particular, convincing models of tumour metastasis have not yet been described, although the recent demonstration

#### Mosaic eye assay

A genetic assay based on the identification of different genetically determined pigmentation phenotypes in adjacent retinal pigment epithelium cells.

of the presence of a lymphatic system in zebrafish<sup>74,75</sup> and the proven utility of zebrafish as a model for studying angiogenesis during development suggest that both blood- and lymph-borne metastasis models might be provided.

Although the mechanistic and pathological correlations between zebrafish tumour models and their murine and human counterparts are interesting, their greatest promise and 'discovery value' stems from the prospect of using the zebrafish models to identify enhancers or suppressors in whole-animal tumour models, in either genetic or small-molecule screens. By using libraries of small molecules that have not been selected for their known targets, such screens make no presumptions about the most effective antitumour approach, and therefore have great potential for identifying new therapeutic targets and treatments<sup>76</sup>. With zebrafish, the logistics and costs of a screen that focuses on one disease are not as prohibitive as in the mouse. The potential of this approach is exemplified by the identification of persynthamide, a chemical modifier of the generalized hyperproliferative (albeit non-malignant) phenotype of the zebrafish *bmyb* mutant *crash and burn*<sup>61,77</sup> (FIG. 2).

**Infection and inflammation.** The mouse is a pre-eminent model for the study of immune function, owing to a large body of knowledge and reagents for cell-biological and molecular studies that have been accumulated over decades. Genetic screens have been undertaken in the mouse to identify genes controlling the immune response (reviewed in REF. 78); however, these screens are limited by aspects of mouse biology such as the difficulty of recognizing relevant embryonic lethal phenotypes<sup>78</sup>. Therefore, the need remains for a genetically tractable system such as the zebrafish to investigate the genes that are involved in host defence mechanisms (for example, by searching for susceptibility and resistance loci for particular pathogens and identifying innate immune response modifier loci). Unlike the other two genetically tractable models for infection, inflammation and immune disease — *Caenorhabditis elegans* and *Drosophila melanogaster* — zebrafish have an adaptive immune system, which is an evolutionary innovation of jawed fish (reviewed in REF. 79).

Zebrafish are susceptible to infection by Gram-positive and Gram-negative bacteria, mycobacteria, protozoa and viruses (reviewed in REF. 80). The zebrafish innate immune response<sup>81</sup> involves phagocytic cells such as macrophages and neutrophils<sup>82–84</sup>, cytokines and their signalling molecules<sup>85,86</sup>, and adaptive humoral and cellular immunity<sup>87,88</sup>. Inflammatory processes can be visualized in transparent zebrafish embryos; furthermore, studies of the cellular components of the inflammatory response have been enhanced by the use of transgenic zebrafish with GFP-marked macrophages<sup>72,89,90</sup>, neutrophils<sup>91,92</sup> and endothelia<sup>72</sup>, and by transgenic GFP-expressing pathogens<sup>93,94</sup>, providing new opportunities for examining the fully integrated host–pathogen interaction quantitatively, in real time and *in vivo*. For example, using wounding as a model of sterile acute inflammation, the participation of transgene-marked fluorescent zebrafish neutrophils in acute inflammation has been studied, formally

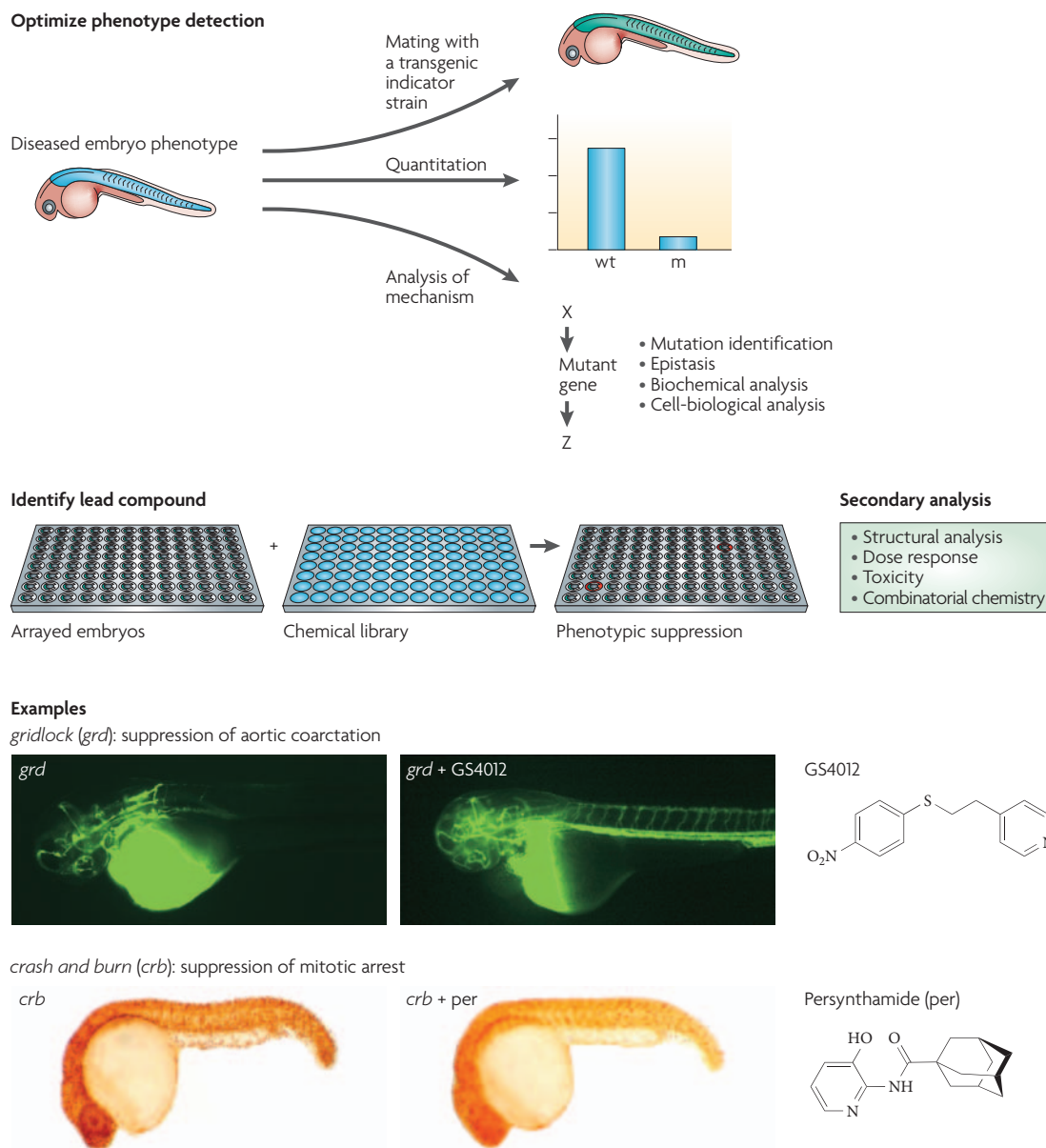
demonstrating that neutrophil emigration from an inflammatory lesion<sup>92</sup> and neutrophil apoptosis<sup>91</sup> are important components of the resolution of inflammatory responses. Similarly, in zebrafish embryos with transgene-marked fluorescent macrophages, the chemotactic response of embryonic macrophages was shown to involve a Rho-kinase-dependent mechanism<sup>90</sup>.

