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

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Amphibians historically have been popular for studies of inner ear development, mainly because of the ease with which embryonic manipulations can be done. *Xenopus*, in particular, provides an excellent model system for studying ear development. The vestibular system of *Xenopus* is very similar to that of humans and, unlike in zebrafish, *Xenopus* have a separate auditory structure. Detailed morphological descriptions of ear development are available in *Xenopus*. Homologues of almost all the molecules involved in mammalian inner ear development have been isolated in *Xenopus* and developing embryos provide an excellent system for gene function assays. Later stages, when the inner ear is differentiated, are very transparent, facilitating *in vivo* observation.

Otic placode induction: The first studies identifying the different embryonic tissues involved in placode induction were done in amphibians. Experiments in *Xenopus* showed that the biasing of the ectoderm to an otic fate begins early in development, at mid-gastrula stages. *Xenopus* has also been important for identifying some of the genes necessary for otic induction such as Sox9 whose mutation in humans can result in campomelic dysplasia, a lethal human disorder characterized by deafness, autosomal XY sex reversal and severe skeletal malformations. Studies in *Xenopus* were some of the first to identify the importance of FGF in otic placode induction.

Axial patterning of the developing inner ear: Sensorineural hearing loss (**SNHL**) is one of the more common birth defects and approximately 20% of these patients have inner ear malformations that are readily visible using radiological examination. Such malformations likely result from defects in inner ear patterning during development. The inner ear is a highly asymmetrical structure with distinct anterior-posterior (A-P) and dorsal-ventral (D-V) axes. Embryonic manipulations in amphibians, where one or more of these axes were switched, demonstrated that A-P axis determination occurs during placode stages and prior to D-V axis determination. In a minority of cases there was an unexpected result: mirror image duplicated (enantiomorphic) inner ears. This observation has remained unexplained until recently when it was discovered in *Xenopus* that half ablations along the A-P axis can result in mirror image duplications at even higher percentages than seen in the rotation studies. The ability to generate mirror-duplicated inner ears provides an assay for studying the molecules and regions of the developing inner ear that are required for normal patterning.

Channels important for hair cell function and inner ear homeostasis: *Xenopus* oocytes are used for studying the physiology of water and ion channels. Identification of the transduction channel of the hair cell, crucial for its mechanosensory function in hearing and balance, has been elusive. Functional analyses of prospective transduction channels often utilize *Xenopus* oocytes. The physiology of the gap junction protein connexin 26 (or GJB2), whose

mutation leads to the most common forms of human genetic deafness, has been studied in homomeric and heteromeric hemichannels using paired oocytes.

Selected References:

Noramly, S., Grainger, R. M., 2002. Determination of the embryonic inner ear. *J Neurobiol.* 53, 100-28.
Represa, J., et al., 1991. The int-2 proto-oncogene is responsible for induction of the inner ear. *Nature*. 353, 561-3.
Saint-Germain, N., et al., 2004. Specification of the otic placode depends on Sox9 function in *Xenopus*. *Development*. 131, 1755-63.
Schlosser, G., Ahrens, K., 2004. Molecular anatomy of placode development in *Xenopus laevis*. *Dev Biol*. 271, 439-66.
Waldman, E. H., et al., 2007. Ablation studies on the developing inner ear reveal a propensity for mirror duplications. *Dev Dyn*. 236, 1237-1248.
White, T. W., et al., 1998. Connexin mutations in deafness. *Nature*. 394, 630-1.
Yntema, C. L., Ear and nose. In: B. H. Willier, et al., Eds., Analysis of Development. Saunders, Philadelphia, 1955, pp. 415-428.

Xenopus grants funded by the Institute:

According to NIH RePORTER Search Tool, in the fiscal year of 2011, the National Institute on Deafness and other Communication Disorders (NIDCD) funded eight grants for projects involving Xenopus. These grants total to $2,617,845.

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
5R01DC001655-17	MECHANISMS OF OLFACTORY TRANSDUCTION	R01	ACHE, BARRY W.	UNIVERSITY OF FLORIDA	$293,356
5T32DC009975-02	TRAINING IN HEARING & COMMUNICATION NEUROSCIENCE	T32	BOTTJER, SARAH W.	UNIVERSITY OF SOUTHERN CALIFORNIA	$279,220
5R01DC004061-08	DEVELOPMENTAL ORIGINS OF THE INNER EAR SENSORY ORGANS	R01	COLLAZO, ANDRES	HOUSE EAR INSTITUTE	$390,113
5R01DC008702-05	CRCNS: COMPUTATIONAL AND EXPERIMENTAL ANALYSIS OF NORADRENERGIC FUNCTION IN EARLY	R01	LINSTER, CHRISTIANE	CORNELL UNIVERSITY ITHACA	$322,223
5R01DC003086-13	MOLECULAR BIOLOGY OF COCHLEAR EFFERENT RECEPTORS	R01	LUEBKE, ANNE E	UNIVERSITY OF ROCHESTER	$332,136
5R01DC007481-05	EYA1 IN EAR DEVELOPMENT AND BRANCHIO-OTO-RENAL SYNDROME	R01	MANALIGOD, JOSE M	UNIVERSITY OF IOWA	$416,815
5R03DC010065-02	TARGETED EXPRESSION OF ATOH1 IN COCHLEAR SUPPORTING CELLS	R03	PARKER, MARK A	EMERSON COLLEGE	$149,342
5R01DC009237-02	INFLUENCE OF MODERATE HEARING LOSS ON AUDITORY PERCEPTION & CORTICAL PROCESSING	R01	SANES, DAN HARVEY	NEW YORK UNIVERSITY	$434,640
				Total	$2,617,845