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

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The NICHD has made a major investment into the study of *Xenopus* as a model vertebrate organism, and there has been a significant profit from this in terms of our understanding of the fundamental mechanisms of vertebrate development that underlie congenital disorders of children. NICHD has also invested in the sequencing of the *Xenopus* genome(s), the development of *Xenopus tropicalis* as a new model, the development of hundreds of EST libraries, and the generation of arrayed expression libraries; along with supporting resources such as databases, and the development of novel genetic, genomic, and bioinformatic techniques. These areas of research will be increasingly combined in the future, and together have placed *Xenopus* in a prime position for the next generation of studies in which experimental embryology and gene targeting will be combined with systems-level analysis.The advantages of *Xenopus* that originally made it one of the most studied model vertebrate organisms will speed the utilization of these novel supporting resources. Using these approaches, we will gain a new and deeper understanding of vertebrate developmental mechanisms, and be positioned to functionally test potential therapeutic reagents for human congenital disorders, using the well-defined developmental pathways of *Xenopus* .

Several properties of *Xenopus* have made it a model vertebrate of choice. These include:

- speed of development. Experiments take days, not weeks or months.
- well worked out pathways of early development,
- abundance of material for biochemistry,
- a reliable fate map during development, allowing the targeting of reagents both into specific tissues and specific organ systems.
- rapid gain and loss of function assays, for specific genes throughout early development.
- the ability to dissect the embryo and graft specific regions from one embryo to another,
- the availability of the oocyte for experimental manipulation,
- the availability of egg lysates to identify cell cycle components,
- the availability of metamorphosis and larval regeneration as models for tissue regeneration,
- a developing set of tools for transgenesis and gene targeting late in development.
- the ability to carry out forward genetics in *Xenopus tropicalis*

These properties have resulted in the fact that most of what we know about vertebrate early development was initiated by studies in *Xenopus*, as well as from related amphibians. Examples include the identification of intercellular signaling as the primary causative agent of early tissue specification and germ layer formation (Nieuwkoop,1985 ; Smith 1986), the natures of the signals involved and their transcriptional targets (reviewed in Heasman 2006), and the maternal transcription factors that initiate these signals (Tao et al. 2005).

As systems biology approaches become routinely available, these will dramatically enhance the ability of *Xenopus* to provide new insights into normal development.:

- **First, the initiation of development.** Although a maternal forward genetic screen has identified some genes important in early development in zebrafish (Abrams and Mullins 2009), it is impossible to carry out a genetic screen that will reveal all the gene regulatory networks active in early patterning of the embryo. However, a screen based on knockdowns of specific maternal mRNAs, followed by genomic/proteomic analysis of the effects on gene expression in early development, could do this. Rapid knockdowns of individual maternal mRNAs can be carried out in *Xenopus* (Torpey et al. 1991). The amount of material available for analysis (57ng polyA$^+$ RNA per gastrula, 20µg non-yolk protein per gastrula), will allow both transcriptome and proteome analyses of the embryos. Furthermore, the ability to dissect the early embryo into its component regions, and graft material from an experimentally manipulated embryo to a control embryo, and vice versa, adds an additional level of discrimination to the analysis of the functions of individual maternal genes (Wylie et al. 1996). Systems level analysis of embryos from such a screen will, in the long term, identify the entire gene regulatory network initiated by maternal transcripts, which controls formation of the basic body axes, early tissue differentiation, and primary germ layer formation.
- **Second, the formation of the organs of the body**. Two properties of *Xenopus* will make it increasingly important in this respect; the fate map, and its lack of growth during early organogenesis. These properties mean that bio-active reagents, combined with lineage tracers to identify the descendants of the injected cell (Gimlich and Gerhart 1984) can be injected into single identifiable blastomeres at early stages, and will give rise to clones of descendants in specific target organs or tissues. Furthermore, their concentrations will not change during development because the *Xenopus* embryo does not grow. This unique property has allowed manipulation of gene expression well into the organogenesis period, in discrete regions of the embryo. Most commonly this has been done using morpholino oligos, first used in *Xenopus* embryos (Heasman et al. 2000). Since this paper appeared, more than300 papers have been published using this technique to identify genes required for differentiation of individual organs (Small et al. 2005), specific morphogenetic movements (Nandadasa et al. 2009, Skoglund et al. 2008), or specific cell processes (Kim et al. 2009). In the future it will be important to extend these studies with the increased level of sophistication allowed by collection of descendant cells by cell sorting, and genome-wide analysis of the effect of gene targeting in specific cell types. The large amount of material available for biochemistry will make it straightforward, for example, to identify target genes, and altered protein associations. Morphogenetic movements are the movements of tissue masses that shape both the whole embryonic body, and its constituent organs. The large size, and ease of dissection, of *Xenopus* embryos allows the embryo to be cut into explants, and imaged with high resolution. This permits the study of sub-cellular events and interactions with the extracellular matrix in real time, and combined with rapid gain and loss of function experiments provides a powerful experimental tool to study morphogenetic movements in the embryo (Nandadasa et al. 2009, Dzamba et al. 2009, Keller et al. 2003).
- **Third, later organogenesis.** The ability to make transgenic lines of *Xenopus* (reviewed in Loeber et al. 2009) allows a major new direction of research in *Xenopus*; late-stage organogenesis. This is perhaps the most difficult area of developmental research in vertebrates, and yet is extremely important in the study of birth defects, many of which occur relatively late in the development

