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:**


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


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:
Colorectal cancer -  Genome Res. 2009.  19, 987-93.
Xenopus egg extracts are used for in vitro biochemical studies:
- BRCA1 - Cell. 2006. 127, 539-552

Xenopus oocytes are used to study gene expression and channel activity:
- Catastrophic cardiac arrhythmia (Long-QT syndrome) - PNAS. 2009. 106, 13082-7.

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-β signal transduction.
- Xenopus egg extracts revealed fundamental aspects of cell division.
- Xenopus embryos were used for studying mucociliary epithelia.
- 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.
- Xenopus embryos linked telomerase to Wnt signaling.
- Xenopus was used for small molecule screens to develop therapeutics.

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 site-specific 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 facilitate 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 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](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](http://www.xenbase.org/community/xenopuswhitepaper.do)
## Appendix

### Xenopus Grants funded by the NIEHS

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Total: $6,085,521