

Moving Into *Xenopus tropicalis*

Abstracts

Saturday June 12, 1999

Session I: Building A Genetic System For The 21st Century

9:00-9:30 a.m.	Introduction to <i>Xenopus tropicalis</i>	Rob Grainger Univ. of Virginia
9:30-10:00 a.m.	Genome Manipulations and Mutants in Amphibians	Bob Tompkins Tulane Univ.
10:00-10:30 a.m.	Genetic Screens for Vertebrate Developmental Genes	Mary Mullins Univ. of Penn.
10:45-11:15 a.m.	Contrasts Between <i>X. tropicalis</i> and <i>X. laevis</i> Major Histocompatibility Complex: the Influence of Polyploidy on Gene Silencing	Martin Flajnik Univ. of Maryland
11:15-11:45 a.m.	Embryology of <i>X. tropicalis</i>	Ray Keller Univ. of Virginia
11:45-12:00 noon	Laboratory Husbandry of <i>X. tropicalis</i>	Nick Hirsch Univ. of Virginia
12:00-1:00 p.m.	Lunch and poster session	

Session II: New Approaches To Manipulating Amphibian Genomes Mechanical Engineering Building Room 205

1:00-1:30 p.m.	Towards Amphibian Developmental Genetics	Len Zon Harvard Univ.
1:30-2:00 p.m.	Transgenesis in <i>Xenopus</i> : Present and Future	Enrique Amaya Wellcome/CRC
2:00-2:30 p.m.	'Gene Trap' Insertional Mutagenesis of <i>X. tropicalis</i>	Odile Bronchain Wellcome/CRC
2:45-3:15 p.m.	Stable Lines and Strategies for Sideways Genetics in <i>X. tropicalis</i>	Lyle Zimmerman Univ. of Virginia
3:15-3:45 p.m.	Transgenic Analysis of Opsin Promoter and Function	Barry Knox SUNY Syracuse
3:45-4:15 p.m.	Saturation of Zebrafish Notochord Mutations: Can new loci be identified in <i>Xenopus tropicalis</i> ?	Derek Stemple NIMR, Mill Hill

Session I: Building A Genetic System For The 21st Century

Rob Grainger

University of Virginia

Introduction To *Xenopus tropicalis*

1. Historical issues concerning *X. laevis* and *X.tropicalis*. The turn to *X. laevis* for developmental studies in the 1960's resulted from the use of this animal for human pregnancy testing: females lay eggs at any time of year in response to human chorionic gonadotropin, found in high levels in the urine of pregnant women. The ability to obtain embryos year-round from a hardy lab animal was a strong motivating factor for its use by embryologists. In these early years, *X. laevis*'s genetics were also extremely important: the 1-nucleolus mutant enabled the identification of ribosomal genes, a major discovery in the field of gene regulation. While *X. tropicalis* has been in many European and American labs at various times in these early days, it was not used extensively. Although there were discussions about the use of *X. tropicalis* for genetic studies in the 1980's, the first major step was not taken until about 10 years ago when Marc Kirschner undertook a major effort to bring animals to the U.S.

2. Why *X. tropicalis*? While *X. laevis* has been extremely useful in defining embryological concepts, there is a strong need to complement this work with genetic studies. The time is right for moving toward a system like *X. tropicalis*, more suited to genetic approaches, and stimulated by the powerful transgenic technology developed by Amaya and Kroll. The transgenic lines that can now be made will transform the field of embryology and add powerful new strategies for evaluating mutagenic screens. A recent meeting at NIH on Non-Mammalian Systems provides a strong boost to this effort by defining goals for NIH that include a serious commitment to genetic and genomic resources for *X. tropicalis*.

3. Meeting goals. There are three primary aims of this meeting. Speakers will present recent data and current and developing technologies that will be helpful to attendees interested in using *X. tropicalis* in their laboratories. In the caucus session in particular, but throughout the meeting, we want to hear the ideas of participants as we begin to develop a community of *X. tropicalis* researchers. Finally, we hope that participants will leave the meeting having a better idea of how they can contribute to this new endeavor.

