

Table of contents

Modification and editing of RNA: historical overview and important facts to remember	1
Henri Grosjean.....	1
Abstract	1
1 Short historical background.....	1
1.1 Discovery of modified nucleosides.....	1
1.2 Discovery of RNA modification enzymes	3
1.3 RNA editing, a new concept	4
1.4 Different mechanisms for RNA modification/editing.....	7
1.5 Localization and temporal order of RNA modification/editing	9
2 Unraveling the functions of RNA modification/editing enzymes	11
2.1 Need to expand the limited RNA vocabulary	11
2.2 Not all nucleosides in RNAs are fully modified/edited	11
2.3 Fine-tuning of RNA structure and function	12
2.4 Few modifying enzymes play a dual role in RNA maturation.....	14
3 Conclusion and further prospects: unravel biological complexity	14
Acknowledgements	15
References	16
 Biosynthesis and function of tRNA wobble modifications	 23
Tsutomu Suzuki	23
Abstract	23
1 Introduction	23
1.1 The wobble rule and the role of RNA modification in decoding	23
1.2 Modified uridines.....	26
1.3 Modified adenosine (inosine)	26
1.4 Modified guanosines.....	27
1.5 Modified cytidines	28
2 Ribonucleome analysis: systematic identification of genes involved in RNA modifications by reverse genetics methods.....	28
2.1 Non-essential RNA modifications	28
2.2 Essential RNA modifications.....	31
3 Biosynthesis of lysidine.....	32
3.1 Identification of an essential gene responsible for lysidine formation.....	32
3.2 <i>In vitro</i> lysidine synthesis by TilS.....	34
3.3 Mechanism of lysidine synthesis	35
3.4 Direct conversion of the amino acid specificity of tRNA ^{Leu} due to the lysidine modification	36
3.5 The lysidine modification is essential for decoding AUA codons <i>in vivo</i>	37

3.6 Recognition of tRNA ^{UUC} by TilS	37
3.7 Evolution of wobble modifications and genetic code assignment of AUA codon	39
4 Wobble modification and subcellular localization of tRNAs.....	40
4.1 Post-transcriptional modifications control the subcellular localization of RNA molecules.....	40
4.2 Role of wobble modifications in the tRNA sorting mechanism in <i>Leishmania tarentolae</i>	41
5 Mitochondrial wobble modifications and human diseases.....	43
5.1 Mitochondrial wobble modifications and the minimal decoding system.....	43
5.2 Biosynthesis of taurinomethyluridines	47
5.3 Role of mitochondrial tRNA-specific 2-thiouridylase (MTU1) in the synthesis of tm ⁵ s ² U	49
5.4 Wobble modification defects in mitochondrial diseases.....	51
5.5 Molecular pathogeneses of mitochondrial diseases	53
6 RNA modification disorders as a cause of human diseases	56
7 Conclusion and outlook.....	58
7.1 Amino acid conjugation involved in RNA modifications.....	58
Acknowledgement.....	59
References.....	60

Editing and modification in trypanosomatids: the reshaping of non-coding RNAs	71
Mary Anne T. Rubio and Juan D. Alfonzo	71
Abstract	71
1 Introduction	71
2 RNA modification and trans-splicing.....	72
3 RNA editing and protein secretion	76
4 Role of modifications on sub-cellular localization of tRNAs	78
5 The story of tRNA ^{TRP} in trypanosomatids: where editing meets modification	81
6 Concluding remarks	83
Acknowledgements	84
References	84

Transfer RNA modifications and modifying enzymes in <i>Saccharomyces cerevisiae</i>.....	87
Marcus J.O. Johansson and Anders S. Byström	87
Abstract	87
1 Introduction	87
2 tRNA maturation	87
3 Modified nucleosides in tRNA	89
4 Genes required for formation of modified nucleosides in tRNA	91
4.1 Pseudouridine (Ψ).....	92
4.2 Dihydrouridine (D)	95

