ZEBRAFISH
Volume 5, Number 1, 2008

DOI: 10.1089/zeb.2008.9996

The Experts Speak

Views on Four Key Questions About Zebrafish Research

Keith C. Cheng and members of the zebrafish community*

Our 2004 "Experts Speak" commentary included a series of well-considered perspectives on the potential contributions of the zebrafish to biology and human health. It is fun to look back on those comments. Since that time, the use of morpholino knockdowns as a reverse genetic tool has become commonplace. Insertional mutagenesis with evolving retroviral and now, transposon-based tools, has made it much easier to clone genes found in mutant screens, though the relative strength of ENU mutagenesis to detect phenotypically interesting mutations in essential genes remains. To challenge the ease of morpholino knock-downs in zebrafish, somatic cell knockdowns of mammalian cell culture cells with RNAi has now become commonplace. Broadly-applicable targeted engineering of animal genomes, including zebrafish, looms tantalizingly on the horizon.

Before Steve Ekker's arrival as our new editor, to take another "snap shot" of our moving field, I was asked to help find out what some senior members of the zebrafish community think about where we are, focusing on four questions (and a "grab bag"of questions). Most striking was the large increase in discussion of relevance of the zebrafish to humans, which validates past and future NIH support for zebrafish research. The answers we received are presented in alphabetical order by investigator last name, with editorial changes in brackets. May these comments inspire an ever-clearer vision of how the zebrafish will contribute to our understanding of the living world.

1. *What do you think are the most significant contributions the zebrafish (and other fish) has made to our understanding of basic biology and medicine that we did not already know from other invertebrate (Drosophila, C. elegans, yeast) and vertebrate (Xenopus, chicken, mouse) model systems? Why?*

Well, the first choice is *in vitro* or *in vivo* model. *In vitro* systems are in general more convenient and are also more favorable from a 3R** perspective, but of course lack the complexity of the *in vivo*'s. The latter spans from insects to primates and the choice of an optimal model involves a

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**Reference to the 3R Research Foundation Switzerland, dedicated to reduction, refinement, and replacement of animal experimentation.

number of circumstances like biological relevance, cost, availability and ethics. Zebrafish obviously has established it self as an excellent compromise, being a vertebrate but still cheap, small, sequenced and "lab friendly". The well-organized zebrafish society with ZFIN and ZIRC on top gives zebrafish the final advantages in its class. This is manifested by the increase in numbers of scientific papers using the zebrafish model, in which the ratio to mouse papers has decreased nearly 10 times over the last 15 years (although there still is a 10^2 gap). Research using the zebrafish as a model has evolved from predominantly developmental biology into biomedicine, functional genomics, applications for eco-tox and drug screening etc., and has even taken the step from academia into industry. Taken all together it suggests the z-model will do good in the years to come.

Peter Aleström
Norwegian School of Veterinary Science
Oslo, Norway

Fish models will be important in studying how morphological differences between populations and how species arose in evolution. Recent research on the changes in the amount of body armor in marine and freshwater sticklebacks has shown that a carefully chosen fish model can be successfully used to identify the genes and mutations that control variation in skeletal armor, fin development and possibly many other traits, including physiological ones.

Regeneration is another field with great potential for zebrafish, which are known to regenerate larval and adult fins, as well as muscle of the body and the heart. Thanks to a number of screens there are numerous mutants that fail during the processes of wound healing and regeneration. Depending on how screens were designed, mutant phenotypes can be temperature-dependent, which allows studying gene function at various stages of the regenerative process by shifting between permissive and restrictive temperature profiles, or might occur as adult viable mutations, suggesting that certain components of the regenerative machinery are not simply reactivated from an embryonic developmental machinery, but are specific to adult organisms. Lastly, transgenic lines that allow induced switching-on and -off of signaling pathways, so far shown to efficiently work for *Wnt*- and *Fgf*-pathways, will surely have a big impact on the field.

Gerrit Begemann
University of Konstanz
Germany

The zebrafish system has provided a number of novel insights into the regulation of embryogenesis; particularly in the area of axis formation, including the role of microRNAs. Identification of the ferroportin gene remains one of the important examples of a discovery in zebrafish that provided information on a much sought-after component of a biological pathway (in this case, iron metabolism), that had immediate clinical relevance. Zebrafish genetics is providing entry points in the understanding of complex traits; this is an area in which we expect the zebrafish to continue to contribute strongly. Contributions are beginning to be made in the area of identification of new drugs, based on relatively straightforward screening strategies in zebrafish, now that reagents are available for reliably marking and monitoring cell compartments.

