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

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It is now well established that both the innate and adaptive immune systems undergo rapid evolution and diversification; consequently, non-mammalian vertebrate animal models that are experimentally tractable alternatives to murine systems are essential, as they will allow us better distinguish important conserved structures and functions from species-specific specializations. In this regard, *Xenopus* offers one of the best comparative models with which to study the immune system.

Indeed, the advantages of the *Xenopus* model systems have been leveraged to advance our understanding of many facets of immunity. These include: humoral and cell-mediated immunity in the context of MHC restricted and unrestricted recognition; ontogeny; phylogeny; and defense against tumors, viruses, fungi and bacteria (reviewed in Pasquier et al., 1989; Robert and Ohta, 2009). *Xenopus* is as valuable as zebrafish for studying the ontogeny of the immune system. Moreover, unlike zebrafish, *Xenopus* has the best characterized immune system outside of mammals and chicken. Furthermore, the *Xenopus* model offers a collection of invaluable research tools including MHC-defined clones, inbred strains, cell lines, and monoclonal antibodies. Finally, the annotated full genome sequence of *X. tropicalis* and its remarkable conservation of gene organization with mammals, as well as ongoing genome mapping and mutagenesis studies in *X. tropicalis* provide a new dimension to the study of immunity The salient features of this amphibian model are summarized below.

Model to study Immunogenetics: The *X. tropicalis* genome has provided compelling evidence for the similarity of gene repertoire in both the adaptive and innate immune systems (Zarrin et al., 2004; Guselnikov et al., 2008). More importantly, it has unveiled the amazing degree of conservation of gene clustering or synteny with mammals, which is far better preserved with *Xenopus* than with any fish species whose genomes have undergone extensive diversification during evolution. Gene synteny is helpful for identifying diverged genes such as immune genes. For example, in *Xenopus* as in mammals CD8 beta retains proximity to CD8 alpha, and CD4 neighbors Lag3 and B protein. Ongoing whole genome mutagenesis will allow one to search for genes critically involved in immune functions.

Xenopus is the only genus where polyploid as well as diploid species exist naturally, and can be artificially produced with various degrees of polyploidy (2N to 8N), enabling an experimental approach to studying the consequences of whole genome duplication (i.e., study the fate of duplicated genes), a subject of major interest nowadays for understanding the origin of the vertebrate genome, as well as the effects of gene dose on host resistance or defense against pathogens. *Xenopus* species can also be cloned using gynogenetic development of diploid eggs coming from interspecies hybrids. These clones can easily be maintained and propagated in the laboratory, and constitute a unique *in vivo* way to study genome regulation. Clones with identical MHC combinations but differences at minor histocompatibilty (H) gene loci provide an excellent biological system to study immune responses in vivo. *X. laevis* is the only species where aneuploid animals can be generated for studying the segregation of immune functions linked to a specific chromosome. In situ hybridization techniques are now available both for chromosome and for whole mounts embryos.

Model to study the development of the immune system: *Xenopus* provides an excellent system to study early ontogeny of the immune system. *Xenopus* has all the lineages of hematopoietic cells that mammals have. However, early developmental stages of *Xenopus* are free of maternal influence, and are easily accessible and amenable to experimentation. This provides an ideal animals model to study early

commitments and fates of myeloid and lymphoid lineages (Suzuki et al., 2004; Marr et al., 2007).

Metamorphosis in *Xenopus* is a truly unique developmental period, in which the larval thymus loses most of its lymphocytes, and a new differentiation occurs from a second wave of stem cell immigration resulting in completely distinct adult immune system. Notably, autoimmunity against the many new adult type proteins needs to be prevented by a new balance of self-tolerance through T cell education (Flajnik et al., 2001). This system has the additional advantage of the accessibility of the thymus early in development. Indeed, thymectomy can be efficiently performed in *Xenopus* at early developmental stages before the migration of stem cells and generate T cell-deficient animals. Therefore, *Xenopus* has been and still is frequently used to study T cell ontogeny, and with the new genomic and genetic technologies it offers new ways to analyze genes and function in a complementary manner.

