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Aquatic Models, Genomics and Chemical Risk Management§

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Abstract

The 5th Aquatic Animal Models for Human Disease meeting follows four previous meetings (Hinton et al., 2009; Schmale et al., 2007; Schmale, 2004; Nairn et al., 2001) in which advances in aquatic animal models for human disease research were reported, and community discussion of future direction was pursued. At this meeting, discussion at a workshop entitled *Bioinformatics and Computational Biology with Web-based Resources* (20 September 2010) led to an important conclusion: Aquatic model research using feral and experimental fish, in combination with web-based access to annotated anatomical atlases and toxicological databases, yield data that advance our understanding of human gene function, and can be used to facilitate environmental management and drug development. We propose here that the effects of genes and environment are best appreciated within an anatomical context - the specifically affected cells and organs in the whole animal. We envision the use of automated, whole-animal imaging at cellular resolution and computational morphometry facilitated by high-performance computing and automated entry into toxicological databases, as anchors for genetic and toxicological data, and as connectors between human and model system data. These principles should be applied to both laboratory and feral fish populations, which have been virtually irreplaceable sentinels for environmental contamination that results in human morbidity and mortality. We conclude that automation, database generation, and web-based accessibility, facilitated by genomic/transcriptomic data and high-performance and cloud computing, will potentiate the unique and potentially key roles that aquatic models play in advancing systems biology, drug development, and environmental risk management.

§Conclusions from the Bioinformatics, Computational Biology and Web-based Resources Session at the 5th Aquatic Animal Models for Human Disease meeting in Corvallis, OR, USA.

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The foundation of this work was presented at the 5th Aquatic Animal Models for Human Disease meeting in Corvallis, Oregon.

Aquatic organisms as sentinels and models

Aquatic organisms are vulnerable to chemicals that come from surrounding terrestrial systems and from direct aquatic inputs derived from human activities (Di Giulio and Hinton, 2008). It has become inarguably clear that anthropogenic activities contribute to the development of neoplasia in wildlife species such as beluga whales and bottom-dwelling fish (McAloose and Newton, 2009). Aquatic species are therefore unique and sensitive sentinels for potential effects of chemical exposures relevant to the environments in which we reside. Aquatic model studies contribute insight into the pathways leading to toxicant-induced tumor formation, including evidence of exposure, host metabolic modification, procarcinogen to carcinogen activation, DNA intercalation and cancer gene mutation (McAloose and Newton, 2009). It is not surprising, therefore, that aquatic organisms are perhaps the most employed organisms for biomonitoring. This research has now moved from a more descriptive phase, necessary to explore those concentrations of single toxicants within water that were not compatible with the life of individual fish, to considerations of sublethal concentrations that do not cause death over the short term but that harm the organism in ways that are mechanistically informative. Biomarkers of exposure, response and genetic susceptibility have been derived from research in this area (Di Giulio and Hinton, 2008).

Aquatic organisms have emerged as valuable models for mechanistic research, and their contributions include findings on the role of nuclear and other receptors in the development of toxicity (Hahn and Hestermann, 2008). Certain species possess novel traits making them strong models for specific diseases. These include zebrafish for cancer, aging, anemia, tissue regeneration, cardiovascular disease and stem cell biology (Amatruda et al., 2002; Gerhard, 2007; North and Zon, 2003; Poss, 2007; White and Zon, 2008); and *Xiphophorus* for mechanistic studies of melanoma (Meierjohann and Scharl, 2006). Zebrafish-based assays are already being used to make preclinical safety decisions in pharmaceutical development (Eimon and Rubinstein, 2009). Genomic sequencing has advanced in at least 11 species of fish, and the aquatic organism research community has a priority list of additional fish for which genome and transcriptome sequencing is relevant to human biology and health (Hinton et al., 2009; unpublished community work).

In light of the importance of research with aquatic models for furthering our understanding of the effects of environmental exposure on human development and disease, we agree that new technology will be needed to accelerate the creation of annotated anatomical atlases and toxicological databases. That work, which includes automated and improved synchrotron-based X-ray imaging of whole animals, will maximize the benefits we derive from high-throughput transcriptomic, proteomic and massively parallel sequencing data. The community-wide discussion that ensued during the *Bioinformatics and Computational Biology with Web-based Resources* workshop centered on two major themes: 1) What are the functions of all the genes in a vertebrate organism, and 2) How do we perform high-throughput measurements of cellular architecture in model organisms in order to discern the effects of environmental toxicants and therapeutic strategies on biological systems?

