

Contents

	Series Editors' Preface	<i>xiii</i>
	Preface	<i>xv</i>
1	Introduction	<i>1</i>
	<i>John Schneekloth Jr. and Martin Pettersson</i>	
	References	<i>4</i>
2	RNA Structure Probing, Dynamics, and Folding	<i>7</i>
	<i>Danny Incarnato</i>	
2.1	Introduction	<i>7</i>
2.1.1	Relevance of RNA Structure in Disease	<i>8</i>
2.1.2	Challenges in Studying RNA Structures	<i>8</i>
2.2	Experimentally Guided RNA Structure Modeling	<i>9</i>
2.2.1	Structural Interrogation of RNA Nucleotides via Chemical Probing	<i>10</i>
2.2.1.1	Limits of RNA Chemical Probing	<i>12</i>
2.2.2	Direct Mapping of RNA–RNA Interactions	<i>14</i>
2.2.2.1	Limits of RNA–RNA Interaction Mapping	<i>16</i>
2.2.3	Mapping Spatially Proximal Nucleotides in RNA molecules	<i>17</i>
2.2.3.1	Limits of Methods for Spatial Proximity Mapping	<i>17</i>
2.3	Dealing with RNA Structure Heterogeneity	<i>19</i>
2.4	Querying RNA–Small Molecule Interactions with Chemical Probing	<i>22</i>
2.5	Conclusions and Future Prospects	<i>22</i>
	References	<i>23</i>
3	High-Resolution Structures of RNA	<i>29</i>
	<i>Lukas Braun, Zahra Alirezaeizanjani, Roberta Tesch, and Hamed Kooshapur</i>	
3.1	Introduction	<i>29</i>
3.2	X-Ray Crystallography	<i>31</i>
3.3	NMR Spectroscopy	<i>34</i>
3.4	Cryo-EM	<i>37</i>
3.5	3D Structure Prediction and Integrative Approaches	<i>39</i>
3.6	Conclusions	<i>43</i>
	Acknowledgments	<i>43</i>
	Conflicts of Interest	<i>43</i>
	References	<i>43</i>

4	Screening and Lead Generation Techniques for RNA Binders	49
	<i>Gary Frey, Emily Garcia Segal, and Neil Lajkiewicz</i>	
4.1	Knowledge-Based Versus Agnostic Screening	49
4.2	Virtual Screening	50
4.3	Screening Methods	51
4.3.1	High-Throughput Screening (HTS)	51
4.3.1.1	Mass Spectrometry	51
4.3.1.2	HTS of RNA Using Direct MS Approaches	52
4.3.1.3	HTS of RNA Using Indirect MS Approaches	54
4.3.1.4	DNA-Encoded Libraries (DELs)	56
4.3.1.5	Microarray Screening	57
4.3.1.6	Fragment-Based Drug Discovery	58
4.3.1.7	Phage Display	63
4.3.2	Orthogonal Methods	63
4.3.2.1	Surface Plasmon Resonance	63
4.3.2.2	Fluorescence-Based Assays	66
4.3.2.3	Microscale Thermophoresis (MST)	70
4.3.2.4	Isothermal Titration Calorimetry (ITC)	70
4.4	Binding Site Identification/Target Engagement	72
4.4.1	Covalent Methods	72
4.4.2	Competition with an Antisense Oligonucleotide (ASO)	74
4.5	Defining SAR and Functional Assays	75
4.5.1	Functional Assays	75
4.5.2	Phenotypic Screens	76
4.6	Identifying a Lead Series	76
4.6.1	Hit Optimization	77
4.6.2	Risdiplam Hit-to-Lead	78
4.6.3	Branaplam Lead Generation	79
4.6.4	Zotatifin Lead Generation	80
4.7	Concluding Thoughts and Outlook	80
	Acknowledgments	81
	References	81
5	Chemical Matter That Binds RNA	93
	<i>Emily G. Swanson Hay, Zhengguo Cai, and Amanda E. Hargrove</i>	
5.1	Introduction	93
5.2	Natural Ligands	94
5.2.1	Aminoglycosides	94
5.2.2	Tetracyclines	95
5.2.3	Macrolides	96
5.2.4	Native Riboswitch Ligands	96
5.3	Commercial Ligands	97
5.3.1	Industrial Libraries	98
5.3.2	Academic Libraries	98

