Published in final edited form as:

Methods Mol Biol. 2012 ; 917: 3–15. doi:10.1007/978-1-61779-992-1_1.

***Xenopus tropicalis* as a Model Organism for Genetics and Genomics: Past, Present and Future**

Robert M. Grainger

Department of Biology, Gilmer Hall, University of Virginia, Charlottesville VA 22904, USA

Robert M. Grainger: rmg9p@virginia.edu

Summary

Xenopus tropicalis was introduced as a model system for genetic, and then genomic research, in the early 1990's, complementing work on the widely used model organism *Xenopus laevis*. Its shorter generation time and diploid genome has facilitated a number of experimental approaches. It has permitted multigenerational experiments (e.g. preparation of transgenic lines and generation of mutant lines) that have added powerful approaches for research by the *Xenopus* community. As a diploid animal, its simpler genome was sequenced before *Xenopus laevis*, and has provided a highly valuable resource indispensable for all *Xenopus* researchers. As more sophisticated transgenic technologies for manipulating gene expression are developed, and mutations, particularly null mutations, are identified in widely studied genes involved in critical cellular and developmental processes, researchers will increasingly turn to *X. tropicalis* for definitive analysis of complex genetic pathways. This chapter describes the historical and conceptual development of *X. tropicalis* as a genetic and genomic model system for higher vertebrate development.

Keywords

Xenopus tropicalis; experimental model system; genetics; natural mutations; mutagenesis screen; genomics; genome sequencing

1. Introduction

Amphibian species have held a prominent position in embryological research for well over a century because their large, abundant, externally developing embryos proved ideal for studying embryological phenomena. Although they share these features with fish embryos, amphibian embryos are more resilient to many surgical manipulations, and, as tetrapods, are more closely related to mammals. However, in other model systems, including fish, the powerful impact of genetic approaches for understanding complex developmental phenomena in the late 20th century left a gap in the analytical approaches available to most developmental biologists using amphibians. While loss of gene function could be elicited by antisense reagents and dominant negative constructs, it became increasingly important to be able to develop genetics in an amphibian system to parse developmental phenomena in a way only feasible by studying mutations. While the amphibian model *Xenopus laevis* has had a very distinguished history in elucidating developmental phenomena [1], its long life cycle and allotetraploid genome made conventional genetic analyses more challenging. Although Zebrafish and other teleosts have been very successful genetic model systems, teleosts too have undergone a genome duplication that complicates both genetic studies and analysis of genome organization. Thus the introduction in the 1990's of *Xenopus tropicalis*, a species closely related to *Xenopus laevis*, but which is diploid and requires a much shorter time to reach sexual maturity, offered the welcome prospect of a model that could provide the power of genetics to the *Xenopus* research community; especially so since it has a

genome organization much like mammals. The development of the *X. tropicalis* system from that time to the present is described here.

2. Early Amphibian Genetic Research

It is important at the outset to highlight that important genetic research has been done in amphibian species before the emergence of *X. tropicalis*. In *X. laevis*, the *anucleolate* mutation leads to the loss of the nucleolus, and was instrumental for identifying the nucleolus as the site of the ribosomal RNA gene locus [2]. In addition, screens of outbred *X. laevis* and animals generated from nuclear transplantation have revealed a number of interesting developmental mutations [3-5]. The Mexican axolotl *Ambystoma mexicanum* has also been a valuable genetic model for which some molecular genetic resources have been generated, for example large collections of ESTs, enabling valuable genomic work [6], despite being an unlikely candidate organism for genome sequencing due to its extremely large genome, like many other amphibians.

