# Impact of the *Xenopus* system on the mission of the 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.

## *Xenopus* grants funded by the Institute:

According to NIH RePORTER Search Tool, in the fiscal year of 2011, the National Institute of General Medical Sciences (NIGMS) funded forty-seven grants for projects involving Xenopus. These grants total to $14,880,910.

## 2011 *Xenopus* White Paper - Community Needs:

***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.

## For complete details of the 2011 *Xenopus* White Paper, please visit

## http://www.xenbase.org/community/xenopuswhitepaper.do

# Appendix

| Project Number | Project Title | Activity | Principal Investigator | Organization Name | Total Cost |
|---|---|---|---|---|---|
| **5K99GM084292-02** | MICROTUBULE POLYMERIZATION AND DEPOLYMERIZATION MECHANISMS BY CONSERVED PROTEINS | K99 | AL-BASSAM, JAWDAT MH | HARVARD UNIVERSITY (MEDICAL SCHOOL) | $72,306 |
| **5R01GM046889-16** | STRUCTURE/FUNCTION OF GAP JUNCTIONS | R01 | BARGIELLO, THADDEUS ANDREW | ALBERT EINSTEIN COL OF MED YESHIVA UNIV | $480,696 |
| **3R01GM030376-30S1** | THE ELECTROPHYSIOLOGICAL STUDIES OF VOLTAGE GATED CHANNELS | R01 | BEZANILLA, FRANCISCO J | UNIVERSITY OF CHICAGO | $341,835 |
| **5R01GM066977-08** | TGFB SIGNALING IN VERTEBRATE MESODERM INDUCTION | R01 | BRIVANLOU, ALI H | ROCKEFELLER UNIVERSITY | $311,788 |
| **3R01GM078502-03S1** | STRUCTURE/FUNCTION ANALYSIS OF THE NA/BICARBONATE COTRANSPORTERS | R01 | CHOI, INYEONG | EMORY UNIVERSITY | $247,488 |
| **3K08GM083216-02S1** | VOLATILE ANESTHETIC REGULATION OF TASK TANDEM PORE POTASSIUM CHANNELS | K08 | COTTEN, JOSEPH F | MASSACHUSETTS GENERAL HOSPITAL | $108,000 |
| **5R01GM075249-05** | ROLES OF CHROMOSOMAL FACTORS IN CHROMOSOME SEGREGATION | R01 | FUNABIKI, HIRONORI | ROCKEFELLER UNIVERSITY | $304,460 |
| **3R01GM067758-06S1** | MECHANISM OF RNA LOCALIZATION IN DROSOPHILA DEVELOPMENT | R01 | GAVIS, ELIZABETH R. | PRINCETON UNIVERSITY | $457,756 |
| **5R01GM023674-33** | CONTROL OF HISTONE FUNCTION | R01 | GRUNSTEIN, MICHAEL | UNIVERSITY OF CALIFORNIA LOS ANGELES | $638,080 |
| **3R01GM052717-14S1** | BIOCHEMISTRY AND REGULATION OF CADHERIN ACTIVITY | R01 | GUMBINER, BARRY M | UNIVERSITY OF VIRGINIA CHARLOTTESVILLE | $234,053 |
| **5R01GM021784-32** | REACTIVE METABOLITES IN DRUG TOXICITY | R01 | HANZLIK, ROBERT P | UNIVERSITY OF KANSAS LAWRENCE | $299,466 |
| **5R01GM057839-09** | STUDYING MITOSIS USING XENOPUS EGG EXTRACTS | R01 | HEALD, REBECCA W | UNIVERSITY OF CALIFORNIA BERKELEY | $297,525 |