Tuberculosis, caused by mycobacteria, provides an example of modelling the host–pathogen interaction in zebrafish. *Mycobacterium marium* is an endemic chronic infection of zebrafish<sup>95</sup>, and conditions have been established for experimental infection of embryos<sup>93</sup> and adults<sup>96</sup>. Whereas the mouse provides a good model of the latent, pathologically inactive phase of human tuberculosis, mycobacteria-infected zebrafish demonstrate active disease with its typical pathology, including caseated granulomas. However, zebrafish seem unable to eradicate the pathogen, despite the involvement of a cell-mediated immune response<sup>96</sup>. Zebrafish mycobacterial infection models therefore provide a basis for undertaking genetic dissections of the host- and pathogen-related determinants of active tuberculosis, that is, infection susceptibility traits and organism virulence determinants. At least in theory, if the fluorescent signal of GFP-marked host or pathogen strains could be quantified, these traits could be examined using QTL approaches.

Although these examples illustrate the opportunities for modelling infectious disease in zebrafish, there remain some potential problems<sup>80</sup>. First, human pathogens cause disease at 37°C, whereas zebrafish are maintained at 28°C; it might not be possible, therefore, to study some pathogens at this lower temperature. This problem could be circumvented by using fish-specific pathogens, but the host–pathogen interactions that could be studied using this approach might be fish-specific. Second, the almost complete lack of monoclonal antibodies to surface antigens of zebrafish immune cells greatly limits cell-biological analyses of these processes. Finally, crucial gaps remain in the comparative physiology of the zebrafish immune system, which might impinge on the validity and usefulness of modelling human infections in zebrafish, for example, the degree to which adaptive immunity and the complex humoral regulation of mammalian granulopoiesis are recapitulated in fish, the identification of dendritic cells in zebrafish, and whether there are fish-specific aspects of immune function.

### Zebrafish disease models and drug discovery

From a clinical perspective, the most practical contribution that a disease model can make is to improve diagnosis and therapy. Efficacious, well tolerated disease therapy is ultimately about selectively suppressing an unwanted phenotype without eliciting side-effects. There is only a limited scope for the use of zebrafish to model non-pharmacological therapeutics, such as those approaches that depend on anatomical modification (for example, surgical approaches) or tissue remodelling, and other models will remain superior for this. By contrast, the intersection of pharmacology and disease modelling in zebrafish promises to be especially productive, and it is particularly in the area of new drug discovery that zebrafish



**Figure 2 | The application of chemical genetics in zebrafish.** Once a zebrafish disease model is identified, an initial phase of phenotypic assessment is required before a chemical-suppression screen can be implemented<sup>97</sup>. Careful quantitation and assessment of the phenotypic penetrance are crucial and can be greatly aided by the use of transgenic indicator strains that express GFP in the affected tissues. Furthermore, information about the molecular, biochemical or cellular role of the gene will help to implicate the target of any small molecule that is identified. Characterized phenotypes can then be screened against small-molecule libraries from various academic and commercial sources (see Further information). Initial screens have suggested that the structural diversity of the library is a key determinant of the likelihood of success. Small groups of zebrafish embryos or larvae are arrayed in multi-well microtitre plates and standard concentrations of small molecules are robotically pipetted into the raising media in individual wells. Throughput is increased if suppression can be assessed directly in the larvae using fluorescent read-outs, or if it can be made quantitative in some way, particularly if the scoring process is suited to automation. Scoring can also be coupled with an immunological or gene-expression assay (for example, the *crash and burn* screen that used the anti-histone H3 antibody) to monitor cell-cycle progression. The active compounds that are identified can undergo a secondary process of validation, dose and toxicity assessment, and can be extended by exploration of analogues generated by combinatorial chemistry, before proceeding to testing in other animal models. The figure outlines two examples (for more details, see the main text): chemical suppression of the *gridlock* zebrafish model of aortic coarctation<sup>100–102</sup>; and chemical suppression of the *crash and burn* zebrafish model of mitotic arrest and genome instability<sup>61,77,105</sup>. m, mutant allele; wt, wild type. Images of the *gridlock* mutant reproduced with permission from *Nature Biotechnology* REF. 100 © (2004) Macmillan Publishers Ltd. Images of the *crash and burn* mutant reproduced with permission from *Nature Chemical Biology* REF. 77 © (2005) Macmillan Publishers Ltd.

provides advantages over other models. Zebrafish is the premier whole-animal vertebrate model for the screening of chemical libraries when searching for lead compounds with a desired therapeutic bioactivity. The recent identification of zebrafish models of genetic and acquired disease has allowed the integration of these models with large-scale small-molecule screens, and has resulted in the identification of new lead therapeutic compounds.

