Impact of the *Xenopus* system on the mission of the NIDCR
Karen J. Liu, PhD. - King’s College London
Hazel Sive, PhD - Whitehead Institute and MIT

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 \textit{Xenopus} lines will contribute to analysis of genes and processes associated with craniofacial abnormalities, especially when combined with chemical screening.

Recent data from \textit{Xenopus} studies pertaining to craniofacial development

Work using \textit{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 \textit{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 \textit{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 \textit{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 \textit{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 \textit{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 \textit{Xenopus} as a whole animal assay system for drug discovery.

Due to the unusual demands of metamorphosis, \textit{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:**

**Neural crest migration:**
Primary mouth formation:

Genetic screens:

Chemical Screens:

Skull development:

*Xenopus* grants funded by the Institute:
According to NIH RePORTER Search Tool, in the fiscal year of 2011, the National Institute of Dental and Craniofacial Research (NIDCR) funded two grants for projects involving *Xenopus*. These grants total to $1,000,854.

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

*Xenopus* egg extracts are used for *in vitro* biochemical studies:
- **BRCA1** – *Cell.* 2006. 127, 539-552

*Xenopus* oocytes are used to study gene expression and channel activity:

**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 our understanding of vertebrate genome organization.

*Xenopus* egg extracts reveal fundamental aspects of cell division.

*Xenopus* reveals new aspects of eu karyotic nuclear structure and function.

*Xenopus* embryos are used for studies of Wnt and TGF-β signal transduction.

*Xenopus* embryos are used for studying mucociliary epithelia.

*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.

*Xenopus* embryos link telomerase to Wnt signaling.

*Xenopus* are used for small molecule screens to develop therapeutics.
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

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