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

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Xenopus has been a classic model system for eye and vision research due to the ease of embryological analysis and manipulation. For example, fundamental insights into retino-tectal connectivity (Sperry), lens induction (Grainger) and retinal cell determination (Harris) have come from work in *Xenopus*. More recently, with the development of modern molecular methodology *Xenopus* has consolidated its role as a unique and vital model for investigating development, physiology and disease of the vertebrate visual system.

Eye Development and Regeneration:

Xenopus is ideal for the study of eye development since histogenesis in the *Xenopus* eye is rapid, with all retinal cell types specified between 1 and 3 days of development. In addition, the eye can be reproducibly targeted by microinjection of blastomeres at early cleavage stages or by in vivo lipofection or electroporation at optic vesicle stages. This allows selective manipulation of gene expression in the eye, with subsequent analysis of effects on optic vesicle patterning and retinal cell fate. This powerful approach has uncovered multiple genes and pathways governing retinal cell fate determination. Important advances range from understanding the importance of basic helix-loop-helix transcription factors in vertebrate retinal cell fate decisions (Kanekar et al., 1997) to the first demonstration that vertebrate homebox proteins act to effect a cellular clock that times the generation of retinal cells (Decembrini et al., 2006). Important achievements in understanding the relevance of signaling pathways to retinal cell fate include the discovery of a novel role for Hedgehog signaling in the transition of stem cell to transient amplifying progenitors (Locker et al., 2006) and the elucidation of the multiple roles that Wnt signaling plays in both embryonic (Van Raay et al., 2005) and post-embryonic eye development (Denayer et al., 2008).

In addition, the development of rapid and efficient methods for generating transgenic animals (Kroll and Amaya, 1996) has led to identification and fine-mapping of multiple eye-specific promoters targeting various cell populations in the developing and mature *Xenopus* eye. For example, promoters for Rx, Pax6, Ath5, X-linked juvenile retinoschisis (RS1) gene and rod opsin have all been mapped in *Xenopus*. These are powerful tools for targeting transgenes to the developing eye and for investigating the mechanisms underlying eye-specific gene regulation.

In *Xenopus*, the eye continues to grow throughout the life of the animal, so there is a true retinal stem cell population present at the margins of the eye in the ciliary marginal zone that drives growth of the eye and can also replace lost or damaged retinal neurons – a feature that is not shared in higher vertebrates. In fact, the cocktail of retinal stem cell/progenitor genes that are sufficient to generate complete functional ectopic eyes from pluripotent ectoderm cells in *Xenopus* has been defined (Viczian et al., 2009). In addition, retinal tissue can be regenerated from animal cap embryonic stem cells (Lan et al, 2009), RPE (Vergara and Del Rio-Tsonis K, 2009) and the lens of the eye can be regenerated from neighboring tissues (reviewed in Beck et al., 2009). Thus, *Xenopus* represents an important model system for understanding retinal stem biology as well as regeneration of ocular tissues.

Retinal Cell Biology & Physiology:

Transgenic methods in *Xenopus* have proved to be a powerful tool for investigating the cell biology of photoreceptors in vivo, in particular for studying protein targeting to photoreceptor outer segments. For example, it was recently shown in *Xenopus* that ankyrin-G binding is necessary and sufficient for targeting of the ®1 subunit of the cyclic nucleotide-gated channel to rod outer segments (Kizhatil et al., 2009). Another study showed that the outer segment serves as a default destination for the trafficking of membrane proteins in photoreceptors (Baker et al.,

2008). The high cone/rod ratio of *Xenopus*, combined with its powerful transgenic methods has proved to be a useful system for investigating rod-cone interactions both in development and disease states (Hamm et al., 2009).

