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

Oliver Wessely, PhD. - Louisiana State University Health Science Center

Xenopus has played a very important role in the mission of NIDDK for a long time. *Xenopus* oocytes have been and still are an invaluable system to study the conductive properties of many channels and transporters expressed on renal epithelial cells. Many recent technological advances such as antisense morpholino oligomers for gene knockdowns, transgenic GFP lines for imaging and the genome information for *X. tropicalis* have promoted *Xenopus* as a valuable model not only to study early embryonic development, but also to investigate organogenesis. This has been realized by NIDDK and projects exploring the pronephric kidney, the pancreas and the liver are among the currently funded grants.

Electrophysiology using *Xenopus* oocytes: *Xenopus* oocytes express a low number of endogenous membrane transporters and channels because they are virtually independent from exogenous nutrients. As such they have been and are the preferred *in vivo* model to characterize channels, receptors and transporters present on renal epithelial cells that are crucially important for kidney function. Oocytes are used to study electrophysiological properties, stoichiometries and the role of post-translational modification. The system is also very amendable to high-throughput screening approaches. As such it has been a powerful tool to perform functional screens for genes encoding ion channels and transporters. In addition to their basic science component these studies have significant impact in respect to human diseases. For example, studies on hypertension have used *Xenopus* oocytes to demonstrate that defects in With no Lysine kinase 4 (WNK4) causes increased activity of the renal transporter molecules NKCC2 and NCC and thereby directly interferes with blood pressure control.

Kidney Development: *Xenopus* embryos due to their aquatic life develop a functional pronephric kidney within 31 hours post fertilization. Thus, *Xenopus* has been established as a valuable animal model to study kidney development. Over the years, it has become evident that the process of kidney development is evolutionary conserved and findings in *Xenopus* are directly applicable to studies in higher vertebrates such as humans and mouse. One of the most recent advances was the realization that *Xenopus* is a powerful model organism to study the patterning of the nephron along its proximal-distal axis. With the availability of the *Xenopus tropicalis* genome it was possible to identify many structural proteins that are specifically expressed in defined segments of the pronephros. This patterning was highly reminiscent to the one found in individual nephrons of the metanephric kidney. It provided a novel angle to understand how transcription factors actually pattern the kidney along its proximal distal axis as illustrated by the recent study on the Iroquois (Irx) gene family. Similarly, the synchronous development of the *Xenopus* pronephros has also provided many novel insights in how kidney progenitors differentiate into their mature counterparts (e.g. the blood-filtering podocyte) or how microRNAs regulate terminal differentiation of the renal epithelial cells.

In addition to understanding the processes that regulate normal kidney development, the pronephric kidney of *Xenopus* is also a valuable tool to study kidney diseases. Knockdown of genes mutated in human forms of Polycystic Kidney Disease result in a “PKD-like” phenotype in *Xenopus* that is used to better understand the molecular mechanisms leading to kidney cyst formation. In particular, the speed of analysis and the nearly unlimited availability of embryos provide an ideal *in vivo* test system to study aspect of Polycystic Kidney Disease that cannot be performed in mouse as easily.

Finally, the *Xenopus* kidney is a great system to study tissue engineering. *Xenopus* was the first organism, where it could be shown that the combined action of Retinoic Acid and Activin can convert primitive ectoderm into a functional kidney that can even be transplanted in nephrectomized *Xenopus* embryos. Ongoing work has extended these studies to several cell types in the kidney and has played an important role in identifying novel kidney-specific genes as well as ways to generate kidney epithelial cells *in vitro*.

Pancreas Development: The formation of the pancreas and the control of islet cell differentiation is one of the most coveted models of lineage specification. It is of high clinical importance due to its disturbance during diabetes. While mouse and chick have been the traditional models to study pancreas formation, the *Xenopus* pancreas has been developed as a viable alternative. Even though there are differences at later stages of pancreas development and its reorganization during metamorphosis, the early pancreas development in *Xenopus* is very similar to that of mice and humans. Many results are directly applicable to mammalian systems. In fact, one of the most important genes in pancreatic development, *Pdx1*, was initially discovered in *Xenopus*. The current research in *Xenopus* pancreas development follows similar avenues as outlined for the kidney. However, one particular interest is directed towards developing a transcriptional network of pancreas development in an effort to understand how early endodermal progenitors are specified first to a pancreatic fate, then to an endocrine fate and finally to a beta cell fate. For this approach *Xenopus* is uniquely suited since combinatorial knockdown studies using antisense morpholino oligomers allow analyses that are much more time-effective than compound mouse mutants.

