Xenopus Community White Paper 2011

Contents:

Executive Summary

***Xenopus*: An essential vertebrate model system for biomedical research:**

Model animals are crucial to advancing biomedical research. Basic studies in vertebrate animals rapidly accelerate our understanding of human health and disease. Among the commonly used model animals, the frog, *Xenopus,* has great impact because of its close evolutionary relationship with mammals. Moreover, the remarkable experimental repertoire of the *Xenopus* system has made it a cornerstone of neurobiology, physiology, molecular biology, cell biology, and developmental biology.

Current NIH investment in research using *Xenopus*:

Consistent with its broad utility, the NIH has made a large and continuing investment in *Xenopus* research. Indeed, a search of the NIH rePORT database for R01 or equivalent grants using the search term "*Xenopus*" returned **678 grants for a total of over $217,000,000** for FY09-10. The NIH has also recently demonstrated its commitment to *Xenopus* community resources by approving $2.5 million to establish the National *Xenopus* Resource in Woods Hole, MA and a similar amount to maintain and expand Xenbase, the *Xenopus* Community's online database.

***Xenopus* as a model system for human disease gene function**

Given the tremendous power of the *Xenopus* system, the pace of new biological discovery by the *Xenopus* Community is vigorous. Using *Xenopus*, we have significantly improved our understanding of human disease genes and their mechanisms of action, justifying the NIH's investment. For example:

Xenopus embryos are used for *in vivo* analysis of gene expression and function:

Congenital Heart Disease – *PNAS* 2011. 108, 2915-2920
CHARGE Syndrome – *Nature* 2010. 463, 958-962.
Bardet-Biedl and Meckel-Gruber Syndromes – *Science* 2010. 329, 1337-1340.
Hereditary hypotrichosis simplex – *Nature* 2010. 464, 1043-1047.
Hutchison-Gilford Progeria – *Dev. Cell* 2010. 19, 413-25.
Cutis laxa – *Nat Genet.* 2009. 41, 1016-21.
Colorectal cancer – *Genome Res.* 2009. 19, 987-93.
Nephronophthisis – *Hum Mol Genet.* 2008. 17, 3655-62; *Nat Genet.* 2005. 37, 537-43.

Xenopus egg extracts are used for *in vitro* biochemical studies:

Fanconi Anemia – *Mol. Cell.* 2009. 35, 704-15; *Science.* 2009, 326, 1698-701.
C-myc oncogene – *Nature*. 2007. 448, 445-51.
BRCA1 – *Cell*. 2006. 127, 539-552

Xenopus oocytes are used to study gene expression and channel activity:

Rapid-onset dystonia-parkinsonsim – *Nature* 2010. 467, 99-102.
Trypanosome transmission – *Nature* 2009. 459, 213-217.
Epilepsy, ataxia, sensorineural deafness – *N Engl J Med.* 2009. 360, 1960-70.
Catastrophic cardiac arrhythmia (Long-QT syndrome) – *PNAS* 2009. 106,13082-7.
Megalencephalic leukoencephalopathy – *Hum Mol Genet.* 2008. 17, 3728-39.

***Xenopus* as a model system for understanding basic biological processes:**

Xenopus also plays a crucial role in elucidating the basic cellular and biochemical mechanisms underlying the entire spectrum of human pathologies. Just a small fraction of the many recent discoveries are highlighted here:

Xenopus contributes to our understanding of vertebrate genome organization.
(*Science.* 2010*. 328, 633-636*).

Xenopus egg extracts reveal fundamental aspects of cell division.
(*Cell.* 2010. 140, 349-359; *Nature*. 2008. 453, 1132-6; *Science*. 2008. 319, 469-72).

Xenopus reveals new aspects of eukaryotic nuclear structure and function.

(*Cell*. 2010. 143, 288-98; *Science. 2010.* 318, 640-643).

Xenopus embryos are used for studies of Wnt and TGF-β signal transduction.
(*Science*. 2010. 327, 459-463; *Cell*. 2009. 136,123-35).

Xenopus embryos are used for studying mucociliary epithelia.
(*Nat Cell. Biol.* 2009 11 1225-32; *Nature*. 2007. 447, 97-101).

Xenopus embryos are used for studying development of the vasculature.
(*Cell*. 2008.135, 1053-64).

Xenopus egg extracts provide key insights into DNA damage responses.
(*Mol Cell.* 2009. 35,704-15; *Cell.* 2008.134, 969-80).

Xenopus embryos link telomerase to Wnt signaling.
(*Nature*. 2009. 460, 66-72).

Xenopus are used for small molecule screens to develop therapeutics.
(*Nat Chem Biol.* 2010. 6, 829-836; *Blood*. 2009. 114, 1110-22; *Nat Chem Biol.* 2008. 4, 119-25).

Despite its demonstrated utility and despite the recent investments by the NIH, *Xenopus* still lacks many resources that are considered entirely essential for other model systems. It is the consensus of the *Xenopus* community that their biomedical research could be greatly accelerated by the development of key resources of use to the entire *Xenopus* research community.

At the 2010 International *Xenopus* Conference, developmental, cell, and molecular biologists gathered to discuss the resources needed and the priority that should be assigned to each. There was broad community-wide consensus that eleven resources are currently needed, and these were prioritized into two categories: Immediate Needs and Essential Resources:

The Immediate Needs of the *Xenopus* research community:

1. Generation of the *Xenopus* ORFeome:
-Will enable genome-wide *in vivo* analyses of gene function.
-Will enable genome-wide *in vivo* analyses of protein localization.
-Will enable, when combined with transgenesis, the first large-scale biochemical determination of protein-protein interactions in specific tissues and at specific embryonic stages.
-Will facilitate more-rapid functional characterization of specific proteins.

2. Improvement of the *Xenopus* genome sequence:
-Will accelerate molecular studies by providing a complete catalogue of *Xenopus* genes.
-Will enable completion of the *Xenopus* ORFeomes.
-Will enable genomic analyses & systems biology approaches for novel gene discovery.
-Will facilitate proteomics approaches and peptide analysis.

Essential Resources for *Xenopus* research community:

In addition to these most-pressing needs, the community has identified nine other Essential Resources that should be developed as soon as possible, so that *Xenopus* biologists can more effectively fulfill the missions of the NIH. The *Xenopus* community considers all of these additional resources to be essential, but understands that priorities must be set, and therefore ranks these as indicated below:

3. **Improvement of long-range contiguity in the *Xenopus laevis* genome**
4. **Improvement of *Xenopus* antibody resources**
5. **Loss of function: Zinc Finger Nucleases/TILLING**
6. **Loss of function: Small inhibitory hairpin RNAs**
7. **Novel loss of function/knockdown/knockout technologies**
8. **Intergenic annotation of the *Xenopus* genome**
9. **Improvements of the *X. tropicalis* genome – long range contiguity**
10. **Additions and improvements to Xenbase: the *Xenopus* Model Organism Database**
11. **Frogbook: A comprehensive resource for methods in *Xenopus* biology**

Community Recommendations for Attaining Resources:

The *Xenopus* Community feels that in order to attain these much needed resources it will be imperative to renew the PAR-09-240/1: "Genetic and Genomic Analyses of *Xenopus*". This mechanism can help to direct funding to the establishment of resources that will accelerate research by the entire community. Development of research resources is essential to the NIH mission, but because such work is not hypothesis-driven, these proposals fare poorly in standard CSR study sections. Moreover, the standard study sections typically lack the depth of expertise that is needed to properly evaluate these proposals. The "Genetics and Genomic Analyses of *Xenopus*" PAR allows for a focused and expert review of resource development proposals, and its renewal will help to ensure a continuing return on the current NIH investment in biomedical research using *Xenopus*.

The *Xenopus* Community also feels that, given the ease with which massive amounts of biological samples can be obtained using this organism, a new PAR to support systems biology using *Xenopus* is warranted. A new PAR in this area would allow all biomedical researchers to exploit the emerging genomic resources for *Xenopus* to perform systems-level analyses *in vivo*, in a vertebrate, and in a cost-effective manner. Such research would generate significant advances into the "New Biology" described below.

Anticipated Gains for Biomedical Research:

Xenopus as an animal model continues to have a broad impact for biomedical research. Given its already long history of large-scale screens of gene function and its broad use in molecular, cell, and developmental biology, the establishment of additional community-wide resources will greatly facilitate the impact of *Xenopus* as a premier vertebrate model for systems-level analyses.