Motivation 1: “What are all the functions of all genes?”

Each gene in an organism can have one or more functions (Jeffery, 2003). A fundamental principle of genetics is that the phenotype of an organism suffering from deficiency in a specific gene, through mutation or knockdown, informs us about gene function. The totality of phenotypes associated with gene deficiencies in each gene in zebrafish was defined as the zebrafish phenome. To address the functions of the more than 20,000 genes in the human genome, the international zebrafish community set the goal of using multiple methods of mutagenesis to produce at least one mutant allele per gene in the next five years, and to phenotype those mutants - they are calling this the “Zebrafish Phenome Project” (<http://www.blsmeetings.net/zebrafish/>). At a March 2010 meeting sponsored by Sanger Center and NHGRI, at NIH, the zebrafish research community discussed potential phenotyping assays. These included physiological and behavioral assays, as well as fluorescence-based morphological assays that require live animals, and will not be covered here. We focus here on an often-neglected topic: the assessment of phenotypes that change the morphological features of cells, tissues, and organs, as assessed using whole, fixed fish. A number of factors were discussed that affect the range of assays needed for this aspect of the zebrafish phenome project. These include the methods of imaging, reproducibility of assays, and throughput of mutant production, sample preparation, computational analysis and visualization.

Motivation 2: “How do we get closer to measuring all effects of therapeutic drugs and of potential toxicants in our environment?”

Advances in the physical and biological sciences have created unprecedented opportunity and risk for society. Among humanity’s achievements is the industrial capability to produce more than 80,000 chemicals for our use. They have contributed to our quality of life and economic advancement of our society. The benefits are, however, associated with known and unknown costs as a result of regulations designed for a past era (reviewed by the Council on Environmental Health, 2011, and by Stone and Delistraty, 2010). Some 3,000 chemicals are produced at a rate of more than a million pounds per year. Many manufactured chemicals are now components of our environment and can be detected in our water, air, and food. Some have negative impact on life in our oceans, and some are now found even in the blood of our newborn children. Environmental factors of anthropogenic origin are implicated in many common conditions such as asthma, cancer, diabetes, hypertension, immune deficiency disorders and Parkinson’s disease; however, the molecular mechanisms underlying these correlations are not well understood. What are the individual effects of each of these chemicals? What are their combinatorial effects? These are challenging questions that available tools are inadequate to answer. How are we going to meet this challenge?

Unanswered societal questions about ecotoxicology

Given the magnitude of our chemical exposures in both number and concentration, we can expect to discover unique and combinatorial effects of chemicals on our biology and biosphere. These new issues have raised important, and presently unanswered questions for society: What is the best way to assess the biological impacts of chemical exposures? How

will we catch up, and then keep up with assessment of chemical exposures? More broadly, how are we going to establish a reasonable balance between industrial productivity, human use benefits, and unanticipated harm as it is discovered? What types of high-dimensional data do we ideally envision that would facilitate ecotoxicological management, and what criteria should be used to set thresholds for action?

Whole-animal phenotyping at multiple developmental stages is necessary because single gene deficiencies and chemical exposures can affect multiple organ systems at different developmental times

Phenotypes caused by single gene deficiencies can cross organ systems in invertebrate and vertebrate model systems, including humans (Zou et al. 2008; Jeffery 2003; Thomas, Canada and Cheng, unpublished data). The fact that we cannot predict which organs may be affected in any particular new mutant means that any survey of gene functions associated with phenome projects needs to cover the entire animal. Some organ systems, especially those having to do with sexual maturity, show their most significant effects at reproductive ages. We therefore need to phenotype animals during embryogenesis, when primordial germ cell migration takes place, and into juvenile stages when sexual differentiation is complete. Phenotyping older animals is relevant because humans with single gene deficiencies often survive into adolescence and adulthood, and because toxic effects can affect sexual maturation. Phenome projects of any model system, including the zebrafish, will therefore need to include analysis at several developmental ages. Due to the availability of light-based phenotyping for early developmental stages and the relative paucity of histologically detectable cell differentiation through day 2, the zebrafish community is interested in pursuing whole-animal morphological studies, based on histology (2D) and microCT (3D). One of the rational sets of ages to screen is two late embryonic stages (such as day 3 and 5), a stage where sex differentiation has begun, and a later stage where sex differentiation is normally complete. The phenotyping pipeline being developed for zebrafish can be adopted to studies of any aquatic organism whose size is suitable, including medaka, and for models with short generation times, it can be applied to the study of potential transgenerational effects of exposures.