4. How to think like a geneticist. Many of us are not trained as geneticists but we hope that talks by several speakers on the zebrafish genetics effort will provide informative background as we begin to design our own strategies for mutagenesis. We are not, however, starting without a history. There are a number of strategies that have been developed for *X. laevis* that will make be extremely helpful in genetic studies of *X. tropicalis*, and the large number of interesting developmental mutants already identified in *X. laevis* provide a strong impetus for us to proceed with great enthusiasm. We are honored that Bob Tompkins, who has made many of these seminal contributions in studies of *X. laevis* is able to join us to start off our meeting.

Bob Tompkins

Tulane University

Genome Manipulations and Mutants in Amphibians

Genetic manipulation of amphibians has a history of several decades, in *Xenopus* as well as other taxa. Manipulations discussed will include mutagenesis, gynogenesis, generation of homozygous diploid frogs, and strategies for maintenance of isogenic clonal populations of *Xenopus*. Many naturally-occurring mutations can be identified in wild-caught *X. laevis*; gynogenesis by suppression of second meiosis (polar body formation) is a useful and efficient shortcut to examining recessive phenotypes. Gene-centromere map distances can be obtained via half-tetrad analysis. Gynogenesis by suppression of first mitotic cleavage is a less efficient process, but can be used to generate isogenic (homozygous diploid) embryos. Viable homozygous diploid animals can be used to establish robust clonal isogenic populations, which have been bred for over seven generations.

Mary Mullins

Univ. of Pennsylvania

Genetic Screens For Vertebrate Developmental Genes

This presentation will cover forward genetics for amphibian embryologists; mechanics of two- and three-generation screens; the pluses and minuses of haploid and gynogenetic screens; different mutagenesis strategies; practical considerations of conducting large-scale mutant hunts; and overview of mutant phenotypes.

Martin Flajnik

Univ. of Maryland

Contrasts Between *X. tropicalis* and *X. laevis* Major Histocompatibility Complex: the Influence of Polyploidy on Gene Silencing

The major histocompatibility complex (MHC) allows the adaptive immune system to discriminate between 'self' and 'non-self.' The MHC encodes molecules called class I and class II that bind to peptides derived from pathogens, followed by 'presentation' of these foreign peptides to antigen-specific lymphocytes. In addition to these 'presentation molecules' other proteins that cleave the proteins derived from pathogens (e.g. the proteasome components Imp2 and Imp7) and transporters that relay peptides from the cytosol to the endoplasmic reticulum (ER) lumen (TAP1 and TAP2) are also encoded in the MHC. Thus, the MHC is a rare example in eukaryotes in which structurally unrelated genes, all involved in a coordinated biological function, are genetically linked. In addition, other immune elements not directly involved in antigen presentation are also encoded by the MHC (e.g. complement components, tumor necrosis factors, heat shock proteins), but it is not known whether there is any significance to these synteny.

Xenopus is an important intermediate in the study of maintenance of linkage of MHC genes over evolutionary time. We have found, primarily in segregation analyses, that composition of the MHC

is quite stable when comparing mammals and amphibians. However, in *X. laevis* several of the mammalian MHC genes were found not to be linked to the functional MHC. Studies in *X. tropicalis* families have suggested that in some cases such 'non-linkage' in *X. laevis* is a derived characteristic, owing to differential silencing after polyploidization. In one case, however, a similar result in *X. tropicalis* and *X. laevis* suggests an ancestral characteristic of MHC-like genes. Finally, studies of MHC in higher order polyploids like *X. ruwenzoriensis* suggests a model for differential silencing of MHC genes as has seemingly occurred in higher order teleosts.

Our results suggest that, whenever possible, *X. tropicalis* should be used as the 'touchstone' to determine whether genes in a representative amphibian are similar to those same genes in of other taxa. Furthermore, comparisons of *X. tropicalis* to the polyploid species of *Xenopus* should allow insight into general mechanisms of gene silencing/reorganization.