The National Research Council and the National Academy of Sciences have recently called on the United States "to launch a new multiagency, multiyear, and multidisciplinary initiative to capitalize on the extraordinary advances recently made in biology". This report (http://www.nap.edu/catalog.php?record_id=12764) recommends the term "New Biology" to describe an approach to research where "physicists, chemists, computer scientists, engineers, mathematicians, and other scientists are integrated into the field of biology." The promise of systems-level analysis in *Xenopus*, combined with its already proven strengths, make *Xenopus* the ideal model organism for pursuing "New Biology."

Specifically, genome improvements will provide *Xenopus* researchers with the ability to perform genome-wide screens for biological activities that will in turn allow the rapid assembly and analysis of gene regulatory networks and their relationship to phenotypes. The ORFeome will greatly facilitate such genome-wide screening by allowing all ORFs to be rapidly analyzed or large numbers of proteins to be tagged for analysis of protein-protein interaction or for *in vivo* visualization. Using extracts and biochemical purification coupled with mass-spectrometry and genomic sequence, protein interactomes can be rapidly identified and validated. *Xenopus* offers a unique resource because it is the only *in vivo* vertebrate animal model that couples vast amounts of biological material and a sequenced genome, thus cell-type specific interactomes can also be identified. Large-scale genetic screens will identify important novel genes in developmental pathways, especially given the relatively simple genome of *X. tropicalis* compared to zebrafish. Finally, the flexibility of both *Xenopus* extracts and embryos make this system ideal for chemical biology screens.

Identifying gene-regulatory networks, interactomes, and novel genes will be only the first steps. The well-established power of *Xenopus* for rapid analysis of gene function will then allow deeply mechanistic analyses to complement the systems-level approaches described above. It is the combination of these characteristics that distinguishes *Xenopus* from other vertebrate model systems such as mouse and zebrafish and allows for a systems-level approach to understanding biological mechanisms. The tremendous impact of the *Xenopus* model cannot be realized, however, without the immediate development of community-wide research resources. This White Paper presents the needed resources, and we look to the NIH for guidance in how to best achieve these goals.

Introduction:

As outlined above in the Executive Summary, *Xenopus* continues to be a critical animal model for understanding the biology underlying human health and disease. *Xenopus* not only provides a remarkably broad experimental platform, but as an amphibian it also bridges the gap between costly mammalian models and the evolutionarily more distant zebrafish model.

The *Xenopus* community strongly believes that the pace of discovery using *Xenopus* could be further accelerated if additional 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.

To identify resources needed by the broad and diverse *Xenopus* community, *Xenopus* researchers met last September during the 13th International *Xenopus* Conference at Lake Louise, Canada. The assembled group of roughly 60 PIs discussed the progress made since the 2009 *Xenopus* White Paper and the new resources needed in 2011.

The 2009 *Xenopus* Community White Paper identified three Immediate Needs: 1) Establishment of the National *Xenopus* Resource at Woods Hole, 2) Expansion of Xenbase (the *Xenopus* model organism database) and 3) Sequencing of the *X. laevis* genome. These resources were considered the most pressing needs of the community at that time. All three of these projects have now been funded.

The success in generating these resources has engendered considerable enthusiasm in the *Xenopus* Community. After a lengthy discussion at the 2010 *Xenopus* Meeting, a new set of priorities was established to further the impact of *Xenopus* as a model organism for studies of human health and disease. Two Immediate Needs were identified along with nine Essential Resources.

The Immediate Needs identified by the *Xenopus* community are:

1) **Generation of the *Xenopus* ORFeome**
2) **Improvement of the *Xenopus* genome sequence**

In addition to these immediate needs, the community has identified nine **Essential Resources** that must be developed in order for *Xenopus* researchers to best capitalize on the prior investments in *Xenopus* research made by the NIH.

These Essential Resource include:

3. **Improvement of long-range contiguity in the *Xenopus laevis* genome**
4. **Improvement of *Xenopus* antibody resources**
5. **Loss of function: Zinc Finger Nucleases/TILLING**
6. **Loss of function: Small inhibitory hairpin RNAs**
7. **Novel loss of function/knockdown/knockout technologies**
8. **Intergenic annotation of the *Xenopus* genome**
9. **Improvements of the *X. tropicalis* genome – long range contiguity**
10. **Additions and improvements to Xenbase: the *Xenopus* Model Organism Database**
11. **Frogbook: A comprehensive resource for methods in *Xenopus* biology**

These goals represent the consensus view of the *Xenopus* community. The priorities were established during an International Conference, and an initial draft document was then prepared by several members of the *Xenopus* community, including molecular, cell, and developmental biologists. (The authors are listed in Appendix 1.) The document was then posted for a period of 6 weeks in order to solicit comments and input from the broader *Xenopus* community. Announcements were made on Xenbase and by emails from Xine, the online *Xenopus* newsletter. During the comment period, ~70 Investigators contributed comments. A full list of signatories (i.e. investigators who have read and agree with the conclusions of this document) is included in Appendix 2. We also direct readers to summaries of the important and continuing contributions of *Xenopus* research to the specific missions of individual NIH Institutes on pages 23-68 of this document.

1. Generation of the *Xenopus* ORFeome.

1A. Summary:

We propose to generate a *Xenopus* ORFeome, which we define as a fully sequenced, validated, complete set of *Xenopus* full-length *cDNAs* for every open reading frame (ORF) encoded in the *Xenopus* genome. These cDNAs will be cloned into a vector system such that any ORF/cDNA can be easily transferred into a diverse array of expression vectors for use in a wide variety of applications. This one set of reagents will be an invaluable resource to the entire *Xenopus* community, regardless of scientific discipline, and will greatly decrease the time and cost to characterize any protein in the myriad of functional assays possible in *Xenopus*, thus elevating the impact of *Xenopus* as a model for human health and disease. These clones will be made available, without restriction, to researchers worldwide. The success of such a resource is emphasized by the utility of similar resources for invertebrate model systems and human ORFs. In regards to *Xenopus*, this resource offers the opportunity to couple the ORFeome with high-throughput *in vivo* functional-genomic screening that is not feasible in any other model system.

1B. Why an ORFeome?

An ORFeome represents a central resource of cloned *Xenopus* ORFs, which can be transferred easily to compatible destination vectors for various functional proteomics studies. ORFeomes facilitate rapid functional characterization of proteins in countless assays. This resource is essential in that the rate limiting steps in protein analyses have changed from the identification of new proteins to the initial functional characterization of entire sets of proteins. As outlined above, *Xenopus* has a great and exceedingly unique set of advantages for screening large sets of putative cDNAs in a high throughput fashion. The existence of an ORFeome will amplify these advantages. Of great importance, there is presently a pressing need to *shorten* the time required for the characterization of proteins in any specific biological process (validation of function, validation of interaction partners, defining subcellular localizaton, etc.). An ORFeome would directly address this need, while saving costs and eliminating duplication of effort by independent investigators.

An ORFeome is especially useful for studies in *Xenopus*, because it is the only vertebrate model system that combines a complete genome, a vast supply of embryonic tissue(s), and rapid and powerful functional bioassays. Furthermore, one of the critical features of *Xenopus* is that it allows for rapid large-scale functional genomic screens based on the ease with which proteins can be expressed *in vivo* (in eggs or embryos) or *in vitro* (in cell-free extracts). In addition, *Xenopus* proteins can also be easily expressed in a temporal and spatial fashion *in vivo* through a variety of means including the targeted injection of blastomeres based on the well-established *Xenopus* fate map, introduction of plasmid cDNAs, and by transgenesis. Thus, *Xenopus* 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 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. Indeed, high-throughput functional genomics studies are a cornerstone of the newly-emerging field of systems biology, and it should be noted that large-scale, genome-wide screens of gene function have already been carried out in *Xenopus* for nearly 20 years, leading to a myriad of important discoveries. Based on these successes, the *Xenopus* Community continues to perform such large-scale unbiased functional genomic screens on an ongoing basis. Thus the availability of an ORFeome in facilitating these approaches is in great demand and will have a significant impact on the community.

