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

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The primary mission of NHGRI is to bring a genomic approach to the translation of genomic sequence information into health benefits. NHGRI has outlined a vision for the future of Genomic Research which encompasses three major themes: (I) Genomics to Biology; (II) Genomics to Health and (III) Genomics to Society. Each one of these themes further defines several grand challenges and research targets for the scientific community aimed at facilitating new achievements that would lead to substantial advances in genomic research and its applications to medicine. Several of the grand challenges outline the need to identify and catalog all the structural and functional components encoded in the human genome and to determine the organization of the genetic and protein networks. Comprehensively research aimed at understanding the building blocks of the human genome will eventually help us to understand how each component contributes to the cellular and organismal phenotype, and how evolutionary variation modifies phenotypes and contributes to susceptibility to disease.

Capabilities developed and optimized for model organisms will contribute substantially to efforts to catalogue, characterize and comprehend the entire set of functional elements encoded in the human genome. Compiling this genome 'parts list' represents an immense challenge that will preoccupy decades of research to come. Even the well-known classes of functional elements, such as protein-coding sequences, still cannot be accurately predicted from sequence information alone. Comparison of genome sequences from evolutionarily diverse species has emerged as a powerful tool for identifying functionally important genomic elements. Initial analyses of available vertebrate genome sequences have revealed many previously unidentified protein-coding sequences. Cross-species sequence comparisons have revealed large numbers of homologies outside of known or predicted protein regions, the majority of which are of unknown function. In particular, since *Xenopus* is a unique biological resource for cell and developmental biology, the advancement of genomic tools and resources for the frog genome will directly contribute to the identification and characterization of novel genes with as of yet unidentified function.

While funding has been allocated for the production of *Xenopus* expression tag sequences (ESTs), full-length *Xenopus* cDNA libraries and *Xenopus* microarrays, additional funding to generate a comprehensive *Xenopuse* ORFeome library will create a powerful resource that would benefit not only members of the *Xenopus* community but also members of the wider community of genomics researchers. The *Xenopus* model system has been at the forefront of expression cloning and functional analysis of protein function via gain-of-function experiments. To obtain insights into human gene function, similar assays can be employed to evaluate human transcript activity in *Xenopus* oocytes. Using evolutionary comparisons, a priority for funding would be for examining human transcripts that are highly orthologous in frog, and examine their putative roles during early embryonic development by gain- and loss- of function. Human and frog expression clones can be tested in parallel in gain-of-function experiments and *Xenopus* morpholinos can be subsequently tested in loss-of-function experiments to determine if such genes play critical roles during embryonic development.

Mammal-to-mammal sequence comparisons have revealed large numbers of homologies in non-coding regions, some of which may play important functions in transcriptional regulation. Functional diversification through transcriptional regulation represents one of the hypotheses for phenotypic differences among species. Comparisons of sequences derived from multiple species, especially those occupying distinct evolutionary positions, could lead to significant refinements in our understanding of the functional importance of conserved sequences, in particular regarding to gene expression patterns. NHGRI has a strong interest in the development of novel tools and approaches for characterizing transcriptional regulatory
elements. The recent successes in *Xenopus* transgenesis provide a unique opportunity for transforming the frog into a new inexpensive and efficient *in vivo* transgenic system that would complement, or even replace the current gold standard of mouse transgenesis. The relative large size of *Xenopus* embryos coupled with external development that allows one to monitor events that occur shortly after fertilization would permit the characterization of embryological events that are almost impossible to study in the mouse. In addition transgenesis will allow later embryological events, such as organogenesis to be amenable to molecular analysis in the frog and combine transgenesis with other molecular or embryological manipulations that are routine in the frog. Funding that would facilitate the development of high throughput transgenic technologies in the frog that increase reliable functional characterization of conserved non-coding elements would be of great value to the entire scientific community.

The *Xenopus* community has already greatly benefited from the recently emerging genetic and genomic resources made available for the *Xenopus Tropicalis* and *Laevis* genomes. Among the non-mammalian model organisms advocated for biomedical research, *Xenopus* continues to be underrepresented, despite its tremendous potential to contribute to the advancement of biomedical research. Future tools and resources will further improve *Xenopus’* ability to contribute to the elucidation of the cellular, molecular and genetic mechanisms that control embryonic development, in particular the following resources gaps would highly parallel and contribute to NHGRI’s mission:

1. ORFeome: comprehensive catalog of all full length *Xenopus* transcripts that can be used in expression assays to determine function in *Xenopus* embryos.
2. Improving transgenic technologies: high throughput assays that can be used for robust regulatory element characterization
4. Develop novel methods for real-time measurement of transcripts and proteins. Improve the ability to monitor multiple protein interactions at the same time to aid in network elucidation and establish the temporal and cellular distribution of proteins.
5. *Xenopus laevis* and *tropicalis* comparisons provide unique opportunity to understand evolutionary variation between two closely related species both at protein and gene regulatory level. Lessons learned from frog could become paradigm for other types of evolutionary events that have separated other species.
6. Use the large emerging collection of mutant frogs to study effects of sequence variation and phenotyping impact. By combining mutagenesis with allelic series can be generated that would provide a valuable resource for the study of single nucleotide effects on potential disease genes.

**References:**


Multiple ING1 and ING2 genes in Xenopus laevis and evidence for differential association of thyroid hormone receptors and ING proteins to their promoters. Wagner MJ, Helbing CC. Biochim Biophys Acta. 2008 Mar; 1779(3):152-63.


**Xenopus Grants funding by the NHGRI**

According to NIH RePORTER Search Tool, in the fiscal year of 2009, the National Human Genome Research Institute (NHGRI) funded 2 grants for projects involving *Xenopus*. These grants total $528,976. 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:

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 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 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](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 NHGRI**

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Total: $528,976