

# Contents

Abstract . . . . .	i
Zusammenfassung . . . . .	iii
Acknowledgements . . . . .	v
List of Figures . . . . .	x
List of Tables . . . . .	xii
<b>1 Introduction</b>	<b>1</b>
1.1 Postranscriptional Gene Regulation . . . . .	1
1.1.1 The Central Dogma of Molecular Biology . . . . .	1
1.1.2 Transcription . . . . .	2
1.1.3 The Splicing Reaction . . . . .	3
1.1.4 Alternative Splicing . . . . .	6
1.1.5 TransSplicing . . . . .	6
1.1.6 Nuclear Processing . . . . .	6
1.1.7 Export of RNA and Localization . . . . .	7
1.1.8 RNA Editing . . . . .	7
1.1.9 Translational Control . . . . .	7
1.1.10 RNA Degradation . . . . .	8
1.1.11 miRNA and siRNA . . . . .	8
1.1.12 RNA and Disease . . . . .	9
1.2 RNA Binding Proteins . . . . .	10
1.2.1 The RNA Recognition Motif - RRM . . . . .	10
1.3 Methods to Study Protein RNA Interactions . . . . .	12
1.3.1 In Vivo Methods . . . . .	12
1.3.2 In Vitro Methods . . . . .	13
1.4 Structural Methods . . . . .	13
1.4.1 NMR Spectroscopy . . . . .	14
1.4.2 X-Ray Crystallography . . . . .	19
1.4.3 Cryo Electron Microscopy . . . . .	19
1.5 Aims of this Thesis . . . . .	21
<b>2 RBM4</b>	<b>22</b>
2.1 Introduction . . . . .	22

2.1.1	Domain Organization of RBM4a, RBM4b and Lark . . . . .	22
2.1.2	Cellular Localization of RBM4a . . . . .	24
2.1.3	The Role of RBM4a in Splicing . . . . .	24
2.1.4	Involvement of RBM4a in Translational Control . . . . .	25
2.1.5	RBM4a and the miRNA Pathway . . . . .	26
2.1.6	Aims of the Project . . . . .	27
2.2	NMR Structural Studies of the RRM Domains . . . . .	27
2.2.1	NMR Structure of RRM1 of RBM4a . . . . .	27
2.2.2	NMR Structure of RRM2 of RBM4a . . . . .	31
2.3	NMR Titration Experiments with RNA . . . . .	34
2.3.1	Overview of RNA Binding Sequences . . . . .	34
2.3.2	Titration Experiment . . . . .	37
2.4	NMR Structural Studies of RBM4 RRM1 in Complex with a SELEX Derived RNA . . . . .	38
2.4.1	RRM1 Complex Structure with a SELEX Derived RNA . . . . .	38
2.4.2	RBM4a Interacts in Vitro with the pri-miRNA 125 Stemloop . . . . .	45
2.4.3	Crystallographic Experiments on the RRM1-stemloop complex . . . . .	46
2.5	Discussion and Outlook . . . . .	50
<b>3</b>	<b>The novel RNA binding domain YTH</b> . . . . .	<b>52</b>
3.1	Introduction . . . . .	52
3.1.1	YT521-B . . . . .	52
3.1.2	The YTH Domain . . . . .	53
3.2	Aims of the Project . . . . .	54
3.3	Preliminary Structure of the YTH Domain of YT521 . . . . .	54
3.4	Titration with a SELEX derived RNA . . . . .	58
3.5	Discussion and Outlook . . . . .	60
<b>4</b>	<b>NMR Based Discovery of Binding Sequences</b> . . . . .	<b>61</b>
4.0.1	Scaffold Independent Analysis . . . . .	61
4.0.2	Aims of the Project . . . . .	62
4.1	Modified Scaffold Independent Analysis . . . . .	63
4.2	Preliminary Structure with the MSIA Derived RNA . . . . .	66
4.2.1	Preliminary Structure . . . . .	66
4.2.2	Challenges Encountered . . . . .	68
4.3	Dimucleotide Scaffold Independent Analysis . . . . .	70
4.4	Discussion and Outlook . . . . .	71
<b>5</b>	<b>Methylated mRNA Recognition by the YTH Domain</b> . . . . .	<b>73</b>
5.1	Introduction . . . . .	73
5.1.1	Adenosine Methylation at the N6 Position . . . . .	73
5.1.2	Methylase . . . . .	74