#### *Drug discovery by chemical screening in zebrafish.*

Chemical genetics is conceptually simple: zebrafish embryos or larvae are arrayed in small groups into 96-well microtitre plates, and small-molecule libraries are robotically dispensed into the raising media<sup>97</sup> (FIG. 2). To take full advantage of the fecundity, rapid development and optical transparency of zebrafish embryos, it must be possible to monitor drug efficacy in embryos or larvae. The end point of such a screen might be as simple as survival (that is, asking if the test molecule is toxic or not), or it might involve direct visual assessment of the severity of a specific phenotype. This approach can be greatly facilitated by using an indicator strain expressing GFP in a tissue that is affected by the pathogenic process. End points might also depend on, or be combined with, refined phenotype evaluations such as immunohistochemistry or whole-mount *in situ* hybridization gene-expression analyses.

Although it is feasible to screen zebrafish phenotypes efficiently on the basis of direct visual inspection, throughput efficiencies are enhanced by screen designs that can be quantitated and automated. An example of this is an automated assay for assessing heart rate using a transgenic zebrafish line with myocardium-restricted fluorescence and a digital videomicroscopy-based, pixel-enumeration, computerized scoring strategy, which can be used in high-throughput settings<sup>98</sup>.

Because the zebrafish drug screening approach uses whole animals, it has the advantage of incorporating both a requirement for bioavailability and an assay of toxicity. This allows the process to leapfrog several hurdles that plague current drug discovery mechanisms that rely on *in vitro* cell lines or biochemical screens. Furthermore, in contrast to traditional biochemical assays that focus on specific molecular targets, a screen that centres on a phenotypic outcome has the advantage of making no presumption about the specific molecular mechanisms that are involved, therefore potentially identifying previously unsuspected proteins and pathways as drug targets. Indeed, identifying the molecular targets of efficacious drugs can be a challenge in phenotype-led drug discovery projects, particularly as regulatory approval for drugs that are developed for therapeutic use in humans currently requires an understanding of not only which target is affected by a drug but also its mechanism of action<sup>99</sup>. Furthermore, it is possible that some hits might not carry cross-species activity into mammals. However, no drug discovery approach provides, from the outset, inherent proof of the feasibility of the proposed pharmacological intervention in achieving the desired disease-suppressing outcome *in vivo* in humans, and these caveats must be viewed in the light of the advantages that zebrafish whole-animal drug discovery approaches offer.

By carrying out screens using transgenic or mutant zebrafish that manifest particular disease phenotypes, disease-suppressing compounds can be identified. FIGURE 2 summarizes this approach and the results of two such chemical screens in zebrafish that have successfully isolated small molecules that suppress a selected disease phenotype<sup>100,101</sup> or pathological mechanism<sup>61,77</sup>.

In the first example, small-molecule screening was used to suppress the *gridlock* (*grl*) mutant phenotype<sup>102</sup>. Homozygous mutant *grl* embryos have no circulation to the posterior trunk and tail because of a localized block to caudal blood flow at the base of the dorsal aorta, the region where the two anterior lateral dorsal aortae merge to form the single midline dorsal aorta. The phenotype of this mutation is highly similar to a common human congenital disorder termed coarctation of the aorta, which is associated with significant abnormalities of the vasculature. The *grl* phenotype is caused by a mutation in the *hey2* gene<sup>103</sup>, which encodes a transcriptional repressor of the bHLH class that drives angioblast differentiation to an arterial fate rather than a venous fate in a dose-dependent manner<sup>104</sup>. Disruption of *hey2* results in the formation of insufficient numbers of arterial cells, and hence aortic deficiency. A phenotype-based small-molecule screen was used to discover two different classes of compound that suppress the coarctation phenotype of *grl* mutants, assaying for rescue by looking for restored blood flow and the survival of larvae using brightfield microscopy<sup>100</sup>. Two structurally related compounds, GS3999 and GS4012, fully suppressed the aortic defect, probably through induction of vascular endothelial growth factor (VEGF) expression. A second screen isolated a structurally unrelated chemical suppressor, GS4898, which suppressed the *grl* phenotype in a similar manner to the earlier identified compounds<sup>101</sup>. GS4898 was shown to be structurally similar to the phosphatidylinositol 3-kinase (PI3K) inhibitor LY29002, which also suppresses the *grl* phenotype, again immediately identifying the mode of action of this compound. Thus, chemical screens not only produce valuable lead compounds for therapy (the compounds identified here have therapeutic potential for instances in which vascular remodelling is required), but are also informative about the basic biological process involved in the defects under study. Whether these leads translate into new therapeutic agents remains to be seen<sup>99</sup>.

In the second example, a similar screening process was used to identify compounds that chemically suppress the phenotype of *crash and burn* (*crb*)<sup>77</sup>. *crb* results from mutations in the ubiquitously expressed transcription factor *bmyb*, which is known to control cell proliferation and has a role in cancer. Cell-cycle progression is blocked in *crb* mutants, resulting in a failure of cells to progress through mitosis<sup>61</sup>. Homozygous *crb* mutants exhibit a large increase in the number of cells that are positive for anti-phosphohistone H3 immunostaining, owing to a failure of cells to progress through G2. This failure of cell-cycle progression is attributed to a reduction in *cyclin B1*, a key cell-cycle regulator. In this chemical screen<sup>77,105</sup>, compounds derived from a structurally

**Coarctation of the aorta**  
A disorder, usually congenital, resulting from a constriction in the thoracic aorta, typically in the vicinity of the ductus arteriosus. Upper-limb hypertension is a clinical characteristic.

diverse, unselected chemical library were tested for their ability to control cell division, and a previously unknown compound that suppressed the *crb* phenotype was identified and named persynthamide.

