Impact of the *Xenopus* system on the mission of the NIEHS

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The mission of the NIEHS is to understand how the environment influences development and progression of human disease, and work done with the *Xenopus* model system is applicable to this mission in many ways. Most notably, various aspects of development can be monitored and modulated in the *Xenopus* embryo, and extracts derived from the eggs and oocytes of *Xenopus laevis* have proven to be a powerful biochemical system for a variety of studies.

Cellular mechanisms for maintaining the fidelity of DNA replication. The environment is a source of many types of DNA damaging agents, and numerous studies have linked defects in the DNA damage response to cancer and other diseases. High fidelity in DNA replication requires the ability to cope with and repair DNA damage encountered before or during the course of DNA replication. Studies using *Xenopus* egg extracts have illuminated the intricacies of DNA replication and how this process is affected by DNA damaging agents and other inhibitors of DNA replication. There are clear advantages to studying this essential cellular process at a biochemical level with the *Xenopus* system, and it is the only known biochemical system that recapitulates key aspects of DNA replication and its regulation in vitro. DNA damage signaling and repair pathways have also been studied in this system, and much progress has been made by taking advantage of the unique ability to manipulate individual steps of replication or DNA damage signaling as well as the nature of the DNA substrates. Furthermore, researchers have taken advantage of the extract system to rapidly and successfully screen for small molecule modulators of the DNA damage response and to define their mechanism of action. Such small molecules have the potential to lead to new therapeutics for the treatment of cancer.

Epigenetics. There are an increasing number of studies which suggest that diseases such as autism and cancer may be influenced by the epigenetic state, which can in turn be influenced by the environment. The *Xenopus* system has been used to study basic mechanisms underlying the inheritance of chromatin structure, as well as the effects of changes in chromatin structure on embryo development.

Selected References:

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Mechanism of replication-coupled DNA interstrand crosslink repair. Räschle M, Knipscheer P, Enoiu M, Angelov T, Sun J, Griffith JD, Ellenberger TE, Schärer OD, Walter JC. *Cell.* 2008 Sep 19;134(6):969-80.

Cdc7-Drf1 kinase links chromosome cohesion to the initiation of DNA replication in *Xenopus* egg extracts. Takahashi TS, Basu A, Bermudez V, Hurwitz J, Walter JC. *Genes Dev.* 2008 Jul 15;22(14):1894-905.

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A forward chemical genetic screen reveals an inhibitor of the Mre11-Rad50-Nbs1 complex.
Dupré A, Boyer-Chatenet L, Sattler RM, Modi AP, Lee JH, Nicolette ML, Kopelovich L, Jasin M, Baer R, Paull TT, Gautier J. *Nat Chem Biol.* 2008 Feb;4(2):119-25.

Initiation of DNA replication in *Xenopus* egg extracts. Arias EE, Walter JC. *Front Biosci.* 2004 Sep 1;9:3029-45..

Localization of MCM2-7, Cdc45, and GINS to the site of DNA unwinding during eukaryotic DNA replication. Pacek M, Tutter AV, Kubota Y, Takisawa H, Walter JC. *Mol Cell.* 2006 Feb 17;21(4):581-7.

DNA damage signaling in early *Xenopus* embryos. Peng A, Lewellyn AL, Maller JL. *Cell Cycle.* 2008 Jan 1;7(1):3-6.

A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling.
Wysocka J, Swigut T, Xiao H, Milne TA, Kwon SY, Landry J, Kauer M, Tackett AJ, Chait BT, Badenhorst P, Wu C, Allis CD. *Nature*. 2006 Jul 6;442(7098):86-90.

Xenopus grants funded by the Institute:

According to NIH RePORTER Search Tool, in the fiscal year of 2011, the National Institute of Environmental Health Sciences (NIEHS) funded seven grants for projects involving Xenopus. These grants total to $2,957,876.

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-parkinsonsim – *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	Principal Investigator	Organization Name	Total Cost
1R15ES016856-01A1	ARSENIC ACCUMULATION BY AQUAGLYCEROPORINS AND PHOSPHATE TRANSPORTERS IN ZEBRAFISH	R15	LIU, ZIJUAN	OAKLAND UNIVERSITY	$222,000
5P50ES015905-04	THE ROLE OF AIRBORNE PAHS AND DEP IN THE PATHOGENESIS OF CHILDHOOD ASTHMA	P50	PERERA, FREDERICA P	COLUMBIA UNIVERSITY HEALTH SCIENCES	$1,977,202
3R15ES011130-03S1	MULTIPLE LOW-AFFINITY ARYL HYDROCARBON RECEPTORS IN THE FROG XENOPUS LAEVIS	R15	POWELL, WADE H	KENYON COLLEGE	$25,000
2R15ES011130-03	MULTIPLE LOW-AFFINITY ARYL HYDROCARBON RECEPTORS IN THE FROG XENOPUS LAEVIS	R15	POWELL, WADE H	KENYON COLLEGE	$184,137
1ZIAES044007-10	PESTICIDES AND PARKINSON S DISEASE IN THE AGRICULTURAL HEALTH STUDY	ZIA	SANDLER, DALE	NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES	$136,587
5R01ES017260-02	ATHEROGENIC MECHANISMS OF ARSENIC	R01	SRIVASTAVA, SANJAY	UNIVERSITY OF LOUISVILLE	$329,670
5K99ES016758-02	DNA DAMAGE INDUCED STRUCTURAL AND DYNAMIC CHANGES AT TELOMERES	K99	WANG, HONG	UNIVERSITY OF PITTSBURGH AT PITTSBURGH	$83,280
				Total	$2,957,876