Preface

During the last century amphibians have served as excellent models for developmental studies, in particular concerning early embryonic development. Since the 1950s (when they were first used in human pregnancy tests), the South-African claw-toed frog *Xenopus laevis* has been the most popular amphibian model organism. *X. laevis* is readily maintained in the laboratory, is commercially available, and can be induced to ovulate and mate at any time of the year (most frog species are seasonal breeders). Thousands of the relatively large and robust *Xenopus* eggs can be produced following a simple injection of the mammalian hormone chorionic gonadotropin. *Xenopus* embryos efficiently translate injected (synthetic) mRNAs, are transparent, develop externally in a simple salt solution, and are characterized by identifiable blastomeres, a reliable fate map, and ease of microinjection, micromanipulation, surgical intervention (grafting), labelling and culturing *in vitro*. Furthermore, it has recently become possible to generate hundreds of stable, non-mosaic transgenic *Xenopus* embryos in a cost-effective and efficient way in a single day [1]. This allows the experimenter to combine the aforementioned traditional advantages of *X. laevis* with the ability to express a gene at any time and in any place, not only for developmental studies but also to examine cell biological and biochemical processes. *X. laevis* has therefore, now been added to the list of (transgenic) model systems used for functional analyses (*C. elegans, Drosophila,* zebrafish and the mouse). *Xenopus tropicalis* is a close, diploid relative of the pseudotetraploid *X. laevis*, and has a short generation time making it attractive for creating permanent transgenic lines and performing molecular-genetic studies. The prospect of being able to perform genetic screens and make mutations in known genes will add an important dimension to the *Xenopus* system. Most anatomical and functional features as well as regulatory pathways are highly conserved between *Xenopus* and mammals, including humans. Therefore, the majority of information revealed from studies on this lower vertebrate will apply to mammalian systems. For these reasons, interest in *Xenopus* as a genomics system has increased, as is apparent from recent community-wide initiatives for the generation of large-scale *Xenopus* expressed sequence tags (ESTs) and genomic sequencing, expression profiling and genetic resources. These research efforts are often coordinated by the *Xenopus* Initiative of the US National Institutes of Health (NIH) that originates from the Human Genome Project via the NIH Non-Mammalian Models Initiative [2]. A search of *Xenopus* in the PubMed database revealed citations for over 28,000 articles (for comparison: mouse, 680,000 and zebrafish, 3,600). Nevertheless, in recent years *Xenopus* has not experienced the great increase in broad interest as seen for other vertebrate model organisms, such as the mouse and zebrafish. This is somewhat surprising since these models have a number of disadvantages when compared to *Xenopus*. For instance, mice produce fewer eggs, have no external embryonic development, it is not possible to generate genetic mouse chimeras (via tissue transplantation), and mouse transgenesis is cumbersome and costly. Zebrafish have a duplicated genome that is not only more divergent from mammals but the duplicated zebrafish genes have also diverged more from each other (a disadvantage for knock-down approaches), since the duplication occurred >400 million years ago [3]. In contrast, *X. tropicalis* is a true diploid and the genome duplication in *X. laevis* occurred ~30 million years ago [4]. Furthermore, both mouse and zebrafish transgenesis are less efficient than in *Xenopus* and often mosaic, necessitating the selection of transgenic lines before analysis. Given these facts, it seemed appropriate to pay more attention to *Xenopus* as a vertebrate model organism and thus, to bring together the latest developments in the *Xenopus* genomics field.