Kathy and Phil Crosier
Department of Molecular Medicine & Pathology
The University of Auckland
New Zealand

I recently watched a video broadcast of a seminar by Nobel-winner Craig Mello. He pointed out the nematode research has won 5 Nobel prizes already; and I suspect #6 (for ageing) is not that far away as well. So how does the zebrafish stack up?

Right now, in my view we as a field are finding good success filling in scientific gaps—identifying important or long-standing genes in topical problems (such as a key iron transporter or a new pigment gene that explains human genetic variation that lead to Caucasians). I found the system developed by Shannon Fisher to functionally identify important regulatory elements not visible using bioinformatics approaches to be an excellent addition to the portfolio of broader zebrafish success.

Other critical work has been building the genetic infrastructure—such as making the *p53* mutant fish by TILLING, or making some key GFP- or RFP-tagged lines that label up key cell types—to directly compare the fish system with that of mammals or other model systems. Successful use of *gal4* and *cre* systems is giving us temporo-spacial control of genes for key experiments. *p53*, *gal4*, *cre*—these are not new developments, but they will be very helpful as we move the field forward.

Stephen Ekker
Mayo Clinic Cancer Center
Rochester, MN

To date, it is likely that the most significant contribution zebrafish has made to basic biology is in understanding early vertebrate development. However, increasingly, zebrafish has become a powerful vertebrate model to study human disease. There has been recent progress in developing zebrafish models to study cancer and inflammation. The power of the zebrafish in the context of these disease models includes the ability to watch cell movement *in vivo*-thereby providing key insight into disease pathogenesis or approaches for therapeutic intervention. It will be very exciting to watch progress in these areas in the coming years.

Anna Huttenlocher
Department of Medical Microbiology and Immunology
University of Wisconsin—Madison
Madison, WI

Identification of the *golden* gene by Keith Cheng.

Koichi Kawakami
National Institute of Genetics
Shizuoka, Japan

There should be no doubt that after many years and many seminal discoveries, the zebrafish has graduated as a model system to study the human disease and genetic disorders and has served on many occasions to elucidate the intricate details of how vertebrate embryos develop. Therefore, in my opinion, pinpointing any one significant contribution is difficult, if not next to impossible, and stating one would likely depend on one's specialty and area of expertise. If polled, however, I am certain most would say it is the genetic studies, i.e., small-to-large-scale forward screens, morpholinos, TILLING, transgenics and genomics that has catapulted the zebrafish system into the mainstream. Now through the power of the genetics (forward and reverse), which people have so eloquently exploited over the last few years by refining techniques and procedures or making reagents available, a lab of any size now has the biological ammunition to make considerable inroads into a plethora of diverse scientific areas. Given the current state of affairs,

I feel the greatest contribution the zebrafish has made to our understanding of biology and medicine relates to the utility of the system and the study of human disease. The zebrafish may not be the panacea, but because of its attributes that we write about in the opening paragraph of our grant applications, the speed by which biological questions pertaining to human health are being answered (the result of high throughput analysis) may be the contributing factor that has in many instances, nudged the zebrafish ahead of other models.

Gregory Kelly
University of Western Ontario
London, Ontario, Canada

The zebrafish provided means to do large-scale screens for mutants, transgenics, regulatory elements in a vertebrate model animal.

An identification of diversity of Nodal genes in zebrafish (cyc, sqt and spaw) illustrated a variety of functions of Nodal beyond its involvement in axis formation and revealed its late roles in eye development upstream of Shh, formation of left-right asymmetry, etc.

Vladimir Korzh
Institute of Molecular and Cell Biology
Proteos, Singapore

Zebrafish have initiated a decentralized, biology-driven, forward genetic dissection of vertebrate development and biology at a scale previously inconceivable in other model vertebrates. Amongst the notable contributions of zebrafish are: (1) basic developmental pathways e.g. *one-eyed-pinhead/cripto*; microRNA-mediated mechanisms for large-scale modification of the transcriptome during development; (2) providing semi-systematic evidence that steps in complex vertebrate organogenesis are also amenable to simple genetic dissection; (3) disease gene discovery e.g. providing evidence for a more generalized role of ribosomal genes in cancer beyond a few rare diseases; the link between *ferroportin/slc40a1* and iron storage diseases.

