

Impact of the *Xenopus* system on the mission 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 mechanics, 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 highly 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.

Selected References:

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According to NIH RePORTER Search Tool, in the fiscal year of 2011, the National Institute of Biomedical Imaging and Bioengineering funded three for projects involving *Xenopus*. These grants total to \$1,624,601.

2011 *Xenopus* White Paper - Community Needs:

Executive Summary

***Xenopus*: An essential vertebrate model system for biomedical research:**

Model animals are crucial to advancing biomedical research. Basic studies in vertebrate animals rapidly accelerate our understanding of human health and disease. Among the commonly used model animals, the frog, *Xenopus*, has great impact because of its close evolutionary relationship with mammals. Moreover, the remarkable experimental repertoire of the *Xenopus* system has made it a cornerstone of neurobiology, physiology, molecular biology, cell biology, and developmental biology.

Current NIH investment in research using *Xenopus*:

Consistent with its broad utility, the NIH has made a large and continuing investment in *Xenopus* research. Indeed, a search of the NIH rePORT database for R01 or equivalent grants using the search term "*Xenopus*" returned **678 grants for a total of over \$217,000,000** for FY09-10. The NIH has also recently demonstrated its commitment to *Xenopus* community resources by approving \$2.5 million to establish the National *Xenopus* Resource in Woods Hole, MA and a similar amount to maintain and expand Xenbase, the *Xenopus* Community's online database.

***Xenopus* as a model system for human disease gene function**

Given the tremendous power of the *Xenopus* system, the pace of new biological discovery by the *Xenopus* Community is vigorous. Using *Xenopus*, we have significantly improved our understanding of human disease genes and their mechanisms of action, justifying the NIH's investment. For example:

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

- Congenital Heart Disease** – *PNAS* 2011. 108, 2915-2920
- CHARGE Syndrome** – *Nature* 2010. 463, 958-962.
- Bardet-Biedl and Meckel-Gruber Syndromes** – *Science* 2010. 329, 1337-1340.
- Hereditary hypotrichosis simplex** – *Nature* 2010. 464, 1043-1047.
- Hutchison-Gilford Progeria** – *Dev. Cell* 2010. 19, 413-25.
- Cutis laxa** – *Nat Genet.* 2009. 41, 1016-21.
- Colorectal cancer** – *Genome Res.* 2009. 19, 987-93.
- Nephronophthisis** – *Hum Mol Genet.* 2008. 17, 3655-62; *Nat Genet.* 2005. 37, 537-43.

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

- Fanconi Anemia** – *Mol. Cell.* 2009. 35, 704-15; *Science.* 2009, 326, 1698-701.
- 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:

- Rapid-onset dystonia-parkinsonism** – *Nature* 2010. 467, 99-102.
- Trypanosome transmission** – *Nature* 2009. 459, 213-217.
- Epilepsy, ataxia, sensorineural deafness** – *N Engl J Med.* 2009. 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 for understanding basic biological processes:**

Xenopus also plays a crucial role in elucidating the basic cellular and biochemical mechanisms underlying the entire spectrum of human pathologies. Just a small fraction of the many recent discoveries are highlighted here:

Xenopus contributes to our understanding of vertebrate genome organization.
(*Science.* 2010. 328, 633-636).

Xenopus egg extracts reveal fundamental aspects of cell division.
(*Cell.* 2010. 140, 349-359; *Nature.* 2008. 453, 1132-6; *Science.* 2008. 319, 469-72).

Xenopus reveals new aspects of eukaryotic nuclear structure and function.
(*Cell.* 2010. 143, 288-98; *Science.* 2010. 318, 640-643).

Xenopus embryos are used for studies of Wnt and TGF- β signal transduction.
(*Science.* 2010. 327, 459-463; *Cell.* 2009. 136, 123-35).

Xenopus embryos are used for studying mucociliary epithelia.
(*Nat Cell. Biol.* 2009 11 1225-32; *Nature.* 2007. 447, 97-101).

Xenopus embryos are used for studying development of the vasculature.
(*Cell.* 2008. 135, 1053-64).

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

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

Xenopus are used for small molecule screens to develop therapeutics.
(*Nat Chem Biol.* 2010. 6, 829-836; *Blood.* 2009. 114, 1110-22; *Nat Chem Biol.* 2008. 4, 119-25).

Despite its demonstrated utility and despite the recent investments by the NIH, *Xenopus* still lacks many resources that are considered entirely essential for other model systems. It is the consensus of the *Xenopus* community that their biomedical research could be greatly

accelerated by the development of key resources of use to the entire *Xenopus* research community.