5.4	Synthetic Ligands	99
5.4.1	Benzimidazoles and Purines	100
5.4.2	Naphthalenes, Quinolines, and Quinazolines	101
5.4.3	Oxazolidinones	102
5.4.4	Amilorides	102
5.4.5	Diphenyl Furan	103
5.4.6	Multivalent Ligands	103
5.5	Computational Tools for the Exploration of Chemical Space	103
5.5.1	Similarity Searches and Principal Component Analysis	104
5.5.2	Additional Machine-Learning Tools	105
5.5.3	Structure-Based Ligand Design	106
5.6	Case Studies in Examining and Expanding RNA-Targeted Chemical Space	106
5.6.1	Using QSAR to Probe RNA-Targeting Small-Molecule Properties	107
5.6.2	Evaluating the Chemical Space of Natural, Synthetic, and Commercial Ligands	108
5.7	Conclusions and Outlook	111
	Acknowledgments	111
	References	111
6	MicroRNAs as Targets for Small-Molecule Binders	119
	<i>Maria Duca</i>	
6.1	Introduction	119
6.2	MicroRNAs	121
6.3	MicroRNAs Biogenesis	122
6.4	Targeting MicroRNAs with Small-Molecule RNA Binders	123
6.4.1	Induction of miRNAs Expression: Tackling the Decrease of Tumor Suppressor miRNAs	124
6.4.2	Inhibition of miRNAs Production: Pre- and Pri-miRNA Binders	125
6.4.2.1	Discovery of miRNAs Inhibitors by Intracellular Assays	125
6.4.2.2	Target-Based <i>In Vitro</i> Assays	127
6.4.2.3	Design of Specific Ligands of Pre- and Pri-miRNAs	131
6.4.2.4	Fragment-Based Drug Design	138
6.4.2.5	DNA-Encoded Libraries (DELs)	139
6.5	Inhibition of RNA-Protein Interactions in miRNAs Pathways	140
6.6	Adding Cleavage Properties to miRNAs Interfering Agents	142
6.7	Conclusions	144
	References	144
7	Pre-mRNA Splicing Modulation	151
	<i>Scott J. Barraza and Matthew G. Woll</i>	
7.1	Introduction	151
7.2	Overview of Splicing Biology	152
7.2.1	The Spliceosome	152
7.2.2	Classes of Alternative Splicing	154

