Impact of the *Xenopus* system on the missions 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embryology and gene targeting will be combined with systems-level analysis.The advantages of *Xenopus* that originally made it one of the most studied model vertebrate organisms will speed the utilization of these novel supporting resources.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).

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Xenopus Grants funding by the NICHD

According to NIH RePORTER Search Tool, in the fiscal year of 2009, the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) **funded 49 grants** for projects involving Xenopus. These grants total **$24,419,094**. 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 NICHD

Project Number	Activity	Project Title	Principal Investigator	Organization	Total
2R01HD032105-11A2	R01	MOLECULAR BASIS OF NEURAL DEVELOPMENT IN XENOPUS	BRIVANLOU, ALI H	ROCKEFELLER UNIVERSITY	$490,288
2P01HD037105-11A1	P01	EXPERIMENTAL SOLUTION OF THE GRN UNDERLYING DEVELOPMENT OF THE CHICK NEURAL CREST	BRONNER-FRASER, MARIANNE	CALIFORNIA INSTITUTE OF TECHNOLOGY	$297,477
3P01HD037105-11A1S2	P01	EXPERIMENTAL SOLUTION OF THE GRN UNDERLYING DEVELOPMENT OF THE CHICK NEURAL CREST	BRONNER-FRASER, MARIANNE	CALIFORNIA INSTITUTE OF TECHNOLOGY	$41,286
1R03HD059995-01	R03	CONTROL OF NUCLEAR THYROID HORMONE SIGNALING BY CELL SURFACE PROTEINS	BUCHHOLZ, DANIEL RICHARD	UNIVERSITY OF CINCINNATI	$78,416
1R03HD060062-01A1	R03	AMPHIBIAN MODELS FOR BODYWALL PATTERNING	BURKE, ANN CAMPBELL	WESLEYAN UNIVERSITY	$78,996
1ZIAHD001501-18	ZIA	PROTEIN-MEDIATED MEMBRANE REMODELING	CHERNOMORDIK, LEONID		$1,027,723
5P01HD048886-03	P01	PATTERNING OF DORSAL RETINA	CHIEN, CHI-BIN	UNIVERSITY OF UTAH	$191,826
3P01HD048886-03S1	P01	PATTERNING OF DORSAL RETINA	CHIEN, CHI-BIN	UNIVERSITY OF UTAH	$19,765
1R01HD056219-01A2	R01	BMP SIGNALING IN VERTEBRATE DEVELOPMENT	CHO, KEN W.Y.	UNIVERSITY OF CALIFORNIA IRVINE	$291,048
5R01HD037976-07	R01	EMBRYONIC SIGNALING REGULATION BY PROPROTEIN CONVERTASES	CHRISTIAN, JAN L	OREGON HEALTH AND SCIENCE UNIVERSITY	$326,314
5R03HD058841-02	R03	ROLE OF SORTLIN IN REGULATING PROTEOLYTIC ACTIVATION OF BMP4 DURING	CHRISTIAN, JAN L	OREGON HEALTH AND SCIENCE UNIVERSITY	$77,000