4.3 5-methyluridine (m ⁵ U).....	95
4.4 5-methoxycarbonylmethyluridine (mcm ⁵ U) and 5-methoxycarbonylmethyl-2-thiouridine (mcm ⁵ s ² U).....	98
4.5 7-methylguanosine (m ⁷ G).....	99
4.6 1-methylguanosine (m ¹ G).....	99
4.7 wybutosine (yW).....	100
4.8 N ² ,N ² -dimethylguanosine (m ₂ ² G).....	100
4.9 5-methylcytidine (m ⁵ C)	101
4.10 N ⁴ -acetylcytidine (ac ⁴ C).....	101
4.11 Inosine (I).....	102
4.12 1-methylinosine (m ¹ I)	102
4.13 1-methyladenosine (m ¹ A)	102
4.14 N ⁶ -isopentenyladenosine (i ⁶ A).....	103
4.15 2'-O-ribosyladenosine (phosphate) (Ar(p)).....	103
4.16 2'-O-methylations	104
5 Phenotypes of tRNA modification mutants.....	104
6 Genetic approaches to study function of modified nucleosides and their modifying enzymes	107
7 Concluding remarks and future prospects	109
Acknowledgements:	110
References	110
Abbreviations	119
Biosynthesis and function of 1-methyladenosine in transfer RNA.....	121
James T. Anderson and Louis Droogmans	121
Abstract	121
1 Introduction	121
2 The m ¹ A methyltransferases (MTases)	123
3 m ¹ A influences tRNA structure	124
3.1 m ¹ A58 and tRNA structure	125
3.2 m ¹ A9 in mitochondrial tRNA structure and function	126
4 m ¹ A58 and HIV replication	128
5 m ¹ A58 function in stabilizing tRNA _i ^{Met} from <i>S. cerevisiae</i>	129
6 Conclusions and perspectives.....	134
Acknowledgements	134
References	135
The biosynthesis and functional roles of methylated nucleosides in eukaryotic mRNA.....	141
Joseph A. Bokar	141
Abstract	141
1 Introduction	141
2 Methylated nucleosides present in eukaryotic mRNA	142
2.1 The 5'-terminal cap structure.....	142

2.2 Biological function of methylated nucleosides within the cap structure	143
2.3 Enzymes involved in cap methylation	146
3 Modified nucleosides at internal positions in eukaryotic mRNA.....	148
3.1 Nucleoside modification by deamination	148
3.3 N6-methyladenosine	149
3.4 Function of m ⁶ A in mRNA	153
4 Characterization and purification of HeLa mRNA N ⁶ -adenosine methyltransferase	156
4.1 Purification and cDNA cloning of the AdoMet-binding subunit....	158
4.2 Further characterization of MT-B	158
4.3 Subnuclear localization of MT-A70 in HeLa cells	159
4.4 MT-A70 is the prototype of a previously undescribed class of putative RNA adenosine methyltransferases in a wide variety of organisms.....	159
5 IME4 is the <i>S. cerevisiae</i> ortholog of MT-A70	161
5.1 m ⁶ A is present in mRNA isolated from sporulating yeast.....	163
5.2 IME4 is necessary for m ⁶ A formation in sporulating yeast	164
6 MT-A70 is critical for viability of mammalian cell lines.....	167
6.1 RNA interference transfection	167
6.2 Loss of MT-A70 leads to HeLa cell apoptosis	168
7 Conclusion	170
References	171

Role of the 5'-cap in the biogenesis of spliceosomal snRNPs.....	179
Achim Dickmanns and Ralf Ficner	179
Abstract	179
1 Introduction	179
2 snRNP biogenesis.....	180
2.1 Transcription of snRNAs	180
2.2 m ⁷ G-dependent nuclear export of UsrnRNAs.....	181
2.3 Assembly of the snRNP core structure (SMN complex)	183
2.4 m ⁷ G-cap hypermethylation	185
2.5 Nuclear import.....	187
2.6 Sub-nuclear localization	188
3 Structural basis for m ⁷ G- and m ₃ G-cap recognition by proteins	190
3.1 Three-dimensional structures of m ⁷ G-cap binding proteins	190
3.2 Three-dimensional structure of the m ₃ G-cap binding domain of human snurportin1	192
4 Conclusions and outlook	194
Acknowledgements	195
References	195

