

Contents

Chapter 1 Ribozymes and RNA Catalysis: Introduction and Primer

David M.J. Lilley and Fritz Eckstein

1.1	What are Ribozymes?	1
1.2	What is the Role of Ribozymes in Cells?	1
1.3	Ribozymes Bring about Significant Rate Enhancements	4
1.4	Why Study Ribozymes?	4
1.5	Folding RNA into the Active Conformation	5
1.6	The Catalytic Resources of RNA – Making a Lot of a Little	6
1.7	Mechanisms and Catalytic Strategies of Ribozymes	7
1.8	Impact of New Methodologies to Study Ribozymes	8
1.9	Finally	8
	References	8

Chapter 2 Proton Transfer in Ribozyme Catalysis

Philip C. Bevilacqua

2.1	Scope of Chapter and Rationale	11
2.2	Overview of Proton Transfer Chemistry	12
2.3	General Considerations for Proton Transfer in RNA Enzymes	17
2.3.1	Classes of Protonation Sites in RNA	17
2.3.2	Driving Forces for pK_a Shifting in RNA	18
2.3.3	Quantitative Contributions of Proton Transfer to RNA Catalysis	19
2.4	Proton Transfer in Small Ribozymes: Five Case Studies	20
2.4.1	Why Small Ribozymes?	20
2.4.2	Proton Transfer in the Hepatitis Delta Virus Ribozyme	22
2.4.3	Proton Transfer in the Hairpin Ribozyme	27
2.4.4	Proton Transfer in the Hammerhead Ribozyme	28

<i>Contents</i>	xiii
2.4.5 Proton Transfer in the VS Ribozyme	29
2.4.6 Proton Transfer in the <i>glmS</i> Ribozyme	30
2.5 Conclusion and Perspectives	31
Acknowledgement	32
References	32
Chapter 3 Finding the Hammerhead Ribozyme Active Site	
<i>Dominic Lambert and John M. Burke</i>	
3.1 Introduction	37
3.2 Background	38
3.3 Experimental Data	40
3.3.1 Mechanistic Hypothesis Leads to Identification and Functional Test of Active Site Components	40
3.3.2 Structural Hypothesis – Large-scale Conformational Changes are Required for Catalysis	41
3.3.3 Molecular Modeling of a Hammerhead Active Fold that Satisfies Structural and Biochemical Constraints	43
3.4 Current Status and Future Prospects	46
Acknowledgements	46
References	46
Chapter 4 Hammerhead Ribozyme Crystal Structures and Catalysis	
<i>William G. Scott</i>	
4.1 Introduction	48
4.2 A Catalytic RNA Prototype	49
4.3 A Small Ribozyme	49
4.4 Chemistry of Phosphodiester Bond Isomerization	50
4.5 Hammerhead Ribozyme Structure	51
4.6 Catalysis in the Crystal	53
4.7 Making Movies from Crystallographic Snapshots	53
4.8 An Ever-growing List of Concerns	55
4.9 Occam’s Razor Can Slit Your Throat	56
4.10 Structure of a Full-length Hammerhead Ribozyme	57
4.11 Do the Minimal and Full-length Hammerhead Crystal Structures have Anything in Common?	59
4.12 How Does the Minimal Hammerhead Work?	60
4.13 A Movie Sequel with a Happy Ending	61
4.14 Concluding Remarks	62
Acknowledgements	62
References	62

Chapter 5 The Hairpin and Varkud Satellite Ribozymes*David M.J. Lilley*

5.1	Nucleolytic Ribozymes	66
5.2	Hairpin Ribozyme	66
5.2.1	Structure of the Hairpin Ribozyme	67
5.2.2	Metal Ion-dependent Folding of the Hairpin Ribozyme	69
5.2.3	Observing the Cleavage and Ligation Activities of the Hairpin Ribozyme	71
5.2.4	Mechanism of the Hairpin Ribozyme	73
5.3	VS Ribozyme	76
5.3.1	Structure of the VS Ribozyme	77
5.3.2	Structure of the Substrate	80
5.3.3	Location of the Substrate	80
5.3.4	Active Site of the VS Ribozyme	82
5.3.5	Candidate Catalytic Nucleobases	82
5.3.6	Mechanism of the VS Ribozyme	84
5.4	Some Striking Similarities between the Hairpin and VS Ribozymes	88
	Acknowledgements	88
	References	88