		EMBRYOGENESIS			
1R15HD057604-01	R15	INVESTIGATING THE ROLE OF LEPTIN IN LARVAL GROWTH AND LIMB DEVELOPMENT IN XENOPUS	CRESPI, ERICA J	VASSAR COLLEGE	$183,773
1ZIAHD001902-15	ZIA	SUMO FAMILY UBIQUITIN-LIKE MODIFIERS IN HIGHER EUKARYOTES	DASSO, MARY C.		$535,580
1ZIAHD008740-08	ZIA	REGULATION OF MITOTIC KINETOCHORES BY THE RAN GTPASE	DASSO, MARY C.		$535,580
1ZIAHD008816-03	ZIA	MITOTIC ROLES OF NUCLEAR PORE COMPLEX (NPC) PROTEINS	DASSO, MARY C.		$459,069
1ZIAHD001002-27	ZIA	GENE EXPRESSION DURING EMBRYONIC DEVELOPMENT OF XENOPUS LAEVIS	DAWID, IGOR		$901,629
1ZIAHD008809-03	ZIA	DEVELOPMENTAL GENETICS IN THE ZEBRAFISH DANIO RERIO	DAWID, IGOR		$1,674,455
5R01HD021502-23	R01	CELL-CELL COMMUNICATION IN XENOPUS DEVELOPMENT	DE ROBERTIS, EDWARD M	UNIVERSITY OF CALIFORNIA LOS ANGELES	$310,407
3R01HD021502-23S1	R01	CELL-CELL COMMUNICATION IN XENOPUS DEVELOPMENT	DE ROBERTIS, EDWARD M	UNIVERSITY OF CALIFORNIA LOS ANGELES	$81,963
2T15HD030269-17	T15	CSHL CELL AND DEVELOPMENTAL BIOLOGY OF XENOPUS	GRODZICKER, TERRI I.	COLD SPRING HARBOR LABORATORY	$59,900
1R15HD060010-01	R15	MRF4 EXPRESSION AND REGULATION IN XENOPUS	HINTERBERGER, TIMOTHY J	UNIVERSITY OF ALASKA ANCHORAGE	$197,600
5R03HD052526-02	R03	THE MECHANISM OF NOTCH SIGNALING PATHWAY IN RADIAL GLIAL DEVELOPMENT	KATO, YOICHI	FLORIDA STATE UNIVERSITY	$73,500
5R37HD025594-20	R37	CELL MOTILITY AND CELL INTERACTIONS DURING NEURULATION	KELLER, RAYMOND E	UNIVERSITY OF VIRGINIA CHARLOTTESVILLE	$332,599
3R37HD025594-20S1	R37	CELL MOTILITY AND CELL INTERACTIONS DURING NEURULATION	KELLER, RAYMOND E	UNIVERSITY OF VIRGINIA CHARLOTTESVILLE	$71,102

5R01HD027262-19	R01	WNT PATHWAY REGULATION IN EMBRYOS AND CELLS	KIMELMAN, DAVID	UNIVERSITY OF WASHINGTON	$279,453
5R01HD054349-03	R01	GROUP II INTRON-BASED GENE TARGETING METHODS FOR XENOPUS	LAMBOWITZ, ALAN M.	UNIVERSITY OF TEXAS AUSTIN	$247,426
4R00HD057298-03	R00	THE FUNCTION OF PIRNAS AND THE PIRNA COMPLEX	LAU, NELSON C	BRANDEIS UNIVERSITY	$233,759
5R01HD035688-10	R01	MATERNAL GENE REGULATION IN EARLY VERTEBRATE DEVELOPMENT	MACNICOL, ANGUS M	UNIVERSITY OF ARKANSAS MED SCIS LTL ROCK	$263,164
1R03HD055321-01A1	R03	NOVEL SIX1 CO-FACTORS AND THEIR ROLE IN PLACODE DEVELOPMENT	NEILSON, KAREN MARY	GEORGE WASHINGTON UNIVERSITY	$78,167
5T32HD007480-13	T32	DEVELOPMENTAL BIOLOGY TRAINING PROGRAM	O'CONNOR, MICHAEL BRENDAN	UNIVERSITY OF MINNESOTA TWIN CITIES	$106,015
5F32HD053213-03	F32	BRIDGING DYNAMIC SIGNALING AND SOMITE MORPHOGENESIS	PRICE, ALIVIA L	SALK INSTITUTE FOR BIOLOGICAL STUDIES	$51,710
5T32HD007312-25	T32	CELL BIOLOGY OF DEVELOPMENT	RICHTER, JOEL D	UNIV OF MASSACHUSETTS MED SCH WORCESTER	$214,039
1R03HD061671-01	R03	INVOLVEMENT OF NONCLASSICAL MHC IN EARLY T CELL ONTOGENY IN XENOPUS	ROBERT, JACQUES	UNIVERSITY OF ROCHESTER	$76,959
5R21HD056444-02	R21	A NOVEL PH DEPENDENT POTASSIUM CHANNEL IN MAMMALIAN SPERM	SANTI, CELIA MARIA	WASHINGTON UNIVERSITY	$190,000
1ZIAHD001006-21	ZIA	PROTEIN/NUCLEIC ACID INTERACTIONS IN VERTEBRATE EMBRYOGENESIS	SARGENT, THOMAS D		$718,651
1R01HD058730-01A1	R01	AGE AND MOLECULAR MECHANISMS CONTRIBUTING TO ANEUPLOIDY IN OOCYTES	LAMPSON, MICHAEL ALTERMAN;SCHULTZ, RICHARD M ;	UNIVERSITY OF PENNSYLVANIA	$329,925
1ZIAHD001901-14	ZIA	MOLECULAR MECHANISM OF THYROID HORMONE RECEPTOR FUNCTION DURING METAMORPHOSIS	SHI, YUN-BO		$857,319

