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

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Xenopus has been a classic model system for eye and vision research due to the ease of embryological analysis and manipulation. For example, fundamental insights into retino-tectal connectivity (Sperry), lens induction (Grainger) and retinal cell determination (Harris) have come from work in *Xenopus*. More recently, with the development of modern molecular methodology *Xenopus* has consolidated its role as a unique and vital model for investigating development, physiology and disease of the vertebrate visual system.

Eye Development and Regeneration:

Xenopus is ideal for the study of eye development since histogenesis in the *Xenopus* eye is rapid, with all retinal cell types specified between 1 and 3 days of development. In addition, the eye can be reproducibly targeted by microinjection of blastomeres at early cleavage stages or by in vivo lipofection or electroporation at optic vesicle stages. This allows selective manipulation of gene expression in the eye, with subsequent analysis of effects on optic vesicle patterning and retinal cell fate. This powerful approach has uncovered multiple genes and pathways governing retinal cell fate determination. Important advances range from understanding the importance of basic helix-loop-helix transcription factors in vertebrate retinal cell fate decisions (Kanekar et al., 1997) to the first demonstration that vertebrate homebox proteins act to effect a cellular clock that times the generation of retinal cells (Decembrini et al., 2006). Important achievements in understanding the relevance of signaling pathways to retinal cell fate include the discovery of a novel role for Hedgehog signaling in the transition of stem cell to transient amplifying progenitors (Locker et al., 2006) and the elucidation of the multiple roles that Wnt signaling plays in both embryonic (Van Raay et al., 2005) and post-embryonic eye development (Denayer et al., 2008).

In addition, the development of rapid and efficient methods for generating transgenic animals (Kroll and Amaya, 1996) has led to identification and fine-mapping of multiple eye-specific promoters targeting various cell populations in the developing and mature *Xenopus* eye. For example, promoters for Rx, Pax6, Ath5, X-linked juvenile retinoschisis (RS1) gene and rod opsin have all been mapped in *Xenopus*. These are powerful tools for targeting transgenes to the developing eye and for investigating the mechanisms underlying eye-specific gene regulation.

In *Xenopus*, the eye continues to grow throughout the life of the animal, so there is a true retinal stem cell population present at the margins of the eye in the ciliary marginal zone that drives growth of the eye and can also replace lost or damaged retinal neurons – a feature that is not shared in higher vertebrates. In fact, the cocktail of retinal stem cell/progenitor genes that are sufficient to generate complete functional ectopic eyes from pluripotent ectoderm cells in *Xenopus* has been defined (Viczian et al., 2009). In addition, retinal tissue can be regenerated from animal cap embryonic stem cells (Lan et al, 2009), RPE (Vergara and Del Rio-Tsonis K, 2009) and the lens of the eye can be regenerated from neighboring tissues (reviewed in Beck et al., 2009). Thus, *Xenopus* represents an important model system for understanding retinal stem biology as well as regeneration of ocular tissues.

Retinal Cell Biology & Physiology:

Transgenic methods in *Xenopus* have proved to be a powerful tool for investigating the cell biology of photoreceptors in vivo, in particular for studying protein targeting to photoreceptor outer segments. For example, it was recently shown in *Xenopus* that ankyrin-G binding is

necessary and sufficient for targeting of the ®1 subunit of the cyclic nucleotide-gated channel to rod outer segments (Kizhatil et al., 2009). Another study showed that the outer segment serves as a default destination for the trafficking of membrane proteins in photoreceptors (Baker et al., 2008). The high cone/rod ratio of *Xenopus*, combined with its powerful transgenic methods has proved to be a useful system for investigating rod-cone interactions both in development and disease states (Hamm et al., 2009).

All levels of the *Xenopus* visual system are amenable to fruitful study, including formation of appropriate connections at central targets. Tremendous advances have also been made in our understanding of retinal axon guidance in *Xenopus*. Recent studies have revealed how local protein synthesis contributes to directional steering of retinal growth cones as they navigate to their target (Leung et al., 2006). In addition, it was recently found that maturation of retinotectal synapses in the developing *Xenopus laevis* optic tectum is regulated by activation of ephrin-B reverse signaling (Lim et al., 2008). Another study investigated the early development and plasticity of local excitatory circuits in the optic tectum of *Xenopus laevis* tadpole, revealing important insights into how the response properties of the tectal network are modulated and optimized (Pratt et al., 2008). Thus connectivity and circuit formation in the visual system have been amenable to fruitful analysis in *Xenopus*.

Circadian oscillator mechanisms have been extensively studied in *Xenopus laevis*. The retina contains the essential components of the clock, and can be selectively manipulated using retinal cell-type-specific promoters to allow molecular dissociation of the circadian clock (Hayasaka et al, 2005).

Modeling Human Disease in Xenopus:

Xenopus is also suitable for modeling certain human ocular disease. For example mutations causing autosomal dominant retinitis pigmentosa (RP) in humans induce rod photoreceptor degeneration in *Xenopus laevis* (Tam and Moritz, 2006). This has led to additional important insights, such as a molecular mechanism for light sensitivity in RP (Tam and Moritz, 2007). These approaches will ultimately open up new avenues for rapidly testing the effects of certain human mutations on gene function in vivo.

