Xenopus Community White Paper 2009

Contents:

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.

Introduction:

As outlined in the Executive Summary, *Xenopus* is an outstanding model for understanding human health and disease, both by elucidating basic fundamental mechanisms of human biology and by the characterization of human disease genes. However, the *Xenopus* community strongly feels that the pace of discovery using *Xenopus* could be further accelerated if additional critical resources were available. The goal of this White Paper is to outline these resources, justify their need, and provide a preliminary plan on how to obtain them.

First, to identify the resources needed by the broad and diverse *Xenopus* community, representatives of the *Xenopus* Community met in June 2009 and again in November 2009 at the MBL in Woods Hole, MA and established three overlapping committees to gather input and set priorities concerning resource needs in the following areas: Developmental Biology, Cell & Molecular Biology, and Genetics/Genomics:

Steering Committees:

Develomental Biology:	*Cell & Molecular Biology:*	*Genetics/Genomics:*
Frank Conlon, Rob Grainger, Richard Harland, Mustafa Khokha, Karen Liu, John Wallingford, Aaron Zorn	Bill Bement, Jean Gautier, Todd Stukenberg, John Wallingford, Yixian Zheng	Frank Conlon, Rob Grainger, Mike Gilchrist, Richard Harland, Mustafa Khokha, Nicolas Pollet, Aaron Zorn

With this new organizational structure, the *Xenopus* Community intends to improve communications between the entire spectrum of *Xenopus* investigators and the NIH.

This White Paper reports on currently un-met resource needs identified by the *Xenopus* community. The initial draft of the document was prepared by members of the Steering Committees, as listed above. The document was posted for a comment period of 6 weeks. During the comment period, over 160 investigators (representing over 80 R01 level grants) read the document, contributed comments, and added their names as signatories. A full list of Investigators who have read and agree with the conclusions of this document is included in Appendix 2. The report includes a review of progress that has been made on the goals of the previous White Paper, the consensus recommendations on additional resources that are currently needed, and proposals to best achieve those goals.

We identified seven community-wide resources that fall into two main categories.

Immediate Needs:

1. *Establishment of the Xenopus Resource and Training Center at the MBL in Woods Hole.*
2. *Expansion and improvement of Xenbase, the Xenopus model organism database.*
3. *Complete sequencing of the Xenopus laevis genome.*

Essential Resources:

4. *Xenopus ORFeome in recombineering vectors.*
5. *Improvement of the X. tropicalis genome sequence.*
6. *Development of novel methods for disrupting gene function in Xenopus*
7. *Generation and Distribution of antibodies for Xenopus research.*

Finally, the important and continuing contributions of *Xenopus* research to the missions of the various Institutes within the NIH are outlined on pages 20-66 of this document.

1. The *Xenopus* Resource and Training Center (XRC) at Woods Hole.

1A. Summary:

While there are centralized resources for other major model organisms (*Drosophila*, mouse and zebrafish), there is no center for *Xenopus* in the United States (US XRC), and such a center is simply essential for continued scientific success. A European Stock Center for *Xenopus* has recently opened in Portsmouth, UK. While valuable for European researchers, the challenges of trans-Atlantic shipping makes it of limited utility for U.S. researchers. The US XRC will provide both a stock center and a training facility to instruct investigators at all levels in the latest technologies and methods in *Xenopus*. The European Center does not have this training focus, which would be a unique and invaluable component of the US XRC.

1B. Why establish a Xenopus stock center?

A primary motivation for creating a *Xenopus* Resource Center (XRC) stems from the recent developments in the *Xenopus* system that further enhance its utility as a model system: 1) the development of a multitude of transgenic, mutant, and inbred lines; 2) the urgent need to find a place to house them; 3) and the pressing need to generate additional lines required by the community (for both *X. laevis* and *X. tropicalis*). In addition, as with researchers working on other model organisms, contemporary biological research has become ever more complex, often requiring not only specialized animal lines, but also requiring the application of diverse technologies, such as advanced imaging, genetic manipulations, high-throughput biochemical approaches, and new genomic tools. The XRC would serve as a Training Center, where investigators or trainees could visit to learn these techniques. Moreover, a *centralized* location for specialized minicourses will be very useful for the *Xenopus* community. Therefore, the XRC has two main missions:

1. Raise, house, and distribute *Xenopus* lines to investigators
2. Hold minicourses where the latest in specialized technology for *Xenopus* can be rapidly disseminated to *Xenopus* researchers.

The pressing need for such a Center has been recognized by *Xenopus* researchers for over 10 years. Researchers have met on a regular basis during the past decade to formulate priorities for community resources in conjunction with NIH officials, generating many of the genetic and genomic initiatives mentioned above. A Resource Center has consistently been recognized as a top priority from this group. The *Xenopus* Community feels strongly that the time is right for establishing the XRC and that the MBL in Woods Hole, MA is the ideal place. The need for the XRC is immediate and would benefit the entire Community of *Xenopus* researchers, facilitating their efforts to use *Xenopus* to better understand human health.

1C. Why Woods Hole?

The community felt that the MBL would be an ideal location for such a national resource, and the MBL readily agreed to serve as the host institution. Subsequent discussions focused on where and when such a facility might be located at the MBL. Between submission of the first P40 application to establish the XRC and the revised application, the MBL received funding to renovate its Loeb Laboratory Building, and it was felt that this was an unparalleled opportunity to create the XRC within newly renovated and purpose-built space on the ground floor of Loeb, where it would have tremendous infrastructural support and be centrally located nearby to both teaching, training, and research facilities, allowing it to easily support both functions in completely remodeled space. Planning for the XRC has thus been carried out in parallel with planning for the Loeb renovation, with the commitment of the MBL to providing this renovated

space in anticipation of establishing this center at the MBL when the Loeb building is completed in 2010.

1D. How to Proceed:

In 2008, a group of *Xenopus* investigators assembled the initial P40 application to fund the proposed XRC, with Robert Grainger as PI and Jonathan Henry as proposed director of the Center. The review of a revised application was highly favorable, with an impact/priority score of 17. While the NIH scoring system used for this application is still new, the feedback we have received is that this is a fundable score, with a final Council decision due in the fall of 2009 or early in 2010.

In discussions with NCRR officials, it has become clear that the proposed budget of the P40 application is sufficiently high ($500,000 for each of five years) that it would be highly advantageous, to assure the funding required for full operation of the Center, to seek some funds from another source. Therefore, a G20 application was submitted in Sept 2009, with Joshua Hamilton of the MBL as PI to provide resources to complete the renovation of Loeb and create the XRC. The *Xenopus* Community feels strongly that funding both the P40 and G20 application to create a XRC is essential for the *Xenopus* Community to move forward both by providing critical animal stocks to the Community as well as disseminating the latest technologies to *Xenopus* users as they become available.

2. Expansion and improvement of Xenbase, the *Xenopus* model organism database.

2A. Summary:

One of the highest priorities of the *Xenopus* community is to maintain and enhance *Xenbase*: the *Xenopus* model organism database. In this post-genomic era, accurately annotated and easily-accessible databases are essential for researchers to integrate vast amounts of sequence, expression and functional data into a meaningful biological synthesis. Such a state-of-the-art database is vital for the acceleration of biomedical research using *Xenopus*.

2B Why expand Xenbase?

With strong support from the *Xenopus* community, as outlined in previous white papers, an R01HD045776 was awarded to Peter Vize in 2005 to develop *Xenbase*. *Xenbase* is now a comprehensive database that provides a portal to inter-related *Xenopus* data including genomic, mRNA, protein and EST sequence, gene expression, function and publications, all of which is highly integrated with NCBI, the JGI-genome browser, and Metazome. *Xenbase* integrates these diverse data and presents them in a simple efficient manner tailored to the needs of the *Xenopus* researcher; something that more general databases do not provide. However, because *Xenbase* uses the GMOD (Generic Model Organism Database) modular schema called Chado it is compatible with, and links to, other model organism databases and is therefore also accessible to the broader biomedical research community. *Xenbase* also provides important services such as overseeing *Xenopus* gene nomenclature and provides weekly annotated data dumps to NCBI, UniProtKB and Metazome. Finally, *Xenbase* hosts a website posting community announcements, news letters, a community white pages as well as providing information on protocols, fate maps and anatomical images.

In addition to *Xenbase* there are a number of other excellent specialized *Xenopus* databases outside of the US that focus on gene expression and EST clustering including: 1) Dr. Mike Gilchrist's full length EST clusters and the *XenMark* image search engine at the NIMR in the UK, 2) Dr. Naoto Ueno's *XDB3* in Japan and 3) Dr. Nicolas Pollet's *Axeldb* in France.

Reciprocal data sharing collaborations exist between these databases and *Xenbase*, with *Xenbase* providing a single common access point integrating all of the data.

As a result of the NICHD funded R01 (which ends in March, 2010), *Xenbase* is now solidly established as the primary *Xenopus* database, and it is essential for the research community. The goals of the *Xenbase* R01 were met with the design of this highly integrated database. What is needed now is to populate the database with the vast array of diverse types of data available in the public domain, much of which is not yet in *Xenbase*. Moving forward it is also clear that there are ways to improve the utility of Xenbase even further by introducing more extensive features.

Specifically the ~15,000 automatically generated gene pages in *Xenbase* now need to be populated with manually curated data including annotated gene expression, gene function, phenotypes and gene-interactions. In addition, the *X. tropicalis* genome assembly predicts that ~10,000 more gene pages remain to be developed. While Xenbase has well-designed automated pipelines for data acquisition, much of the more sophisticated data that researchers need such as literature, 3-D gene expression and phenotypes all require manual curation, which is well beyond the capacity of the current *Xenbase* staff. In addition the community has identified a number of areas where further improvements to *Xenbase* are needed to meet the ever-accelerating demands of *Xenopus* research.

2C. How to proceed?

Continued funding is required to maintain *Xenbase* operations and to further develop this critical resource. The Xenbase project has clearly moved beyond the R01 mechanism. Therefore, to meet the goals of the community, a **Multi-PI P41 application (HD 064556) was submitted by Drs. Peter Vize and Aaron Zorn in May 2009 in response to the PA-08-180 to maintain and improve *Xenbase*.** The Aims of the P41 application are:

1) Maintain *Xenbase* and curate *Xenopus* data (literature and gene expression).
2) Enhance the functionality of *Xenbase* by introducing a phenotypes feature.
3) Support new content on *Xenbase* (support proteomics data including protein function and protein-protein interaction data, new genome browser, and Wiki)
4) Continue and expand collaborative and service efforts (provide *Xenopus* data to other databases including NCBI, UniProtK, Mascot and Tornado)

The Xenbase P41 (HD06556) application was reviewed in October 2009; it received an excellent priority score of 20 and as of Nov. 09 is awaiting a funding decision. The *Xenopus* Community strongly endorses the continued development of Xenbase and feels that funding of the P41 application is essential to continue to make this critical resource available to *Xenopus* users and to improve it.

2D. What are the anticipated outcomes?

The first outcome of sustained Xenbase funding will be to maintain operations of this critical resource, which is used by most *Xenopus* research labs on a daily basis. The loss of Xenbase would be a major set back to the community. The enhanced funding requested in the P41 application will also allow Xenbase to develop important new features. As a result Xenbase will provide the broad *Xenopus* research community (cell and developmental biologists, neurobiologists and physiologists) with a single web-based portal to search many diverse and inter-related data sets that are uniquely tailored to their specific needs, all of which will be seamless linking to orthologous data sets in humans and other model organisms. As such Xenbase will increasingly facilitate the next generation of “system biology” type analyses. A final important outcome of the enhanced Xenbase will be its increasing service role in providing

curated up to date data to other more general data bases (NCBI). This has the added benefit of make Xenopus data more widely visible and accessible to the larger biomedical community.

3. Sequencing of the *Xenopus laevis* genome.

3A Summary:

A high-quality *Xenopus laevis* genome assembly is essential for the advancement of research in molecular, cell, and developmental biology and will allow researchers to take full advantage of the strengths of the *Xenopus* system. Moreover, an *X. laevis* genome assembly will promote the integration of genomic, biochemical, and cell biological approaches in a way that is not feasible in any other vertebrate model system. As such, it is among the very highest priorities across the *Xenopus* community.

3B. Why Sequence Xenopus laevis?

Given the large number of researchers that use *Xenopus laevis* for studies in cell, molecular, and developmental biology, as well as the key contributions from research on *Xenopus* to our understanding of cell cycle control, gene regulation, and cell signaling in early development, it is perhaps surprising that the *X. laevis* genome has not yet been sequenced. In 2002, when *X. tropicalis* was chosen for sequencing by the Department of Energy's Joint Genome Institute, sequencing the genome of *X. laevis* appeared to be too costly, given its larger size and genomic complexity compared to that of *X. tropicalis*. Indeed, at the time, it was uncertain whether the alloalleles in the genome could be separately assembled, or whether the tetraploidy would confound a high quality draft assembly. In contrast, *X. tropicalis* is diploid and has one of the smallest genomes amongst the tetrapods at $1.5x10^9$ bp. Moreover, ongoing genetic analyses in *X. tropicalis* have been greatly aided by the availability of genomic sequence.

Sequencing and assembly of the *X. laevis* genome is now feasible, in view of recent advances in sequencing technology, advances in genome assembly, and the evidence that the alloalleles are substantially different in sequence so that they will be easily separated. Although *X. laevis* researchers have been able to use *X. tropicalis* genomic sequence to identify complete gene sets, determine orthologous tetrapod genes especially given the remarkable synteny between frogs and mammals, and identify putative *cis*-regulatory modules based on the conservation of non-coding sequences, *X. laevis* genomic sequence would provide critical information that cannot be obtained from the *X. tropicalis* genome. In the long term, sequencing and assembly of the *X. laevis* genome will advance research using this model system in several specific ways.