Liver Development: Another organ system that has recently found more attention in *Xenopus* is the liver. The liver is an essential organ, yet the molecular basis of liver development is still poorly understood. Therefore, liver transplantation is often the only option for life threatening liver malfunctions. In an effort to develop alternative treatment options such as tissue replacement therapies from stem cells, the processes involved in hepatic tissue specification and the initial patterning of the foregut domain that will give rise to the liver are of high interest. Using the advantages of *Xenopus* it was recently shown that liver development relies on canonical and noncanonical Wnt signaling. Both pathways are necessary, but their activities have to be coordinated correctly to promote proper outgrowth of the liver bud.

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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 Diabetes and Digestive and Kidney Diseases (NIDDK) funded thirty-one grants for projects involving Xenopus. These grants total to $6,882,566.

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 or 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
3R01DK062348-06S1	CNS ACTION OF APPETITE SUPPRESSANT AMINOSTEROL	R01	AHIMA, REXFORD S	UNIVERSITY OF PENNSYLVANIA	$18,270
5R01DK081128-02	A GENETIC MODEL OF VESICOURETERAL REFLUX AND REFLUX NEPHROPATHY	R01	BATES, CARLTON MATTHEW	UNIVERSITY OF PITTSBURGH AT PITTSBURGH	$365,959
5K08DK068226-06	SODIUM CHLORIDE COTRANSPORTER REGULATION BY WNK KINASE	K08	CAI, HUI	EMORY UNIVERSITY	$128,250
5R01DK073380-05	EXOCYTOSIS AND COUPLED ENDOCYTOSIS IN NEUROENDOCRINE CELLS	R01	CASTLE, JOHN DAVID	UNIVERSITY OF VIRGINIA CHARLOTTESVILLE	$291,172
5R21DK080431-02	ISOLATION AND CHARACTERIZATION OF RAT KIDNEY ACTIVE UREA TRANSPORTER	R21	CHEN, GUANGPING	EMORY UNIVERSITY	$193,750
7K08DK074595-05	SIGNALING PATHWAYS AND EXPANSION OF HEMATOPOIETIC STEM CELLS IN ZEBRAFISH	K08	DE JONG, JILL L	UNIVERSITY OF CHICAGO	$129,060
1K01DK087816-01	THE ROLE OF VISCERAL FATTY ACIDS IN RELATION TO INSULIN RESISTANCE	K01	FOSTER, MICHELLE T	UNIVERSITY OF CINCINNATI	$123,798
3R01DK032753-25A1S1	MECHANISMS OF TRANSPORT IN PROXIMAL AND DISTAL TUBULES	R01	GUGGINO, WILLIAM B.	JOHNS HOPKINS UNIVERSITY	$16,400
5R01DK059913-09	NONGENOMIC STEROID SIGNALING IN OOCYTES	R01	HAMMES, STEPHEN R	UNIVERSITY OF ROCHESTER	$300,470
5K08DK067245-06	MECHANISMS OF REGULATION OF ANION EXCHANGER SLC26A6	K08	HASSAN, HATIM A	UNIVERSITY OF CHICAGO	$142,020

