

# Table of Contents

<b>Abstract</b>	vii
<b>Zusammenfassung</b>	ix
<b>Abbreviations</b>	xvii
<b>Symbols</b>	xix
<b>1 Circadian Clocks: An Overview</b>	1
1.1 A Chronology of Chronobiology . . . . .	2
1.2 Properties of Circadian Rhythms . . . . .	6
1.3 The Mammalian Circadian System . . . . .	9
1.3.1 Organization of the mammalian circadian system . . . . .	10
1.3.2 The molecular clock machinery in mammals . . . . .	13
1.3.3 Non transcriptional rhythms . . . . .	17
1.4 Conservation of Circadian Rhythms . . . . .	18
1.4.1 The Big Bang of circadian clocks . . . . .	18
1.4.2 Segregation of incompatible processes . . . . .	20
1.4.3 How to measure fitness? . . . . .	21
1.5 Time for Chronomedicine . . . . .	22
1.5.1 Clock in modern societies . . . . .	22
1.5.2 Disrupted clocks at the molecular level . . . . .	24
1.5.3 Medicine's secret ingredient: time . . . . .	25
1.6 Future Perspectives: Uchronia vs. Dyschronia . . . . .	26
1.7 Work Overview . . . . .	27
<b>2 Compensation in Circadian Clocks</b>	31
2.1 Compensation in Circadian Clocks: A Brief Overview . . . . .	31
2.1.1 Temperature compensation: a well-known property of clocks . . . . .	31
2.1.2 Metabolic compensation: hints to its existence . . . . .	33
2.2 Hypothesis: Circadian Compensation . . . . .	37
2.3 Results: Towards a More General Concept of Circadian Compensation . . . . .	38
2.3.1 Knockdown of metabolic genes affects oscillation parameters . . . . .	39

2.3.2 Knockdown of metabolic genes affects period compensation against temperature steps . . . . .	43
2.3.3 Low amplitude oscillations are associated with high period variability	46
2.3.4 Oscillation parameters differ in different reporter cells . . . . .	46
2.4 Discussion . . . . .	48
2.4.1 Temperature compensation is modulated by metabolism . . . . .	49
2.4.2 Metabolism regulates the mammalian clockwork . . . . .	50
2.4.3 Does temperature increase damping? . . . . .	51
2.4.4 Different reporters, different circadian parameters? . . . . .	51
2.4.5 Concluding remarks . . . . .	52
<b>3 Modeling Circadian Redox Oscillations</b>	<b>55</b>
3.1 Redox Oscillations: A Brief Overview . . . . .	55
3.1.1 Redox homeostasis is central to life . . . . .	55
3.1.2 Biochemistry of peroxiredoxins . . . . .	57
3.1.3 A day in the life of peroxiredoxins . . . . .	58
3.2 Aim: In Search of a Model for Redox Oscillations . . . . .	60
3.3 How to Model Clocks? From Equations to Oscillations . . . . .	60
3.3.1 Fundamentals of modeling: some important terms . . . . .	61
3.3.2 Benefits of modeling . . . . .	66
3.4 Results: A Robust Model for Circadian Redox Oscillations . . . . .	66
3.4.1 A novel kinetic deterministic model for circadian redox oscillations	67
3.4.2 The core Prx3-SO <sub>2</sub> H/Srx circadian oscillator: A 3-ODE model .	68
3.4.3 Design principles of the redox oscillator: fast A inactivation followed by a slow, delayed negative feedback loop . . . . .	70
3.4.4 A stochastic amplitude-phase model for circadian redox oscillations	74
3.4.5 Stochastic oscillators can entrain, respond to pulses and synchronize via mean-field coupling . . . . .	76
3.5 Discussion . . . . .	81
3.5.1 A novel model for circadian redox oscillations . . . . .	81
3.5.2 Alternative views on the nature of the negative feedback in the redox oscillator . . . . .	83
3.5.3 Crosstalk between Prx and TTFL rhythms in eukaryotes . . . . .	84
3.5.4 Model predictions . . . . .	85
3.5.5 Concluding remarks . . . . .	85
<b>4 Coupling Redox and TTFL Rhythms</b>	<b>89</b>
4.1 Crosstalk Between Redox and TTFL Timing Systems: A Brief Overview	89
4.1.1 Circadian redox rhythms . . . . .	89
4.1.2 Indications of redox regulation of TTFL rhythms . . . . .	90
4.1.3 Indications of TTFL regulation of redox rhythms . . . . .	92
4.1.4 Physiological relevance of the TTFL-redox crosstalk . . . . .	94