Lead compounds that are identified in such screens require further assessment for activity in mammalian systems. It is likely that lead compounds themselves will not have optimal bioavailability, specificity, therapeutic indices and dose-response relationships, and hence will almost certainly benefit from further medicinal chemical modification as part of their development into marketable therapeutic drugs. Nevertheless, it is clear that zebrafish screens have potential for identifying compounds for further characterization and development.

### Conclusions

Our goal has been to draw on evidence provided by diverse examples to demonstrate the scope for zebrafish to model a wide range of human diseases, both in early and adult life, and to highlight the particular strengths of this model organism for biomedical research. No disease model is perfect. Using zebrafish to model human disease will almost certainly always require acceptance of a greater degree of abstraction than is required when working with mammalian models. There will be cases in which gene function has diverged between zebrafish and humans sufficiently to undermine any phenotypic similarity between a fish mutant and the corresponding human syndrome. There will also be aspects of zebrafish physiology that complicate some disease models. However, despite these caveats, the examples that we have provided show that zebrafish can be used to model diseases that affect many different organ systems, that are due to a range of different pathologies, and that result in diverse phenotypic or clinical consequences for the whole organism.

Currently, modelling a disease in embryonic or larval (rather than adult) zebrafish takes most advantage of the strengths of this model organism. However, experimentation with adult animals is also proving rewarding (for example, an age-related decline in cognitive function has been modelled in zebrafish<sup>40</sup>) and this will be a growing area. However, a better understanding of the comparative anatomy and physiology of adult zebrafish will be required, and several projects are underway to bridge this gap. Another ongoing challenge is to transfer the technical advantages of working with embryonic zebrafish into advantages for working with the adult organism, although there are now examples of this.

A further challenge will be to maintain the ongoing development of community-based resources for zebrafish research. To date, zebrafish researchers have taken a community-minded approach, and have invested heavily in stock centres, information and database resources, and the free exchange of materials and strains (see [The Zebrafish Model Organism Database \(ZFIN\)](#) and the [Zebrafish International Resource Center](#) web sites). However, the ongoing exponential increase in the use and demand for zebrafish resources will outstrip the ability of community-based resources to provide access to key material for all who work with this model. The main pressure points will be stock centres and the sequencing of the zebrafish genome, both of which will require continued ongoing investment to provide the necessary quality of material and sufficient access to it.

With ongoing investment in zebrafish research, zebrafish models of disease offer experimental strengths that can work alongside and along with other human disease models to improve the understanding of disease pathogenesis and ultimately develop new, effective therapies for a wide range of human diseases.

- Nolan, P. M. *et al.* A systematic, genome-wide, phenotype-driven mutagenesis programme for gene function studies in the mouse. *Nature Genet.* **25**, 440–443 (2000).
- Hrabe de Angelis, M. H. *et al.* Genome-wide, large-scale production of mutant mice by ENU mutagenesis. *Nature Genet.* **25**, 444–447 (2000).
- Michaud, E. J. *et al.* Efficient gene-driven germ-line point mutagenesis of C57BL/6J mice. *BMC Genomics* **6**, 164 (2005).
- Carpinelli, M. R. *et al.* Suppressor screen in *Mpl*<sup>+/−</sup> mice: *c-Myb* mutation causes supraphysiological production of platelets in the absence of thrombopoietin signaling. *Proc. Natl Acad. Sci. USA* **101**, 6553–6558 (2004).
- Coghill, E. L. *et al.* A gene-driven approach to the identification of ENU mutants in the mouse. *Nature Genet.* **30**, 255–256 (2002).
- Quwailid, M. M. *et al.* A gene-driven ENU-based approach to generating an allelic series in any gene. *Mamm. Genome* **15**, 585–591 (2004).
- Roosen-Runge, E. Observations of the early development of the zebrafish. *Brachydanio rerio*. *Anat. Rec.* **70**, S103 (1937).
- Laale, H. W. The biology and use of zebrafish, *Brachydanio rerio* in fisheries research. A literature review. *J. Fish. Biol.* **10**, 121–174 (1977).
- 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).
- Chakrabarti, S., Streisinger, G., Singer, F. & Walker, C. Frequency of  $\gamma$ -ray induced specific locus and recessive lethal mutations in mature germ cells of the zebrafish, *Brachydanio rerio*. *Genetics* **103**, 109–123 (1983).
- Walker, C. & Streisinger, G. Induction of mutations by  $\gamma$ -rays in pregonial germ cells of zebrafish embryos. *Genetics* **103**, 125–136 (1983).
- Grunwald, D. J. & Streisinger, G. Induction of recessive lethal and specific locus mutations in the zebrafish with ethyl nitrosourea. *Genet. Res.* **59**, 103–116 (1992).
- Solnica-Krezel, L., Schier, A. F. & Driever, W. Efficient recovery of ENU-induced mutations from the zebrafish germline. *Genetics* **136**, 1401–1420 (1994).
- Stuart, G. W., McMurray, J. V. & Westerfield, M. Replication, integration and stable germ-line transmission of foreign sequences injected into early zebrafish embryos. *Development* **103**, 403–412 (1988).
- Streisinger, G., Singer, F., Walker, C., Knauber, D. & Dower, N. Segregation analyses and gene-centromere distances in zebrafish. *Genetics* **112**, 311–319 (1986).
- Driever, W. *et al.* A genetic screen for mutations affecting embryogenesis in zebrafish. *Development* **123**, 37–46 (1996).
- 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). **References 16 and 17 are back-to-back publications of the first two large ENU mutagenesis forward-genetic screens in zebrafish, resulting in over a thousand mutants with specific developmental phenotypes.**
- Amsterdam, A. *et al.* A large-scale insertional mutagenesis screen in zebrafish. *Genes Dev.* **13**, 2713–2724 (1999). **Describes the first large-scale insertional mutagenesis screen in zebrafish.**
- Amsterdam, A. & Hopkins, N. Mutagenesis strategies in zebrafish for identifying genes involved in development and disease. *Trends Genet.* **22**, 473–478 (2006).
- Udvardi, A. J. & Linney, E. Windows into development: historic, current, and future perspectives on transgenic zebrafish. *Dev. Biol.* **256**, 1–17 (2003).
- Wienholds, E., Schulte-Merker, S., Walderich, B. & Plasterk, R. H. Target-selected inactivation of the zebrafish *rag1* gene. *Science* **297**, 99–102 (2002). **The first report of TILLING for a zebrafish mutant allele.**
- Russell, W. L. *et al.* Specific-locus test shows ethylnitrosourea to be the most potent mutagen in the mouse. *Proc. Natl Acad. Sci. USA* **76**, 5818–5819 (1979).
- Amsterdam, A. *et al.* Identification of 315 genes essential for early zebrafish development. *Proc. Natl Acad. Sci. USA* **101**, 12792–12797 (2004).
- Bassett, D. I. *et al.* Dystrophin is required for the formation of stable muscle attachments in the zebrafish embryo. *Development* **130**, 5851–5860 (2003).
- Wang, H., Long, Q., Marty, S. D., Sassa, S. & Lin, S. A zebrafish model for hepatocellular carcinoma. *Nature Genet.* **20**, 239–243 (1998).
- Childs, S. *et al.* Zebrafish *dracula* encodes ferrochelatase and its mutation provides a model for erythropoietic protoporphyria. *Curr. Biol.* **10**, 1001–1004 (2000).