Phenotyping at cell resolution is necessary to discern pathophysiological mechanisms

One hundred and fifty years ago, based on the discovery that all organisms are comprised of cells, Virchow proposed that disease is a cellular process (Virchow 1858) that can often be characterized by the microscopic study of stained tissue sections. This principle forms the existing gold standard of diagnosis in diagnostic pathology. Our modernized version of those criteria is supplemented with protein- and gene-specific assays (oncogene mutations, receptors). The zebrafish has been a darling of developmental biologists due to its transparency and rapid extra-corporal development.

How do we image whole zebrafish at cell resolution through its life span?

Imaging of the entire embryonic zebrafish is possible at cell resolution using laser-based microscopy (Megason and Fraser 2003, Huisken et al., 2004). Due to light scatter associated with tissues of more than about 200 microns and blocking of light by pigmented cells, laser-based methods of imaging are limited to the first days of development. Another commonly used imaging mode is MRI, whose resolution is determined in part by the field strength of the magnet. MRI can be used for whole adults, and therefore can serve as a point of reference for other imaging methods, but even with long imaging times and high field strength, it does not presently seem likely that MRI imaging is able to achieve the 1 micron voxel dimension required for different cell types to be recognized (Neuberger, Cheng and Clark, unpublished).

Micron-scale computed tomography (microCT) is a method by which 3D specimens are fixed and stained with heavy metals, and series of hundreds to over a thousand images are taken at each of multiple angles. The 3D volume is then computed from those individual images (Stock, 2009). Contrast agents are needed for imaging soft tissues (Betz et al. 2007, Metscher, 2009). Free-standing machines, which are based on “cone-beam” technology, have not yet achieved the resolutions needed for cell resolution or scanning speeds needed for high-resolution and do not seem to be on a trajectory to achieve imaging times short enough for high throughput (Clark and Cheng, unpublished), but higher energy (>10keV) synchrotron-based microCT is capable of high resolutions, and with work, may be able to achieve the required throughput. Increasing size of the field of view (where one can fit the organism) diminishes resolution; so optimizing the balance between these factors is one of the challenges of this field. Improvements in scintillator and detector sensitivity and size, together with modifications to X-ray and light optics, may make it possible to achieve the resolution, field of view, and speed needed for high-throughput imaging.

Automated, high-throughput, whole-animal imaging, annotation and visualization will be required to assess structure information for all organs, to increase consistency, and to minimize error

Here, we discuss aspects of an ideal way to define anatomic abnormalities for tens of thousands of genes and chemical exposures. Consider for the moment 10,000 units (gene deficiencies or chemical exposures at each exposure data point). Several ages need to be imaged per unit, and at least 3 experimental and 3 control individuals imaged per age. First, we will need standardized methods of choice of samples and controls, fixation, staining, and embedding. Histology, which is destructive, is today's standard (Giulio and Hinton, 2008), but if automated, can be potentially pursued in high throughput (Sabaliauskas et al. 2006, Cheung et al. 2011). Since 3D imaging provides information that 2D does not, it would be ideal to also have a rapid method of 3D imaging at similar resolutions. It would be better yet if imaged specimens could be saved for future imaging at higher resolutions either by choice or with improvements in technology. Preliminary experiments with plastic embedding (Clark, Copper and Cheng, unpublished) suggest that this will be possible, so let us assume that we are able to achieve whole-animal, 3D snapshots at cell resolution. The 3D