This Current Genomics Hot Topic issue deals with various aspects of *X. laevis/tropicalis* as a genomics system, including genomic and cDNA library construction and sequencing, bioinformatics, microarray studies, and (insertional) mutagenesis and misexpression approaches. A limiting step for the use of *X. tropicalis* has been the availability of high-quality cDNA libraries. Bruce Blumberg and colleagues (University of California in Irvine, CA, USA) have recently generated normalized, full-length enriched cDNA libraries from *X. tropicalis* that will allow functional studies and the identification of transcription units from the genomic sequence (article by Peng *et al*.). Paul Richardson and colleagues (US Department of Energy Joint Genome Institute, Walnut Creek, CA, USA) lead the *X. tropicalis* sequencing project, and aim to produce a high-quality sequence (8x coverage) and annotation in 2005. Their efforts are described in the second article of this issue (Richardson and Chapman). The *X. laevis* and *X. tropicalis* EST sequencing projects are currently underway with 294,924 ESTs available for *laevis* and 170,927 ESTs for *tropicalis*, as of July 25, 2003. Initially, *Xenopus* EST sequencing was relatively slow but the recent efforts have placed *Xenopus* high in the species ranking of available EST sequences. The availability of the ESTs is crucial for genome-wide expression screens and the construction of *Xenopus* UniGene sets (i.e. forming clusters of unique gene sequences) for efficient microarray analysis and allowing 'digital (*in silico*) differential display'. Having the sequence of the *Xenopus* genome will enable identification of conserved DNA regulatory elements, which can then be directly assayed in an experimental system. The genome sequencing effort will also provide a

vital resource in mapping and cloning mutant genes in *X. tropicalis*. In addition, the *Xenopus* genome sequence will add important insights into vertebrate evolution, since amphibians occupy a key phylogenetic position between mammals and fish. In both their ontogeny and phylogeny, most amphibians are quasiterrestrial with a double life in aquatic and land environments. For example, the amphibian limb skeleton clearly resembles that of mammals, but is very different from the fin of fish. A further interesting aspect of the availability of the amphibian genome may be the possibility of developing high-throughput tools to be used in toxicological and environmental research (*Xenopus* toxicogenomics), since frogs are considered to be good indicators of environmental damage caused by pollution. The main website resource with a compendium of useful *Xenopus* data is Peter Vize's Xenbase (University of Calgary, Alberta, Canada) and includes sections on genetics and genomics, as described in the article by Bowes and Vize. A large-scale gene-expression analysis was performed by Ken Cho and colleagues (University of California in Irvine, CA, USA) using *Xenopus* microarrays containing 42,000 sequenced cDNAs prepared from embryos at the neurula stage of development, to address several key aspects of *Xenopus* embryogenesis (Peiffer *et al*.). The article by Takuya Nakayama and Rob Grainger (University of Virginia, Charlottesville, VA, USA) presents the main features of *X. tropicalis*, in particular its genetic possibilities. *X. tropicalis* is a small, fast-growing frog with the only diploid genome among the 14 *Xenopus* species and one of the smallest genomes (~1,700 million base pairs or half the size of that of *X. laevis*) among amphibians (>4,500 species), simplifying genetic studies. This amphibian may thus, make fundamental contributions to vertebrate comparative and functional genomics. In general, mutations in model organisms are either being identified in wild-caught animals, or introduced by chemical mutagenesis and gene-trapping strategies. Michelle Hamlet and Paul Mead (St. Jude Children's Research Hospital, Memphis, TN, USA) adapted the transgenesis technique in order to trap genes and describe transposons to increase the rate of gene trapping (article by Hamlet and Mead). Many early-developmental studies involve microinjection of mRNAs, antibodies, or (morpholino) antisense oligonucleotides but these (macro)molecules are degraded with time (transient expression). The generation of stable and non-mosaic transgenic *Xenopus* embryos, tadpoles and frogs has allowed cell- and developmental stage-specific transgene expression, and the transgene is reliably transferred from the parent (F0) to subsequent generations. Often the green fluorescent protein GFP is used as the reporter to study transgene expression in the living embryo. In the last article (Dirks *et al*.), Ron Dirks and colleagues (University of Nijmegen, The Netherlands) describe efforts to accomplish in *X. laevis* transgene-driven RNA interference and the use of stable, cell-specific transgene expression to study the role of proteins of unknown function (Functional Genomics).

Finally, I would like to take the opportunity to thank Current Genomics Editor-in-Chief Christian Néri for his invitation to act as a Guest Editor, Sadia Masoom for editorial assistance and all colleagues for their contributions to this Hot Topic issue on *Xenopus* Genomics. As will be apparent from the information presented in the various articles of this issue, it is conceivable that *Xenopus* will develop into an attractive alternative of other model organisms, including zebrafish and mouse, to study the functioning of the vertebrate genome.

References

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