Impact of the *Xenopus* system on the missions of the NIBIB

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The *Xenopus* models system has made major contributions to the mission of the NIBIB, most notably because *Xenopus* embryos provide a unique platform (i.e. *fast and cheap*) for the elucidation of the multi-scale principles of morphogenesis and tissue self-assembly. Due to their relatively simple culture conditions and low cost, *Xenopus* has been a rich source of material to test new imaging tools and understand basic principles of tissue mechaniscs, regeneration, growth, and remodeling. Additional resources for cross-disciplinary training and increasing the access to molecular tools would accelerate the use of *Xenopus* and make the cost-of-entry lower for engineers frustrated by the complex culture conditions needed to study of mammalian organogenesis.

Xenopus is an outstanding, proven test-bed for studying key concepts and the principles underlying tissue engineering outlined in the 2007 Multi-Agency Tissue Engineering Sciences (MATES; http://tissueengineering.gov) report "Advancing Tissue Science and Engineering, a Foundation for the Future." Current strategies for engineering tissues focus on providing compatible artificial scaffolds but lack mechanistic understanding of what cells do once they occupy and begin to remodel these artificial scaffolds. *Xenopus* studies provide just such a mechanistic framework guiding developmental biology to understand how cell identity can be controlled and manipulated to produce organ-specific differentiated tissues. For instance, fully functioning hearts can be generated from embryonic tissues "reprogrammed" to differentiate into heart progenitor cells. Studies on tail regeneration can provide clues to cellular and tissue mechanisms that are absent in humans.

In contrast to mammalian model systems, *Xenopus* provides a higly tractable experimental model and can provide tissue engineers with hands-on experience during advanced cross-disciplinary training. Simple experimental systems are essential to provide tissue engineers with real biology experience. Often, the challenges of using mammalian tissues and cells is too great an obstacle for tissue engineers eager to develop new technologies. Furthermore, animal care facilities, equipment, and resources needed to work with frog embryos are low. For instance, animal care costs are less than $0.03/day for each frog. Furthermore, temperature controlled incubators can be very inexpensive compared to heated CO2 incubators. *Xenopus* embryos can be cultured in low cost saline-type media rather than high cost 50% fetal rat serum needed for mouse embryo culture.

Xenopus has been a crucial resource for the development of novel imaging modalities, new sample preparations, and for testing new image processing tools. Whole animal histology and live embryo imaging using magnetic resonance interferometry (MRI) where the 3D architecture is preserved provide insights into the growth and movements of tissues normally hidden from view in the embryo. New imaging tools such as optical coherence tomography (OCT) and micro-computed tomography (microCT) are developed, used, and validated with *Xenopus* tissues as a first step toward adoption for clinical use. Lastly, large embryonic *Xenopus* cells allow live studies of protein dynamics and reveal the cell and tissue mechanics needed to sculpt functional tissues.

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2009 *Xenopus* White Paper – Community Needs

Executive Summary

Xenopus - a crucial model organism for biomedical research:

Experiments in model animals are a cornerstone of biomedical research and have a massive impact on our understanding of human health and disease. The frog, *Xenopus,* is a widely used and crucial vertebrate model organism that offers a unique combination of three powerful advantages: strong conservation of essential biological mechanisms, a remarkable experimental repertoire, and unparalleled cost-effectiveness when compared to murine or other mammalian models.

In fact, for many experimental applications, *Xenopus* is the only viable model system. For example, in cell and molecular biology, *Xenopus* extracts allow for individual components of the cell cycle or DNA replication/repair machinery to be analyzed in a manner that cannot be recapitulated *in vivo* or in cell culture. For developmental biology, no other model system allows for high-throughput genomic/proteomic screening and at the same time allows for transplant/explant analysis (i.e. "experimental embryology"). The *Xenopus* oocyte is unique as a system for studying channel physiology using the patch-clamp and as a system for protein expression. Finally, *Xenopus* is the only vertebrate model that readily produces enough biological material for biochemical purification from eggs, intact embryos, or isolated embryonic tissues. The combination of these characteristics offers a wide range of experimental opportunities for studies using the *Xenopus* system in contrast to other vertebrates such as the mouse or zebrafish.