3. The search for an amphibian genetic model system

Those of us who were thinking about a genetically tractable alternative to *X. laevis* in the early 1990's were also discussing potential model systems. For example, in my own laboratory I was exploring *Rana pipiens* as a system. It is possible to generate androgenetic haploid embryos in this species by mechanical removal of the egg nucleus from the very large eggs of this species [7] and from these embryos cell lines had been made and studied extensively for somatic cell genetics [8]. Such cells could be a source of material for mutagenesis, for example by homologous recombination, and the nuclei from such cells used to reprogram development by transplantation back into the egg. The possibility of transplanting mutant haploid nuclei back into the egg had been discussed in a 1970 paper by Freed and Metzger-Freed [8], based on the previously published nuclear transplantation work of Briggs and King in *Rana* [9], work that was followed by the extensive studies on this subject by Gurdon and colleagues using *Xenopus laevis* [10]. The *Rana* system has the disadvantages, however, of not being so easily used to generate embryos year-round as *Xenopus*, nor does it have a large core of researchers working on cell and developmental problems. *Hymenochirus*, a genus of small rapidly developing pipid frogs, relatively close relatives of *X. tropicalis* [11], was also being discussed but is distant enough from *Xenopus* and studied by few researchers so that they were unlikely to be adopted by the *Xenopus* community.

4. Introduction of *Xenopus tropicalis* as a genetic model system

Xenopus tropicalis was in some ways a very obvious choice for a new amphibian genetic model. This West African species is an evolutionarily close relative of the South African species *X. laevis*, having diverged approximately 50 Mya [12]. This close relationship to *X. laevis* would mean that it was likely to share developmental features, and have conserved gene sequences and functions and thus be attractive to the large group of researchers working on *X. laevis* [13]. Indeed many features are shared by the two systems [14, 15], including gene expression patterns. However, there is considerable remodeling of the post-tetraploid transcriptome in *X. laevis* [16] that provides an advantage for *X. tropicalis* in comparative gene function and regulation studies, where the diploid gene structure and function is more likely to be conserved with mammalian species. *X. tropicalis* is, as well, the only known diploid species in the *Xenopus* genus.

Before the 1990's research on *X. tropicalis* was primarily restricted to studies on the evolutionary and cytogenetic relationships among the various *Xenopus* species (e.g.[11, 17]). While *X. tropicalis* were used and housed in several laboratories, and held in the Swiss

repository for *Xenopus* lines at the University of Geneva (D. Runniger, personal communication), prior to 1990, they were not widely available nor used for genetic screens. In 1990 a small group of *X. tropicalis* originally collected from the Ivory Coast were brought from the Geneva Center to the U.S. by Marc Kirschner (University of California, San Francisco), and shortly thereafter, a much larger population was imported from Nigeria by the Kirschner lab (M. Kirschner and E. Amaya, personal communication). It was difficult at that time to import animals from Africa and, after exhausting conventional contacts with academic institutions in Nigeria, Marc Kirschner serendipitously tracked down and enlisted the aid of a sympathetic Nigerian tribal leader, formerly an academic in the U.S., to import Nigerian *X. tropicalis*. The animals of both Ivory Coast and Nigerian strains were raised under the supervision of Enrique Amaya in facilities at UC San Francisco and UC Berkeley.

These two strains from California were transported to my laboratory shortly thereafter and we initiated inbreeding of the two *X. tropicalis* lines with the intent of developing lines more suitable for stable genetic screens, and for possibly mapping of genes by crossing of divergent lines. At the same time, we also imported other wild caught populations of *X. tropicalis* from Nigeria that we thought might provide useful genetic diversity in future experiments. The animals from the Kirschner/Amaya collection and my laboratory have been distributed around the world, and other lines were acquired as well, for example one line imported by the Asashima laboratory in Japan that is somewhat diverged from the Nigerian/Ivory Coast group [18].

5. Early genetic screens for natural mutations

The wild caught animals in our laboratory were the first to be used in a genetic screen [19], presuming that these outbred animals would be a natural source of genetic variation that would reveal mutations. This allowed us to begin to do screening immediately while we were still perfecting methods for inducing mutagenesis for later screens. The first screen took advantage of being able to generate gynogenetically-derived offspring in *Xenopus* (and other amphibians) to greatly facilitate rapidly identifying mutations [20]. This procedure entails generating haploid embryos carrying only maternal genetic material by fertilizing with U.V. irradiated sperm, which activate embryonic development but do not contribute genetically to the embryo. Such haploid embryos would develop abnormally, but they can be diploidized by preventing release of the second polar body by high pressure, or more simply by a cold shock protocol developed in my laboratory (modified after ref. [21]; see Subsection 3.2. in Chapter 5). Such embryos develop as diploid animals but if eggs carry a developmental mutation, segregation of maternal chromatid pairs reveals recessive mutations in a fraction of the gynogenetic embryos. This method saves a great deal of time, and animal housing space, when compared to the classical three generation screening method of interbreeding offspring of potential heterozygote carrier animals used in many systems for identifying recessive mutations.

