Impact of the *Xenopus* system on the missions of the 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:

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 funding by the NIAAA

According to NIH RePORTER Search Tool, in the fiscal year of 2009, the National Institute of Alcohol Abuse and Alcoholism (NIAAA) **funded 11 grants** for projects involving *Xenopus*. These grants total **$5,568,831**. See appendix for a complete list.

2009 *Xenopus* White Paper – Community Needs

Executive Summary

Xenopus - a crucial model organism for biomedical research:

Experiments in model animals are a cornerstone of biomedical research and have a massive impact on our understanding of human health and disease. The frog, *Xenopus,* is a widely used and crucial vertebrate model organism that offers a unique combination of three powerful advantages: strong conservation of essential biological mechanisms, a remarkable experimental repertoire, and unparalleled cost-effectiveness when compared to murine or other mammalian models.

In fact, for many experimental applications, *Xenopus* is the only viable model system. For example, in cell and molecular biology, *Xenopus* extracts allow for individual components of the cell cycle or DNA replication/repair machinery to be analyzed in a manner that cannot be recapitulated *in vivo* or in cell culture. For developmental biology, no other model system allows for high-throughput genomic/proteomic screening and at the same time allows for transplant/explant analysis (i.e. "experimental embryology"). The *Xenopus* oocyte is unique as a system for studying channel physiology using the patch-clamp and as a system for protein expression. Finally, *Xenopus* is the only vertebrate model that readily produces enough biological material for biochemical purification from eggs, intact embryos, or isolated embryonic tissues. The combination of these characteristics offers a wide range of experimental opportunities for studies using the *Xenopus* system in contrast to other vertebrates such as the mouse or zebrafish.

NIH Investment in *Xenopus:*

The NIH has made a substantial and continuing investment in *Xenopus* research. Indeed, a search of the NIH rePORT database for R01's or equivalent grants using the search term "*Xenopus*" returned **427 grants for a total cost of $127,583,776** for FY08 and FY09. Despite this investment in individuals' research, the *Xenopus* community lacks many resources that are considered entirely essential for other model systems, including a complete genome sequence, stock and training centers, and a comprehensive model organism database.

Xenopus as a Model System and Human Disease:

Given the tremendous advantages of the *Xenopus* system, the pace of new biological discovery by the *Xenopus* Community is brisk. Using *Xenopus*, we have significantly improved our understanding of human disease genes and their mechanisms, justifying the NIH's investment in *Xenopus*. Below we provide examples of just a few of the human health discoveries made in the last two years:

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

Nephronophthisis - *Hum Mol Genet.* 2008. 17, 3655-62; *Nat Genet. 2005*. 37, 537-43.
Cutis laxa - *Nat Genet.* 2009. 41, 1016-21.
Meckel-Gruber syndrome - *Am J Hum Genet.* 2008. 82, 959-70.
Colorectal cancer - *Genome Res.* 2009. 19, 987-93.

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

Fanconi Anemia - *Mol. Cell.* 2009. 35, 704-15; *J Biol Chem.* 2009, 284, 25560-8.
C-myc oncogene - *Nature*. 2007. 448, 445-51.
BRCA1 - *Cell*. 2006. 127, 539-552

Xenopus oocytes are used to study gene expression and channel activity:
Trypanosome transmission - *Nature* 2009. 459, 213-217.
Epilepsy, ataxia, sensorineural deafness - *N Engl J Med.* 360, 1960-70.
Catastrophic cardiac arrhythmia (Long-QT syndrome) - *PNAS* 2009. 106,13082-7.
Megalencephalic leukoencephalopathy - *Hum Mol Genet.* 2008. 17, 3728-39.

