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Keeping two animal systems in one lab – a frog plus fish case study

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Sections

Summary

For two decades, my lab has been studying development using two vertebrate animals, the frog *Xenopus* and the zebrafish, *Danio*. This has been both productive and challenging. The initial rationale for the choice was to compare the same process in two species, as a means to find commonalities that may carry through all vertebrates. As time progressed, however, each species has become exploited for its specific attributes, more than for comparative studies. Maintaining two species simultaneously has been challenging, as has the division of research between the two and making sure that lab members know both systems well enough to communicate productively. Other significant issues concern funding for disparate research, figuring out how to make contributions to both fish and frog communities, and being accepted as a member of two communities. I discuss whether this dual allegiance has been a good idea.

Introduction: why fish and frog?

A long time ago, in 1991, as I was setting up my own lab, I decided to include an additional animal model in our research. Since an undergraduate, I had used the frog *Xenopus laevis* as a tool to study developmental questions. Frog embryos are large enough to manipulate by transplant and explant assays, and until the last decade, essentially everything known about vertebrate development came from studies in amphibians, primarily *Xenopus*. However, certain assays were frustrating in *Xenopus*. There were no embryonic mutants, and loss of function assays had to be performed by expression of dominant negative constructs or by antibody injection. There was the promise of antisense ***(1)***, but nothing usable. Promoter analysis was very difficult, as transient transgenics made by DNA injection expressed only in a highly mosaic fashion, and as *X. laevis* has a generation time of 2 years, stable transgenics were difficult to make and seemed no better. Nonetheless, almost everything that was known about early vertebrate development had come from amphibian embryos (for example, ***(2,3,4,5,6)***, rev. in ***(7)***), due to the ease of explanting and transplanting embryonic tissue, and the ability to obtain large numbers of embryos for biochemical or molecular assays. These attributes made *Xenopus* very attractive.

In 1991, the zebrafish, *Danio rerio,* had yielded some information about early mesodermal and neural development (for example, ***(8,9,10)***), but the attraction of the system was its promise. Pre-eminent among the vertebrates, the zebrafish could readily be used for forward genetic screens, yielding mutants and identifying genes required for vertebrate development. Interesting mutants already existed (for example, ***(11,12)***), and massive zygotic mutant screens were underway. Preparation of transgenic lines was not established, but was being worked on ***(13)***. All this promised a system that was more tractable than the frog at identifying genes required for development, and at assaying true loss of function effects. The drawback to the fish is that the embryo is small, and transparent, which is great for imaging, but tough for microdissection-based assays, as one cannot readily distinguish specific regions, and once these are removed, they can vanish easily in the petri dish. Thus, explant assays, so valuable in the frog, had not been developed for the fish. In addition, the zebrafish fate map is not as sterotypical as that of the frog embryo ***(14,15)***, further complicating embryological assays. Nonetheless, it was clear that the zebrafish was becoming a very important vertebrate system.

One thing that bothered me about both frogs and fish was their evolutionary distance from mammals, and whether what we learned in frogs would extend to mammals. Amphibians and teleosts diverged more than 200my ago, frogs and mammals, about 150my ago. As the distance between fish and frogs is very great, and it seemed therefore, that if one identified a process conserved in both *Xenopus* and zebrafish, it was more likely to be conserved throughout the vertebrates than one identified in frogs or fish alone.

These considerations made the power of frogs and fish a compelling dual system in which to perform both embryological and genetic assays, and so we set up both systems to address the molecular basis of nervous system determination and patterning. **The overarching rationale was that asking the same questions in two animal systems, was a powerful way to compare and define conserved principles of vertebrate neural development.**

The progression of our research using two models

Our initial analyses using zebrafish were entirely comparative with those in *Xenopus*. We had isolated a set of genes expressed very early during *Xenopus* neural patterning, and compared expression patterns and time of specification of forebrain-expressed genes in fish and frog. This yielded several good papers, although each paper used either the frog ***(16,17)*** or fish ***(18,19)***, not both. If we did the studies today, they would compare species in a single paper.