First, *X. laevis* sequence would provide a complete gene set to deconvolute peptide fragments for proteomics work. This is especially relevant to the many *Xenopus* researchers investigating the biochemistry of cell cycle regulation. Because of its massive size, the *X. laevis* oocyte/egg is the premier vertebrate system for biochemical analysis. However, even small changes in protein sequence (as is present between *X. tropicalis* and *X. laevis*) can significantly change mass spectrometry data, making it difficult to identify the isolated *X. laevis* protein. Second, *X. laevis* genomic sequence would be extremely useful for designing morpholino oligonucleotides for loss-of-function studies, both to target the A and B alloalleles in this allotetraploid organism and also to design splice-blocking morpholinos. Third, genome sequencing for *X. laevis* would provide a comprehensive set of untranslated regions (UTRs), which would greatly aid studies of translational control. Here again, *X. laevis* is the chief model system for the analysis of translational control in vertebrates. Fourth, *X. laevis* genome sequence would allow investigators to monitor DNA transactions occurring at a specific genomic locus. This includes assessing the consequences of targeted DNA damage (coupled with chromatin immunoprecipitation -ChIP) or monitoring DNA replication pattern and timing (DNA

combing coupled with FISH). In addition, genome-wide ChIP would provide invaluable information on the distribution of transcription factors, replication and repair proteins and chromatin modifications during development and during the cell cycle. Fifth, *X. laevis* genome sequence would be critical for identifying genes for noncoding RNAs, which is necessary to understand their biogenesis and mode of action; these findings in turn would contribute to studies of chromatin organization, as well as transcriptional and translational regulation. Finally, the *X. laevis* genome has undergone a genome duplication sufficiently recently to provide a unique opportunity to study the effects of genome duplication on gene expression and evolution. Genomic sequencing of the duplicated genome would provide critical insights into these processes.

3C. How to proceed?

Sequencing and assembly of the *X. laevis* genome is a multi-step process. While in the past, the size and complexity of the *X. laevis* genome may have seemed intractable, advances in sequencing technology offer new strategies. Moreover, the *X. tropicalis* genome provides a template for the assembly of a *X. laevis* sequence, and further improvements to the *X. tropicalis* genome will aid in *X. laevis* genome assembly (see below). Focused efforts are now underway in the US, Japan, and elsewhere to carry out specific aspects and/or test strategies for *X. laevis* genome sequencing, and continuing coordination and communication among participating laboratories are essential.

Below we outline a multi-step approach to *X. laevis* genome sequencing:

1. **Transcriptome Sequencing**
   One critical goal of sequencing the *X. laevis* genome is to identify all the ORFs in order to create an ORFeome and provide critical protein sequence information for deconvoluting mass spectrometry data. One rapid and cost-effective strategy would be to use massively parallel sequencing strategies to assemble the transcriptome from critical stage-specific libraries.
2. **Gene-based Assembly**
   As read length improves for platforms such as Illumina and 454, it will be possible to sequence *X. laevis* genome DNA directly using these platforms and then aligning to the *X. tropicalis* genome. For example, using the Illumina platform, sequence can be generated at approximately 200kb/$1, a cost which is plummeting rapidly. The sequence reads in this platform were initially very short (20-30 bp) but are now at 75 bp/read with paired ends. At this read length, *X. laevis* sequence information can then be aligned to *X. tropicalis* where exons are expected to have a 90-94% identity based on mRNA alignments. This alignment of *X. laevis* reads onto the *X. tropicalis* genome, together with comparisons with full-length *X. laevis* cDNA sequences and transcriptome sequence assemblies, would greatly simplify the assembly of *X. laevis* sequence. Then paralogous sequences can be deconvoluted from these alignments to determine the two copies that result from genome duplication.
   At a minimum, a preliminary draft sequence based on the strategies outlined above would lead to an assembly of the *X. laevis* exon sequence as well as approximately 100bp of intron-exon boundary. The assembly is very likely to progress sufficiently far into adjacent intronic, or non-coding sequence to permit a gene-scale assembly, resolving the alloalleles fully. Such an assembly would provide the critical exomic information necessary for peptide deconvolution and morpholino design as well as important information on genome duplication events.
3. **Complete Sequencing and Assembly of the *X. laevis* genome**
   Strategies to generate long-range assembly would presumably concentrate on BAC sequencing and alignment with both the exon-based sequences and the *X. tropicalis* assembly.

Although with sufficient coordination, much of this work can be carried out by groups of investigators from the *Xenopus* community, the process would greatly benefit from the input and participation of an established genome center, particularly in the areas of long-range assembly, data integration, and dissemination of the genome assembly to commonly used databases.

4. Generation of a *Xenopus* ORFeome in recombineering vectors.

4A. Summary:

We propose to generate a *Xenopus* ORFeome, which we define as a fully sequenced, validated set of *Xenopus cDNA* clones containing one each of every open reading frame (ORF) encoded in the genome, in a format in which any ORF sequence can be easily transferred into a diverse array of expression vectors. This one set of reagents will greatly decrease the time *to characterize* any protein in the myriad of functional assays carried out by the entire *Xenopus* community. But most importantly, an ORFeome set would allow high-throughput *in vivo* functional-genomic screening in manner currently not feasible, but would take advantage of a particular strength of the *Xenopus* system. These clones are to be made available, without restriction, to researchers worldwide.

4B. Why an ORFeome?

ORFeomes facilitate rapid functional characterization of proteins. In current research pipelines, the rate limiting steps have changed from the identification of new proteins in a process to the initial functional characterizations of entire sets of such candidates. This is because new methodologies for high throughput screens and proteomics have greatly increased our ability to identify new proteins that are involved in a given process. As outlined above, *Xenopus* has great advantages for screening, so the ORFeome will only amplify these advantages. But more importantly, there is a pressing need to *shorten* the time required for the characterization of proteins in any specific biological process (validating function, validating interaction partners, establishing subcellular localizaton, etc.). An ORFeome would directly address this need, as it would significantly decrease the time required to generate the various reagents needed to test the role of any protein in the genome in a very large number of assays. Because it is so easily manipulated for rapid protein characterization, the rate-limiting step in *Xenopus* is often producing the correct expression clone.

An ORFeome is especially useful for studies in *Xenopus*, because one of the key assets of this model system is rapid and powerful functional genomics. Indeed, since high-throughput functional genomics studies are a cornerstone of the newly-emerging field of systems biology, it should be noted that large-scale, genome-wide screens of gene function have been carried out in *Xenopus* for nearly 20 years, leading to a myriad of important discoveries. The *Xenopus* Community performs such large-scale functional genomic screens on an ongoing basis and a clone set that will allow new flexibility and greater genomic coverage in performing these screens is in great demand.

The critical feature of *Xenopus* that allows for rapid large-scale functional genomic screens is the ease with which proteins can be expressed in *Xenopus in vivo* (in eggs or embryos) or *in vitro* (in cell-free extracts) by the introduction of *in vitro* transcribed mRNA. Proteins can also be easily expressed with temporal and spatial control *in vivo* by targeted injection based on the well-established *Xenopus* fate map, introduction of plasmid cDNAs, or by transgenesis. *Xenopus* thus provides a simple, very rapid, and very cost-effective system for protein expression studies.

Importantly, protein expression in *Xenopus* facilitates not only misexpression studies, but also can be combined with epitope- or fluorescent protein-tagged constructs, thereby allowing imaging-based protein localization studies and biochemical protein-protein interaction studies.

Finally, since *Xenopus* oocytes and embryos readily yield massive amounts of biological material, generation of the *Xenopus* ORFeome would propel *Xenopus* to the premier system for high-throughput proteomics.

*4C. Why a **Xenopus** ORFeome?*

While proteins from other species can be effectively expressed in *Xenopus*, scientific rigor demands that we perform *Xenopus* experiments with *Xenopus* proteins. From a more practical standpoint, consider the case where an experiment using a human protein in *Xenopus* gives a totally unexpected result or contradicts previous data (as has been documented by many members of the *Xenopus* Community including authors of this White Paper [Wallingford, Stukenberg]). In every such case, the investigator must then obtain the *Xenopus* protein and rule out the heterospecific protein as a cause for the confusing result. We expect proteins to co-evolve and that tight interactions between species-specific proteins may be different across different species. In fact, by comparing the evolutionary similarities and differences between human and frog proteins, we can identify domains that lead to functional differences allowing us to better understand both human and frog biology. However, it is essential to build a *Xenopus* ORFeome to do so.

An additional need met by generation of a *Xenopus* ORFeome is that currently, many important proteins cannot be studied efficiently, as no full-length clones exist. Indeed, authors of this White Paper have repeatedly found proteins of interest for which there is no available full-length clone, despite the >1 million *Xenopus* ESTs.

Finally, a very important benefit of the *Xenopus* ORFeome will be the ready availability of clones for all *Xenopus* genes for *in vivo* gene expression analysis by *in situ* hybridization. The first step in characterizing a protein is to define where it is expressed, and the ORFeome will provide an important reagent for those analyses.

4F. How should we proceed?

To facilitate expression studies, one priority identified in the 2006 *Xenopus* white paper was to generate collections of full-length cDNAs for *X. laevis* and *X. tropicalis* genes in expression-ready vectors such as CS2+ or CS10X. As part of the trans-NIH MGC-CGAP project, the *Xenopus* Gene collection (XGC) was established. At the conclusion of that project in September of 2008 ~9,500 *laevis* and ~7,300 *tropicalis* full-length cDNA clones had been re-arrayed and completely sequenced. This represents approximately one third of the *Xenopus* transcriptome. Individual clones and re-arrayed plates are now available from authorized distributors such as Open Biosystems.

While this has been an immensely valuable resource, not all of the clones are in expression ready vectors (in fact, more than 12 different plasmid vectors have been used). In order to realize the full potential of this resource, the community feels that the critical next step will be to develop a *complete* collection of full-length Open Reading Frame (ORF) clones and that these should be in a standardized, multifunctional GATEWAY vectors.

It is essential to generate the ORF clones in GATEWAY “entry” vectors (or similar technology) so that they can be shuttled in a high-throughput manner into a variety of other modified “destination” vectors many of which will be developed specifically by the Community, thus dramatically increasing the versatility of the ORFeome resource.

We envision a large array of destination vectors, including:

N- and C- terminal epitope tags (myc, flag, his)
N- and C- terminal purification tags (GST, TAP, LAP)
N- and C- terminal fluorescent protein tags (eGFP, mCherry)
Transgenesis vectors (for insertion of ORF clones downstream of commonly-used promoters for expression in specific tissues).

A project of this scale must be done in collaboration with experts who have the knowledge and infrastructure to do it right. Groups who have completed the *C. elegans* and human ORFeomes have established a pipeline for collaboration (http://www.orfeomecollaboration.org/), and we would seek to coordinate the *Xenopus* ORFeome project with this group and with the NIH. Alternatively, the ORFeome could be generated via a fee-for-service agreement with Invitrogen (maker of the Gateway system).

As a starting point, we would explore the possibility that the current Full-length clone set for both *laevis* and *tropicalis* should be PCR amplified and transferred to Gateway vectors. In addition, unrepresented ORFs predicted from the *Xenopus* genome would be cloned and added to the collection. Given that the *tropicalis* genome is now complete, generation of unrepresented ORFs should begin with *tropicalis*, and *laevis* to follow as the genome (or transcriptome) data becomes available.

5. Improvement of the *X. tropicalis* genome sequence and annotation

5A. Summary:

The *X. tropicalis* genome has been extremely useful to *Xenopus* Community members worldwide. Improving the *X. tropicalis* genome assembly, in terms of both local and long-range contiguity, will significantly increase the number of complete gene loci further making it even more useful for *Xenopus* investigators.

5B. Why improve the X. tropicalis genome?

The Department of Energy's Joint Genome Institute (JGI), acknowledging that *Xenopus* occupies a unique position among vertebrates, expressed interest in sequencing the *Xenopus tropicalis* genome. A white paper was submitted with strong Community support for a sequencing effort, and sequencing began in 2002. The JGI has carried out sequencing of the *X. tropicalis* genome using the DNA of a single seventh generation inbred N strain female. This sequencing effort has produced an outstanding resource for *Xenopus* researchers and the *Xenopus* Community applauds their crucial contribution to our field.

The *X. tropicalis* draft genome sequence described here was produced from ~7.6-fold redundant random shotgun sampling of genomic DNA. The Xentr4 assembly spans about 1.51 Gbp of scaffolds, including gaps between contigs, with half of the assembled sequence contained in 272 scaffolds ranging in size from 1.56 to 7.82 Mb. Ubiquitous long tandem arrays of ~30-200 bp repetitive elements and incomplete coverage from the partial-digest BAC libraries limited the range of the sequence assembly. Nevertheless, 95% of known genes are recovered in the assembly, attesting to its relative completeness in genic regions. Using a first generation genetic map derived from ~2,200 microsatellite markers {Sater and Wells, unpublished results} and taking advantage of extensive conservation of gene order between frog and human, nearly 1 Gb of the assembly can be assigned to chromosomes, enabling map-based cloning approaches. The genome sequence has been complemented by NIH- and internationally-funded cDNA sequencing projects resulting in nearly two million *Xenopus* ESTs (two-thirds from *X. tropicalis*, one-third from *X. laevis*) from a diverse sampling of developmental stages and adult organs and tissues. This has facilitated genome annotation and enabled studies of alternative splicing, digital expression profiling, and microarray design.

While the *X. tropicalis* genome project has been very successful to date, there are still some significant limitations with the sequence data and genome annotation. For example, we estimate that the mean distance between in-scaffold gaps is 8.6k, which, in combination with the large number of smaller scaffolds, suggests that significant numbers of genes will be poorly modeled or missing due to fragmentation of the assembly. An analysis of the 28,704 Ensembl

transcripts generated by gene modeling on this assembly (last updated Jan. 2009) suggests that 14,417 gene models (50%) have one or both ends of the open-reading frame truncated or ill-defined, and an additional 8,926 (31%) have a complete open reading frame but are missing one or both UTRs. The number of genes that are missing from the assembly or are not modeled is not known. We can estimate these from EST assembled mRNA sequences, where we find that about 5% of the EST assembled sequence fails to find a match on the genome sequence. This leads us to believe that there may be up to several thousand genes of the expected tetrapod gene set that are missing or cannot be modeled from the current assembly. Resolving the incomplete gene models and identifying the missing genes are essential for properly defining the full complement of *Xenopus* genes, which in turn is essential for assembling a complete collection of full-length clones (see ORFeome). Thus improved genome sequence and gene annotation are critical to the *Xenopus* community.

The *X. tropicalis* genome also lacks long-range contiguity. This is an acute problem for the developing *Xenopus* genetics community. In forward genetic screens without genome contiguity, it becomes difficult to identify additional linkage from a loosely linked marker in order to identify the mutated gene. Improving long-range contiguity of the *X. tropicalis* genome will facilitate *Xenopus* genetics significantly. For mapping resolution, cloning of mutations from forward genetics is dependent entirely on adequate numbers of meioses. In *Xenopus*, meiotic resolution is deeper than any other vertebrate genetic system (a single ovulation from a female can produce >2000 informative meiotic events). As a result, positional cloning in *Xenopus* should be relatively rapid and straightforward, potentially far simpler than in the mouse provided that genome contiguity is improved.