All levels of the *Xenopus* visual system are amenable to fruitful study, including formation of appropriate connections at central targets. Tremendous advances have also been made in our understanding of retinal axon guidance in *Xenopus*. Recent studies have revealed how local protein synthesis contributes to directional steering of retinal growth cones as they navigate to their target (Leung et al., 2006). In addition, it was recently found that maturation of retinotectal synapses in the developing *Xenopus laevis* optic tectum is regulated by activation of ephrin-B reverse signaling (Lim et al., 2008). Another study investigated the early development and plasticity of local excitatory circuits in the optic tectum of *Xenopus laevis* tadpole, revealing important insights into how the response properties of the tectal network are modulated and optimized (Pratt et al., 2008). Thus connectivity and circuit formation in the visual system have been amenable to fruitful analysis in *Xenopus*.

Circadian oscillator mechanisms have been extensively studied in *Xenopus laevis*. The retina contains the essential components of the clock, and can be selectively manipulated using retinal cell-type-specific promoters to allow molecular dissociation of the circadian clock (Hayasaka et al, 2005).

Modeling Human Disease in Xenopus:

Xenopus is also suitable for modeling certain human ocular disease. For example mutations causing autosomal dominant retinitis pigmentosa (RP) in humans induce rod photoreceptor degeneration in *Xenopus laevis* (Tam and Moritz, 2006). This has led to additional important insights, such as a molecular mechanism for light sensitivity in RP (Tam and Moritz, 2007). These approaches will ultimately open up new avenues for rapidly testing the effects of certain human mutations on gene function in vivo.

Selected references:

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Xenopus grants funded by the Institute:

According to NIH RePORTER Search Tool, in the fiscal year of 2011, the National Energy Institute (NEI) funded ten grants for projects involving Xenopus. These grants total to $2,876,799.

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	Activity	Project Title	Principal Investigator	Organization Name	Total Cost
1ZIEEY000487-02	ZIE	MEDICAL RETINA FELLOWSHIP	CHEW, EMILY Y	NATIONAL EYE INSTITUTE	$243,488
3R01EY015788-06S1	R01	MECHANISMS OF VISUAL MAP DEVELOPMENT IN THE SUPERIOR COLLICULUS	CRAIR, MICHAEL	YALE UNIVERSITY	$299,240
5R01EY015163-05	R01	TRANSGENIC STUDIES OF VERTEBRATE RETINAL DEVELOPMENT	DOWLING, JOHN E	HARVARD UNIVERSITY	$359,709
3R01EY013246-07S1	R01	MOLECULAR SCAFFOLDING FOR PHOTORECEPTOR OUTER SEGMENT STRUCTURE AND RENEWAL.	GOLDBERG, ANDREW FX	OAKLAND UNIVERSITY	$21,161
3R01EY012085-11S1	R01	INTERCELLULAR COMMUNIATION IN THE EYE LENS	JIANG, JEAN X	UNIVERSITY OF TEXAS HLTH SCI CTR SAN ANT	$302,637
2R15EY016027-02	R15	ISWI REMODELING COMPLEXES IN EYE DEVELOPMENT	KREBS, JOCELYN E	UNIVERSITY OF ALASKA ANCHORAGE	$205,484
1ZIAEY000420-07	ZIA	AG13764 AND AG13711 REVERSES VEGF-INDUCED CHOROIDAL NEOVASCULARIZATION IN RAT EYE	MILLER, SHELDON	NATIONAL EYE INSTITUTE	$46,825
5R01EY006891-19	R01	MEMBRANE BIOSYNTHESIS IN NORMAL AND DYSTROPHIC RETINA	PAPERMASTER, DAVID S	UNIVERSITY OF CONNECTICUT SCH OF MED/DNT	$462,014
3R01EY016094-04S1	R01	DEVELOPMENT OF NANOSCALE NEUROMODULATING PLATFORMS	PEPPERBERG, DAVID R	UNIVERSITY OF ILLINOIS AT CHICAGO	$530,341
5R01EY002966-31	R01	NEURAL VISUAL CODING: IMAGE TO OBJECT REPRESENTATION	VON DER HEYDT, RUDIGER	JOHNS HOPKINS UNIVERSITY	$405,900
				Total	$2,876,799