27. Piotrowski, T. *et al.* The zebrafish *van gogh* mutation disrupts *tbx1*, which is involved in the DiGeorge deletion syndrome in humans. *Development* **130**, 5043–5052 (2003).
28. Paw, B. H. *et al.* Cell-specific mitotic defect and dyserythropoiesis associated with erythroid band 3 deficiency. *Nature Genet.* **34**, 59–64 (2003).
29. Sun, Z. *et al.* A genetic screen in zebrafish identifies cilia genes as a principal cause of cystic kidney. *Development* **131**, 4085–4093 (2004).
30. Bahadori, R. *et al.* The zebrafish *fade out* mutant: a novel genetic model for Hermansky–Pudlak syndrome. *Invest. Ophthalmol. Vis. Sci.* **47**, 4523–4531 (2006).
31. Siu, B. L. *et al.* Familial dilated cardiomyopathy locus maps to chromosome 2q31. *Circulation* **99**, 1022–1026 (1999).
32. Xu, X. *et al.* Cardiomyopathy in zebrafish due to mutation in an alternatively spliced exon of titin. *Nature Genet.* **30**, 205–209 (2002).
33. Gerull, B. *et al.* Mutations of *TTN*, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy. *Nature Genet.* **30**, 201–204 (2002).
34. Haramis, A. P. *et al.* Adenomatous polyposis coli-deficient zebrafish are susceptible to digestive tract neoplasia. *EMBO Rep.* **7**, 444–449 (2006).
35. Kato, S. *et al.* A computer image processing system for quantification of zebrafish behavior. *J. Neurosci. Methods* **134**, 1–7 (2004).
36. Levin, E. D., Bencan, Z. & Cerutti, D. T. Anxiolytic effects of nicotine in zebrafish. *Physiol. Behav.* **54**–58 (2006).
37. Levin, E. D. & Chen, E. Nicotinic involvement in memory function in zebrafish. *Neurotoxicol. Teratol.* **26**, 731–735 (2004).
38. Levin, E. D., Chrysanthis, E., Yacisin, K. & Linney, E. Chlorpyrifos exposure of developing zebrafish: effects on survival and long-term effects on response latency and spatial discrimination. *Neurotoxicol. Teratol.* **25**, 51–57 (2003).
39. Darland, T. & Dowling, J. E. Behavioral screening for cocaine sensitivity in mutagenized zebrafish. *Proc. Natl Acad. Sci. USA* **98**, 11691–11696 (2001).
40. Yu, L., Tucci, V., Kishi, S. & Zhdanova, I. V. Cognitive aging in zebrafish. *PLoS ONE* **1**, e14 (2006).
41. Lockwood, B., Bjerke, S., Kobayashi, K. & Guo, S. Acute effects of alcohol on larval zebrafish: a genetic system for large-scale screening. *Pharmacol. Biochem. Behav.* **77**, 647–654 (2004).
42. McCallum, C. M., Comai, L., Greene, E. A. & Henikoff, S. Targeted screening for induced mutations. *Nature Biotechnol.* **18**, 455–457 (2000).
43. Wienholds, E. *et al.* Efficient target-selected mutagenesis in zebrafish. *Genome Res.* **13**, 2700–2707 (2003).
44. Sood, R. *et al.* Methods for reverse genetic screening in zebrafish by resequencing and TILLING. *Methods* **39**, 220–227 (2006).
45. Berghmans, S. *et al.* *tp53* mutant zebrafish develop malignant peripheral nerve sheath tumors. *Proc. Natl Acad. Sci. USA* **102**, 407–412 (2005).
46. Hurlstone, A. F. *et al.* The Wnt/beta-catenin pathway regulates cardiac valve formation. *Nature* **425**, 633–637 (2003).
47. Hammerschmidt, M., Blader, P. & Strahle, U. Strategies to perturb zebrafish development. *Methods Cell Biol.* **59**, 87–115 (1999).
48. Nasevicius, A. & Ekker, S. C. Effective targeted gene ‘knockdown’ in zebrafish. *Nature Genet.* **26**, 216–220 (2000).  
**This paper demonstrated the utility of morpholino antisense oligonucleotides for transient knockdown of gene expression in zebrafish.**
49. Ando, H., Furuta, T., Tsien, R. Y. & Okamoto, H. Photo-mediated gene activation using caged RNA/DNA in zebrafish embryos. *Nature Genet.* **28**, 317–325 (2001).
50. Winkler, C. *et al.* Reduced U snRNP assembly causes motor axon degeneration in an animal model for spinal muscular atrophy. *Genes Dev.* **19**, 2320–2330 (2005).
51. Brownlie, A. *et al.* Positional cloning of the zebrafish *sauternes* gene: a model for congenital sideroblastic anaemia. *Nature Genet.* **20**, 244–250 (1998).
52. Fan, L. & Collodi, P. Zebrafish embryonic stem cells. *Methods Enzymol.* **418**, 64–677 (2006).
53. Langenau, D. M. *et al.* Cre/lox-regulated transgenic zebrafish model with conditional myc-induced T cell acute lymphoblastic leukemia. *Proc. Natl Acad. Sci. USA* **102**, 6068–6073 (2005).
54. Kawakami, K. Transgenesis and gene trap methods in zebrafish by using the *Tol2* transposable element. *Methods Cell Biol.* **77**, 201–222 (2004).
55. Balciunas, D. *et al.* Harnessing a high cargo-capacity transposon for genetic applications in vertebrates. *PLoS Genet.* **2**, e169 (2006).
56. Beckwith, L. G., Moore, J. L., Tsao-Wu, G. S., Harshbarger, J. C. & Cheng, K. C. Ethylnitrosourea induces neoplasia in zebrafish (*Danio rerio*). *Lab. Invest.* **80**, 379–385 (2000).
57. Spitsbergen, J. M. *et al.* Neoplasia in zebrafish (*Danio rerio*) treated with N-methyl-N'-nitro-N-nitrosoguanidine by three exposure routes at different developmental stages. *Toxicol. Pathol.* **28**, 716–725 (2000).
58. Mizgireu, I. V. & Revskoy, S. Y. Transplantable tumor lines generated in clonal zebrafish. *Cancer Res.* **66**, 3120–3125 (2006).
59. Moore, J. L., Rush, L. M., Breneman, C., Mohideen, M. A. & Cheng, K. C. Zebrafish genomic instability mutants and cancer susceptibility. *Genetics* **174**, 585–600 (2006).
60. Shepard, J. L. *et al.* A mutation in separase causes genome instability and increased susceptibility to epithelial cancer. *Genes Dev.* **21**, 55–59 (2007).
61. Shepard, J. L. *et al.* A zebrafish *bmyb* mutation causes genome instability and increased cancer susceptibility. *Proc. Natl Acad. Sci. USA* **102**, 13194–13199 (2005).
62. Spitsbergen, J. M. & Kent, M. L. The state of the art of the zebrafish model for toxicology and toxicologic pathology research — advantages and current limitations. *Toxicol. Pathol.* **31**, S62–S87 (2003).
63. Langenau, D. M. *et al.* Myc-induced T cell leukemia in transgenic zebrafish. *Science* **299**, 887–890 (2003).
64. Langenau, D. M. *et al.* Suppression of apoptosis by *bcl-2* overexpression in lymphoid cells of transgenic zebrafish. *Blood* **105**, 3278–3285 (2005).
65. Sabaawy, H. E. *et al.* TEL–AML1 transgenic zebrafish model of precursor B cell acute lymphoblastic leukemia. *Proc. Natl Acad. Sci. USA* **103**, 15166–15171 (2006).
66. Chen, J. *et al.* NOTCH1-induced T-cell leukemia in transgenic zebrafish. *Leukemia* **21**, 462–471 (2007).
