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

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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.

Selected References:

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Pandur P, Läsche M, Eisenberg LM, Kühl M. (2002) Wnt-11 activation of a non-canonical Wnt signalling pathway is required for cardiogenesis. *Nature*. 418:636-41.

Schneider VA, Mercola M. (2001) Wnt antagonism initiates cardiogenesis in *Xenopus laevis*. *Genes Dev.* 15:304-15.

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Xenopus Grants funding by the NHLBI

According to NIH RePORTER Search Tool, in the fiscal year of 2009, the National Heart, Blood, and Lung Institute (NHLBI) **funded 19 grants** for projects involving *Xenopus*. These grants total **$6,886,476**. 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 NHLBI

Project Number	Activity	Project Title	Principal Investigator	Organization	Total
5R01HL086964-02	R01	THE ROLE OF NDRG4 IN MYOCARDIAL DEVELOPMENT	BALDWIN, H. SCOTT	VANDERBILT UNIVERSITY	$383,750
5R01HL062248-08	R01	INDUCTION AND SPECIFICATION OF HEMATOPOIETIC MESODERM	BARON, MARGARET H.	MOUNT SINAI SCHOOL OF MEDICINE OF NYU	$423,750
5R01HL084464-03	R01	CONNEXINS AND HEART FUNCTION	BUKAUSKAS, FELIKSAS	ALBERT EINSTEIN COL OF MED YESHIVA UNIV	$415,000
5R01HL089641-02	R01	TBX5 AND CARDIAC PROLIFERATION	CONLON, FRANK LEO	UNIVERSITY OF NORTH CAROLINA CHAPEL HILL	$363,108
7R01HL064282-10	R01	FUNCTION OF GATA FACTORS IN CARDIOGENESIS	EVANS, TODD R	WEILL MEDICAL COLLEGE OF CORNELL UNIV	$78,142
5R01HL036783-22	R01	NA/K PUMP CURRENT IN ISOLATED HEART CELLS	GADSBY, DAVID C	ROCKEFELLER UNIVERSITY	$338,000
5K18HL092231-02	K18	THE BIOLOGY OF ZEBRAFISH HEMATOPOIETIC STEM CELLS	HANDIN, ROBERT I	BRIGHAM AND WOMEN'S HOSPITAL	$298,857
5R01HL087017-04	R01	REGULATION OF LUNG EPITHELIAL SODIUM CHANNELS BY CGMP	JI, HONG-LONG	UNIVERSITY OF TEXAS HLTH CTR AT TYLER	$275,000
3R01HL087017-04S1	R01	REGULATION OF LUNG EPITHELIAL SODIUM CHANNELS BY CGMP	JI, HONG-LONG	UNIVERSITY OF TEXAS HLTH CTR AT TYLER	$204,647
1F30HL096279-01	F30	GPCR-BASED REGULATION OF THE HERG POTASSIUM CHANNEL BIOSYNTHESIS AND FUNCTION	KRISHNAN, YAMINI A.	ALBERT EINSTEIN COL OF MED YESHIVA UNIV	$46,176

1ZIAHL002540-15	ZIA	EXPRESSION, STRUCTURE/FUNCTION, REGULATION, AND ROLES OF PDE3 ISOFORMS	MANGANIELLO, VINCENT		$1,638,160
2R01HL031197-24A1	R01	MECHANISMS OF ENAC INHIBITION BY REPLICATING INFLUENZA VIRUS: ROLE OF M2 PROTEIN	MATALON, SADIS	UNIVERSITY OF ALABAMA AT BIRMINGHAM	$392,056
1R21HL092513-01A1	R21	CREATION OF STEM CELLS BY NUCLEAR REPROGRAMMING	MCGARRY, THOMAS J	NORTHWESTERN UNIVERSITY	$228,750
5P01HL034322-23	P01	MOLECULAR AND PROTEIN CORE	O'NEAL, WANDA K	UNIVERSITY OF NORTH CAROLINA CHAPEL HILL	$204,157
5K02HL086737-03	K02	DEVELOPMENT OF XENOPUS AS A MODEL TO STUDY CONGENITAL HEART DISEASE	RAMSDELL, ANN F	UNIVERSITY OF SOUTH CAROLINA AT COLUMBIA	$103,929
5R01HL071806-07	R01	HYPERTENSION AND COLLAGEN: EFFECT OF AC-SDKP	RHALEB, NOUR-EDDINE	HENRY FORD HEALTH SYSTEM	$362,500
1R01HL089902-01A2	R01	THE ROLE OF CYSTEINE RICH PROTEIN2 BINDING PROTEIN IN CARDIOVASCULAR DEVELOPMENT	SCHWARTZ, ROBERT JOEL	TEXAS A&M UNIVERSITY HEALTH SCIENCE CTR	$366,250
4R37HL076795-06	R37	CALMODULIN/CA CHANNEL PHYSIOLOGY IN HEART	YUE, DAVID T	JOHNS HOPKINS UNIVERSITY	$427,792
5R01HL068854-08	R01	PATHOGENESIS OF HERG MUTATIONS IN HUMAN LONG QT SYNDROME	ZHOU, ZHENGFENG	OREGON HEALTH AND SCIENCE UNIVERSITY	$336,452
				Total	$6,886,476