Moreover, long-range contiguity of the genome would be highly valuable for other resources. The *X. laevis* genome sequencing project will rely on such long-range contiguity for assembly and annotation. In addition, Chip-seq projects aimed at deciphering gene regulatory networks require long-range contiguity in order to be most successful.

Finally, annotation of gene models is essential but labor-intensive. A correct gene name needs to be assigned to each gene model as well as the structure of the gene (exon-intron boundaries, UTRs, etc) and biological information (expression patterns, activity, function). As many as half of all the predicted *X. tropicalis* gene models (~15,000) have only been machine identified based on syntenic relationships with mammalian genomes. These gene models require more supporting data and proper annotation. In addition many gene loci have not yet been correctly identified, and the large numbers of uninformative, systematic IDs still in use significantly hampers the analysis of microarray and deep sequencing data, as this requires time-consuming manual identification of interesting target genes.

5C. How should we proceed?

We propose several complementary approaches to improve *X. tropicalis* genome sequence assembly and annotation.

1. Transcriptome sequencing. Advances in massively parallel sequencing now allow the in-depth analyses of the mRNA transcriptome (RNA-seq), without the biases inherent to traditional cDNA cloning sequencing. This would help in a number of ways: 1) Mapping transcripts to genome can help resolve incorrect gene models, particularly 5' ends of genes [for example Developmental Cell 2009, 17: 425-434] 2) RNA-seq will provide a complete transcriptome sequence, including alternatively spliced transcripts which is essential for the ORFeome resource 3) Sequencing different *X. tropicalis* strains will identify SNPs that could be used in genetic mapping

2. **Short range contiguity** (targeted gap filling and mRNA sequencing)

A complete collection of *Xenopus tropicalis* gene models and/or transcript sequences is needed for other high-priority projects (*Xenopus laevis* genome, ORFeome). A complete collection of intact gene models is currently hindered by the high density of gaps in the genome assembly. Although we anticipate that new genome assemblies will improve the number of complete gene models over time, alternate approaches will accelerate the process. We propose two types of targeted approaches: (A) strategies that target gaps in the genome assembly to improve gene modeling, and (B) strategies that can efficiently provide full-length mRNA sequences for missing genes.

There are probably too many gaps in the genome to sequence across all of them, but only a small proportion of these gaps disrupt gene models. This smaller set of gaps can be identified bioinformatically, and the sequence recovered, for example, by shotgun sequencing a gap-spanning fosmid or BAC, or direct PCR sequencing across the gap, or other strategies.

The shortfall in full-length mRNA sequences could be tackled in two ways: (1) further bioinformatic review of EST clusters and EST sequences to identify existing putative full-length clones and then full-length sequencing of these clones possibly by next generation sequencing methods to reduce costs. (2) leveraging of the steady accumulation of RNA-seq data to identify likely 3' UTR reads in gene loci without full-length mRNAs. These 3'UTR sequences can then be used to design reverse transcription primers to build low complexity cDNA libraries, where shallow sequencing should yield a high proportion of the missing gene sequences.

3. Long-range contiguity

Analysis by Jeremy Schmutz of finished BAC sequences indicates that there are many *Xenopus* specific repeats scattered throughout the genome that lead to breaks in the genomic assembly.

Because of the large numbers of these repeat-induced breaks, the *Xenopus* Community recognizes that generating a *X. tropicalis* genome with "Bermuda" quality sequence is an expensive and significant challenge. However, we have identified a number of alternative, less expensive strategies that should vastly improve genome contiguity:

A. **BAC end sequencing** - extensive BAC end sequencing would provide additional contiguity information. A number of *X. tropicalis* BAC libraries have been made including a sheared library that would provide useful information for joining scaffolds. Currently, there is relatively shallow BAC end coverage of the *X. tropicalis* genome.
B. **SNP map** - the establishment of a SNP map could be used to harness the power of *Xenopus* meioses to make a high-resolution meiotic map. Since the meiotic map would be based on genomic sequence, meiotic mapping could then be used to order and orient the genomic scaffolds into a contiguous super-assembly. As described above, next generation sequencing could rapidly and inexpensively identify SNPs and provide linkage information.
C. **Cytogenetics** - Performing FISH using cDNA or genomic probes can provide additional long-range contiguity and provide the required integration with the *Xenopus* chromosome complement.

BAC Sequencing - Additional BAC resources and BAC sequencing are essential. The *X. tropicalis* genome is particularly shallow in BAC sequencing which complicates the assembly. BAC end sequencing would improve long-range contiguity. In addition, sequencing of selected BACs would resolve particularly fragmented regions of the genome. Collections of BAC end sequences should enable the selection of individual BACs, and pooling strategies in combination with next generation sequencing can then be used for further sequencing. These BAC libraries would have added value in that they would be an important resource for studying DNA *cis*-regulatory sequences with rapid *Xenopus* transgenics.

4. **Genome Data Integration** – Additional resources would be needed to integrate data from the transcriptome sequencing, BAC sequencing, meiotic mapping and HAPPY mapping to produce improved genome assemblies as well as to resolve the many incomplete gene models. Coordination through a genome advisory board is essential to plan future assemblies that include all available sequence data and dissemination to public repositories (UCSC, ENSEMBL, Xenbase).

5. Genome Annotation - Improved annotation of the *X. tropicalis* genome is essential. Gene models require names, correct exon-intron boundaries, transcription start sites, translation start sites, poly-adenylation signals, etc. In fact, the lack of annotation makes the interpretation of genomics information more difficult; this is especially problematic since microarray, deep-seq, and proteomic information can be so readily obtained in *Xenopus*. Because many of the gene models continue to have uninformative automatically generated gene model IDs, analyzing genomics or proteomics data can be laborious if not prohibitive. Since effective annotation of sequence information requires dedicated curation teams, we propose that resources be made available to annotate the *Xenopus* genome. This team would need to work in close conjunction with genome assembly groups as well as other nomenclature committees (HUGO) and Xenbase where gene annotation would be maintained, continually updated, and integrated. Finally, this genome curation team would provide an interface between centralized Genome browsers (JGI, ENSEMBL, etc.) and the *Xenopus* community, allowing individual researchers to report and suggest corrections to genome mis-assemblies and incomplete annotations. This is critical as there is currently no means by which this information can be vetted and systematically introduced into the larger genome browsers.

6. Capitalizing on loss-of-function methods in *Xenopus*

6A. Summary

Xenopus has been a powerful system for high-throughput dissection of vertebrate gene activities, largely via gain-of-function methods such as injection of synthetic mRNA. In order to make *Xenopus* even more effective for analyzing vertebrate gene function, integrating both gain and loss of function methods along with genomics resources is essential. Recent technological advances in loss of function methods have made this a reality in *Xenopus* and should be further developed.

6B. Why develop loss-of-function tools in Xenopus?

Historically, the ease of injecting its embryos and/or oocytes with RNA or DNA established *Xenopus* as the pre-eminent high-throughput system for analysis of vertebrate gene function. This gain-of-function (GOF) approach continues to have a broad range of applications, from simple expression of chimeric fluorescent proteins to visualize intracellular protein dynamics to whole-transcriptome screens for novel gene activities. Whole-transcriptome screens in *Xenopus* are unique because they can be done *in vivo* using pools of synthetic mRNAs injected into fertilized eggs and then assayed for a particular molecular or embryological phenotype. These screens can be highly efficient, filtering through thousands of mRNAs quickly and will likely become even more powerful with emerging genomics resources and assays.

While GOF tools can quickly determine gene activities *in vivo*, loss-of-function (LOF) tools are required to determine the necessity of gene function for particular cellular and developmental processes. Combining LOF tools, genomics, and GOF assays in a single system has extraordinary potential for deciphering gene regulatory networks and protein interactomes.

Traditionally, *Xenopus* investigators performed loss of function studies by injecting "dominant-negative" constructs or expressing known antagonists or chemical inhibitors. While these methods can often be highly effective, these technologies do have some significant limitations. Two new avenues, morpholino oligonucleotides and *X. tropicalis* genetics, have recently emerged as powerful new strategies for the disruption of gene function. Morpholino oligonucleotides (MOs) are now widely used by the *Xenopus* Community and are highly effective. These antisense oligonucleotides are injected into *Xenopus* and lead to depletion of the target protein. MOs have revolutionized much of *Xenopus* biology by allowing the depletion of not only single but multiple proteins simultaneously.

Nevertheless, MOs have disadvantages. The primary disadvantage is that non-specific effects can occur and controls for these nonspecific effects can be challenging. In addition, later embryonic phenotypes are particularly problematic since MOs can lose efficacy at later stages, and it can be difficult to rescue these later stage phenotypes via mRNA injection. Nevertheless, MOs have revolutionized *Xenopus* biology and led to a number of significant insights. Therefore, given the power of loss of function in *Xenopus* for dissecting molecular mechanisms, generating additional loss of function technologies is essential.

Genetics has recently emerged as a powerful tool in *Xenopus* biology. The diploid species *Xenopus tropicalis* is an effective genetic model in which loss-of-function phenotypes can be analyzed with the full range of molecular, embryological and genomic tools transferred from *X. laevis*. In addition, the *X. tropicalis* genome is relatively simple compared to that of teleosts suggesting that different phenotypes may be accessible in frog genetics compared to that in zebrafish. Pilot forward genetic screens have uncovered a number of informative mutant phenotypes. Cloning of these mutant phenotypes has been facilitated by genomic sequence and a preliminary meiotic map as well as syntenic relationships between frog and more polished mammalian genomes. Strategies for rapid low-resolution meiotic mapping have been established (Khokha *et al* 2009) which take advantage of the simple genomic structure and the ease of haploid genetics including gynogenesis. Positional cloning efforts are also greatly facilitated by the massive number of meioses in *Xenopus* (many thousands from a single mating). Positional cloning has identified a number of disease models ranging from cardiac development and function (*mya6* null allele, human cardiac hypertrophy (Abu-Daya et al 2009) to copper metabolism (a splicing deficiency in *ATP7a* typical of Menkes Disease, Zimmerman group).

Forward genetics is extremely useful, since it can lead to discovery of novel gene functions without bias towards pre-existing sequence information. However, because so many experiments can be done only in *Xenopus*, tools to delete known genes (i.e. reverse genetics) would also greatly aid in understanding biological mechanisms. One method for generating mutations in known genes is called TILLING (Targeting Induced Local Lesions IN Genomes). Genomic DNA samples are obtained from a mutagenized population (*TILLING* library), then exons of specific genes are amplified and sequenced to identify individual mutation carriers, which are in turn isolated and bred to uncover associated phenotypes. With reasonable mutagenesis efficiency, a modest-sized *TILLING* library can provide an allelic series of mutations in most genes. The revolution in high-throughput sequencing technology has made this approach increasingly economical for identifying mutants. With support from the *Xenopus* Genetics and Genomics PA, a community pilot TILLING project is underway. Preliminary results have been encouraging, with mutations identified in two genes, *Rax* (Grainger group) and *noggin2* (Harland group), which produced homozygous phenotypes consistent with those described in other models or by morpholino knockdown. In the case of *noggin2*, MO injections revealed a cardiac phenotype that was difficult to rescue by targeted injection of noggin2 and so the specificity of the phenotype was uncertain. Gratifyingly, the noggin2 mutant also revealed this cardiac phenotype.

6C. How should we proceed?

Recognizing the power of loss of function studies in *Xenopus*, the Community feels that additional genetic and non-genetic methods should be developed. It is important to note that many of these loss of function resources have been initiated with support from the *Xenopus* Genetics and Genomics Program Announcement as well as an RFA for Developing the Potential of *Xenopus tropicalis* as a Genetic Model. These funding opportunities are critical for developing these technologies, and the *Xenopus* Community strongly feels that continued PAs and RFAs are essential for further technological advancement.

TILLING: In pilot efforts, TILLING has already identified mutations in known genes. Additional support is required to further optimize mutagenesis methods and exploit new massively parallel sequencing methods. Harnessing these new technologies to improve TILLING will reduce the cost of reverse genetics and allow additional mutants to be identified. *TILLING* is likely to be transformative for the *Xenopus* field in a number of ways. First, true null alleles may be critical for the analysis of gene regulatory networks and protein interactomes where incomplete knockdown may be insufficient to understand the perturbations in the system. Alternatively, hypomorphic alleles have been very useful in other genetics systems and may allow for new insights into gene functions. Second, genetics facilitates the study of later gene functions in organogenesis and differentiation that cannot be efficiently targeted by MOs or dominant negative expression. Finally, mutations in known genes will allow experiments that can be uniquely performed in *Xenopus*.

Novel loss-of-function methods*:* Additional avenues for manipulating gene function must also be developed. These include non-genetic techniques for affecting specific genes such as small antisense RNAs (siRNA, etc), small-molecule "chemical genetics" screens, as well as alternative genetic strategies. Insertional mutagenesis, by simplifying cloning of disrupted genes, can greatly reinforce forward genetics, and an insertional mutant with a dramatic forelimb ablation phenotype has already been identified (*xenopus de milo*, Zimmerman group). Conditional control of transgene expression via cre-lox or other recombinase systems has been demonstrated (Waldner et al 2006), and becomes increasingly powerful when combined with null mutant backgrounds. Homologous recombination strategies and targeted gene disruption, for example, using zinc finger nucleases need to be explored along with other strategies.

Forward Genetics: With NIH support, significant resources have been made available to facilitate forward genetic screens. This includes genomic sequence and a preliminary meiotic map. With these resources, pilot forward genetic screens have identified many interesting phenotypes. The screens to date have been small in scale and far from saturation. Therefore, additional forward genetic screening is likely to identify additional interesting phenotypes and should be continued. In addition, sophisticated screening methods for particular phenotypes (ie transgenic lines that highlight specific structures or cellular processes) should be developed and implemented in forward genetic screens. Once mutants are identified, they can then be deeply analyzed with all the advantages of the *Xenopus* system.

7. The generation of an antibody resource for *Xenopus* epitopes

Summary:

Although genomic and genetic resources in *Xenopus* have come a long way in the past decade, the availability of antibodies (Abs) specific to *Xenopus* proteins is limited. The generation of Abs would facilitate essentially all aspects of research in *Xenopus*, including developmental, cellular, molecular and neural biology and immunology. These Abs would be generated in a systematic manner based on community need and would be curated and distributed via collaboration with the European Xenopus Resource Centre.