7.3	Pharmacological Mechanisms of Splicing Modulation	155
7.3.1	<i>Cis</i> - and <i>Trans</i> -Regulatory Elements (Splicing Factors)	155
7.3.1.1	Stabilization of <i>Cis</i> -Regulatory Elements	156
7.3.1.2	Destabilization of <i>Cis</i> -Regulatory Elements	158
7.3.1.3	Inhibition of <i>Cis</i> -Regulatory RNA–Protein Interactions	158
7.3.1.4	Inhibition of <i>Trans</i> -Regulatory Elements	160
7.3.1.5	Degradation of <i>Trans</i> -Regulatory Elements	161
7.3.1.6	Inhibition of <i>Trans</i> -Regulatory Element Protein–Protein Interactions (PPIs)	162
7.3.1.7	Stabilization of <i>Trans</i> -Regulatory Element RNA–Protein Interactions (RPIs)	165
7.3.2	Kinases and Phosphatases	165
7.3.2.1	Challenges in Targeting Kinases	167
7.3.2.2	Inhibition of Kinases	168
7.3.2.3	Activation and Degradation of Kinases	168
7.3.2.4	Inhibition and Activation of Protein Phosphatases	169
7.3.3	Epigenetic Writers and Erasers	172
7.3.3.1	Inhibition of Epigenetic Writers	172
7.3.4	RNA Helicases	174
7.3.5	Drugging the Spliceosome	175
7.3.5.1	Inhibition of U2 snRNP Recognition of the 3′-Splice Site	176
7.3.5.2	E7107	176
7.3.5.3	H3B-8800	177
7.3.5.4	Stabilizers of U1 snRNP Recognition of the 5′-Splice Site	180
7.3.5.5	Introduction to Spinal Muscular Atrophy (SMA)	180
7.3.5.6	Risdiplam (Evrysdi®)	183
7.4	Future Outlook	186
	References	188
8	Prospects for Riboswitches in Drug Development	203
	<i>Michael G. Mohsen and Ronald R. Breaker</i>	
8.1	Introduction	203
8.1.1	The Known Landscape of Riboswitches	203
8.1.2	Riboswitches in Drug Development	203
8.1.3	The Need for Novel Antibiotics	205
8.2	Riboswitches as Drug Targets	207
8.2.1	Why Target Riboswitches?	207
8.2.2	Features of a Druggable Riboswitch	208
8.2.3	Riboswitch-Targeted Drugs	208
8.2.3.1	Small Molecules Targeting FMN Riboswitches	208
8.2.3.2	Other Riboswitches Targeted in Proof-of-Principle Demonstrations	209
8.2.4	Barriers and Future Developments	210
8.3	Riboswitches as Tools for Antibiotic Drug Development	210
8.3.1	Riboswitches as Biosensors	210
8.3.2	A Riboswitch-Based Fluoride Sensor Illuminates Agonists of Fluoride Toxicity	211

- 8.3.3 A Riboswitch-Based ZTP Sensor Identifies Inhibitors of Folate Biosynthesis 211
- 8.3.4 A Riboswitch-Based SAH Sensor Reveals an Inhibitor of SAH Nucleosidase 212
- 8.3.5 Barriers and Future Developments 213
- 8.4 Application of Riboswitches in Gene Therapy 213
- 8.4.1 Considerations for Designer Riboswitches 213
- 8.4.2 Eukaryotic Expression Platforms 214
- 8.4.3 Barriers and Future Developments 216
- 8.5 Concluding Remarks 217
- Acknowledgment 218
- References 218

- 9 Small Molecules That Degrade RNA 227**
- Noah A. Springer, Samantha M. Meyer, Amirhossein Taghavi, Jessica L. Childs-Disney, and Matthew D. Disney*
- 9.1 Antisense Oligonucleotide Degraders 227
- 9.2 Small-Molecule Direct Degraders 228
- 9.2.1 *N*-Hydroxypyridine-2(1*H*)-thione (*N*-HPT) Conjugates 229
- 9.2.2 Bleomycin 229
- 9.2.3 Bleomycin Conjugates 231
- 9.2.3.1 Bleomycin Degraders Targeting the r(CUG) Repeat Expansion That Causes DM1 231
- 9.2.3.2 Bleomycin Degraders Targeting r(CCUG) Repeat Expansion that Causes DM2 233
- 9.2.3.3 Bleomycin Degraders Targeting Oncogenic Precursor microRNAs 233
- 9.2.3.4 Conclusions and Outlook for Bleomycin-Based Direct Degraders 234
- 9.3 Ribonuclease Targeting Chimeras (RiboTACs) 235
- 9.3.1 RNase L is an Endogenous Endoribonuclease That Functions as Part of the Innate Immune Response 236
- 9.3.2 First-Generation RiboTACs Targeting Oncogenic miRNAs 236
- 9.3.3 Small-Molecule-Based RiboTACs 239
- 9.3.4 Comparison of Bleomycin-Based Direct Degraders and RiboTACs 242
- 9.3.5 Discovery of Additional Small-Molecule RNase L Activators 242
- 9.3.6 Conclusions and Outlook for RiboTACs 243
- 9.4 Summary and Outlook for Small-Molecule RNA Degraders 244
- References 246