Chapter 6 Catalytic Mechanism of the HDV Ribozyme*Selene Koo, Thaddeus Novak and Joseph A. Piccirilli*

6.1	Introduction	92
6.1.1	Hepatitis Delta Virus Biology	92
6.1.2	Cleavage Reactions of Small Ribozymes	93
6.2	HDV Ribozyme Structure	95
6.2.1	Determination of Crystal Structures	95
6.2.2	Structure Overview	97
6.2.3	Active Site	97
6.3	Catalytic Strategies for RNA Cleavage	99
6.4	The Active Site Nucleobase: C75	100
6.4.1	Exogenous Base Rescue Reactions	101
6.4.2	Role of C75 in HDV Catalysis	103
6.4.3	Resolving the Kinetic Ambiguity	105
6.4.3.1	Reaction in the Absence of Divalent Cations	105
6.4.3.2	Sulfur Substitution of the Leaving Group	106
6.5	Metal Ions in the HDV Ribozyme	108
6.5.1	Structural Metal Ions	108
6.5.2	Catalytic Metal Ions	111

6.6	Contributions of Non-active-site Structures to Catalysis	112
6.7	Dynamics in HDV Function	113
6.8	Varieties of Experimental Systems	115
6.9	Models for HDV Catalysis	117
6.10	Conclusion	119
	Acknowledgements	120
	References	120
Chapter 7	Mammalian Self-Cleaving Ribozymes	
	<i>Andrej Lupták and Jack W. Szostak</i>	
7.1	Introduction	123
7.2	General Features of Small Self-cleaving Sequences	124
7.3	Genome-wide Selection of Self-cleaving Ribozymes	124
7.4	<i>CPEB3</i> Ribozyme	125
7.4.1	Expression of the <i>CPEB3</i> Ribozyme	126
7.4.2	Structural Features of the <i>CPEB3</i> and HDV Ribozymes	127
7.4.3	Linkage of HDV to the Human Transcriptome	129
7.5	Possible Biological Roles of Self-cleaving Ribozymes	130
7.6	Closing Remarks	131
	References	131
Chapter 8	The Structure and Action of <i>glmS</i> Ribozymes	
	<i>Kristian H. Link and Ronald R. Breaker</i>	
8.1	Introduction	134
8.2	Biochemical Characteristics of <i>glmS</i> Ribozymes	136
8.2.1	Divalent Metal Ions Support Structure and Not Chemistry	136
8.2.2	Ligand Specificity of <i>glmS</i> Ribozymes	137
8.2.3	Evidence for a Coenzyme Role for GlcN6P	139
8.3	Atomic-resolution Structure of <i>glmS</i> Ribozymes	141
8.3.1	Secondary and Tertiary Structures of <i>glmS</i> Ribozymes	141
8.3.2	Metabolite Recognition by <i>glmS</i> Ribozymes	143
8.4	Mechanism of <i>glmS</i> Ribozyme Self-cleavage	145
8.5	Can <i>glmS</i> Ribozymes be Drug Targets?	148
8.6	Conclusions	149
	References	150

Chapter 9 A Structural Analysis of Ribonuclease P

Steven M. Marquez, Donald Evans, Alexei V. Kazantsev and Norman R. Pace

9.1	Introduction	153
9.2	Chemistry of RNase P RNA	155
9.2.1	Universal	155
9.2.2	S_N2 -type Reaction	155
9.2.3	pH-Dependence of the Reaction: Hydroxide Ion as the Nucleophile	157
9.2.4	Metal Ions in Catalysis	157
9.3	Phylogenetic Variation and Structure of RNase P RNA	158
9.4	Early Studies of the RNase P RNA Structure	159
9.5	Crystallographic Studies of Bacterial RNase P RNAs	160
9.6	Modeling an RNase P RNA:tRNA Complex	162
9.7	Modeling the Bacterial RNase P Holoenzyme	163
9.8	Substrate Recognition	165
9.9	Archaeal and Eucaryal Holoenzymes – More Proteins	166
9.10	Concluding Remarks	170
	Acknowledgements	171
	References	171

