
CONTENTS

<i>PREFACE</i>	xv
<i>ACKNOWLEDGMENTS</i>	xix
<i>CONTRIBUTORS</i>	xxi

PART I *DIVERSIFICATION OF THE PROTEOME THROUGH RNA AND DNA EDITING*

CHAPTER 1 <i>DIVERSIFYING EXON CODE THROUGH A-TO-I RNA EDITING</i>	3
1.1 Introduction and Background	3
1.1.1 Initial Discovery and Context of A-to-I RNA Editing and ADARs	4
1.1.2 Important Cases of Recoding by A-to-I Modification in Pre-mRNA	5
1.1.3 Cis-Acting Features for A-to-I Editing	14
1.1.4 Properties of the A-to-I Editing Machinery	15
1.2 Main Questions in the Field and Approaches	17
1.2.1 Biochemical Versus Computational Approaches	17
1.2.2 Editing of miRNA Sequences	21
1.3 Future Directions: Evolution of Editing Sites and Machinery	23
References	24
CHAPTER 2 <i>ANTIBODY GENE DIVERSIFICATION BY AID-CATALYZED DNA EDITING</i>	31
2.1 Introduction	31
2.2 Before AID	32
2.2.1 Without DNA (Darkness) and with DNA (Light)	32
2.2.2 Prominent Early Models for Antibody Diversification	32
2.2.3 How Protein Sequencing Technology Enabled an Understanding of Antibody Diversity	34
2.2.4 Somatic DNA Rearrangements Underpin V(D)J Joining and Create the Primary Antibody Repertoire	37
2.2.5 Additional Antibody Diversity by Somatic Hypermutation (and Gene Conversion in Some Animals)	40
2.2.6 Altering Antibody Function by Class Switch Recombination (Isotype Switching)	40
2.3 After AID	41
2.3.1 A Novel Deaminase Is Required for CSR, SHM, and IGC	41

2.3.2	AID Is a DNA Cytosine Deaminase that Directly Triggers Antibody Diversification	43
2.3.3	The Importance of Uracil Bases in DNA <i>In Vivo</i>	45
2.3.4	Processing of AID-induced Lesions: The Molecular Mechanism of Somatic-Hypermutation	48
2.3.5	Processing of AID-induced Lesions: The Molecular Mechanism of Immunoglobulin Gene Conversion	49
2.3.6	Processing of AID-induced Lesions: The Molecular Mechanism of Class Switch Recombination	50
2.4	Hot Areas and Speculations	53
2.4.1	Immunodeficiency Syndromes Caused by Defects in AID-Mediated Ig Gene Diversification	53
2.4.2	Regulating the DNA Mutator Activity of AID	54
2.4.3	Misregulation of AID and Cancer	57
2.4.4	AID Is But One Member of a Much Larger Family of Polynucleotide Deaminases	58
2.5	Conclusions	60
	Acknowledgments	60
	References	61

CHAPTER 3 *PROTEIN-PROTEIN AND RNA-PROTEIN INTERACTIONS IN U-INSERTION/DELETION RNA EDITING COMPLEXES*

71

3.1	A Bizarre Phenomenon and its <i>Raison D'être</i>	71
3.2	The Catalytic Mechanism and Machinery	73
3.3	Extent of U-Insertion/Deletion RNA Editing in <i>Trypanosoma</i> and <i>Leishmania</i> Species	75
3.4	Functional Studies of Editing Complex Subunits	76
3.4.1	REN1, REN2, and MP67. Endonuclease Homologs	80
3.4.2	REX1 and REX2. Exonuclease Homologs	83
3.4.3	RET2. TUTase	85
3.4.4	REL1 and REL2. Ligase Homologs	85
3.4.5	MP81, MP63, MP42, MP46, MP44, MP24, MP18. Structural Components	87
3.5	RNA-Protein Interactions: Isolated Subunits and Assembled Editing Complexes	92
3.5.1	MP42	92
3.5.2	MP24	93
3.5.3	RNA-Protein Interactions in Assembled Editing Complexes	93
3.6	Concluding Remarks	96
	Acknowledgments	96
	References	96