Then, we moved on to the hindbrain, and here, something interesting happened. The question of how the hindbrain is set aside and patterned had been started in *Xenopus* ***(20)***. However, we had isolated, by subtractive cloning, a set of genes expressed specifically in the zebrafish hindbrain ***(21)***, and most of these had not yet been isolated in frog. Fish hindbrain mutants had already given very interesting information (rev. in ***(22)***). So the zebrafish hindbrain project moved rapidly, with analyses of new hindbrain gene function ***(23)***, as well as analysis using a *vhnf1* mutant and Fgf signaling ***(24)***. The fish studies moved ahead of the frog, and a catch up game with frog did not seem

useful, or a fair project for a student or postdoc, who would get less novel publications than the authors of the fish studies.

During the fish hindbrain study, which involved looking at the brain a lot, we started thinking about brain morphology. This led us to the fascinating question of why the vertebrate nervous system is tubular, what the cavities (brain ventricles) are for, and later, why the tube bends (rev. in ***(25)***). From the outset, it was clear that the fish was a much better system than frog with which to address questions of brain morphogenesis and brain ventricle formation – there were mutants already, and now the transparency of the fish was very useful. We could make amazing live movies of the brain cells changing shape, and look in the mutants to see what had gone wrong ***(26,27,28)***. It was clear that this was going to be a productive approach, and that it did not include the frog, at least in our lab.

At the same time, we had, for a long time, studied the extreme anterior of the embryo, in *Xenopus*. We had productively studied the cement gland, an amphibian-specific anterior organ (many years of work rev. in ***(29)***), but then moved dorsally, to the primary mouth, which is highly conserved. In the frog, we were able to determine which cells contribute to the primary mouth, which tissue interactions are necessary, and began to study which factors were required, identifying Wnt antagonists as pivotal ***(30,31)***. There was no way that this study could have been done in fish – the primary mouth (stomodeal) region is difficult to image as the eyes are in the way, the germ layers in the fish are not distinct without lineage-specific gene markers, which are not available, and face transplant assays ***(31)***, which have been crucial in figuring out whether specific gene function is required locally in *Xenopus*, are not possible in fish.

The distinct attributes of each species were reinforced by an early project we performed, to ask whether frog explant techniques could be applied to the zebrafish. This was very challenging because the fish embryo is small and transparent, but two talented postdocs succeeded in isolating and culturing embryonic explants and performing ectoderm/mesendoderm induction assays, to show that neural induction occurs in the fish ***(18,19)***. A later paper compared frog and fish neural specification, emphasizing the usefulness of having parallel techniques available, and of comparative studies ***(32)***. Developing these techniques was a tour de force, but the assays are difficult, and still, *Xenopus* is a much simpler system for this approach.

Overall, these experiences added up to a move away from directly comparative studies, rather using the attributes of each species to address specific questions. **Thus, the initial rationale of fish/frog comparison led to useful insight; however, more recent studies have used each species for its greatest attributes**.

Advantages and challenges of fish + frog

Overall, having two animal species as experimental tools has been good, but not really for the reasons I thought. As discussed above, the notion of comparative studies turned out to be more cumbersome than the original rationale suggested, although this approach remains very important. The greatest advantage, I think, is that we have been able to study a much broader array of questions in two systems, than we would with one. This outcome arose due to technical considerations. Both fish and frog methods have improved enormously in the last two decades: including efficient methods to make stable

or transient transgenics (for example, ***(33,34,35)***), facilitating promoter analysis and tissue-specific or inducible gene expression; and antisense morpholino-modified oligonucleotides which have been extremely useful in both systems (rev. in ***(36)***). However, the genetic power of the fish remains supreme, even with the promise of mutants from *X. tropicalis*. Thus, *X. laevis* and *D. rerio* remain species with distinct attributes, which can be combined to address a single question, or applied to different questions that make use of the distinct attributes.