NIH Investment in *Xenopus:*

The NIH has made a substantial and continuing investment in *Xenopus* research. Indeed, a search of the NIH rePORT database for R01's or equivalent grants using the search term "*Xenopus*" returned **427 grants for a total cost of $127,583,776** for FY08 and FY09. Despite this investment in individuals' research, the *Xenopus* community lacks many resources that are considered entirely essential for other model systems, including a complete genome sequence, stock and training centers, and a comprehensive model organism database.

Xenopus as a Model System and Human Disease:

Given the tremendous advantages of the *Xenopus* system, the pace of new biological discovery by the *Xenopus* Community is brisk. Using *Xenopus*, we have significantly improved our understanding of human disease genes and their mechanisms, justifying the NIH's investment in *Xenopus*. Below we provide examples of just a few of the human health discoveries made in the last two years:

Xenopus embryos are used for *in vivo* analysis of gene expression and function:

Nephronophthisis - *Hum Mol Genet.* 2008. 17, 3655-62; *Nat Genet. 2005*. 37, 537-43.
Cutis laxa - *Nat Genet.* 2009. 41, 1016-21.
Meckel-Gruber syndrome - *Am J Hum Genet.* 2008. 82, 959-70.
Colorectal cancer - *Genome Res.* 2009. 19, 987-93.

Xenopus egg extracts are used for *in vitro* biochemical studies:

Fanconi Anemia - *Mol. Cell.* 2009. 35, 704-15; *J Biol Chem.* 2009, 284, 25560-8.
C-myc oncogene - *Nature*. 2007. 448, 445-51.
BRCA1 - *Cell*. 2006. 127, 539-552

Xenopus oocytes are used to study gene expression and channel activity:

Trypanosome transmission - *Nature* 2009. 459, 213-217.
Epilepsy, ataxia, sensorineural deafness - *N Engl J Med.* 360, 1960-70.
Catastrophic cardiac arrhythmia (Long-QT syndrome) - *PNAS* 2009. 106,13082-7.

Megalencephalic leukoencephalopathy - *Hum Mol Genet.* 2008. 17, 3728-39.

Xenopus as a Model System and Basic Biological Processes:

Xenopus also plays a crucial role in elucidating the basic cellular and biochemical mechanisms underlying the entire spectrum of human pathologies. Again only a few of the many discoveries in the last two years are highlighted here:

Xenopus embryos were used for studies of TGF-® signal transduction.
(*Cell*. 2009. 136,123-35; *Science*. 2007. 315, 840-3).

Xenopus egg extracts revealed fundamental aspects of cell division.
(*Nature*. 2008. 453, 1132-6; *Science*. 2008. 319, 469-72).

Xenopus embryos were used for studying mucociliary epithelia.
(*Nat Genet.* 2008. 40, 871-9; *Nature*. 2007. 447, 97-101).

Xenopus embryos were used for studying development of the vasculature.
(*Cell*. 2008.135, 1053-64).

Xenopus egg extracts provided key insight into DNA damage responses.
(*Mol Cell.* 2009. 35,704-15; *Cell.* 2008. 134, 969-80).

Xenopus embryos linked telomerase to Wnt signaling.
(*Nature*. 2009. 460, 66-72).

Xenopus was used for small molecule screens to develop therapeutics.
(Nat Chem Biol. 2008. 4, 119-25; Blood. 2009. 114, 1110-22).

Immediate Needs of the *Xenopus* Community:

It is the consensus of the *Xenopus* community that their biomedical research could be greatly accelerated by the development of key resources that are currently lacking. These resources would be of use to the entire *Xenopus* research community. In this White Paper, the community identifies seven resources in two categories: Three Immediate Needs and four Essential Resources:

The **Immediate Needs** are a common set of key resources that were identified as the most pressing by three committees established to identify needed resources across the broad and diverse *Xenopus* community. There is a broad, community-wide consensus that these resources would have an immediate impact on all *Xenopus* users and should be assigned the highest priority in order to accelerate the pace of biomedical research using *Xenopus* as a model system.