*1C. Why a **Xenopus** ORFeome?*

While proteins from other species can be effectively expressed in *Xenopus*, scientific rigor demands that we perform *Xenopus* experiments using *Xenopus* proteins. From a 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 possibility that use of a heterospecific protein as the cause for the confusing result. It is well known that genes to co-evolve, and hence their protein products co-evolve, and thus interaction strengths between proteins may have species-specific requirements. 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 the 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.

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.

Finally, the issue of time and cost cannot be overstated. *Xenopus* researchers spend an enormous effort cloning *Xenopus* genes for their genomics and proteomics efforts and then transferring those clones into different vectors for a variety of assays. This consumes NIH dollars and researchers time. Additionally, the research of other investigators is delayed in requesting clones necessary for their experiments. An ORFeome would greatly reduce the time, energy, and money spent in obtaining needed clones. In addition, research would be facilitated by a library of expression vectors with different attributes that further exploit the power of *Xenopus*. With an ORFeome, these new vectors would be readily usable to include a host of different genes and biomedical research would continue to accelerate.

1D. How should we proceed?

A priority identified in the 2006 *Xenopus* White Paper to facilitate expression studies was the generation of collections of full-length cDNAs for *X. laevis* and *X. tropicalis* genes in expression-ready vectors; i.e. pCS2+ or pCS10X. 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 predicted *Xenopus* genes. 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 clones are in expression vectors (in fact, more than 12 different plasmid vectors were 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 standardized, multifunctional GATEWAY vector.

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, etc.)
- N- and C- terminal protein purification tags (GST, TAP, LAP))
- N- and C- terminal fluorescent protein tags (eGFP, mCherry, etc.)
- 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 *X. laevis* and *X. tropicalis* should be PCR amplified in an error free fashion 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 *X. tropicalis* genome is now complete, generation of unrepresented ORFs should begin with *X. tropicalis*, with *X. laevis* to follow as its genome (or transcriptome) becomes available.

Proposed Funding: The Xenopus ORFeome should be funded via user-initiated grants through the PAR - Genetic and Genomic Analyses of Xenopus.

2. Improvement of the *Xenopus* genome sequence.

2A. Summary:

The *X. tropicalis* genome is the only amphibian genome currently sequenced and assembled. Efforts are now underway to begin sequencing of the *X. laevis* genome. These genomic resources have made a huge impact. Nonetheless, these resources need significant improvement in order to realize their true potential for advancing biomedical research employing *Xenopus*. Improving the *Xenopus* genomes will also positively impact other *Xenopus* white paper priorities such as the ORFeome and novel loss of function/knockdown/knockout technologies.

Recognizing that *Xenopus* genome improvements will need to be garnered by individual R01 grants rather than the efforts of a large sequencing center, we have split *Xenopus* genome improvements into multiple different resources including:

Immediate Need #2 – Improvement of the *Xenopus* genome sequence.
Essential Resource #3 – Improvement of long-range contiguity in the *X. laevis* genome sequence.
Essential Resource #8 – Intergenic annotation of the Xenopus genome.
Essential Resource #9 – Improvement of the X. tropicalis genome – long range contiguity.

In the current section, we focus on the Immediate Need for improving the *Xenopus* gene sequence, transcriptome assembly, and annotation.

2B. Why improve the Xenopus tropicalis gene sequence?

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. This sequencing effort has produced an outstanding resource for *Xenopus* researchers and the sequence, assembly and annotation has had a tremendous impact on the 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 (~66%) 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 tremendously successful and has great impact, there are significant limitations associated with the draft sequence data and its genome annotation. A major goal of genome sequencing is to identify gene models across the entire genome. However, due to the large number of in-scaffold gaps and the large number of smaller scaffolds, significant numbers of genes are 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. Therefore, 80% of gene models are incomplete. The number of genes that are missing from the assembly or are not modeled is not known. We can obtain a minimal estimate of 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. Thus, 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.

Recently, a new genome sequence assembly by Jeremy Schmutz and Jerry Jenkins at the Hudson Alpha Institute of Biotechnology has been generated. This genome appears to have many advantages over the previous version. First, contig gaps have fallen from 10% to 5% of the genome, and by incorporating meiotic map information and synteny, chromosome sized assemblies have been generated. While this is a significant improvement in the *X. tropicalis* genome, a substantial number of gaps remain that compromise gene models.

The impact of incomplete gene models on *Xenopus* research has multiple effects. First, incomplete gene models complicate novel gene discovery. Gene models that have accurate annotation of the 5'UTR, CDS, and 3' UTR allow the identification of proximal promoters and regulatory sequences necessary for building gene regulatory networks. Lack of 5' ends of genes complicate the identification of translational start and early exon-intron boundaries that are necessary for loss of function morpholino oligonucleotides. Incomplete gene models significantly complicate genomics efforts or systems biology approaches that aim to look at whole genome interactomes because the genome is incomplete. Creating expression constructs for gain of function experiments simply cannot be done without complete gene models.

Finally, annotation of gene models is essential in order to effectively interpret genomics data. 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). Nearly half of all the predicted *X. tropicalis* gene models (~12,000) 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 currently only have uninformative automatically generated gene model IDs which significantly hampers the analysis of microarray and deep sequencing data, as this requires time-consuming manual identification of interesting target genes. In the absence of good annotation, the interpretation of genomics information is more difficult; this is especially problematic since microarray, deep sequencing, and proteomic information can be so readily obtained in *Xenopus*.

2C. How should we proceed?

We propose several complementary approaches to improve *Xenopus* gene sequence 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-based sequencing. This would help in a number of ways: 1) Mapping transcripts to the genome will aid in resolving incorrect gene models, particularly the 5' ends of genes [for example *Dev 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 inbred *X. tropicalis* strains will identify SNPs that will be used in genetic mapping. Both *X. laevis* and *X. tropicalis* transcriptome sequencing and assembly should be actively pursued.

2. Gene Gap Closure (targeted gap filling and mRNA sequencing)

A complete collection of *X. tropicalis* gene models and/or transcript sequences is needed for other high-priority projects (*X. laevis* genome, ORFeome). The high density of gaps in the genome assembly hinders the complete collection of intact gene models. Although we anticipate that new genome assemblies will improve the number of complete gene models, 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 many gaps in the genome, but only a small proportion of these gaps disrupt gene models. We propose to target sequencing to these gaps and close them in order to provide continuous sequence through gene models. Strategies that should work and should be tried include targeted fosmid or BAC sequencing, sequence-capture sequencing, or PCR amplifying across gaps and sequencing. Given the revolution in massively parallel, sequencing platforms, generating tremendous depth of sequencing necessary for assembly is vastly less costly and should be employed.

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. Genome Data Integration

Additional resources will be needed to integrate data from transcriptome sequencing, gap closure sequencing, and other sequence resources, to produce improved genome assemblies and to resolve the many incomplete gene models. A *Xenopus* Genome Steering Committee has been established with a diverse group of PIs, and includes of a panel of bioinformaticists to create new assemblies.

4. 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. Since effective annotation of sequence information requires dedicated curation teams, we propose that resources be made available to better annotate the *Xenopus* genome. This team would need to work in conjunction with genome assembly groups as well as other nomenclature committees (HUGO Gene Nomenclature Committee) 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 (UCSC, 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.

Proposed Funding: The Improvement of the Xenopus genome sequence should be funded via user-initiated grants through the PAR - Genetic and Genomic Analyses of Xenopus.

3. Improvement of long-range contiguity in the *X. 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. An effort to generate a draft genome has already been funded. However, further improvements are needed to realize the full potential of the *Xenopus laevis* genome including further improvements to gene models, deconvolution of alloalleles, identification of regulatory intergenic sequences, and improving our understanding of genome duplications and the evolution of duplicated genes. We group these improvements into the general term "long-range contiguity".

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 laevis* to our understanding

of cell cycle control, gene regulation, and cell signaling in early development, it is surprising that the *X. laevis* genome has not *already* 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 allotetraploidy would confound a high quality assembly. In contrast, *X. tropicalis* is diploid and has one of the smallest genomes amongst the tetrapods at $1.5x10^9$ bp.

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, sequencing and assembly of the *X. laevis* genome is now feasible. Indeed, a UC-Berkeley team led by Richard Harland and Daniel Rokhsar has secured funding to begin sequencing using Illumina's short read sequencing technology. In the long term, sequencing and assembly of the *X. laevis* genome will advance research using this model system in several specific ways.