References

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- Nonaka, M et al. (1997) Evolution of the proteasome subunits Imp2 and Imp7: cDNA cloning and linkage analysis with MHC in lower vertebrates. *Journal of Immunology* 159:734.
- Salter-Cid, L et al. (1996) Ring 3 is linked to the *Xenopus* major histocompatibility complex. *Immunogenetics* 44:397.
- Flajnik, M.F. et al. 1993. A novel type of class I organization in vertebrates: A large family of non-MHC-linked class I genes is expressed at the RNA level in the amphibian *Xenopus*. *EMBO J* 12:4385.

Ray Keller and Dave Shook

Univ. of Virginia

Embryology of *X. tropicalis*

- A. Manipulating *tropicalis*: personal impressions
 - effects of size
 - tissue texture under the eyebrow hair
 - explants and grafts

- B. Similarities and differences between *tropicalis* and *laevis*
 - variation and consistency of process, size, proportions
 - head mesoderm behavior
 - developmental rate

- C. transgenic lines for studying morphogenesis

- D. On a background of parameters that vary among amphibians:
 - marginal zone position and yolk
 - fate maps
 - mesoderm formation: ingression and relamination

Nick Hirsch

Univ. of Virginia

Laboratory husbandry of *X. tropicalis*

A clear understanding of the best method to grow and maintain *X. tropicalis* in the laboratory is a necessary prerequisite to its widespread use as a genetic model system. For the purposes of animal husbandry, the *tropicalis* life cycle can be divided into three phases: early embryo, tadpole and froglet. We have conducted a number of experiments to determine the optimal environmental conditions for each of these phases which will result in a minimum time to sexual maturity (MTSM) for each animal. These conditions include: low salt media for tadpoles, low population density, clean water and frequent feeding of a well-balanced diet which includes live food. We are currently investigating different types of housing, including the use of a recirculating water system to minimize the amount of hands-on maintenance required. However, further optimization of these conditions, and the study of alternative husbandry techniques, is particularly important now - at the early stages of research on *X. tropicalis* - and is critical to future experimental success.

Session II: New Approaches To Manipulating Amphibian Genomes

Len Zon

Harvard University

Towards Amphibian Developmental Genetics: Lessons From Zebrafish and the *Xenopus tropicalis* Genome Project

Our expectation for the *Xenopus tropicalis* system is that gene traps and mutants would be available to study in several years. Perhaps one of the most useful aspects of the *Xenopus tropicalis* will be to utilize embryonic explants from mutant embryos to look at inductive systems. For future approaches, it is critical to invest in the genomics of the system. This includes the genetic map, radiation hybrid panels, making use of chromosomal synteny between the vertebrates, as well as a functional genomics approach.

As a first step, it is critical to establish the genome size as well as to evaluate the rate of meiotic recombination in *tropicalis*. A polymorphic strain must be found to allow genetic mapping. Genetic markers placed on the *tropicalis* map would include microsatellite markers, AFLPs, and cloned genes using single strand conformational polymorphism. In the zebrafish, this has been done and over 3,000 markers are placed on the genetic map at this time. Radiation hybrid panels are a key resource for the *tropicalis* community. This is the easiest way to map in a vertebrate species. A *tropicalis* cell line will be fused to a hamster cell line. This will allow co-localization between mutant map positions and candidate clones. A grant should be sought to fund this *X. tropicalis* genome project. In the zebrafish, there is a current proposal to increase the marker density to a 1cM map, to sequence 100,000 EST sequences, to provide radiation hybrid panel mapping for 5,000-10,000 genes, and to obtain deletion mutants throughout the entire genome. Additional resources are being sought for the zebrafish for the years 2000 and 2001.

A functional genomics approach has recently been utilized by Bernard Thisse in which random in situ hybridization studies in zebrafish have been used to study genes expressed in particular tissues. This has provided ample markers as well as candidates for mutants. Such a similar approach would be

helpful for the *tropicalis* community. In the end, candidate cloning is a very powerful approach, but positional cloning will often yield new genes of interest. The positional cloning is done using a polymorphic strain. In *Xenopus tropicalis* it would be critical to use amplified fragment length polymorphism studies to establish a chromosomal walk. BAC, PAC, and YAC libraries will be necessary for chromosomal walking through a locus. In the zebrafish, we have developed a hybridization technique that will allow the isolation of the gene. Very often chromosomal synteny is extremely useful for the positional cloning project, and ultimately, the gene that is rescued can be tested functionally by injecting the wild-type copy into mutant embryo.