Graham Lieschke
Cancer and Haematology Division
The Walter and Eliza Hall Institute of Medical Research
Parkville, Victoria, Australia

I reckon that the most noticeable contributions that the fish model organisms (zebrafish and medaka) have made to basic biology and medicine are at two levels: at a methodological level they have shown that forward genetic screens are feasible even within the borders of a single laboratory, are efficient in finding genes responsible for developmental and disease phenotypes, can be applied easily to study biologically important events ranging from subcellular (see the Zon's lab screen for mutations affecting DNA replication) to organogenesis (most screens) and behaviour. Another methodological achievement is the possibility of combining the forward genetic screens with the superior "in vivo" imaging capabilities of zebrafish transgenic lines. All this is different from invertebrates, where organogenesis cannot be addressed in full, and from vertebrates, where forward genetic screens are either not feasible, or too demanding. At the level of discoveries instead, there are so many that it will be unfair to categorize them, but I believe that the most important aspect is the fact that in the last 5 years (i.e., since zebrafish have grown past the stage of "good developmental tools") the fish models have become increasingly suitable for studying human diseases, useful to address all sorts of biological questions in organ formation

and pathology, and successful for performing drug screens. These qualities make the fish models a "must" for all disease studies where we want to extend the relevance of a biological process to a whole organism.

Marina Mione
Firc Institute of Molecular Oncology
Milan, Italy

Clearly, some of the more significant advancements have come from the analyses of embryonic lethal mutations in genes in zebrafish, which have been difficult, if not impossible, to study in mouse models. However, in the last 5–10 years there has been an upsurge of experimental work analyzing host-pathogen interactions during infectious disease using zebrafish as a model host. Many new insights have emerged regarding the interaction of the pathogen with the host immune system and the subversion tactics that have evolved to allow the pathogen to survive in this environment. This work is complimented by an exciting new field of developmental research in zebrafish in the analyses of the developing immune system. Results from this area of research revealed that the zebrafish immune system is amazingly similar to that of humans. One of the extraordinary advantages of working with the zebrafish that has been highlighted from this early work is that while the innate immune system is developed within 24 hours post-fertilization, the adaptive immune system is not fully functional until after 4 weeks. This allows analyses of innate immune system functions during infection in isolation from the adaptive immune system. However, response to infection can then be analyzed in the same model host after development of the adaptive immune system, allowing dissection of the individual functions as well as analysis of the interplay between the two systems.

Melody N. Neely
Wayne State University School of Medicine
Detroit, MI

The biggest impact of zebrafish research has been undoubtedly on developmental genetics. By being able to do detailed embryology and genetics in the same vertebrate organism is still the biggest selling point for zebrafish. It is difficult to single out specific areas where the zebrafish has uniquely contributed. Embryonic signaling pathways, especially the Nodal pathway and the role of gradients in vertebrate development are among the most visible contributions of zebrafish. Hematopoiesis, cardiac development and the evolutionary role of genome duplications also come to mind. We are at the stage where the field is still preoccupied with gene identification, which will be the prerequisite for mechanistic studies in the near future that will have an immense impact on most fields of biology.

Stephan Neuhauss
University of Zurich, Institute of Zoology
Switzerland

There are two main areas where fish can make unprecedented contributions. They are related to imaging and to drug screening.

The demonstration of *in vivo* germ cell migration in zebrafish by Erez Raz' group is an unparalleled accomplishment. Similarly, recent work from Yosuke Takahama's laboratory in medaka and from Philippe Hebomel's lab in zebrafish show for the first time in vivo hematopoietic precursor migration between different developmental hematopoietic organs and from there to the

thymus, where T cells develop. In addition, small molecule drug screens, such as those carried out in Randy Peterson's and Len Zon's labs, start to identify new, potentially clinically useful medications, and are difficult to carry out in other vertebrates. Finally, while feasible in mouse, discovery of new disease genes has been achieved efficiently using phenotype-driven approaches in zebrafish; particularly those pertaining to normal and malignant blood formation cannot be duplicated in invertebrates.