CHAPTER 4 *MACHINERY OF RNA EDITING IN PLANT ORGANELLES*

99

4.1	Introduction	99
4.2	Mechanism of Target Recognition	100
4.3	PPR Protein is a <i>Trans</i> -Factor in Plastids	101
4.4	How Can the Model Be Generalized to Plant RNA Editing?	104
4.5	Can Closely Located Editing Sites Share a <i>Trans</i> -Factor?	105

4.6	Is a <i>Trans</i> -Factor Specific to a Single <i>Cis</i> -Element?	106
4.7	Mechanism Determining the Efficiency of RNA Editing	107
4.8	Co-Evolution of <i>Trans</i> -Factors and Editing Sites	109
4.9	What is an Editing Enzyme?	110
4.10	A Model of Editing Machinery in Plastids	112
4.11	Future Directions	114
	Acknowledgments	114
	References	114

PART II *FUNCTIONAL COORDINATION OF RNA EDITING WITH OTHER CELLULAR MECHANISMS*

CHAPTER 5	<i>TRANSFER RNA EDITING ENZYMES; AT THE CROSSROADS OF AFFINITY AND SPECIFICITY</i>	123
------------------	--	-----

5.1	Introduction: Structural Versus Functional tRNA Editing	123
5.2	Transfer RNA Editing for Structure	124
5.2.1	C-to-U Editing of the tRNA Backbone	124
5.2.2	A-to-I Editing and Modification at Position 37 and 57 of tRNAs	126
5.3	Transfer RNA Editing for Function	127
5.3.1	The Lysidine Story	127
5.3.2	Nucleotide Additions at the Ends of tRNAs	130
5.3.3	C-to-U Editing in Marsupials and Trypanosomatids	133
5.3.4	A-to-I Editing of tRNAs in Yeast and Bacteria	137
5.3.5	Double Editing in Trypanosomatids	139
5.4	The Transfer RNA Editing Enzymes of Trypanosomatids: A Special Case of Catalytic Flexibility	140
5.5	Complex Formation by Transfer RNA Editing Enzymes: A Model for the Regulation of Editing Activity	141
5.6	Concluding Remarks: Evolution of Transfer RNA Editing Deaminases: Affinity Versus Specificity	142
	References	143

CHAPTER 6	<i>A-TO-I EDITING AS A CO-TRANSCRIPTIONAL RNA PROCESSING EVENT</i>	146
------------------	--	-----

6.1	Introduction	146
6.1.1	Overview of Co-transcriptional Pre-mRNA Processing	147
6.1.2	Localization of the ADAR Proteins	148
6.1.3	A-to-I Editing as a Pre-mRNA Processing Event	149
6.2	Main Questions in the Field and Approaches	149
6.2.1	Why Are Edited Sites Often Situated Close to Exon/Intron Border?	149
6.2.2	The Potential of A-to-I Editing in Changing the Transcriptome	151
6.2.3	RNA Editing, the Influence on Pre-mRNA Splicing and Vice Versa	152
6.3	Can Editing Influence the Fate of a Messenger RNA in Other Ways?	156
6.3.1	Editing and Its Potential Effect on RNA Export	156
6.3.2	Editing as a Modulator of RNA Stability	156
6.3.3	Editing and Its Influence on Polyadenylation	157
6.4	Prospectives for Future Research	158
	References	159

