

Meeting report

Genomics and embryology in amphibians

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A report on the Eighth Biannual *Xenopus* Conference, Estes Park, Colorado, August 16-20, 2000.

Although *Xenopus* is well known for its contributions to development and cell biology, it has been a genetically challenged organism. The Eighth Biannual *Xenopus* Conference showed that this disability has an excellent prognosis. Genetics, transgenics and genomics were a prominent theme of the meeting, which was dedicated to John Gurdon (Wellcome/CRC Institute, Cambridge, UK) who, together with Igor Dawid (National Institute of Child Health and Development (NICHD), National Institutes of Health (NIH), Bethesda, USA), initiated the meeting series 16 years ago. Among Gurdon's many accomplishments, presented by former student Eddy M. De Robertis (Howard Hughes Medical Institute, University of California, Los Angeles, USA), was the first cloning of a vertebrate, nearly 30 years before the generation of the cloned sheep Dolly. A notable achievement of the meeting was the coalescence of the *Xenopus* community toward supporting a recent NIH funding initiative, The Trans-NIH *Xenopus* Initiative [<http://www.nih.gov/science/models/xenopus/reports/index.html>]. Interest was particularly keen in the genomic approaches being taken by a growing number of labs.

Sequencing and genomics

In the past year, efforts to apply genomic approaches to the study of *Xenopus laevis* have increased dramatically, and a survey of this effort was presented by Bruce Blumberg (University of California, Irvine, USA). *Xenopus* currently ranks eighth in terms of the total number of expressed sequence tag (EST) sequences that are publicly available in GenBank, with 48,000 submissions to public databases (50-fold less than mouse and human, however). These sequences come from 29 different cDNA libraries representing all the early

stages of development and a number of differentiated organs. The pattern of growth of this sequence resource is presented in Figure 1. The trend is set to continue under the NIH 'Initiatives for Model Systems', which now includes *Xenopus* (see below). To generate a Unigene [<http://www.ncbi.nlm.nih.gov/UniGene/index.html>] set, that is, a non-redundant set of sequences that overlap, each of which represents a unique gene, we have clustered the *Xenopus* sequences; the results are presented in Figure 2. A majority of the clones are unique sequences (13,500), whereas 2,500 and 1,000 clusters contain two or three members, respectively. The output of clustering will be used to prepare annotated full-length cDNA databases through a community-wide effort modeled after the *Drosophila* consortium. Complementing these efforts are ongoing radiation-hybrid mapping, amplified fragment-

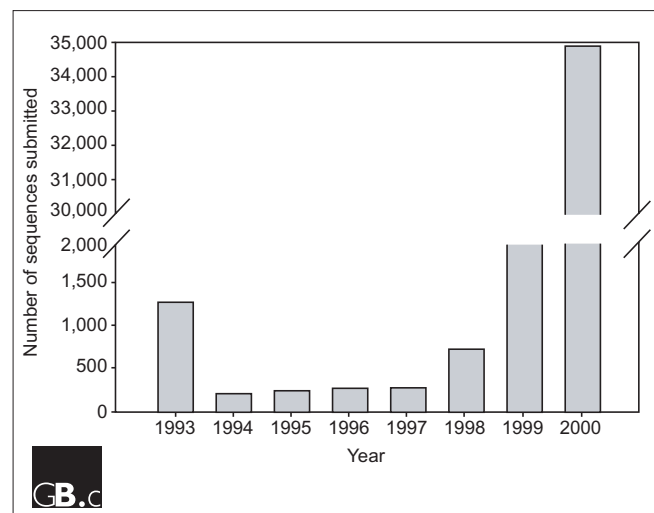


Figure 1
Growth of *Xenopus* sequence information in GenBank as of June 2000.

