Impact of the *Xenopus* system on the mission of the NIMH

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

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

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

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

bipolar disorder associated with mutations in the *DISC1* gene, the *Xenopus* system will remain an important tool to advance our basic understanding of mental illnesses and to translate these basic discoveries to the treatment of psychiatric disorders.

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

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

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

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

treatments. Similarly, the molecular basis of memory and learning pathways are readily addressed in *Xenopus*, since the larvae are readily trained at many stages of development and amenable to surgical, pharmacological, and genetic intervention.

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

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Xenopus grants funded by the Institute:

According to NIH RePORTER Search Tool, in the fiscal year of 2011, the National Institute of Mental Health (NIMH) funded nine grants for projects involving Xenopus. These grants total to $3,247,050.

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
5K01MH074148-05	PARENTAL HELP-SEEKING FOR CHILDREN'S DISRUPTIVE BEHAVIOR	K01	BURKE, JEFFREY DAVID	UNIVERSITY OF PITTSBURGH AT PITTSBURGH	$145,129
5R01MH062723-10	FUNCTION OF THE PET-1 ETS FACTOR IN THE MAMMALIAN 5-HT SYSTEM	R01	DENERIS, EVAN S	CASE WESTERN RESERVE UNIVERSITY	$318,792
1F32MH092116-01	ROLE OF HISTONE ACETYLTRANSFERASES IN MEMORY STORAGE AND SYNAPTIC PLASTICITY	F32	ESTEVEZ, MARCEL ANDRE	UNIVERSITY OF PENNSYLVANIA	$54,974
5F31MH083395-03	MECHANISMS BY WHICH NMDA RECEPTOR ANTIBODIES MEDIATE A NOVEL AUTOIMMUNE DISEASE	F31	GLEICHMAN, AMY J.	UNIVERSITY OF PENNSYLVANIA	$29,136
1ZIAMH002841-07	TESTING WHETHER THE ENZYME GSK-3 IS A THERAPEUTICALLY RELEVANT TARGET OF LITHIUM	ZIA	HERKENHAM, MILES A	NATIONAL INSTITUTE OF MENTAL HEALTH	$158,234
5K23MH073091-05	FMRI ASSESSMENT OF ATTENTION AND MEMORY IN PTSD	K23	MOREY, RAJENDRA A	DUKE UNIVERSITY	$167,066
1ZIAMH002240-24	NEUROBIOLOGY OF DISRUPTIVE BEHAVIOR DISORDERS	ZIA	RAPOPORT, JUDITH L.	NATIONAL INSTITUTE OF MENTAL HEALTH	$1,362,116
5R01MH074702-05	MECHANISMS OF CALCIUM TRANSIENTS IN NEURONAL DEVELOPMENT	R01	SPITZER, NICHOLAS CANADAY	UNIVERSITY OF CALIFORNIA SAN DIEGO	$316,150
5R01MH079974-03	SMI DUAL ELIGIBLES AND MEDICARE PART D: IMPACT ON MEDICATIONS CONTINUITY AND OUTC	R01	STEINWACHS, DONALD MICHAEL	JOHNS HOPKINS UNIVERSITY	$695,453
				Total	$3,247,050