ofindividual tissues or organs. The generation of new transgenic lines (Yergeau et al. 2009), the application of Cre-mediated gene targeting (Rankin et al. 2009), and novel reporter proteins (Waldner et al. 2009), will be essential in the continued development of tools to study late organogenesis. Stable transgenic lines of *Xenopus* will have enormous potential, because of their long life span, and the numbers of eggs, and thus experimental tissue, they can generate. In addition, the generation and mapping of specific mutations in *Xenopus tropicalis* has begun to allow genetic analysis organogenesis in *Xenopus* (Abu-Daya et al. 2009). All of the techniques mentioned above can be applied to embryos from these lines, allowing a level of sophistication of analysis that simply does not exist in any other organism. This will be particularly useful for the functional analysis of specific mutations that have been shown to cause congenital disorders in humans**.**

- **Fourth, metamorphosis and tissue regeneration.** NICHD has supported work on metamorphosis and nuclear hormone action, and this area will continue to provide valuable insights into hormone induced tissue remodeling. This and the regenerative power of the tadpole will focus future attention on later organogenesis and the signaling underlying tissue homeostasis (Beck et al., 2009). The continuing development of transgenic technologies will dramatically help this area of research.

Analysis of fundamental cellular processes applicable to many aspects of biology and disease. The nature of *Xenopus* development, and the properties described above, have made it a model, not just for vertebrate developmental mechanisms, but also for universal mechanisms. Examples of this include the enormous advances made on our understanding of the cell cycle in *Xenopus* (Mochida et al. 2009), basic mechanisms of the Wnt signaling pathway (Cha et al. 2008), the specific role of signal inhibition in development (Smith and Harland 1992, Lee et al. 2006), the generation of form by changes in cell shape (Rolo et al. 2009), the identification of pluripotency mechanisms in the oocyte (Gurdon and Melton 2008), the fact that vertebrates can be cloned from individual cell nuclei (Gurdon 2006), the discovery that morpholino oligos can be used to block gene expression in embryos (Heasman et al. 2000), and many more. In the future, it will continue to provide new information on these topics. It will also provide an extremely sensitive assay for novel compounds that act as agonists and antagonists for developmental pathways, since the readout of these pathways is known with some detail in *Xenopus*, and given the large amount of tissue available, can be easily quantitated. *Xenopus* embryos offer the most rigorous model available for fast throughput screens like this.

Novel mechanisms of development identified in *Xenopus* are applicable more generally to both normal development in other species, and to disease processes. Examples include ectodermin, an attenuator of TGFbeta signaling in *Xenopus* (Dupont et al. 2005) also plays a role in limiting the antimitogenic effects of Smad4 in tumor cells. Other modifiers of intercellular signaling such as noggin, identified in *Xenopus*, have also been found to function in mammals, and to be mutated in children with congenital deformities (Hwang and Wu 2007). One of the most useful roles of the *Xenopus* embryo, in addition to its function in rapidly producing new knowledge of development, will be in translational studies. As our knowledge increases of the precise mutations that cause developmental disorders in children, so the effects of such mutations on specific developmental pathways can be

characterized in this well-understood developmental system. Screening of small molecules for roles as agonists and antagonists of specific steps in these pathways will offer potential therapies in the future (Wheeler & Brandli 2009).

Selected References

Abrams, E.W., and Mullins, M.C. (2009) Early zebrafish development: it's in the maternal genes. *Curr. Opin. Genet. Dev.* 19, 396-403.