Role of a conserved pseudouridine in U2 snRNA on the structural and electrostatic features of the spliceosomal pre-mRNA branch site	205
Nancy L. Greenbaum	205
Abstract	205
1 Introduction	205
1.1 The spliceosome	205
1.2 Modified bases in structural RNAs	206
2 Structure of branch site duplexes	209
2.1 Structural features of the unmodified branch site helix	209
2.2 Structural features of the ψ -modified branch site helix	210
3 Stabilization of the ψ -modified pre-mRNA branch site helix by interactions with water molecules	212
3.1 Global interactions with solvent	212
3.2 Local interactions with water	213
4 Global features of the ψ -modified branch site helix	214
4.1 Electrostatic surface features of the ψ -dependent branch site helix	214
5 Extrapolation from the branch site duplex to the native context	216
6 Functional role of ψ in the branch site	217
7 Outlook.....	217
Acknowledgements	218
References	218
Mechanisms and functions of RNA-guided RNA modification	223
Yi-Tao Yu, Rebecca M. Terns, and Michael P. Terns	223
Abstract	223
1 Introduction	223
2 Discovery of eukaryotic snoRNAs that guide rRNA modifications.....	224
2.1 Early studies of snoRNAs	225
2.2 Two classes of snoRNAs—box C/D and box H/ACA snRNAs	226
2.3 Discovery that Box C/D snoRNAs guide rRNA 2'- <i>O</i> -methylation	226
2.4 Discovery that Box H/ACA snoRNAs guide rRNA pseudouridylation	228
2.5 Toward identification of complete sets of rRNA modification guide snoRNAs	228
3 RNAs also guide the pseudouridylation and 2'- <i>O</i> -methylation of snRNAs	229
4 sno/scaRNAs may also guide mRNA modifications.....	232
5 Small RNA–guided RNA modification of rRNA and tRNA in archaea	233
6 Gene organization and biosynthesis of snoRNAs	234
7 Modification guide RNAs function as RNA-protein complexes.....	235
7.1 Protein components of methylation guide RNP.....	236

7.2 Protein components of pseudouridylation guide RNPs	236
7.3 Evolutionary relationships between archaeal and eukaryotic modification guide RNPs.....	237
8 Assembly and structural organization of modification guide RNPs	239
8.1 Methylation guide RNP structure	239
8.2 Pseudouridylation guide RNP structure.....	243
9 Function of pseudouridylation and 2'- <i>O</i> -methylation.....	246
9.1 rRNA modifications occur primarily in functionally important regions of the ribosome	247
9.2 rRNA modifications in the peptidyl transferase center contribute to ribosome function and cell growth	247
9.3 Spliceosomal snRNA modifications are required for pre-mRNA splicing	248
9.4 How do modified nucleotides contribute to RNA function?	250
9.5 Are RNA modifications reversible?.....	250
10 Concluding remarks	251
Acknowledgments.....	251
References.....	252

Conserved ribosomal RNA modification and their putative roles in ribosome biogenesis and translation.....

Bruno Lapeyre	263
Abstract	263
1 Introduction.....	263
2 Pseudouridylation conserved in bacteria and eukaryotes.....	267
3 Base modification and their enzymes.....	268
4 2'- <i>O</i> -ribose methylations conserved in bacteria and eukaryotes.....	270
5 The outstanding case of Spb1p: an essential site-specific enzyme in a world of snoRNA-guided modifications	271
5.1 The universally conserved U _a m is catalyzed by the site-specific MTase RrmJ in bacteria.....	272
5.2 <i>S. cerevisiae</i> possesses three homologs of RrmJ	272
6 Perspectives: how can modifications extend the ability of RNA molecules	278
Acknowledgements	279
References.....	279

Nucleotide methylations in rRNA that confer resistance to ribosome-targeting antibiotics

Stephen Douthwaite, Dominique Fourmy, and Satoko Yoshizawa	285
Abstract	285
1 Introduction.....	285
2 Ribosomal RNA modifications	286
3 The antibiotic resistance rRNA methyltransferases	287
4 Resistance to antibiotics targeting the small subunit rRNA	289
4.1 The case of kasugamycin resistance	290