In summary, the *Xenopus tropicalis* will be a premier system to study development and disease. The key to success resides in activism. It is very important to make it easy for people to use the *Xenopus tropicalis* system and to obtain adequate funds for a genome initiative. It is critical for the community to collaborate and to ask for lots of advice from zebrafish, human, mouse, drosophila, worm and yeast genome efforts. Most of all, there should be an effort to highlight the advantages of the *tropicalis* system such as explant assays on mutant tissues.

Enrique Amaya

Wellcome/CRC

Transgenesis in *Xenopus*: Past, Present and Future

1. History and motivation for development of amphibian transgenesis; introduction to Restriction Enzyme Mediated Integration (REMI);
2. Promoter studies using cross-species approaches.
3. Mis-expression studies, leading to discussion of binary expression systems (GAL4) in *laevis* and *tropicalis*.
4. Introduction to REMI insertional mutagenesis.

Odile Bronchain

Wellcome/CRC

'Gene Trap' Insertional Mutagenesis of *X. tropicalis* and *X. laevis*

1. Gene trapping in *Xenopus* embryos via REMI transgenesis.
2. Cloning trapped genes using 5' RACE PCR
3. Gene trapping in *Xenopus tropicalis*
4. Advantages and disadvantages of the REMI technique for insertional mutagenesis.

5. Alternative ways to mediate random insertions: Transposition in *Xenopus*

Lyle Zimmerman

Univ. of Virginia

Stable Transgenic Lines and Strategies for Sideways Genetics in *X. tropicalis*

1. Experimental embryology and transgenesis in *Xenopus* The development of a transgenic procedure by Kroll and Amaya (REMI) is one of the most important technical innovations in the last decade of amphibian embryological research. *X. laevis*' long generation time has limited experimental design to analysis of the primary products of transgenesis, which are not always produced with high efficiency. The shorter lifecycle of *Xenopus tropicalis* makes multigeneration experimental designs feasible. By utilizing *in vivo* detection of gene expression in transgenic reporter strains, novel insights can be obtained in established embryological model systems.

2. Using stable transgenics to study the timing of differentiation Studies of mesoderm differentiation suggest that an intrinsic 'clock' regulates the timing of mesodermal marker gene expression following specification. We have examined this issue in another model system for early embryonic induction and differentiation, lens ectoderm, and obtained a different result. Our data, using transplantation and explantation of specified lens ectoderm from the progeny of γ -crystallin-GFP transgenic animals, demonstrate the importance of extrinsic environmental cues: onset of reporter gene expression is delayed by transplantation to younger hosts or explantation. Assaying specific gene expression in families of embryos bearing identical GFP reporter transgenes greatly simplifies this analysis, since onset of expression can be compared *in vivo* between controls and experimentals.

3. Moving beyond GFP Using tissue-specific promoters to drive experimental transgenes theoretically permits a greater degree of temporal and spatial control of expression. Binary systems based on promoter-transactivator (e.g. GAL4) and responder-effector (e.g. UAS-experimental gene) components may provide a way to reliably mis-express a variety of gene products. Generating separate transactivator and effector stable transgenic lines avoids lethal misexpression effects, and different transactivator and effector lines can be crossed to create new experimental combinations. A greater degree of temporal control may be obtained by the use of inducible transactivator fusions, e.g. GAL4-steroid receptor constructs. The *tropicalis* genetics community should prioritize a stock center for distribution of reporter, transactivator, and effector strains.

4. 'Sideways' genetics While amphibian forward genetics (chemical or insertional random mutagenesis) is still in its infancy, and reverse genetics (gene targeting) is over a distant horizon, overexpression of dominant interfering alleles and other specific antagonists via mRNA injection has been well-utilized in *Xenopus*. The combination of this 'sideways' genetic approach with transgenesis, especially binary inducible strategies, may make it feasible to block important gene functions at specific stages throughout later development.