In this initial screen a wide range of phenotypes was observed in mutations generated from several outbred lines. These include very broad defects, e.g. affecting left-right asymmetry, as well as alterations in specific tissues, e.g. in the inner ear. Fig.1 shows one of these mutations, *little pitcher*, in which the inner ear fails to develop any internal structure, including the otoconia characteristic of the early amphibian inner ear; embryos swim in circles due to failure to develop appropriate inner ear structures needed for balance. It is also valuable to note that, although *Xenopus* embryos are not transparent at pre-tadpole stages (as are zebrafish embryos), they become transparent as organogenesis proceeds. As can be seen in Fig. 1, embryos are indeed transparent at five days, when organ systems are well defined and subtle morphological defects can be recognized.

A second mutation identified during a gynogenetic screen of wild caught animals, *cataract*, shows an opacity in the lens area (Fig. 1) resulting from a greatly reduced lens size. Whether this defect is due to a lesion in a gene acting within the lens, or due to failure in inductive signals from adjacent tissues, can be determined because one can so readily make tissue chimeras in *Xenopus* (e.g. combining mutant lens tissue and wildtype inducers, or vice versa), as was done, for example, in other work on lens determination [22].

Inbreeding of *X. tropicalis* lines was being performed by Tim Grammer and Mustafa Khokha in the Harland laboratory (U.C. Berkeley) in parallel to our inbreeding efforts, and they described a group of mutations revealed during the inbreeding process [23]. In addition this paper discusses husbandry methodology for raising *X. tropicalis*, which has gradually been refined in several laboratories so that it is now a routine process for researchers and discussed in detail in Chapters 2 and 3.

6. *X. tropicalis* mutagenesis screens

Mutagenesis using inbred lines as starting material has now been performed in several laboratories using the classic mutagen *N*-nitroso-*N*-ethylurea (ENU). In my group we have used spermatogonial mutagenesis (*see* Subsection 3.4.2. in Chapter 5), in which males are injected with ENU and F1 mutagenized animals are used to identify mutations by gynogenesis. Similar spermatogonial mutagenesis has been used in many model systems [24, 25]. In the Zimmerman lab, mature sperm were mutagenized (*see* Subsection 3.4.1. in Chapter 5), in a manner similar to what has been done in zebrafish [26], a procedure that results in very high mutagenesis rates, though first generation embryos generated in this manner are genetically mosaic since mutagenesis is DNA strand-specific when performed in this way and require sibling crosses in subsequent generations to generate conventional mutant lines. Using this protocol, the first large ENU screen was published (also using gynogenesis to identify mutant phenotypes), revealing 29 mutations in a wide diversity of organ systems [27]. One mutant in this group, *xenopus de milo* (*xdm*) is mentioned here (and illustrated in Fig. 1) because of its striking effect on forelimb formation. This mutation illustrates an obvious advantage of *Xenopus tropicalis* as a genetic system that can serve as a model for tetrapod-specific developmental features. In addition, limb regeneration in amphibians is one of the most carefully examined examples of regeneration in any organ system, and the genetic and transgenic approaches so well suited to *Xenopus tropicalis* should be great assets that have broad relevance to the current surge of interest in regenerative medicine [28].

Mapping and cloning of *X. tropicalis* mutants has been facilitated by genomic resources, described below, particularly the availability of a reliable genetic map based on simple sequence length polymorphisms (SSLPs) [29]. In addition, the initial steps of mapping genetic lesions in *X. tropicalis* is facilitated by gynogenetic screening, because the frequency of the appearance of a recessive mutation in a gynogenetically-derived embryo population depends on its distance from the centromere [30] (*also see* Subsection 3.7.4 in Chapter 5). Gynogenetic mapping has also been used to develop methods to quickly identify the chromosome on which a particular mutation lies [31].