reconstructions would include thousands of possible slices through the animal in each orthogonal direction (transverse, coronal, and sagittal). Assuming we analyze 5 images in each direction, we would need to analyze 2,700,000 images (10,000 units $\times$ 3 ages $\times$ 6 individuals per age $\times$ 15 slides). With these numbers, not only are there not enough pathologists to interpret the images (Barthold, 2007), but also, it is highly likely that differences in training, fatigue, and experience would result in significant human error. In addition, the terms used by pathologists are neither quantitative, nor readily accessible computationally. Ideally, we will solve the demands of reproducibility, quantitative analysis, and computational accessibility by developing methods of high-throughput sample handling, imaging and image analysis for both 2D histology and 3D analysis. Experience indicates that 3D image segmentation, analysis, visualization, and interpretation at cell resolutions, for whole animals, will require high-performance computing, and is under discussion by a growing group of investigators. The terminology used will need to fit within anatomic and phenotypic ontology guidelines (available for zebrafish from www.zfin.org). The ability to achieve these improvements will require an unprecedented level of collaborative activity between established geneticists, toxicologists, histopathologists, physicists, engineers, computer scientists, and bioinformaticians to develop the hardware and software required for such large-scale projects. This interdisciplinary breadth is expected to be a challenge. Large cross-agency investments in hardware and people will be needed to create the infrastructure.

Web-based interfaces with phenomic data

The file sizes associated with whole-animal imaging at cell resolution, whether single microscope slide scans at high resolution or 3D scans, are in the range of tens of gigabytes (Cheng, Peckins, Copper, and Clark, unpublished observations). Even with improvements in computer technology and web speed, it will be impractical for each user to download such data, in real time, for analysis. The computational demands of all these steps in the process lie in the realm of high performance computing (Peckins, Xin, and Cheng, unpublished observations). Integration of data modes will need to take into account slight to major differences in resolution, file formats, and means of software-drive manipulation. Coordinated effort across multiple laboratories will be necessary to integrate these critical tasks. The anticipated volumes of phenomic data, which can be expected to reach petabytes with 3D information (e.g. for 10,000 units), indicate that investigators, regulators, and trainees will need to build or use data-farms and take advantage of the ubiquity of web-based resources. Cloud computing is an obvious potential contributor to this end.

Integration of bioinformatics and computational approaches

Computational and bioinformatics tools, and gene-chemical-disease databases will play an important role in meeting the challenge by enabling the testing of novel gene $\times$ environment hypotheses in genetically tractable aquatic model organisms. This work can be pursued at a fraction of the cost of using mammalian model organisms for the same purpose. Significant evolutionary similarities between fish and humans suggest that the relevance to human health will be significant. We know, for example, that human skin color genes can rescue functional deficiencies in zebrafish, and that zebrafish genes can serve as an entry point for

the discovery of human genes of great relevance to humans (Lamason et al., 2005, Tsatskheladze and Cheng, unpublished).

Discovery through databases

Bioinformatics and computational approaches are being taken to uncover potentially harmful interactions between complex chemical mixtures and biological systems. The growing number of web-based resources and databases reflect the increasing need to aggregate, analyze and integrate diverse data sets in many areas of biological research. Information from diverse areas relevant to toxicity testing and mechanism-based environmental health research has grown exponentially in recent years. This growth has led to the development of creative database solutions to provide tools for hypothesis generation, to connect otherwise disconnected datasets, and to create opportunities for new fields of inquiry (Mattingly 2009). Although several publicly available databases focus on the actions of chemicals, drugs or small molecules, few focus on environmental factors and address the emerging need for mechanism-based studies to understand disease etiologies. ToxRefDB and the Aggregated Computational Toxicology Resource (ACToR) are being developed by the Environmental Protection Agency to house data profiles from their high-throughput screening assays for chemicals prioritized by the ToxCast™ program (Judson et al. 2008). The Chemical Effects in Biological Systems (CEBS) is a public repository for environmentally relevant microarray, proteomic, clinical chemistry, hematology and histopathology data (Waters et al. 2008). The Comparative Toxicogenomics Database (CTD) is unique among other publicly available databases by virtue of its combination of inclusion of environmental chemicals and pharmaceutical agents, manual curation of data sets from the peer-reviewed literature, mechanistic and disease information with diverse cross-species representation, and integration of functional and pathway data with chemicals (Davis et al., 2011). CTD promotes understanding about the effects of chemical factors on human health. To achieve this goal, it provides manually curated chemical-gene/protein interactions from vertebrates and invertebrates as well as chemical-disease and gene-disease relationships. These data are integrated with select public data sets to enable development of testable hypotheses about chemical mechanisms of action and disease etiologies. Research groups are using CTD to formulate new hypotheses, discover previously unknown connections between disease pathways and chemical exposures, and perform high-level meta-analyses of multiple datasets to discern genetic and environmental pathways leading to complex diseases (Mattingly et al., 2009; Gohlke et al., 2009). This capacity would be significantly augmented by expanding CTD curation to include comprehensive phenotypes at varying levels of granularity and in diverse systems. These phenotypes will be integrated with cross-species chemical-gene interaction data to enable users to identify correlations between molecular changes and a broad range of effects – some of which may precede the onset of disease. These correlations will provide new opportunities for identifying potential biomarkers of exposure and targets for new therapeutic interventions, gaining insights into events involved in the progression of environmental diseases and informing extrapolation between diverse model organisms, including aquatic species, and human.

