0	Abstract				
	0.1	English	abstract	8	
	0.2	Zusam	menfassung	9	
1	Chapter One – Introduction to microRNA biology				
	1.1	1 Ideas are rarely new 1			
	1.2	What a	re microRNAs?	11	
		1.2.1	Early discoveries	11	
		1.2.2	Biogenesis	14	
		1.2.3	microRNAs continue to be discovered	15	
	1.3	RNAi c	liscovery and siRNAs in functional genomics	18	
	1.4	MicroR	NA target prediction	19	
	1.5	Function	ons of microRNAs	25	
	1.6	Global	effects on proteins by microRNAs	26	
	1.7	System	ns biology of microRNA targeting –		
		a quan	titative approach	27	
2	Chap	oter Two	o – microRNA discovery and target gene prediction	28	
	2.1	Synops	sis	28	
	2.2	microR	NA gene discovery by cloning and bioinformatics	28	
		2.2.1	Cloning	28	
		2.2.2	What was the computational challenge?	29	
		2.2.3	Computational pipeline	29	
		2.2.4	Classes of D. melanogaster small RNAs	30	
		2.2.5	microRNA discovery	32	
		2.2.6	Discovery of Repeat Associated silencing		
			RNAs - rasiRNAs	37	
	2.3	microRN	NA targets in Drosophila melanogaster	39	
		2.3.1	Background to target prediction	39	
		2.3.2	Architecture of microRNA target sites	40	
		2.3.3	Number and location of binding sites in the target mRNA	41	
		2.3.4	siRNA studies demonstrate cooperativity of target sites	42	
		2.3.5	Conservation of microRNAs and implications for		
			target sites	43	
		2.3.6	Design of the target prediction algorithm, miRanda	45	
		2.3.7	Assumptions with the miRanda algorithm		
		2.3.8	Free energy of RNA duplex	51	



		2.3.9	Conservation rules	52
		2.3.10	Benchmarking and validation	52
	2.4	microR	NA target prediction in D. melanogaster using miRanda	54
		2.4.1	Collecting sequences and running miRanda	54
		2.4.2	Validation	56
		2.4.3	Results: Scope of microRNA regulation	59
		2.4.4	Predicted processes and pathways targeted by	
			Drosophila microRNAs	61
		2.4.5	microRNAs targeting Hox genes and body axis	
			specification	63
		2.4.6	microRNAs targeting ecdysone signaling and	
			developmental timing	64
		2.4.7	microRNAs targeting development of the nervous system	66
	2.5	Summa	ary	68
3	Chap	ter Thr	ee – Human microRNA target prediction	70
	3.1	Synops	sis	70
	3.2	Backgr	ound	71
		3.2.1	Why predict the targets of human microRNAs?	71
		3.2.2	Target sites	73
		3.2.3 A	Algorithms	74
	3.3	Human	microRNA targets: Methods	75
		3.3.1	MicroRNA sequence acquisition	75
		3.3.2	UTR orthology and alignment	76
		3.3.3	microRNA target prediction	78
		3.3.4	Functional analysis of predicted microRNA targets	79
		3.3.5	Validation	80
			3.3.5.1 Known target sites	80
			3.3.5.2 Estimate of false positives	80
		3.3.6	mRNA s associated with RNA binding protein FMRP	82
	3.4	Results		82
		3.4.1	Prediction of human microRNA targets	82
		3.4.2	Validation of Target Predictions	
		3.4.2.1	Agreement with known targets	
			Estimate of false positives.	
			Indirect experimental support: FMRP-associated mRNAs	

