Impact of the *Xenopus* system on the missions 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:

Polycomb proteins remain bound to chromatin and DNA during DNA replication in vitro. Francis NJ, Follmer NE, Simon MD, Aghia G, Butler JD. *Cell.* 2009 Apr 3;137(1):110-22.

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.

The structural determinants of checkpoint activation. MacDougall CA, Byun TS, Van C, Yee MC, Cimprich KA. *Genes Dev.* 2007 Apr 15;21(8):898-903.

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 funding by the NIEHS

According to NIH RePORTER Search Tool, in the fiscal year of 2009, the National Institute of Environmental Health Sciences (NIEHS) **funded 15 grants** for projects involving *Xenopus*. These grants total **$6,085,521**. See appendix for a complete list.

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

Appendix

Xenopus Grants funded by the NIEHS

Project Number	Activity	Project Title	Principal Investigator	Organization	Total
1R01ES017217-01A2	R01	MDIG GENE AND HISTONE DEMETHYLATION IN LUNG CANCER	CHEN, FEI NONE	UNIVERSITY OF KENTUCKY	$296,663
5R01ES016486-08	R01	REGULATION OF THE DNA DAMAGE RESPONSE	CIMPRICH, KARLENE A	STANFORD UNIVERSITY	$324,064
5F30ES016504-03	F30	REGULATION OF WNT SIGNAL TRANSDUCTION BY FLAVONOIDS.	HANSON, ALISON HANSON	VANDERBILT UNIVERSITY	$25,739
4R00ES017044-03	R00	METAL-REGULATORY FACTOR 1 (MTF-1) ROLE IN DEVELOPMENT AND STRESS RESPONSE	JENNY, MATTHEW J.	UNIVERSITY OF ALABAMA IN TUSCALOOSA	$240,708
1R15ES016856-01A1	R15	ARSENIC ACCUMULATION BY AQUAGLYCEROPORINS AND PHOSPHATE TRANSPORTERS IN ZEBRAFISH	LIU, ZIJUAN	OAKLAND UNIVERSITY	$222,000
3R15ES016856-01A1S2	R15	ARSENIC ACCUMULATION BY AQUAGLYCEROPORINS AND PHOSPHATE TRANSPORTERS IN ZEBRAFISH	LIU, ZIJUAN	OAKLAND UNIVERSITY	$15,700
1ZIAES048002-22	ZIA	STATISTICAL MODELS IN TOXICOLOGY AND BIOCHEMISTRY	PORTIER, CHRISTOPHER J		$1,606,782
3R15ES011130-03S1	R15	MULTIPLE LOW-AFFINITY ARYL HYDROCARBON RECEPTORS IN THE FROG XENOPUS LAEVIS	POWELL, WADE H	KENYON COLLEGE	$25,000

5P01ES011624-07	P01	RESEARCH PROJECT 2: ROLE OF CK2 AND THE WNT SIGNALING PATHWAY IN THE PROGRESSION	SELDIN, DAVID C	BOSTON UNIVERSITY MEDICAL CAMPUS	$281,384
5R01ES004106-23	R01	DNA REPAIR IN A HORMONE RESPONSIVE GENE	SMERDON, MICHAEL J	WASHINGTON STATE UNIVERSITY	$320,908
5R01ES013686-03	R01	MOLECULAR DETERMINANTS OF PYRETHROID NEUROTOXICITY	SODERLUND, DAVID M	CORNELL UNIVERSITY ITHACA	$256,564
3P42ES007373-15S1	P42	PROJECT 8: ARSENIC AND THE UBIQUITIN-LYSOSOMAL PATHWAY	STANTON, BRUCE A.	DARTMOUTH COLLEGE	$17,002
3P42ES007373-15S2	P42	PROJECT 8: ARSENIC AND THE UBIQUITIN-LYSOSOMAL PATHWAY	STANTON, BRUCE A.	DARTMOUTH COLLEGE	$48,106
2R01ES010845-06A1	R01	METABOLISM AND TOXICITY OF ARSENIC IN THE HUMAN LIVER	STYBLO, MIROSLAV	UNIVERSITY OF NORTH CAROLINA CHAPEL HILL	$340,472
1ZIAES090089-13	ZIA	MODULATION OF NEURONAL CHANNELS AND RECEPTORS IN THE BRAIN	YAKEL, JERREL L		$2,064,429
				Total	$6,085,521