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Xenopus Grants funding by the NEI

According to NIH RePORTER Search Tool, in the fiscal year of 2009, the National Energy Institute (NEI) **funded 41 grants** for projects involving *Xenopus*. These grants total **$13,565,485**. 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 NEI

Project Number	Activity	Project Title	Principal Investigator	Organization	Total
5R01EY010843-16	R01	REGULATION OF RETINAL CGMP PHOSPHODIESTERASES	ARTEMYEV, NIKOLAI O	UNIVERSITY OF IOWA	$370,972
3R01EY010843-16S1	R01	REGULATION OF RETINAL CGMP PHOSPHODIESTERASES	ARTEMYEV, NIKOLAI O	UNIVERSITY OF IOWA	$54,082
2R01EY012682-10	R01	MOLECULAR MECHANISM OF PHOTORECEPTOR G PROTEIN SIGNALING	ARTEMYEV, NIKOLAI O	UNIVERSITY OF IOWA	$367,000
7R01EY011261-14	R01	ACTIVITY DEPENDENT CONTROL OF VISUAL SYSTEM DEVELOPMENT	CLINE, HOLLIS T	SCRIPPS RESEARCH INSTITUTE	$509,927
3R01EY011261-14S1	R01	ACTIVITY DEPENDENT CONTROL OF VISUAL SYSTEM DEVELOPMENT	CLINE, HOLLIS T	SCRIPPS RESEARCH INSTITUTE	$185,209
5R01EY011912-12	R01	TROPHIC INTERACTIONS DURING VISUAL SYSTEM DEVELOPMENT	COHEN-CORY, SUSANA	UNIVERSITY OF CALIFORNIA IRVINE	$375,105
2R01EY015788-06	R01	MECHANISMS OF VISUAL MAP DEVELOPMENT IN THE SUPERIOR COLLICULUS	CRAIR, MICHAEL	YALE UNIVERSITY	$413,750
3R01EY015788-06S1	R01	MECHANISMS OF VISUAL MAP DEVELOPMENT IN THE SUPERIOR COLLICULUS	CRAIR, MICHAEL	YALE UNIVERSITY	$299,240
5F32EY018066-04	F32	PRESYNAPTIC REGULATION OF DENDRITIC STRUCTURAL DYNAMICS	DEMAS, JAMES ANTHONY	SCRIPPS RESEARCH INSTITUTE	$53,354
5R01EY013246-08	R01	MOLECULAR SCAFFOLDING FOR PHOTORECEPTOR OUTER SEGMENT STRUCTURE AND RENEWAL.	GOLDBERG, ANDREW FX	OAKLAND UNIVERSITY	$360,138

3R01EY013246-07S1	R01	MOLECULAR SCAFFOLDING FOR PHOTORECEPTOR OUTER SEGMENT STRUCTURE AND RENEWAL.	GOLDBERG, ANDREW FX	OAKLAND UNIVERSITY	$21,161
5R01EY013849-07	R01	CATARACTOGENESIS, CONNEXIN MUTANTS AND GENETIC MODIFIERS	GONG, XIAOHUA	UNIVERSITY OF CALIFORNIA BERKELEY	$380,000
3R01EY013849-07S1	R01	CATARACTOGENESIS, CONNEXIN MUTANTS AND GENETIC MODIFIERS	GONG, XIAOHUA	UNIVERSITY OF CALIFORNIA BERKELEY	$310,000
5R01EY009412-15	R01	INTERPHOTORECEPTOR RETINOID BINDING PROTEIN: STRUCTURE AND FUNCTION	GHOSH, DEBASHIS ;GONZALEZ-FERNANDEZ, FEDERICO ;	STATE UNIVERSITY OF NEW YORK AT BUFFALO	$394,396
5R01EY017400-04	R01	GENETIC CONTROL OF EARLY RETINAL DEVELOPMENT	GRAINGER, ROBERT M	UNIVERSITY OF VIRGINIA CHARLOTTESVILLE	$366,433
1R01EY018000-01A2	R01	GENOMIC SURVEY OF CIS-REGULATORY ELEMENT FUNCTION BY HIGH-THROUGHPUT TRANSGENESIS	GRAINGER, ROBERT M	UNIVERSITY OF VIRGINIA CHARLOTTESVILLE	$355,746
2R01EY005661-24	R01	STRUCTURE AND FUNCTION OF LENS CHANNELS	HALL, JAMES EWBANK	UNIVERSITY OF CALIFORNIA IRVINE	$482,795
5R01EY009844-14	R01	MOLECULAR AND CELLULAR BASIS OF LENS DEVELOPMENT	HENRY, JONATHAN J	UNIVERSITY OF ILLINOIS URBANA-CHAMPAIGN	$283,483
5R01EY012085-12	R01	INTERCELLULAR COMMUNIATION IN THE EYE LENS	JIANG, JEAN X	UNIVERSITY OF TEXAS HLTH SCI CTR SAN ANT	$365,000
3R01EY012085-11S1	R01	INTERCELLULAR COMMUNIATION IN THE EYE LENS	JIANG, JEAN X	UNIVERSITY OF TEXAS HLTH SCI CTR SAN ANT	$302,637
5R01EY012975-08	R01	MOLECULAR MECHANISMS OF PHOTORECEPTOR FUNCTION	KNOX, BARRY E	UPSTATE MEDICAL UNIVERSITY	$314,000
5R01EY018168-03	R01	NOVEL MECHANISM OF INDUCTION OF EYE TISSUE: KATP CHANNEL MODULATION	LEVIN, MICHAEL	TUFTS UNIVERSITY MEDFORD	$338,153

