PREFACE

IN 1986, Rob Benne's research group published their finding of a posttranscriptional process in which mitochondrial messenger RNAs were altered by uridine insertions and deletions, a process he referred to as RNA editing. The finding explained a paradox that the mitochondrial genome of protozoa such as Trypanosomes encoded a scarcity of proteins and many of the genes appeared to have disrupted open reading frames or lacked a translational start codon. Benne's publication took the scientific community by surprise. The known mechanisms for nucleotide modification in RNA and alternative mRNA splicing could simply not accommodate the finding that Trypanosoma mitochondrial mRNAs contained multiple insertions of one or more non-genomically encoded uridines with no apparent consensus flanking sequence at the sites of insertion.

By the early 1990s, several forms of insertion/deletion and base modification editing had been described in amoeba, flagellates, Physarum, mammalian viruses, plants, and the kidney, intestine, liver, and neuronal tissues of mammals. However, many in the scientific community remained unaware of this emerging frontier and the sporadic nature of the identification of editing in different organisms, tissues, and organelles, and the diversity of editing mechanisms led others to question the significance that editing mechanisms would have in understanding cellular systems. For these early years the field collectively had an orphan status, finding outlets for its new discoveries largely in "catch-all" sessions at diverse scientific society meetings.

Beginning in 1994, RNA editing realized solidarity through three international conferences on RNA editing and modification organized independently by Harold Smith and Steve Hajduk (1994, Albany Conference, Rensselaerville, NY, USA), Glenn Bjork, Ted Maden, and Henri Grosjean (1994, EMBO Workshop, Aussois, France), and Paul Sloof and Rob Benne (1996, EMBO Workshop, Maastricht, The Netherlands). The first text dedicated to the topic of RNA editing was edited by Rob Benne in 1993.* The inaugural Gordon Research Conference dedicated to RNA editing and modification was led by Smith and co-chaired by Jonatha Gott and Maureen Hanson in 1997. By 1998, many of the RNA editing systems that are known today had been identified, and it was at this time that Grosjean and Benne co-edited a comprehensive text on RNA modification and editing.† The field has grown rapidly and gathered momentum as we learn how RNA and DNA editing mechanisms influence, and are influenced by, other biochemical pathways in the cell.

RNA Editing: The Alteration of Protein Coding Sequences of RNA, Benne, R. (ed.), Ellis Horwood Series in Molecular Biology, Prentice Hall, Englewood Cliffs, NJ, 1993.

†*Modification and Editing of RNA*, Grosjean, H., and Benne, R. (eds.), *American Society of Microbiology Press, Washington, D.C., 1997.*

The topic of this book is RNA and DNA editing. The chapters were written from the perspective of the *next generation* of investigators who were formerly trainees in the field or have been newly drawn to it. The authors suggest open questions to pursue while evaluating the context of discoveries and methodologies that have led researchers to this threshold. The vitality of this text lies in its cutting-edge perspective and in its fresh introspective treatment of the progress to date. The target audience of the book are not only the aficionados of the field, but also academics and members of the private sector who are seeking to learn about the field and explore its new applications.

RNA editing is a process in which the nucleotide sequence of RNA is altered from the genomic code. Editing is accomplished through nucleotide insertion, nucleotide deletion, or base modification. It is distinguished from other forms of RNA modification in that the consequence of RNA editing is a change in the diversity and/or abundance of proteins expressed in the proteomes of organisms, their tissues, or organelles. RNA modifications that diversify RNA function or produce a gain or loss of RNA function are also considered editing. Within this rubric, numerous alterations to nucleotides have been documented affecting coding and noncoding sequences of messenger RNAs (mRNAs) as well as transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), and spliceosomal RNAs (UsnRNAs).

As might be anticipated, coordination of editing activity is essential relative to other cellular pathways involving RNA such as transcription, RNA processing, and translation. Our appreciation of this regulation has grown through the characterization of the biological occurrence of RNA editing and the macromolecules that contribute to editing mechanisms. In this regard, the factors involved in RNA substrate recognition and catalysis are diverse, ranging from lone enzymes with both substrate recognition and catalytic activity to macromolecular complexes containing both protein and small RNAs as guides for substrate recognition and multiple proteins to carry out and coordinate editing activity. In A-to-I and C-to-U base modification editing, one editing factor or editosome serves multiple sites. In other systems, such as plant organellar C-to-U editing and organellar guide RNA-dependent mRNA, UsnRNA, and rRNA editing and modification, there is more complexity and a large number of site-specific editing factors.

A recent development in the field is the identification of select members in the family of cytidine deaminase editing enzymes that use single-stranded DNA as a substrate. DNA editing is mutagenic and is responsible for diversification of the genomic coding capacity for immunoglobulins and also serves in antiviral host defense. Another exciting discovery is that A-to-I RNA editing can regulate the production of interference RNA (RNAi) and thereby may constitute an important cellular mechanism for modulating the abundance of individual sequences within the transcriptome. A-to-I RNA editing also can modulate gene silencing through RNAi-dependent regulation of the specificity and activity of the machinery involved in DNA and histone modification, leading to chromatin remodeling.