4.2 Resistance by methylation of the small subunit rRNA	290
5 Resistance by methylation of the large subunit rRNA	294
5.1 Thiopeptide antibiotics.....	294
5.2 Orthosomycin antibiotics	295
5.3 MLS _B antibiotics.....	296
6 Synergistic effects of dual rRNA methylations	298
7 Conclusion and future perspectives.....	299
Acknowledgements	300
References	300
 Translational Recoding and RNA Modifications.....	309
Olivier Namy, Fran�ois Lecointe, Henri Grosjean, and Jean-Pierre Rousset.....	309
Abstract	309
1 Introduction	309
1.1 Recoding events	309
1.2 The stimulatory recoding signals	310
1.3 Modified nucleotides in RNA and decoding.....	311
1.4 Complexity of the decoding process within the ribosome	314
1.5 Testing the roles of modified nucleotides of RNA in recoding	314
2 Influence of modified tRNA nucleotides in frameshifting	316
2.1 Programmed +1 frameshifting in bacteria	316
2.2 Programmed +1 frameshifting in Eukarya	318
2.3 Programmed -1 frameshifting in Bacteria and Eukarya.....	320
3 Modified nucleotides in tRNA also affect stop codon readthrough efficiency	323
4 Conclusions and Perspectives	326
4.1 Decoding rules of recoding process are special	328
4.2 <i>Trans</i> -recoding elements are complex and difficult to identify	328
4.3 Role of modified nucleotides in both tRNA and rRNA	330
Acknowledgements	331
References	331
Data bases.....	340
 Adenosine to inosine RNA editing in animal cells	341
Barry Hoopengardner, Mary A. O'Connell, Robert Reenan, and Liam P. Keegan	341
Abstract	341
1 Introduction: ADAR RNA editing in vertebrates	341
1.1 Functional studies on vertebrate ADAR2	345
1.2 Functional studies on vertebrate ADAR1	346
1.3 Other <i>ADAR</i> genes in vertebrates.....	349
1.4 Searches for edited transcripts in human cells	350
2 RNA editing in <i>Drosophila</i>	351
2.1 Edited transcripts in <i>Drosophila</i> : from serendipity to systematic identification	353

3 RNA editing in squid	356
4 RNA editing in <i>C. elegans</i>	356
Conclusion	357
Acknowledgments.....	359
References.....	359
 Mammalian C to U editing	 365
Harold C. Smith, Joseph E. Wedekind, Kefang Xie, and Mark P. Sowden...	365
Abstract	365
1 Introduction.....	365
2 Site-specific <i>apoB</i> mRNA editing: the basic facts	366
3 Characteristics of the RNA substrate	367
4 APOBEC-1.....	369
4.1 Requirement of APOBEC-1 for C to U mRNA editing.....	369
4.2 Catalytic residues, RNA binding and oligomerization of APOBEC-1	370
4.3 Post-translational modification of APOBEC-1	372
4.4 The conserved deaminase fold.....	373
4.5 Comparative models of APOBEC-1 and AID	373
4.6 APOBEC-1 and dC to dU DNA mutation	376
4.7 APOBEC-1 and neoplasia	377
5 Auxiliary proteins.....	378
5.1 Emergence of the C to U editosome concept	378
5.2 The editosome	379
5.3 APOBEC-1 complementation factor (ACF)	379
5.4 Other auxiliary proteins	380
6 Subcellular distribution of editing factors	381
6.1 APOBEC-1	381
6.2 ACF	382
6.3 Regulation of apoB mRNA stability	383
7 Regulation of apoB mRNA editing	383
8 Prospective for APOBEC-1 and APOBEC-1 related proteins	385
Acknowledgements	388
References.....	388
 Transfer RNA modifications and DNA editing in HIV-1 reverse transcription	 401
Roland Marquet and Frédéric Dardel	401
Abstract	401
1 Introduction.....	401
2 tRNA modification and HIV-1 reverse transcription	402
2.1 Function of the modified nucleotides of tRNA.....	402
2.2 Selective uptake of primer tRNA into the viral particle	404
2.3 Initiation of reverse transcription.....	405
2.4 Plus strand strong stop synthesis	410
3 DNA editing and HIV-1 reverse transcription	411

3.1 Vif: a viral infectivity factor that counteracts a cellular restriction factor	411
3.2 Cytosine deamination as an innate antiviral activity.....	412
3.3 Vif neutralizes APOBEC3G	416
4 Conclusions	419
Acknowledgements	420
References	420
Um34 in selenocysteine tRNA is required for the expression of stress-related selenoproteins in mammals	431
Bradley A. Carlson, Xue-Ming Xu, Vadim N. Gladyshev, and Dolph L. Hatfield	431
Abstract	431
1 Introduction	431
2 Sec tRNA ^{[Ser]Sec}	432
3 Generation of mouse models.....	433
3.1 Selective rescue of selenoprotein expression.....	435
4 Discussion and concluding remarks	436
References	437
Index	439