67. Patton, E. E. *et al.* BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. *Curr. Biol.* **15**, 249–254 (2005).
68. Topczewska, J. M. *et al.* Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. *Nature Med.* **12**, 925–932 (2006).
69. Haldi, M., Ton, C., Seng, W. L. & McGrath, P. Human melanoma cells transplanted into zebrafish proliferate, migrate, produce melanin, form masses and stimulate angiogenesis in zebrafish. *Angiogenesis* **9**, 139–151 (2006).
70. Lamason, R. L. *et al.* SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. *Science* **310**, 1782–1786 (2005).
71. Tsai, T., Vu, C. & Henson, D. E. Cutaneous, ocular and visceral melanoma in African Americans and Caucasians. *Melanoma Res.* **15**, 213–217 (2005).
72. Lawson, N. D. & Weinstein, B. M. *In vivo* imaging of embryonic vascular development using transgenic zebrafish. *Dev. Biol.* **248**, 307–318 (2002).
73. Huang, C. C., Lawson, N. D., Weinstein, B. M. & Johnson, S. L. *reg6* is required for branching morphogenesis during blood vessel regeneration in zebrafish caudal fins. *Dev. Biol.* **264**, 263–274 (2003).
74. Kuchler, A. M. *et al.* Development of the zebrafish lymphatic system requires VEGFC signaling. *Curr. Biol.* **16**, 1244–1248 (2006).
75. Yaniv, K. *et al.* Live imaging of lymphatic development in the zebrafish. *Nature Med.* **12**, 711–716 (2006).
76. Stern, H. M. & Zon, L. I. Cancer genetics and drug discovery in the zebrafish. *Nature Rev. Cancer* **3**, 533–539 (2003).
77. Stern, H. M. *et al.* Small molecules that delay S phase suppress a zebrafish *bmyb* mutant. *Nature Chem. Biol.* **1**, 366–370 (2005).
78. Nelms, K. A. & Goodnow, C. C. Genome-wide ENU mutagenesis to reveal immune regulators. *Immunity* **15**, 409–418 (2001).
79. Kasahara, M., Suzuki, T. & Pasquier, L. D. On the origins of the adaptive immune system: novel insights from invertebrates and cold-blooded vertebrates. *Trends Immunol.* **25**, 105–111 (2004).
80. van der Sar, A. M., Appelmelk, B. J., Vandenbroucke-Grauls, C. M. & Bitter, W. A star with stripes: zebrafish as an infection model. *Trends Microbiol.* **12**, 451–457 (2004).
81. Meijer, A. H. *et al.* Transcriptome profiling of adult zebrafish at the late stage of chronic tuberculosis due to *Mycobacterium marinum* infection. *Mol. Immunol.* **42**, 1185–1203 (2005).
82. Herbolme, P., Thisse, B. & Thisse, C. Ontogeny and behaviour of early macrophages in the zebrafish embryo. *Development* **126**, 3735–3745 (1999).
83. Lieschke, G. J., Oates, A. C., Crowhurst, M. O., Ward, A. C. & Layton, J. E. Morphologic and functional characterization of granulocytes and macrophages in embryonic and adult zebrafish. *Blood* **98**, 3087–3096 (2001).
84. Bennett, C. M. *et al.* Myelopoiesis in the zebrafish, *Danio rerio*. *Blood* **98**, 643–651 (2001).
85. Altmann, S. M., Mellon, M. T., Distel, D. L. & Kim, C. H. Molecular and functional analysis of an interferon gene from the zebrafish, *Danio rerio*. *J. Virol.* **77**, 1992–2002 (2003).
86. Oates, A. C. *et al.* Zebrafish *stat3* is expressed in restricted tissues during embryogenesis and *stat1* rescues cytokine signaling in a *STAT1*-deficient human cell line. *Dev. Dyn.* **215**, 352–370 (1999).
87. Schorpp, M. *et al.* Conserved functions of Ikaros in vertebrate lymphocyte development: genetic evidence for distinct larval and adult phases of T cell development and two lineages of B cells in zebrafish. *J. Immunol.* **177**, 2463–2476 (2006).
88. Traver, D. *et al.* Transplantation and *in vivo* imaging of multilineage engraftment in zebrafish bloodless mutants. *Nature Immunol.* **4**, 1238–1246 (2003).
89. Ward, A. C. *et al.* The zebrafish *spi1* promoter drives myeloid-specific expression in stable transgenic fish. *Blood* **102**, 3238–3240 (2003).
90. Redd, M. J., Kelly, G., Dunn, G., Way, M. & Martin, P. Imaging macrophage chemotaxis *in vivo*: studies of microtubule function in zebrafish wound inflammation. *Cell Motil. Cytoskeleton* **63**, 415–422 (2006).
91. Renshaw, S. A. E. *et al.* A transgenic zebrafish model of neutrophilic inflammation. *Blood* **108**, 3976–3978 (2006).
92. Mathias, J. R. *et al.* Resolution of inflammation by retrograde chemotaxis of neutrophils in transgenic zebrafish. *J. Leukoc. Biol.* (2006).  
**References 90–92 demonstrate the use of transgenic zebrafish with fluorescent leukocytes to study cellular processes in acute inflammation *in vivo*.**
93. Davis, J. M. *et al.* Real-time visualization of mycobacterium–macrophage interactions leading to initiation of granuloma formation in zebrafish embryos. *Immunity* **17**, 693–702 (2002).
94. van der Sar, A. M. *et al.* Zebrafish embryos as a model host for the real time analysis of *Salmonella typhimurium* infections. *Cell Microbiol.* **5**, 601–611 (2003).
95. Watral, V. & Kent, M. L. Pathogenesis of *Mycobacterium* spp. in zebrafish (*Danio rerio*) from research facilities. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **145**, 55–60 (2006).
96. Swaim, L. E. *et al.* *Mycobacterium marinum* infection of adult zebrafish causes caseating granulomatous tuberculosis and is moderated by adaptive immunity. *Infect. Immun.* **74**, 6108–6117 (2006).
97. Peterson, R. T., Link, B. A., Dowling, J. E. & Schreiber, S. L. Small molecule developmental screens reveal the logic and timing of vertebrate development. *Proc. Natl Acad. Sci. USA* **97**, 12965–12969 (2000).  
**The first demonstration of the feasibility of using zebrafish embryos for chemical library bioactivity screening.**
98. Burns, C. G. *et al.* High-throughput assay for small molecules that modulate zebrafish embryonic heart rate. *Nature Chem. Biol.* **1**, 263–264 (2005).  
**The first description of a whole-animal based high-throughput screening tool for a dynamic physiological function using fluorescent zebrafish.**
99. Margolis, J. & Plowman, G. D. Overcoming the gridlock in discovery research. *Nature Biotechnol.* **22**, 522–524 (2004).
100. Peterson, R. T. *et al.* Chemical suppression of a genetic mutation in a zebrafish model of aortic coarctation. *Nature Biotechnol.* **22**, 595–599 (2004).
101. Hong, C. C., Peterson, Q. P., Hong, J. Y. & Peterson, R. T. Artery/vein specification is governed by opposing phosphatidylinositol-3 kinase and MAP kinase/ERK signaling. *Curr. Biol.* **16**, 1366–1372 (2006).