Model to study immune tolerance. *Xenopus* serves as an exciting comparative model to explore self-tolerance because of the ease with which allotolerance to minor H-Ags on adult skin grafts can be induced just prior or during metamorphosis that is the transitional animal undergoes a temporary period of altered immunoregulation (Flajnik et al., 2001). During this period, one can experimentally induce long-lasting specific non-deletional ("split") anergic-like tolerance to minor H-Ags that persists after metamorphosis. MHC genes are also differentially regulated in larvae and adults. The change in MHC gene regulation during metamorphosis, the new histogenesis in the thymus, and the ease with which one can experimentally manipulate larvae (e.g., thymectomy, blocking or accelerating metamorphosis) allows one to address questions about MHC restriction, autoimmunity, and the development of self-tolerance that can not be easily studied in other animal models.

Model to study tumor immunity: *Xenopus* is the only amphibian genus where series of true lymphoid tumors have been discovered and cell lines have been obtained, thereby opening up new avenues for tumor biology and the isolation and characterization of membrane proteins. In particular, distinct immune systems of larvae and adults, and the ease of manipulating their maturation during metamorphosis provides a unique to investigate *in vivo* the possible influence of the immune system on the selection of more aggressive tumor. *Xenopus* has also significantly helped to demonstrate the importance of certain heat shock proteins such as hsp70 in anti-tumor immune responses. It provides a natural *in vivo* model to dissect the contribution of innate (pro-inflammatory) and adaptive (MHC class I restricted T cell) arm of the immune system in hsp-mediated anti-tumor responses (Goyos et al., 2007). As such *Xenopus* is an important comparative tumor immunity model that can contribute to designing more efficient immunotherapeutic approaches to control cancer.

Model to study vascular and lymphatic transdifferentiation and regeneration. The *Xenopus* tadpole has recently emerged as a very powerful system for tissue and vasculature regeneration research (Slak et al., 2008). Within 7-10 days following amputation, a completely new functional tail, with all its tissue types (including muscles, spinal cord, etc) regenerates in this system. Formation, maintenance and regeneration of lymphatics and blood vessel have become a major area of investigation in their own right, as well as owing to on immune function and immune responses (Ny et al., 2005; 2008; Fukazawa et al., 2009)

Model *to study immune responses to important emerging infectious diseases*: *Xenopus* provides a powerful laboratory model to study immunity to important emerging infectious diseases caused by a chitrid fungus and by ranaviruses (*Iridoviridae*). The

recognized threat of these emerging wildlife diseases on global biodiversity, which ultimately impacts on human health, makes it urgent to better understand host-pathogen interactions in vertebrates other than mammals. Because of the extent to which knowledge has already been acquired, as well as the availability of tools including microarrays and genomic information, *Xenopus* is an ideal model for such studies. For example, comparison between susceptible tadpoles and resistant adults to ranaviral infection, and between susceptible *X. tropicalis* and resistant *X. laevis* to chytrid fungal infection, provide ways to elucidate virulence and immune escape mechanisms that are of high fundamental relevance (Morales and Robert, 2007; Rosenblum et al., 2009). The unique antimicrobial peptides in skin secretions produced by *Xenopus* are very potent against HIV and many human gram negative and positive bacteria, and therefore are of high biomedical interest. Available genomic information will provide further insight about the regulation and evolution of the genes encoding these proteins (Zasloff, 2002).

Generation and maintenance of animal and tools: Invaluable research tools for *X. laevis* including monoclonal antibodies (mAbs), antisera, cell lines, genomic, cDNA, and EST libraries have been accumulated since 1976 and are maintained for the scientific community in a research resource funded by NIAID. This resource also maintains MHC-defined and clones that permit classic adoptive transfer and transplantation manipulations (e.g., skin grafting) as in mice. Unlike mice, however, they also permit transfer of tissues and cells between larva and adult. Material and animals have been provided for more than 40 laboratories worldwide. Recently, inbred strains of *X. tropicalis* have also been established.

Several transgenesis techniques are now operational for both *X. laevis* and *X. tropicalis*, and transgenic lines with fluorescence reporter genes specifically expressed by myeloid cells are available (ref. Other transgenic lines are under development. A relatively large panel of mAbs including anti-MHC, and anti-B, T, NK and general leukocyte markers are available for *X. laevis* and more are currently being generated using novel technologies such as phage displays of single chain Abs. Generation of *Xenopus* -specific Abs is among the priorities identified by the *Xenopus* community. The combined use of transgenic lines with cell types expressing fluorescence reporter genes and flow cytometry cell sorting using available mAbs to isolate specific cell subsets with the possibility of transferring these cells to embryos or adult recipients will make *Xenopus* an even more valuable model in the next decade.

