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Eunice Kennedy Shriver National Institute of Child Health and Human Development

Scientific Vision Workshop on Development

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Workshop White Paper

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I. INTRODUCTION/BACKGROUND

Developmental biology is in the midst of an exciting transformation because of paradigm shifting advances in technology and an exponentially growing store of accessible genomic information. To devise strategies to translate these opportunities into new discoveries relevant to human health, NICHD sponsored a "scientific vision" workshop held on February 9-10, 2011 in Bethesda, Maryland with about 60 discussants representing diverse research and technology areas. To launch discussion, nine invited speakers presented their views of what new opportunities for developmental biology might arise in the next decade in nine different areas central to understanding development. Given the breadth of the field, not all important topics could be covered by the speakers so the opportunities for research in additional areas were addressed in the breakout sessions. The speakers addressed:

- Imaging technologies: Dr. Jennifer Lippincott-Schwartz discussed advances in microscopy that use super-resolution to reveal activities that occur in living cells.
- Systems biology: Dr. Arthur Lander described a research strategy that exploits computational and theoretical approaches to interrogate complex biological problems in development such as the function of gene regulatory networks.
- Cell biological approaches: Dr. Dan Kiehart highlighted the importance of using biophysical, biochemical, molecular and genetic approaches to interrogate developmental processes that occur at the cellular level, by making more precise force, tension, and time measurements for example.
- Discovery approaches for identifying genes and pathways: Dr. Alexandra Joyner provided current and future approaches using the mouse model as a way to understand mammalian development.
- Epigenomic analysis of pluripotent and lineage-committed cells: Dr. Bing Ren discussed genome-wide approaches for identifying histone modifications and DNA methylation states of different cell types.
- Cell specification, differentiation, and morphogenesis: Dr. Mary Mullins covered the zebrafish as a vertebrate model for discovery using forward genetics, including approaches for discovery of maternal information in the egg.
- Organogenesis: Dr. Didier Stanier provided a vision of how modern approaches would penetrate the complex mechanisms of organ formation in vertebrates.
- Regenerative medicine and regeneration: Dr. Kristin Baldwin described opportunities for using reprogramming techniques, model organisms and endogenous adult and embryonic stem cells to identify regenerative mechanisms and to model human diseases *in vitro*.
- Signal encoding and single-cell dynamics: Dr. Michael Elowitz showed how theory and experimentation can be combined to reveal mechanisms at a molecular level using bottom up and top down approaches.

Following these presentations workshop participants were asked to consider three questions as they deliberated:

1. How does development work?
2. How can tools be improved to ask and answer that question?
3. How can the workshop help advance the mission of NICHD?

The participants discussed how recent technological and conceptual advances could be best translated into knowledge about poorly understood aspects of development, key areas for rapid discovery and how best to link discoveries in model systems to clinical applications with particular consideration of which areas could most benefit by support from NICHD. All were in agreement that information flow is more important now than ever before. At every level of the discussion it was repeated that the scientific community must find better ways to share databases, to archive and make available DNA sequence, transcriptome, proteome, and other large datasets in ways that permit productive cross-comparisons. After deliberations that covered a wide-ranging number of topics, the following projected opportunities and technology advances were agreed upon as the coming decade's most promising prospects for basic developmental discovery.

II. SCIENTIFIC OPPORTUNITIES

1. **Harness the power of regenerative medicine and direct reprogramming.**
   Recent advances in generating human and mouse embryonic and induced pluripotent stem cells open the door to direct modeling of human disease and provide accessible new methods to study mammalian development and the function of particular cell types *in vitro.* For example, it is now possible to generate important cell types such as neurons, blood cells and heart cells directly from fibroblasts in only a few days. These advances and the relative simplicity of direct reprogramming and directed differentiation from pluripotent stem cells will inspire the development of new *in vitro* systems to study aspects of normal embryonic development and to identify the cellular consequences of genetic or environmental based dysfunctions that give rise to developmental defects or lead to adult onset disease. Therefore a critical area of research is to harness the power of stem cells, regenerative medicine and reprogramming of cell types (via forced gene expression, RNA/protein transduction or chemical methods) to generate a diverse array of differentiated and multipotent cell types. These cells will be useful for modeling disease and understanding normal developmental processes. Ultimately these cell types and the knowledge gained from *in vitro* studies will establish safe yet effective methods for organ regeneration or repair, either by cell replacement therapy or by chemical or other modification of cellular pathways to restore damaged cells to a functional state *in vivo*.

