
Preface

The process whereby a single cell, the fertilized egg, develops into an adult has fascinated for centuries. Great progress in understanding that process, however, has been made in the last two decades, when the techniques of molecular biology have become available to developmental biologists. By applying these techniques, the exact nature of many of the interactions responsible for forming the body pattern are now being revealed in detail. Such studies are a large, and it seems ever-expanding, part of most life-science groups. It is at newcomers to this field that this book is primarily aimed.

A number of different plants and animals serve as common model organisms for developmental studies. In *Molecular Methods in Developmental Biology: Xenopus and Zebrafish*, a range of the molecular methods applicable to two of these organisms are described, these are the South African clawed frog, *Xenopus laevis*, and the zebrafish, *Brachydanio rerio*. The embryos of both of these species develop rapidly and externally, making them particularly suited to investigations of early vertebrate development. However, both *Xenopus* and zebrafish have their own advantages and disadvantages. *Xenopus* have large, robust embryos that can be manipulated surgically with ease, but their pseudotetraploidy and long generation time make them unsuitable candidates for genetics. This disadvantage may soon be overcome by using the diploid *Xenopus tropicalis*, and early experiments are already underway. The transparent embryos of zebrafish render them well-suited for *in situ* hybridization and immunohistochemistry, and good for observing mutations in genetic screens. Zebrafish are, however, quite difficult to keep and somewhat prone to disease. Since both organisms are suited to similar studies, the techniques applied to them overlap considerably. A number of laboratories also use both models, thus this methods manual includes experimental approaches that can be applied to both species. Indeed, some of these approaches are identical for *Xenopus* and zebrafish, for example, those for analysis of steady-state mRNA levels by RT-PCR or RNase protection. However, when there are substantial differences, as in whole-mount *in situ* hybridization, two methods are described.

The first two chapters deal with tissue explant and transplant techniques. These have been included not only because they represent one of the major

uses of these embryos, but also because they are mainly used in conjunction with the molecular techniques described here, for instance, in ectopic protein expression or RNA analysis. This combination of molecular techniques and embryo explants has proven extremely powerful, especially in the study of secreted signaling molecules with a role in development.

When one begins a developmental investigation, possibly with a novel or poorly defined protein to study, discovering the role of this gene and its product within an embryo is often the goal. Quantitative and spatial analysis of mRNA, the subject of the next four chapters (Chaps. 3–6), is often the starting point when attempting to investigate the developmental function of a newly discovered gene or protein. If these studies reveal that an mRNA is expressed in restricted regions of the embryo during the early stages of development, or at discrete embryonic or larval stages, this is often an indicator that the gene and its product will repay further analysis. Genes so expressed may well have a role in controlling development, and studying their regulation may add to our knowledge of how the pathways underlying embryo patterning are controlled. Although such mRNA analysis is useful, it is important to bear in mind that the expression of mRNA and protein do not necessarily correlate. There are many examples of translational control in developmental systems. Also, proteins which are expressed maternally may be present outside the cells that contain their cognate mRNA. There are a number of examples (both unreported and within the literature) where stable proteins, which are very widely expressed quite late in development as a result of maternal transcription, show very restricted mRNA expression if assayed by *in situ* hybridization. Ensuring that RNA analysis does not mislead in this way requires that protein expression is analyzed by immunohistochemistry, the subject of the two next chapters (Chaps. 7 and 8).

Analyzing mRNA and protein expression gives very limited information about the function of a given gene in the embryo. However, perturbing the level or activity of a test protein will in many cases produce data that define its role. One current weakness of both *Xenopus* and zebrafish as developmental models is that gene inactivation by homologous recombination, a technique that has led to the elucidation of the function of many genes in mice, is not available. Although the ablation of a particular gene product cannot be achieved, the over- or ectopic expression of a protein is relatively straightforward in embryos of both the species described here. Thus the lack of the ability to make specific mutants has in part been overcome by the expression of ingenious variants of the endogenous proteins under study. These dominant interfering mutant proteins disrupt the activity of their endogenous counterparts, and effectively test

gene inactivation. The techniques and experimental design underlying this type of approach are described in Chaps. 9–12.

All the techniques described up to that point in *Molecular Methods in Developmental Biology: Xenopus and Zebrafish* are useful for studying proteins, whatever their function. The final five chapters are more specialised (Chap. 12 contains both expression and promoter analysis methods). These deal with the analysis of transcription control in early development. Such analysis is particularly appropriate in *Xenopus* and zebrafish as, especially in the former, the large size and number of embryos available make viable the biochemical analysis of transcription factors and their interactions with DNA. Such analysis is extremely difficult at the early stages of development in other model organisms.

Though *Molecular Methods in Development Biology: Xenopus and Zebrafish* is primarily aimed at newcomers to the field, we have wherever possible obtained authors for each chapter who are still working at the bench and continually developing their techniques. Thus we hope that experienced workers may also find it useful. As with almost every methods manual it has been impossible to include all the techniques used to study *Xenopus* and zebrafish development. For example, zebrafish genetics are not touched upon here—there are many excellent treatments of positional cloning techniques available. Nonetheless the methods presented will provide a sound basis for the majority of studies. Other resources are available to both *Xenopus* and zebrafish developmental biologists on the internet; good starting points are the *Xenopus* molecular marker resource maintained by the Vize lab at the University of Texas (<http://vize222.zo.utexas.edu/>) or the zebrafish fishnet maintained by institute of neuroscience at the University of Oregon (<http://zfish.uoregon.edu/>).

Finally, many thanks to all the authors who managed to cope with yet another pressure in their hectic research careers, to the staff at Humana press for their patience and help, and to my family for putting up with even more paper than usual strewn around the house. May all your experiments be successful!

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