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

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Craniofacial abnormalities are among the most prevalent birth defects, occurring in 1/700 live births, and present a tremendous medical and social burden. Furthermore, oral and dental health issues affect a majority of the population. Much current understanding of human craniofacial development comes from patient studies and will be immensely facilitated by studies in selected animal models, including *Xenopus*.

Overall, it appears that vertebrate craniofacial development is well conserved. Patterning of facial structures requires complex interactions between different tissue types, from the initial specification of the germ layers through morphogenesis of the facial prominences to the integration of the skeletal elements, muscles, nerves and other tissues. These processes begin very early in gestation and continue throughout life. A number of craniofacial abnormalities, including cleft palate, frontonasal dysplasia and DiGeorge syndrome, can be traced to abnormal development of a migratory, pluripotent population of cells called the neural crest. Therefore, defining the etiology of these pathologies requires an understanding of the mechanisms of neural crest formation, migration and plasticity.

Methodology useful to analysis of craniofacial development and abnormalities

Xenopus is one of the most accessible vertebrate model systems for analysis of craniofacial development. In particular, developing craniofacial structures are more readily visible in *Xenopus* than in any other vertebrate model, primarily because *Xenopus* embryos develop externally to the mother, allowing analyses of the earliest stages, and facilitating live imaging at single cell resolution. Amongst vertebrate models developing externally, *Xenopus* is more useful for craniofacial analysis than the zebrafish system, as *Xenopus* embryos are larger and easier to dissect, and the developing facial region is more accessible to imaging than the equivalent region in fish. Explants and transplants have been routine for decades; this, combined with the large clutch size (hundreds vs dozens in zebrafish) allows easy reproducibility. The ease of gain- and loss-of-function experiments in *Xenopus* has led to discoveries fundamental to biology, including Nobel Prize winning work on the cell cycle (Medicine, 2001) and water channels (Chemistry, 2003). Furthermore, experimental analyses have explored topics as varied as transcriptional control, chromatin accessibility, RNA processing, protein translation, pharmacology and synaptic plasticity. As more human mutations are uncovered, new genes, with unclear functions, will be implicated in craniofacial development.

Xenopus embryos are one of the simplest and most economical models in which to study gene function in an intact animal. Mutant alleles can be readily expressed *in vivo*; the large clutch size then allows reproducible, statistically significant phenotypic and biochemical readouts. The recent development of forward and reverse genetics in *Xenopus tropicalis* will result in new insights into craniofacial development. Several ongoing mutagenesis screens (Yale University, USA; Sanger Center, UK; National Institute of Medical Research, UK) have already produced multiple carriers of craniofacial mutations. In complementary studies, a TILLING (targeting induced local lesions in genomes) strategy is being used to identify mutations in known genes. These banks of mutations can then be used in combination with well-established embryological and molecular approaches. The availability of chemical libraries also makes *Xenopus* an extremely attractive system for studying craniofacial anomalies. *Xenopus* embryos are aquatic and can be arrayed in multi-well dishes, allowing automation of chemical screens. Libraries of small molecules can simply be added to the media, and tadpoles can then be assayed for morphological changes visually. Finally, the increased availability of

transgenic *Xenopus* lines will contribute to analysis of genes and processes associated with craniofacial abnormalities, especially when combined with chemical screening.

Recent data from *Xenopus* studies pertaining to craniofacial development

Work using *Xenopus laevis* embryos has contributed tremendously to knowledge of early steps in craniofacial development. Most notable are studies on the early induction of the neural crest. Functional studies have defined the molecules and signal transduction processes important for cell-cell and tissue-tissue interactions during neural crest development (including BMPs, FGFs and Wnts). Mechanisms underlying migration of neural crest cells can also be studied in *Xenopus*: recent work includes the molecular basis of contact inhibition and directional migration of neural crest cells. These kinds of studies are important for understanding craniofacial defects resulting from abnormal neural crest development.

Another use of *Xenopus* has been to analyze development of the primary mouth (or stomodeum) - the first opening between the pharynx and the outside of the embryo. Multiple craniofacial defects are likely to be caused by defects in this region. Recent work in *Xenopus* defined a set of steps leading to primary mouth opening, where the earliest step is local dissolution of the basement membrane. Further analysis showed that local expression of the Wnt inhibitors, Frb1 and Crescent, is necessary for basement membrane breakdown in this region. Basement membrane remodeling is essential for normal development of most organs, and pivotal in metastasis, and these unprecedented findings have proven *Xenopus* a pioneer organism, yet again.

With regard to chemical screening, a recent study identified multiple compounds affecting cell migration. By combining chemical structure predictions and enzymatic assays using *Xenopus* lysates, the authors identified an activity that inhibited matrix metalloproteinases (MMPs). They then performed loss-of-function analyses; by knocking down several MMPs, confirming the drug target. Finally, they were able to extrapolate their findings to a human melanoma cell line, illustrating the ease of using *Xenopus* as a whole animal assay system for drug discovery.