2. **Discover mechanisms that explain how genomic variability and the environment contribute to phenotypic outcome.**
   Clear evidence supports the role of both environmental exposure and genotypic variability as important and essential influences on the observed phenotype of any developmental process. Despite these data, little is known about the mechanisms underlying their effects. Research is needed in experimental and clinical situations to explore and comprehend the entire exposome (environmental exposures), including nutrition, during embryonic development. Methods are needed to quantify this exposure, to delineate this within specific developmental windows and to directly relate the outcomes to genetic variability across the entire genome relevant to developmental processes.

3. **Develop methods to utilize human genetic and phenotyping data as a starting point to inform experimental research in model organisms.**
   These advances will reverse the traditional direction of research where model organism data was used to inform research in humans. Because of advances derived from the human genome project, human genetic and phenotyping data offer a valuable new resource to inform experimental research in model systems and to uncover differences between the gene repertoires of humans compared to model organisms.

4. **Integrate information from diverse organisms, including humans, to enhance our knowledge of conserved aspects of basic developmental processes.**
   Model organisms (worm, fly, frog, fish, mouse, etc.) have greatly enhanced our understanding of basic developmental processes. However, the wealth of information obtained from studies of diverse organisms, including humans, has not been fully integrated across species. In many cases the available information does not exist in a form that is useful for scientists in the field. To obtain a complete understanding of developmental processes, especially those relevant to human development, a common platform for integration of information is needed.

   Closely related genes that function in highly conserved regulatory pathways must be collated, bringing together information from across the spectrum of organisms to significantly advance our knowledge of processes such as pattern formation and organogenesis. The most important benefit to be derived from this integrated and systems level approach to data compilation is to provide models that will guide future research.

5. **Delineate how linear information encoded in a genome is translated into 3-dimensional forms over time.**
   The mechanisms by which one-dimensional information is transformed into 3-dimensional patterned structures over time are largely unknown. The grand challenge of explaining how embryos and animals acquire a 3-dimensional pattern remains foundational for developmental biology. Research in this area will require the collaboration of scientists from diverse fields and disciplines and the integration of the biological, physical, quantitative and computational sciences. This area is likely to be one of the major breakthroughs of the coming decade.

   Training physicists to understand biology and training biologists to understand physics will require new training programs to meld the disciplines. Simulation models, both highly undetermined and highly determined models (such as models that incorporate evolution and are predictive) are essential and will require quantitative data and broad-based informatics support. These developments will make it possible to supplement current experimental approaches using animal models with mathematical and computational modeling (see section on Training below).

6. **Understand the sequences in the genome that affect developmental processes.**
   Work in the past decade has greatly increased our knowledge of the specific genes involved in developmental processes. Nevertheless, there is a clear need for a systematic approach to precisely define the role of the distinct genomic elements in development. Annotating the genome sequence of well understood experimental model organisms would provide a much needed blueprint for work exploring the role of epigenetic control and non-transcribed and/or translated sequences in development. Such blueprints will also be essential for comprehending clinical genetic information in the context of complex disorders of development leading to human birth defects.

7. **Obtain a better understanding of how cell biology integrates with developmental processes (signaling pathways, cytoskeleton, etc).**
   Major advances are being made in the field of cell biology but many of these advances have not been uniformly used to advance our understanding of developmental processes. Signaling pathways that control cytoskeletal dynamics for example are understood at a level that allows one to explain complex processes such as cell migration and cell shape changes but this information has not been put to full use to understand pattern formation in the embryo. In a similar way, cellular pruning by apoptosis is well understood but much of this knowledge has not been applied to the developing embryo. Extracellular matrix, cell adhesion and signaling are critical for differentiated cells but are equally important for developing embryos. A better understanding of how cell biology integrates with developmental processes is needed. New tools such as protein tags that allow identification of protein expression and better technology for reporters of mechanical properties are needed. Cell biological studies of developing architecture and pattern are needed. Systems-level approaches that allow one to understand the role of particular circuit modules in pattern formation and other developmental processes will advance developmental studies of complex structures and movements. These understandings will come from perturbation and analysis of developing embryos alone as well as application of new high-throughput and imaging techniques currently available or on the horizon. Better exchange of information, additional databases, and new animal models for drug testing, device and surgical technique development are needed as tools for this opportunity.

