CONTENTS

PREFACE 1

INTRODUCTION 3

CHAPTER ONE: THE MASTER ELEMENTS OF CONTROL 13

Components of the Switch 16
  DNA 16
  RNA Polymerase 17
  The Repressor 17
  Cro 19

The Action of Repressor and Cro 20
  Negative Control 20
  Positive Control 20
  Cooperativity of Repressor Binding 22

Induction—Flipping the Switch 24

Cooperativity—Switch Stability and Sensitivity 27

The Effect of Autoregulation 30

Other Cases 30

CHAPTER TWO: PROTEIN-DNA INTERACTIONS AND GENE CONTROL 33

The Operator 33

Repressor 35

Cro 39

Amino Acid-Base Pair Interactions 41

The Promoter 44

Gene Control 45

CHAPTER THREE: CONTROL CIRCUITS—SETTING THE SWITCH 49

A Brief Overview of λ Growth 50
- The Genetic Map 50
- Circularization 51
- Gene Expression 52
  - Very early
  - Early
  - Late
- Integration 53

Control of Transcription 54
- Very Early 54
- Early 54
- Late Lytic 56
- Late Lysogenic 57

The Decision 58

Control of Integration and Excision 59
- Case 1 – Establishing Lysogeny 59
- Case 2 – Lytic Growth 60
- Case 3 – Induction 61

Other Phages 61

The SOS Response 62

λ Pathways and Cell Development 63
- Regulatory Genes 63
- Switches 65
- Patterns of Gene Expression 66
- Mechanisms 66

CHAPTER FOUR: HOW DO WE KNOW—THE KEY EXPERIMENTS 69

The Repressor Idea 69
- Clear and Virulent Mutants 69
  - Observations
  - Explanation
- Immunity and Heteroimmunity 70
  - Observations
  - Explanation
- Asymmetry in Bacterial Mating 72
  - Observations
  - Explanation

The Repressor Problem in the Early 1960s 73

Repressor Isolation and DNA-binding 74

Making More Repressor 76

The Claims of Chapters One and Two 77

- The repressor is composed of two globular domains held together by a linker of some 40 amino acids 77
- The repressor dimerizes, largely through interaction between its carboxyl domains 79
- A repressor dimer binds, through its amino domains, to a 17 base pair operator site 79
  - A single operator binds one dimer of repressor
  - Dimers form before DNA binding
  - The amino domains contact DNA
- There are three 17 base pair repressor binding sites in the right operator. At each site repressor and Cro bind along the same face of the helix 85
  - Chemical probes
  - Operator mutations
  - Binding to supercoiled and linear DNA
- Repressor binds to three sites in O_R with alternate pairwise cooperativity. The cooperativity is mediated by interactions between carboxyl domains of adjacent dimers 87
- In a lysogen repressor is typically bound to O_R1 and O_R2. The bound repressors turn off rightward transcription of *cro* and stimulate leftward transcription of *cI*. At higher concentrations, repressor binds to O_R3 to turn off transcription of *cI* 88
- Cro binds first to O_R3, then to O_R1 and O_R2, thereby first turning off P_{RM}, then P_R 93
  - Some background about Cro
  - Cro *in vivo*
  - Cro *in vitro*
- RecA cleaves repressor to trigger induction 96
- When Cro is bound at O_R3 the switch is thrown 97
- Repressor and Cro bind to the operator as shown in Figures 2.6, 2.8, and 2.10 97
  - Crystallography
  - The 'helix swap' experiment
  - The role of the arm of λ repressor
  - Specific amino acid-base pair contacts

Repressor activates transcription of *cI* by binding to O_R2 and contacting polymerase with its amino domain 101
  Positive control mutants
  Positive control *in vitro*

Conclusion 104

Appendix One: Designing an Efficient DNA-binding Protein 109

Synopsis 109
Specific and Non-specific Binding 109
Increasing Specificity 111
  Increasing protein concentration
  Improving specificity directly
  Using cooperativity
DNA-Protein Interaction in Eukaryotes 114

Appendix Two: Strong and Weak Interactions 117

Appendix Three: Control of Transcription in Eukaryotes and Prokaryotes—A Common Mechanism 119