

Easy passage: Germline transgenesis in frogs

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Amphibians have been a favored model organism for studies of early development for over a century. The African frog *Xenopus laevis*, the amphibian most widely used today, owes much of its popularity to its robustness and facility of husbandry, the ready availability of oocytes and eggs in large quantities, and the ease with which cell and tissue manipulations can be performed. These features allow many embryological experiments that are difficult or not possible in other model organisms. Compared with other vertebrate embryos, *Xenopus* embryos are large and accessible for manipulation from fertilization onward, and many of the early patterning processes have proven generalizable to all vertebrates.

The great limitation of the frog has been in genetic manipulability. Ectopic expression of injected RNA is very simple and has yielded a wealth of information. However, RNA injections are only practical for global expression studies early in embryogenesis. For many purposes, transgenes in which spatial and temporal expression can be precisely targeted are much more useful. Recently, a rapid, efficient, and simple approach for transgenesis was developed, allowing the production of more than 1,000 transgenic *Xenopus* embryos per day (1). Until now, this transgenesis approach has been mainly used for transient expression during early development; there has not been an effort to examine whether introduced genes can be passed through the germline. The paper by Marsh-Armstrong *et al.* published in this issue of PNAS (2) demonstrates that transgenesis can indeed be used to produce lines of genetically manipulated frogs. This approach and the transgenic lines that are generated will serve as an invaluable resource for early developmental studies.

The transgenic method for *Xenopus* relies on transplantation of a genetically manipulated sperm nucleus into an unfertilized egg. Demembrated sperm nuclei are briefly incubated with linearized plasmid DNA in the presence of *Xenopus* egg extract (which partially decondenses the sperm chromatin) and a restriction enzyme that is thought to facilitate integration by causing breaks in the sperm chromatin. Transplantation of these manipu-

lated sperm nuclei into unfertilized eggs results in a high frequency of embryos (usually $\approx 60\text{--}80\%$ of embryos produced) that integrate transgenes into the genome and express them with the proper temporal and spatial fidelity. As plasmid DNA integrates before first cleavage, the embryos produced are not chimeric, and breeding is not required to obtain integrated plasmid in every embryonic cell. In this respect, the approach is better than those for other vertebrates, such as mouse and fish.

Since its development, transgenesis in *Xenopus* has proven useful for both misexpression experiments (1–5) and for studies of transcriptional regulation (6–10). For example, promoter studies are rapid and inexpensive in *Xenopus* compared with mouse; a large number of transgenic *Xenopus* embryos containing many different plasmids can be made within 1 day and analyzed in subsequent days without passage through the germline. Limitations of analyzing embryos derived directly from the transgenesis procedure are that some fraction develop abnormally and that different numbers of plasmid copies are integrated into each embryo leading to variability in the transgene expression level. These features of transgenesis may complicate the interpretation of some experiments.

In the paper by Marsh-Armstrong *et al.* (2), the transgenesis approach described above was used to create transgenic embryos that were raised to sexual maturity and were bred to create transgenic lines. In all 19 tested cases, transgenic first generation (F0) founder animals were found to reliably produce progeny that carried and expressed the transgene. The frequency of transgenic progeny ranged from 50 to 98%, consistent with integration of plasmid at one to four sites in the genome of founder animals. Significantly, no silencing of transgene expression was observed in F1 progeny whereas gene silencing has been a major problem for transgenesis in both mouse and fish. This obviates the need to produce one or more additional generations to obtain transgenic frog lines that express with high fidelity. A variety of promoters from different sources (viral, rat, and *Xenopus*) maintained the expected expression pat-

tern in transgenic F1 progeny, and the expression level was equivalent for all progeny derived from a founder animal with integration at a single site. Thus, expression level variability and morphological abnormalities resulting from the transgenesis procedure were effectively eliminated in these transgenic lines. Additionally, the authors found that two different plasmids introduced into transgenics usually cointegrate in the F0 animal and later faithfully cosegregate in F1 progeny. These results show that F1 transgenic lines expressing one or more plasmids can be produced easily and at high efficiency.

One important consequence of this work is that large numbers of transgenic embryos expressing promoter-reporter plasmids can be produced from transgenic lines of frogs. These embryos will be a major resource for several embryological applications.