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|---|---|---|---|---|---|
| **5R01GM019629-38** | GENETIC REGULATION OF PHOSPHOLIPID SYNTHESIS IN YEAST | R01 | HENRY, SUSAN ARMSTRONG | CORNELL UNIVERSITY ITHACA | $502,485 |
| **5R01GM072754-06** | MECHANISMS OF CENTROSOME REPRODUCTION IN ANIMAL CELLS | R01 | HINCHCLIFFE, EDWARD H | UNIVERSITY OF MINNESOTA TWIN CITIES | $254,722 |
| **5R01GM079427-18** | MOLECULAR PHYSIOLOGY OF VOLTAGE-GATED ION CHANNELS | R01 | HORN, RICHARD J | THOMAS JEFFERSON UNIVERSITY | $368,737 |
| **5R01GM050806-16** | REGULATION OF DNA REPLICATION IN S. POMBE | R01 | KELLY, THOMAS J | SLOAN-KETTERING INSTITUTE FOR CANCER RES | $441,477 |
| **5R01GM033932-23** | ESTABLISHING GERM CELL FATE IN XENOPUS | R01 | KING, MARY LOU | UNIVERSITY OF MIAMI SCHOOL OF MEDICINE | $349,123 |
| **3R01GM066492-07S1** | CHEMICAL GENETIC AND BIOCHEMICAL STUDIES OF MITOTIC PROTEOLYSIS | R01 | KING, RANDALL W | HARVARD UNIVERSITY (MEDICAL SCHOOL) | $199,678 |
| **3R01GM080333-03S1** | CONTROL OF CASPASE ACTIVATION IN APOPTOSIS | R01 | KORNBLUTH, SALLY A | DUKE UNIVERSITY | $225,036 |
| **3R01GM066815-07S1** | TRANSCRIPTIONAL REGULATION BY GEMININ | R01 | KROLL, KRISTEN L | WASHINGTON UNIVERSITY | $283,295 |
| **3R01GM081635-03S1** | BIOCHEMICAL RECONSTITUTION OF HETEROTRIMERIC G PROTEINS IN THE WNT PATHWAY | R01 | LEE, ETHAN | VANDERBILT UNIVERSITY | $59,134 |
| **5R01GM061829-10** | REGULATION OF CALCIUM SIGNALING DURING OOGENESIS | R01 | MACHACA, KHALED | WEILL MEDICAL COLLEGE OF CORNELL UNIV | $214,718 |
| **5T32GM008443-17** | THE GENETIC ARCHITECTURE OF QUANTITATIVE TRAITS | T32 | MACKAY, TRUDY F | NORTH CAROLINA STATE UNIVERSITY RALEIGH | $142,538 |
| **3R01GM088790-05A1S1** | FUNCTIONAL ARCHITECTURE OF IP3-EVOKED LOCAL CA2+ SIGNALS | R01 | MARCHANT, JONATHAN S | UNIVERSITY OF MINNESOTA TWIN CITIES | $224,769 |
| **1U01GM094622-01** | PARTNERSHIP FOR HIGH-THROUGHPUT ENABLED BIOLOGY OF THE MITOCHONDRIAL | U01 | MARKLEY, JOHN L | UNIVERSITY OF WISCONSIN MADISON | $1,792,845 |