Xenopus as a Model System and Basic Biological Processes:

Xenopus also plays a crucial role in elucidating the basic cellular and biochemical mechanisms underlying the entire spectrum of human pathologies. Again only a few of the many discoveries in the last two years are highlighted here:

Xenopus embryos were used for studies of TGF-® signal transduction.
(*Cell*. 2009. 136,123-35; *Science*. 2007. 315, 840-3).
Xenopus egg extracts revealed fundamental aspects of cell division.
(*Nature*. 2008. 453, 1132-6; *Science*. 2008. 319, 469-72).
Xenopus embryos were used for studying mucociliary epithelia.
(*Nat Genet.* 2008. 40, 871-9; *Nature*. 2007. 447, 97-101).
Xenopus embryos were used for studying development of the vasculature.
(*Cell*. 2008.135, 1053-64).
Xenopus egg extracts provided key insight into DNA damage responses.
(*Mol Cell.* 2009. 35,704-15; *Cell.* 2008. 134, 969-80).
Xenopus embryos linked telomerase to Wnt signaling.
(*Nature*. 2009. 460, 66-72).
Xenopus was used for small molecule screens to develop therapeutics.
(Nat Chem Biol. 2008. 4, 119-25; Blood. 2009. 114, 1110-22).

Immediate Needs of the *Xenopus* Community:

It is the consensus of the *Xenopus* community that their biomedical research could be greatly accelerated by the development of key resources that are currently lacking. These resources would be of use to the entire *Xenopus* research community. In this White Paper, the community identifies seven resources in two categories: Three Immediate Needs and four Essential Resources:

The **Immediate Needs** are a common set of key resources that were identified as the most pressing by three committees established to identify needed resources across the broad and diverse *Xenopus* community. There is a broad, community-wide consensus that these resources would have an immediate impact on all *Xenopus* users and should be assigned the highest priority in order to accelerate the pace of biomedical research using *Xenopus* as a model system.

These Immediate Needs and the resulting improvements in biomedical research are as follows:

1. *Establishment of the Xenopus Resource and Training Center at the MBL in Woods Hole.*
   -Will allow rapid distribution of transgenic *Xenopus laevis* lines expressing fluorescent reporters and tagged proteins (for example histone-RFP for visualizing the mitotic spindle or organ specific GFP in embryos)
   -Will allow centralized generation, housing, and distribution of genetically modified *X. tropicalis* lines, including both mutants and transgenics.
   -Will allow both current investigators and the next generation of researchers to get hands-on training in *Xenopus*-based biomedical research methods (including cell, molecular, and developmental methods).
2. *Expansion and improvement of Xenbase, a Xenopus model organism database.*
   -Maintain and curate data for the essential primary database for *Xenopus* researchers.
   -Enhance the functionality of *Xenbase* by introducing a phenotypes feature.

-Support new content on *Xenbase*, including proteomics support, a new genome browser, and Wiki for *Xenopus* methods.
-Continue and expand collaborative and service efforts (e.g. provide *Xenopus* data to other databases including NCBI, UniProtK, Mascot and Tornado).

3. *Complete sequencing of the Xenopus laevis genome.*
-Will allow the deconvolution of data in mass-spectrometry-based proteomic studies.
-Will facilitate identification of conserved gene regulatory regions to build gene-regulatory networks.
-Will facilitate site-specifc studies of DNA transaction (repair and replication)
-Will facilitate identification of all ORFs to build an ORFeome for rapid functional characterization of genes
-Will facilitate the design of morpholino oligonucleotides for gene depletion studies
-Will faciliate the analysis of chromatin-immunoprecipitations to identify DNA-bound to transcription factors and DNA modifications.

Essential Resources Needed by the *Xenopus* Community:

In addition to these immediate, community-wide needs, the committees identified four **Essential Resources** that should be developed as soon as possible, so that *Xenopus* biologists can more effectively fulfill the missions of the NIH. The *Xenopus* community considers all four of these additional resources to be essential, but understands that priorities must be set, and ranks these behind the Immediate Needs. These Essential Resources are as follows:

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

Anticipated Gains for Biomedical Research:

Xenopus is a crucial model organism for biomedical research. With the development of large-scale community-wide resources, *Xenopus* is poised to be become the premier vertebrate model for systems-level approaches to understanding biological mechanisms in cell, molecular, and developmental biology.