These methods have now permitted for the first time the positional cloning of a number of mutant genes in the past two years. This includes mutations in cardiac myosin *myh6* (resulting in a no heartbeat phenotype)[32], in the secreted integrin ligand nephronectin (resulting in missing forelimbs) [33], and in *unc45b* (resulting in paralysis) [34]. Mutations identified in these screens appear to be commonly linked to human disease syndromes (e.g. [32]).

7. Development of the *X. tropicalis* research community and resources for genetic and genomic studies

Research on the genetic potential of *X. tropicalis* in the U.S. began in a few labs in the San Francisco area, as discussed above, in the early 1990's. While no formal meetings of the groups that studied this animal model occurred until several years later, there were informal gatherings, including the "First International *X. tropicalis* Meeting" in late 1992, an event that was held at one of the meetings of the Bay Area Research on Frogs (BARF) group that met during this period. Fig. 2 shows the participants of this meeting, which merits highlighting here because the meeting included many of those who have played a key role in the development of *X. tropicalis* as a model system, and, more generally, contributed in very important ways to the development of *Xenopus* more broadly as a major system for studies of cell and developmental biology.

While work on *X. tropicalis* began in earnest during the 1990's in several laboratories, with transgenic lines created in this more rapidly developing *Xenopus* species, and enhancer/gene trap experiments underway [13], there was still only a small group working on this species, and no centralized efforts to generate community-wide resources nor for disseminating information about this new system, or more broadly about new technologies for *Xenopus* in general. The establishment of the Cold Spring Harbor *Xenopus* Development Course by Hazel Sive and Richard Harland in 1994 was a productive step in that direction and resulted in the publication of a widely used manual of experimental technology for *Xenopus* [35].

An important turn of events occurred in 1999 when Harold Varmus, then Director of the U. S. National Institutes of Health (NIH), convened a meeting focused on Non-Mammalian Model Systems to establish priorities for centralized resources needed to develop these model organisms. Included in this group were representatives from the yeast, *C. elegans*, *Drosophila*, zebrafish and *Xenopus* communities. Janet Heasman and I convened the *Xenopus* group and we were told by the organizers of this meeting that a primary reason *Xenopus* was included in the meeting because of the prospect of the development of genetic tools in this system, tools which were already a mainstay in the research of the other systems represented at the meeting. After surveying the research community, the recommendations of the *Xenopus* breakout committee [36] included genomic resources for producing ESTs, full-length cDNA sequencing, and microarray development. Regarding *X. tropicalis*, support was recommended for establishing its viability as a genetic system by support of several genetic strategies, development of large-insert DNA libraries, development of a genetic map, and sequencing of its genome. Regarding centralized resource centers, support of a database and an animal stock center and training center was recommended. The meeting helped to stabilize NIH infrastructure and support for *Xenopus* resources, which have grown significantly since this time [36, 37]. Interestingly, and of great importance for the *Xenopus* research community, essentially all of the recommended resources from the 1999 meeting have now been realized.

Shortly following the 1999 meeting, the first large meeting of *Xenopus* researchers focusing on *X. tropicalis* research was held at the University of Virginia as a satellite meeting accompanying the Society for Developmental Biology meeting in Charlottesville. The meeting covered the history of genetics in *Xenopus*, strategies for performing genetic screens, transgenic strategies for studying gene regulation and for mutagenesis, review of new husbandry methods for raising *X. tropicalis* [38].

8. Sequencing of the *X. tropicalis* genome

A key milestone in *X. tropicalis* research (and for the *Xenopus* community at large) was the sequencing of the *X. tropicalis* genome, undertaken by the Department of Energy's Joint Genome Institute (JGI) in 2001. *X. tropicalis* was an obvious choice for an amphibian genome sequencing project at the time, because of its diploid genome, contrasting the allotetraploidy of the *X. laevis* genome. By this time, the inbreeding of *X. tropicalis* lines was far enough along in our laboratory that a suitable animal was available for this project, and complementary work to produce BAC libraries important for both genome assembly and as a resource for projects examining gene organization. In addition to providing the first several generations of the *X. tropicalis* genome, resulting in publication of the work in 2010 [12], the JGI sponsored other genomically based efforts including identification of a very large number of ESTs. This work has provided the primary platform for research in genetics and genomics in *Xenopus*. The genome sequencing project confirmed that indeed *X. tropicalis* has a diploid genome, and showed that its genome has a remarkable degree of synteny with mammalian genomes, often in stretches of a hundred genes or more, far greater than that seen between fish and mammal. The genome organization is discussed further in Chapter 4. While the efforts of *X. laevis* researchers to have the genome of this widely used animal met with frustration early on, in large part because of the perceived difficulty in sequencing and assembling its allotetraploid genome, the *X. tropicalis* project has been extraordinarily useful for all *Xenopus* researchers, and important as a foundation for the new *X. laevis* genome project, now finally underway.

