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

Jaques Robert, PhD – University of Rochester Medical School

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.

Selected references:

1. Du Pasquier L, Schwager J, Flajnik MF. 1989. The immune system of *Xenopus*. Annu Rev Immunol 7:251-275.
2. Flajnik MF, Kasahara M. 2001. Comparative genomics of the MHC: glimpses into the evolution of the adaptive immune system. Immunity 15:351-362.
3. Fitzgerald KA, Palsson-McDermott EM, Bowie AG, Jefferies CA, Mansell AS, Brady G, Brint E, Dunne A, Gray P, Harte MT, McMurray D, Smith DE, Sims JE, Bird TA, O'Neill LA. 2001. Mal (MyD88-adapter-like) is required for Toll-like receptor-4 signal transduction. Nature 413:78-83.
4. Fukazawa T, Naora Y, Kunieda T, Kubo T. 2009.Suppression of the immune response potentiates tadpole tail regeneration during the refractory period. Development. 136:2323-2327.
5. Goyos A, Guselnikov S, Chida AS, Sniderhan LF, Maggirwar SB, Nedelkovska H, Robert J. 2007. Involvement of nonclassical MHC class Ib molecules in heat shock protein-mediated anti-tumor responses. Eur J Immunol. 37:1494-501.

6. Guselnikov SV, Ramanayake T, Erilova AY, Mechetina LV, Najakshin AM, Robert J, Taranin AV. 2008. The *Xenopus* FcR family demonstrates continually high diversification of paired receptors in vertebrate evolution. BMC Evol Biol. 8:148.
7. Marr S, Morales H, Bottaro A, Cooper M, Flajnik M, Robert J. 2007. Localization and differential expression of activation-induced cytidine deaminase in the amphibian *Xenopus* upon antigen stimulation and during early development. J Immunol. 179:6783-6789.
8. Morales, H.D. and Robert, J. (2007). *In vivo* characterization of primary and secondary anti-ranavirus CD8 T cell responses in *Xenopus laevis*. J. Virol. 81:2240-2248.
9. Ny A, Koch M, Schneider M, Neven E, Tong RT, Maity S, Fischer C, Plaisance S, Lambrechts D, Héligon C, Terclavers S, Ciesiolka M, Kälin R, Man WY, Senn I, Wyns S, Lupu F, Brändli A, Vleminckx K, Collen D, Dewerchin M, Conway EM, Moons L, Jain RK, Carmeliet P. 2005. A genetic *Xenopus laevis* tadpole model to study lymphangiogenesis. Nat Med. 11:998-1004.
10. Ny A, Koch M, Vandevelde W, Schneider M, Fischer C, Diez-Juan A, Neven E, Geudens I, Maity S, Moons L, Plaisance S, Lambrechts D, Carmeliet P, Dewerchin M. (2008). Role of VEGF-D and VEGFR-3 in developmental lymphangiogenesis, a chemicogenetic study in *Xenopus* tadpoles. Blood. 112:1740-1749.
11. Ohta Y, Goetz W, Hossain MZ, Nonaka M, Flajnik MF. 2006. Ancestral organization of the MHC revealed in the amphibian *Xenopus*. J Immunol 176:3674-3685.
12. Ohta Y, Flajnik M. 2006. IgD, like IgM, is a primordial immunoglobulin class perpetuated in most jawed vertebrates. Proc Natl Acad Sci U S A 103:10723-10728.
13. Robert J, Cohen N. 1998. Evolution of immune surveillance and tumor immunity: studies in *Xenopus*. Immunol Rev 166: 231-243.
14. Robert, J. and Ohta, Y. 2009. Comparative and developmental study of the immune system in *Xenopus*. Dev. Dyn. 238:1249–1270.
15. Rosenblum EB, Poorten T, Settles M, Murdoch G, Robert **J**, Maddox N, and Eisen MB. (2009). Genome wide transcriptional response of *Silurana (Xenopus) tropicalis* to infection with the deadly Chytrid fungus.
16. Slack, J.M., G. Lin, and Y. Chen, The *Xenopus* tadpole: a new model for regeneration research. Cell Mol Life Sci, 2008. 65(1): p. 54-63.
17. Silva AB, Aw D, Palmer DB. 2006. Evolutionary conservation of neuropeptide expression in the thymus of different species. Immunology. 118(1):131-40.
18. Smith SJ, Kotecha S, Towers N, Latinkic BV, Mohun TJ. 2002. XPOX2-peroxidase expression and the XLURP-1 promoter reveal the site of embryonic myeloid cell development in *Xenopus*. Mech Dev 117:173-186
19. Suzuki M, Takamura Y, Maéno M, Tochinai S, Iyaguchi D, Tanaka I, Nishihira J, Ishibashi T. 2004 *Xenopus laevis* macrophage migration inhibitory factor is essential for axis formation and neural development. J Biol Chem. 279:21406-21414.
20. VanCompernolle SE, Taylor RJ, Oswald-Richter K, Jiang J, Youree BE, Bowie JH, Tyler MJ, Conlon JM, Wade D, Aiken C, Dermody TS, KewalRamani VN, Rollins-Smith LA, Unutmaz D. 2005. Antimicrobial peptides from amphibian skin potently inhibit human immunodeficiency virus infection and transfer of virus from dendritic cells to T cells. J Virol. 79:11598-11606.
21. Zarrin AA, Alt FW, Chaudhuri J, Stokes N, Kaushal D, Du Pasquier L, Tian M. 2004. An evolutionarily conserved target motif for immunoglobulin class-switch recombination. Nat Immunol.5:1275-1281.
22. Zasloff, M. 2002. Antimicrobial peptides of multicellular organisms, Nature 415:389-395.