Given these considerations, RNA and DNA editing will be discussed in four thematic areas to provide a contextual map for this field. Part I, "Diversification of the Proteome through RNA and DNA Editing," highlights how editing regulates protein expression through A-to-I base modification of mRNA, dC-to-dU modification of immunoglobulin genes for somatic hypermutation and immunoglobulin class switch

recombination, guide RNA-dependent uridine insertion and deletion editing of mitochondrial RNA, and C-to-U and U-to-C mRNA editing in plant chloroplast and mitochondrial transcripts. This chapter explores the question "Why are nucleic acid sequences edited instead of encoded genomically?" through discussion of the occurrence of editing sites within transcriptomes and their distribution within individual RNAs. Depending on the biological system, editing can be seen through the lens of diversification, repair, or mutagenesis. Paramount in these discussions are mechanisms that govern RNA editing site selectivity and specificity and restrict the chromosomal domains targeted for DNA editing. Regulation depends on the temporal control of site-specific editing factor expression, subcellular localization, their interaction with nucleic acids, and the composition of individual editosomes. The reader will appreciate how diversity in *cis*- and *trans*-acting factors in different species, or in different organelles within the same species, contributes to different patterns of editing activity and thereby enables plasticity in each biological system.

Part II, "Functional Coordination of RNA Editing with Other Cellular Mechanisms," brings to the forefront why RNA and DNA editing is essential for cell survival and adaptation. This section profiles base modification of RNA and DNA and guided RNA editing as examples where cells require editing to produce functional tRNAs, process rRNA, splice pre-mRNA, regulate the stability of mRNAs, and control RNAi and viral infectivity. In some instances, editing at different sites within the same RNA is interdependent and requires coordination of the activity of different editosomes or transport of editing enzymes or their substrates within the cell and its organelles. In other examples, RNA editing site selectivity is coordinated through the interaction of A-to-I editing enzymes with the C-terminus or RNA polymerase II. In this way, editing factors have immediate access to nascent transcripts and can carry out editing before pre-mRNA splicing deletes introns that participate in RNA secondary structure necessary for editing site recognition. Transcription also makes available single-stranded DNA within chromosomes that can be targeted for mutational DNA editing leading to diversification of the genomic sequences encoding the variable regions of antibodies (as described in the prior section). Reverse transcription, coupled to RNase H activity, also regulates editing activity by exposing single-stranded viral DNA during replication for mutagenic DNA editing as a form of host defense.

The global role of RNA editing in cellular regulation is emphasized in this section of the book through several examples. Modification editing of U2 spliceosomal RNA is essential for U2-snRNP splice site binding specificity and spliceosome activity. The stability of select mRNAs is affected by binding of the factors responsible for C-to-U mRNA editing in mammalian cells to AU-rich elements in mRNA. And, modulation of RNAi production by A-to-I RNA editing is described as a mechanism for regulating gene silencing by affecting the specificity and activity of the enzymes that carry out DNA and histone modifications. The exquisite level of integration of editing with other biochemical pathways and cellular functions described in all of the chapters will lead the reader to the inescapable conclusion that RNA and DNA editing have significant roles in biology that includes, and goes well beyond, codon sequence changes and reading frame alterations.

A long-sought goal in the field has been to use our understanding of editing sites and editing factors to discover novel editing substrates and new biological roles for

editing. Part III, "Predictive Studies," underscores the power of computational approaches in identifying novel editing sites and predicting the biological consequences of editing at these sites. Historically, computational analyses have been used sporadically to validate sequences as having been edited; however, computational methods have developed to the point where comparative sequence analyses enable genome wide predictions of edited mRNA sequences. Computational approaches have also advanced comparative phylogenic analyses of edited sequences. These studies have provided unique insights into the origins of editing systems, their evolution, and an understanding of the conserved, minimally essential functional domains within editing factors.

Highly related to these discussions is Part IV, "Structural Approaches," which is the final section of the text. Structural biology is an enabling technology for basic science, biomedical research, and drug development. The structural basis for function is more conserved in many instances than is primary nucleotide or amino acid sequence. Comparative structural analyses have been vital in predicting RNA secondary structure of the substrates for A-to-I editing and guide RNAs as well as the functional folds within enzymes in both A-to-I and C-to-U families of deaminases. Comparative structural analysis suggests conserved protein folds and implicate, in some instances, ancient phylogenic origins for components of editing machinery. Importantly, computational and structural studies suggest the reaction chemistry that enzymes catalyze, and they aim to predict the physical constraints in macromolecules that determine substrate and editing site specificity.

The selection of chapters and organization of the book was conceived with multiple purposes in mind. The text serves as a reference for background information in the field. It provides an opportunity for the newest contributors to the editing field to express their vision for the future. The perspectives voiced by these authors are anticipated to be provocative and are intended to motivate discussion, lead to new experiments, and promote collaboration. Finally, this book is intended to promote new hypotheses and models to springboard the next generation of discovery in the field.

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