9. The Future of Research utilizing *X. tropicalis*

X. tropicalis genetics is still a relatively new direction for the *Xenopus* community, though significant technological progress has been made, allowing forward genetic screens to be performed productively and identification of mutant genes by positional cloning to be accomplished effectively and relatively quickly. Of note is that many of the genes identified in screens in *X. tropicalis* bear on human diseases or syndromes (L. Zimmerman, pers. comm.). It is not clear whether there are the resources to undertake the large-scale screens performed in the zebrafish community in the 1990's. However, more focused screens, for example utilizing transgenic reporter lines to identify mutations in particular organ systems, offer a bright future for forward genetics even on a smaller scale. Novel insights from the *Xenopus* system are being made in many areas (proteomics, signal transduction, organogenesis, etc.) and provide unique areas where genetic analysis in the future will add important dimensions to what can be done in this, or any other system.

The challenges *Xenopus* researchers have had to face in setting up facilities suited to the genetic and transgenic manipulations that can be accomplished with *X. tropicalis* highlight the importance of a key community resource. The two large centers, The European *Xenopus* Resource Center in the U.K. and the National *Xenopus* Resource in the U.S., provide sites for housing mutant lines, generating transgenic lines, and in general fostering new genetic technologies that will be vital for the development of genetics in *Xenopus*. They will provide efficient mechanisms for disseminating both animals and technologies to the community at large that would be difficult or impossible to establish in individual laboratories.

Many researchers look toward *X. tropicalis* as a system that will foster technologies to identify mutations in genes already known to be of high interest for studies in cell and developmental biology. As discussed earlier, there are at present no methods as yet developed in *Xenopus* for targeted gene inactivation as in the mouse. However, projects are underway to identify genes by screening for mutations in populations of mutagenized animals by high throughput sequencing (a process often referred to as TILLING [39], see

also Subsection 3.6 in Chapter 5), a strategy now being pursued in a number of genetically tractable model organisms [40, 41]. Mutations have now been discovered in *X. tropicalis* by this methodology (R. Grainger, S. Carruthers, D. Stemple, unpublished), which promises to become even more efficient and less expensive, as high throughput sequencing methods continue to improve. Other methods for gene inactivation in *X. tropicalis*, e.g. mutations induced by zinc-finger nucleases ([42] *see also* Chapter 7) and studies using RNA interference [43, 44], are showing promise as well.

X. tropicalis will also continue to contribute important biological insights because of the efforts to improve the sequence of its genome, and the utility of this genomic information. For example, analysis of the structure and function of enhancers, many of which can be a megabase or possibly further from the genes they regulate, is greatly aided by the long-range assembly available in *X. tropicalis*. This allows rapid identification of highly conserved elements (by comparative genomics), which can then be studying using the powerful transgenic methods available in *Xenopus* ([45], *see also* Chapter 15). Similarly, analysis of genome-wide epigenetic changes is ideally suited to *X. tropicalis* ([46], *see also* Chapter 17). These genomic approaches in *X. tropicalis*, combined with analysis of mutations in key genes in this species, will lead to novel insights about key gene regulatory networks that regulate complex cellular and developmental events.

Acknowledgments

The author gratefully acknowledges contributions to developing the *X. tropicalis* system from lab members Lyle Zimmerman, Nicolas Hirsch, Selina Noramly, Jei Chae, Hui Wang, Hong Jin, Hajime Ogino, Takuya Nakayama, Marilyn Fisher, Margaret Fish and Matthew Etzell. Research on *X. tropicalis* was supported by grants to R.M.G. from the National Institutes of Health RR013221, EY019000 and EY017400. Grants to R.M.G. from NIH also support a National *Xenopus* Resource (RR025867) and National TILLING Resource (HD065713).