Conclusion

We have reviewed here the outcomes of a community-wide discussion of existing and envisioned tools that will be necessary for us as a society to tackle the important issues of mechanisms of disease and environmental toxicity. It is clearly within the interest of society's health that aquatic models continue to be used for environmental monitoring, that digitally and computationally accessible output would be an important and useful improvement to current practice, and that additional work is urgently needed to address the high-throughput definition of phenomes for aquatic model organisms such as the zebrafish and medaka. The expected experimental output has the potential to transform our ability to not only better understand gene function, but also to assist us in drug development and to prioritize societal responses to chemical toxicity.

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References

- Amatruda JF, Shepard JL, Stern HM, Zon LI. Zebrafish as a cancer model system. *Cancer Cell*. 2002; 3:229–231. [PubMed: 12086858]
- Barthold S, Borowsky A, Brayton C, Bronson R, Cardiff R, Griffey S, Ince T, Nitikin A, Sundberg J, Valli V. From whence will they come? A perspective on the acute shortage of pathologists in biomedical research. *J Vet. Diag. Invest.* 2007; 19:455–456.
- Betz O, Wegst U, Weide D, Heethoff M, Helpen L, Lee WK, Cloetens P. J. *Microscopy*. 2007; 227:51–71.
- Cheung NKM, Hinton DE, Au DWT. Development of high-throughput embryo histoarray for quantitative molecular profiling in uniformly orientated medaka (*Oryzias latipes*) embryos. *Comp Biochem Physiol C Toxicol Pharmacol*. 2011 In Press.
- Council on Environmental Health. Policy Statement—Chemical-Management Policy: Prioritizing Children's Health. *Pediatrics*. 2011; 127:983–990. [PubMed: 21518722]
- Davis AP, King BL, Mockus S, Murphy CG, Saraceni-Richards C, Rosenstein M, Wieggers T, Mattingly CJ. The Comparative Toxicogenomics Database: update. *Nucleic Acids Res*. 2011; 39(Database issue):D1067–D1072. [PubMed: 20864448]
- Di Giulio, RT.; Hinton, DE., editors. *The Toxicology of Fishes*. Boca Raton: CRC Press, Taylor and Francis Group; 2008. p. 1071
- Gerhard GS. Small laboratory fish as models for aging research. *Ageing Res. Rev.* 2007; 6:64–72. [PubMed: 17392037]
- Gohlke JM, Thomas R, Zhang Y, Rosenstein MC, Davis AP, Murphy C, Becker KG, Mattingly CJ, Portier CJ. Genetic and environmental pathways to complex diseases. *BMC Syst Biol*. 2009; 3:46–60. [PubMed: 19416532]
- Hahn, ME.; Hestermann, EV. Receptor-Mediated Mechanisms of Toxicity. In: Di Giulio, RT.; Hinton, DE., editors. *The Toxicology of Fishes*. Boca Raton: CRC Press, Taylor and Francis Group; 2008. p. 1071
- Hinton, DE.; Hardman, RC.; Kullman, SW.; Law, JM.; Schmale, MC.; Walter, RB.; Winn, RN.; Yoder, JA., editors. *Comp Biochem Physiol, Part C; Aquatic Animal Models of Human Disease*:

