Impact of the *Xenopus* system on the mission 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:

Albert CM, Nam EG, Rimm EB, Jin HW, Hajjar RJ, Hunter DJ, MacRae CA, Ellinor PT. (2008) Cardiac sodium channel gene variants and sudden cardiac death in women. *Circulation*. 117:16-23.

Amaya E, Stein PA, Musci TJ, Kirschner MW (1993) FGF signalling in the early specification of mesoderm in *Xenopus*. *Development*. 118: 477-87.

Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, Potenza D, Moya A, Borggrefe M, Breithardt G, Ortiz-Lopez R, Wang Z, Antzelevitch C, O'Brien RE, Schulze-Bahr E, Keating MT, Towbin JA, Wang Q. (1998) Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature*. 392:293-6.

Kalin RE, Banziger-Tobler NE, Detmar M, Brandli AW. (2009) An in vivo chemical library screen in Xenopus tadpoles reveals novel pathways involved in angiogenesis and lymphangiogenesis. *Blood* 114:1110-1122

Nof E, Luria D, Brass D, Marek D, Lahat H, Reznik-Wolf H, Pras E, Dascal N, Eldar M, Glikson M. (2007) Point mutation in the HCN4 cardiac ion channel pore affecting synthesis, trafficking, and functional expression is associated with familial asymptomatic sinus bradycardia. *Circulation*. 116:463-70.

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.

Seebohm G, Strutz-Seebohm N, Ureche ON, Henrion U, Baltaev R, Mack AF, Korniychuk G, Steinke K, Tapken D, Pfeufer A, Kääb S, Bucci C, Attali B, Merot J, Tavare JM, Hoppe UC, Sanguinetti MC, Lang F. (2008) Long QT syndrome-associated mutations in KCNQ1 and KCNE1 subunits disrupt normal endosomal recycling of IKs channels. *Circ Res.* 103:1451-7.

Shi Y, Katsev S, Cai C, Evans S. (2000) BMP signaling is required for heart formation in vertebrates. *Dev Biol.* 224:226-37.

Xenopus grants funded by the Institute:

According to NIH RePORTER Search Tool, in the fiscal year of 2011, the National Heart, Blood, and Lung Institute (NHLBI) funded seventeen grants for projects involving Xenopus. These grants total to $6,456,710.

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
1R01HL101161-01	STRESS, GENE-ENVIRONMENT INTERACTION AND CARDIOVASCULAR DISEASE	R01	DIEZ ROUX, ANA	UNIVERSITY OF MICHIGAN AT ANN ARBOR	$1,046,175
5R01HL086644-04	COMPUTER MODELING OF PERLECAN AS A VASCULAR REGULATOR	R01	FANNON, MICHAEL W	UNIVERSITY OF KENTUCKY	$330,960
5K18HL092231-02	THE BIOLOGY OF ZEBRAFISH HEMATOPOIETIC STEM CELLS	K18	HANDIN, ROBERT	BRIGHAM AND WOMEN'S HOSPITAL	$298,857
5R01HL083015-05	LINKING SPATIAL VARIATIONS IN SHEAR STRESS WITH OXIDATIVE STRESS	R01	HSIAI, TZUNG K	UNIVERSITY OF SOUTHERN CALIFORNIA	$391,501
5R01HL091958-03	NEUTROPHIL ACCUMULATION IN BACTERIAL PNEUMONIA	R01	JEYASEELAN, SAMITHAMBY	LOUISIANA STATE UNIV A&M COL BATON ROUGE	$334,643
3R01HL087017-04S1	REGULATION OF LUNG EPITHELIAL SODIUM CHANNELS BY CGMP	R01	JI, HONG-LONG	UNIVERSITY OF TEXAS HLTH CTR AT TYLER	$204,647
1ZIAHL005401-19	IL-2 FAMILY CYTOKINES AND THEIR RECEPTORS-- BIOLOGY OF THE IL-2 SYSTEM	ZIA	LEONARD, WARREN	NATIONAL HEART, LUNG, AND BLOOD INSTITUTE	$675,999
5R01HL088664-04	SYNTHETIC LETHAL TARGETING OF P53 IN MYELODYSPLASIA	R01	LOOK, A THOMAS	DANA-FARBER CANCER INSTITUTE	$427,500
2T32HL079995-06A1	TRAINING IN VASCULAR BIOLOGY AND MEDICINE	T32	MARCH, KEITH LEONARD	INDIANA UNIV-PURDUE UNIV AT INDIANAPOLIS	$321,697

5R01HL051854-17	LUNG FLUID BALANCE AND MESENCHYMAL STEM CELLS	R01	MATTHAY, MICHAEL A.	UNIVERSITY OF CALIFORNIA SAN FRANCISCO	$386,250
1R15HL079972-01A1	MECHANISMS OF SERUM ELECTROLYTE EFFECTS ON BLOCK OF HERG	R15	MILLER, ALAN G.	TOURO UNIVERSITY OF CALIFORNIA	$248,550
5R01HL064641-11	MOLECULAR PHYSIOLOGY OF RESPIRATORY MUSCLES	R01	MILLER, JEFFREY BOONE	BOSTON BIOMEDICAL RESEARCH INSTITUTE	$554,245
2R01HL075826-05A1	THE ROLE OF CREB IN NORMAL MYELOPOIESIS AND LEUKEMOGENESIS	R01	SAKAMOTO, KATHLEEN MIHO	UNIVERSITY OF CALIFORNIA LOS ANGELES	$379,965
5R01HL070738-07	ADULT HSC PROVIDE 'HEMANGIOBLAST' ACTIVITY	R01	SCOTT, EDWARD	UNIVERSITY OF FLORIDA	$366,250
5T32HL094290-02	TRAINING IN FUNCTIONAL LIPIDOMICS IN CARDIOVASCULAR AND RESPIRATORY DISEASES	T32	SPIEGEL, SARAH	VIRGINIA COMMONWEALTH UNIVERSITY	$266,788
1F31HL103102-01	ENGINEERING STEM CELL NICHES TO GUIDE DIFFERENTIATION	F31	TSOU, DANIELLE	UNIVERSITY OF CALIFORNIA BERKELEY	$28,559
5U01HL075409-08	CAMP CONTINUATION STUDY/PHASE 3	U01	WILLIAMS, PAUL	ASTHMA, INC.	$194,124
				Total	$6,456,710