Bibliography

1. Harland RM, Grainger RM. *Xenopus* research: metamorphosed by genetics and genomics. Trends Genet. 2011
2. Brown DD, Gurdon JB. Absence of Ribosomal Rna Synthesis in the Anucleolate Mutant of *Xenopus laevis*. Proc Natl Acad Sci U S A. 1964; 51:139–46. [PubMed: 14106673]
3. Krotoski DM, Reinschmidt DC, Tompkins R. Developmental mutants isolated from wild-caught *Xenopus laevis* by gynogenesis and inbreeding. Journal of Experimental Zoology. 1985; 233(3): 443–9. [PubMed: 3973558]
4. Droin A. The developmental mutants of *Xenopus*. Int J Dev Biol. 1992; 36(4):455–64. [PubMed: 1295559]
5. Graf JD, Kobel HR. Genetics of *Xenopus laevis*. Methods Cell Biol. 1991; 36:19–34. [PubMed: 1811134]
6. Voss SR, Epperlein HH, Tanaka EM. *Ambystoma mexicanum*, the axolotl: a versatile amphibian model for regeneration, development, and evolution studies. Cold Spring Harb Protoc. 2009; 2009(8):pdb emo128. [PubMed: 20147230]
7. Porter KR. Androgenetic development of the egg of *Rana pipiens*. Biol Bull. 1939; 77:233–257.
8. Freed JJ, Mezger-Freed L. Stable haploid cultured cell lines from frog embryos. Proc Natl Acad Sci U S A. 1970; 65(2):337–44. [PubMed: 5263768]
9. Briggs R, King TJ. Transplantation of Living Nuclei From Blastula Cells into Enucleated Frogs' Eggs. Proc Natl Acad Sci U S A. 1952; 38(5):455–63. [PubMed: 16589125]
10. Gurdon JB, Byrne JA. The first half-century of nuclear transplantation. Proc Natl Acad Sci U S A. 2003; 100(14):8048–52. [PubMed: 12821779]
11. de Sa RO, Hillis DM. Phylogenetic relationships of the pipid frogs *Xenopus* and *Silurana*: an integration of ribosomal DNA and morphology. Mol Biol Evol. 1990; 7(4):365–76. [PubMed: 2385174]