In summary, *Xenopus* provides a unique, versatile, non-mammalian model with which to investigate important contemporary issues of immunity such as, ontogeny of immunity, self-tolerance, autoimmunity, tumor immunity, and adaptation of host immune defenses to emerging pathogens. The recent genomic and genetic technologies developed in *Xenopus* has the potential to make *Xenopus* a one of the most powerful and innovative comparative models for immunological and biomedical research.

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Xenopus grants funded by the Institute:

According to NIH RePORTER Search Tool, in the fiscal year of 2011, the National Institute of Allergy and Infectious Diseases (NIAID) funded fourteen grants for projects involving Xenopus. These grants total to $7,762,913

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
5F31AI080183-02	EFFECTS OF A CHLAMYDIAL ATP-CONSUMING ENZYME ON THE INFLAMMASOME	F31	AVILA, MARIA LUISA	UNIVERSITY OF CALIFORNIA, MERCED	$32,522
5K22AI073888-02	ORIGINS OF SPECIALIZED MUCOSAL LYMPHOCYTE SUBSETS AND IMMUNOGLOBULIN ISOTYPES	K22	CRISCITIELLO, MICHAEL FREDERICK	TEXAS AGRILIFE RESEARCH	$108,000
5R01AI027877-20	ONTOGENY AND PHYLOGENY OF THE MHC	R01	FLAJNIK, MARTIN F.	UNIVERSITY OF MARYLAND BALTIMORE	$385,484
Contract	GENOMIC SEQUENCING CENTERS FOR INFECTIOUS DISEASES	N01	FRASER-LEGGETT, CLAIRE	UNIVERSITY OF MARYLAND BALTIMORE	$96,000
Contract	AWARD OF PART B - IN VITRO TESTING RESOURCES FOR AIDS THERAPEUTICS DEVELOPMENT	N01	HEIL, MARINTHA	SOUTHERN RESEARCH INSTITUTE	$2,802,585
1ZIAAI000914-09	GENERATION AND CHARACTERIZATION OF ROTAVIRUS VIRUS-LIKE PARTICLE (VLP) VACCINES	ZIA	HOSHINO, YASUTAKA	NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES	$226,171
1ZIAAI001099-02	T-CELL RECEPTOR REPERTOIRES IN IMMUNE REGULATION AND RESPONSE	ZIA	MILNER, JOSHUA	NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES	$402,156
5R01AI057695-05	SNPS IN HANDLING OF SMALL POX ANTIVIRALS AND OTHER DRUGS	R01	NIGAM, SANJAY	UNIVERSITY OF CALIFORNIA SAN DIEGO	$329,612
5R01AI047833-10	NOTCH REGULATION OF HEMATOPOETIC CELL FATES	R01	PEAR, WARREN	UNIVERSITY OF PENNSYLVANIA	$371,317

5R21AI084711-02	A NEW INSIGHT INTO HIV-1 LATENCY THROUGH A NOVEL IN VITRO SYSTEM	R21	ROMERIO, FABIO	UNIVERSITY OF MARYLAND BALTIMORE	$191,836
5R01AI049512-08	PURINE PATHWAYS AND INHIBITOR DESIGN IN PLASMODIUM	R01	SCHRAMM, VERN L.	ALBERT EINSTEIN COL OF MED YESHIVA UNIV	$490,845
3UH2AI083266-01S2	THE HUMAN VIROME IN CHILDREN AND ITS RELATIONSHIP TO FEBRILE ILLNESS	UH2	STORCH, GREGORY A.	WASHINGTON UNIVERSITY	$250,000
1ZIAAI000354-28	IMMUNOREGULATORY DEFECTS IN INFLAMMATORY BOWEL DISEASE	ZIA	STROBER, WARREN	NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES	$959,273
Contract	NONHUMAN PRIMATE BREEDING COLONY	N01	WESTERGAARD, GREG	ALPHA GENESIS, INC.	$1,117,112
				Total	$7,762,913