5. Pilot chemical mutagenesis of *X. tropicalis* We are in the process of conducting a pilot *N*-nitroso-*N*-ethylurea (ENU) chemical mutagenesis on *X. tropicalis* spermatogonia, and are currently breeding candidate heterozygous progeny of mutagenized animals. While no mutants have yet been

isolated, we have obtained useful ENU toxicity data. We are screening F2 haploids and raising F2 families to sib-cross and screen F3 progeny. Collaborators are invited to help house, raise, and screen F2 families.

6. For Consideration: MultiReporter (MR) Strain Generation and Mutagenesis

Goal: to facilitate forward genetic screens by amplifying a wild-type "search image" of gene expression upon which to detect variants. This is a candidate for a multi-lab collaboration.

Strategy: Develop isogenic strains homozygous for several robust fluorescent reporter transgenes. Ideally, three or four transgenic strains, each bearing three or four tissue-specific transgenes, would be parallel substrates for chemical mutagenesis and screening.

Special considerations: For this strategy to be most useful, transgenes should be homozygous. Use of isogenic 'homozygous diploid' strains, if robust, simplifies genomic analysis. Gynogenesis by suppression of first cleavage helps attain these goals, but requires sex-reversal strategies for maintaining breeding clones. This strategy is designed with collaborations in mind.

<u>generation</u>	<u>purpose</u>	<u>useful byproducts</u>
F0	cleavage suppression isogenic founders need sex-reversed ZZ females reproduce by gynogenesis	uncover recessive mutations?
F1	expand clone (sex-reverse half); males for sperm nuclei, females for eggs reproduce by REMI, introduce transgenes	genomic DNA for large-insert library
F2	multireporter founders, hemizygous for transgenes; reproduce by gynogenesis (suppress 1st cleavage)	promoter mapping; single-transgene reporter lines
F3	homozygous MR founders reproduce by gynogenesis, sex-reverse half	
F4	expanded clones; mutagenize males, cross to sib females sex reverse half	Standard 2- or 3- generation screen from here on down
F5	heterozygous for mutations; sex-reverse half	
F6	screen haploids, gynogenetic diploids; 'outcross' to same isogenic MR background and raise families (sex-reverse half)	phenotype?
F7	inbreed families, screen	phenotype?

Barry Knox

SUNY Syracuse

Transgenic Analysis of Opsin Promoter and Function

The photoreceptors of the vertebrate retina express a large number of proteins that are involved in the process of light transduction. These genes appear to be coordinately regulated at the level of transcription, with rod and cone-specific isoforms. The mechanisms that regulate gene expression in a rod/cone-specific fashion have been difficult to address using traditional approaches and remain unknown. We report here that transgenic frog embryos can be used to study cell-specific expression in the retina.

We have used a 5.5 kb 5' upstream fragment from the *Xenopus* principal rod opsin gene controlling a reporter gene, green fluorescent protein (GFP), to produce numerous independent transgenic *Xenopus*. High-level, rod-specific expression of green fluorescent protein was also observed in *Xenopus* carrying transgenes with -508/+41 promoter fragment, similar to transgenic animals produced with the 5.5 kb fragment. We find that this construct drives expression only in the retina and pineal, which is apparent by 4 days post-nuclear injection. Moreover, the expression from these construct is limited to principal rod cells. These results illustrate the advantages of transgenic *Xenopus* for retinal promoter analysis and for the expression of proteins with dominant phenotypes.