12. Hellsten U, Khokha MK, Grammer TC, Harland RM, Richardson P, Rokhsar DS. Accelerated gene evolution and subfunctionalization in the pseudotetraploid frog *Xenopus laevis*. *BMC Biol.* 2007; 5:31. [PubMed: 17651506]
13. Amaya E, Offield MF, Grainger RM. Frog genetics: *Xenopus tropicalis* jumps into the future. *Trends in Genetics.* 1998; 14(7):253–5. [PubMed: 9676522]
14. Hirsch N, Zimmerman LB, Grainger RM. *Xenopus*, the next generation: *X. tropicalis* genetics and genomics. *Dev Dyn.* 2002; 225(4):422–33. [PubMed: 12454920]
15. Khokha MK, Chung C, Bustamante EL, Gaw LW, Trott KA, Yeh J, et al. Techniques and probes for the study of *Xenopus tropicalis* development. *Dev Dyn.* 2002; 225(4):499–510. [PubMed: 12454926]
16. Morin RD, Chang E, Petrescu A, Liao N, Griffith M, Chow W, et al. Sequencing and analysis of 10,967 full-length cDNA clones from *Xenopus laevis* and *Xenopus tropicalis* reveals post-tetraploidization transcriptome remodeling. *Genome Res.* 2006; 16(6):796–803. [PubMed: 16672307]
17. Tymowska J. Karyotype analysis of *Xenopus tropicalis* Gray, Pipidae. *Cytogenet Cell Genet.* 1973; 12(5):297–304. [PubMed: 4780767]
18. Kashiwagi K, Kashiwagi A, Kurabayashi A, Hanada H, Nakajima K, Okada M, et al. *Xenopus tropicalis*: an ideal experimental animal in amphibia. *Exp Anim.* 2010; 59(4):395–405. [PubMed: 20660986]
19. Noramly S, Zimmerman L, Cox A, Aloise R, Fisher M, Grainger RM. A gynogenetic screen to isolate naturally occurring recessive mutations in *Xenopus tropicalis*. *Mech Dev.* 2005; 122(3):273–87. [PubMed: 15763208]
20. Tompkins R. Triploid and gynogenetic diploid *Xenopus laevis*. *Journal of Experimental Zoology.* 1978; 203:251–56.
21. Kawahara H. Production of triploid and gynogenetic diploid *Xenopus* by cold treatment. *Develop Growth and Differ.* 1978; 20(3):227–236.
22. Henry JJ, Grainger RM. Early tissue interactions leading to embryonic lens formation in *Xenopus laevis*. *Developmental Biology.* 1990; 141:149–163. [PubMed: 2390999]
23. Grammer TC, Khokha MK, Lane MA, Lam K, Harland RM. Identification of mutants in inbred *Xenopus tropicalis*. *Mech Dev.* 2005; 122(3):263–72. [PubMed: 15763207]
24. Mullins MC, Hammerschmidt M, Haffter P, Nusslein-Volhard C. Large-scale mutagenesis in the zebrafish: in search of genes controlling development in a vertebrate. *Curr Biol.* 1994; 4(3):189–202. [PubMed: 7922324]
25. Justice MJ, Noveroske JK, Weber JS, Zheng B, Bradley A. Mouse ENU mutagenesis. *Hum Mol Genet.* 1999; 8(10):1955–63. [PubMed: 10469849]
26. Riley BB, Grunwald DJ. Efficient induction of point mutations allowing recovery of specific locus mutations in zebrafish. *Proc Natl Acad Sci U S A.* 1995; 92(13):5997–6001. [PubMed: 7597068]
27. 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; 2(6):e91. [PubMed: 16789825]
28. Beck CW, Izpisua Belmonte JC, Christen B. Beyond early development: *Xenopus* as an emerging model for the study of regenerative mechanisms. *Dev Dyn.* 2009; 238(6):1226–48. [PubMed: 19280606]
29. Wells DE, Gutierrez L, Xu Z, Krylov V, Macha J, Blankenburg KP, et al. A genetic map of *Xenopus tropicalis*. *Dev Biol.* 2011; 354(1):1–8. [PubMed: 21458440]
30. Reinschmidt D, Friedman J, Hauth J, Ratner E, Cohen M, Miller M, et al. Gene-centromere mapping in *Xenopus laevis*. *Journal of Heredity.* 1985; 76(5):345–7. [PubMed: 4056365]
31. Khokha MK, Krylov V, Reilly MJ, Gall JG, Bhattacharya D, Cheung CY, et al. Rapid gynogenetic mapping of *Xenopus tropicalis* mutations to chromosomes. *Dev Dyn.* 2009; 238(6):1398–46. [PubMed: 19441086]
32. Abu-Daya A, Sater AK, Wells DE, Mohun TJ, Zimmerman LB. Absence of heartbeat in the *Xenopus tropicalis* mutation muzak is caused by a nonsense mutation in cardiac myosin myh6. *Dev Biol.* 2009; 336(1):20–9. [PubMed: 19769958]

