

---

## Contents

<b>Methods and Platforms for the Quantification of Splice Variants' Expression .....</b>	<b>1</b>
Laurent Bracco, Emeline Throo, Olivier Cochet, Richard Einstein, Florence Maurier	
1 Introduction.....	1
2 General Principles: Specificity is the Key Issue.....	3
3 Low/Medium Throughput Techniques.....	4
3.1 RT-PCR-Based Platforms.....	4
3.1.1 Semi-quantitative RT-PCR .....	5
3.1.2 Quantitative RT-PCR.....	6
3.2 Alternative Technologies to RT-PCR .....	8
3.2.1 The Ligase Chain Reaction .....	8
3.2.2 The RNA-Invasive Cleavage Assay .....	9
3.3 Conclusion.....	10
4 High-Throughput Analysis of Alternative Splicing Using Microarrays .....	11
4.1 Introduction .....	11
4.2 Microarray Configuration and Probe Design .....	11
4.3 Labeling Protocols.....	15
4.4 Data Analysis .....	15
4.5 Commercially Available Products .....	17
4.5.1 Custom Arrays .....	17
4.5.2 Catalog Arrays .....	17
4.6 Conclusion.....	18
5 The Detection and Quantification of Splice Variants at the Protein Level .....	18
6 Conclusion .....	19
References.....	20
 <b>Pre-mRNA Missplicing as a Cause of Human Disease .....</b>	<b>27</b>
Tatyana Novoyatleva, Yesheng Tang, Ilona Rafalska, Stefan Stamm	
1 Importance of Alternative Splicing for Gene Regulation.....	27
1.1 Splice Sites are Selected Through Combinatorial Control .....	28
2 Human Diseases Caused by Mutation in Splicing Signals .....	30
2.1 Mutation of Cis-acting Elements.....	30
2.2 Examples of Diseases .....	31
3 Changes of Trans Factors Associated with Diseases.....	35
4 Human Diseases Associated with Aberrant Splice Site Selection Without Obvious Mutations .....	36

5	Treatment of Diseases Caused by Missplicing .....	37
5.1	Gene Transfer Methods .....	37
5.2	Low Molecular Weight Drugs .....	38
5.3	Diagnostics .....	38
6	Conclusions.....	39
	References.....	39
	<b>Alternative Splicing: Therapeutic Target and Tool .....</b>	<b>47</b>
	Mariano A. Garcia-Blanco	
1	Introduction .....	47
1.1	Pre-messenger RNA Splicing and Disease .....	48
2	Conventional Therapies that Have Impact on Splicing .....	49
3	Oligonucleotide Drugs and Splicing.....	52
3.1	Making Antisense of Splicing.....	53
3.2	Enhanced Antisense.....	55
4	Exon-Specific RNA Interference.....	56
5	RNA-Based Corrective Therapy .....	56
6	Conclusion .....	58
	References.....	59
	<b>SR Proteins as Potential Targets for Therapy .....</b>	<b>65</b>
	Johann Soret, Mathieu Gabut, Jamal Tazi	
1	Introduction .....	65
1.1	The Role of SR Proteins in Constitutive and Alternative Splicing .....	66
1.2	SR Proteins Regulate Alternative Splicing in a Dose-Dependent Way .....	67
1.3	SR Protein Activity is Regulated by Phosphorylation of Their RS Domain .....	67
1.4	Involvement of SR Proteins in Human Diseases .....	68
1.5	Unforeseen Modulation of Alternative Splicing by Various Molecules .....	69
2	General Modulation of SR Proteins' Expression Level.....	70
2.1	Modulation of Splicing by Histone Deacetylase Inhibitors.....	70
2.2	Modulation of Splicing by Aclarubicin .....	71
3	General Modulation of SR Proteins' Phosphorylation Level.....	72
3.1	Modulation of Phosphatase Activity .....	72
3.2	Modulation of Kinase Activity .....	72
4	Modulation of Specific SR Proteins' Expression Level.....	75
4.1	Overexpression of SR Proteins .....	75
4.2	Down-Regulation of SR Proteins' Expression.....	76
5	Selective Modulation of SR Proteins' Activity.....	77
6	Concluding Remarks.....	78
	References.....	79