8. **Integration of how nutrition and metabolism affects developmental processes including cell migration, development timing, and other processes.**
   While evidence from folic acid supplementation in humans clearly reveals the critical role of defined nutrients in specific developmental processes, a much clearer understanding of the role of nutrition and metabolism in development is needed. Work in this area should focus on specific cellular and molecular processes critical to development, such as cell migration, developmental timing, circadian rhythms and gene transcription. Experimental models should be explored where nutritional and metabolic processes can be carefully controlled and phenotypic outcomes readily observed.

9. **Vertebrate genomes will be functionally annotated through characterization of developmental phenotypes in mutant models.**
   The revolution in next generation sequencing and gene expression analysis has led to an increased understanding of how many genes and functional RNAs are transcribed in different tissues of model organisms. Similarly, human genome sequencing and expression analyses are providing increasing numbers of candidate genes that are responsible for congenital abnormalities and disease. One critical data set that could accelerate our understanding of development and disease would be to have an annotated database of the consequence of mutating each gene or small RNA element in the genome, in multiple model organisms. Therefore in ten years, an achievable goal would be that the complete transcriptome of the mouse, zebrafish and perhaps other genetically tractable vertebrates would be functionally annotated.

10. **Better understanding of how developmental processes have been conserved and have diverged during evolution.**
    Opportunities from many genome-sequencing projects will allow a better understanding of how developmental processes have been conserved or have diverged over time. Not only will there be an ability to determine the degree of variation extant within a species but seminal insights will be gained on how some processes are conserved while others diverge through evolutionary time. Further, these analyses will identify multigenic cassettes that provide conserved function for processes shared by many organisms. As biologists, we tend to look for commonalities in processes. However, it is also useful to promote science that looks across species for fundamental mechanisms that have changed, such as processes associated with regeneration. Such studies could inform studies in other disciplines, such as regenerative medicine.

TECHNOLOGY

Here we consider how best to apply recent technological advances to the study of development, as well as outline areas where additional advances could help to better achieve the goals of the NICHD.

1. **Imaging and microscopy**
   The pace of innovation in microscopy is high. Already many technologies exist that could accelerate studies across developmental systems, but limited access to new expensive machines and image processing software can prevent researchers from using these tools or obtaining critical preliminary data to support future studies. Better means to provide access to new and next-generation microscopes is important. These include newer generation spectral scanning confocal microscopes, multiphoton microscopes, super-resolution microscopes, tools for serial or automated electron microscopy, large-scale imaging of intact organisms, embryos or organs by selective plane illumination microscopy (SPIM) or other approaches, better time-lapse or 4-dimensional imaging over timescales relevant to developmental processes (hours, days, or weeks). Simultaneous multiscale imaging, for example, of individual molecules at synapses in the context of an intact brain, or imaging specific chromosomal loci or conformations in multiple cells in an intact developing embryo, will

also greatly enhance our understanding of developmental processes. Fiber optic approaches to *in vivo* imaging of organs or developing embryos in intact model organisms and adaptation of light-emitting diode (LED) technologies will also further advance imaging. Each of these tools must be coupled with accessible and affordable image processing software and data storage. Mechanisms to share the data and view it in "movies" or in three and four dimensions need to be developed. Finally, means to couple imaging approaches with manipulations of organisms via small molecules or biophysical methods should be developed.