Nikolaus S. Trede
Department of Pediatrics, University of Utah
Salt Lake City, UT

2. *What resources are the most important to develop and improve to make zebrafish or other fish models even more useful?*

The zebrafish has significant advantages as a fish model that would be greatly enhanced by improved access to the genome. This is manifested by the development of microarray technologies, with tiling arrays and the coming ultra-high throughput sequencing. A well-known bottleneck in exploring the potential from the increase in genome wide information is, in addition to storage of data, access to user-friendly bioinformatics. I think perhaps the latter will be the more important resource.

Peter Aleström
Norwegian School of Veterinary Science
Oslo, Norway

Many interesting evolutionary and developmental phenomena in fish, like brood pouch development in seahorses, colour patterns in guppies, or caudal fin patterning in male swordtails, to name but a few, are difficult to perturb genetically. Swordtails, platyfish and guppies are livebearers with internal fertilization, which poses considerable, but probably not unsolvable challenges to in vitro fertilization and the establishment of transgenic animals. Clearly, efficient methods need to be developed or adapted from other models to transform gonads or germ cells in order to make transgenic livebearers a reality. In parallel, fish like guppies and swordtails can be used for quantitative trait analyses (QTLs), as hybrids of species with divergent traits (guppies from different predator environments; swordtails and the swordless platy species) do form fertile and viable hybrids. The association of traits and genes requires extensive genomic resources (genomic and cDNA libraries, genetic linkage maps with closely spaced markers), whose development, and improvement of available resources, needs to be intensified if these fish models are to make a splash in the Evodevo field.

Gerrit Begemann
University of Konstanz
Germany

There are a number of things that the zebrafish community should strongly consider to move the zebrafish forward as a genetic model system for the analysis of complex phenotypes. We need sequenced genomes from several common wild-type laboratory strains. We know there are interesting phenotypic differences between strains and comparative genomics can reveal the putative causal genes. We need a SNP chip for rapid analysis of genetic linkage and several inbred

homozygous strains that can be sustained by natural sibling crosses to increase the efficiency of our genetic analyses.

Michael J. Carvan, III
Great Lakes WATER Institute
University of Wisconsin—Milwaukee
Milwaukee, WI

Right now, many expression patterns are only available in 2D, and at lower resolution than is possible. I would love to see a catalogue of 3D expression patterns in the zebrafish as a large evolutionary step in Systems Biology. To add the detail of protein expression, we would have to generate a resource that would greatly accelerate zebrafish research—antibodies for all zebrafish proteins. This would help us correlate RNA expression with protein-expression, and allow us to add critical details such as subcellular localization. The man-on-the-moon project would be to know the expression patterns of all proteins through the lifespan of this vertebrae animal—a first.

High resolution, high-throughput imaging and imaging analysis has the potential to revolutionize Systems Biology and toxicology. The latter has huge potential impact on the future of environmental testing and acceleration of drug development.

Interdisciplinary research involving complex trait analysis in humans is much more powerful when combined with zebrafish functional assays (expression and knock-down analysis)—combining analysis of complex traits and "omics" approaches with genetic models is an approach that has been called "Systems Genetics." This approach has great potential using the zebrafish.

For cloning mutant genes emerging from in ENU screens, commercially-available and cost-effective SNP-based mapping and region-specific genome sequencing will greatly help.

Keith C. Cheng
Jake Gittlen Cancer Research Foundation
Penn State College of Medicine
Hershey, PA

Conditional and inducible expression systems; increased numbers of knockouts from either TILLING or retroviral gene insertion; increased numbers of full length cDNAs; comprehensive sets of antibody reagents especially to cell surface receptors, transcription factors and signalling molecules.

Kathy and Phil Crosier
Department of Molecular Medicine & Pathology
The University of Auckland
New Zealand

Our DNA genome is on track to be very high quality, indeed. But our transcriptome is still incomplete. I think we should focus on shooting for at least 80% full-length coverage, and we should encourage the development of a rich library that includes isoform variants.

Stephen Ekker
Mayo Clinic Cancer Center
Rochester, MN

The most important resources to develop in zebrafish are targeted knock out/knock in technologies. In particular, tissue specific knock-outs will be critical. These methods will be essential for the further development of zebrafish models of human disease and to provide progress in understanding vertebrate development. Other reagents that are needed are antibodies.

Anna Huttenlocher
Department of Medical Microbiology and Immunology
University of Wisconsin—Madison
Madison, WI

A stock of fish lines in which all of the genes are mutated (probably by insertional mutagenesis).