At the 2010 International *Xenopus* Conference, developmental, cell, and molecular biologists gathered to discuss the resources needed and the priority that should be assigned to each. There was broad community-wide consensus that eleven resources are currently needed, and these were prioritized into two categories: Immediate Needs and Essential Resources:

The Immediate Needs of the *Xenopus* research community:

1. Generation of the *Xenopus* ORFeome:

- Will enable genome-wide *in vivo* analyses of gene function.
- Will enable genome-wide *in vivo* analyses of protein localization.
- Will enable, when combined with transgenesis, the first large-scale biochemical determination of protein-protein interactions in specific tissues and at specific embryonic stages.
- Will facilitate more-rapid functional characterization of specific proteins.

2. Improvement of the *Xenopus* genome sequence:

- Will accelerate molecular studies by providing a complete catalogue of *Xenopus* genes.
- Will enable completion of the *Xenopus* ORFeomes.
- Will enable genomic analyses & systems biology approaches for novel gene discovery.
- Will facilitate proteomics approaches and peptide analysis.

Essential Resources for *Xenopus* research community:

In addition to these most-pressing needs, the community has identified nine other 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 of these additional resources to be essential, but understands that priorities must be set, and therefore ranks these as indicated below:

3. Improvement of long-range contiguity in the *Xenopus laevis* genome
4. Improvement of *Xenopus* antibody resources
5. Loss of function: Zinc Finger Nucleases/TILLING
6. Loss of function: Small inhibitory hairpin RNAs
7. Novel loss of function/knockdown/knockout technologies
8. Intergenic annotation of the *Xenopus* genome
9. Improvements of the *X. tropicalis* genome – long range contiguity
10. Additions and improvements to Xenbase: the *Xenopus* Model Organism Database
11. Frogbook: A comprehensive resource for methods in *Xenopus* biology

Community Recommendations for Attaining Resources:

The *Xenopus* Community feels that in order to attain these much needed resources it will be imperative to renew the PAR-09-240/1: “Genetic and Genomic Analyses of *Xenopus*”. This mechanism can help to direct funding to the establishment of resources that will accelerate research by the entire community. Development of research resources is essential to the NIH mission, but because such work is not hypothesis-driven, these proposals fare poorly in standard CSR study sections. Moreover, the standard study sections typically lack the depth of expertise that is needed to properly evaluate these proposals. The “Genetics and Genomic Analyses of *Xenopus*” PAR allows for a focused and expert review of resource development proposals, and its renewal will help to ensure a continuing return on the current NIH investment in biomedical research using *Xenopus*.

The *Xenopus* Community also feels that, given the ease with which massive amounts of biological samples can be obtained using this organism, a new PAR to support systems biology using *Xenopus* is warranted. A new PAR in this area would allow all biomedical researchers to exploit the emerging genomic resources for *Xenopus* to perform systems-level analyses *in vivo*, in a vertebrate, and in a cost-effective manner. Such research would generate significant advances into the “New Biology” described below.

Anticipated Gains for Biomedical Research:

Xenopus as an animal model continues to have a broad impact for biomedical research. Given its already long history of large-scale screens of gene function and its broad use in molecular, cell, and developmental biology, the establishment of additional community-wide resources will greatly facilitate the impact of *Xenopus* as a premier vertebrate model for systems-level analyses.

The National Research Council and the National Academy of Sciences have recently called on the United 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 “New Biology.”

Specifically, 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 and their relationship to phenotypes. 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. *Xenopus* offers a unique resource because it is the only *in vivo* vertebrate animal model that couples vast amounts of biological material and a sequenced genome, thus 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 gene-regulatory networks, interactomes, and novel genes will be only the first steps. 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 impact 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 2011 *Xenopus* White Paper,
please visit**

<http://www.xenbase.org/community/xenopuswhitepaper.do>

Appendix

Project Number	Project Title	Activity	Contact Principal Investigator	Organization Name	Total Cost
1K01EB010171-01	IN VIVO STUDIES OF CREATINE KINASE KINETICS BY 31P MAGNETIZATION TRANSFER MRS	K01	BASHIR, ADIL	WASHINGTON UNIVERSITY	\$120,095
5R01EB009355-02	IN VIVO WITH AN ATOMIC MAGNETOMETER	R01	SAVUKOV, IGOR M	LOS ALAMOS NAT SECTY-LOS ALAMOS NAT LAB	\$363,829
5R01EB007969-03	A MICROSURGICAL ASSISTANT SYSTEM	R01	TAYLOR, RUSSELL H	JOHNS HOPKINS UNIVERSITY	\$1,140,677
					\$1,624,601