The Use of Antibodies in *Xenopus* Research

Cell biologists using *Xenopus* egg extracts to study a wide range of events such as nuclear formation, DNA replication and repair and entry into mitosis have long used antibodies, both to monitor subcellular changes and to deplete proteins to probe functionality. By contrast, developmental biologists using *Xenopus* as a model system have generally tracked gene expression by *in situ* hybridization. Antibodies to developmentally important proteins are quite rare. Where they do exist, they have often been raised to other species and fortuitously cross-react. However, it is becoming increasingly clear that mRNA level is only one measure of gene expression; many, many developmentally important proteins are regulated at the level of post-translational stability/modification, and this can only be determined if protein levels are assessed using antibodies.

Why do we need a *Xenopus* antibody initiative?

Antibodies are needed for all aspects of *Xenopus* research. Presently, almost no *Xenopus*-specific Abs are commercially available. Although, some commercial Abs against highly conserved heterologous proteins (e.g., hsp70) or motifs (e.g., double phosphorylated ERK epitope, acetylated tubulin) cross-react with *Xenopus proteins*, many other Abs do not work at all or display poor affinity or high non-specific background. This White Paper therefore supports the generation of large numbers of antibodies against proteins of broad interest to the community according to the need of the different fields of study and investigators. This development will be crucial for exploiting the advantages of the *Xenopus* system to its full potential as biomedical model.

How to proceed?

Initially, a Wiki page will be set up in conjunction with Xenbase. This will initially record successes and failures with existing commercial antibodies so that the community can share information regading their utility. The analysis to which they have been applied will also be recorded eg ChIP, IP, CoIP immunofluorescence. Equally important, particularly for polyclonal antibodies required for immunodepletion, these antibodies are a finite resource; therefore, we need a database detailing which antigen was successfully used to generate a functional antibody and a repository for protein expression constructs used to generate recombinant proteins used for inoculation. These resources should aid generation of a similar antiserum. A priority list for antibodies needed by the Community and for what type of experiment will be generated.

We envision this community-wide antibody initiative to have three main components: (1) Input from the annual *Xenopus* Resources Development meeting held at XRC, Woods Hole to evaluate and prioritize requests from investigators; (2) The community would be encouraged to submit RO1s specifically to address the production of these reagents. (3) Resource Center involvement to maintain and provide mAbs to the community upon request (e.g. EXRC in Portsmouth)

Many different approaches can be taken for the production of immunological reagents; the choice depends on the technique in which they will be utilized. For biochemical analyses and techniques involving precipitation it is often advantageous to generate polyclonal antibodies which will contain components to a number of different epitopes. These will be generated either from bacterial fusion proteins or by DNA immunizations in eukaryotic systems depending on whether correct protein modifications are required in the antigen. For other applications, mouse monoclonal antibodies (mAbs) or mono-specific single chain Ab fragment (scFv-Abs) from phage display technology could be generated; these Abs would have a unique antigen specificity requiring further characterization and assessment of binding affinity.

A major goal of the Community is to create an ORFeome library for *Xenopus* in GATEWAY vectors (see above). Design of a series of expression vectors would allow the expression of tagged proteins with the shuttled ORFeome sequences could greatly facilitate the production of a source of immunogen.

What are the outcomes?

It would be expected that this priority would result in the generation of a large number of antibodies of differing types depending on their application. For example, for any developing system involving a gene regulatory network, a series of polyclonal antibodies could be developed for ChIP, and monoclonal antibodies or scFc could be generated for high specificity imaging or FACS analysis. Furthermore a panel of cell biological reagents could be generated for particular cellular components, chromatin modifications or signaling pathway components which could be used in fixed cell/embryo and real time imaging, depletion experiments or IP to analyze inter-molecular complexes. The detailed focus of the R01s would depend on the particular interests of the PI, but there would be an understanding that reagents generated under this priority would be made available to the *Xenopus* community.

National Institute of General Medical Sciences (NIGMS)

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The NIGMS "supports basic research that increases understanding of life processes and lays the foundation for advances in disease diagnosis, treatment, and prevention" (http://www.nigms.nih.gov/Initiatives/). Experiments in model animals are a cornerstone of such fundamental biomedical research and they play a particularly important role in the mission of the NIGMS.

The frog, *Xenopus,* is a widely used and crucial vertebrate model organism that is unique for its combination of its experimental tractability and its close evolutionary relationship with humans. *Xenopus* is an essential tool for *in vivo* studies in molecular, cell, and developmental biology of vertebrate animals. However, the enormous breadth of *Xenopus* research stems from the additional fact that cell-free extracts made from *Xenopus* are a premier *in vitro* system for studies of fundamental aspects of cell and molecular biology. Thus, *Xenopus* is the only vertebrate model system that allows for high-throughput *in vivo* analyses of gene function and high-throughput biochemistry. Finally, it should be borne in mind that *Xenopus* oocytes are a leading system for studies of ion transport and channel physiology.

Because of its diverse applications, *Xenopus* research is funded by nearly all Institutes within the NIH. However, the NIGMS remains by far the largest source of funding for *Xenopus* research. In this statement, we provide a summary of the crucial contributions made by *Xenopus* research to the mission of the NIGMS. We start with recent contributions of *Xenopus* to the study of known human disease genes. We follow this with a selection of examples that illustrate the huge impact that recent *Xenopus* research has had on our understanding of fundamental biological processes. Finally, we summarize very briefly the long and rich history which formed the foundation for myriad current advances being made *Xenopus* research to our understanding of the biology underlying human disease.

I. Direct investigation of human disease genes using *Xenopus*:

The NIGMS funds research that "lays the foundation for advances in disease diagnosis, treatment, and prevention" (http://www.nigms.nih.gov/Initiatives/). Therefore, it is notably that all modes of *Xenopus* research (embryos, cell-free, extracts, and oocytes) are now commonly and widely used in direct study of human disease genes.

Xenopus embryos for in vivo studies of human disease gene function: *Xenopus* embryos are large and easily manipulated, and moreover, many hundreds of embryos can be obtained in a single day. It is not surprising, then, that *Xenopus* was the first vertebrate animal for which methods were developed that allowed rapid analysis of gene function using misexpression (by mRNA injection; *Nature.* 1971. 233, 177-82). Indeed, injection of mRNA in *Xenopus* led to the cloning of interferon (*PNAS.* 1975. 72, 4881-4885). Moreover, the use of morpholino-antisense oligonucleotides for gene knockdowns in vertebrates, which is now the state-of-the-art, was first developed by Janet Heasman using *Xenopus* (*Dev. Biol.* 2000. 222, 124-34.).

In recent years these approaches have played in important role in studies of human disease genes. The mechanism of action for several genes mutated in human cystic kidney disorders (e.g. nephronophthisis) have been extensively studied in *Xenopus* embryos, shedding

new light on the link between these disorders, ciliogenesis and Wnt signaling (*Hum Mol Genet.* 2008. 17, 3655-62). *Xenopus* embryos have also provided a rapid test bed for validating newly-discovered disease genes. For example, studies in *Xenopus* confirmed and elucidated the role PYCR1 in cutis laxa with progeroid features **(***Nat Genet.* 2009. 41, 1016-21).

Transgenic Xenopus for studying transcriptional regulation of human disease genes: *Xenopus* embryos develop rapidly, and so transgenesis in *Xenopus* is a rapid and effective method for analyzing genomic regulatory sequences. In a recent study, mutations in the SMAD7 locus were revealed to associate with human colorectal cancer. Interestingly, the mutations lay in conserved, but non-coding sequences, suggesting that these mutations impacted the patterns of SMAD7 transcription. To test this hypothesis, the authors used *Xenopus* transgenics, and revealed that this genomic region drove expression of GFP in the hindgut. Moreover, transgenics made with the mutant version of this region displayed substantially less expression in the hindgut (*Genome Res.* 2009. 19, 987-93.).

Xenopus cell-free extracts for biochemical studies of proteins encoded by human disease genes: A unique advantage of the *Xenopus* system is that cytosolic extracts contain both soluble cytoplasmic and nuclear proteins (including chromatin proteins). This is in contrast to cellular extracts prepared from somatic cells with already distinct cellular compartments. *Xenopus* egg extracts have provided innumerable insights into the basic biology of cells with particular impact on cell division and the DNA transactions associated with it.

Studies in *Xenopus* egg extracts have also yielded critical insights into the mechanism of action of human disease genes associated with genetic instability and elevated cancer risk, such as ATM (Ataxia telangiectasia), BRCA1 (Inherited Breast and Ovarian cancer), Nbs1 (Nijmegen Breakage Syndrome), RecQL4 (Rothmund-Thomson Syndrome), c-Myc oncogene and FANC proteins (Fanconi anemia) (*Cell.* 2006, 127, 539-52; *Nat. Cell Biol*. 2007. 9, 1311-18; *Mol. Cell.* 2009. 35, 704-15; *J Biol Chem.* 2009, 284, 25560-8; *Nature*. 2007. 448, 445-51).

Xenopus oocytes for studies of gene expression and channel activity related to human disease: Yet another strength of *Xenopus*, and another strength that is simply not matched by any other vertebrate model system, is the ability to rapidly and easily assay the activity of channel and transporter proteins using expression in oocytes. This application has also led to important insights into human disease, including studies related to trypanosome transmission (*Nature* 2009. 459, 213-217), Epilepsy with ataxia and sensorineural deafness (*N Engl J Med.* 360, 1960-70), Catastrophic cardiac arrhythmia (Long-QT syndrome; *PNAS* 2009. 106,13082-7) and Megalencephalic leukoencephalopathy (*Hum Mol Genet.* 2008. 17, 3728-39).

II. Investigation of fundamental biological processes using *Xenopus*:

In addition to applied studies directed at the mechanisms of known human disease genes, the NIGMS very broadly supports “basic research that increases understanding of life processes” (http://www.nigms.nih.gov/Initiatives/). It is this area where *Xenopus* has made its most substantive and wide-ranging contributions.

To name only a few areas of study in which *Xenopus* has had a large impact in the last two years:

Signal transduction: *Xenopus* embryos and cell-free extracts are widely used for basic research in signal transduction. In just the last few years, *Xenopus* embryos have provided crucial insights into the mechanisms of TGF-® and Wnt signal transduction. For example, *Xenopus* embryos were used to identify the enzymes that control ubiquitination of smad-4 (*Cell*.

2009. 136,123-35), and also to demonstrate direct links between TGF-® superfamily signaling pathways and other important networks, such as the MAP kinase pathway (*Science*. 2007. 315, 840-3) and the Wnt pathway (*Cell*. 2007. 131, 980-993). Moreover, new methods using egg extracts revealed novel, important targets of the Wnt/GSK3 destruction complex (*PNAS*. 2009. 106, 5165-5170).

Cell division: *Xenopus* egg extracts have allowed the study of many complicated cellular events in a test tube. Because egg cytosol can support successive cycling between mitosis and interphase *in vitro*, it has been critical to diverse studies of cell division. For example, the small GTPase Ran was first found to regulate interphase nuclear transport, but *Xenopus* egg extracts revealed the critical role of Ran GTPase in mitosis independent of its role in interphase nuclear transport (*Nature*. 2006 440, 697-701). Similarly, the cell-free extracts were used to model nuclear envelope assembly from chromatin, revealing the function of RanGTPase in regulating nuclear envelope reassembly after mitosis (*Science* 2006 311, 1887-1893). More recently, using *Xenopus* egg extracts, it was possible to demonstrate the mitosis-specific function of the nuclear lamin B in regulating spindle morphogenesis (*Nat. Cell Biol.* 2009. 11, 247-256) and to identify new proteins that mediate kinetochore attachment to microtubules (*Cell*. 2007. 130, 893-905.

Embryonic development: *Xenopus* embryos are so widely used in developmental biology that it is impossible to quickly summarize the myriad of important advances made by *Xenopus* research in recent years. A very short list would include:

- Epigenetics of cell fate specification (*Dev. Cell.* 2009. 17, 425-434),
- microRNAs in germ layer patterning and eye development (*Dev. Cell.* 2009. 16, 517-527*; Genes & Dev.* 2009. 23, 1046-1051)
- Link between Wnt signaling and telomerase (*Nature*. 2009. 460, 66-72),
- Development of the vasculature (*Cell*. 2008.135, 1053-64),
- Gut morphogenesis (*Genes & Dev.* 2008. 22, 3050-3063),
- Contact inhibition and neural crest cell migration (*Nature*. 2008. 146, 957-961).

Initiation of DNA replication: *Xenopus* cell-free extracts also support the synchronous assembly and the activation of origins of DNA replication. They have been instrumental in characterizing the biochemical function of the pre-replicative complex, including MCM proteins (*Mol. Cell*. 2008. 32, 862-9; *EMBO J.* 2009. 28, 3005-14).

Response to DNA damage: Cell-free extracts have been instrumental to unravel the signaling pathways that are activated in response to DNA double-strand breaks (ATM), replication fork stalling (ATR) or DNA interstrand crosslinks (FA proteins and ATR). Notably, several mechanisms and components of these signal transduction pathways were first identified in *Xenopus* (*Mol Cell.* 2009. 35,704-15; *Cell.* 2008. 134, 969-80; *Genes Dev.* 2007. 21, 898-903).

Apoptosis: *Xenopus* oocytes provide a tractable model for biochemical studies of apoptosis. Recently, oocytes were used recently to study the biochemical mechanisms of caspase-2 activation; importantly, this mechanism turns out to be conserved in mammals (*Dev Cell.* 2009. 16, 856-66).

Regenerative medicine: In recent years, there has been tremendous interest in developmental biology stoked by the promise of regenerative medicine. *Xenopus* has played a role here as well. For example, it has been found that expression of seven transcription factors

in pluripotent *Xenopus* cells rendered those cells able to develop into functional eyes when implanted into *Xenopus* embryos, providing potential insights into the repair of retinal degeneration or damage (*PLoS Biology*. 2009. 7, e1000174.).

In a vastly different study*, Xenopus* embryos was used to study the effects of tissue tension on morphogenesis (*Dev Cell.* 2009. 16, 421-432.), an issue that will be critical for *in vitro* tissue engineering.

Physiology: The directional beating of multi-ciliated cells is essential to development and homeostasis in the central nervous system, the airway, and the oviduct. Interestingly, the multi-ciliated cells of the *Xenopus* epidermis have recently been developed as the first *in vivo* test-bed for live-cell studies of such ciliated tissues, and these studies have provided important insights into the biomechanical and molecular control of directional beating (*Nat Genet.* 2008. 40, 871-9; *Nature*. 2007. 447, 97-101).