Chapter 10 Group I Introns: Biochemical and Crystallographic Characterization of the Active Site Structure

Barbara L. Golden

10.1	Group I Intron Origins	178
10.2	Group I Intron Self-splicing	178
10.3	What has Changed in Group I Intron Knowledge in the Last Decade	181
10.4	Structure of Group I Introns	181
10.5	Crystallography of Group I Introns	182
10.5.1	<i>Tetrahymena</i> LSU P4-P6 Domain	182
10.5.2	<i>Tetrahymena</i> Intron Catalytic Core	183
10.5.3	Twort orf142-I2 Ribozyme	183
10.5.4	<i>Azoarcus</i> sp. BBH72 tRNA ^{Ile} Intron	184
10.6	Structural Basis for Group I Intron Self-splicing	184
10.6.1	Recognition of the 5'-Splice Site	185
10.6.2	Does the Ribozyme Undergo Conformational Changes upon P1 Docking?	186
10.6.3	A Binding Pocket for Guanosine	187
10.6.4	Packed Stacks	189

<i>Contents</i>		xvii
10.7	Biochemical Characterization of the Structure	191
10.7.1	Metal Ion Binding and Specificity Switches	191
10.7.2	Identification of Ligands to the Catalytic Metal Ions	192
10.7.3	Correlation with Metal Ion Binding Sites within the Crystal Structures	193
10.7.4	Nucleotide Analog Interference Techniques	194
10.8	What Makes a Catalytic Site?	196
10.9	Back to the Origins	197
	References	198

Chapter 11 Group II Introns: Catalysts for Splicing, Genomic Change and Evolution

Anna Marie Pyle

11.1	Introduction: The Place of Group II Introns Among the Family of Ribozymes	201
11.2	The Basic Reactions of Group II Introns	201
11.3	The Biological Significance of Group II Introns	204
11.3.1	Evolutionary Significance	204
11.3.2	Significance and Prevalence in Modern Genomes	204
11.3.3	The Potential Utility of Group II Introns	204
11.4	Domains and Parts: The Anatomy of a Group II Intron	205
11.4.1	Domain 1	206
11.4.2	Domain 2	206
11.4.3	Domain 3	206
11.4.4	Domain 4	206
11.4.5	Domain 5	206
11.4.6	Domain 6	207
11.4.7	Other Domains and Insertions	207
11.4.8	Alternative Structural Organization and Split Introns	208
11.5	A Big, Complicated Family: The Diversity of Group II Introns	208
11.6	Group II Intron Tertiary Structure	209
11.7	Group II Intron Folding Mechanisms	211
11.7.1	A Slow, Direct Path to the Native State	211
11.7.2	A Folding Control Element in the Center of D1	212
11.7.3	Proteins and Group II Intron Folding	212
11.8	Setting the Stage for Catalysis: Proximity of the Splice Sites and Branch-site	213

11.8.1	Recognition of Exons and Ribozyme Substrates	213
11.8.2	Branch-site Recognition and the Coordination Loop	213
11.9	A Single Active-site for Group II Intron Catalysis	215
11.10	The Group II Intron Active-site: What are the Players?	216
11.10.1	Active-site Players in D1 and Surrounding Linker Regions	217
11.10.2	Domain 3 and the J2/3 Linker	217
11.10.3	Domain 5: Structural and Catalytic Regions	218
11.11	The Chemical Mechanism of Group II Intron Catalysis	219
11.12	Proteins and Group II Intron Function	221
11.12.1	Maturases	221
11.12.2	CRM-domain Plant Proteins	221
11.12.3	ATPase Proteins	221
11.13	Group II Introns and Their Many Hypothetical Relatives	222
11.14	Group II Introns: RNA Processing Enzymes, Transposons, or Tiny Living Things?	223
	References	223

Chapter 12 The GIR1 Branching Ribozyme

Henrik Nielsen, Bertrand Beckert, Benoit Masquida and Steinar D. Johansen

12.1	Introduction	229
12.2	Distribution and Structural Organization of Twin-ribozyme Introns	231
12.3	Biological Context	234
12.3.1	Three Processing Pathways of a Twin-ribozyme Intron	234
12.3.2	Processing of the <i>I-DirI</i> mRNA	235
12.3.3	Conformational Switching in GIR1	236
12.4	Biochemical Characterization	238
12.4.1	GIR1 Catalyzes Three Different Reactions	239
12.4.2	Characterization of the Branching Reaction	240
12.4.3	Biochemistry of GIR1	240
12.5	Modelling the Structure of GIR1	241
12.5.1	Overall Structure	242
12.5.2	Coaxially Stacked Helices	242
12.5.3	Junctions and Tertiary Interactions Involving Peripheral Elements	245