Sequencing the *X. laevis* genome using Illumina's short read sequencing technology will produce an assembly that will allow the construction of many gene models. One approach is to use the assembled *X. tropicalis* genome to act as a template for aligning and then assembling the *X. laevis* sequence. However, an independent assembly is highly desirable in case genome changes have occurred since the time of divergence of these species that may provide clues on the evolution of vertebrate genomes. Even with short reads and the genome duplication, *de novo* independent assembly of the *X. laevis* genome is now feasible. However, due to the short reads and relatively small insert sizes required by the Illumina platform, gaps are inevitable and will hinder building a complete set of gene models and contiguity of intergenic sequence. Therefore, improving the *X. laevis* genome by generating long-range contiguity is an important goal. It will enable a number of different research avenues in *Xenopus*.

First, *X. laevis* sequence would provide a complete gene set to deconvolute peptide fragments for proteomics work. This is especially relevant to the many researchers using *X. laevis* to investigate 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 are 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 animal model 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 will 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 approaches. Moreover, the present draft *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. To this end, a *Xenopus* Genome Steering Committee has been established and will coordinate efforts to effectively produce and improve the *X. laevis* genome.

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. This strategy has already been addressed in the second Immediate Need but is relevant here also.

2. Gene-based Assembly
   Short read Illumina sequencing is ongoing and preliminary assemblies are being generated. At a minimum, a preliminary draft sequence should 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
   One limitation that greatly hampers genomic assembly using short read massively parallel sequencing technologies is that the insert length between paired-end reads is relatively short (on the order of a few hundred base pairs). This limitation is inherent to its “bridge” amplification technology. However, new sequencing methodologies have been generated to sequence mate-pair libraries with inserts of a few kilobases, which will allow bridging across common repetitive elements and improve assembly. These mate pair libraries can take advantage of short read massively parallel sequencing technologies and therefore can be relatively inexpensive. New, single molecule, long-read, sequencing technologies are also gaining traction and such long-read sequences could revolutionize sequence assembly. Lastly, sequencing long-insert libraries such as fosmids and BACs will certainly improve the genome as well.

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.

Proposed Funding: The Improvement of the long-range contiguity in the Xenopus laevis genome should be funded via user-initiated grants through the PAR - Genetic and Genomic Analyses of Xenopus.

4. Improvement of *Xenopus* antibody resources.

4A. Summary:

Although genomic and genetic resources in *Xenopus* have been advanced in the past decade, the availability of antibodies (Abs) specific to *Xenopus* proteins is limited. The generation of a wide range of Abs would facilitate all aspects of research in *Xenopus*, including developmental, cellular, molecular, neurobiology and immunology. These Abs need to be generated in a systematic manner and prioritized based on community need and input. Once an Ab has been developed and validated, the Ab would then be curated and distributed via collaboration with the European *Xenopus* Resource Centre.

4B. Why develop antibody resources for Xenopus?

Xenopus has been at the forefront of cell, molecular, and developmental biology for many decades. However with regard to its use for *in vivo* cell biological and biochemical approaches, the broader impact of the *Xenopus* model system has been compromised by the lack of high affinity, high specificity antibodies. Outside of the field of cell cycle control, DNA repair and cytoskeletal regulation, relatively few *Xenopus*-specific antibodies have been generated, and while there are some notable exceptions, there is relatively low cross-reactivity with existing or commercial antibodies. Therefore, there is a widespread and acutely perceived need to improve both access and development of Abs for the *Xenopus* community, and in parallel, to have an open access database of antibodies tested by the *Xenopus* community.

To address this need Drs. Anna Philpott and Chenbei Chang carried out a broad survey at the 13th International *Xenopus* Meeting in Canada in 2010, canvassing all delegates to determine the need to develop better antibody resources and databases: 90% of respondents considered the improvement of antibody resources vital to their research. Within this group, 49 different antibodies had been purchased. We additionally asked how many non-*Xenopus*-specific antibodies had been purchased, in the hope of cross-reaction to *Xenopus* in the last year. This number was a staggering 204, with many respondents stating that the antibodies had not worked. This represents a massive waste of time and financial resources.

This White Paper therefore supports the generation of large numbers of high-specificity, high-affinity antibodies against *Xenopus* proteins. This resource will be essential for exploiting the advantages of the *Xenopus* system to its full impact as a biomedical model.

4C. How to proceed?

We propose both an interim approach and a long-term approach. In the short term, Drs. Anna Philpott and Chenbei Chang are undertaking a community-wide project to collect and organize data on antibodies generated by community members. Secondly, they are collecting and organizing data on commercially-available antibodies raised against other species that have been tested for cross-reactivity to *Xenopus*. This data will be curated and uploaded to Xenbase. This will facilitate research by identifying successfully used reagents and also save considerable resources by preventing the purchase of antibodies that have already been shown to not work in *Xenopus*. This survey of *Xenopus* PIs, to be conducted for the first time in early 2011, will then be undertaken annually to keep this database up-to-date. Moreover, we will cross-reference the antibody database with Xenbase gene-specific pages to seamlessly indicate antibody resources available to proteins of interest. Additional input will be solicited annually at the *Xenopus* Resources Development meetings held at National *Xenopus* Resource at the Marine Biology Laboratory (MBL) in Woods Hole, MA.

In the longer term, the *Xenopus* community must generate antibodies to *Xenopus* antigens. These antibodies must then be made widely available. Monoclonal antibodies could be lodged with the Developmental Studies Hybridoma Bank. Alternatively, a proportion of polyclonal sera (often better for cell biology studies) could be stored at and distributed from the European *Xenopus* Stock Centre or the new National *Xenopus* Resource at the MBL.

4D. Anticipated outcomes:

It would be expected that this priority would result in the generation of a large number of antibodies. These antibodies would be of wide application. For example, for gene regulatory network studies, a series of polyclonal antibodies could be developed for ChIP, and monoclonal or scFc single-chain Fc antibodies 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 that could be used in fixed cell/embryo and real time imaging, depletion experiments or IP to analyze inter-molecular complexes.

Proposed Funding: We envision that the development of new antibody resources should be achieved by a combination of four mechanisms:

-Large-scale antibody production should be supported by user-initiated grants via the Xenopus Genetics and Genomics PAR

- The community should actively pursue public/private partnerships with antibody companies via the SBIR and/or STTR funding opportunities at the NIH.

-All individual researchers should add additional funding requests to their grant applications with the expressed intent of raising antibodies to Xenopus antigens, a proportion of which would be made available to the wider community.

-The community should encourage commercial companies to both make Xenopus-specific antibodies and also to include Xenopus as a species against which its other antibodies are tested.

5. Loss of function: Zinc Finger Nucleases and TILLING

5A. Summary

Xenopus has been a powerful system for high-throughput dissection of vertebrate gene activities for two decades, initially via gain-of-function methods such as injection of synthetic mRNA. To facilitate the use and impact of *Xenopus* as a model system, loss of function technologies must continue to be developed, advanced, and integrated with genomic resources. Recent technological advances in loss of function methods such as Zinc Finger Nucleases and TILLING have made this a reality in *Xenopus* and should be further developed.

5B. Why develop loss of function tools in Xenopus such as Zinc Finger Nucleases and TILLING?

Historically, the ease of injecting *Xenopus* embryos and/or oocytes with RNA or DNA established *Xenopus* as the pre-eminent high-throughput system for analysis of vertebrate gene function *in vivo*. Gain-of-function (GOF) approaches continue 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. These procedures and technologies are unique to the *Xenopus* model system. For example, whole-transcriptome screens in *Xenopus* can be performed *in vivo* with pools of synthetic mRNAs, which are specific for a molecular or biological function and thus, produce a distinct embryological phenotype. These screens are highly efficient, allowing a single lab to filter through thousands of mRNAs quickly and efficiently. Moreover, these types of approaches will only have greater impact for the study of human health and disease when coupled 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 in a particular biological process. *Xenopus* is unique in its ability to rapidly combine LOF tools, genomics, and GOF assays in a single system. Thus, *Xenopus* provides and exceptional platform for *in vivo*, systems-level analyses of vertebrate biology.

Traditionally, morpholino-oligonucleotides (MOs) were described in *Xenopus* and have been widely used by the *Xenopus* Community mainly since these antisense oligonucleotides can be easily injected into *Xenopus* and efficiently lead to depletion of a target protein. Though MOs have revolutionized *in vivo* studies in vertebrate animals by allowing very rapid depletion of multiple proteins simultaneously in the same animal, MOs do have some disadvantages. For example, later embryonic phenotypes are particularly problematic since MOs can lose efficacy at later stages do to effective dilution of their concentration with cell divisions. Because loss of function approaches are essential, generating additional loss of function technologies for *Xenopus* is paramount.