5R01EY005477-25	R01	RETINAL GANGLION CELLS: ION CHANNELS & TRANSMITTERS	LIPTON, STUART A	BURNHAM INSTITUTE FOR MEDICAL RESEARCH	$463,653
1F31EY019843-01	F31	CHARACTERIZATION OF DIVERSE MELANOPSIN-EXPRESSING CELLS IN ZEBRAFISH	MATOSCRUZ, VANESSA	CARNEGIE INSTITUTION OF WASHINGTON, D.C.	$30,955
5R01EY016807-03	R01	CIRCADIAN PHOTOENTRAINMENT IN MAMMALS	PANDA, SATCHIDANANDA	SALK INSTITUTE FOR BIOLOGICAL STUDIES	$478,750
5R01EY006891-19	R01	MEMBRANE BIOSYNTHESIS IN NORMAL AND DYSTROPHIC RETINA	PAPERMASTER, DAVID S	UNIVERSITY OF CONNECTICUT SCH OF MED/DNT	$462,014
5R00EY018085-04	R00	TOWARDS A STRUCTURAL AND TEMPORAL UNDERSTANDING OF PHOTOTRANSDUCTION	PARK, PAUL S	CASE WESTERN RESERVE UNIVERSITY	$249,000
3R00EY018085-04S1	R00	TOWARDS A STRUCTURAL AND TEMPORAL UNDERSTANDING OF PHOTOTRANSDUCTION	PARK, PAUL S	CASE WESTERN RESERVE UNIVERSITY	$80,484
5R01EY016094-04	R01	DEVELOPMENT OF NANOSCALE NEUROMODULATING PLATFORMS	PEPPERBERG, DAVID R	UNIVERSITY OF ILLINOIS AT CHICAGO	$1,248,803
3R01EY016094-04S1	R01	DEVELOPMENT OF NANOSCALE NEUROMODULATING PLATFORMS	PEPPERBERG, DAVID R	UNIVERSITY OF ILLINOIS AT CHICAGO	$530,341
5R01EY017809-10	R01	RETINAL NEURONS AFFERENT TO THE CIRCADIAN SYSTEM	PICKARD, GARY EDWARD	UNIVERSITY OF NEBRASKA LINCOLN	$372,652
2R01EY014979-05A2	R01	DEVELOPMENT AND PLASTICITY OF A RETINOTECTAL SYSTEM	POO, MU-MING	UNIVERSITY OF CALIFORNIA BERKELEY	$333,031
5F32EY018981-02	F32	CNG CHANNEL GATING MOVEMENTS MONITORED VIA FLUORESCENE QUENCHING	PULJUNG, MICHAEL C	UNIVERSITY OF WASHINGTON	$53,354
2R01EY011105-14	R01	CHEMICAL ARCHITECTURE OF RETINAL CIRCUITS	VARDI, NOGA	UNIVERSITY OF PENNSYLVANIA	$590,453

1R21EY019758-01	R21	THE ROLE OF PHOSPHORYLATION IN PHOTORECEPTOR CELL BIOLOGY	WEISS, ELLEN RUTH	UNIVERSITY OF NORTH CAROLINA CHAPEL HILL	$185,000
5R01EY011900-12	R01	RGS PROTEIN FUNCTION IN MAMMALIAN RETINA	WENSEL, THEODORE G	BAYLOR COLLEGE OF MEDICINE	$364,125
5T32EY007001-34	T32	RESEARCH TRAINING IN VISUAL SCIENCES	WU, SAMUEL M.	BAYLOR COLLEGE OF MEDICINE	$193,315
5R01EY006837-22	R01	PHOTOTRANSDUCTION AND SIGNALING IN PHOTORECEPTORS	YAU, KING-WAI	JOHNS HOPKINS UNIVERSITY	$410,000
5R01EY018141-03	R01	ENZYMATIC AND MOTOR PROPERTIES OF MYOSIN III	YENGO, CHRISTOPHER M	PENNSYLVANIA STATE UNIV HERSHEY MED CTR	$257,926
3R21EY018111-02S1	R21	DEVELOPMENT OF A MODEL SYSTEM FOR PRESYNAPTIC STUDY	ZENISEK, DAVID PAUL	YALE UNIVERSITY	$84,298
5R01EY017964-03	R01	GENETIC REGULATORY NETWORK CONTROLLING VERTEBRATE EYE FORMATION	ZUBER, MICHAEL E	UPSTATE MEDICAL UNIVERSITY	$274,750
				Total:	$13,565,485