<b>Misregulation of Tau Alternative Splicing in Neurodegeneration and Dementia .....</b>	<b>89</b>
Athena Andreadis	
1 Brief Overview of Constitutive and Alternative Splicing .....	89
2 Structure, Transcripts, and Alternative Splicing of the Tau Gene .....	90
3 Tau Splicing and Neurodegeneration, Prologue .....	93
4 Commonalities in the Splicing Regulation of Tau Exons 2 and 10.....	95
4.1 Exon 2 – Splicing Regulation and the Possible Connection to DM 1 .....	95
4.2 Exon 10 – Splicing Regulation and the Established Connection to Tauopathies.....	97
5 Tau Connection to Disease, Epilogue.....	100
References.....	102
<b>Spinal Muscular Atrophy and Therapeutic Prospects .....</b>	<b>109</b>
Brunhilde Wirth, Lars Brichta, Eric Hahnen	
1 Clinical Picture of Spinal Muscular Atrophy (SMA) .....	109
2 Molecular Basis of SMA .....	110
2.1 SMN, the SMA Determining Gene .....	111
2.2 Alternative Splicing of SMN Transcripts.....	112
2.2.1 Splicing Regulation of SMN Exon 7 .....	112
2.3 The SMN Protein Function.....	116
2.4 SMN1 Gene Mutations in SMA Patients .....	117
2.5 Influence of SMN2 Copy Number on the SMA Phenotype .....	117
2.6 Evidence for Further Genes Modifying the Disease Severity .....	118
3 Therapeutic Prospects for SMA .....	119
3.1 Drugs that Increase the SMN Protein Level .....	119
3.1.1 Histone Deacetylase (HDAC) Inhibitors that Increase the SMN-RNA/Protein .....	120
3.2 Correction of Exon Skipping by Synthetic Small Molecules.....	122
3.3 A First Causal Therapy for SMA Patients? .....	122
4 Conclusions and Perspectives .....	123
References.....	124
<b>Misregulation of Alternative Splicing Causes Pathogenesis in Myotonic Dystrophy .....</b>	<b>133</b>
N. Muge Kuyumcu-Martinez, Thomas A. Cooper	
1 Myotonic Dystrophy .....	133
2 Repeat Instability .....	134
3 Mechanism of DM Pathogenesis .....	135

3.1	Loss of Function of DMPK.....	136
3.2	Loss of Function of Surrounding Genes .....	137
3.3	RNA “Gain of Function” Hypothesis.....	137
3.3.1	Misregulation of Alternative Splicing.....	138
3.4	Mechanisms of Misregulated Alternative Splicing.....	142
3.4.1	Increased CUG-BP1 Splicing Activity.....	143
3.4.2	Sequestration of MBNL Proteins.....	144
3.4.3	Sequestration of Other RNA Binding Proteins .....	147
3.4.4	Transcriptional Interference .....	147
3.4.5	Muscle Differentiation Defects and Altered Translation Regulation .....	148
	References.....	149