These Immediate Needs and the resulting improvements in biomedical research are as follows:

1. *Establishment of the Xenopus Resource and Training Center at the MBL in Woods Hole.*
   - Will allow rapid distribution of transgenic *Xenopus laevis* lines expressing fluorescent reporters and tagged proteins (for example histone-RFP for visualizing the mitotic spindle or organ specific GFP in embryos)
   - Will allow centralized generation, housing, and distribution of genetically modified *X. tropicalis* lines, including both mutants and transgenics.
   - Will allow both current investigators and the next generation of researchers to get hands-on training in *Xenopus*-based biomedical research methods (including cell, molecular, and developmental methods).
2. *Expansion and improvement of Xenbase, a Xenopus model organism database.*
   - Maintain and curate data for the essential primary database for *Xenopus* researchers.
   - Enhance the functionality of *Xenbase* by introducing a phenotypes feature.
   - Support new content on *Xenbase*, including proteomics support, a new genome browser, and Wiki for *Xenopus* methods.
   - Continue and expand collaborative and service efforts (e.g. provide *Xenopus* data to other databases including NCBI, UniProtK, Mascot and Tornado).
3. *Complete sequencing of the Xenopus laevis genome.*
   - Will allow the deconvolution of data in mass-spectrometry-based proteomic studies.
   - Will facilitate identification of conserved gene regulatory regions to build gene-regulatory networks.

-Will facilitate site-specifc studies of DNA transaction (repair and replication)
-Will facilitate identification of all ORFs to build an ORFeome for rapid functional characterization of genes
-Will facilitate the design of morpholino oligonucleotides for gene depletion studies
-Will faciliate the analysis of chromatin-immunoprecipitations to identify DNA-bound to transcription factors and DNA modifications.

Essential Resources Needed by the *Xenopus* Community:

In addition to these immediate, community-wide needs, the committees identified four **Essential Resources** that should be developed as soon as possible, so that *Xenopus* biologists can more effectively fulfill the missions of the NIH. The *Xenopus* community considers all four of these additional resources to be essential, but understands that priorities must be set, and ranks these behind the Immediate Needs. These Essential Resources are as follows:

4. *Xenopus ORFeome in recombineering vectors.*
5. *Improvement of the X. tropicalis genome sequence and annotation*
6. *Development of methods for disrupting gene function in Xenopus.*
7. *Generation and Distribution of antibodies for Xenopus research.*

Anticipated Gains for Biomedical Research:

Xenopus is a crucial model organism for biomedical research. With the development of large-scale community-wide resources, *Xenopus* is poised to be become the premier vertebrate model for systems-level approaches to understanding biological mechanisms in cell, molecular, and developmental biology.

The National Research Council and the National Academy of Sciences have recently called on the Unites States “to launch a new multiagency, multiyear, and multidisciplinary initiative to capitalize on the extraordinary advances recently made in biology”. This report (http://www.nap.edu/catalog.php?record_id=12764) recommends the term "new biology" to describe an approach to research where “physicists, chemists, computer scientists, engineers, mathematicians, and other scientists are integrated into the field of biology.” The promise of systems-level analysis in *Xenopus*, combined with its already proven strengths, make *Xenopus* the ideal model organism for pursuing this “new biology.”

Genome improvements will provide *Xenopus* researchers with the ability to perform genome-wide screens for biological activities that will in turn allow the rapid assembly and analysis of gene regulatory networks. The ORFeome will greatly facilitate such genome-wide screening by allowing all ORFs to be rapidly analyzed or large numbers of proteins to be tagged for analysis of protein-protein interaction or for *in vivo* visualization. Using extracts and biochemical purification coupled with mass-spectrometry and genomic sequence, protein interactomes can be rapidly identified and validated. Because *Xenopus* can be so easily manipulated and because vast amounts of biological material can be generated, cell-type specific interactomes can also be identified. Large-scale genetic screens will identify important novel genes in developmental pathways, especially given the relatively simple genome of *X. tropicalis* compared to zebrafish. Finally, the flexibility of both *Xenopus* extracts and embryos make this system ideal for chemical biology screens. Identifying these gene-regulatory networks, interactomes, and novel genes will be only the first steps, of course. The well-established power of *Xenopus* for rapid analysis of gene function will then allow deeply mechanistic analyses to complement the systems-level approaches described above.

It is the combination of these characteristics that distinguishes *Xenopus* from other vertebrate model systems such as mouse and zebrafish and allows for a systems-level approach to understanding biological mechanisms. The tremendous promise of the *Xenopus* model cannot be realized, however, without the immediate development of community-wide research resources. This White Paper presents the needed resources, and we look to the NIH for guidance in how to best achieve these goals.

For complete details of the 2009 Xenopus White Paper, please visit
http://www.xenbase.org/community/xenopuswhitepaper.do