5.1.3	Demethylase . . . . .	74
5.1.4	Analysis of Binding Sites by High Throughput Sequencing and Proteomics . . . . .	75
5.2	Aims of the Project . . . . .	76
5.3	Comparison of the Methylation Consensus with YTH Binding Sequences . . . . .	77
5.4	The YTH domain is a Methylated Adenosine Binder . . . . .	77
5.5	Preliminary Structure of the YTH domain in Complex with Methylated RNA . . . . .	77
5.6	Discussion and Outlook . . . . .	82
<b>6</b>	<b>Generation of Small Labeled RNAs</b>	<b>83</b>
6.1	Introduction . . . . .	83
6.1.1	Use of Small Labeled RNAs . . . . .	83
6.1.2	Methods for Sequence Specific RNA Cleavage . . . . .	84
6.1.3	CSY4 . . . . .	85
6.1.4	Aims of the Project . . . . .	87
6.2	Results . . . . .	87
6.2.1	General Considerations . . . . .	87
6.2.2	Substrate RNA Production . . . . .	88
6.2.3	Protein Production . . . . .	90
6.2.4	First Cleavage Experiments . . . . .	93
6.2.5	Sequence Specificity . . . . .	94
6.2.6	Large Scale Cleavage . . . . .	95
6.2.7	NMR spectra of Cleavage Derived UGACAC . . . . .	95
6.3	Discussion and Outlook . . . . .	98
<b>7</b>	<b>Outlook</b>	<b>100</b>
<b>8</b>	<b>Materials and Methods</b>	<b>102</b>
8.1	Molecular Biology . . . . .	102
8.1.1	Concentration Determination . . . . .	102
8.1.2	DNA Oligonucleotides . . . . .	102
8.1.3	Gel Electrophoresis . . . . .	102
8.1.4	DNA Handling . . . . .	103
8.1.5	Vectors . . . . .	103
8.1.6	RNA Preparation . . . . .	103
8.1.7	Protein Production . . . . .	104
8.1.8	DSF . . . . .	106
8.1.9	Crystallography . . . . .	106
8.2	NMR Data acquisition, Processing and Interpretation . . . . .	106
8.2.1	Spectra Acquisition and Processing . . . . .	106
8.2.2	Measurement Conditions and Constructs . . . . .	107
8.2.3	Acquired Spectra . . . . .	107
8.2.4	Processing of Spectra . . . . .	108

---

8.2.5	Spectra Analysis and Chemical Shift Assignment	108
8.3	Structure Calculation	109
8.3.1	AtnosCandidCyana	109
8.3.2	Cyana NOE Assign	109
8.3.3	Cyana	109
8.3.4	Amber	110
8.4	Additional Software	110
<b>9</b>	<b>Appendix</b>	<b>111</b>
9.1	RBM4 RRM Domains	111
9.1.1	RRM1 Chemical Shifts	111
9.1.2	RRM2 Chemical Shifts	113
9.2	RBM4-RRM1-Stemloop Complex	115
9.2.1	RRM1 Domain Chemical Shifts	115
9.2.2	Stemloop Chemical Shifts	117
9.2.3	Intermolecular NOE Derived Restraints	118
9.2.4	IntraRNA NOE Derived Restraints	121
9.3	YTH Domain YT521	124
9.3.1	YTH Domain Chemical Shifts	124
9.4	YTH domain YT521 in Complex with UGACAC	127
9.4.1	YTH Domain Chemical Shifts	127
9.4.2	UGACAC Chemical Shifts	130
9.4.3	Intermolecular NOE Derived Restraints	130
9.4.4	Intramolecular NOE derived RNA restraints	132
9.5	YTH domain YT521 in Complex with UGm6ACAC	132
9.5.1	YTH Domain Chemical Shifts	132
9.5.2	UGm6ACAC Chemical Shifts	136
9.5.3	Intermolecular NOE Derived Restraints	136
9.5.4	Intramolecular RNA NOE Derived Restraints	137
	References	139
	Curriculum Vitae	159