2. Biosensors and biomanipulators

Great strides have been made in developmental biology by linking specific proteins to fluorescent reporters such as green fluorescent protein (GFP). While these toolkits have expanded, advances in multicolor imaging and chemical genomic approaches offer several exciting possibilities for improved detection of nearly any biologic molecule or process, as well as manipulation of gene expression or protein function in appropriate timescales or with reversible methods. Specific tools that could be developed and applied in the next decade include:

a. Genetically encoded reporters for signal transduction pathways, gene expression, epigenetic changes, cell structure changes, intercellular communication, apoptosis, numbers of cell divisions, senescence, cytoplasmic distribution after cell division, protein-protein associations, metabolic states of cells, reactive oxygen species and differences in subcellular pH or concentrations of calcium.
b. Brighter tags in more colors for individual molecules that could be used in parallel to assess protein and/or protein-DNA complexes in an intact cell or organ.
c. Chemical tags for particular proteins, protein classes, RNA species or DNA species.
d. Chemical and genetic tools to manipulate specific proteins, genes, RNAs or pathways, in specific cell types, at particular times, ideally reversibly and with limited toxicity.
e. Biophysical tools to measure and manipulate cells and tissues using force, polarity, shear, spring constants, flow and other parameters of biophysical consequence on a nanoscale and organ scale level.
f. More quantitative methods to measure relative protein abundance and enzyme function at the subcellular and single cell level, such as mass spectrometry with increased sensitivity or based on cell position in a tissue section.

3. Applied next generation nucleic acid sequencing

It is expected that the cost of sequencing DNA molecules will continue to drop, making it possible to assess the genomic sequence of many or all humans and of an increasing number of model organisms. RNA-Seq, chromatin immunoprecipitation followed by sequencing (ChIP-Seq), and similar approaches will also allow us to identify the whole transcriptome and epigenome of individual cell types in many organisms. One roadblock to these studies is sample size requirement, particularly for studies of developmental processes in which only a few cells at a time may be undergoing fate transitions, or be present at all (i.e., in the early embryo). Furthermore, these experiments generate an extraordinary amount of data, which is difficult for classically trained developmental biologists to process, interpret and handle. Important advances required to harness the power of sequencing include:

a. Improved means to analyze, store and share sequencing data
b. Improved sequencing of small samples or individual cells, perhaps via improved unbiased amplification methods, single molecule sequencing with reduced loss or cell type-specific tags for RNA or DNA to allow subsets of cells to be sequenced from an intact organism or tissue.
c. Decreased cost and dissemination of technologic advances.

4. Bioinformatic and Computational Tools

Advances in imaging, DNA sequencing, proteomics, metabolomics, high-throughput screening for small molecules, and biophysical studies all require increasingly sophisticated quantitative and computational analytic skills, which previously were not as essential to developmental studies. Participants agreed that improved open access analysis tools are required for each of these areas, and for bottom-up and top-down computational modeling of biologic processes. New methods to integrate and cross-reference multiple “omics” data sets are required as are methods to image large and small structures and compare them across multiple samples. Researchers and students with computational skills should be recruited to the field, or persons already in the field provided training to allow these advances to lead to new insights into systems level biologic processes as well as theoretical and biophysical studies of cells and organs during development. Finally, linking human mutation data sets from diseased individuals to databases of knockouts or RNA knockdowns in model organisms would accelerate discovery.

5. Community Resources

Technological advances and the growing impact of system-wide studies are making it critical to establish community-wide resources to link the growing store of knowledge about human genes implicated in specific developmental disorders with appropriate studies in model systems or using human or other mammalian cell types *in vitro*. Two types of resources were suggested to achieve this goal.

a. Biorepositories: In the past, model systems (invertebrates - worm, flies and vertebrates, frogs, mice, fish) have taught us a great deal about embryonic development. This is a critical issue for childhood disease as many children are born with congenital malformations that range from limb malformations to neural tube defects to congenital heart disease. With the revolution in human genetics, we can now investigate children with congenital malformations and determine the mutated genes. This becomes a new avenue for gene discovery that can then be thoroughly investigated in model systems, an extraordinary opportunity for learning about embryonic development that is completely new and is only now feasible. Therefore, we need to engage children’s hospitals and pediatric and obstetrics departments to identify children with congenital malformations and then collect DNA and tissue samples from those children and from parents. These samples need to be banked along with phenotyping information. The latter is critical as extremely rare patients with congenital malformations are likely to be highly informative along with common malformations. Therefore, wide access to investigators is also critical. The NICHD should develop standard human investigation committee (HIC) protocols so institutional review board (IRB) approval can be identical across institutions, enabling sample collection at one institution and analysis at another. It is critical that we investigate each child with congenital malformations in an attempt to better understand this disease, which may enable genetic counseling, genetic testing, changes in patient management, better outcomes, and screens for cures.