33. Abu-Daya A, Nishimoto S, Fairclough L, Mohun TJ, Logan MP, Zimmerman LB. The secreted integrin ligand nephronectin is necessary for forelimb formation in *Xenopus tropicalis*. *Dev Biol.* 2011; 349(2):204–12. [PubMed: 20977901]
34. Geach TJ, Zimmerman LB. Paralysis and delayed Z-disc formation in the *Xenopus tropicalis* unc45b mutant dicky ticker. *BMC Dev Biol.* 2010; 10:75. [PubMed: 20637071]
35. Sive, HL.; Grainger, RM.; Harland, RM. Early Development of *Xenopus laevis*: A Laboratory Manual. Cold Spring Harbor, New York: Cold Spring Harbor Press; 2000.
36. Klein SL, Strausberg RL, Wagner L, Pontius J, Clifton SW, Richardson P. Genetic and genomic tools for *Xenopus* research: The NIH *Xenopus* initiative. *Dev Dyn.* 2002; 225(4):384–91. [PubMed: 12454917]
37. Klein SL, Gerhard DS, Wagner L, Richardson P, Schriml LM, Sater AK, et al. Resources for genetic and genomic studies of *Xenopus*. *Methods Mol Biol.* 2006; 322:1–16. [PubMed: 16739712]
38. Vogel G. Frog is a prince of a new model organism. *Science.* 1999; 285(5424):25. [PubMed: 10428694]
39. Stemple DL. TILLING--a high-throughput harvest for functional genomics. *Nat Rev Genet.* 2004; 5(2):145–50. [PubMed: 14726927]
40. Winkler S, Schwabedissen A, Backasch D, Bokel C, Seidel C, Bonisch S, et al. Target-selected mutant screen by TILLING in *Drosophila*. *Genome Res.* 2005; 15(5):718–23. [PubMed: 15867432]
41. Moens CB, Donn TM, Wolf-Saxon ER, Ma TP. Reverse genetics in zebrafish by TILLING. *Brief Funct Genomic Proteomic.* 2008; 7(6):454–9. [PubMed: 19028802]
42. Young JJ, Cherone JM, Doyon Y, Ankoudinova I, Faraji FM, Lee AH, et al. Efficient targeted gene disruption in the soma and germ line of the frog *Xenopus tropicalis* using engineered zinc-finger nucleases. *Proc Natl Acad Sci U S A.* 2011; 108(17):7052–7. [PubMed: 21471457]
43. Lund E, Sheets MD, Imboden SB, Dahlberg JE. Limiting Ago protein restricts RNAi and microRNA biogenesis during early development in *Xenopus laevis*. *Genes Dev.* 2011; 25(11): 1121–31. [PubMed: 21576259]
44. Chen CM, Chiu SL, Shen W, Cline HT. Co-expression of Argonaute2 Enhances Short Hairpin RNA-induced RNA Interference in *Xenopus* CNS Neurons In Vivo. *Front Neurosci.* 2009; 3:63. [PubMed: 20582287]
45. Ogino H, Fisher M, Grainger RM. Convergence of a head-field selector Otx2 and Notch signaling: a mechanism for lens specification. *Development.* 2008; 135(2):249–58. [PubMed: 18057103]
46. Akkers RC, van Heeringen SJ, Jacobi UG, Janssen-Megens EM, Francoijs KJ, Stunnenberg HG, et al. A hierarchy of H3K4me3 and H3K27me3 acquisition in spatial gene regulation in *Xenopus* embryos. *Dev Cell.* 2009; 17(3):425–34. [PubMed: 19758566]

Figure 1.

Images of *X. tropicalis* developmental mutants. Two *X. tropicalis* mutants identified during a gynogenetic screen of wild caught animals are shown in A-F. A and B, images of wild type and mutant *little pitcher* embryos, respectively. Otoconia are missing in the mutant inner ear (demarcated by the black arrow in wildtype embryos) and the inner ear vesicle is enlarged (indicated by dotted circle). C and D, images of wild type and mutant *cataract* embryos, respectively. The lens area appears very cloudy in the mutant embryos (white arrow). Sections of wild type and mutant embryos in E and F, respectively, reveal a highly reduced lens size in *cataract* embryos (black arrows). G. Metamorphosing *xenopus de milo* (*xdm*) froglets (two mutant; one wildtype). The striking absence of forelimbs illustrates the kind of mutation that is likely to be particularly insightful for clarifying tetrapod development. This mutation was found to interrupt the *nephronectin* gene, which produces a small integrin ligand, now clearly implicated in forelimb development. Image courtesy of L. Zimmerman (National Institute for Medical Research, Mill Hill, London).

Figure 2.

Participants in a Bay Area Regional Frog meeting in late 1992 in which the prospects for the *X. tropicalis* system were discussed. Participants included, from left to right, Marc Kirschner, Jeremy Green, Pascal Stein, Andre Brandli, Tabitha Doniach, William Smith, Kristen Kroll, Michael Sheets, Richard Harland, Enrique Amaya, John Gerhart, Robert Davis and Lyle Zimmerman.