CHAPTER 7	<i>STUDYING AND WORKING WITH RIBONUCLEOPROTEINS THAT CATALYZE H/ACA GUIDED RNA MODIFICATION</i>	162
7.1	Introduction	162
7.2	Discovery of Complex (RNA-guided) Pseudouridine Synthases	164
7.3	Approaches and Challenges	164
7.4	RNP Reconstitution	166
7.5	Lessons from Archaeal H/ACA RNPs	167
7.6	Biogenesis of Eukaryotic H/ACA RNPs	168
7.7	Debate on Dyskeratosis Congenita	168
7.8	Importance and Future of H/ACA RNPs	169
	Acknowledgments	170
	References	171
CHAPTER 8	<i>FUNCTIONAL ROLES OF SPLICEOSOMAL snRNA MODIFICATIONS IN PRE-mRNA SPLICING</i>	175
8.1	Introduction	175
8.2	Modified Nucleotides in Spliceosomal snRNAs	176
8.3	Functional Analysis of Spliceosomal snRNA Modifications	179
8.4	Modified Nucleotides of U2 snRNA are Important for Pre-mRNA Splicing	180
8.5	U2 Modifications Contribute to snRNP Biogenesis and Spliceosome Assembly	182
8.6	Genetic Analysis of U2 Modification in Yeast	183
8.7	Cytotoxicity Associated with 5FU Treatment is a Result of Inhibition on Pseudouridylation and Splicing	184
8.8	Biophysical Analysis of U2 snRNA Modification	185
8.9	Concluding Remarks	186
	References	186
CHAPTER 9	<i>A ROLE FOR A-TO-I EDITING IN GENE SILENCING</i>	190
9.1	Expression of Double-Stranded RNA in Cells	190
9.2	The Activity of ADAR in the Nucleus	191
9.3	Alternative Fates of Edited RNAs in the Nucleus	192
9.4	A Possible Connection Between RNA Editing and Gene Silencing	193
9.4.1	Heterochromatin	193
9.4.2	RNAi-Directed Heterochromatin Formation	194
9.4.3	Connections Between RNAi and dsRNA Editing	196
9.4.4	Vigilin	196
9.4.5	Recognition of RNA by Vigilins	197
9.4.6	The Vigilin Complex	198
9.5	A Model for the Nuclear Function of Vigilin	199
	References	200
CHAPTER 10	<i>BIOLOGICAL IMPLICATIONS AND BROADER-RANGE FUNCTIONS FOR APOBEC-1 AND APOBEC-1 COMPLEMENTATION FACTOR (ACF)</i>	203
10.1	Overview	203

10.2	Background to Our Current Understanding of C-to-U Editing of ApoB mRNA: Canonical Functions for Apobec-1 and ACF	204
10.2.1	Role of Cis-Acting Elements	204
10.2.2	Identification and Characterization of <i>Trans</i> -Acting Factors	206
10.3	Current Understanding of Apobec-1 and ACF: Structure-Function and Genetic Regulation	209
10.3.1	Apobec-1: Structure-Function Relationships	209
10.3.2	Functions of Apobec-1 Beyond apoB mRNA Editing	210
10.3.3	Apobec-1: Genetic Regulation and Gain- and Loss-of-Function	212
10.3.4	ACF: Structure-Function Relationships	214
10.3.5	Intersections of Apobec-1 and ACF Regulation in the Modulation of C-to-U RNA Editing	216
10.4	Implications and Broad-Range Function for Apobec-1 and ACF: Future Directions and Overarching Questions	218
10.4.1	Apobec-1	218
10.4.2	ACF	220
10.5	Conclusions	224
	Acknowledgments	224
	References	224
CHAPTER 11 ANTIVIRAL FUNCTION OF APOBEC3 CYTIDINE DEAMINASES		231
11.1	Explanation of Vif Phenotype Uncovers a Unique Innate Resistance to HIV-1 Infection	231
11.2	Antiviral Functionality of the APOBEC3 Family of Proteins	232
11.2.1	Mechanism of Action	232
11.2.2	APOBEC3 Proteins and the Prevention of Zoonosis	235
11.2.3	<i>In Vivo</i> Correlations Between APOBEC3 Expression and Disease Course	236
11.3	The Battle for Control: Viral Suppression of the APOBEC3 Proteins	237
11.3.1	The Hijacking of the Proteasomal Degradation Pathways	237
11.3.2	Virion Exclusion of the APOBECs via a Viral-Dependent Mechanism	238
11.4	Cellular Function and Regulation of the APOBEC3 Family	238
11.4.1	Guardians of the Genome: APOBEC3-Mediated Suppression of Cellular Retroelements	238
11.4.2	Subcellular Localization (Sequestration)	239
11.4.3	Control of the Expression of the APOBEC3 Family is Exerted Transcriptionally	240
11.5	Research Questions and the Hope of Therapeutic Manipulation of the APOBEC3 Family	243
11.5.1	The "Alternative Function"	244
11.5.2	Protein Partitioning/Subcellular Localization	245
11.5.3	Protein Cofactors and Posttranslational Modifications	245
11.5.4	Therapeutic Potential	246
	References	247

PART III PREDICTIVE STRUCTURES

CHAPTER 12 A-TO-I EDITING OF ALU REPEATS		257
12.1	Background	257
12.1.1	Indirect Evidence for Abundant A-to-I Editing	257

