METHODS IN MOLECULAR BIOLOGY™

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MicroRNAs in Development

Methods and Protocols

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Preface

How do a lion or an orchid develop from a single cell? Answering this question in detail has fascinated developmental biologists for a long time. Plants and animals can have simple or very complex body organization but they all derive from a single cell, the fertilised egg. This cell divides and the progeny cells divide many–many times to build the entire body but the genetic information does not change during these cell divisions. Therefore, all our cells contain the same genetic information but there are many different tissues with specialised functions in our body. These tissues are different from each other because a different set of proteins are present in the cells that make up a certain tissue. The reason for this is that only a certain set of genes are active in each cell. Gene expression is a relatively complex process; therefore, it can be regulated at several layers. First the chromosomal DNA is transcribed into mRNA and this step is regulated by various mechanisms. For example, transcription factor proteins can activate or suppress the transcription and certain modifications of the DNA and the histon proteins that package the DNA also regulate transcription. The mRNAs are then processed and translocated to the cytoplasm where they are translated into proteins. Accumulation level of a protein can be regulated at the mRNA processing stage, mRNA stability level and through the half-life of the protein itself. One of the most recently recognised regulatory layers involves short RNAs to regulate the translation efficiency of mRNAs. These short RNAs are called microRNAs (miRNAs) since these molecules are very short, only 21–24 nucleotides.

The first miRNA (*lin-4*) was discovered in 1993, although it was not called a miRNA [1]. The next miRNA (*let-7*) was discovered 7 years later [2], but at that time these molecules were called small temporal RNAs because of their specific expression pattern during certain developmental transitions. The name miRNA was coined in the following year when three groups identified many short RNAs similar to *lin-4* and *let-7* in different organisms and because some of them were expressed all the time, the name "short temporal" was changed to miRNA [3–5]. miRNAs soon became one of the most intensively studied subjects in molecular biology. It is now clear that many mammalian genes are regulated by miRNAs, therefore understanding the role of miRNAs in development and disease is an important but difficult task.

One could say that working with miRNAs is not different from working with other RNAs. However, the very small size of miRNAs often requires specific techniques to study them and standard protocols (that are used for mRNA analysis) either cannot be used or important modifications have to be made. This book describes protocols for investigating miRNAs in plant and animal development. The chapters fall into three sections. Chapters 1–6 describe various techniques to detect and profile miRNA expression either spatially or at different time points. In situ hybridisation can establish where the miRNAs are expressed and northern blot, qPCR, deep sequencing, and array can be used to profile the expression of miRNAs at different developmental stages. Deep sequencing also has the potential

to discover new miRNAs. Chapters 7–10 are protocols to manipulate the activity of miRNAs in various organisms. These approaches are very useful to learn more about the function of miRNAs in developmental processes. Finally Chapters 11–15 describe different methods to identify and validate miRNA targets in animals and plants.

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