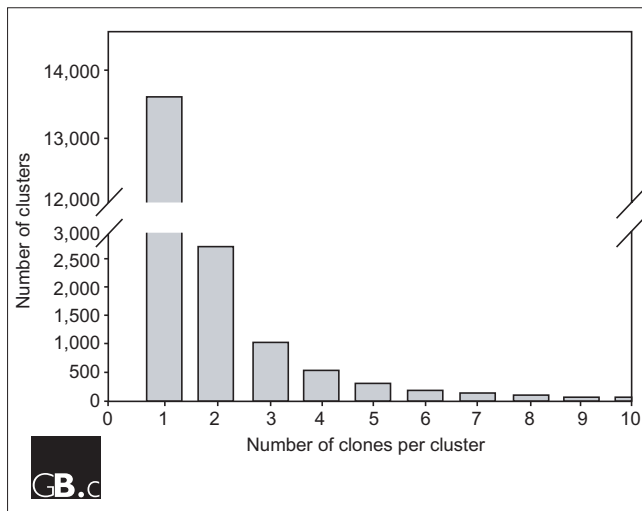


Figure 2
Cluster analysis of publicly available *Xenopus* sequences. Sequences deposited in public databases up to June 2000 (30,152 sequences) were clustered using an unpublished method developed by Jason Goncalves (University of Toronto, Canada). Each cluster represents a set of unique sequences, which in many cases represents an individual gene but does not currently distinguish between alternate splice variants.

length polymorphism, and genetic marker projects. The results of ongoing large-scale expression-screening approaches are available online (Axelddb [<http://www.dkfz-heidelberg.de/abt0135/axelddb.htm>]) and were presented by Nicholas Pollet (Deutsche Krebsforschungszentrum, Heidelberg, Germany). Peter Vize (University of Texas, Austin, USA) presented the updated Xenbase [<http://www.xenbase.org/Xenbase.html>] *Xenopus* database resource and highlighted the need to integrate the information from the various databases.

Transgenics and genetics

Another exciting aspect of the conference was hearing the latest information and advances in the transgenic field. Several labs, in particular those of Robert Grainger (University of Virginia, Charlottesville, USA), Enrique Amaya (Wellcome/CRC, Cambridge, UK) and Don Brown (Carnegie Institution of Washington, Baltimore, USA), have recently focused on bringing genetics to the *Xenopus* community, as demonstrated by the variety of transgenic animals described in their talks. Multitudes of different lines are currently being developed. Many of these are targeted at specific promoters, such as *Otx*, *crystallin*, *N-tubulin*, human cytomegalovirus (CMV) and cardiac actin, driving green fluorescent protein (GFP). An example of an F2 generation tadpole expressing the microtubule-associated protein tau fused to GFP (tau-GFP) from the CMV promoter generated by Nick Marsh-Armstrong, (Carnegie Institution of Washington, Baltimore,

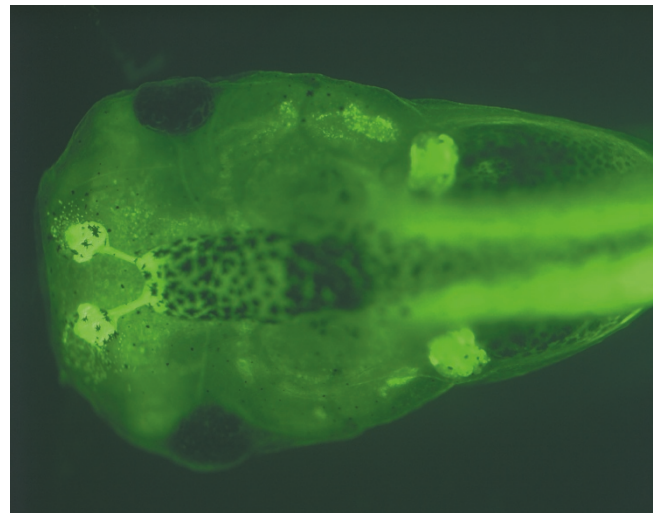


Figure 3
A stage 45 F2 generation transgenic *Xenopus* tadpole, transgenic for tau-GFP. Accumulation of signal is observed in the olfactory bulb and nasal pits. The parent line was prepared by Nick Marsh-Armstrong (Carnegie Institution of Washington, Baltimore, USA). Photograph courtesy of Paris Skourides (Laboratory of Molecular Vertebrate Embryology, Rockefeller University, New York, USA).