12.1.2	Early Screens for A-to-I Editing Targets	258
12.1.3	The <i>Alu</i> Repeats	259
12.2	Computational Detection of A-to-I Editing	260
12.2.1	Sifting through db EST	260
12.2.2	Clusters of Mismatches in RNAs	262
12.2.3	Numbers of Editing Sites Detected	265
12.2.4	Characterization of the Edited Transcripts	265
12.2.5	Looking for Conserved Polymorphism Sites	267
12.2.6	Additional Potential Targets for Abundant A-to-I Editing	267
12.3	Editing in Other Organisms	268
12.3.1	Uniqueness of the <i>Alu</i> Repeat	269
12.3.2	Predicting Editing Sites from Genomic Data	270
12.4	Biological Role of <i>Alu</i> Editing	272
12.4.1	Possible Regulatory Roles	272
12.4.2	<i>Alu</i> Editing and miRNA	272
12.4.3	Alternative Splicing and <i>Alu</i> Editing	273
12.5	Concluding Remarks	275
	References	276

CHAPTER 13 RNA EDITING IN DINOFLAGELLATES AND ITS IMPLICATIONS FOR THE EVOLUTIONARY HISTORY OF THE EDITING MACHINERY

280

13.1	Introduction	280
13.2	Inferred RNA Editing in Dinoflagellates	283
13.2.1	<i>cob</i> and <i>cox1</i> mRNA	283
13.2.2	Chloroplast Transcripts	288
13.3	Biochemical Characteristics of Editing	289
13.3.1	Unusually Diverse Types of Editing	289
13.3.2	Varying Editing Density and Discrete Distribution of Editing Events in Coding Sequences	293
13.3.3	Markedly Nonrandom Distribution in the Type of Codon Edited and in the Position of the Editing Site Within Codons	294
13.4	Consequences of Editing	297
13.5	Phylogenetic Trend	301
13.6	Implications for the Origin and Evolution of the RNA Editing Machinery	304
	Acknowledgment	306
	References	306

PART IV STRUCTURAL APPROACHES

CHAPTER 14 THE BOX C/D RNPs: EVOLUTIONARILY ANCIENT NUCLEOTIDE MODIFICATION COMPLEXES

313

14.1	Introduction	313
14.2	Diversity of Box C/D RNA Populations	314
14.2.1	Box C/D RNA Nomenclature	314
14.2.2	Box C/D RNA Structure	315

14.2.3	Diversity of Box C/D RNA Populations	316
14.2.4	Box C/D RNA Identification	316
14.3	Box C/D RNA Functions and Target RNAs	319
14.3.1	Folding and Cleavage of Pre-rRNA	319
14.3.2	2'-O-Methylation of Diverse RNA Targets	319
14.3.3	Additional Roles and Targets for Box C/D RNAs	320
14.4	Box C/D RNP Structure and Nucleotide Methylation Function	321
14.4.1	Eukaryotic Box C/D Core Proteins and snoRNP Structure	321
14.4.2	Archaeal Box C/D Core Proteins and <i>In Vitro</i> sRNP Assembly	322
14.4.3	Emerging Core Protein and RNP Crystal Structures	322
14.4.4	Investigating Methylation Function Using <i>In Vitro</i> Assembled Archaeal Box C/D sRNP	323
14.5	Box C/D RNP Biogenesis	323
14.5.1	Genomic Organization of Eukaryotic Box C/D snoRNA Genes	323
14.5.2	Independently Transcribed and Intronic Eukaryotic Box C/D snoRNA Genes	324
14.5.3	Archaeal Box C/D sRNA Genes	324
14.5.4	Transcription and Processing of Independently Transcribed Box C/D snoRNAs	325
14.5.5	Transcription and Processing of Intronic Box C/D snoRNAs	326
14.5.6	Box C/D snoRNP Transport	327
14.6	Future Directions and Experimental Challenges	327
14.6.1	Box C/D RNA Diversity, Targets, and Functions	327
14.6.2	Box C/D RNP Structure and Methylation Function	328
14.6.3	Box C/D RNP Biogenesis	330
	Acknowledgments	331
	References	331