Koichi Kawakami
National Institute of Genetics
Shizuoka, Japan

In my research, antibodies, either for immunoblot analysis, immunoprecipitation or whole mount immunocytochemistry, have been a nemesis. Many antibodies directed against mouse, chicken or human proteins either do not work or give spurious or inconsistent results. More and more companies now include pertinent information as to whether or not their antibodies cross-react with the zebrafish protein in question or its usefulness for a specific technique (kudos to them), but of course this is often reserved for the more "popular" antibodies. The availability and production of antibodies specific to zebrafish proteins, not to mention the numerous isoforms if they exist, are well behind what I would have expected at this stage. A battery of zebrafish-specific antibodies would be ideal, but it might take a Herculean effort to convince companies/government agencies to invest in such a large research and development undertaking.

Gregory Kelly
University of Western Ontario
London, Ontario, Canada

An availability of a complete genome sequence is very important for many efforts to become more efficient.

Vladimir Korzh
Institute of Molecular and Cell Biology
Proteos, Singapore

Panels of randomly-generated mutants sharing phenotypes of interest are the biological *tour-de-force* of zebrafish and expediting the process of turning these mutants into identified mutations in genes would be invaluable. Hence: (1) completion of the Zebrafish Genome Sequencing Project to provide a reliable reference sequence; (2) annotation of genome sequence; (3) high density SNP mapping resources. In the area of leukocyte biology and host defense, a more complete understanding of fish leukocyte subsets and panel of antibody reagents for their identification would be a big help. The development of at least rudimentary hematopoietic cell culture techniques would greatly facilitate understanding the biology of mutants. Vigorous, inbred, syngeneic fish would

permit transplantation assays in adults. (There are big votes here at the lab-bench for a fully-automated dechorionation machine.)

Graham Lieschke
Cancer and Haematology Division
The Walter and Eliza Hall Institute of Medical Research
Parkville, Victoria, Australia

No doubt that we should put extra effort into developing techniques (ES cells, homologous recombination) that will make gene replacement easy in zebrafish. This is the most urgent need for making the zebrafish the most accessible and complete model for genetic studies.

Resources such as antisera, expression libraries, and other proteomic tools may change dramatically our way to study protein function: ideally we would like to express modified proteins in a whole organism and study their post-translational modifications and interactions in the context of different tissues, going from structure to function in a single experiment. This may become possible using the zebrafish in the future if we develop the tools to improve the use of the zebrafish in biochemical and structural studies.

Marina Mione
Institute of Molecular Oncology
Milan, Italy

Many of the reagents that are commonly available for mouse or human analyses are not yet available for zebrafish research. Although many new antibodies for developmental research are becoming available for zebrafish, there is a scarcity of reagents for signal transduction or immune system functions. The availability of more transgenic strains of zebrafish would also greatly improve the types of experiments that could be performed. Although the use of morpholinos in embryos has proven quite effective, this does not work well in adult fish, therefore a more stable form of genetic mutation or replacement would be desirable.

Melody N. Neely
Wayne State University School of Medicine
Detroit, MI

The zebrafish community has been very successful in building openly available resources. A pressing need is the complete assembly and annotation of the zebrafish genome. Another important resource is an expanded stock center(s) making mutant lines and characterized transgenic lines available for the community. In order to use the full potential of the zebrafish as an integrative vertebrate model there is a need to develop and standardize physiological and behavioral assays. This is an important requirement to study biology and diseases beyond early development.

Stephan Neuhauss
University of Zurich, Institute of Zoology
Switzerland

The development of three main areas would greatly improve and accelerate zebrafish research. For the zebrafish community at large establishment of embryonic stem cell lines for knock-out and knock-in experiments would be on top of the list. Another generally important advance would be establishment of reliable cell culture systems. We currently lack many of the growth

factors that are necessary to sustain cells in long-term culture. For hematologists and immunologists in the zebrafish field generation of a panel of monoclonal antibodies that can be used to identify phenotypic and functional subgroups of hematopoietic cells would be a huge asset.

Nikolaus S. Trede
Department of Pediatrics, University of Utah
Salt Lake City, UT

3. *What exciting progress is reasonable to hope for in your area of zebrafish research?*

One major break-through will be to finally join the mouse model by getting access to *in vitro* cultures of ESCs and targeted mutagenesis—the focused research area of ***Zebrafish*** Editor Paul Collodi's lab for many years.