III. Use of *Xenopus* for small molecule screens to develop novel therapeutics.

Because huge amounts of material are easily obtained, all modalities of *Xenopus* research are now being used for small-molecule based screens.

Chemical genetics of vascular growth in Xenopus tadpoles: Given the important role of neovascularization in cancer progression, *Xenopus* embryos were recently used to identify new small molecules inhibitors of blood vessel growth. Notably, compounds identified in *Xenopus* were effective in mice (*Blood*. 2009. 114, 1110-22; *Blood*. 2008. 112, 1740-9).

In vivo testing of potential endocrine disruptors in transgenic Xenopus embryos: Endocrine disrupting chemical released into the environment are pose a potential public health risk, but our ability to identify such compounds in vitro vastly outstrips our ability to monitor the in vivo effects of such chemicals. A high-throughput assay for thyroid disruption has recently been developed using transgenic *Xenopus* embryos (*Environ. Sci. Technol.* 2007. 41, 5908-14).

Small molecule screens in Xenopus egg extracts: Egg extracts provide ready analysis of molecular biological processes and can rapidly screened. This approach was used to identify novel inhibitors of proteasome-mediated protein degradation and DNA repair enzymes (*Nat Chem Biol.* 2008. 4, 119-25; *Int. J. Cancer*. 2009. 124, 783-92).

IV. A long history of research laid the foundation for the myriad recent contributions of *Xenopus* to biomedical science.

In addition to its current wide usage in diverse areas of biology, we feel that it is also worth summarizing the some of the landmark discoveries that come to mind when thinking about the contributions of *Xenopus* to the NIH.

1950's

- The discovery that somatic nuclei are totipotential, from which present excitement about nuclear reprogramming and stem cells arises (Gurdon et al., 1958).

1960's

- 1969: The discovery that the nucleolar organizer encodes the ribosomal RNA genes (Brown and Gurdon, 1969).

- 1968: Selective DNA amplification of rDNA in oogenesis (Brown and Dawid, 1968; Gall, 1968).
- Mitochondrial DNA exists and is inherited from the mother (Dawid, 1966).

1970's

- The isolation of the first eukaryotic genes by equilibrium density centrifugation in the form of rRNA and 5S genes (Birnstiel et al., 1968; Brown et al., 1971).
- The first eukaryotic translation system by oocyte mRNA microinjection (Gurdon et al., 1971).
- The first transcription and translation system for cloned genes (Brown and Gurdon, 1977; De Robertis and Mertz, 1977).
- Discovery of MPF, a meiosis maturation promoting factor that provided the key to the elucidation of the cell cycle (Wasserman and Masui, 1976).
- First system for electrophysiological studies on cloned membrane channels and receptors (Kusano et al., 1977).
- Identification of nuclear targeting signal sequences in the mature sequence of nuclear proteins (De Robertis et al., 1978).

1980's

- The isolation of the first eukaryotic transcription factor, TFIIIA (Engelke et al., 1980).
- First in vitro system for nuclear and chromosome assembly (Lohka and Masui, 1983).
- Discovery of the first Hox gene homologue in vertebrates (Carrasco et al., 1984).
- Mesoderm induction is mediated by members of the TGF-beta family of growth factors (Smith, 1987).
- Cell cycle progression is regulated through protein degradation of cyclins via ubiquitinylation (Murray et al., 1989).

1990's

- Realization that Homeobox genes direct gastrulation morphogenetic movements (Niehrs et al., 1993).
- Molecular nature of Spemann's organizer: cell-cell signals are regulated by secreted growth factors antagonists such as Noggin, Gremlin, Follistatin, Chordin, Cerberus, Frzb and Dickkopf (reviewed by Harland and Gerhart, 1997).
- Identification of the cell-cell signals that cause induction and patterning of the Central Nervous System (Zimmerman et al., 1996; Piccolo et al., 1996).

These and many other past discoveries would more than justify a re-dedication of the NIGMS's efforts to the acceleration and promotion of biomedical research using *Xenopus*. But as the document above makes clear, the current, sustained contributions made by this system are such that *Xenopus* should be considered one of the most promising post-genomic systems for research in Cell and Molecular Biology.

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Common molecules control key events in both embryonic development and cancer, and elucidating the molecular mechanisms via which such factors regulate normal development provides important insight into how their misregulation contributes to tumor formation and progression. *Xenopus laevis* embryos are a powerful system in which to investigate the molecular mechanisms underlying gene function, organogenesis, and disease. All stages of development are accessible to experimental manipulation in embryos and a major advantage of this system is the ease with which gene expression and signaling pathways can be perturbed. Furthermore, *Xenopus* embryos are large and easy to obtain in large numbers, facilitating the collection of material for biochemical studies and proteomics. Their external development also makes them ideal for chemical genetics and drug discovery screens aimed at the development and evaluation of putative chemotherapeutics. Thus, *Xenopus* provides a series of advantages not readily available in other vertebrate systems and remains an important area of investment for the continued development of tools to advance studies using this model organism.

Among the studies in *Xenopus* of high relevance to cancer are those aimed at understanding the vertebrate neural crest and its derivatives. A number of cancers of great clinical significance are neural crest-derived, including neuroblastoma, melanoma, and gliomas. Interestingly, a number of identified molecular mediators of neural crest development appear to be mis-regulated in human cancers, including c-myc, and Snail family proteins. In particular, the molecules that control the Epithelial-Mesenchymal Transition (EMT) and invasive behavior of neural crest cells have been co-opted by epithelial tumors to mediate metastasis and *Xenopus* has become a powerful model for understanding the mis-regulation of these molecules during tumor progression. Similarly the *Xenopus* system has recently provided evidence that the cancer-associated Wilms Tumor Suppressor protein WTX is a required component of the ß-catenin destruction complex which is mis-regulated in a broad range of tumors.

Beyond whole embryo studies, cell-free extracts derived from *Xenopus laevis* eggs have provided a powerful and biochemically tractable system for the study of the cell cycle under physiological and stressed conditions. This is the only cell-free system that recapitulates all DNA transactions associated with cell cycle progression and the response to DNA damage (DNA replication, chromosome segregation, DNA repair and checkpoints). Of particular relevance to cancer, the *Xenopus* egg extract system has been instrumental to the study of the DNA damage response and of DNA replication in the maintenance of genome integrity. In response to DNA damage or to a block to DNA replication, S phase is delayed to allow DNA repair processes to occur as well as to ensure the completion of DNA replication prior to the start of mitosis. The molecular bases of these checkpoint pathways that influence DNA replication were unraveled using *Xenopus* cell-free extracts. These extracts allows us to study DNA lesion-specific signaling. It was shown that DNA double-strand breaks activate the ATM kinase leading to the Cdc25-dependent inhibition of Cdk2. Similarly, it was demonstrated that DNA polymerase stalling triggered by aphidicolin or by UV lesions activates ATR resulting in the Chk1-dependent inhibition of Cdk1. More recently, these extracts have been instrumental to the study of complex DNA lesions such as inter-strand crosslinks. *Xenopus* cell-free extracts have also provided models to study the biochemical bases of several cancer-prone diseases associated with mutations in ATM (Ataxia telangiectasia), BRCA1 (Inherited Breast and Ovarian cancer), Nbs1 (Nijmegen Breakage Syndrome) and FANC proteins (Fanconi anemia). Finally, preliminary studies indicate that *Xenopus* cell-free extracts could be used successfully to identify small molecules that modulate the DNA damage response with potential

chemosensitizing properties for cancer therapy. Thus studies in *Xenopus* continue to provide essential insights into basic cellular pathways that are essential to the maintenance of genomic stability and the prevention of tumor formation.

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National Eye Institute (NEI)

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Xenopus has been a classic model system for eye and vision research due to the ease of embryological analysis and manipulation. For example, fundamental insights into retino-tectal connectivity (Sperry), lens induction (Grainger) and retinal cell determination (Harris) have come from work in *Xenopus*. More recently, with the development of modern molecular methodology *Xenopus* has consolidated its role as a unique and vital model for investigating development, physiology and disease of the vertebrate visual system.

Eye Development and Regeneration:

Xenopus is ideal for the study of eye development since histogenesis in the *Xenopus* eye is rapid, with all retinal cell types specified between 1 and 3 days of development. In addition, the eye can be reproducibly targeted by microinjection of blastomeres at early cleavage stages or by in vivo lipofection or electroporation at optic vesicle stages. This allows selective manipulation of gene expression in the eye, with subsequent analysis of effects on optic vesicle patterning and retinal cell fate. This powerful approach has uncovered multiple genes and pathways governing retinal cell fate determination. Important advances range from understanding the importance of basic helix-loop-helix transcription factors in vertebrate retinal cell fate decisions (Kanekar et al., 1997) to the first demonstration that vertebrate homebox proteins act to effect a cellular clock that times the generation of retinal cells (Decembrini et al., 2006). Important achievements in understanding the relevance of signaling pathways to retinal cell fate include the discovery of a novel role for Hedgehog signaling in the transition of stem cell to transient amplifying progenitors (Locker et al., 2006) and the elucidation of the multiple roles that Wnt signaling plays in both embryonic (Van Raay et al., 2005) and post-embryonic eye development (Denayer et al., 2008).

In addition, the development of rapid and efficient methods for generating transgenic animals (Kroll and Amaya, 1996) has led to identification and fine-mapping of multiple eye-specific promoters targeting various cell populations in the developing and mature *Xenopus* eye. For example, promoters for Rx, Pax6, Ath5, X-linked juvenile retinoschisis (RS1) gene and rod opsin have all been mapped in *Xenopus*. These are powerful tools for targeting transgenes to the developing eye and for investigating the mechanisms underlying eye-specific gene regulation.

In *Xenopus*, the eye continues to grow throughout the life of the animal, so there is a true retinal stem cell population present at the margins of the eye in the ciliary marginal zone that drives growth of the eye and can also replace lost or damaged retinal neurons – a feature that is not shared in higher vertebrates. In fact, the cocktail of retinal stem cell/progenitor genes that are sufficient to generate complete functional ectopic eyes from pluripotent ectoderm cells in *Xenopus* has been defined (Viczian et al., 2009). In addition, retinal tissue can be regenerated from animal cap embryonic stem cells (Lan et al, 2009), RPE (Vergara and Del Rio-Tsonis K, 2009) and the lens of the eye can be regenerated from neighboring tissues (reviewed in Beck et al., 2009). Thus, *Xenopus* represents an important model system for understanding retinal stem biology as well as regeneration of ocular tissues.

Retinal Cell Biology & Physiology:

Transgenic methods in *Xenopus* have proved to be a powerful tool for investigating the cell biology of photoreceptors in vivo, in particular for studying protein targeting to photoreceptor outer segments. For example, it was recently shown in *Xenopus* that ankyrin-G binding is necessary and sufficient for targeting of the ®1 subunit of the cyclic nucleotide-gated channel to

rod outer segments (Kizhatil et al., 2009). Another study showed that the outer segment serves as a default destination for the trafficking of membrane proteins in photoreceptors (Baker et al., 2008). The high cone/rod ratio of *Xenopus*, combined with its powerful transgenic methods has proved to be a useful system for investigating rod-cone interactions both in development and disease states (Hamm et al., 2009).

All levels of the *Xenopus* visual system are amenable to fruitful study, including formation of appropriate connections at central targets. Tremendous advances have also been made in our understanding of retinal axon guidance in *Xenopus*. Recent studies have revealed how local protein synthesis contributes to directional steering of retinal growth cones as they navigate to their target (Leung et al., 2006). In addition, it was recently found that maturation of retinotectal synapses in the developing *Xenopus laevis* optic tectum is regulated by activation of ephrin-B reverse signaling (Lim et al., 2008). Another study investigated the early development and plasticity of local excitatory circuits in the optic tectum of *Xenopus laevis* tadpole, revealing important insights into how the response properties of the tectal network are modulated and optimized (Pratt et al., 2008). Thus connectivity and circuit formation in the visual system have been amenable to fruitful analysis in *Xenopus*.

Circadian oscillator mechanisms have been extensively studied in *Xenopus laevis*. The retina contains the essential components of the clock, and can be selectively manipulated using retinal cell-type-specific promoters to allow molecular dissociation of the circadian clock (Hayasaka et al, 2005).

Modeling Human Disease in Xenopus:

Xenopus is also suitable for modeling certain human ocular disease. For example mutations causing autosomal dominant retinitis pigmentosa (RP) in humans induce rod photoreceptor degeneration in *Xenopus laevis* (Tam and Moritz, 2006). This has led to additional important insights, such as a molecular mechanism for light sensitivity in RP (Tam and Moritz, 2007). These approaches will ultimately open up new avenues for rapidly testing the effects of certain human mutations on gene function in vivo.

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The *Xenopus* system has been instrumental in advancing our understandng of the basic biology of the cardiovascular system. The *Xenopus* embryo develops a fully functional cardiovascular system, complete with beating heart and circulating blood cells, within approximately 72 hours of fertilization. The extreme rapidity of this process and the fact that development occurs in plain view, outside of the mother, makes the *Xenopus* embryo an ideal system for study of the cellular and molecular mechanisms regulating cardiovascular development.

Cellular mechanisms regulating heart development: In vertebrates, the first instructional signals leading to development of the myocardium occur during gastrulation. Additional signaling between tissues is required for maintenance and expansion of precardiac tissue and then for differentiation of myocardial cells. Understanding this series of signaling events will provide the best approach for directed differentiation of embryonic stem cells towards cardiomyocytes. *Xenopus* embryonic tissues are uniquely accessible for the study of heart development and much of our knowledge of essential cellular signaling pathways has been derived from this system. For example, the importance for cardiac development of FGF, BMP, Wnt11 and inhibition of canonical Wnt signaling all were first described in *Xenopus*. Each of these pathways has been utilized for differentiation of human ES cells into cardiomyocytes. Future studies using *Xenopus* will provide further insights into the fundamental biological processes underlying myocardial differentiation.

Cellular physiology of cardiac ion channels: The *Xenopus* oocyte is the preferred expression system for analysis of cardiac ion channel function. This system has proven to be invaluable for analysis of mutant ion channels detected in human patients with cardiovascular defects ranging from sudden infant death to arrythmias. In 2008 alone, more than 50 publications made use of *Xenopus* oocytes for analysis of cardiac-specific ion channels.

