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

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Common molecules control key events in both embryonic development and cancer, and elucidating the molecular mechanisms via which such factors regulate normal development provides important insight into how their misregulation contributes to tumor formation and progression. *Xenopus laevis* embryos are a powerful system in which to investigate the molecular mechanisms underlying gene function, organogenesis, and disease. All stages of development are accessible to experimental manipulation in embryos and a major advantage of this system is the ease with which gene expression and signaling pathways can be perturbed. Furthermore, *Xenopus* embryos are large and easy to obtain in large numbers, facilitating the collection of material for biochemical studies and proteomics. Their external development also makes them ideal for chemical genetics and drug discovery screens aimed at the development and evaluation of putative chemotherapeutics. Thus, *Xenopus* provides a series of advantages not readily available in other vertebrate systems and remains an important area of investment for the continued development of tools to advance studies using this model organism.

Among the studies in *Xenopus* of high relevance to cancer are those aimed at understanding the vertebrate neural crest and its derivatives. A number of cancers of great clinical significance are neural crest-derived, including neuroblastoma, melanoma, and gliomas. Interestingly, a number of identified molecular mediators of neural crest development appear to be mis-regulated in human cancers, including c-myc, and Snail family proteins. In particular, the molecules that control the Epithelial-Mesenchymal Transition (EMT) and invasive behavior of neural crest cells have been co-opted by epithelial tumors to mediate metastasis and *Xenopus* has become a powerful model for understanding the mis-regulation of these molecules during tumor progression. Similarly the *Xenopus* system has recently provided evidence that the cancer-associated Wilms Tumor Suppressor protein WTX is a required component of the ß-catenin destruction complex which is mis-regulated in a broad range of tumors.

Beyond whole embryo studies, cell-free extracts derived from *Xenopus laevis* eggs have provided a powerful and biochemically tractable system for the study of the cell cycle under physiological and stressed conditions. This is the only cell-free system that recapitulates all DNA transactions associated with cell cycle progression and the response to DNA damage (DNA replication, chromosome segregation, DNA repair and checkpoints). Of particular relevance to cancer, the *Xenopus* egg extract system has been instrumental to the study of the DNA damage response and of DNA replication in the maintenance of genome integrity. In response to DNA damage or to a block to DNA replication, S phase is delayed to allow DNA repair processes to occur as well as to ensure the completion of DNA replication prior to the start of mitosis. The molecular bases of these checkpoint pathways that influence DNA replication were unraveled using *Xenopus* cell-free extracts. These extracts allows us to study DNA lesion-specific signaling. It was shown that DNA double-strand breaks activate the ATM kinase leading to the Cdc25-dependent inhibition of Cdk2. Similarly, it was demonstrated that DNA polymerase stalling triggered by aphidicolin or by UV lesions activates ATR resulting in the Chk1-dependent inhibition of Cdk1. More recently, these extracts have been instrumental to the study of complex DNA lesions such as inter-strand crosslinks. *Xenopus* cell-free extracts have also provided models to study the biochemical bases of

several cancer-prone diseases associated with mutations in ATM (Ataxia telangiectasia), BRCA1 (Inherited Breast and Ovarian cancer), Nbs1 (Nijmegen Breakage Syndrome) and FANC proteins (Fanconi anemia). Finally, preliminary studies indicate that *Xenopus* cell-free extracts could be used successfully to identify small molecules that modulate the DNA damage response with potential chemosensitizing properties for cancer therapy. Thus studies in *Xenopus* continue to provide essential insights into basic cellular pathways that are essential to the maintenance of genomic stability and the prevention of tumor formation.

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Xenopus grants funded by the Institute:

According to NIH RePORTER Search Tool, in the fiscal year of 2011, the National Center Institute (NCI) funded twenty-seven grants for projects involving Xenopus. These grants total to $7,489,124.