Genetics has recently emerged as a powerful tool in *Xenopus* biology. The diploid species *X. 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, since it has not undergone duplication, is relatively simple compared to that of teleost fishes. Therefore phenotypes in frogs may be more relevant to mammals than many of those obtained in zebrafish. Based on these observations, pilot forward genetic screens in *Xenopus* were conducted and have led to a number of informative mutant phenotypes. Moreover, cloning of genes responsible for these mutant phenotypes has been facilitated by novel *Xenopus* strategies such as rapid low-resolution meiotic mapping, which takes advantage of the simple genomic structure and the ease of haploid genetics including gynogenesis in *Xenopus*. These types of approaches have higher resolution in *Xenopus* due to the massive number of meioses available for genetic analysis in a single mating (many thousands). Collectively, positional cloning in

Xenopus has already had a significant impact on human health and disease through the identification and characterization of mutations in a number of disease models.

Complementing these approaches, reverse genetics generates mutations in known genes for analysis. A number of approaches have been demonstrated in *Xenopus* as well as other systems. Both Zinc Finger Nucleases and TILLING are able to generate lesions in DNA that can lead to loss of function (discussed below). Small inhibitory hairpin RNAs can also be used to knockdown gene function (Essential Resource #6). Finally, novel methods for loss of function need to be explored and further developed (Essential Resource #7). Adding these loss-of-function strategies to the remarkable experimental repertoire of *Xenopus* will significantly enhance our ability to elucidate the underlying biology.

5C. Why Zinc Finger Nucleases (ZFNs)?

The broad representation of zinc finger motifs in specific DNA binding proteins and their well-understood structure has led to a thorough understanding of the way that these proteins interact specifically with DNA. From this understanding a number of groups in both the pharmaceutical industry and in NIH funded laboratories have built libraries of oligonucleotides encoding zinc finger motifs that can be combinatorially arranged to target specific sequences. Ultimately, with the growth of these libraries, it should be possible to target almost any DNA sequence with high specificity. In order to induce a lesion in DNA, multiple zinc fingers are linked to a dimeric endonuclease domain of a restriction enzyme; the zinc fingers provide specific DNA binding, and the nuclease creates a double stranded break in the target. Following endonucleolytic cleavage, nucleotides are removed from the ends or are added by endogenous nuclease and polymerase activities, prior to non-homologous end joining. This process frequently results in a mutation in the sequence. To date, ZF Nucleases have been used to target chromosomal loci in cultured cells and in a variety of embryos, including mammalian, fish and a variety of invertebrates.

In work shortly to be resubmitted for publication, the effectiveness of ZFNs has now been verified in *X. tropicalis*, where a variety of mutations of varying allelic strength have been recovered and propagated through the germline. While the experiments to date have demonstrated the proof of principle, the approach should be broadened so that a variety of selected genes might be targeted with the nucleases, as a fast track to making targeted allelic series of mutations in those genes.

5D. Why TILLING?

TILLING (Targeting Induced Local Lesions in Genomes) employs a panel of randomly mutagenized animals, and then sequences desired loci to identify deleterious mutations in genes of interest. In pilot efforts, TILLING has already identified mutations in known *Xenopus* genes (such as *r*ax and noggin2). The power of TILLING is that, given a large enough panel of mutagenized animals, it offers the possibility of generating multiple different alleles in any given gene. Therefore, the limitations of the TILLING method are a large enough panel of efficiently mutagenized animals and an inexpensive detection method. This is complimentary to ZFNs where the limitation remains the ability to design ZFNs that are specific to the desired locus.

ZFNs and TILLING will be transformative for the *Xenopus* model system. First, true null alleles will be critical for the analysis of any gene's function, including gene regulatory networks and protein interactomes where incomplete knockdown may be insufficient to understand the perturbations in the system. Coupling these mutations with the technologies for large-scale GOF screens (see above) is an approach that is unique to *Xenopus*. Complimenting these approaches, the generation of hypomorphic alleles will allow for new insights into gene functions. Second, genetics facilitates the study of later gene functions in organogenesis and differentiation that often 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*. Expanding our ability to perform LOF experiments on genes expressed later in development, or in the adult, promises to transform the use of *Xenopus* as a model system.

5E. How to proceed?

1. Zinc Finger Nucleases:

Currently the effective ZFNs have been made in collaboration with Sangamo Pharmaceuticals, and the technology has been licensed to Sigma. The cost of the reagents from this private enterprise is remarkably high, so we argue that the *Xenopus* community should take advantage of public efforts to build such libraries of ZFNs. We suggest that a pilot project should be undertaken with the NIH-funded effort directed at making mutations in the zebrafish, and led by Keith Joung, Randall Peterson, Joanna Yeh and Jeffry Sander. Following proof of principle, the community should seek funding for an expanded effort, either in collaboration with the zebrafish effort, or taking advantage of technology developed by the zebrafish group, which will complement the other loss of function approaches outlined in the current White Paper. The technique is currently the only reliable way of making targeted lesions in the genome, without extensive selection from a library of mutagenized animals (as in TILLING), and thus this targeted approach has the advantage of effectiveness in making a specific mutant line.

2. TILLING:

Two efforts to develop TILLING have been funded via the PAR: Genetic and Genomic Analysis in *Xenopus*. These efforts are now optimizing mutagenesis methods and exploiting new massively parallel sequencing methods. Harnessing these new technologies to improve TILLING will reduce the cost of reverse genetics and elevate the scientific impact of such an approach.

Proposed Funding: Both Zinc Finger Nucleases and TILLING should be funded via user-initiated grants through the PAR - Genetic and Genomic Analyses of Xenopus.

6. Loss of function: Small inhibitory hairpin RNAs

6A. Summary

Although a number of reverse genetics methodologies for *Xenopus* are being developed, one technique that has genome-wide and community-advancing potential is RNA interference (RNAi), an endogenous gene silencing phenomenon that broadly regulates gene expression. In the post-genomic era, geneticists have already harnessed the power of RNAi to knock down nearly every protein-coding gene in model invertebrates like nematodes and fruit flies. RNAi knockdown strains very often phenocopy the genetic null mutant, and allow study of essential genes in later developmental stages, which would otherwise be missed from standard mutational analysis. RNAi in invertebrates is so robust and simple that many individual labs routinely and independently apply this approach to their genes of interest. Scientists studying vertebrates, however, are still in need of transgenic RNAi tools.

RNAi has revolutionized genetic screens and functional genomics in invertebrates, yet the wide application of RNAi in vertebrates is largely restricted to *in vitro* cell culture. Successful use of RNAi in vertebrate animal models has been elusive, probably due to an incomplete understanding of the activity of the pathway during vertebrate development. To overcome this obstacle, next-generation RNAi technologies tailored for transgenic RNAi in vertebrates can be most efficiently and cost-effectively developed in *X. tropicalis* due to the ease of transgenesis and certain ideal characteristics of this animal (a tetrapod) and its genome. RNAi implementation in *Xenopus* will greatly accelerate functional characterization of genes implicated in roles for human development and disease, and provide a ready resource for individual laboratories to more productively gain insights into genes under investigation.

6B. Why develop inhibitory small hairpin RNAs in Xenopus?

The effector units of RNAi are small RNAs (20-24 bp) that guide Argonaute nucleases to target messages. Specific complementary base pairing between the small RNA and the target message results in mRNA degradation. Invertebrates can utilize long double-stranded RNAs (dsRNAs) that serve as precursors for small RNAs. However, an innate immunity pathway in vertebrate cells recognizes dsRNAs as viruses and initiates cell death and clearance. On the other hand, microRNAs (miRNAs) are endogenous small RNAs that regulate endogenous genes and come from a small hairpin-like precursor, which avoids triggering vertebrate innate immunity.

