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

Adron Harris, PhD - University of Texas at Austin

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:

**Selected references**:

Ethanol's molecular targets. Harris RA, Trudell JR, Mihic SJ. Sci Signal. 2008 Jul 15;1(28):re7.

GABRG1 and GABRA2 as independent predictors for alcoholism in two populations. Enoch MA, Hodgkinson CA, Yuan Q, Albaugh B, Virkkunen M, Goldman D. Neuropsychopharmacology. 2009 Apr;34(5):1245-54.

Low-dose alcohol actions on alpha4beta3delta GABAA receptors are reversed by the behavioral alcohol antagonist Ro15-4513. Wallner M, Hanchar HJ, Olsen RW. Proc Natl Acad Sci U S A. 2006 May 30;103(22):8540-5.

Association of GABRA2 with drug dependence in the collaborative study of the genetics of alcoholism sample. Agrawal A, Edenberg HJ, Foroud T, Bierut LJ, Dunne G, Hinrichs AL, Nurnberger JI, Crowe R, Kuperman S, Schuckit MA, Begleiter H, Porjesz B, Dick DM. Behav Genet. 2006 Sep;36(5):640-50.

Mutations of gamma-aminobutyric acid and glycine receptors change alcohol cutoff: evidence for an alcohol receptor? Wick MJ, Mihic SJ, Ueno S, Mascia MP, Trudell JR, Brozowski SJ, Ye Q, Harrison NL, Harris RA. Proc Natl Acad Sci U S A. 1998 May 26;95(11):6504-9.

Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. Mihic SJ, Ye Q, Wick MJ, Koltchine VV, Krasowski MD, Finn SE, Mascia MP, Valenzuela CF, Hanson KK, Greenblatt EP, Harris RA, Harrison NL. Nature. 1997 Sep 25;389(6649):385-9.

## *Xenopus* grants funded by the Institute:

According to NIH RePORTER Search Tool, in the fiscal year of 2011the National Institute of Alcohol Abuse and Alcoholism (NIAAA) funded five grants for projects involving Xenopus. These grants total to $1,601,276.

# 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 |
|---|---|---|---|---|---|
| **1R01AA018673-01A1** | AUTOMATING BEHAVIORAL CODING VIA TEXT-MINING AND SPEECH SIGNAL PROCESSING | R01 | ATKINS, DAVID CHARLES | UNIVERSITY OF WASHINGTON | $629,070 |
| **5R03AA017928-02** | LONGITUDINAL TRENDS IN ALCOHOL USE AND AUDS AMONG RURAL STIMULANT USERS | R03 | BORDERS, TYRONE FINLEY | UNIVERSITY OF ARKANSAS MED SCIS LTL ROCK | $71,775 |
| **5R01AA013922-05** | SITES AND MECHANISMS OF ETHANOL ACTION IN P2X RECEPTORS | R01 | DAVIES, DARYL L | UNIVERSITY OF SOUTHERN CALIFORNIA | $312,263 |
| **3R01AA012153-08S1** | SHORT-CHAIN DEHYDROGENASES IN RETINOL/STEROL METABOLISM | R01 | KEDISHVILI, NATALIA Y | UNIVERSITY OF ALABAMA AT BIRMINGHAM | $341,418 |
| **1R21AA017545-01A1** | REGULATION OF MCP-1 AND CHEMOKINE RECEPTOR 2 (CCR2) IN ALCOHOLIC LIVER DISEASE | R21 | MANDREKAR, PRANOTI | UNIV OF MASSACHUSETTS MED SCH WORCESTER | $246,750 |
| | | | | Total | $1,601,276 |