102. 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).
103. Zhong, T. P., Rosenber, 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).
104. Zhong, T. P., Childs, S., Leu, J. P. & Fishman, M. C. Gridlock signalling pathway fashions the first embryonic artery. *Nature* **414**, 216–220 (2001).
105. Murphey, R. D., Stern, H. M., Straub, C. T. & Zon, L. I. A chemical genetic screen for cell cycle inhibitors in zebrafish embryos. *Chem. Biol. Drug Des.* **68**, 213–219 (2006).
106. Imamura, S. & Kishi, S. Molecular cloning and functional characterization of zebrafish ATM. *Int. J. Biochem. Cell Biol.* **37**, 1105–1116 (2005).
107. Amsterdam, A. *et al.* Many ribosomal protein genes are cancer genes in zebrafish. *PLoS Biol.* **2**, e139 (2004).
- A new hypothesis about a mechanism of carcinogenesis arising out of the broad unbiased genomic overview of a large-scale insertional mutagenesis screen.**
108. Bayliss, P. E. *et al.* Chemical modulation of receptor signaling inhibits regenerative angiogenesis in adult zebrafish. *Nature Chem. Biol.* **2**, 265–273 (2006).
109. Rawls, J. F. & Johnson, S. L. Zebrafish kit mutation reveals primary and secondary regulation of melanocyte development during fin stripe regeneration. *Development* **127**, 3715–3724 (2000).
110. Poss, K. D., Wilson, L. G. & Keating, M. T. Heart regeneration in zebrafish. *Science* **298**, 2188–2190 (2002).
111. Lepilina, A. *et al.* A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration. *Cell* **127**, 607–619 (2006).
112. Traver, D. *et al.* Effects of lethal irradiation in zebrafish and rescue by hematopoietic cell transplantation. *Blood* **104**, 1298–1305 (2004).
113. Langenau, D. M. *et al.* *In vivo* tracking of T cell development, ablation, and engraftment in transgenic zebrafish. *Proc. Natl Acad. Sci. USA* **101**, 7369–7374 (2004).
114. Donovan, A. *et al.* Positional cloning of zebrafish *ferroportin 1* identifies a conserved vertebrate iron exporter. *Nature* **403**, 776–781 (2000).
115. Fleming, R. E. & Sly, W. S. Ferroportin mutation in autosomal dominant hemochromatosis: loss of function, gain in understanding. *J. Clin. Invest.* **108**, 521–522 (2001).
116. van der Ven, L. T., van den Brandhof, E. J., Vos, J. H., Power, D. M. & Wester, P. W. Effects of the antithyroid agent propylthiouracil in a partial life cycle assay with zebrafish. *Environ. Sci. Technol.* **40**, 74–81 (2006).
117. Elsalini, O. A. & Rohr, K. B. Phenylthiourea disrupts thyroid function in developing zebrafish. *Dev. Genes Evol.* **212**, 593–598 (2003).
118. Lam, S. H., Sin, Y. M., Gong, Z. & Lam, T. J. Effects of thyroid hormone on the development of immune system in zebrafish. *Gen. Comp. Endocrinol.* **142**, 325–335 (2005).
119. Biga, P. R. & Goetz, F. W. Zebrafish and *giant danio* as models for muscle growth: determinate vs. indeterminate growth as determined by morphometric analysis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **291**, R1327–R1337 (2006).
120. Goldsmith, M. I., Iovine, M. K., O'Reilly-Pol, T. & Johnson, S. L. A developmental transition in growth control during zebrafish caudal fin development. *Dev. Biol.* **296**, 450–457 (2006).
121. Jagadeeswaran, P. & Sheehan, J. P. Analysis of blood coagulation in the zebrafish. *Blood Cells Mol. Dis.* **25**, 239–249 (1999).
122. Spence, R., Jordan, W. C. & Smith, C. Genetic analysis of male reproductive success in relation to density in the zebrafish, *Danio rerio*. *Front. Zool.* **3**, 5 (2006).
123. Amanuma, K., Takeda, H., Amanuma, H. & Aoki, Y. Transgenic zebrafish for detecting mutations caused by compounds in aquatic environments. *Nature Biotechnol.* **18**, 62–65 (2000).
124. Yabu, T. *et al.* Thalidomide-induced antiangiogenic action is mediated by ceramide through depletion of VEGF receptors, and is antagonized by sphingosine-1-phosphate. *Blood* **106**, 125–134 (2005).
125. Liu, F. J., Wang, J. S. & Theodorakis, C. W. Thyrotoxicity of sodium arsenate, sodium perchlorate, and their mixture in zebrafish *Danio rerio*. *Environ. Sci. Technol.* **40**, 3429–3436 (2006).