An unexpected advantage is that our group members become familiar with two models, and with a little effort can become facile with both. Several former lab members have switched systems, more easily than would be possible without the two animal exposure. Further, use of a technique in one species in our lab often inspires researchers working on the other system to rapidly try out the technique.

On the challenging side is the issue of maintaining healthy colonies of two species. *Danio* and *Xenopus* may both be aquatic, but they have their own water quality and temperature requirements, food needs, and techniques of embryo collection. We successfully raise some *X. laevis* to adulthood, but raising enough zebrafish to keep the group stocked is a continual and huge task. Separate technicians for each animal have been necessary, and separate animal rooms are essential. Where one aquatic system goes wrong frequently, two do so even more frequently! As anyone who works with aquatic species knows, a disaster of temperature change, or lack of proper feeding can lead to no or poor embryos for protracted periods, and this is amplified when two species are used. On the flip side, exchange between the animal managers of each species is synergistic and beneficial.

Another challenge is the need for a large enough group to have a critical mass of investigators using each system, both to get the research done, and to share techniques and responsibilities for the animals. Associated with that is the challenge of securing funding for separate lines of research, in separate animal systems. This increases the need for proven technical expertise in the particular system. For example, although expertise in making transgenic frogs bodes well for success in the fish, it is nowhere near as valuable as having actually prepared transgenic fish. An ongoing challenge, has been participating extensively in two communities, and mostly, ensuring that the group is viewed as committed to the fish or frog community. In the case of frog, multiple *Xenopus* investigators moved entirely to zebrafish, and we are one of the very few groups who added fish, but also stayed with the frog. We have tried to emphasize our commitment to both communities, but the perception of not fully participating has been frustrating at times.

In sum, having two animals in the lab has clear advantages: the ability to ask diverse questions, exposure of lab members to the practicalities and techniques of more than one system. The challenges include extensive husbandry required, the need to be facile with techniques in both species and the challenge of participating in two different animal communities.

I would do it again. Circle YES/NO.

Here's the question. Would I have pursued both fish and frog models, had I known the challenges involved? I love the gentle frogs, large enough to hold and to encourage to lay their eggs. I love frog embryos, which are really beautiful, where their lack of

transparency makes the changing parts of the embryo readily visible. I can't imagine not working with these embryos. The extreme anterior projects we have worked on are fascinating, and if anything, I would devote more time to these if I did it again. On the other hand, fish have grown on me. The adults are small and don't have the personality of frogs. One has to look very hard at the embryos to see their features. But when the cells are GFP labeled, and the imaging is done right, cells moving, changing shape or dividing are easy to see, deep within the living brain, and the embryos are very wonderful. We could not have performed the primary mouth study in fish. Period. Conversely, we could not have performed the brain study in frogs. Certainly, we could have focused on just one question, but that is not my style – the pull of so much interesting biology waiting to be explored is too strong. So, circle YES for me, please.

Last thoughts

Finally, if you are thinking of starting another animal system in your lab, here are some questions that may help you explore whether this is really the path you want to take.

1. Why do you want a second animal system in your lab?
2. Could you collaborate with another group working on the second system, rather than maintain both systems yourself?
3. Which two animals juxtapose effectively in terms of the questions you are addressing? Should they both be vertebrates, or would an invertebrate be useful?
4. Which two animal species juxtapose effectively in terms of the husbandry involved? Would expertise be shared between the two?
5. Can one animal caretaker maintain both systems?
6. How do you plan to split research between the two systems?
7. How would you ensure that a small group of researchers working on one animal system connect with others working on the same system?
8. Would research questions in the two systems be overlapping, or distinct?
9. If distinct, do you have sufficient funding and personnel to make a scientific contribution to each project?
10. What strategies would you employ to optimize the contribution of your group to the communities of each animal model?

And, if you have the energy to go for two systems, best of luck!

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