To understand the mechanisms that control this cell-specific transcription, the *Xenopus* rhodopsin 5' regulatory region have been further characterized using a novel in vivo promoter assay based on transient transfection of *Xenopus* embryos and transgenic tadpoles. Activity from the mutants indicated that the interaction of a negative regulatory region (-233 to -202) with at least two downstream positive regulatory regions (-170 to -145, -145 to -127) function in concert with a core promoter (-127 to +41) to direct transcription. These data demonstrate the *Xenopus* opsin promoter is more compact than mammalian homologues, yet shares several conserved features. Targeted disruption of functional and conserved elements were generated in the context of an otherwise unaltered *Xenopus* rhodopsin proximal promoter (-503/+41). Targeted disruption of XOP4 (-233/-203) and Ret 1(-136/-122) regions caused an increase in luciferase expression in transfected heads. Selective disruption of other regions such as XOP3 (-170/-146), NRE (-120/-109), BAT-1b(-98/-91) and Ret 4 (-52/-38) did not affect the levels of reporter expression in transfected heads. The selective changes in individual elements did not cause expression of reporter in non retinal cell types. The targeted disruption of specific regions do not alter the rod cell specific expression in transgenic *Xenopus*. The basal region of the rhodopsin promoter (-36/+41) was functionally interchangeable with heterologous basal promoters, indicating that critical elements needed for correct cell specific expression reside in sequences upstream of the basal region. Hybrid promoter experiments with multiple copies of the conserved Ret 1 in the context of the endogenous basal promoter demonstrate the requirement of additional regulatory sequences for rhodopsin promoter function. Rod cell specific expression of rhodopsin appears to be regulated by proximal promoter sequences by multiple conserved regulatory elements with redundant functions.

The nearly spherical lens of the *Xenopus* eye can be used as an optical element for imaging the retina. We were able to employ the lens to obtain high-resolution images with a low-power stereo microscope using the farfield radiation pattern, that has long been used to image *Drosophila* photoreceptors. In our transgenic *Xenopus*, the fluorescence from the rod cells produces a situation analogous to antidromic illumination of *Drosophila* photoreceptors. In the dissecting microscope image, individual discrete fluorescent disks in the focal plane reveal the location of rod cells in the observed patch of retina. Using a confocal microscope, we were able to image rod cells in their

entirety with subcellular resolution. To do this, we observed anesthetized transgenic tadpoles through the center of the lens using a long working distance objective and reconstructed the image of individual cells. The synaptic layer (S), inner (IS) and outer (OS) segments can easily be distinguished. The outer segment has considerably less GFP than the other parts of the rod cell, because the soluble GFP partitions to the cytoplasm, which is reduced in the outer segment compared to the inner segment or soma. Our results suggest that high-resolution protein trafficking studies will be possible with GFP-tagged proteins, such as rhodopsin. The entire image collection took 12 minutes after which the tadpoles easily recover from anesthesia.

Derek Stemple, Richard Gibbons, Masa Tada, and Jim Smith NIMR
Saturation of Zebrafish Notochord Mutations: Can new loci be identified in
***Xenopus tropicalis*?**

Using results of two large-scale screens for mutations affecting embryogenesis in zebrafish we have estimated the degree of saturation for each screen and for the notochord and brain phenotypic groups from combined results of both screens. The estimation method uses a Poisson distribution to model the frequency of mutations. Implicit in the estimation method is the assumption that all loci are equally mutable. While the assumption is obviously incorrect the estimation does, however, provide an upper-bound to the degree of saturation. We estimate the Boston screen to be at most 58% saturated and the Tübingen screen to be at most 79% saturated. The brain and notochord phenotypic groups provide a strikingly different picture. Combining data from both the Tübingen and Boston screens, we estimate the brain phenotypic group to be only 42% saturated. By contrast the estimation method predicts that the notochord phenotypic group is completely saturated.

Recent evidence concerning the genome complexity of zebrafish indicates that teleosts have undergone a genome-wide duplication relative to tetrapods. Additionally, certain genes, such as *sonic hedgehog*, that when mutated in mice produce a severe notochord mutant phenotype, produce a relatively mild mutant phenotype in zebrafish. Taken together this evidence and the low number of notochord loci that were identified in the two screens suggest additional screens in another vertebrate organism will be necessary to identify the majority of loci important for notochord formation.