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

Genome improvements will provide *Xenopus* researchers with the ability to perform genome-wide screens for biological activities that will in turn allow the rapid assembly and analysis of gene regulatory networks. The ORFeome will greatly facilitate such genome-wide screening by allowing all ORFs to be rapidly analyzed or large numbers of proteins to be tagged for analysis of protein-protein interaction or for *in vivo* visualization. Using extracts and biochemical purification coupled with mass-spectrometry and genomic sequence, protein interactomes can be rapidly identified and validated. Because *Xenopus* can be so easily manipulated and because vast amounts of biological material can be generated, cell-type specific interactomes can also be identified. Large-scale genetic screens will identify important novel genes in developmental pathways, especially given the relatively simple genome of *X. tropicalis* compared to zebrafish. Finally, the flexibility of both *Xenopus* extracts and embryos make this system ideal for chemical biology screens. Identifying these gene-regulatory networks, interactomes, and novel genes will be only the first steps, of course. The well-established power of *Xenopus* for rapid analysis of gene function will then allow deeply mechanistic analyses to complement the systems-level approaches described above.

It is the combination of these characteristics that distinguishes *Xenopus* from other vertebrate model systems such as mouse and zebrafish and allows for a systems-level approach to understanding biological mechanisms. The tremendous promise of the *Xenopus* model cannot be realized, however, without the immediate development of community-wide research resources. This White Paper presents the needed resources, and we look to the NIH for guidance in how to best achieve these goals.

For complete details of the 2009 Xenopus White Paper, please visit
http://www.xenbase.org/community/xenopuswhitepaper.do

Appendix

Xenopus Grants funded by the NIAAA

Project Number	Activity	Project Title	Principal Investigator	Organization	Total
5K01AA017243-02	K01	SITES OF ETHANOL ACTION IN PURINERGIC P2X4 RECEPTORS	ASATRYAN, LIANA	UNIVERSITY OF SOUTHERN CALIFORNIA	$119,828
1ZIGAA000600-01	ZIG	OFFICE OF LABORATORY ANIMAL SCIENCE	BARNES, ANDREA		$3,017,450
5R01AA005846-23	R01	INTRACELLULAR STUDY OF ETHANOL EFFECTS ON BRAIN NEURONS	BRODIE, MARK S	UNIVERSITY OF ILLINOIS AT CHICAGO	$409,147
5R01AA013922-05	R01	SITES AND MECHANISMS OF ETHANOL ACTION IN P2X RECEPTORS	DAVIES, DARYL L	UNIVERSITY OF SOUTHERN CALIFORNIA	$312,263
1R01AA017889-01A1	R01	TRANSLATIONAL STUDIES OF NICOTINIC RECEPTOR GENES: ALCOHOL AND NICOTINE BEHAVIORS	EHRINGER, MARISSA A	UNIVERSITY OF COLORADO AT BOULDER	$571,931
2R01AA006399-27A1	R01	ALCOHOL ACTIONS-MOLECULAR TARGETS ON BRAIN PROTEINS	HARRIS, ROBERT ADRON	UNIVERSITY OF TEXAS AUSTIN	$433,661
3R01AA012153-08S1	R01	SHORT-CHAIN DEHYDROGENASES IN RETINOL/STEROL METABOLISM	KEDISHVILI, NATALIA Y	UNIVERSITY OF ALABAMA AT BIRMINGHAM	$341,418
5R01AA014697-04	R01	THE DOPAMINE TRANSPORTER: A NOVEL SITE OF ETHANOL ACTION	MAYFIELD, ROY D	UNIVERSITY OF TEXAS AUSTIN	$220,325
1R03AA018197-01	R03	IN VITRO SCREENING OF NOVEL THERAPEUTIC AGENTS FOR THE TREATMENT OF ALCOHOL ABUSE	MIHIC, S JOHN	UNIVERSITY OF TEXAS AUSTIN	$72,100
5F31AA017029-03	F31	IMPORTANT SITES OF ETHANOL ACTION IN PURINERGIC (P2X) RECEPTORS	POPOVA, MAYA	UNIVERSITY OF SOUTHERN CALIFORNIA	$41,176
1F31AA017802-01A1	F31	SINGLE CHANNEL CHARACTERIZATION OF ETHANOL ACTION ON THE GLYCINE RECEPTOR	WELSH, BRIAN T	UNIVERSITY OF TEXAS AUSTIN	$29,532
				Total	$5,568,831