Current transgene-based RNAi technologies for vertebrate cells express small hairpin RNAs (shRNAs) for which the specific element that is double-stranded has homology to a target gene. These hairpin vectors can

effectively silence a target gene in cell culture; however shRNA design is still not fully understood because many shRNAs mysteriously fail. Thus, screens in vertebrate cells and vendors selling shRNAs must employ multiple shRNA designs against a single gene, not only to control for off-target effects but also because of inherent shRNA variability. Finally, transgenic shRNA approaches in mouse tissues and transgenic mice lines have been reported but are rare not only because of shRNA variability but also because shRNA expression may incur toxicity by interfering with the endogenous miRNA pathway. In other words, while cell cultures can tolerate high levels of shRNAs, developing embryos and animals may have lower tolerance for shRNAs.

Despite past limitations, several recent discoveries now suggest a ripe potential for making transgenic RNAi a robust and transferrable technology to *Xenopus* and vertebrate researchers. First, transgenesis vectors and protocols for *X. tropicalis* continue to improve with different modes of regulation and widespread tissue expression. Second, RNAi biologists believe shRNA design parameters can be refined to further mimic endogenous miRNAs to improve success rate and reduce shRNA variability and toxicity. Third, several lines of evidence suggest that co-expression of extra factors from the RNAi pathway during transgenesis may buffer shRNAs from antagonizing the miRNA pathway, thus improving the tolerance of animals for RNAi-mediated knockdown of target genes. Should the technology for transgenic RNAi in *Xenopus* be achieved, the utility and large impact for functional genomics studies will be obvious.

6C. How should we proceed?

Next-generation designs for shRNAs incorporating the newest data on miRNA characteristics are under development (Lau lab, Brandeis University, and Grainger lab, University of Virginia). As proof of principle, new shRNA designs will be developed for a set of genes with well-characterized developmental roles in *Xenopus*. In conjunction with *Xenopus* cDNAs, next-generation shRNAs will be initially evaluated for specificity and efficacy in gene knockdown studies by transient transfection in cell culture. The shRNAs cloned into *Xenopus* transgenesis vectors allow for quick validation in cell culture, while promising shRNA vectors will ultimately be microinjected and integrated into developing *X. tropicalis* embryos. Initial mosaicism from P0 animals will be expected and inspected for tolerance of shRNA vectors, while F1 animals from germ-line transmitting founders will provide the clearest test of transgenic RNAi efficacy.

In addition to shRNA design improvements, co-delivered RNAi factors are being tested to augment the RNAi pathway so that embryonic cells can accommodate shRNAs by buffering shRNAs from affecting endogenous miRNA systems. This will require optimization of transgenesis vectors to become multi-functional: expressing a fluorescent reporter for verification/tracking of transgene integration, the extra RNAi factor, and the shRNA. Constitutive promoters driving transgene expression are being tested first, but clearly tissue-specific and conditionally tunable promoters can be incorporated for greater genetic control. Taking a page from *Drosophila*, a binary system of transgene expression similar to the GAL4-UAS method could be applied here and has been used in *Xenopus* transgenesis. The major repositories of *Drosophila* transgenic RNAi strains already employ the GAL4-UAS system, allowing for remarkable spatiotemporal silencing of gene function.

Once transgenic RNAi vectors and shRNA designs for *Xenopus* begin to exhibit robustness in generating expected phenotypes from target gene knockdowns, these vectors and design parameters can be widely distributed to the research community. Algorithms for optimal shRNA design can be automated so that any researcher can access a database of shRNA templates for any gene and construct their own set of transgenic RNAi vectors. The *Xenopus* stock center could be a training resource and might be employed to distribute stable RNAi strains to researchers for future experiments, including performing synthetic gene interaction studies in the RNAi background. Myriad of applications could extend from the development of this technology in *Xenopus*, including translating what is learned in *Xenopus* transgenic RNAi to the mouse and other vertebrates important for biomedical research.

Proposed Funding: Development of Small Inhibitory Hairpin RNAs in Xenopus should be funded via user-initiated grants through the PAR - Genetic and Genomic Analyses of Xenopus.

7. Novel loss of function/knockdown/knockout technologies

7A. Summary:

Current loss of function methods have revolutionized *Xenopus* as a model for understanding human disease. However, the experimental repertoire of *Xenopus* is vast and tools to effectively knockdown or knockout genes in different experimental conditions continue to have certain limitations. Therefore, developing novel knockout or knockdown technologies remains a critical goal for *Xenopus* biologists.

7B. Why develop novel loss-of-function tools in Xenopus?

As described in the previous two sections, loss of function methodologies will significantly advance the use of *Xenopus* to understand basic human biology and disease mechanisms. Because there are so many different experimental techniques used to study biology with *Xenopus*, additional loss of function techniques are important to develop. Greater flexibility for knockout and knockdown technology will allow *Xenopus* investigators to fully exploit loss of function in *Xenopus* oocytes, cell free extracts, developing embryos and adults.

7C. How should we proceed?

Recognizing the power of loss of function studies in *Xenopus*, the Community feels that additional genetic and non-genetic methods are required. It is important to note that many 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 *X. 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.

It is of vital importance that additional avenues for manipulating gene function are developed. These include non-genetic techniques for affecting specific genes including small-molecule "chemical genetics" screens, and alternative genetic strategies, i.e. homologous recombination strategies for targeted gene disruption and insertional mutagenesis methods. An additional example of emerging technologies in the *Xenopus* system is the conditional control of transgene expression via Cre-lox or other recombinase systems.

Proposed Funding: Novel loss of function/knockdown/knockout technologies in Xenopus should be funded via user-initiated grants through the PAR - Genetic and Genomic Analyses of Xenopus.

8. Intergenic annotation of the *Xenopus* genome

8A. Summary:

Annotation of the *X. tropicalis* genome has been mainly gene-centered. However, intergenic annotation is essential for placing genes in their chromosomal context, for genome-wide and systems-level analyses of gene activity and regulation. This undertaking is also essential for the use of phylogenetic footprinting and other biomedical research that exploits enhancer-sequences for targeted expression in specific cell types or stages of development.

8B. Why intergenic annotation?

The *X. tropicalis* genome has great potential to expedite analyses of the cellular and molecular basis of vertebrate development and the developmental origins of congenital disease. However, the utility of the genome for both gene-centered and systems level approaches rests on the knowledge infrastructure for this genome. Annotation of the genome has been predominantly focused on expressed sequences, identified by corresponding ESTs or predicted by homology to known genes. But equally important is the ***annotation of intergenic and intragenic genomic elements*** that affect gene expression and higher order chromatin structure and that are ***pivotal to understanding the regulation of developmental processes***. These non-coding genomic elements include enhancers, insulators, repetitive and parasitic elements, and telomeric and pericentric heterochromatin. Identification of these elements is important; epigenetic modifications mediate phenotypic variability (Feinberg, 2007) and microdeletions of regulatory sequences such as enhancers are found in congenital disease (Kouwenhoven et al. 2010).

There are five principal types of chromatin (Filion et al. 2010) and the local configuration of the genome in chromatin-structure affects all DNA-based processes in the cell. Chromatin state is indicative of a wide variety of molecular processes which has been highlighted by the ENCODE project. Gene-distal DNAseI hypersensitivity sites (enhancers and insulators) for example, have characteristic histone modifications (ENCODE Project Consortium, 2007; Heintzman et al. 2007, 2009). These modifications can also be used for the discovery of large non-coding RNAs (Guttman et al. 2009). It is also possible to apply chromatin interaction analysis in combination with paired-end tag (ChIA-PET) sequencing (Fullwood et al. 2009) to uncover looping interactions between regulatory regions and promoters, establishing regulatory connectivity over long genomic distances. This would solve several problems in the analysis of gene regulation. For most genes the enhancers are unknown. Moreover, it often is assumed that binding events of transcription factors generally influence the nearest gene. However from recent analyses in human cells it is clear this need not be true. Enhancers can regulate multiple genes and proximity does not imply a regulatory interaction.

Knowledge of non-coding genomic elements can be applied in numerous settings. In particular, intergenic annotation will help researchers in the following ways:

1. A genome-wide atlas of developmentally important enhancers will allow researchers to target the expression of transgenes to specific cells at specific stages of development. One could select a gene with the desired expression pattern from the expression database at Xenbase, find the responsible enhancers in the genome enhancer resource, clone the enhancer(s) and perform the experiment.
2. Using epigenome reference maps, transgene integrations can be more rapidly evaluated and characterized with respect to position-effect variegation.
3. The influence of repetitive DNA on gene regulation would become more transparent.
4. The identification of enhancers and their interactions with genes would facilitate functional analyses of transcription factors. For ChIP-PCR analyses it is important to know where the enhancers of a gene-of-interest are located. In ChIP-sequencing experiments it is important to know what genes are regulated by particular binding events. The looping interactions between enhancers and promoters are therefore important for unambiguous analyses of the role of transcription factors in development and for efforts to dissect gene-regulatory networks.