There are several features of the amphibian *Xenopus tropicalis* that make it an attractive organism for future screens. First, *X. tropicalis* has been maintained for many generations under laboratory conditions. They are able to produce large clutches of embryos without significant seasonal variation. The life cycle of *X. tropicalis* is similar to that of zebrafish (~5 months). Finally, there is good evidence that an insertional mutagenesis approach will be effective for the genetic and molecular identification of loci important for development.

Before undertaking a large-scale screen it will be important to establish the genome complexity of *X. tropicalis*. To this end we have initiated several studies to determine the number of genes in several characteristic gene families. Our initial genomic southern blot studies probing with the *X. laevis Bix1* gene indicate that there are half the number of *Bix1* related genes in *X. tropicalis* as there are in *X. laevis*. We are currently extending these studies to include other genes such as the *Engrailed* and *Hox* gene families.

Finally, in an effort to generate resources for the genetic analysis of *X. tropicalis* development we have begun a collaborative project with L. Zimmerman, R. Grainger and colleagues to identify polymorphic strains of *X. tropicalis*. Using the amplified fragment length polymorphism (AFLP) method for DNA fingerprinting we have found that one strain of laboratory *X. tropicalis* called Swiss

(originally collected from the Ivory Coast), is significantly polymorphic to another laboratory strain called Nigerian. These two strains of frog can now be used to generate a genetic linkage map for *X. tropicalis*.

Peter Vize

Univ. of Texas

Database Resources For *X. tropicalis*

X. tropicalis may provide more than an opportunity to perform genetics in amphibians: it may also serve as a point of common interest that could bring together the disparate frog community. A vertebrate genetic screen will require resources beyond those to which we are accustomed in each isolating and characterizing small numbers of genes. Community wide effort would ensure that the *tropicalis* system has the opportunity to reach its full potential.

If genetic screens are to be a community project there will need to be a community resource at which shared data is accessible to all. The success of such a site will depend on community participation, and participation will require individual rewards. We, and other databases, have learned that getting data from researchers is the most difficult aspect of database construction. This isn't that surprising, and one of the main problems has been nicely summed up by the designers of the zebrafish database:

"Perhaps the most confounding factor in designing shared data resources for scientific communities is the tension between information sharing and secrecy. Because research geneticists are all essentially working on the same problem (i.e., connecting genetic code with biological characteristics) and because experiments are extremely time- and effort-intensive, sharing of information as soon as it becomes available is highly desirable. At the same time, the success of individual scientists hinges on publishing unique scientific results, motivating researchers to keep information to themselves. A viable solution to this dilemma must maintain proper accreditation of work while making new results available soon after they are discovered." (Doerry et al., <http://zfish.uoregon.edu/ZDB/PAPERS/Web97/web97-final.htm>).

The other problem is effort vs. reward, as it has in the past taken quite a bit of trouble to have your data published on the web. After going through the tortuous process of manuscript submittal, trashing, resubmission etc. and finally getting data published many labs simply don't bother to resubmit the data to the *Xenopus* database. Few people reference the database so there is little reward for having gone to the trouble of submission.

The fish community has put a large amount of effort into designing a database that uses "Participatory Design", meaning that the users helped programmers design a system that worked for them. If it's easy to submit data people are more likely to actually do so. On the fish site labs have a password that gives them access to their own data pages, and individual pages are managed by individual labs.

The other component we need to address is effort (small) vs. reward (large). If the web site serves as the only publication source for critical information web publishing will serve a similar function to the scientific literature. If we as a community adopt the referencing of a central database in matters relating to transgenes, mutants and mapping data people will want to be the first to supply information. This will result in more information being available and the whole process snowballing.

We are presently discussing the redesign of the *Xenopus* database. I would like to propose that we make use of the valuable lessons learned by the fish community and contribute to the creation of a centralized, participant designed, easy to use, and easy to understand *Xenopus* database.

Please visit the fish site at <http://zfish.uoregon.edu/ZFIN/> and let us know what you think. Direct feedback to Peter Vize, Randy Moon, Enrique Amaya or Sally Moody, the temporary members of the web group. Finally if you would like to participate in the creation of management of *Xenopus* web resources, please let us know.