Peter Aleström
Norwegian School of Veterinary Science
Oslo, Norway

Further models of cancer and inflammation in zebrafish. Greater insights into processes of organogenesis.

Kathy and Phil Crosier
Department of Molecular Medicine & Pathology
The University of Auckland
New Zealand

Insertional mutagenesis using transposons seems very promising. At the moment, the current methods look to be nicely complementing ENU and retroviral success in the zebrafish while offering the opportunity to conduct new screens not possible with these earlier tools. I look forward to watching the advances unfold new areas for our favorite model—such as the genetics of behavior—research areas now possible due to these technical advances.

Stephen Ekker
Mayo Clinic Cancer Center
Rochester, MN

Researchers are just beginning to model inflammatory disease in zebrafish—there will likely be exciting progress in this area over the next 5-10 years. The ability to image leukocyte trafficking has already revealed novel mechanisms involved in the resolution of inflammation. The challenge will be to identify the molecular mechanisms that contribute to leukocyte recruitment and resolution of inflammation using tools that are readily available in zebrafish including chemical and genetic screens and targeted morpholinos.

Anna Huttenlocher
Department of Medical Microbiology and Immunology
University of Wisconsin—Madison
Madison, WI

Development of a methodology for a small lab to perform saturation insertional mutagenesis.

Koichi Kawakami
National Institute of Genetics
Shizuoka, Japan

Over the next few years and with the help of strong industrial partners, I expect exciting progress to be made in the area of applied research. Like many, I am not only interested in signaling pathways and the crosstalk between pathways that pattern the zebrafish early embryo, but also in what happens when these pathways are disrupted (pharmacologically or via environmental toxins) and if the resulting phenotype(s) are reminiscent of those associated with a human disease. An attractive strategy would be to use this approach with morpholino screens to identify key proteins associated with or in opposition to the pathological condition or disorder. Towards that end, a successful partnership would create a situation that would yield data to satisfy my interest in revealing how these pathways specify development, but would also fulfill the interest of an industrial partner whose goal is to produce a marketable therapeutic product relevant to human health and disease. Nevertheless, this strategy costs money and a dedicated team, but when the team sees the zebrafish as the "embryo" and the other, backing the project, sees it as the "test tube" the benefits and spinoffs to both are not only expected to be exciting, but also immeasurable.

Gregory Kelly
University of Western Ontario
London, Ontario, Canada

An improvement of bioimaging at a single cell level will provide means for in vivo observation of morphogenetic processes in most cell lineages, tissues and organs.

Vladimir Korzh
Institute of Molecular and Cell Biology
Proteos, Singapore

Live cell imaging of leukocyte behaviours in embryos and adults promises to provide a much more sophisticated morphological basis for understanding the in vivo role of these cells in acute inflammation and host defense. Combined with the development of reproducible and characterized inflammation and infection models, this sets the stage for discovery of new anti-inflammatory and anti-infective therapeutic approaches, heading ultimately for new first-in-class pharmaceuticals. The biology of regeneration is a particularly novel area amenable to study in zebrafish; understanding the role of leukocytes in shaping tissues and organs during regeneration is a largely unexplored field.

Graham Lieschke
Cancer and Haematology Division
The Walter and Eliza Hall Institute of Medical Research
Parkville, Victoria, Australia

My area of research is cancer and human genetic diseases, and we use the zebrafish as a model. While a few years ago the use of the zebrafish as a model for human diseases was restricted to a few labs and driven by funding opportunities, nowadays many more researchers are approaching this area with the genuine belief that we can really make the difference not only by providing supporting evidence, but especially through new insights and novel approaches. The short-term

progress in this area that I wish for is the full integration of the zebrafish model in the current protocols of medical research, as a necessary complement to in vitro studies. [Long term], I would like to see the establishment of an international resource center where zebrafish cancer (and other disease) models can be employed routinely for anticancer drug screens. Indeed, chemical screens are among the most important developments in which the zebrafish has unique advantages. Extending the use of the zebrafish in these screens beyond the currently limited number of companies and labs will allow the zebrafish to reach its full potential for the benefit of population health.