### **Redirecting Splicing to Address Dystrophin Mutations:**

#### **Molecular By-pass Surgery .....161**

Stephen D. Wilton, Susan Fletcher

1	The Dystrophin Gene and Duchenne Muscular Dystrophy .....	161
1.1	The Dystrophin Gene and Products .....	161
1.2	Duchenne and Becker Muscular Dystrophy .....	164
1.3	Dystrophin Mutations .....	165
1.4	Atypical Mutations: Exceptions to the Reading Frame Hypothesis.....	169
1.5	Revertant Fibres .....	170
2	Splicing .....	171
2.1	Mechanism of Pre-mRNA Processing .....	171
2.2	Alternative Splicing.....	172
2.3	Antisense Oligonucleotide Modification of Splicing Patterns .....	174
2.4	Antisense Oligonucleotide Chemistries .....	175
2.5	Candidate Genes for Splicing Intervention .....	175
3	Molecular By-pass Surgery for Duchenne Muscular Dystrophy .....	176
3.1	The Dystrophin Gene and Redirected Splicing.....	176
3.2	Antisense Studies in Animal Models of Muscular Dystrophy .....	179
3.3	Human Dystrophin Gene Studies .....	181
3.4	Antisense Oligonucleotide Delivery .....	184
4	Clinical trials .....	185
4.1	Fine-Mapping Dystrophin Mutations .....	186
4.2	Trial Design .....	186
5	The Future .....	188
	References.....	189

<b>Altered Splicing in Prelamin A-Associated Premature Aging Phenotypes.....</b>	<b>199</b>
Annachiara De Sandre-Giovannoli, Nicolas Lévy	
1 Altered Lamin A/C Function and Disease: the “Laminopathies” .....	199
1.1 <i>LMNA</i> -Encoding Lamins A/C, Fundamental Nuclear “Bricks” .....	200
1.1.1 Involvement in Nuclear Structure, Morphology, and Resistance to Stress .....	203
1.1.2 Regulation of Gene Expression .....	203
1.2 The “Laminopathies” .....	204
2 Altered Lamin A Splicing and Progeroid Syndromes .....	209
2.1 Hutchinson-Gilford Progeria Syndrome .....	210
2.2 Restrictive Dermopathy .....	215
2.2.1 Lamin-Linked RD .....	215
2.2.2 <i>ZMPSTE24</i> -Linked RD .....	217
3 Hypothetical Pathophysiological Mechanisms Involved in Laminopathies and Progeroid Syndromes Linked to <i>LMNA</i> and <i>ZMPSTE24</i> in Relation to Animal Models Possible Therapeutic Strategies .....	218
4 Conclusions and Future Prospects .....	221
References .....	223
<b>Splicing Modulation as a Modifier of the CFTR Function.....</b>	<b>233</b>
Malka Nissim-Rafinia, Batsheva Kerem	
1 The CF Disease and the CFTR Gene .....	233
2 Spectrum of Splicing Mutations in the CFTR Gene.....	234
2.1 Splicing Mutations Leading to Complete Aberrant Splicing.....	234
2.2 Splicing Mutations Leading to Partial Generation of Correct Splicing .....	235
2.2.1 The 3849+10 kb C→T Mutation .....	235
2.2.2 The 2789+5 G→A Mutation .....	237
2.2.3 The 3272–26 A→G Mutation.....	237
2.2.4 The IVS8–5T Allele.....	237
2.3 Exonic Point Mutations Leading to Aberrant Splicing .....	238
3 Correlation Between Levels of Correctly Spliced RNA and Disease Severity .....	239
4 Splicing Modulation by Splicing Factors .....	241
4.1 Splicing Modulation of CFTR Minigenes Carrying Splicing Mutations.....	241

---

4.1.1 The 3849+10 kb C→T Minigene .....	242
4.1.2 The IVS8–5T Minigene .....	242
4.1.3 Exon 9 Minigene Carrying the A455E Mutation .....	243
4.1.4 Exon 12 Minigenes Carrying the D565G and G576A Mutations .....	244
4.1.5 Exon 13 Minigenes .....	244
4.2 Splicing Modulation of Endogenous CFTR Allele Carrying 3849+10 Kb C→T Splicing Mutations and Restoration of the CFTR Function .....	245
5 Splicing Modulation by Small Molecules .....	248
6 Splicing Modulation by Antisense Oligonucleotides .....	249
References .....	250
<b>Index .....</b>	<b>255</b>

Alternative Splicing and Disease

Jeanteur, P. (Ed.)

2006, XII, 257 p. 26 illus., 2 illus. in color., Hardcover

ISBN: 978-3-540-34448-3