Such lines will, for example, allow us to identify and manipulate particular cell populations undergoing cell fate decisions in living embryos much earlier and more precisely than was previously possible. In all vertebrate model organisms, embryological approaches have relied on relatively crude morphological and/or spatial landmarks for identifying particular cell populations for manipulation. Furthermore, it has not been possible to simply visualize cell commitment events in living embryos as they occurred. Rather, analysis has required sampling of the embryo or explanted tissue at a particular time by molecular or microscopic analysis.

The merger of green fluorescent protein (GFP) technology with promoter plasmids in transgenic embryos now allows specific cell populations to be marked by GFP as inductive processes occur in live embryos in real time. These cell populations can then be precisely identified and explanted, transplanted, or otherwise manipulated. This approach has already proven practical with several early promoters. Combining the ability to visualize inductive events as they occur in developing embryos with the embryological ma-

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nipulability of *Xenopus* and its accessibility at all stages should allow experimental manipulation that would currently be difficult in any vertebrate model system.

One use of lines of transgenic frog embryos containing promoter-reporter plasmids is as a source of responding tissue to screen cDNA libraries for genes with particular inductive activities. Much of our knowledge about embryonic induction in vertebrates is derived from work in *Xenopus*. Explanted tissue can develop into varied cell types, including muscle, epidermis, nerve, neural crest, and endoderm in response to injected cRNAs. Using such responding tissue taken from transgenic embryos would allow many cRNAs to be assayed by rapid, visual observation for a particular inductive activity. For example, responding tissue taken from a transgenic embryos containing a muscle promoter:GFP plasmid would fluoresce in response to a muscle inducing signal. Currently, such screens require laborious and time-consuming molecular assays such as reverse transcription-PCR to determine whether an inductive response has occurred. This limits the number of cDNAs that can be screened by using such a method. Using transgenic tissue greatly simplifies such a screen and should make screening entire cDNA libraries using this sort of approach feasible.

Additionally, embryos from transgenic lines that express promoter-reporter plasmids will serve as a source of eggs and embryos for genetic screening. The use of transgenic eggs or embryos containing a

tissue-specific promoter:GFP reporter plasmid for mutagenesis should allow defects in a particular structure to be easily visualized by changes in the pattern of fluorescence and defective embryos to be selected. Such genetic approaches, relying on both classical and insertional mutagenesis strategies, are underway and will rely on a close relative of *Xenopus laevis*, *Xenopus tropicalis*, which has a genome size and generation time similar to zebrafish (11, 12). Germline transmission of transgenes has also recently been obtained in *X. tropicalis* (12).

In this study, Marsh-Armstrong *et al.* (2) observed that two different plasmids cointegrate and cosegregate in F0 embryos and F1 progeny. This observation also permits the co-expression of a plasmid that perturbs development along with a promoter-reporter plasmid. Live transgenic embryos can then be identified early in development by expression of the promoter-GFP reporter plasmid, and mutant cells or tissues can be removed, used in embryological manipulations of any kind, or observed *in vivo* during manifestation of effects caused by the added gene. One can use this technology to make chimeric embryos containing, in principle, any combination of mutant and wild-type tissues.

Likewise, co-introduction of plasmids will permit several inducible gene expression schemes to allow precise control over the times and places of expression in an experiment. One could turn on gene expression at any developmental timepoint

(either throughout the embryo or in a specific tissue) by using an inducible system. For example, the yeast transcription factor GAL4 can be made inducibly active by fusing it to the ligand-binding domain of the glucocorticoid receptor (GR) (13). Two plasmids are then introduced into transgenic embryos, one driving constitutive or tissue-specific expression of this GAL4-GR fusion and one consisting of the GAL4 promoter driving the gene of interest. GAL4 remains in an inactive state until the GR ligand dexamethasone is added, then binds the GAL4 promoter to drive inducible expression. The feasibility of this type of dual-plasmid GAL4 system and of GR-protein fusions in *Xenopus* embryos has already been demonstrated (4, 13).

At this stage of biological investigation, the scale of experiments can be as important as the experimental design itself. With sufficient effort, virtually any animal can be manipulated to express foreign DNA. Yet the power of the frog is the robustness of the system, the simplicity of culture, and the applicability to tissue and organ differentiation in all vertebrates. The experiments of Marsh-Armstrong *et al.* (2) are another milestone in the development of *Xenopus* as a system for experimental vertebrate embryology. This work extends the utility of the frog beyond the earliest stages to any stage in development. It will reinforce those features of the frog that have made it a major system of embryological study for over a hundred years. It's a great way to start the next millennium.

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