Abu-Daya, A., Sater, A.K., Wells, D.E., Mohun. T.J., and Zimmerman, L.B. (2009) Absence of heartbeat in the *Xenopus tropicalis* mutation muzak is caused by a nonsense mutation in cardiac myosin myh6. *Dev Biol* (Epub).

Beck, C. W., Izpisua Belmonte, J. C. and Christen, B. (2009). Beyond early development: *Xenopus* as an emerging model for the study of regenerative mechanisms. *Dev Dyn* 238, 1226-48.

Cha SW, Tadjuidje E, Tao Q, Wylie C, and Heasman J. (2008) Wnt5a and Wnt11 interact in a maternal Dkk1-regulated fashion to activate both canonical and non-canonical signaling in *Xenopus* axis formation. *Development* 135, 3719-29.

Dupont, S., Zacchigna, L., Cordenonsi, M., Soligo, S., Adorno, M., Rugge, M., and Piccolo, S. (2005) Germ layer specification and control of cell growth by Ectodermin, a Smad4 ubiquitin ligase. *Cell* 121, 87-99.

Dzamba BJ, Jakab KR, Marsden M, Schwartz MA, and DeSimone DW. (2009) Cadherin adhesion, tissue tension, and noncanonical Wnt signaling regulate fibronectin matrix organization. *Dev Cell* 16, 421-32

Gimlich, R., and Gerhart, J. (1984) Early cellular interactions promote embryonic axis formation in *Xenopus laevis*. *Dev Biol* 104, 117-30.

Gurdon, J.B. (2006) From nuclear transfer to nuclear reprogramming: the reversal of cell differentiation. *Annu Rev Cell Dev Biol*. 22, 1-22

Gurdon, J.B., and Melton, D.A. (2008) Nuclear reprogramming in cells. *Science* 322, 1811-15.

Heasman, J. (2006) Patterning the early *Xenopus* embryo. *Development* 133, 1205-17

Heasman, J., Kofron M., and Wylie C. (2000) Beta catenin signaling activity dissected in the early *Xenopus* embryo: a novel antisense approach. *Dev Biol*. 222, 124-34

Hwang, C.H., and Wu, D.K. (2008) Noggin heterozygous mice: an animal model for congenital conductive hearing loss in humans. *Hum. Mol. Genet.* 17, 844-53

Keller, R., Davidson, L. A. and Shook, D. R. (2003). How we are shaped: the biomechanics of gastrulation. *Differentiation* 71, 171-205.

Kim, H., Cheong, S.M., Ryu, J., Jung, H.J., Jho, E.H., and Han, J.K. (2009) *Xenopus* Wntless and the retromer complex cooperate to regulate XWnt4 secretion. *Mol Cell Biol*, 29, 2118-28

Lee, H.X., Ambrosio, A.L., Reversade, B., and De Robertis, E.M. (2006) Embryonic dorsal-ventral signaling: secreted frizzled-related proteins as inhibitors of tolloid proteinases. *Cell*, 124, 147-59.

Loeber, J., Pan, F.C., and Pieler, T. (2009) Generation of transgenic frogs. *Methods Mol Biol* 561, 65-72.

Mochida, S., Ikeo, S., Gannon, J., and Hunt, T. (2009) Regulated activity of PP2A-B55 delta is crucial for controlling entry into and exit from mitosis in *Xenopus* egg extracts. *EMBO J.* 28, 2777-85.

Nandadasa, S., Tao, Q., Menon, N.R., Heasman, J., and Wylie, C. (2009) N- and E-cadherins in *Xenopus* are specifically required in the neural and non-neural ectoderm, respectively, for F-actin assembly and morphogenetic movements. *Development* 136, 1327-38.