USA) is shown in Figure 3. Currently, other lines are being prepared that will express dominant-negative forms of designated genes, or ectopically express specific genes in targeted areas using a variety of tissue specific promoters. Nick Marsh-Armstrong, formerly of Don Brown's lab, has recently demonstrated that the first generation of transgenics, once sexually mature, exhibited transgene expression in the F2 progeny. This has emboldened other researchers to initiate transgenesis in their own labs.

Many labs are also using *Xenopus tropicalis* for their transgenic approaches. The advantage of *X. tropicalis* is the short time taken for the frogs to become sexually mature (2-3 months, compared with 12 months for *X. laevis*). The Grainger lab reported the preparation of F4 and F5 inbred strains in *X. tropicalis* and are well on the way to the desired F7 strain. Several laboratories are also using the transgenic approach to carry out a large-scale gene-trap screen, including screens using transposable elements, in both *Xenopus laevis* and *X. tropicalis*. Mutagenesis screens using gynogenesis are currently being developed; early phenotypes already observed include neural-crest, ear and tail patterning defects (poster presented by Lyle Zimmerman, University of Virginia, Charlottesville, USA).

Microarrays

As a complement to sequencing efforts, our group at The Rockefeller University and Cho's group (University of California, Irvine, USA) reported the preparation of cDNA

microarrays containing 5,000 and 1,400 genes, respectively. In addition to preparing the arrays, we have developed a bioinformatic system to allow the analysis and storage of the data. Using a web interface, the array data are linked directly to a sequence information database (*Xenopus* Microarray Project [<http://arrays.rockefeller.edu/xenopus>]). This has allowed the submission and analysis of microarray experiments by researchers around the world. The availability of these tools will allow the rapid examination of gene expression changes during development in this previously genetically challenged organism.

Outstanding issues

One of the last remaining concerns for the application of large-scale genetic screens in *Xenopus* has been the issue of ploidy. While it is accepted that *X. laevis* is pseudo-tetraploid, *X. tropicalis* has been regarded as a diploid organism. This issue is important in the light of the recent evidence showing non-diploid nature of the zebrafish, which makes genetic approaches more difficult. Karyotyping of *X. tropicalis* reveals ten chromosome pairs that are not very dimorphic (preliminary results presented by Amy Sater, University of Houston, USA). DNA content determinations reveal that *X. tropicalis* contains about half the amount of DNA per cell as is in *X. laevis*. Of the limited set of genes examined to date (including the major histocompatibility complex, MHC), only a single copy has been identified in *X. tropicalis* whereas two copies can be found in *X. laevis*. Although little is known about the organization of the Hox genes, data concerning duplications in these important genes, which are present in four clusters in mammals, will bear directly on the question of ploidy. Discussions at the meeting led to community-wide support for comparison of *X. tropicalis* and *X. laevis* by Southern blot using existing *X. laevis* probes. To this end, genomic samples have been prepared by Zimmerman and provided to interested labs (currently 16 participants). This effort should allow the question of ploidy to be addressed and should help finally bring the power of genetics to *Xenopus*.

The Trans-NIH initiative

The NIH has included *Xenopus* as one of eight model organisms on which they are funding multicenter research. This was the result of a process begun with the Non-Mammalian Model Initiative in February 1999. Steve Klein (NICHD, NIH, Bethesda, USA), presented the community-led proposal that has been submitted to the NIH for including support for *Xenopus* genomic and genetic research in their earmarked funding. He also presented the formation of The Trans-NIH *Xenopus* Initiative [<http://www.nih.gov/science/models/xenopus/reports/index.html>], which will oversee the cross-institute initiative.

For more than 100 years, the study of amphibians has been at the forefront of experimental embryology. More recently,

much of the amphibian work has been based on *Xenopus*, and this meeting showed that *Xenopus* can be used for genetic and genomic approaches - the future of molecular embryology. The next century promises to be as bright as the last.

Acknowledgements

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