Marina Mione
Institute of Molecular Oncology
Milan, Italy

Using the knowledge we have already gained from the infectious disease studies along with the highly successful techniques of forward genetics and high-throughput screens, I think we will be able to easily identify zebrafish strains that are more resistant to infectious disease. This will lead us in new directions for the development of therapeutic strategies to bolster specific targets of the immune system during infection, instead of trying to just eliminate the pathogen with a new antibiotic, which has proven to be only a short-term solution with the continuing emergence of antibiotic resistant strains. This experimental strategy may also identify new signaling pathways used by the immune system that are not currently known. Since the immune response and the clinical presentation of infectious disease is very similar in zebrafish and humans, both of these outcomes would be directly relevant to human disease.

Melody N. Neely
Wayne State University School of Medicine
Detroit, MI

The transparency of the zebrafish larvae lends itself ideally for optical recordings. In the near future, techniques will be developed that will allow researchers to study the activity of a neuronal ensemble in vivo, possibly even in the behaving animal. The continuing improvement of voltage sensitive dyes that can be genetically encoded and thereby transgenically expressed in characterized neurons will finally make the zebrafish an attractive model for neurophysiologists . . . Additionally, the identification of cell-type specific regulatory elements will make it possible to express gene products of choice in specific neurons, enabling direct genetic manipulation of neural circuits in combination with behavioral read-outs.

Stephan Neuhauss
University of Zurich, Institute of Zoology
Switzerland

A number of zebrafish leukemia models have been established, based on mis-expression of proto-oncogenes. These models have been established partly for the purpose of identifying genetic modifiers through subsequent forward genetic screens. The recent identification of leukemia-promoting mutations in a phenotype-driven screen from our laboratory brings modifier screens a step closer to reality. A case in point is the demonstration of the modifying effect of Bcl-2 overexpression in Notch-1 mediated T cell leukemia from Tom Look's lab.

Nikolaus S. Trede
Department of Pediatrics, University of Utah
Salt Lake City, UT

4. *What big breakthroughs in genetic or imaging technology might be expected to help revolutionize the power of zebrafish as a model in the next several years?*

I would welcome alternative methods for targeted gene inactivation. Although TILLING is very powerful and should become more affordable in the future, alternatives, such as efforts to express RNAi in transgenic animals under the control of inducible switches, may be a step in the right direction, as long as this kind of RNA-interference provides more target specificity than injection of dsRNA. Advances in stem cell biology should encourage further efforts to develop mouse-style reverse genetics to be utilized in zebrafish or medaka.

Gerrit Begemann
University of Konstanz
Germany

Targeted mutagenesis using double stranded break repair is an exciting possibility in zebrafish, which would potentially allow us to target any mutation or engineered construct anywhere in the genome, opening up a myriad of ways to answer questions about gene function and to set up new interesting genetic screens.

With work, microanatomical characterization of mutant phenotypes can become routine within a few years. Computational analysis of those changes will greatly enhance the value of those analyses.

Keith C. Cheng
Penn State College of Medicine
Hershey, PA

Developing systems that allow genetic tools to be used more effectively in adult zebrafish.

Kathy and Phil Crosier
Department of Molecular Medicine & Pathology
The University of Auckland
New Zealand

I think there is a real chance at ES-like cells and homologous recombination for the zebrafish. Image processing technology to allow one to semi-automate small molecule screening of transgenic zebrafish lines seems like something potentially soon that could have a strong impact in the field.

Stephen Ekker
Mayo Clinic Cancer Center
Rochester, MN

In terms of imaging, in vivo analysis of signaling will be an area of exciting progress in zebrafish over the next 10 years. The use of FRET and other imaging technology *in vivo* will revolutionize the power of zebrafish to study development and disease.

Anna Huttenlocher
Department of Medical Microbiology and Immunology
University of Wisconsin—Madison
Madison, WI

Targeting by homologous recombination.

Koichi Kawakami
National Institute of Genetics
Shizuoka, Japan

An identification of most specific regulatory sequences (promoters and enhancers) and application of new fluorescent proteins for gene tagging or manipulation in combination with wide use of various inducible systems of gene expression will provide convenient tools to manipulate gene activity and observe an outcome of such manipulation in vivo.

Vladimir Korzh
Institute of Molecular and Cell Biology
Proteos, Singapore

Computerized, quantitative imaging of stereotypic zebrafish behaviours present the prospect of genetic screens for adult behaviour-determining genes with an efficiency and scale not possible in other vertebrate species. Improving the techniques for deep light-microscopic imaging of adult zebrafish, or developing other deep imaging technologies with improved resolution, would enhance the model's utility. The ability to trace the position and fate of multiple individual cells and their progeny in one animal, without loss of their identity and ontogeny, would enable new questions to be asked about programming, stocasticism and plasticity in development.