4.2	Aim: Coupling Redox and TTFL Oscillations . . . . .	95
4.3	How to Achieve Clock Synchronization? Coupling from a Theoretical Perspective . . . . .	96
4.3.1	A brief introduction to coupled oscillator theory . . . . .	96
4.3.2	Coupled oscillators in the context of the circadian clock . . . . .	97
4.4	Results: Towards a Coupled Model of Redox-TTFL Circadian Oscillations	98
4.4.1	Choice and reproduction of TTFL models . . . . .	98
4.4.2	Entrainment of the TTFL by a periodic redox Zeitgeber input: Unidirectional redox→TTFL coupling . . . . .	100
4.4.3	Entrainment of the redox oscillator by a periodic TTFL input: Unidirectional TTFL→redox coupling . . . . .	102
4.4.4	Mutual coupling of TTFL and redox oscillators: Bidirectional TTFL↔redox coupling . . . . .	106
4.5	Discussion . . . . .	107
4.5.1	Control of the redox:TTFL synchronization ratios . . . . .	107
4.5.2	Tissue-specificity of redox oscillators and coupling nodes . . . . .	108
4.5.3	Concluding remarks and outlook . . . . .	109
<b>5</b>	<b>General Discussion and Outlook</b>	<b>113</b>
5.1	Redox and TTFL Clocks: State of the Art . . . . .	113
5.1.1	Metabolic clocks: Temporal separation of cellular metabolism . . . . .	114
5.1.2	Redox balance as the mediator between cellular metabolism and canonical circadian timekeeping? . . . . .	115
5.1.3	Reciprocal TTFL-redox crosstalk, in both directions . . . . .	116
5.2	Key Findings Discussed . . . . .	116
5.2.1	Metabolism and the clock: A means for circadian resilience? . . . . .	117
5.2.2	Modeling Prx/Srx circadian redox oscillations . . . . .	118
5.2.3	Coupling between redox and TTFL clocks . . . . .	120
5.3	Limitations and Perspectives . . . . .	121
5.3.1	Can we speak about redox “clocks”? . . . . .	121
5.3.2	Future perspectives . . . . .	122
5.3.3	Interlocked vs. dissociated feedback loops: Circadian physiology vs. pathophysiology? . . . . .	123
5.3.4	Modeling limitations . . . . .	125
5.4	Final Conclusions and Open Questions . . . . .	126
<b>6</b>	<b>Materials and Methods</b>	<b>129</b>
6.1	Cell culture . . . . .	129
6.2	RNAi screen . . . . .	130
6.3	Processing of bioluminescent data . . . . .	132
6.4	Simulations of Ordinary Differential Equations . . . . .	136
6.5	Simulations of Stochastic Differential Equations . . . . .	136

---

**TABLE OF CONTENTS**

---

<b>References</b>	<b>141</b>
<b>A Appendix A</b>	
A.1 Knockdown of metabolic genes affects rhythm parameters in <i>Per2-luc</i> U-2 OS cells . . . . .	X
A.2 Knockdown of metabolic genes affects period compensation against temperature steps in <i>Per2-luc</i> U-2 OS cells . . . . .	XII
A.3 Period lengths are stable across oscillations . . . . .	XII
<b>B Appendix B</b>	
B.1 Detailed Model of Redox Oscillations . . . . .	XVII
B.2 Model Simplification . . . . .	XVIII
B.3 Bifurcation Analyses of the Core 3-ODE Redox Oscillator Model . . . . .	XXII
B.4 Control Analysis of the Core 3-ODE Redox Oscillator Model . . . . .	XXII
B.5 Responses of an Ensemble of Noisy Oscillators to External Signals . . . . .	XXIII
<b>C Appendix C</b>	
C.1 Models of the Mammalian Circadian TTFL Clockwork . . . . .	XXVII
C.1.1 Relógio model of the canonical TTFL . . . . .	XXVIII
C.1.2 Redox regulation of the Relógio model of the canonical TTFL . . . . .	XXX
C.1.3 Almeida model of the canonical TTFL . . . . .	XXXII
C.1.4 Redox regulation of the Almeida model of the canonical TTFL	XXXIII
C.2 Complex nonlinear phenomena in the Almeida model . . . . .	XXXIII
C.3 Periodic forcing of the kinetic minimal redox oscillator model . . . . .	XXXV
C.4 Mutual coupling of redox and TTFL systems . . . . .	XXXVI
<b>Publications and Distinctions</b>	
Publications . . . . .	XXXIX
Conference Contributions . . . . .	XXXIX
Awards . . . . .	XL
Professional experiences . . . . .	XLI
<b>Acknowledgments</b>	
	XLIII