Molecular and cellular regulation of blood vessel development: Understanding of the regulation of blood vessel development is essential for designing strategies for treatment of human diseases, ranging from inhibition of tumor angiogenesis to stimulation of vessel growth in diabetic limbs. The *Xenopus* model has provided insights into multiple aspects of blood vessel growth and regression. Furthermore, *Xenopus* embryos provide an important vertebrate system for high throughput detection of small molecule inhibitors of angiogenesis. Continuing advances in live imaging techniques will ensure that *Xenopus* continues to contribute to understanding of blood vessel formation during embryogenesis.

Analysis of cardiovascular gene regulation: *Xenopus* provides one of the simplest, fastest and most economical methods for generation of transgenic embryos. The high efficiency of the procedure allows extremely rapid in vivo studies of cardiac gene regulation. Due to the high conservation of transcriptional regulatory mechanisms, this information gained in the *Xenopus* embryo will be relevant for understanding gene regulatory pathways involved in human cardiovascular disease in adults and underlying congenital cardiovascular defects.

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National Human Genome Research Institute (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 *Xenopus*e 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 trangenesis 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
3. Chip-Seq technologies. Development of chromatin immunoprecipitation assays in *Xenopus* for identifying transcription factor DNA targets.
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.

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National Institute for Allergy and Infectious Disease (NIAID)

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It is now well established that both the innate and adaptive immune systems undergo rapid evolution and diversification; consequently, non-mammalian vertebrate animal models that are experimentally tractable alternatives to murine systems are essential, as they will allow us better distinguish important conserved structures and functions from species-specific specializations. In this regard, *Xenopus* offers one of the best comparative models with which to study the immune system.

Indeed, the advantages of the *Xenopus* model systems have been leveraged to advance our understanding of many facets of immunity. These include: humoral and cell-mediated immunity in the context of MHC restricted and unrestricted recognition; ontogeny; phylogeny; and defense against tumors, viruses, fungi and bacteria (reviewed in Pasquier et al., 1989; Robert and Ohta, 2009). *Xenopus* is as valuable as zebrafish for studying the ontogeny of the immune system. Moreover, unlike zebrafish, *Xenopus* has the best characterized immune system outside of mammals and chicken. Furthermore, the *Xenopus* model offers a collection of invaluable research tools including MHC-defined clones, inbred strains, cell lines, and monoclonal antibodies. Finally, the annotated full genome sequence of *X. tropicalis* and its remarkable conservation of gene organization with mammals, as well as ongoing genome mapping and mutagenesis studies in *X. tropicalis* provide a new dimension to the study of immunity The salient features of this amphibian model are summarized below.

Model to study Immunogenetics: The *X. tropicalis* genome has provided compelling evidence for the similarity of gene repertoire in both the adaptive and innate immune systems (Zarrin et al., 2004; Guselnikov et al., 2008). More importantly, it has unveiled the amazing degree of conservation of gene clustering or synteny with mammals, which is far better preserved with *Xenopus* than with any fish species whose genomes have undergone extensive diversification during evolution. Gene synteny is helpful for identifying diverged genes such as immune genes. For example, in *Xenopus* as in mammals CD8 beta retains proximity to CD8 alpha, and CD4 neighbors Lag3 and B protein. Ongoing whole genome mutagenesis will allow one to search for genes critically involved in immune functions.

Xenopus is the only genus where polyploid as well as diploid species exist naturally, and can be artificially produced with various degrees of polyploidy (2N to 8N), enabling an experimental approach to studying the consequences of whole genome duplication (i.e., study the fate of duplicated genes), a subject of major interest nowadays for understanding the origin of the vertebrate genome, as well as the effects of gene dose on host resistance or defense against pathogens. *Xenopus* species can also be cloned using gynogenetic development of diploid eggs coming from interspecies hybrids. These clones can easily be maintained and propagated in the laboratory, and constitute a unique *in vivo* way to study genome regulation. Clones with identical MHC combinations but differences at minor histocompatibilty (H) gene loci provide an excellent biological system to study immune responses in vivo. *X. laevis* is the only species where aneuploid animals can be generated for studying the segregation of immune functions linked to a specific chromosome. In situ hybridization techniques are now available both for chromosome and for whole mounts embryos.

Model to study the development of the immune system: *Xenopus* provides an excellent system to study early ontogeny of the immune system. *Xenopus* has all the lineages of hematopoietic cells that mammals have. However, early developmental stages of *Xenopus* are free of maternal influence, and are easily accessible and amenable to experimentation. This provides an ideal animals model to study early commitments and fates of myeloid and lymphoid lineages (Suzuki et al., 2004; Marr et al., 2007).

Metamorphosis in *Xenopus* is a truly unique developmental period, in which the larval thymus loses most of its lymphocytes, and a new differentiation occurs from a second wave of stem cell immigration resulting in completely distinct adult immune system. Notably, autoimmunity against the many new adult type proteins needs to be prevented by a new balance of self-tolerance through T cell education (Flajnik et al., 2001). This system has the additional advantage of the accessibility of the thymus early in development. Indeed, thymectomy can be efficiently performed in *Xenopus* at early developmental stages before the migration of stem cells and generate T cell-deficient animals. Therefore, *Xenopus* has been and still is frequently used to study T cell ontogeny, and with the new genomic and genetic technologies it offers new ways to analyze genes and function in a complementary manner.

Model to study immune tolerance. *Xenopus* serves as an exciting comparative model to explore self-tolerance because of the ease with which allotolerance to minor H-Ags on adult skin grafts can be induced just prior or during metamorphosis that is the transitional animal undergoes a temporary period of altered immunoregulation (Flajnik et al., 2001). During this period, one can experimentally induce long-lasting specific non-deletional ("split") anergic-like tolerance to minor H-Ags that persists after metamorphosis. MHC genes are also differentially regulated in larvae and adults. The change in MHC gene regulation during metamorphosis, the new histogenesis in the thymus, and the ease with which one can experimentally manipulate larvae (e.g., thymectomy, blocking or accelerating metamorphosis) allows one to address questions about MHC restriction, autoimmunity, and the development of self-tolerance that can not be easily studied in other animal models.

Model to study tumor immunity: *Xenopus* is the only amphibian genus where series of true lymphoid tumors have been discovered and cell lines have been obtained, thereby opening up new avenues for tumor biology and the isolation and characterization of membrane proteins. In particular, distinct immune systems of larvae and adults, and the ease of manipulating their maturation during metamorphosis provides a unique to investigate *in vivo* the possible influence of the immune system on the selection of more aggressive tumor. *Xenopus* has also significantly helped to demonstrate the importance of certain heat shock proteins such as hsp70 in anti-tumor immune responses. It provides a natural *in vivo* model to dissect the contribution of innate (pro-inflammatory) and adaptive (MHC class I restricted T cell) arm of the immune system in hsp-mediated anti-tumor responses (Goyos et al., 2007). As such *Xenopus* is an important comparative tumor immunity model that can contribute to designing more efficient immunotherapeutic approaches to control cancer.

Model to study vascular and lymphatic transdifferentiation and regeneration. The *Xenopus* tadpole has recently emerged as a very powerful system for tissue and vasculature regeneration research (Slak et al., 2008). Within 7-10 days following amputation, a completely new functional tail, with all its tissue types (including muscles, spinal cord, etc) regenerates in this system. Formation, maintenance and regeneration of lymphatics and blood vessel have become a major area of investigation in their own right, as well as owing to on immune function and immune responses (Ny et al., 2005; 2008; Fukazawa et al., 2009)

Model *to study immune responses to important emerging infectious diseases*: *Xenopus* provides a powerful laboratory model to study immunity to important emerging infectious diseases caused by a chitrid fungus and by ranaviruses (*Iridoviridae*). The recognized threat of these emerging wildlife diseases on global biodiversity, which ultimately impacts on human health, makes it urgent to better understand host-pathogen interactions in vertebrates other than mammals. Because of the extent to which knowledge has already been acquired, as well as the availability of tools including microarrays and genomic information, *Xenopus* is an

ideal model for such studies. For example, comparison between susceptible tadpoles and resistant adults to ranaviral infection, and between susceptible *X. tropicalis* and resistant *X. laevis* to chytrid fungal infection, provide ways to elucidate virulence and immune escape mechanisms that are of high fundamental relevance (Morales and Robert, 2007; Rosenblum et al., 2009). The unique antimicrobial peptides in skin secretions produced by *Xenopus* are very potent against HIV and many human gram negative and positive bacteria, and therefore are of high biomedical interest. Available genomic information will provide further insight about the regulation and evolution of the genes encoding these proteins (Zasloff, 2002).

Generation and maintenance of animal and tools: Invaluable research tools for *X. laevis* including monoclonal antibodies (mAbs), antisera, cell lines, genomic, cDNA, and EST libraries have been accumulated since 1976 and are maintained for the scientific community in a research resource funded by NIAID. This resource also maintains MHC-defined and clones that permit classic adoptive transfer and transplantation manipulations (e.g., skin grafting) as in mice. Unlike mice, however, they also permit transfer of tissues and cells between larva and adult. Material and animals have been provided for more than 40 laboratories worldwide. Recently, inbred strains of *X. tropicalis* have also been established.

Several transgenesis techniques are now operational for both *X. laevis* and *X. tropicalis*, and transgenic lines with fluorescence reporter genes specifically expressed by myeloid cells are available (ref. Other transgenic lines are under development. A relatively large panel of mAbs including anti-MHC, and anti-B, T, NK and general leukocyte markers are available for *X. laevis* and more are currently being generated using novel technologies such as phage displays of single chain Abs. Generation of *Xenopus* -specific Abs is among the priorities identified by the *Xenopus* community. The combined use of transgenic lines with cell types expressing fluorescence reporter genes and flow cytometry cell sorting using available mAbs to isolate specific cell subsets with the possibility of transferring these cells to embryos or adult recipients will make *Xenopus* an even more valuable model in the next decade.

In summary, *Xenopus* provides a unique, versatile, non-mammalian model with which to investigate important contemporary issues of immunity such as, ontogeny of immunity, self-tolerance, autoimmunity, tumor immunity, and adaptation of host immune defenses to emerging pathogens. The recent genomic and genetic technologies developed in *Xenopus* has the potential to make *Xenopus* a one of the most powerful and innovative comparative models for immunological and biomedical research.

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National Institute of Alcohol Abuse and Alcoholism (NIAAA)

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A key question in alcohol research is the sensitivity of proteins to modulation by ethanol. Because this is a small molecule with low potency, defining the multiple targets responsible for its wide range of biological actions. The *Xenopus* oocyte expression system has been critical for defining proteins sensitive to alcohol and elucidating molecular sties of action on these proteins. In brief, a number of investigators have expressed proteins (primarily brain proteins) in *Xenopus* oocytes and used site-directed mutagenesis to define protein regions critical for alcohol actions. Several of the human genes coding these proteins (members of the GABA receptor family) have emerged as leading candidates for genetic predisposition to alcoholism (and abuse of other drugs) in multiple human populations, thus showing the translational value of the basic research that has been carried out in *Xenopus* ooctyes. One current limitation of this system is that posttranslational modification of these proteins, particularly by protein phosphorylation, may be important for alcohol actions. Thus, the field needs more detailed knowledge of the enzymology of *Xenopus* oocytes, particularly the sequence of all genes coding for components of the posttranslational machinery. The proposed *Xenopus* projects will be very valuable for future studies using *Xenopus* oocytes for alcoholism, and other neuroscience, research. Representative publications about the use of *Xenopus* oocytes in alcoholism research, and the implications of this research for human genetics, are given below:

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National Institute of Biomedical Imaging and Bioengineering (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 mechaniscs, 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 higly 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.

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Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

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The NICHD has made a major investment into the study of *Xenopus* as a model vertebrate organism, and there has been a significant profit from this in terms of our understanding of the fundamental mechanisms of vertebrate development that underlie congenital disorders of children. NICHD has also invested in the sequencing of the *Xenopus* genome(s), the development of *Xenopus tropicalis* as a new model, the development of hundreds of EST libraries, and the generation of arrayed expression libraries; along with supporting resources such as databases, and the development of novel genetic, genomic, and bioinformatic techniques. These areas of research will be increasingly combined in the future, and together have placed *Xenopus* in a prime position for the next generation of studies in which experimental embryology and gene targeting will be combined with systems-level analysis.The advantages of *Xenopus* that originally made it one of the most studied model vertebrate organisms will speed the utilization of these novel supporting resources. Using these approaches, we will gain a new and deeper understanding of vertebrate developmental mechanisms, and be positioned to functionally test potential therapeutic reagents for human congenital disorders, using the well-defined developmental pathways of *Xenopus* .

Several properties of *Xenopus* have made it a model vertebrate of choice. These include:

- speed of development. Experiments take days, not weeks or months.
- well worked out pathways of early development,
- abundance of material for biochemistry,
- a reliable fate map during development, allowing the targeting of reagents both into specific tissues and specific organ systems.
- rapid gain and loss of function assays, for specific genes throughout early development.
- the ability to dissect the embryo and graft specific regions from one embryo to another,
- the availability of the oocyte for experimental manipulation,
- the availability of egg lysates to identify cell cycle components,
- the availability of metamorphosis and larval regeneration as models for tissue regeneration,
- a developing set of tools for transgenesis and gene targeting late in development.
- the ability to carry out forward genetics in *Xenopus tropicalis*

These properties have resulted in the fact that most of what we know about vertebrate early development was initiated by studies in *Xenopus*, as well as from related amphibians. Examples include the identification of intercellular signaling as the primary causative agent of early tissue specification and germ layer formation (Nieuwkoop,1985 ; Smith 1986), the natures of the signals involved and their transcriptional targets (reviewed in Heasman 2006), and the maternal transcription factors that initiate these signals (Tao et al. 2005).

As systems biology approaches become routinely available, these will dramatically enhance the ability of *Xenopus* to provide new insights into normal development.:

- **First, the initiation of development.** Although a maternal forward genetic screen has identified some genes important in early development in zebrafish (Abrams and Mullins 2009), it is impossible to carry out a genetic screen that will reveal all the gene regulatory networks active in early patterning of the embryo. However, a screen based on knockdowns of specific maternal mRNAs, followed by genomic/proteomic analysis of

the effects on gene expression in early development, could do this. Rapid knockdowns of individual maternal mRNAs can be carried out in *Xenopus* (Torpey et al. 1991). The amount of material available for analysis (57ng polyA$^+$ RNA per gastrula, 20µg non-yolk protein per gastrula), will allow both transcriptome and proteome analyses of the embryos. Furthermore, the ability to dissect the early embryo into its component regions, and graft material from an experimentally manipulated embryo to a control embryo, and vice versa, adds an additional level of discrimination to the analysis of the functions of individual maternal genes (Wylie et al. 1996). Systems level analysis of embryos from such a screen will, in the long term, identify the entire gene regulatory network initiated by maternal transcripts, which controls formation of the basic body axes, early tissue differentiation, and primary germ layer formation.