5R00DK077441-04	A NOVEL TOOL TO UNDERSTAND INSULIN PRODUCTION AND FAILURE IN PANCREATIC BETA-CELL	R00	HODISH, ISRAEL	UNIVERSITY OF MICHIGAN AT ANN ARBOR	$249,000
5R01DK069575-05	MOLECULAR BASIS OF ANTIDIABETOGENIC HORMONE ACTION	R01	HOLZ, GEORGE G	UPSTATE MEDICAL UNIVERSITY	$266,562
3R01DK064572-07S1	MECHANISMS FOR ALTERED GLUCOSE HOMEOSTASIS DURING HAART	R01	HRUZ, PAUL W	WASHINGTON UNIVERSITY	$38,195
5R01DK081472-02	BETA-CELL PROLIFERATION	R01	HUSSAIN, MEHBOOB A	JOHNS HOPKINS UNIVERSITY	$459,853
5R01DK073932-04	REGULATION OF SMAD2 SIGNALING BY SMP1 PHOSPHATASE	R01	LIN, XIA	BAYLOR COLLEGE OF MEDICINE	$250,890
3R01DK080047-02S2	DIVALENT METAL-ION TRANSPORTER DMT1 AND ITS ROLE IN INTESTINAL METAL-ION UPTAKE	R01	MACKENZIE, BRYAN	UNIVERSITY OF CINCINNATI	$35,846
1R56DK083785-01A1	SODIUM-CHLORIDE CO-TRANSPORTER REGULATION IN THE KIDNEY	R56	MCDONOUGH, ALICIA A.	UNIVERSITY OF SOUTHERN CALIFORNIA	$172,692
1R01DK081147-01A1	PEROXYNITRITE, PROTEIN NITRATION AND ADVANCED DIABETIC NEUROPATHY	R01	OBROSOVA, IRINA G	LSU PENNINGTON BIOMEDICAL RESEARCH CTR	$386,528
1R56DK085692-01	ELYS-MCM2 INTERACTIONS DURING INTESTINAL PROGENITOR CELL REPLICATION STRESS	R56	PACK, MICHAEL A	UNIVERSITY OF PENNSYLVANIA	$393,400
5R01DK056695-09	SGK REGULATION OF EPITHELIAL SODIUM TRANSPORT	R01	PEARCE, DAVID	UNIVERSITY OF CALIFORNIA SAN FRANCISCO	$298,375
3R01DK056695-08S1	SGK REGULATION OF EPITHELIAL SODIUM TRANSPORT	R01	PEARCE, DAVID	UNIVERSITY OF CALIFORNIA SAN FRANCISCO	$109,574
3K01DK080194-02S1	AN EPITHELIAL MODEL FOR V-TYPE	K01	PIERMARINI, PETER M	CORNELL UNIVERSITY	$54,000

	H+-ATPASE-DRIVEN ACID-BASE TRANSPORT			ITHACA	
4R00DK081610-03	OLFACTORY PROTEINS IN THE KIDNEY AND REGULATION OF GLOMERULAR FILTRATION RATE	R00	PLUZNICK, JENNIFER L	JOHNS HOPKINS UNIVERSITY	$249,000
5R01DK077276-04	IN VIVO INTERACTIONS OF FGF-23, KLOTHO AND VITAMIN D	R01	RAZZAQUE, MOHAMMED S	HARVARD UNIVERSITY (MEDICAL SCHOOL)	$290,328
5R01DK081324-03	AN INTERGENERATIONAL CBPR INTERVENTION TO REDUCE APPALACHIAN HEALTH DISPARITIES	R01	SCHOENBERG, NANCY E	UNIVERSITY OF KENTUCKY	$616,081
5R01DK037274-25	ROLE OF PACS PROTEINS IN POLYCYSTIN-2 TRAFFICKING AND ADPKD	R01	THOMAS, GARY	OREGON HEALTH AND SCIENCE UNIVERSITY	$322,798
5R01DK082890-02	MAL2 REGULATION OF HEPATIC PROTEIN TRAFFICKING: MECHANISMS AND BINDING PARTNERS	R01	TUMA, PAMELA L	CATHOLIC UNIVERSITY OF AMERICA	$191,211
1R01DK087789-01	TOLL-LIKE RECEPTOR SIGNALING IN THE ESOPHAGEAL EPITHELIUM	R01	WANG, MEI-LUN	CHILDRENS HOSPITAL OF PHILADELPHIA	$411,250
5R01DK068258-05	PURINERGIC NEUROGENIC MUCOSAL SECRETION	R01	WOOD, JACKIE D.	OHIO STATE UNIVERSITY	$277,835
3R01DK080823-01A1S1	MAMMALIAN FOREGUT AND LIVER DEVELOPMENT	R01	ZORN, AARON M	CHILDREN'S HOSPITAL MED CTR (CINCINNATI)	$99,999
				Total	$6,882,566