# Impact of the *Xenopus* system on the missions 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.

**Selected References.**


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# *Xenopus* Grants funding by the NCI

According to NIH RePORTER Search Tool, in the fiscal year of 2009, the National Center Institute (NCI) **funded 24 grants** for projects involving *Xenopus*. These grants total **$10,047,657**. 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 NCI

| 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 |
| **5R01CA123238-03** | R01 | NON-CANONICAL WNT/DISHEVELLED SIGNALING AND CANCER CELL MALIGNANCY | BROWN, ANTHONY M.C. | WEILL MEDICAL COLLEGE OF CORNELL UNIV | $319,200 |
| **1R13CA141843-01** | R13 | GENETIC RECOMBINATION & GENOME REARRANGEMENTS | COX, MICHAEL M. | FEDERATION OF AMER SOC FOR EXPER BIOLOGY | $15,000 |
| **1ZIABC010006-08** | ZIA | MECHANISMS OF CROSS-TALK BETWEEN EPHRINB AND ALTERNATE SIGNALING PATHWAYS | DAAR, IRA | | $593,214 |
| **1ZIABC010958-02** | ZIA | SIGNALING MECHANISMS OF EPHRINB1 IN CELL ADHESION, MIGRATION AND INVASION | DAAR, IRA | | $593,214 |
| **5R01CA092245-08** | R01 | REGULATION OF THE DNA DAMAGE RESPONSE BY THE MRN-ATM PATHWAY | GAUTIER, JEAN | COLUMBIA UNIVERSITY HEALTH SCIENCES | $285,442 |
| **3R01CA092245-08S1** | R01 | REGULATION OF THE DNA DAMAGE RESPONSE BY THE MRN-ATM PATHWAY | GAUTIER, JEAN | COLUMBIA UNIVERSITY HEALTH SCIENCES | $344,934 |
| **2R01CA082621-11** | R01 | PROTON-COUPLED FOLATE/ANTIFOLATE TRANSPORT | GOLDMAN, ISRAEL DAVID | ALBERT EINSTEIN COL OF MED YESHIVA UNIV | $526,718 |
| **2R01CA031760-27A1** | R01 | INTERACTIONS BETWEEN INTERMEDIATE FILAMENTS AND NUCLEUS | GOLDMAN, ROBERT D | NORTHWESTERN UNIVERSITY | $376,170 |
| **5R01CA106569-04** | R01 | REGULATION OF BETA-CATENIN SIGNALING BY TYROSINE PHOSPHORYLATION | HE, TONG-CHUAN | UNIVERSITY OF CHICAGO | $260,136 |

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|---|---|---|---|---|---|
| **1ZIABC010549-07** | ZIA | ZEBRAFISH MODELS OF CANCER | HICKSTEIN, DENNIS | | $1,223,960 |
| **5R01CA112005-05** | R01 | FUNCTION OF MYELOID TRANSLOCATION GENE RELATED-1 | HIEBERT, SCOTT | VANDERBILT UNIVERSITY | $329,190 |
| **5R01CA112775-12** | R01 | FUNCTIONAL ANALYSIS OF THE FANCONI PATHWAY | HOATLIN, MAUREEN E | OREGON HEALTH AND SCIENCE UNIVERSITY | $273,823 |
| **5R01CA116402-03** | R01 | CHECKPOINT KINASE CHK1 IN CANCER BIOLOGY AND THERAPY | HUNTER, TONY R. | SALK INSTITUTE FOR BIOLOGICAL STUDIES | $363,850 |
| **2R01CA080100-11A1** | R01 | SOMATIC CELL CYCLE REGULATION BY PHOSPHORYLATION | HUNTER, TONY R. | SALK INSTITUTE FOR BIOLOGICAL STUDIES | $720,345 |
| **5R01CA114058-05** | R01 | TRANSCRIPTIONAL REGULATION OF NC PRECURSOR FORMATION | LABONNE, CAROLE B | NORTHWESTERN UNIVERSITY | $243,714 |
| **1ZIABC010761-04** | ZIA | STUDIES OF PROTEINS WITH IMPORTANT ROLES IN IMMUNOLOGY ANDOR CANCER BIOLOGY | LUBKOWSKI, JACEK T | | $661,102 |
| **5R01CA082845-10** | R01 | BIOLOGICAL ROLES OF THE PROLYL ISOMERASE, PIN1 | MEANS, ANTHONY R | DUKE UNIVERSITY | $310,291 |
| **5R01CA138143-02** | R01 | CHARACTERIZATION OF JNK IN CELL CYCLE CONTROL | RONAI, ZE'EV A | BURNHAM INSTITUTE FOR MEDICAL RESEARCH | $396,325 |
| **1ZIABC009003-27** | ZIA | THE ROLE OF CRIPTO IN THE PATHOGENESIS OF BREAST AND COLON CANCER | SALOMON, DAVID | | $1,145,609 |
| **1R01CA139395-01A1** | R01 | WNT SIGNALING | WU, DIANQING | YALE UNIVERSITY | $408,109 |
| **5P30CA006927-47** | P30 | CORE--LABORATORY ANIMAL FACILITY | YOUNG, ROBERT C | FOX CHASE CANCER CENTER | $415,548 |
| **3P30CA006927-47S5** | P30 | CORE--LABORATORY ANIMAL FACILITY | YOUNG, ROBERT C | FOX CHASE CANCER CENTER | $2,419 |
| **3P30CA006927-47S4** | P30 | CORE--LABORATORY ANIMAL FACILITY | YOUNG, ROBERT C | FOX CHASE CANCER CENTER | $67,219 |
| | | | | Total | $10,047,657 |