Intergenic annotation based on chromatin state is feasible in *Xenopus* (Akkers et al. 2009). Generating this type of annotation will expedite analysis of gene regulatory mechanisms, enable development of experimental tools and applications based on enhancer function, and stimulate systems biology approaches of vertebrate development, thereby enhancing the utility of this genome in biomedical research.

6C. How should we proceed?

We propose to generate intergenic annotation of the *X. tropicalis* genome using a combination of experimental and bioinformatic approaches to generate the following community resources. As the *X. laevis* genomic sequence is assembled, similar analysis on this genome will also prove highly important:

1. Epigenome reference maps.

Advances in sequencing and progress by groups participating in the ENCODE and NIH Roadmap Epigenomics projects allow the characterization of the epigenomic landscape of *Xenopus* embryos in a targeted and cost-efficient way. Capitalizing on these projects, it is possible to use selected histone modification and DNA methylation profiles (six to ten selected modifications) to index the *X. tropicalis* genome for different euchromatin and heterochromatin subtypes. The reference maps generated for a range of developmental stages (covering representative stages from blastula, gastrula, neurula and tailbud stages) will allow reliable bioinformatic predictions regarding enhancers, promoter-proximal regulatory elements, unstable yet expressed non-coding RNAs, repressed and active transposable elements, telomeric and centromeric heterochromatin, and different types of facultative heterochromatin and stably repressed constitutive heterochromatin.

2. Enhancer resource.

Histone modification profiles, in particular those of histone H3 lysine 4 mono- and trimethylation, can be

used to reliably predict distal regulatory regions. For the enhancer resource, it is essential that the enhancer predictions are validated in enhancer assays for robust identification. Furthermore, the long-range interactions of enhancers with promoters need to be determined by genome-wide crosslinking-based approaches such as ChIA-PET. Establishing enhancer connectivity is important to increase the practical utility of the enhancer resource for the community.

Proposed Funding: Intergenic Annotation of the Xenopus genome should be funded via user-initiated grants through the PAR - Genetic and Genomic Analyses of Xenopus.

9. Improvement of the *X. tropicalis* genome – long range contiguity

9A. Summary:

The *X. tropicalis* genome is a powerful resource for numerous genomics applications. However, long-range contiguity remains a challenge. Improvements in long-range contiguity of the *X. tropicalis* genome would potentiate many different methodologies such as intergenic annotation and genetics.

9B. Why improve the long-range contiguity of the X. tropicalis genome?

The current genome assembly (JGI v4.1) consists of 19,759 scaffolds, with many internal gaps of variable estimated sizes. The total genomic sequence in scaffolds is 1,514 MB (contigs plus estimated gaps) and the total contig sequence is 1,360 MB, revealing that ~10% of the genome lies in gaps. 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. A new genome assembly has been completed and is currently undergoing automated annotation. This genome appears significantly better. The total number of scaffolds has fallen from 19,759 to 10,315 and the total number of contigs has fallen from 191,480 to 59,701. A further assembly using synteny and the meiotic map has generated a chromosome-sized assembly. However, substantial portions of this genome are inferred based on synteny to chicken and polished mammalian genomes. Therefore, experimental validation of these assemblies is essential.

Improvement of long-range contiguity is essential to facilitate different methodologies in *Xenopus*. Since many enhancers can be found distal to various genes, long-range contiguity is critical for analyzing gene regulatory networks and other genomics applications such as chromosomal immunoprecipitation. Long-range contiguity greatly facilitates genetics where fragmentation of the genome hampers positional cloning experiments despite the extraordinary meiotic power of *Xenopus* afforded by the thousands of meioses sampled in a single *Xenopus* fertilization.

9C. How should we proceed?

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:

1. gPET/mate-pair sequencing. The next generation sequencing technologies have substantially reduced the cost of sequencing. However, they are limited for generating long-range contiguity because they require small insert sizes between paired-end reads. However, gPET/mate-pair sequencing allows for joining distal genomic pieces that are amenable to short read sequencing. Since the insert size between joined pieces is known, these gPET tags can then provide contiguity information and can be generated to significant depth. Genomic distance between paired reads can be from 2-20kb depending on the methods used and can effectively bridge repetitive elements and join breaks in scaffolds.
2. 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.

3. BAC end sequencing/BAC sequencing. Additional BAC resources and BAC sequencing are essential. The *X. tropicalis* genome is particularly shallow in BAC end sequencing which complicates the assembly. 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. 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 will 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. Cytogenetics - Performing FISH using cDNA or genomic probes will provide additional long-range contiguity and provide the required integration with the *Xenopus* chromosome complement.

Proposed Funding: Improvement of the long-range contiguity of the X. tropicalis genome should be funded via user-initiated grants through the PAR - Genetic and Genomic Analyses of Xenopus.

10. Additions and improvements to Xenbase, the *Xenopus* model organism database

10A. Summary:

Xenbase, the internet-based X*enopus* model organism database, continues to be an essential resource and an important priority for the *Xenopus* research community. Model organisms provide valuable tools for studying human disease and in this post-genomic era, versatile databases are essential for researchers to integrate vast amounts of sequence, expression and functional data into a meaningful biological synthesis. Improving Xenbase will provide *Xenopus* researchers with better database tools to analyze larger and larger biological datasets.

10B. Why improve Xenbase?

Xenbase provides an internet portal to inter-related diverse types of *Xenopus* data including genomic, mRNA and protein sequence, gene expression and function, all of which is highly integrated with NCBI, JGI, ENSEMBL and other model organism and human disease databases. 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, protocols, educational material and it acts as a clearinghouse for community resources (e.g. clones and transgenic lines). Thus Xenbase has an immense impact for *Xenopus* researchers and the broader biomedical community.

10C. How should we proceed?

With strong community support a NICHD grant (P41 HD064556, PIs Zorn and Vize) was recently awarded to maintain Xenbase and enhance the annotation of literature and gene expression patterns. Moving forward it will be important to continue Xenbase funding and to expand its capabilities as technologies evolve and new types of data and resources become available. This will require additional funds to develop software, pipelines and annotation teams to support these new data. One strategy to accomplish this would be for new community resource proposals, such as the ORFeome, *X. laevis* genome sequencing and various NextGen transcriptome projects, to incorporate Xenbase as an integral part of their plan. In this way projects will be able to leverage Xenbase’s infrastructure and maximize the visibility and accessibility of the new resource/data. In the future, it will also be important to develop new database tools to better utilize the rich gene function data already present in decades of *Xenopus* literature. For example, the community strongly supports the development of phenotype tools that use standardized cross-species ontology enabling the comparison of *Xenopus* gene-phenotypes to gene mutations/phenotypes in humans and other organism, thus providing powerful tools to identify and characterize human disease models. These tools would be broadly applicable and could be funded through collaborative projects with other model organism databases.

11. Frogbook: A comprehensive resource for methods in *Xenopus* biology

11A. Summary:

The use of online technologies and social media has seen comparatively slow adoption by the biology research community. However, one area that has seen a high level of community involvement and use has been the creation of online collections of resources for particular research disciplines, particularly those centered around the use of a common model organism. Examples of this include Wormbase/Wormbook/WormAtlas, Flybase, and Xenbase. These sorts of resources are expensive to build and once built, are often endangered by a lack of available funds for a dedicated staff, maintenance, and updates. The Frogbook project is an attempt to find a new way to build these community resources in a less expensive and more readily-sustainable manner. Instead of building from the ground up, Frogbook will take advantage of the already existing infrastructure of Cold Spring Harbor Laboratory (CSHL).

11B. Why Frogbook?

Growth of the internet has greatly altered the way research scientists gather necessary information. Younger scientists no longer reach for the bookshelf to find laboratory manuals or primers describing the biology of their model organism. Instead, internet search engines such as Google have become the method of choice for gathering knowledge. Simultaneously, research communities have been experimenting with new technologies to create resources and online gathering points for scientists with common interests. Modern resources include sequence databases, anatomical atlases, and manuals of laboratory techniques. Many of these resources revolve around groups using common laboratory model organism, such as Xenbase. Recently, animal specific online resources such as Wormbook have been developed.