CHAPTER 15 STRUCTURAL FEATURES OF THE ADAR FAMILY OF ENZYMES AND THEIR SUBSTRATES

340

15.1	ADAR Enzymes	340
15.2	Overview and Functions of ADARs	341
15.2.1	Double-Stranded RNA Binding Domains (dsRBDs)	344
15.2.2	<i>Xenopus laevis</i> Xlrbpa	345
15.2.3	ADAR2 dsRBD1 and dsRBD2	346
15.2.4	Deaminase Domain	346
15.2.5	Z α and Z β Domains	347
15.2.6	Z α Structure	349
15.2.7	Z β Structure	351
15.2.8	Structural Comparison of Z α and Z β	354
15.3	Conclusions	354
15.4	Substrates	355
15.4.1	Overview and General Features	355
15.5	Double-Stranded RNA Targets and Structural Features	355
15.5.1	Site-Selective A-to-I Editing	355
15.5.2	Structural Features of Site-Selective A-to-I Editing	358
15.5.3	Promiscuous Editing	359

- 15.6 Single-stranded RNA Targets 361
- 15.7 Z-DNA and Z-RNA Targets 361
- 15.8 Future Directions 362
- References 362

CHAPTER 16 CHEMISTRY, PHYLOGENY, AND THREE-DIMENSIONAL STRUCTURE OF THE APOBEC PROTEIN FAMILY

369

- 16.1 Introduction to Nucleic Acid Deamination with Implications for Biological Activity 369
- 16.2 The Chemistry of the Zinc-Dependent Deaminase Amino Acid Signature Motif 370
- 16.3 The ZDD Signature Motif Implies a Specific Three-Dimensional Arrangement of Amino Acids 371
- 16.4 Rationale for a Combined Structural and Phylogenetic Approach to Understand APOBEC Evolution 373
 - 16.4.1 The Starting Point for Structural and Phylogenetic Analyses of APOBEC Family Members 375
 - 16.4.2 The CDA Superfamily: Overview of Conserved Fold Topology in the Core and Common Variations 379
 - 16.4.3 Comparison of the Common CDA Superfamily Core Reveals Broad Peripheral Diversification 381
- 16.5 Modes of Oligomerization 382
 - 16.5.1 Free Nucleotide Cytidine Deaminases (CDA): Strand $\beta 5$ Antiparallel to Strand $\beta 4$ 382
 - 16.5.2 Cytosine Deaminase, Guanine Deaminase, and TadA: Strand $\beta 5$ Parallel to $\beta 4$ 382
 - 16.5.3 Deoxycytidylate Deaminases (T4, *N. e*) and APOBEC2: Strand $\beta 5$ Parallel to $\beta 4$ 383
 - 16.5.4 Multidomain Enzymes RibG and ADAR2 of the CDA Superfamily: Strand $\beta 5$ Parallel to $\beta 4$ 387
- 16.6 Modes of Substrate Interaction 388
 - 16.6.1 Tetrameric fnCDAs Favor Flexible Flaps: RNA Editing and the Case of CddI from Yeast 390
 - 16.6.2 A Topological Transformation Obstructs Active Site Accessibility in Dimeric Deaminases that Bind Bases 391
 - 16.6.3 Substrate Selection by Polynucleotide Editing Enzymes Remains Elusive 391
- 16.7 The APOBEC Family: Insights into a Structurally Underrepresented Family 392
 - 16.7.1 Activation-Induced Deaminase (AID)—an Ancient Enzyme with Essential Roles in Adaptive Immunity 395
 - 16.7.2 APOBEC2—A Divergent Ancestral Protein of Unknown Function 396
 - 16.7.3 APOBEC1—The Historical Archetype of C-to-U Editing Enzymes 397
 - 16.7.4 APOBEC4—Pushing the Envelope of APOBEC Boundaries 399
- 16.8 APOBEC3—Radiative Expansion of Proteins Involved in Viral Defense 400
 - 16.8.1 Mechanisms of Primate-Specific Expansion of the APOBEC3 Proteins 402
 - 16.8.2 Alternative Methods to Obtain Structure: The Molecular Envelope of APOBEC3G by Small-Angle X-Ray Scattering 405
 - 16.8.3 Characterization of Structural Changes in APOBEC3G Morphology in the Presence of RNA 406

16.8.4 Positive Selection Exerted on the APOBEC3 Family	408
16.9 Conclusions and Future Prospects	410
References	411