Preface

The sequencing and analysis of numerous genomes, and more recently transcriptomes, has shown that RNA is much more than a recording tape used to transmit genetic information from DNA to proteins. Although most human DNA is transcribed into RNA, only 1–2% of the DNA contains information directly encoding proteins, the biomolecules that orchestrate most of the cell's functions. Owing to its chemical properties and reactivity, and the lack of a fully complementary strand, RNA is very versatile: as a container for genetic information, as a tool that can regulate gene expression, as a catalyst for chemical reactions and even as a scaffold for protein complexes. RNA is therefore central not only to the basic processes of gene expression (DNA → RNA → protein), but it also has widespread roles in controlling gene expression at all levels.

Of the RNA-based mechanisms to regulate gene expression, alternative splicing has the most direct influence on the proteins formed. Alternative splicing enables the vast majority of human genes to encode more than one protein. In some instances a single gene can even create thousands of functionally distinct proteins. Much progress has been made in understanding the basic mechanism of pre-mRNA splicing and the rules that control alternative exon selection. The study of model organisms has demonstrated clearly that the correct regulation of alternative splicing is important for the proper functioning of cells and organisms, and this is underscored by evidence from the clinic. Changes in alternative splicing, caused either by mutations or alterations of regulatory factors can result in human diseases. An ever increasing catalogue of splicing-related pathologies attests to the importance of alternative splicing for human health.

The past few years have seen an encouraging start towards meeting major challenges such as predicting from genomic DNA sequence alone how splice sites will be recognized and regulated in different tissues, or the therapeutic manipulation of splicing in animal models of human disease. But, despite this considerable progress, much exciting work remains to be done before we can fully comprehend alternative pre-mRNA splicing.

As alternative pre-mRNA splicing impacts upon many areas in biology and medicine, scientists working on the subject come from different disciplines, ranging from medical geneticists to biochemists, molecular biologists and bioinformaticians. To bring these groups together, a European Alternative Splicing Network (**EURASNET**) was initiated in 2005 and continued until its formal winding-up in early 2011. EURASNET was a consortium of 43 research groups from Europe, Israel and Argentina, funded by the European Commission through its VIth Framework programme from 2006 to 2011. EURASNET has contributed to the integration of research efforts and dissemination of knowledge in the field of alternative pre-mRNA splicing. Its network activities were not restricted to research, but were also aimed at informing other scientists of alternative splicing. EURASNET organized numerous workshops and practical 'hands-on' courses all over Europe. Many of the protocols described in this book were tested in these settings.

One of the key missions of EURASNET was to reach out across scientific disciplines and make people aware of the importance of alternative splicing. In this spirit, we encourage post-doctoral fellows and graduate students to contact the authors of the various chapters, listed in the list of contributors, if they see any opportunity for collaboration. A simple Internet search or a visit to the EURASNET site (www.eruasnet.info) will reveal their current e-mail addresses.

Despite the importance of alternative splicing as a fundamental biological phenomenon and as a physiological mechanism which impacts on human health, to date there has been no single volume summarizing the current state of knowledge about alternative splicing, as well as detailing the experimental protocols for its analysis. This book aims to make good this deficit, giving an overview of both the theory and practice of alternative splicing. Its intendedreadership includes graduate students, post-doctoral fellows working in life sciences, medical practitioners who encounter aberrant alternative splicing in patients, and established investigators from other fields.

The book consists of two major parts: The first one provides a brief theoretical introduction that gives a short overview of alternative splicing and cites key papers in the field for more in-depth information. The second part is a collection of experimental protocols that are used in the field of alternative splicing. We envisage the protocols not as 'cookbook-recipes', but as guides for experiments that allow investigators to understand the procedures. Therefore, each protocol has a theoretical introduction explaining the background of the experiments, a list for troubleshooting and an example of an experiment. Each protocol is preceded by a graphic outline of the procedure that concisely summarizes the method and lists theexpected outcome of the experiment and the scientific questions that can be asked. We hope that this feature will allow readers to quickly find the experimental tools necessary for their projects and that it will stimulateinterest in looking at other techniques. The protocolsare arranged into six groups according to the scientific question that they address, which together with the theoretical introduction subdivide the book into seven parts. To help in orientation, these parts are marked by sidebars in Isaac Newton's rainbow colors.

The protocols are generally advanced, and a basic knowledge of molecular biology and RNA methods is necessary. Excellent textbooks that cover these topics are listed in Chapter 12 (Stamm), which can serve as a further entry point.

We would like to thank the European Commission for their vision and support, which has promoted collaboration and the establishment of durable links that have changed the landscape of research in alternative splicing and its medical impact. We are also grateful to the members of the scientific advisory board of EURASNET for their constant encouragement and critical input: Joan Steitz, Mariano-Garcia Blanco, James Dahlberg, Witek Filipowicz, Adrian Krainer, Michael Rosbash and Robert Singer.

Many thanks to Anne Chassin du Guerny, Andrea Zschaege, Nitin Vashisht and Andreas Sendtko from Wiley for their excellent support in publishing this book.

Finally, we are indebted to Jason Scroggin and Akari Takebayashi who teach at the University of Kentucky College of Design and are principals of Design Office Takebayashi Scroggin (http://dots-ky.com) for unifying the graphic design of the book, for getting into molecular biology and for their patience with endless revisions.

We hope that the book will help newcomers, especially medically oriented investigators, to understand the fascinating world of alternative splicing. We envisage that the protocols will allow experiments that push the field forward and that the background information will stimulate the improvement of existing protocols and development of new procedures.

October 2011

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