Nieuwkoop, P.D. (1985) Inductive interactions in early amphibian development and their general nature. *J. Embryol Exp Morph 89 (Suppl)* 333-47
Rankin, S.A., Hasebe, T., Zorn, A.M., and Buchholz, D.R. (2009) Improved Cre reporter transgenic *Xenopus*. *Dev Dyn* 238, 2401-8.
Rolo A, Skoglund P, and Keller R. (2009) Morphogenetic movements driving neural tube closure in *Xenopus* require myosin IIB. *Dev Biol* 327, 327-38.
Skoglund, P. Rolo, A., Chen, X., Gumbiner, B., and Keller, R. (2008) Convergence and extension at gastrulation require a myosin IIB-dependent cortical actin network. *Development* 135, 2435-44.
Small, E.M., Warkman, A.S., Wang, D.Z., Sutherland, L.B., Olson, E.N., and Krieg, P.A. (2005) Myocardin is sufficient and necessary for cardiac gene expression in *Xenopus*. *Development* 132, 987-97.
Smith, W.C., and Harland, R.M. (1992) Expression cloning of noggin, a new dorsalizing factor localized to the Spemann organizer in *Xenopus*. *Cell* 70, 829-40.
Tao, Q., Yokota, C., Puck, H., Kofron, M., Birsoy, B., Yan, D., Asashima, M., Wylie, C., Lin, X., and Heasman, J. (2005) Maternal Wnt11 activates the canonical wnt signaling pathway required for axis formation in *Xenopus* embryos. *Cell* 120, 857-71.
Torpey, N., Wylie, C., and Heasman, J. (1992) Function of maternal cytokeratin in *Xenopus* development. *Nature* 357, 413-5.
Waldner, C., Roose, M., and Ryffel, G.U. (2009) Red fluorescent *Xenopus laevis*: a new tool for grafting analysis. *BMC Dev Biol* 9, 37
Wheeler GN, Brändli AW. (2009) Simple vertebrate models for chemical genetics and drug discovery screens: Lessons from zebrafish and Xenopus. Dev. Dyn. 238:1287-1308
Wylie C, Kofron M, Payne C, Anderson R, Hosobuchi M, Joseph E, and Heasman J. (1996) Maternal beta-catenin establishes a 'dorsal signal' in early *Xenopus* embryos. *Development* 122, 2987-96.
Yergeau DA, Johnson Hamlet MR, Kuliyev E, Zhu H, Doherty JR, Archer TD, Subhawong AP, Valentine MB, Kelley CM, and Mead PE. (2009) Transgenesis in *Xenopus* using the Sleeping Beauty transposon system. *Dev Dyn* 238, 1727-43.

Xenopus grants funded by the Institute:

According to NIH RePORTER Search Tool, in the fiscal year of 2011, the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) funded ten grants for projects involving Xenopus. These grants total to $3,690,370.

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
5P01HD039530-09	DEVELOPMENT OF LITERACY IN SPANISH-SPEAKING CHILDREN	P01	AUGUST, DIANE LOUISE	CENTER FOR APPLIED LINGUISTICS	$1,030,317
5R01HD046737-07	ACTIVATION OF HUMAN PLACENTAL HORMONAL EXPRESSION	R01	COOKE, NANCY E.	UNIVERSITY OF PENNSYLVANIA	$399,828
1R15HD057604-01	INVESTIGATING THE ROLE OF LEPTIN IN LARVAL GROWTH AND LIMB DEVELOPMENT IN XENOPUS	R15	CRESPI, ERICA J	VASSAR COLLEGE	$183,773
5R03HD056021-02	THE ECONOMICS OF NATURAL DISASTERS AND EPIDEMICS	R03	DATAR, ASHLESHA	RAND CORPORATION	$93,742
1R15HD059070-01	SUPPRESSION OF TADPOLE LIMBS: A MODEL FOR ORGAN SIZE REGULATION	R15	ELINSON, RICHARD P	DUQUESNE UNIVERSITY	$208,098
1R15HD060010-01	MRF4 EXPRESSION AND REGULATION IN XENOPUS	R15	HINTERBERGER, TIMOTHY J	UNIVERSITY OF ALASKA ANCHORAGE	$197,600
3R37HD025594-20S1	CELL MOTILITY AND CELL INTERACTIONS DURING NEURULATION	R37	KELLER, RAYMOND E	UNIVERSITY OF VIRGINIA CHARLOTTESVILLE	$71,102
1ZIAHD008837-04	PINEAL REGULATION: MOLECULAR	ZIA	KLEIN, DAVID	EUNICE KENNEDY SHRIVER NATIONAL	$306,456

	BASIS OF DEVELOPMENT			INSTITUTE OF CHILD HEALTH & HUMAN DEVELOPMENT	
5R01HD041590-09	GENETIC CONTROL OF MYELINATION BY EGR2 AND NAB PROTEINS	R01	SVAREN, JOHN P	UNIVERSITY OF WISCONSIN MADISON	$293,764
1P41HD064556-01	XENBASE: A XENOPUS MODEL ORGANISM DATABASE	P41	ZORN, AARON M	CHILDREN'S HOSPITAL MED CTR (CINCINNATI)	$905,690
				Total	$3,690,370