b. 4D Atlas of Development in Multiple Model Organisms with many dimensions of information: Studies of human disorders will be limited to genetic analyses and studies that may be performed in human models *in vitro*. It is unlikely that these studies will lead to a greater understanding of normal developmental processes than can be achieved in model systems. Studies leading to a fundamental understanding of the generation of all cell types, or the formation of intact organs or morphologic features are more easily carried out in model organisms than in a human. Comparative approaches, therefore, are important and would be greatly aided by a systematic compilation of a 4D atlas of normal development for each model. These atlases should include establishing the organization and identity of the cell types present from fertilization to adulthood at the level of cellular composition of tissues and organs. They should also provide information on cellular morphology and microenvironment; patterns of gene expression (RNA and protein) that distinguish each cell type; signaling pathways between cells; epigenetic markers for cell types and cell fate transition; and cell biological mechanisms employed for morphogenetic changes.

ADDITIONAL RESEARCH NEEDED

In addition to the scientific opportunities listed above, research is needed to address many questions, including the following:

- How to determine gene function in the face of genetic redundancy.
- How to measure and manipulate genetic and epigenetic variation at single cell resolution.
- How to address the genetic basis of complex heritable birth defects.

TRANSLATIONAL RESEARCH NEEDED

- How to use developmental models to identify therapies.
- Which are the best animal models for specific developmental disorders?
- How do we decode pre- and peri-natal genetic information?

Translating fundamental knowledge of development into useful tools for prevention and treatment of human birth defects remains an important long-term goal of all basic scientific work on development. Work will be needed to continue to identify models where such knowledge can be addressed with newer tools for drug discovery including high-throughput screening and chemical genomics that can be translated into novel therapeutic tools. Multiple developmental models will continue to be needed and efforts should focus on which models may be most critical for these translational studies. For example, planaria or zebrafish may be most useful for regenerative work and drug discovery whereas primate models may be needed in exploring approaches to developmental events leading to abnormalities in cognition and/or behavior. Perhaps most importantly, all such work must be taken in the context of perinatal genetic information allowing for maternal, paternal and fetal genomic influences. Here work is needed in defining the genetic information encoding the most likely therapeutic targets and the rational approaches to drug design and application.

TRAINING

Progress in the biological sciences and in particular, developmental biology, will require scientists with expertise in computational biology, bioinformatics, biophysics and imaging. In addition, developmental biologists will need to develop the skills required to collaborate with scientists from the physical, quantitative and computational sciences.

Graduate training programs are, and will remain, at the core of our strength in forwarding research in development. At the same time, traditional graduate programs are not sufficient to provide the kind of training needed, given the rapid technical and scientific advances. Training for these new specialized and emerging areas are often best provided in an intensive, hands-on multi-week lab course such as the summer courses offered at the Marine Biological Laboratory, in Woods Hole, MA, and Cold Spring Harbor, NY. These courses assemble the best experts in the field as instructors and the latest equipment (either provided by vendors or brought to the site from research labs). Unfortunately, these summer courses are over-subscribed and cannot accommodate the number of scientists needing training. Therefore, new courses and training opportunities are needed, including online courses and workshops organized by professional societies. These training opportunities should encourage and promote cross-disciplinary collaborations.

III. CONCLUSION

A number of promising scientific opportunities in developmental biology are on the brink of major breakthroughs. These breakthroughs, both foundational and transformative, will occur only if the scientific community establishes better ways to share information and make available the rich trove of information in databases of DNA sequences, transcriptomes, proteomes, and other -omes. The key to success will be the development of new and improved technologies and training programs that prepare future developmental biologists with expertise in cross disciplines including the physical, quantitative and computational sciences. These research opportunities, when taken together, will lead to major advances in the scientific mission of the NICHD and in public health.

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