While funding has been available for building these valuable information sharing resources, maintaining and updating them has proven a difficult economic venture, as noted in this Nature article:

http://www.nature.com/nature/journal/v462/n7271/full/462252a.html

Building these resources from the ground up will be an expensive project. These resources often see an initial burst of enthusiasm and contribution but then are unable to continue at a high level of active participation and growth. While members of the community readily volunteer to provide their expertise and create content, these research scientists cannot spare the necessary time to manage a project on this scale. A dedicated editor and staff are often needed to guide the initial creation of material and to continuously solicit new material to keep the resource current with the field.

11C. How to proceed?

Rather than incurring the expenses of creating a new resource from scratch, we propose to take advantage of the infrastructure already in place at Cold Spring Harbor Laboratory to minimize startup costs, take advantage of staff already in place and reduce maintenance overhead by creating the resource under the umbrella of one of CSHL Press' journals. The reasons here are two fold. First, CSHL Press already handles publication of the standard book of *Xenopus* protocols (Sive et al., 2000. Early Development of *Xenopus laevis*: A laboratory Manual). Second, the CSH Protocols journal already hosts nearly 100 additional protocols using *Xenopus*.

While previous efforts have focused on developmental biology with *Xenopus*, an effort must also be made to collect and organize methods related cell and molecular biology, studies with *Xenopus* egg extracts, and physiology studies with *Xenopus* oocytes.

The proposed resource will be aimed at the *Xenopus* research community and will be freely accessible to all. The end goal will be to have this resource fully integrated with Xenbase.

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 experimental tractability and 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 notable 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, thousands 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* transgenesis, 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 recent 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 Smad4 (*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 chemicals released into the environment 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.

1950s

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

1960s

- The discovery that the nucleolar organizer encodes the ribosomal RNA genes (Brown and Gurdon, 1969).
- Selective DNA amplification of rDNA in oogenesis (Brown and Dawid, 1968; Gall, 1968).
- Mitochondrial DNA exists and is inherited from the mother (Dawid, 1966).

1970s

- 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).

1980s

- 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 ubiquitination (Murray et al., 1989).

1990s

- 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 accelerate and promote 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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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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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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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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National Institute on Deafness and Other Communication Disorders (NIDCD)

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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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National Institute of Dental and Craniofacial Research (NIDCR)

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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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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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Cognition and behavior; neuroendocrine regulation

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Appendix 1 - Authors of the 2011 *Xenopus* Community White Paper

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Matt Guille	
Barry Gumbiner	5R01GM052717 3R37GM037432
Raymond Habas	3R01GM078172 1R01GM086377
James Hanken	
Richard Harland	1R01HD065705 1R313HD066961 5R21DC0102102 5R01GM042341 5R01GM049346 5R01GM086321
Adron Harris	2R01AA006399
Bill Harris	5R01HL089590 5P20RR017662
Rebecca Hartley	
Xi He	5R01GM057603 2R01GM074241
Rebecca Heald	1DPOD000818 2R01GM065232 2R01GM057839 5P41RR011823
Janet Heasman	1R01GM084951
John Heikkila	
Jon Henry	5R01EY009844
P. Renee Hew	
Tim Hinterberger	1R15HD060010
Maureen Hoatlin	

Thomas Holleman	
Christine Holt	
Stefan Hoppler	
Marko Horb	5R01DK077197
Tim Hunt	
Harry Isaacs	
Laura Itzhaki	
Antone Jacobson	
Wei Jang	
Thomas Jessell	2R37NS033245
Liz Jones	
Peter Jones	7R01AR055877
Phil (Eugene) Jones	
Ken Kao	
Raymond Keller	5R37HD025594
Darcy Kelley	5T32HD007430 5R01NS023684
Daniel Kessler	5R01AG025282 5R01GM064768
Mustafa Khokha	1R01DE018825 1R21RR025168 5R01DE018824
Mary Lou King	5R01GM033932
Chris Kintner	5T32HD007495 5R01GM076507 1R01GM096021
Atsushi Kitayama	
Peter Klein	5R01MH058324
Mike Klymkowski	5R01GM084133
Laurent Kodjabachian	
Sally Kornbluth	5R01CA102707 1R01CA153216 5R01GM067225 5R01GM080333 5R01GM088175
Paul Krieg	1R01HL093694
Kris Kroll	5R01GM066815
Michael Kuhl	
Carole Labonne	5R01CA114058 1R01GM077288
Alan Lambowitz	5R01HD054349 5R01GM037949 5R01GM037951
Branko Latinkic	
Ethan Lee	5R01GM081635
Daniel Levy	
Karen Liu	
Gabriela Loots	5R01HG003963 5R01DK075730

Laura Ann Lowery	5F32NS063512
James Maller	
Forbes Manson	
Kathrin Marheineke	
Nicholas Marsh Armstrong	5R01DK071683 5P41RR004050
William Marzluff	5R01CA065773 5R90DA023417 5T90DA022857 5R01GM058921 3R01GM029832
Roberto Mayor	
Andre Mazabraud	
Pierre McCrea	1R13CA137955 5R01GM052112
Sarah McFarlane	
Paul Mead	5R01MH079381
Brian Mitchell	1R01GM089970
Ann Miller	5R01GM085302
Tim Mohun	
Anne-Helene Monsoro-Burq	
Sally Moody	
Paul Mueller	
Isabelle Neant	
Christof Niers	
Shin-ichi Ohnuma	
Nancy Papalopulu	
Robert Patient	
Benjamin Peng	
Muriel Perron	
Stefano Piccolo	
Isabel Phillip	
Anna Philpott	
Tomas Pieler	
Nicholas Pollet	
Mu-Ming Poo	5R01EY014979
Susannah Rankin	5P20RR016478
Angie Ribera	2P30NS048154 2R01NS025217 5R01NS038937 5T32NS007083
Jacques Robert	5R01CA108982 5R24AI059830 5R03HD061671
Dan Rokhsar	1R01HD065705 5R01GM086321 2T32HG000047
Ralph Rupp	

Edward Ruthazer	
Gerhart Ryffel	
Margaret Saha	1R15NS067566
Jean-Pierre Saint-Jeannet	
Amy Sater	
Elba Serrano	5R25GM061222 5P50GM068762
Colin Sharpe	
David Shechter	
Michael Sheets	
Yun-Bo Shih	1ZIAHD001901 1ZIAHD008858 1ZUAHD008859
Chris Showell	
Hazel Sive	1S10RR027044 1R01DE021109
Paris Skourides	
Jonathan Slack	1R01DK080747 5R01GM088500
Jim Smith	
Ann Marie Sokac	
Sergei Sokol	2R01HD031247 5R01GM077592 5R01NS040972 3R01NS040972
Nick Spitzer	5R01MH074702 5R01NS015918 5R01NS057690 5T32NS007220
Nancy Standart	
Tim Stearns	5R01GM052022
Herbert Steinbeisser	
Todd Stukenberg	5R01GM063045 5R01GM081576
Atsushi Suzuki	
Shuji Takahashi	
Gerald Thomsen	1R03HD064908 5R01GM080462 5T32GM007964

	5R01GM076599
Vance Trudeau	
Naoto Ueno	
Katharine Ullman	5R01GM061275
Gert Veenstra	5R01HD054356
Peter Vize	1P41HD064556
Claire Walczak	1R43DE019593 3R01GM059618
Christoph Waldner	
John Wallingford	5R01GM074104 5R01GM086627
Johannes Walter	5R01GM062267 5R01GM080676 5R01HL098316
Daniel Weinstein	5R01GM061671
Dan Weeks	2R01GM069944
Oliver Wessely	5R01DK080745
Grant Wheeler	
Jeffrey White	5F30DK082121
Malcolm Whitman	5R01HD029468 1R01GM089885
Chris Wright	5T32HD007502 1U01DK089750 5R01GM056238
Christopher Wylie	5R01HD044764 2R01HD045737 5R01HD060578
Renee Yew	
Joel Yisraeli	
Joseph Yost	5U01HL066292 5P01HD048886
Zhongshen You	
Yixian Zheng	1R21AA0220060 5R01GM056312
Lyle Zimmerman	
Michael Zuber	5R01EY017964 1R21AI092313 2R01GM045898