1ZIAHD008858-02	ZIA	REGULATION OF STEM CELL DEVELOPMENT DURING TISSUE REMODELING	SHI, YUN-BO		$214,330
1ZIAHD008859-02	ZIA	REGULATION AND FUNCTION OF MATRIX METALLOPROTEINASES DURING FROG METAMORPHOSIS	SHI, YUN-BO		$357,216
5R01HD031247-15	R01	WNT SIGNAL TRANSDUCTION DURING XENOPUS DEVELOPMENT	SOKOL, SERGEIY	MOUNT SINAI SCHOOL OF MEDICINE OF NYU	$284,341
5R01HD054354-03	R01	A XENOPUS TROPICALIS MUTANT RESOURCE	STEMPLE, DEREK L	SANGER INSTITUTE	$401,712
1ZIAHD000195-16	ZIA	INTRACELLULAR SIGNALING IN ENDOCRINE CELLS	STOJILKOVIC, STANKO S.		$1,212,577
1ZIGHD008806-03	ZIG	RESEARCH ANIMAL MANAGEMENT BRANCH	STRATAKIS, CONSTANTINE A.		$8,096,437
5R01HD054356-03	R01	INTERROGATING THE XENOPUS TROPICALIS GENOME USING TILE PATH MICROARRAYS	VEENSTRA, GERT JAN C.	STICHTING KATHOLIEKE UNIVERSITEIT	$209,034
5R01HD045776-05	R01	XENBASE: A XENOPUS MODEL ORGANISM DATABASE	VIZE, PETER D	UNIVERSITY OF CALGARY	$361,802
1R03HD057334-01A2	R03	TARGETS OF A CYTIDINE DEAMINASE REQUIRED FOR LEFT-RIGHT AXIS IN XENOPUS	VONICA, ALIN	ROCKEFELLER UNIVERSITY	$84,500
5R01HD029468-16	R01	REGULATION OF XENOPUS EMBRYONIC DEVELOPMENT BY TGFBETA SUPERFAMILY LIGANDS AND SM	WHITMAN, MALCOLM R.	HARVARD UNIVERSITY (MEDICAL SCHOOL)	$424,795
2R01HD044764-06	R01	CADHERIN-BASED ACTIN ASSEMBLY IN THE XENOPUS EMBRYO	WYLIE, CHRISTOPHER C	CHILDREN'S HOSPITAL MED CTR (CINCINNATI)	$311,250
5P01HD029587-16	P01	MOLECULAR ANALYSIS OF NMDA RECEPTOR MODULATORY SITES	ZHANG, DONGXIAN	BURNHAM INSTITUTE FOR MEDICAL RESEARCH	$477,217
				Total	$24,419,094