- **Second, the formation of the organs of the body**. Two properties of *Xenopus* will make it increasingly important in this respect; the fate map, and its lack of growth during early organogenesis. These properties mean that bio-active reagents, combined with lineage tracers to identify the descendants of the injected cell (Gimlich and Gerhart 1984) can be injected into single identifiable blastomeres at early stages, and will give rise to clones of descendants in specific target organs or tissues. Furthermore, their concentrations will not change during development because the *Xenopus* embryo does not grow. This unique property has allowed manipulation of gene expression well into the organogenesis period, in discrete regions of the embryo. Most commonly this has been done using morpholino oligos, first used in *Xenopus* embryos (Heasman et al. 2000). Since this paper appeared, more than300 papers have been published using this technique to identify genes required for differentiation of individual organs (Small et al. 2005), specific morphogenetic movements (Nandadasa et al. 2009, Skoglund et al. 2008), or specific cell processes (Kim et al. 2009). In the future it will be important to extend these studies with the increased level of sophistication allowed by collection of descendant cells by cell sorting, and genome-wide analysis of the effect of gene targeting in specific cell types. The large amount of material available for biochemistry will make it straightforward, for example, to identify target genes, and altered protein associations. Morphogenetic movements are the movements of tissue masses that shape both the whole embryonic body, and its constituent organs. The large size, and ease of dissection, of *Xenopus* embryos allows the embryo to be cut into explants, and imaged with high resolution. This permits the study of sub-cellular events and interactions with the extracellular matrix in real time, and combined with rapid gain and loss of function experiments provides a powerful experimental tool to study morphogenetic movements in the embryo (Nandadasa et al. 2009, Dzamba et al. 2009, Keller et al. 2003).
- **Third, later organogenesis.** The ability to make transgenic lines of *Xenopus* (reviewed in Loeber et al. 2009) allows a major new direction of research in *Xenopus*; late-stage organogenesis. This is perhaps the most difficult area of developmental research in vertebrates, and yet is extremely important in the study of birth defects, many of which occur relatively late in the development ofindividual tissues or organs. The generation of new transgenic lines (Yergeau et al. 2009), the application of Cre-mediated gene targeting (Rankin et al. 2009), and novel reporter proteins (Waldner et al. 2009), will be essential in the continued development of tools to study late organogenesis. Stable transgenic lines of *Xenopus* will have enormous potential, because of their long life span, and the numbers of eggs, and thus experimental tissue, they can generate. In addition, the generation and mapping of specific mutations in *Xenopus tropicalis* has begun to allow genetic analysis organogenesis in *Xenopus* (Abu-Daya et al. 2009). All of the techniques mentioned above can be applied to embryos from these lines, allowing a level of sophistication of analysis that simply does not exist in any other organism. This

will be particularly useful for the functional analysis of specific mutations that have been shown to cause congenital disorders in humans**.**

- **Fourth, metamorphosis and tissue regeneration.** NICHD has supported work on metamorphosis and nuclear hormone action, and this area will continue to provide valuable insights into hormone induced tissue remodeling. This and the regenerative power of the tadpole will focus future attention on later organogenesis and the signaling underlying tissue homeostasis (Beck et al., 2009). The continuing development of transgenic technologies will dramatically help this area of research.

Analysis of fundamental cellular processes applicable to many aspects of biology and disease. The nature of *Xenopus* development, and the properties described above, have made it a model, not just for vertebrate developmental mechanisms, but also for universal mechanisms. Examples of this include the enormous advances made on our understanding of the cell cycle in *Xenopus* (Mochida et al. 2009), basic mechanisms of the Wnt signaling pathway (Cha et al. 2008), the specific role of signal inhibition in development (Smith and Harland 1992, Lee et al. 2006), the generation of form by changes in cell shape (Rolo et al. 2009), the identification of pluripotency mechanisms in the oocyte (Gurdon and Melton 2008), the fact that vertebrates can be cloned from individual cell nuclei (Gurdon 2006), the discovery that morpholino oligos can be used to block gene expression in embryos (Heasman et al. 2000), and many more. In the future, it will continue to provide new information on these topics. It will also provide an extremely sensitive assay for novel compounds that act as agonists and antagonists for developmental pathways, since the readout of these pathways is known with some detail in *Xenopus*, and given the large amount of tissue available, can be easily quantitated. *Xenopus* embryos offer the most rigorous model available for fast throughput screens like this.

Novel mechanisms of development identified in *Xenopus* are applicable more generally to both normal development in other species, and to disease processes. Examples include ectodermin, an attenuator of TGFbeta signaling in *Xenopus* (Dupont et al. 2005) also plays a role in limiting the antimitogenic effects of Smad4 in tumor cells. Other modifiers of intercellular signaling such as noggin, identified in *Xenopus*, have also been found to function in mammals, and to be mutated in children with congenital deformities (Hwang and Wu 2007). One of the most useful roles of the *Xenopus* embryo, in addition to its function in rapidly producing new knowledge of development, will be in translational studies. As our knowledge increases of the precise mutations that cause developmental disorders in children, so the effects of such mutations on specific developmental pathways can be characterized in this well-understood developmental system. Screening of small molecules for roles as agonists and antagonists of specific steps in these pathways will offer potential therapies in the future (Wheeler & Brandli 2009).

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Amphibians historically have been popular for studies of inner ear development, mainly because of the ease with which embryonic manipulations can be done. *Xenopus*, in particular, provides an excellent model system for studying ear development. The vestibular system of *Xenopus* is very similar to that of humans and, unlike in zebrafish, *Xenopus* have a separate auditory structure. Detailed morphological descriptions of ear development are available in *Xenopus*. Homologues of almost all the molecules involved in mammalian inner ear development have been isolated in *Xenopus* and developing embryos provide an excellent system for gene function assays. Later stages, when the inner ear is differentiated, are very transparent, facilitating *in vivo* observation.

Otic placode induction: The first studies identifying the different embryonic tissues involved in placode induction were done in amphibians. Experiments in *Xenopus* showed that the biasing of the ectoderm to an otic fate begins early in development, at mid-gastrula stages. *Xenopus* has also been important for identifying some of the genes necessary for otic induction such as Sox9 whose mutation in humans can result in campomelic dysplasia, a lethal human disorder characterized by deafness, autosomal XY sex reversal and severe skeletal malformations. Studies in *Xenopus* were some of the first to identify the importance of FGF in otic placode induction.

Axial patterning of the developing inner ear: Sensorineural hearing loss (**SNHL**) is one of the more common birth defects and approximately 20% of these patients have inner ear malformations that are readily visible using radiological examination. Such malformations likely result from defects in inner ear patterning during development. The inner ear is a highly asymmetrical structure with distinct anterior-posterior (A-P) and dorsal-ventral (D-V) axes. Embryonic manipulations in amphibians, where one or more of these axes were switched, demonstrated that A-P axis determination occurs during placode stages and prior to D-V axis determination. In a minority of cases there was an unexpected result: mirror image duplicated (enantiomorphic) inner ears. This observation has remained unexplained until recently when it was discovered in *Xenopus* that half ablations along the A-P axis can result in mirror image duplications at even higher percentages than seen in the rotation studies. The ability to generate mirror-duplicated inner ears provides an assay for studying the molecules and regions of the developing inner ear that are required for normal patterning.

Channels important for hair cell function and inner ear homeostasis: *Xenopus* oocytes are used for studying the physiology of water and ion channels. Identification of the transduction channel of the hair cell, crucial for its mechanosensory function in hearing and balance, has been elusive. Functional analyses of prospective transduction channels often utilize *Xenopus* oocytes. The physiology of the gap junction protein connexin 26 (or GJB2), whose mutation leads to the most common forms of

human genetic deafness, has been studied in homomeric and heteromeric hemichannels using paired oocytes.

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Craniofacial abnormalities are among the most prevalent birth defects, occurring in 1/700 live births, and present a tremendous medical and social burden. Furthermore, oral and dental health issues affect a majority of the population. Much current understanding of human craniofacial development comes from patient studies and will be immensely facilitated by studies in selected animal models, including *Xenopus*.

Overall, it appears that vertebrate craniofacial development is well conserved. Patterning of facial structures requires complex interactions between different tissue types, from the initial specification of the germ layers through morphogenesis of the facial prominences to the integration of the skeletal elements, muscles, nerves and other tissues. These processes begin very early in gestation and continue throughout life. A number of craniofacial abnormalities, including cleft palate, frontonasal dysplasia and DiGeorge syndrome, can be traced to abnormal development of a migratory, pluripotent population of cells called the neural crest. Therefore, defining the etiology of these pathologies requires an understanding of the mechanisms of neural crest formation, migration and plasticity.

Methodology useful to analysis of craniofacial development and abnormalities

Xenopus is one of the most accessible vertebrate model systems for analysis of craniofacial development. In particular, developing craniofacial structures are more readily visible in *Xenopus* than in any other vertebrate model, primarily because *Xenopus* embryos develop externally to the mother, allowing analyses of the earliest stages, and facilitating live imaging at single cell resolution. Amongst vertebrate models developing externally, *Xenopus* is more useful for craniofacial analysis than the zebrafish system, as *Xenopus* embryos are larger and easier to dissect, and the developing facial region is more accessible to imaging than the equivalent region in fish. Explants and transplants have been routine for decades; this, combined with the large clutch size (hundreds vs dozens in zebrafish) allows easy reproducibility. The ease of gain- and loss-of-function experiments in *Xenopus* has led to discoveries fundamental to biology, including Nobel Prize winning work on the cell cycle (Medicine, 2001) and water channels (Chemistry, 2003). Furthermore, experimental analyses have explored topics as varied as transcriptional control, chromatin accessibility, RNA processing, protein translation, pharmacology and synaptic plasticity. As more human mutations are uncovered, new genes, with unclear functions, will be implicated in craniofacial development.

Xenopus embryos are one of the simplest and most economical models in which to study gene function in an intact animal. Mutant alleles can be readily expressed *in vivo*; the large clutch size then allows reproducible, statistically significant phenotypic and biochemical readouts. The recent development of forward and reverse genetics in *Xenopus tropicalis* will result in new insights into craniofacial development. Several ongoing mutagenesis screens (Yale University, USA; Sanger Center, UK; National Institute of Medical Research, UK) have already produced multiple carriers of craniofacial mutations. In complementary studies, a TILLING (targeting induced local lesions in genomes) strategy is being used to identify mutations in known genes. These banks of mutations can then be used in combination with well-established embryological and molecular approaches. The availability of chemical libraries also makes *Xenopus* an extremely attractive system for studying craniofacial anomalies. *Xenopus* embryos are aquatic and can be arrayed in multi-well dishes, allowing automation of chemical screens. Libraries of small molecules can simply be added to the media, and tadpoles can then be assayed for morphological changes visually. Finally, the increased availability of

transgenic *Xenopus* lines will contribute to analysis of genes and processes associated with craniofacial abnormalities, especially when combined with chemical screening.

Recent data from *Xenopus* studies pertaining to craniofacial development

Work using *Xenopus laevis* embryos has contributed tremendously to knowledge of early steps in craniofacial development. Most notable are studies on the early induction of the neural crest. Functional studies have defined the molecules and signal transduction processes important for cell-cell and tissue-tissue interactions during neural crest development (including BMPs, FGFs and Wnts). Mechanisms underlying migration of neural crest cells can also be studied in *Xenopus*: recent work includes the molecular basis of contact inhibition and directional migration of neural crest cells. These kinds of studies are important for understanding craniofacial defects resulting from abnormal neural crest development.

Another use of *Xenopus* has been to analyze development of the primary mouth (or stomodeum) - the first opening between the pharynx and the outside of the embryo. Multiple craniofacial defects are likely to be caused by defects in this region. Recent work in *Xenopus* defined a set of steps leading to primary mouth opening, where the earliest step is local dissolution of the basement membrane. Further analysis showed that local expression of the Wnt inhibitors, Frb1 and Crescent, is necessary for basement membrane breakdown in this region. Basement membrane remodeling is essential for normal development of most organs, and pivotal in metastasis, and these unprecedented findings have proven *Xenopus* a pioneer organism, yet again.

With regard to chemical screening, a recent study identified multiple compounds affecting cell migration. By combining chemical structure predictions and enzymatic assays using *Xenopus* lysates, the authors identified an activity that inhibited matrix metalloproteinases (MMPs). They then performed loss-of-function analyses; by knocking down several MMPs, confirming the drug target. Finally, they were able to extrapolate their findings to a human melanoma cell line, illustrating the ease of using *Xenopus* as a whole animal assay system for drug discovery.

Due to the unusual demands of metamorphosis, *Xenopus* also provides a fascinating example of developmental plasticity. Craniofacial alterations during metamorphosis are similar to changes that occur in regeneration, remodeling and wound healing. Thus, studying these transitions may be extremely informative. Recent studies have begun applying molecular tools to these questions.

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National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

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Xenopus has played a very important role in the mission of NIDDK for a long time. *Xenopus* oocytes have been and still are an invaluable system to study the conductive properties of many channels and transporters expressed on renal epithelial cells. Many recent technological advances such as antisense morpholino oligomers for gene knockdowns, transgenic GFP lines for imaging and the genome information for *X. tropicalis* have promoted *Xenopus* as a valuable model not only to study early embryonic development, but also to investigate organogenesis. This has been realized by NIDDK and projects exploring the pronephric kidney, the pancreas and the liver are among the currently funded grants.

Electrophysiology using *Xenopus* oocytes: *Xenopus* oocytes express a low number of endogenous membrane transporters and channels because they are virtually independent from exogenous nutrients. As such they have been and are the preferred *in vivo* model to characterize channels, receptors and transporters present on renal epithelial cells that are crucially important for kidney function. Oocytes are used to study electrophysiological properties, stoichiometries and the role of post-translational modification. The system is also very amendable to high-throughput screening approaches. As such it has been a powerful tool to perform functional screens for genes encoding ion channels and transporters. In addition to their basic science component these studies have significant impact in respect to human diseases. For example, studies on hypertension have used *Xenopus* oocytes to demonstrate that defects in With no Lysine kinase 4 (WNK4) causes increased activity of the renal transporter molecules NKCC2 and NCC and thereby directly interferes with blood pressure control.