12.5.4	The Active Site	245
12.6	Phylogenetic Considerations	247
12.7	Concluding Remarks	248
	References	249
Chapter 13	Is the Spliceosome a Ribozyme?	
	<i>Dipali G. Sashital and Samuel E. Butcher</i>	
13.1	Introduction	253
13.2	Similarity to Group II Self-splicing Introns	253
13.3	Role of snRNA in the Spliceosome Active Site	255
13.4	Conformation of the U2-U6 Complex and Parallels to Group II Intron Structures	260
13.5	RNA-mediated Regulation in the Spliceosome	262
	References	266
Chapter 14	Peptidyl Transferase Mechanism: The Ribosome as a Ribozyme	
	<i>Marina V. Rodnina</i>	
14.1	Introduction: Historical Background	270
14.2	The Ribosome	271
14.3	Peptidyl Transfer Reaction	272
14.3.1	Characteristics of the Reaction off the Ribosome	273
14.3.2	Enzymology of the Peptidyl Transfer Reaction	274
14.3.2.1	Potential Mechanisms of Rate Acceleration by the Ribosome	274
14.3.2.2	Experimental Approaches to Reaction on the Ribosome	275
14.3.2.3	pH-Rate Profiles	277
14.3.2.4	Activation Parameters	278
14.4	The Active Site	279
14.4.1	Structures of the Reaction Intermediates	281
14.4.2	Conformational Rearrangements of the Active Site	282
14.4.2.1	Induced Fit	282
14.4.2.2	Role of the P-site Substrate	283
14.4.2.3	Conformational Flexibility of the Active Site	284
14.4.3	Probing the Catalytic Mechanism: Effects of Base Substitutions	285
14.4.4	Importance of the 2'-OH of A76 of the P-site tRNA	286
14.5	Conclusions and Evolutionary Considerations	287
	References	288

Chapter 15 Folding Mechanisms of Group I Ribozymes*Sarah A. Woodson and Prashanth Rangan*

15.1	Introduction	295
15.2	Multi-domain Architecture of Group I Ribozymes	296
15.3	RNA Folding Problem	297
	15.3.1 Hierarchical Folding of tRNA	297
	15.3.2 Coupling of Secondary and Tertiary Structure	298
15.4	Late Events: Formation of Tertiary Domains in the <i>Tetrahymena</i> Ribozyme	298
	15.4.1 Time-resolved Footprinting of Intermediates	298
	15.4.2 Misfolding of the Intron Core	300
	15.4.3 Peripheral Stability Elements	300
15.5	Kinetic Partitioning among Parallel Folding Pathways	301
	15.5.1 Theory and Experiment	301
	15.5.2 Single Molecule Folding Studies	301
	15.5.3 Estimating the Flux through Footprinting Intermediates	302
	15.5.4 Kinetic Partitioning <i>In Vivo</i>	302
15.6	Early Events: Counterion-dependent RNA Collapse	302
	15.6.1 Compact Non-native Form of bI5 Ribozyme	303
	15.6.2 Small Angle X-ray Scattering of <i>Tetrahymena</i> Ribozyme	303
	15.6.3 Native-like Folding Intermediates in the <i>Azoarcus</i> Ribozyme	304
	15.6.4 Early Folding Intermediates of the P4-P6 RNA	305
15.7	Counterions and Folding of Group I Ribozymes	305
	15.7.1 Metal Ions and RNA Folding	305
	15.7.2 Valence and Size of Counterions Matter	306
	15.7.3 Specific Metal Ion Coordination and Folding	307
15.8	Protein-dependent Folding of Group I Ribozymes	307
	15.8.1 Stabilization of RNA Tertiary Structure	308
	15.8.2 Stimulation of Refolding by RNA Chaperones	308
15.9	Conclusion	309
	References	309
	Subject Index	315