3.5	Overvie	ew of mammalian microRNA target genes	89	
	3.5.1	More than 2,000 mammalian targets		
	3.5.2	One-to-many and Many-to-one		
	3.5.3	Functional analysis		
3.6		cargo mRNAs regulated by miRNAs		
	3.6.1	Overview of microRNA targeted FMRP cargo		
	3.6.2	Alzheimer's disease amyloid protein.		
	3.6.3	PSD95 and synaptic processes		
3.7	Components of RNPs Regulated by miRNAs			
	3.7.1	FMRP-associated proteins		
	3.7.2	RISC		
	3.7.3	Other RNPs		
		Elav proteins		
		Cytoplasmic Polyadenylation Binding Proteins		
		Regulated by miRNAs	100	
3.8	Targets	s of Cancer-Related microRNAs		
	3.8.1	The E2F family are predicted targets of miR-17-5p		
3.9	How fa	r are microRNA:mRNA relationships conserved		
	in evolution?			
	3.9.1	Targets Conserved between Mammals and Fish		
	3.9.2	Targets Conserved between Vertebrates and Flies		
3.10	Investigation of "unusual" kinds of microRNA targeting10			
		Target Sites in Protein-Coding Sequences		
		Target Sites with near perfect matches in cDNAs		
		Comparison of miRNA Target Prediction Methods		
	3.10.4	•		
3.11	Discuss	sion		
	3.11.1	How Widespread Is the Regulation of Translation		
		by miRNA?	111	
	3.11.2	How conserved in evolution aree microRNA targets?		
	3.11.3	Principles of microRNA regulation - Multiplicity and		
		cooperativity ?	112	
	3.11.4	Implications for mechanisms of microRNA action		
	3.11.5			
	3.11.6	Scope of microRNA regulation		

4	4 Chapter Four – Competition and Saturation in microRNA				
	regulation117				
	4.1	, ,	sis1′		
	4.2	Backgr	round11	17	
	4.3	Metho	ds12	20	
		4.3.1	mRNA and protein experimental datasets12	20	
		4.3.2	Target prediction12	22	
		4.3.3	Endogenous miRNA expression12	22	
		4.3.4	Kolomogorov-Smirnov statistics12	23	
		4.3.5	Notation12	23	
		4.3.6	Regression analysis to model expression12	24	
		4.3.7	Forward stepwise regression analysis12	24	
		4.3.8	Cell cycle and cancer genes analysis12	25	
	4.4	Results	s12	26	
		4.4.1	Targets of endogenous miRNAs are upregulated after		
			miRNA transfection12	26	
		4.4.2	siRNA transfections display the same effect as		
			miRNA transfections13	30	
		4.4.3	Attenuated knockdown of si/miRNA targets containing		
			endogenous sites13	31	
		4.4.4	A quantitative model resolves the endogenous		
			miRNA profile13	32	
		4.4.5	The competition effect has a dose response	34	
		4.4.6	Evidence for a transitory saturation effect13		
		4.4.7	Overexpression of Argonaute shows no saturation effect13	37	
		4.4.8	miRNA inhibition may cause upregulation of other		
			endogenous targets1	37	
		4.4.9	microRNAs regulate cancer associated genes and		
			these are dysregualted during small RNA transfections13	39	
	4.5	Summa	ary14	41	
5	Chap	ter Fiv	e – Target abundance dilutes microRNA		
	•		activity1	44	
	5.1 Synopsis				
	5.2	·			
	5.3	•			

		5.3.1	Quantification of transcript abundance	146
		5.3.2	Quantification of target down-regulation	147
		5.3.3	Representative set of independent microRNA	
			experiments	147
		5.3.4	Down-regulation of primary siRNA targets	148
	5.4	Results	and Discussion	148
		5.4.1	Target abundance affects	
			average down-regulation by small RNAs	148
		5.4.2	Down-regulation by transfected microRNAs	
			is a function of target abundance	152
		5.4.3	Pairwise analysis controls for artifacts	154
		5.4.4	Target abundance affects primary target and off-target	
			average down-regulation by siRNAs	156
		5.4.5	Michaelis-Menten kinetics describes total transcripts	
			degraded	157
		5.4.6	Conclusions	158
6	Char	oter Six	- Conclusions and Future work	160
	6.1		is	
	6.2		ary of Thesis	
		6.2.1.1	The discovery of microRNAs, microRNA target predictio	n
			in Drosophila melanogaster	160
		6.2.1.2	miRanda, microRNA target and function prediction	
			in human	161
		6.2.1.3	A system-level approach to microRNA function	163
	6.3		directions	
		6.3.1	Quantitative, functional target prediction	164
		6.3.2	Combinatorics of RNA binding proteins and microRNAs	
			in gene regulation	165
		6.3.3	Do microRNAs as a whole regulate specific kinds of gene	
			more than others?	
		6.3.4	Therapeutics and siRNAs – the future	168
	6.4	Summa	ary	169
7	Refe	rences.		170
			Vitae	
9 Publikationsliste			liste	