Kidney Development: *Xenopus* embryos due to their aquatic life develop a functional pronephric kidney within 31 hours post fertilization. Thus, *Xenopus* has been established as a valuable animal model to study kidney development. Over the years, it has become evident that the process of kidney development is evolutionary conserved and findings in *Xenopus* are directly applicable to studies in higher vertebrates such as humans and mouse. One of the most recent advances was the realization that *Xenopus* is a powerful model organism to study the patterning of the nephron along its proximal-distal axis. With the availability of the *Xenopus tropicalis* genome it was possible to identify many structural proteins that are specifically expressed in defined segments of the pronephros. This patterning was highly reminiscent to the one found in individual nephrons of the metanephric kidney. It provided a novel angle to understand how transcription factors actually pattern the kidney along its proximal distal axis as illustrated by the recent study on the Iroquois (Irx) gene family. Similarly, the synchronous development of the *Xenopus* pronephros has also provided many novel insights in how kidney progenitors differentiate into their mature counterparts (e.g. the blood-filtering podocyte) or how microRNAs regulate terminal differentiation of the renal epithelial cells.

In addition to understanding the processes that regulate normal kidney development, the pronephric kidney of *Xenopus* is also a valuable tool to study kidney diseases. Knockdown of genes mutated in human forms of Polycystic Kidney Disease result in a “PKD-like” phenotype in *Xenopus* that is used to better understand the molecular mechanisms leading to kidney cyst formation. In particular, the speed of analysis and the nearly unlimited availability of embryos provide an ideal *in vivo* test system to study aspect of Polycystic Kidney Disease that cannot be performed in mouse as easily.

Finally, the *Xenopus* kidney is a great system to study tissue engineering. *Xenopus* was the first organism, where it could be shown that the combined action of Retinoic Acid and Activin can convert primitive ectoderm into a functional kidney that can even be transplanted in

nephrectomized *Xenopus* embryos. Ongoing work has extended these studies to several cell types in the kidney and has played an important role in identifying novel kidney-specific genes as well as ways to generate kidney epithelial cells *in vitro*.

Pancreas Development: The formation of the pancreas and the control of islet cell differentiation is one of the most coveted models of lineage specification. It is of high clinical importance due to its disturbance during diabetes. While mouse and chick have been the traditional models to study pancreas formation, the *Xenopus* pancreas has been developed as a viable alternative. Even though there are differences at later stages of pancreas development and its reorganization during metamorphosis, the early pancreas development in *Xenopus* is very similar to that of mice and humans. Many results are directly applicable to mammalian systems. In fact, one of the most important genes in pancreatic development, *Pdx1*, was initially discovered in *Xenopus*. The current research in *Xenopus* pancreas development follows similar avenues as outlined for the kidney. However, one particular interest is directed towards developing a transcriptional network of pancreas development in an effort to understand how early endodermal progenitors are specified first to a pancreatic fate, then to an endocrine fate and finally to a beta cell fate. For this approach *Xenopus* is uniquely suited since combinatorial knockdown studies using antisense morpholino oligomers allow analyses that are much more time-effective than compound mouse mutants.

Liver Development: Another organ system that has recently found more attention in *Xenopus* is the liver. The liver is an essential organ, yet the molecular basis of liver development is still poorly understood. Therefore, liver transplantation is often the only option for life threatening liver malfunctions. In an effort to develop alternative treatment options such as tissue replacement therapies from stem cells, the processes involved in hepatic tissue specification and the initial patterning of the foregut domain that will give rise to the liver are of high interest. Using the advantages of *Xenopus* it was recently shown that liver development relies on canonical and noncanonical Wnt signaling. Both pathways are necessary, but their activities have to be coordinated correctly to promote proper outgrowth of the liver bud.

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National Library of Medicine (NLM)

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NLM resources such as the National Center for Biotechnology Information (NCBI) play a central role in the daily life of most biomedical scientists. Key resources at the NCBI include PubMed, Entrez Gene, UniGene, OMIM and the various sequence and molecular biology databases. *Xenopus* data plays an important role in the functionality of many of these central resources due to its place in the phylogenetic tree, bridging aquatic models such as the zebrafish and pufferfish and terrestrial vertebrates such as mouse and man. As the suite of experimental techniques available in *Xenopus* is unique it also serves as a methodological bridge between animal model systems and human biology. *Xenopus* is the only amphibian with both large scale genomic resources and a rich heritage of experimental data on the role of genes during embryonic development and the only amphibian used extensively for high throughput microinjection screens.

Xenopus data and the *Xenopus* community helps achieve the goals of the NLM by providing annotated data on *Xenopus* development, anatomy and gene structure and function to the NCBI. This is achieved through the *Xenopus* model organism database, Xenbase (NIH R01 HD045776) generating output files used by NCBI services such as Entrez Gene. There are currently over 10,000 Entrez gene records generated from Xenbase data imports. The community provides raw data through sequence submissions and scientific publications. Bioinformatics is essential to associating model organisms data to human biology and disease and this is one of the major goals of the NLM/NCBI. Gene function can be tested in via unique microinjection approaches such as pooled mRNA screens in *Xenopus* and serve as a bridge between genetic data from more simple models and less complete functional information in more complex mammalian systems. These approaches have led to the discovery of many novel genes that play essential roles in human health.

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National Institute of Environmental Health Sciences (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.

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National Institute of Mental Health (NIMH)
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Contributions of research in *Xenopus* to the understanding of major psychiatric and neurodegenerative disorders: The *Xenopus* system has led to, and continues to lead to, fundamental advances in understanding the mechanisms of mood stabilizing drugs. Lithium is the most effective and widely used treatment for bipolar disorder, a mood disorder that affects more than 2 million Americans and more than 50 million people worldwide, and yet the mechanism of lithium action remains uncertain. Lithium also disrupts the early development of *Xenopus* embryos, and this robust phenotype has been used extensively to explore the molecular mechanisms of lithium action. One of the most actively investigated mechanisms for lithium action is the inositol depletion hypothesis, and some of the strongest and most frequently cited support for this hypothesis comes from seminal papers using *Xenopus*, including the classic work from Busa and Gimlich, who provided the strongest in vivo data to date showing that exogenous inositol can reverse effects of lithium on phosphatidylinositol signaling. Their findings provide a cornerstone of the inositol depletion hypothesis.

The NIMH also supports research in *Xenopus* that led to the discovery that lithium inhibits the signaling kinase GSK-3 and thereby activates Wnt and neurotrophin/RTK signaling pathways. This discovery provided a compelling alternative mechanism for the developmental effects of lithium in *Xenopus*, directly led to extensive research on the role of GSK-3 in neuronal signaling in mammalian systems, including humans, and led to clinical trials applying GSK-3 inhibitors for neuropsychiatric and neurodegenerative disorders. NIMH funded research in *Xenopus* also led to studies on lithium and GSK-3 in neuronal regeneration, mammalian behavior, Alzheimer's disease, and other neuropsychiatric disease models.

NIMH funded research in *Xenopus* also directly contributed to the discovery that another widely prescribed mood stabilizing and antiepileptic medication, valproic acid, is a direct inhibitor of histone deacetylases (HDACs). These findings are immediately relevant to the mission of the NIMH, but have also had an important impact on research outside the Institute's mandate, including the development of over 40 clinical trials (see http://clinicaltrials.gov/ and search "valproic acid") using valproic acid to treat neurodegenerative, neuromuscular, and neoplastic disorders, and potentially to activate latent HIV in the treatment of AIDS. Inhibition of HDACs also provides a compelling molecular mechanism for the devastating birth defects associated with the use of valproic acid in humans during pregnancy.

Future Directions for the use of *Xenopus* in research on signaling in psychiatric and neurodegenerative disorders: *Xenopus* is an ideal system for future studies on the mechanisms of mood stabilizer drug action, as *Xenopus* embryos and oocytes provide readily accessible, in vivo systems to query the effects of both small molecules and gene products on canonical signaling pathways, including Wnt, TGF-ß/BMP, and FGF pathways, that have been worked out to a great extent in this model system. *Xenopus* oocytes are widely used vehicles for the study of ion channels and G protein coupled receptors that mediate neurotransmitter signaling, and have been one of the classical systems to study cell cycle regulators, posttranscriptional regulation of RNA, and the analysis of small RNA species. *Xenopus* embryos have been, and continue to be, an essential model system for characterizing the molecular mechanisms of Wnt and TGFß signaling. As these pathways are now believed to be important in the pathogenesis of major psychiatric disorders in humans, including schizophrenia and bipolar disorder associated with mutations in the *DISC1* gene, the *Xenopus* system will

remain an important tool to advance our basic understanding of mental illnesses and to translate these basic discoveries to the treatment of psychiatric disorders.

Xenopus as a model for understanding neurodevelopment and behavior: Of high priority to NIMH objectives is the mechanistic understanding of the links between genetics, nervous system structure as established during embryogenesis, and behavior. *Xenopus* is an ideal vertebrate model system for this purpose because it is uniquely amenable to state-of-the-art functional approaches that target every step along the genetics-behavior axis.

Xenopus is a very popular system for neurodevelopmental studies, with a plethora of information available on the molecular genetics of patterning of the CNS and peripheral innervation. It is also very easy to perturb gene function via gain- and loss-of-function approaches (morpholinos, RNAi, dominant negative and mutant construct misexpression). Likewise, many of the antibodies and RNA probes revealing specific components of the sensory and nervous systems are available and work well in Xenopus. Thus, not only are the mechanics of neural structure being unraveled in this system, but any protein of interest (e.g., candidates for human diseases or syndromes) can rapidly and inexpensively be tested. Because the frog embryo can be manipulated from before fertilization, and completes all of its developmental events *in vitro*, it is a model system in which every aspect of nervous system development and behavior can be tracked (and modulated), from the earliest stages of neural induction through to mature animal social behavior.

Moreover, *Xenopus* possesses unique advantages for this work. First, the neurophysiology community routinely tests ion channel, neurotransmitter, and related proteins in the *Xenopus* oocytes assay, which makes a huge toolkit of well-characterized constructs available that have already been tested to a high level of mechanistic detail in this system (Adams et al., 2006; Levin et al., 2002). This also means that not only can biophysical factors (long-term transmembrane voltage gradients etc.) be studied in addition to secreted factors/ECM, but pre-nervous and nervous morphogenetic roles of small molecule neurotransmitters are readily addressed (Levin et al., 2006). Second, unlike in the zebrafish embryo, early *Xenopus* blastomeres have a determined fate-map (Dale and Slack, 1987; Moody, 1987), which means that specific regions of the nervous system can be targeted by microinjection. For example, one can target one side of the brain with a specific mRNA leaving the contralateral side of the animal as an internal control. This is particularly useful for characterization of brain laterality (Wassersug et al., 1999; Wassersug and Yamashita, 2002), a fascinating topic of high relevance to a number of NIMH priority areas.

Most importantly, *Xenopus* is a model system that provides unique opportunities in cognitive science and ethology. *Xenopus laevis* larvae have been a popular behavioral system for investigation of responses to light and gravity, in individual behaviors and schooling (Copp and McKenzie, 1984; Jamieson and Roberts, 2000; Katz et al., 1981; Lum et al., 1982; Moriya et al., 1996; Pronych et al., 1996; Roberts, 1978; Rot-Nikcevic and Wassersug, 2004; Wassersug and Hessler, 1971). Unlike zebrafish and similar model systems, *Xenopus* tadpoles exhibit complex and rich behavioral patterns as larvae, performing schooling and conspecific recognition within 1 week of fertilization. Thus, *Xenopus* tadpoles can be analyzed for behavior, sensory abilities, learning/memory, and social interactions. These are highly sophisticated animals and yet are small enough to be easily amenable high-throughput automated behavioral analysis technology (Hicks et al., 2006). Thus, the effects of neurotoxins, or putative nootropics (drugs that augment memory or learning rate) can easily be characterized in animals that are mutant, wild-type, or modified by mRNA microinjection or pharmacological treatments. Similarly, the molecular basis of memory and learning pathways are readily addressed in *Xenopus*, since the larvae are readily trained at many stages of development and amenable to surgical, pharmacological, and genetic intervention.

Nearly all of the NIMH priority areas can be advanced significantly by segments of the *Xenopus* community, due to this vertebrate model system's combination of accessibility to molecular-genetic, biophysical, and pharmacological approaches and rich behavioral repertoire that will help us with the exciting and biomedically-crucial task of understanding how embryogenesis ultimately gives rise to coherent behavior and cognitive abilities.

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National Institute of Neurological Disorder and Stroke (NINDS)

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The mission of the NINDS is to reduce the burden of neurological disease. This mission is supported by a robust portfolio of basic research efforts aimed at understanding the structure and activities of the brain, knowledge essential for diagnosing and treating human brain disease. Some important areas of NINDS basic research include: biology of the cells of the nervous system, brain and nervous system development, genetics of the brain, cognition and behavior, neurodegeneration, brain plasticity and repair, neural signaling, learning and memory, motor control and integration, sensory function, and neural channels, synapses, and circuits.

As an overview, because of the ease with which its developing and adult nervous system can be studied, *Xenopus* has been an important model system for understanding brain function. Among the most prominent early examples of general biological insights were Gurdon's studies demonstrating that a cell nucleus from embryonic intestine could drive development of an entire embryo, using nuclear transplantation at the one-cell stage[1]. The use of informative (e.g. animal cap) assays for early tissues interactions (induction) demonstrated that this model system is an engine for gene discovery in neural development[2]. Important insights into synapse formation and refinement came from studies of neuromuscular junctions, especially those in very early development[3] and from the establishment of topographic maps in the retinotectal system[4]. Our understanding of ion channel function in the nervous system has been heavily dependent on expression in *Xenopus* oocytes[5]. Neuroendocrine discoveries included the identification and isolation of melanocyte stimulating hormone[6]. These historical strengths have been followed by a series of very important new discoveries, falling within the NINDS mission, whose insights would have been much more difficult, or impossible, to obtain with other systems. Selected examples from the recent literature (2006 – 2009) are given below.

The fundamental contributions of research in *Xenopus* is documented in the recent papers listed below that were selected to illustrate the facilitation of NINDS mission objectives through use of this model system. The topics range widely and the contributions are substantive and highly visible.

Cell biology of neurons

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CNS genetics; *Xenopus* models of neurological disease

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Cognition and behavior; neuroendocrine regulation

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Acknowledgement: The Contributors to the 2009 Xenopus White paper are deeply indebted to Lisa Palmieri for her administrative assistance.

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