### Acknowledgements

We thank W. Alexander, J. Horsfield, J. Heath, J. Postlethwait and R. Currie for their helpful comments on aspects of the manuscript. G.J.L. gratefully acknowledges the support of the Department of Clinical Haematology and Medical Oncology and the Bone Marrow Research Laboratories at the Royal Melbourne Hospital, Australia. G.J.L.'s work using zebrafish to model haematological disease is supported by the National Health and Medical Research Council (NHMRC, Australia) and the National Institutes of Health (NIH, USA). P.D.C.'s work on using zebrafish to model muscular dystrophy is supported by the Muscular Dystrophy Association (USA), the Human Frontier Science Program and NHMRC.

### Competing interests statement

The authors declare no competing financial interests.

### DATABASES

The following terms in this article are linked online to:

**Entrez Gene:** <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>  
[apc|bmyb|BRAF|cyclin B1|dystrophin|hey2|p53|rag1|TTN|ttn](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene)  
**OMIM:** <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>  
 Duchenne muscular dystrophy

### FURTHER INFORMATION

**Graham Lieschke's homepage:** <http://www.wehi.edu.au/facweb/indexresearch.php?id=57>  
**Peter Currie's homepage:** <http://www.victorchang.edu.au/research/ProfPeterCurrie.cfm?cid=73>  
**Sanger Centre:** <http://www.sanger.ac.uk>  
**The Danio rerio Sequencing Project:** [http://www.sanger.ac.uk/Projects/D\\_rerio/](http://www.sanger.ac.uk/Projects/D_rerio/)  
**The Zebrafish Model Organism Database (ZFIN):** <http://zfin.org>  
**Trans-NIH Zebrafish Initiative:** <http://www.nih.gov/science/models/zebrafish/>  
**Zebrafish International Resource Center:** <http://zebrafish.org/zirc/home/guide.php>  
**ZF-MODELS: Zebrafish Models for Human Development and Disease:** <http://www.zf-models.org>  
**Znomics:** <http://www.znomics.com/index.shtml>  
 Access to this links box is available online.