- 10 Approaches to the Identification of Molecules Altering Programmed Ribosomal Frameshifting in Viruses 253**
- Elinore A. VanGraafeiland, Diego M. Arévalo, and Benjamin L. Miller*
- 10.1 Introduction 253
- 10.2 Mechanisms of Frameshifting 256
- 10.3 Targeting Frameshifting in HIV 257
- 10.4 Targeting Frameshifting in SARS-CoV-1 and SARS-CoV-2 263
- 10.5 Conclusions 274
- References 274

11	RNA–Protein Interactions: A New Approach for Drugging RNA Biology	281
	<i>Dalia M. Soueid and Amanda L. Garner</i>	
11.1	Molecular Basis of RNA–Protein Interactions	282
11.1.1	RNA Recognition Motifs (RRMs)	282
11.1.2	Double-Stranded RNA-Binding Domains (dsRBD)	286
11.1.3	Zinc Finger (ZnF) Domains	287
11.1.4	K Homology (KH) Domains	289
11.1.5	Other RBDs	290
11.2	Regulation and Dysregulation of RNA–Protein Interactions	290
11.2.1	Poor Quality Control Leads to Over- and Underproduction of RBPs	292
11.2.2	RBPs Become Out of Control, mRNA Processing Gets a Makeover (and Hates It)	294
11.2.3	RBP Shuttling of mRNA Becomes Askew	294
11.2.4	The RBP is Lost and Wreaks Havoc on the Cell	295
11.2.5	RBPs Dictate Which mRNAs are Translated, Favoring their Toxic Friends	295
11.2.6	RBPs and RNA Become Very Clique-y, Form Their Own Complex and Cause Stress to the Rest of the Cell	296
11.3	Experimental Methods to Detect and Screen for Small Molecules that Modulate RNA–Protein Interactions	297
11.3.1	<i>In vitro</i> Fluorescence-Based Assays	297
11.3.2	<i>In vitro</i> Chemiluminescence-Based Assays	297
11.3.2.1	Cell-Based RPI Detection Assays	300
11.3.3	Cell-Based RNA–Protein Interaction Screening	301
11.4	Closing Remarks	302
	References	303
12	Drugging the Epitranscriptome	321
	<i>Tanner W. Eggert and Ralph E. Kleiner</i>	
12.1	Introduction	321
12.2	Modifications on mRNA: <i>N</i> ⁶ -Methyladenosine, Pseudouridine, and Inosine	325
12.2.1	<i>N</i> ⁶ -Methyladenosine (m ⁶ A)	325
12.2.2	Pseudouridine (Ψ)	327
12.2.3	Inosine (I)	328
12.3	Modifications on tRNA and rRNA	330
12.3.1	tRNA Modifications	330
12.3.2	rRNA Modifications	334
12.4	Concluding Remarks	335
	References	336

13	Outlook	355
	<i>Christopher R. Fullenkamp, Xiao Liang, Martin Pettersson, and John Schneekloth Jr.</i>	
13.1	Introduction	355
13.2	Target Selection: Identification of the Most Promising RNA Intervention Points	357
13.3	Development of Robust Biophysical Methods, Alternative Strategies for Target Engagement, and Accurate and Reliable Functional Models	358
13.3.1	Biophysical Methods for Interrogating Small Molecule–RNA Interactions	358
13.3.2	Cellular Target Engagement Methods	360
13.3.3	Unique Challenges Faced in the Development of Functional Assays for Studying Small Molecule–RNA Interactions	364
13.4	Acquisition of High-Resolution RNA and RNA–Ligand Structures is Needed to Enable the Development and Validation of Computational Tools for RNA–Small Molecule Therapeutic Discovery	367
13.4.1	RNA Structure Prediction	367
13.4.2	Computational Tools for Hit Optimization	369
13.4.3	Implementation of Molecular Dynamics Simulations, Machine Learning, and AI Tools to Interrogate RNA–Small Molecule Interactions	371
13.5	Deposition of Small Molecule–RNA Interaction Data with Rigorous Experimental Protocols and Controls is Needed	373
13.6	Outlook: The Future of Small Molecule-Based RNA Therapeutics is Bright	375
	References	376
	Index	385