Graham Lieschke
Cancer and Haematology Division
The Walter and Eliza Hall Institute of Medical Research
Parkville, Victoria, Australia

Together with gene replacement, the use of transposons has already started a revolution in zebrafish genetics. I hope that the efforts of several labs, including those of Kawakami, Chien, Lawson, Korzh and many others will bring zebrafish genetic resources up to the same level of Drosophila genetics, with a library of live, transgenic lines carrying transposable/replaceable elements throughout the genome. This will undoubtedly require national and international funding, as the benefits of this research is enormous for the community and for science in general, but is not immediately lucrative. Imaging techniques will continue to progress as they are technology-driven: the aim now is to be able to image (and manipulate) without harming the embryos, very fast subcellular and molecular events in the context of a whole organism. Transgenic zebrafish with GFP-fusion proteins as reporters are ideal for this!

Marina Mione
Institute of Molecular Oncology
Milan, Italy

The development of conventional knockout technology for making targeted gene deletions or replacements in the zebrafish has been problematic thus far, but is clearly going to happen in the near future. The completion of the zebrafish genome sequencing has provided a more focused picture of the zebrafish system. The combination of this new resource and the eventual devel-

opment of a targeted knockout technology will greatly propel the science forward through the ability to target specific genes that are identified in the genome.

Melody N. Neely
Wayne State University School of Medicine
Detroit, MI

The availability of more and more vertebrate genome sequences will offer new avenues for comparative genomics. Here the zebrafish will play a central role, especially in the context of functional and evolutionary genomics.

Stephan Neuhauss
University of Zurich, Institute of Zoology
Switzerland

As far as genetic innovations are concerned, zinc-finger nuclease mediated targeted gene inactivation is on the horizon, and may (in the absence of embryonic stem cell lines) be a much-needed substitute for conventional knock-out (and possibly knock-in) procedures.

For imaging, autofluorescence has been a constant nuisance for zebrafish researchers. We have experimented with multispectral imaging (MSI), a technology developed by CRI Inc. (Woburn, MA). This technology performs spectral analysis of every pixel in an image and allows to highlight only pixels that represent the spectrum of a particular fluorochrome and to subtract all other pixels with different spectra. We have achieved very nice results with this technology in embryos and adult zebrafish.

Nikolaus S. Trede
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Grab bag: ***Do you have any other expert opinions to share here? For example, are there other genetic model systems that you are aware of that lie in the wings with unique strengths we should be thinking about? What up-and-coming fish or other genetic models might we expect to become a significant contributor to broad or specific areas of biological and biomedical sciences?***

Zebrafish are not very interesting for Evodevo-style research, but other teleosts have evolved a variety of amazing morphologies. Specifically in cichlids there is a multitude of wonderful colour patterns important for camouflage and reproduction, like egg-spots on male anal fins in some species. These patterns are relevant for mating success, and as they have been modified to differ in closely related species, it will be fascinating to analyze the molecular nature of changes to the underlying molecular mechanisms in a phylogenetic framework. Methods for producing transgenic cichlids are established and eggs of certain suitable cichlid species are amenable to genetic manipulation, although the rather long generation times of 6-9 months make high demands on the time available for experiments and lab space. Fish also show considerable variation in the shapes and modifications of fins. Several species have developed specializations of anal or pelvic fins towards copulatory organs (gonopodia, pelvic claspers). Male adult swordtails develop a sword, a conspicuously coloured elongation of the caudal fin that evolved as a sexually selected trait. In these and other examples it will be important to understand how fin ray growth is modified by molecular pathways and if the same pathways are modified when elongated or

otherwise modified fins evolve in different species. Since fin rays are mainly made of bone, such studies will also be of biomedical importance, to understand and model bone growth in development and regeneration.

Gerrit Begemann
University of Konstanz
Germany

An introduction of fish species which adults are transparent as a model species will help to move the whole field towards analysis of physiology, cancer research, aging, etc.

Vladimir Korzh
Institute of Molecular and Cell Biology
Proteos, Singapore

[. . .] planaria has revolutionized research in regeneration biology.

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