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| | PROTEOME | | | | |
| **5R01GM078247-04** | BEYOND GFP AND AEQUORIN: OCEAN-WIDE STUDY OF FLUORESCENT AND LUMINOUS PROTEINS | R01 | MATZ, MIKHAIL V | UNIVERSITY OF TEXAS AUSTIN | $291,735 |
| **3R01GM066270-07S1** | MOLECULAR STRUCTURE AND FUNCTION OF THE HUMAN KINETOCHORE OUTER PLATE | R01 | MCEWEN, BRUCE F | WADSWORTH CENTER | $71,546 |
| **5R01GM086854-11** | NITRIC OXIDE SIGNALING IN HYPOXIA AND IMMUNITY IN DROSOPHILA | R01 | O'FARRELL, PATRICK H | UNIVERSITY OF CALIFORNIA SAN FRANCISCO | $337,871 |
| **5R25GM062881-07** | MSBRS RISE PROGRAM AT HASKELL INDIAN NATIONS UNIVERSITY | R25 | O'MALLEY, JAMES DENNIS | HASKELL INDIAN NATIONS UNIVERSITY | $441,375 |
| **5SC2GM084789-03** | NOVEL MECHANISMS OF EGFR GENE EXPRESSION REGULATION | SC2 | PEREZ-TORRES, MARIANELA | UNIVERSITY OF PUERTO RICO MED SCIENCES | $75,000 |
| **3R01GM083025-02S1** | SPECIFICITY OF EFFECTOR ACTIVATION BY RHO FAMILY GTPASES | R01 | PETERSON, JEFFREY R | INSTITUTE FOR CANCER RESEARCH | $229,307 |
| **5R01GM084104-02** | MOLECULAR MECHANISMS OF GENETIC RECOMBINATION IN MAMMALS | R01 | PETUKHOVA, GALINA V. | HENRY M. JACKSON FDN FOR THE ADV MIL/MED | $286,100 |
| **5R01GM059975-09** | FUNCTIONAL ANALYSIS OF VERTEBRATE NUCLEAR TRANSPORT | R01 | POWERS, MAUREEN A. | EMORY UNIVERSITY | $309,966 |
| **5R01GM076112-04** | BUILDING A SYSTEMS-LEVEL VIEW OF CELL CYCLE CHECKPOINTS | R01 | SIBLE, JILL C | VIRGINIA POLYTECHNIC INST AND ST UNIV | $222,162 |
| **3R01GM030758-28S2** | CENTROSOME REDUPLICATION AND CONSEQUENCES OF CLEAVAGE FAILURE/PROLONGED MITOSIS | R01 | SLUDER, GREENFIELD | UNIV OF MASSACHUSETTS MED SCH WORCESTER | $186,599 |
| **5R01GM084318-03** | PATHOADAPTIVE EVOLUTION OF SALMONELLA | R01 | SOKURENKO, EVGENI VENIAMINOVIC | UNIVERSITY OF WASHINGTON | $543,602 |
| **5R01GM076599-04** | TRAF4 IN TGF-BETA SIGNALING AND | R01 | THOMSEN, GERALD H | STATE UNIVERSITY NEW | $290,606 |

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| | EMBRYONIC DEVELOPMENT | | | YORK STONY BROOK | |
| **5SC3GM084771-02** | MOLECULAR ANALYSIS OF ORIT-INDEPENDENT TRANSFER IN THE GONOCOCCAL R-PLASMIDS | SC3 | TORRES-BAUZA, LUIS | UNIVERSITY OF PUERTO RICO MED SCIENCES | $112,500 |
| **5R01GM074104-05** | MECHANISM OF VERTEBRATE NEURAL TUBE MORPHOGENESIS | R01 | WALLINGFORD, JOHN B | UNIVERSITY OF TEXAS AUSTIN | $280,998 |
| **5R01GM062267-09** | PROPERTIES OF THE EUKARYOTIC REPLICATIVE DNA HELICASE | R01 | WALTER, JOHANNES | HARVARD UNIVERSITY (MEDICAL SCHOOL) | $337,398 |
| **3R01GM081489-02S1** | ROLE OF UBP-M AND H2A DEUBIQUITINATION IN CHROMATIN AND CELLULAR FUNCTION | R01 | WANG, HENGBIN | UNIVERSITY OF ALABAMA AT BIRMINGHAM | $202,813 |
| **3R01GM050942-13S1** | FUNCTION OF 3'UTRS | R01 | WICKENS, MARVIN P. | UNIVERSITY OF WISCONSIN MADISON | $473,266 |
| **5R01GM079139-03** | MICROTUBULE DYNAMICS DURING CELL POLARITY AND MIGRATION | R01 | WITTMANN, TORSTEN | UNIVERSITY OF CALIFORNIA SAN FRANCISCO | $282,202 |
| **3R01GM073863-04S1** | RNA QUALITY CONTROL AND ENVIRONMENTAL STRESS | R01 | WOLIN, SANDRA L. | YALE UNIVERSITY | $78,408 |
| **5R01GM073863-04** | RNA QUALITY CONTROL AND ENVIRONMENTAL STRESS | R01 | WOLIN, SANDRA L. | YALE UNIVERSITY | $399,154 |
| **5R01GM061542-09** | REGULATION OF THE ANAPHASE-PROMOTING COMPLEX BY THE SPINDLE CHECKPOINT | R01 | YU, HONGTAO | UNIVERSITY OF TEXAS SW MED CTR/DALLAS | $304,894 |
| **3R01GM084879-02S1** | EVOLUTION OF SODIUM CHANNEL GENES | R01 | ZAKON, HAROLD H | UNIVERSITY OF TEXAS AUSTIN | $271,398 |
| | | | | Total | $14,880,910 |