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	Total
5R21CA132046-02	R21	DETECTION OF MELANOMA BY CANINE OLFACTORY RECEPTORS.	ABAFFY, TATJANA	UNIVERSITY OF MIAMI SCHOOL OF MEDICINE	$172,125
5R01CA125103-04	R01	B-RAF REGULATION OF THE CELL CYCLE IN MELANOMA	APLIN, ANDREW E	THOMAS JEFFERSON UNIVERSITY	$256,470
5R01CA116195-06	R01	ADJUVANT TRIAL WITH SOY AFTER RADICAL PROSTATECTOMY	BOSLAND, MAARTEN C	UNIVERSITY OF ILLINOIS AT CHICAGO	$251,751
1ZIABC011295-01	R01	OPTIMIZING THE GRAFT VERSUS LEUKEMIA EFFECT FOR PEDIATRIC ALL	FRY, TERRY	DIVISION OF BASIC SCIENCES - NCI	$330,006
3R01CA092245-08S1	R01	REGULATION OF THE DNA DAMAGE RESPONSE BY THE MRN-ATM PATHWAY	GAUTIER, JEAN	COLUMBIA UNIVERSITY HEALTH SCIENCES	$344,934
5R01CA118761-04	R01	P53 AND PATHWAYS OF APOPTOSIS	GEORGE, DONNA L	UNIVERSITY OF PENNSYLVANIA	$299,250
5R01CA111482-05	R01	PDK1 AND PPARDELTA SIGNALING IN MAMMARY TUMORIGENESIS	GLAZER, ROBERT I	GEORGETOWN UNIVERSITY	$266,540
5R01CA106588-07	R01	THE MOLECULAR ACTIONS OF IMATINIB MESYLATE IN GISTS	GODWIN, ANDREW K	FOX CHASE CANCER CENTER	$433,446
5R03CA141199-02	R03	IDENTIFICATION OF BIOACTIVE MARINE NATURAL PRODUCTS THAT INHIBIT MAST CELLS IMPLI	GUZMAN, ESTHER AMALIA	FLORIDA ATLANTIC UNIVERSITY	$71,250
5U54CA126518-05	U54	INFILTRATING GLIOMAS ACT TO RECRUIT STEM CELLS	HOLLAND, ERIC C	SLOAN-KETTERING INSTITUTE FOR CANCER RES	$407,571
1U54CA156732-01	U54	RESOURCE/INFRASTRUCTURE DEVELOPMENT CORE	JOFFE, STEVE	DANA-FARBER CANCER INSTITUTE	$56,109
1R01CA142724-01A1	R01	ROLE OF FOXM1 IN LUNG CANCER MICROENVIRONMENT	KALIN, TANYA	CHILDREN'S HOSPITAL MED CTR (CINCINNATI)	$316,438

5R01CA114058-05	R01	TRANSCRIPTIONAL REGULATION OF NC PRECURSOR FORMATION	LABONNE, CAROLE	NORTHWESTERN UNIVERSITY	$243,714
5R01CA133086-02	R01	MOLECULAR TARGET FOR LIVER CANCER DIAGNOSIS AND THERAPY	LIU, CHEN	UNIVERSITY OF FLORIDA	$303,988
5R01CA082845-10	R01	BIOLOGICAL ROLES OF THE PROLYL ISOMERASE, PIN1	MEANS, ANTHONY R	DUKE UNIVERSITY	$310,291
2P01CA109901-06	P01	BIOSTATISTICS AND BIOINFORMATICS CORE	NEUBERG, DONNA S	DANA-FARBER CANCER INSTITUTE	$45,372
5K22CA124578-02	K22	AGAP2 REGULATES MEMBRANE TRAFFICKING AND MIGARATION OF GLIOBLASTOMA CELLS	NIE, ZHONGZHEN	UNIVERSITY OF FLORIDA	$192,024
5R01CA129096-04	R01	STATEWIDE COMMUNICATION TO REACH DIVERSE LOW INCOME WOMEN	PASICK, RENA JOY	UNIVERSITY OF CALIFORNIA SAN FRANCISCO	$423,805
261200900075C-1-0-1	Contract	SBIR TOPIC 257, INSTRUMENTS AND DEVICES THAT PRESERVE MOLECULAR PROFILES IN TUMO	SCHAYOWITZ, ADAM BRENT	BIOMARKER STRATEGIES, LLC	$1,011,413
5R01CA130888-02	R01	THE GABA-B RECEPTOR IS A NOVEL DRUG TARGET FOR PANCREATIC CANCER	SCHULLER, HILDEGARD M	UNIVERSITY OF TENNESSEE KNOXVILLE	$272,655
5R01CA109366-05	R01	DEVELOPMENT OF ANTI-CXCR4 COMPOUNDS AS ANTI-METASTATIC, ANGIOGENIC DRUG	SHIM, HYUNSUK	EMORY UNIVERSITY	$263,700
5T32CA009035-35	T32	TRAINING PROGRAM IN CANCER RESEARCH	SKALKA, ANNA MARIE	INSTITUTE FOR CANCER RESEARCH	$481,011
1P50CA148596-01	P50	TRAINING AND CAREER DEVELOPMENT	SORENSEN, GLORIAN C	HARVARD UNIVERSITY (SCH OF PUBLIC HLTH)	$21,805
1Z1ABC010973-03	Z1A	ANALYSES OF HUMAN T REGULATORY CELLS	TSANG, KWONG YOK	DIVISION OF BASIC SCIENCES - NCI	$337,799
261201000125C-0-0-1	Contract	TAS::75 0849::TAS	WANG, Y. ANDREW	OCEAN NANOTECH, LLC	$150,000

5R01CA117895-04	R01	TARGETING RAR-BETA2-LED GENE PATHWAY IN PREVENTION OF ESOPHAGEAL CANCER	XU, XIAOCHUN	UNIVERSITY OF TEXAS MD ANDERSON CAN CTR	$248,978
1U01CA142565-01	U01	PET-MRI FOR ASSESSING TREATMENT RESPONSE IN BREAST CANCER CLINICAL TRIALS	YANKEELOV, THOMAS E	VANDERBILT UNIVERSITY	$416,680
				Total	$7,489,124