Due to the unusual demands of metamorphosis, *Xenopus* also provides a fascinating example of developmental plasticity. Craniofacial alterations during metamorphosis are similar to changes that occur in regeneration, remodeling and wound healing. Thus, studying these transitions may be extremely informative. Recent studies have begun applying molecular tools to these questions.

Selected references

Neural crest induction:

Monsoro-Burq AH, Wang E, Harland R. Msx1 and Pax3 cooperate to mediate FGF8 and WNT signals during *Xenopus* neural crest induction. *Dev Cell.* 2005. Feb;8(2):167-78.

Neural crest migration:

Carmona-Fontaine C, Matthews HK, Kuriyama S, Moreno M, Dunn GA, Parsons M, Stern CD, Mayor R. Contact inhibition of locomotion in vivo controls neural crest directional migration. *Nature*. 2008 Dec 18;456(7224):957-61. Epub 2008 Dec 10.

Primary mouth formation:

Dickinson A. and Sive H. The Wnt antagonists, Frzb-1 and Crescent regulate primary mouth formation by modulating the basement membrane. *Development* 2009 Apr;136(7):1071-81. Epub 2009 Feb 18.

Genetic screens:

Grammer TC, Khokha MK, Lane MA, Lam K, Harland RM. Identification of mutants in inbred *Xenopus tropicalis*. *Mech Dev.* 2005 Mar;122(3):263-72.

Goda T, Abu-Daya A, Carruthers S, Clark MD, Stemple DL, Zimmerman LB. Genetic screens for mutations affecting development of *Xenopus tropicalis*. *PLoS Genet.* 2006 Jun;2(6):e91.

Chemical Screens:

Tomlinson ML, Guan P, Morris RJ, Fidock MD, Rejzek M, Garcia-Morales C, Field RA, Wheeler GN. A chemical genomic approach identifies matrix metalloproteinases as playing an essential and specific role in *Xenopus* melanophore migration. *Chem Biol.* 2009 Jan 30;16(1):93-104.

Skull development:

Gross JB, Hanken J. Cranial neural crest contributes to the bony skull vault in adult *Xenopus laevis*: insights from cell labeling studies. *J Exp Zoolog B Mol Dev Evol.* 2005 Mar 15;304(2):169-76.

Slater BJ, Liu KJ, Kwan MD, Quarto N, Longaker MT. Cranial osteogenesis and suture morphology in *Xenopus laevis*: a unique model system for studying craniofacial development. *PLoS One.* 2009;4(1):e3914.

Xenopus Grants funding by the NIDCR

According to NIH RePORTER Search Tool, in the fiscal year of 2009, the National Institute of Dental and Craniofacial Research (NIDCR) **funded 8 grants** for projects involving *Xenopus*. These grants total **$1,748,299**. 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 NIDCR

Project Number	Activity	Project Title	Principal Investigator	Organization	Total
5K22DE016633-06	K22	DUCTIN'S ROLE IN CRANIOFACIAL PATTERNING AND DEVELOPMENT	ADAMS, DANY SPENCER	TUFTS UNIVERSITY MEDFORD	$135,000
5R01DE016289-04	R01	MECHANISM OF XENOPUS CRANIAL NEURAL CREST CELL MIGRATION	ALFANDARI, DOMINIQUE R	UNIVERSITY OF MASSACHUSETTS AMHERST	$328,003
5R01DE017911-03	R01	EVOLUTIONARY ORIGIN OF VERTEBRATE NEURAL CREST GENE NETWORKS	BRONNER-FRASER, MARIANNE	CALIFORNIA INSTITUTE OF TECHNOLOGY	$388,529
3R01DE017911-02S1	R01	EVOLUTIONARY ORIGIN OF VERTEBRATE NEURAL CREST GENE NETWORKS	BRONNER-FRASER, MARIANNE	CALIFORNIA INSTITUTE OF TECHNOLOGY	$29,853
3R01DE017911-03S1	R01	EVOLUTIONARY ORIGIN OF VERTEBRATE NEURAL CREST GENE NETWORKS	BRONNER-FRASER, MARIANNE	CALIFORNIA INSTITUTE OF TECHNOLOGY	$91,125
5R01DE013849-09	R01	GENETIC STUDIES OF ODDD	JABS, ETHYLIN WANG	MOUNT SINAI SCHOOL OF MEDICINE OF NYU	$351,407
5R01DE018824-02	R01	CHARACTERIZATION AND CLONING OF X. TROPICALIS CRANIOFACIAL MUTANTS	KHOKHA, MUSTAFA K	YALE UNIVERSITY	$324,380
3R01DE018824-02S1	R01	CHARACTERIZATION AND CLONING OF X. TROPICALIS CRANIOFACIAL MUTANTS	KHOKHA, MUSTAFA K	YALE UNIVERSITY	$100,002
				Total	$1,748,299