- Selected Papers and Recommendations from the 4th Conference; January 31–February 3; Durham, NC, USA. 2008. p. 121–128.
- Huisken J, Swoger J, Del Bene F, Wittbrodt J, Stelzer EHK. Optical sectioning deep inside live embryos by selective plane illumination microscopy. *Science*. 2004; 305:1007–1009. [PubMed: 15310904]
- Jeffery CJ. Multifunctional proteins: examples of gene sharing. *Annals Med*. 2003; 35:28–35.
- Judson R, Richard A, Dix D, Houck K, Elloumi F, Martin M, Cathey T, Transue TR, Spencer R, Wolf M. ACToR--Aggregated Computational Toxicology Resource. *Toxicol Appl Pharmacol* 15. 2008; 233(1):7–13.
- Lamason RL, Mohideen M-AP, Mest JR, Wong AC, Norton HL, Aros MC, Jurynek MJ, Mao X, Humphreville VR, Humbert JE, Sinha S, Moore JL, Jagadeeswaran P, Ning G, Makalowska I, Zhao W, McKeigue PM, O'Donnell D, Kittles R, Parra EJ, Mangini NJ, Grunwald DJ, Shriver MD, Canfield VA, Cheng KC. SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. *Science*. 2005; 310:1782–1786. [PubMed: 16357253]
- Mattingly CJ. Chemical databases for environmental health and clinical research. *Toxicol Lett*. 2009; 186:62–65. [PubMed: 18996453]
- Mattingly CJ, Hampton TH, Brothers KM, Griffin NE, Planchart A. Perturbation of defense pathways by low-dose arsenic exposure in zebrafish embryos. *Environ Health Perspect*. 2009; 117:981–987. [PubMed: 19590694]
- Megason SG, Fraser SE. Digitizing life at the level of the cell: high-performance laser-scanning microscopy and image analysis for in toto imaging of development. *Mech. Dev*. 2003; 120:1407–1420. [PubMed: 14623446]
- Meierjohann S, Schartl M. From Mendelian to molecular genetics: The Xiphophorus melanoma model. *Trends Genet*. 2006; 22:654–661. [PubMed: 17034900]
- Metscher BD. MicroCT for developmental biology: A versatile tool for high-contrast 3D imaging at histological resolutions. *Dev. Dyn*. 2009; 238:632–640. [PubMed: 19235724]
- Nairn RS, Schmale MC, Stegeman J, Winn RN, Walter RB. Aquaria fish models of human disease: Reports and recommendations from the working groups. *Mar. Biotechnol*. 2001; 3:S249–S258. [PubMed: 14961321]
- North TE, Zon LI. Modeling human hematopoietic and cardiovascular diseases in zebrafish. *Dev. Dyn*. 2003; 228:568–583. [PubMed: 14579393]
- Poss KD. Getting to the heart of regeneration in zebrafish. *Semin. Cell Dev. Biol*. 2007; 18:36–45. [PubMed: 17178459]
- Sabalianskas NA, Foutz CA, Mest JR, Budgeon LR, Sidor A, Gershenson J, Joshi S, Cheng KC. High-throughput zebrafish histology. *Methods*. 2006; 39:246–254. [PubMed: 16870470]
- Schmale MC. Aquatic animal models of human disease. *Comp. Biochem. Physiol Part C: Toxicol Pharmacol*. 2004; 138:229–231.
- Schmale MC, Nairn RS, Winn RN. Aquatic animal models of human disease. *Comp Biochem Physiol C: Toxicol Pharmacol*. 2007; 145:1–4. [PubMed: 17198764]
- Stock, SR. *MicroComputed Tomography: Methodology and Applications*. CRC Press; 2009.
- Virchow, R. *Die Cellular Pathologie*. Berlin: August Hirschwald; 1858.
- Waters M, Stasiewicz S, Merrick BA, Tomer K, Bushel P, Paules R, Stegman N, Nehls G, Yost KJ, Johnson CH, Gustafson SF, Xirasagar S, Xiao N, Huang CC, Boyer P, Chan DD, Pan Q, Gong H, Taylor J, Choi D, Rashid A, Ahmed A, Howle R, Selkirk J, Tennant R, Fostel J. CEBS--Chemical Effects in Biological Systems: a public data repository integrating study design and toxicity data with microarray and proteomics data. *Nucleic Acids Res*. 2008; 36:D892–D900. [PubMed: 17962311]
- White RM, Zon LI. Melanocytes in development, regeneration, and cancer. *Cell Stem Cell*. 2008; 3:242–252. [PubMed: 18786412]
- Zou L, Sriswasdi S, Ross B, Missiuro PV, Liu J, Ge H. Systematic analysis of pleiotropy in *C. elegans* early embryogenesis. *PLoS Comput Biol*. 2008; 4:e1000003. [PubMed: 18463698]