Session 3: Emerging Technologies

Micron-Scale Synchrotron X-Ray Tomography as a Tool for Pancellular 3-D Assessment of Cellular and Tissue Architecture

Keith Cheng, MD, PhD
Department of Pathology, College of Medicine, Penn State University

Global populations are exposed voluntarily and involuntarily to potentially tens of thousands of chemicals and other environmental factors, including thousands that are exempted by law from testing. Toxicological assessment requires a comprehensive knowledge of the toxicity of these chemicals with respect to cell type and effect on tissue architecture, but no existing imaging methodology provides all of that information. We have been working towards high-throughput, system-wide assessment of toxicity that is capable of detecting changes in any cell type at cell resolution (requiring voxel dimensions of ~1 micron). For reference, human CT and MRI voxel dimensions are ~1 mm. A practical means of achieving cell resolution in optically opaque tissues has been elusive.[1] Light-based methods of imaging cannot be used due to tissue opacity. MRI has insufficient resolution. Histological analysis is limited by plane of section and sampling. Based on the hypothesis that the monochromaticity and flux of synchrotron-based x-rays would provide the resolution an imaging speed needed for large-scale imaging, we have used Argonne National Laboratory's 2-BM tomographic imaging facility to image fixed and stained biological specimens in the millimeter size range, focusing on whole animal specimens of a powerful vertebrate model, zebrafish. We have generated the first pancellular, postorganogenesis, whole animal images (~100GB each) at cell resolution. From these images it is possible to identify and characterize virtually every cell type contributing to soft tissue architecture. Visualizations of specific structures including nerve tracts, vessels, and organs can be optimized by choice of stain and by digital adjustment of slab thickness, slice angles, and grey value histograms. Broad community accessibility and full-organism scans are envisioned through creation of cloud computing-assisted synchrotron stations (beamlines) dedicated to biological samples. The development of automated imaging and computational phenotyping will enable new "big data" approaches to the integrated assessment of toxicological risk.[2.]

All studies are IACUC compliant.

References

1. Cheng KC, Xin X, Clark DP, and La Riviere P (2011) Whole-animal imaging, gene function, and the Zebrafish Phenome Project. *Current Opinion in Genetics & Development* 2011, 21:620–629.
2. Cheng KC, Hinton D., Mattingly CJ, and Planchart A (2012) Aquatic models, genomics and chemical risk management. *Comparative Biochemistry and Physiology, Part C*, 155 (2012) 169–173.

Evolution's Experiments: Use of Teleost Diversity to Mine the Genetic Regulation of Development, Physiology, and Behavior

Matthew Harris, PhD
Children's Hospital Boston

The zebrafish has become a powerful model for genetic and experimental analyses to understand the regulation of development, physiology and behavior of vertebrates. Zebrafish are members of a larger class of vertebrates that represent over 30,000 species that have adapted to a wide range of environments. The varying evolutionary history among fishes and their specific adaptations provide unique test cases in which to understand how developmental and physiological systems respond to change, both environmental and genetic. I will discuss work we have done to combine advances in sequencing technology and refined mutagenesis approaches to systematically study the genetic regulation of development in three, diverse fresh water teleosts in parallel. This comparative approach allows for a broad analysis of gene function and uncovers unique roles of 'conserved' signaling mechanisms that would not be predicted by analysis in one model alone.

Rapid Identification and Characterization of Neuromodulator Chemicals Using an Embryonic Zebrafish System

David M. Reif, PhD
Department of Biological Sciences, Bioinformatics Research Center, North Carolina State University

New strategies are needed to address the data gap between the bioactivity of chemicals in commerce and the environmental versus existing hazard information. This data gap is especially wide when considering toxicological endpoints that are difficult to measure using purely *in vitro* systems, such as abnormal behavior. We describe an embryonic zebrafish system where behavior can be both experimentally perturbed (5 doses of chemical and 1-second pulses of light) and measured (accompanying real-time, spontaneous movement) in a high-throughput fashion. This system is applied to characterize the behavioral response to 1060 ToxCast™ chemicals as hyperactive or suppressive to movement of 24 hours postfertilization (hpf) zebrafish embryos subjected to intermittent pulses of light. By dividing the experimental interval according to light pulses (background = prior to first pulse; excitatory = after first pulse; refractory = after second pulse), we observe movement responses that can classify neuromodulator chemicals as eliciting light-independent movement alterations, light-dependent photomotor responses, or both. The chemicals can be further subdivided into a discrete number of clusters based upon hypo- and/or hyperactivity patterns across light intervals. These early behavioral responses are predictive (relative risk $p < 0.05$) of 17 specific developmental abnormalities (including notochord defects) and mortality measured at 5 days postfertilization (dpf). Therefore, this system can provide for rapid characterization of chemical-elicited behavioral responses at an early developmental stage that are indicative of significant downstream hazard. As an integrative measure of normal development, significant alterations in movement highlight neuromodulator chemicals representing several modes of action.

CRISPR-Cas9 systems and genome editing applications

Rodolphe Barrangou, PhD
Department of Food, Bioprocessing and Nutrition Sciences,
College of Agriculture and Life Sciences, North Carolina State University

Clustered regularly interspaced short palindromic repeats (CRISPR) and associated proteins (Cas) comprise the CRISPR-Cas system, which confers adaptive immunity against invasive elements in many bacteria and most archaea. CRISPR-mediated immunization occurs through the uptake of DNA from invasive genetic elements such as plasmids and viruses, followed by its integration into CRISPR loci. These loci are subsequently transcribed and processed into small interfering RNAs that guide nucleases for specific cleavage of complementary sequences. Although CRISPR-Cas systems have historically been used for bacterial genotyping and harnessed to enhance immunity against viruses in bacteria of industrial interest, recent focus has been on Cas9-based applications for genome editing. The signature protein of Type II CRISPR-Cas systems, the Cas9 endonuclease, can be reprogrammed by small guide RNAs (sgRNAs) to generate sequence-specific double stranded DNA breaks. This sgRNA:Cas9 technology has shown unprecedented potential and flexibility for genome editing, and can be repurposed for numerous DNA targeting applications including surgical genome editing, DNA imaging, large genetic screens and transcriptional control, in a wide range of cell types and model systems. Collectively, the potential of Cas9:sgRNA ribonucleoprotein complexes has open new avenues for CRISPR technologies in the genomic studies of a wide range of organisms of industrial and translational interest.