Xenopus Grants funding by the NIAID

According to NIH RePORTER Search Tool, in the fiscal year of 2009, the National Institute of Allergy and Infectious Diseases (NIAID) **funded 10 grants** for projects involving *Xenopus*. These grants total **$2,408,946**. 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 NIAID

Project Number	Activity	Project Title	Principal Investigator	Organization	Total
5R01AI072068-02	R01	NOVEL REGULATORS OF T CELL MRNA DECAY	BOHJANEN, PAUL R	UNIVERSITY OF MINNESOTA TWIN CITIES	$332,384
5K22AI073888-02	K22	ORIGINS OF SPECIALIZED MUCOSAL LYMPHOCYTE SUBSETS AND IMMUNOGLOBULIN ISOTYPES	CRISCITIELLO, MICHAEL FREDERICK	TEXAS AGRILIFE RESEARCH	$108,000
3R01AI027877-20S1	R01	ONTOGENY AND PHYLOGENY OF THE MHC	FLAJNIK, MARTIN F.	UNIVERSITY OF MARYLAND BALTIMORE	$77,273
5R01AI046454-10	R01	ACTIN PEDESTAL FORMATION BY EHEC O157:H7	LEONG, JOHN M	UNIV OF MASSACHUSETTS MED SCH WORCESTER	$546,416
5R01AI057695-05	R01	SNPS IN HANDLING OF SMALL POX ANTIVIRALS AND OTHER DRUGS	NIGAM, SANJAY K	UNIVERSITY OF CALIFORNIA SAN DIEGO	$329,612
3R01AI057695-05S2	R01	SNPS IN HANDLING OF SMALL POX ANTIVIRALS AND OTHER DRUGS	NIGAM, SANJAY K	UNIVERSITY OF CALIFORNIA SAN DIEGO	$120,488
2R24AI059830-06	R24	A XENOPUS LAEVIS RESEARCH RESOURCE FOR IMMUNOBIOLOGY	ROBERT, JACQUES	UNIVERSITY OF ROCHESTER	$269,354
3R24AI059830-06S1	R24	A XENOPUS LAEVIS RESEARCH RESOURCE FOR IMMUNOBIOLOGY	ROBERT, JACQUES	UNIVERSITY OF ROCHESTER	$113,194
2R01AI026765-19A2	R01	FUNCTIONS OF 5' NCRS OF PICORNAVIRUS AND CELLULAR MRNAS	SEMLER, BERT L.	UNIVERSITY OF CALIFORNIA IRVINE	$369,666
1R01AI080754-01A1	R01	THE PLASMODIUM 2TM AND PHIST PROTEIN FAMILIES	TEMPLETON, THOMAS J	WEILL MEDICAL COLLEGE OF CORNELL UNIV	$413,951
				Total	$2,680,338