

# Contents

|                                                                                                                       |          |
|-----------------------------------------------------------------------------------------------------------------------|----------|
| <i>Preface</i>                                                                                                        | v        |
| <i>About the Authors</i>                                                                                              | ix       |
| <b>Chapter 1 History of DNA Polymerases</b>                                                                           | <b>1</b> |
| 1.1 Imaging an enzyme that assembles the nucleotides into DNA                                                         | 1        |
| 1.1.1 DNA polymerase activity in extracts of <i>Escherichia coli</i>                                                  | 5        |
| 1.1.2 <i>Escherichia coli</i> DNA polymerase can synthesize DNA with genetic activity: creating life in the test tube | 9        |
| 1.1.3 Bacteria contain many DNA polymerases                                                                           | 11       |
| 1.1.4 How is a new DNA chain started? Discontinuous DNA synthesis and the need for a RNA primer                       | 13       |
| 1.1.5 RNA priming as a mechanism for initiation: DNA primase                                                          | 14       |

|                                                                                                                                                                       |    |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| 1.2 Late 1960s to early 1970s DNA replication shows its complexity                                                                                                    | 15 |
| 1.2.1 The DNA structure is much more complex, rich of conformational flexibility and thus full of functional potentialities than the one proposed by Watson and Crick | 15 |
| 1.2.2 DNA binding proteins, DNA helicases, DNA topoisomerases                                                                                                         | 17 |
| 1.3 Concluding remarks, parts 1.1–1.2                                                                                                                                 | 18 |
| 1.4 Multiple DNA polymerases in eukaryotic cells: DNA polymerases $\alpha$ , $\beta$ and $\gamma$ as the first ones                                                   | 19 |
| 1.4.1 DNA polymerase $\alpha$                                                                                                                                         | 21 |
| 1.4.2 DNA polymerase $\beta$                                                                                                                                          | 22 |
| 1.4.3 Lack of relationship between high- and low-molecular-weight DNA polymerases                                                                                     | 23 |
| 1.4.4 1975: First nomenclature system for eukaryotic DNA polymerases                                                                                                  | 24 |
| 1.4.5 DNA polymerase $\gamma$                                                                                                                                         | 25 |
| 1.5 Early attempts to ascribe an <i>in vivo</i> function to DNA polymerases $\alpha$ , $\beta$ and $\gamma$                                                           | 27 |
| 1.5.1 Positive correlation of DNA polymerase $\alpha$ with cellular DNA replication and development                                                                   | 27 |
| 1.5.2 DNA polymerase $\gamma$ is the mitochondrial DNA polymerase and replicates mitochondrial DNA                                                                    | 30 |
| 1.5.3 Further evidence for a major involvement of DNA polymerase $\alpha$ in DNA replication and of DNA polymerase $\beta$ in DNA repair                              | 32 |
| 1.6 DNA polymerases $\delta$ and $\epsilon$                                                                                                                           | 36 |
| 1.7 Yeast DNA polymerases                                                                                                                                             | 38 |
| 1.7.1 Revised nomenclature for eukaryotic DNA polymerases                                                                                                             | 40 |

|                                                                           |           |
|---------------------------------------------------------------------------|-----------|
| 1.8 Plant cell DNA polymerases                                            | 40        |
| 1.9 Virus-induced DNA polymerases                                         | 43        |
| 1.9.1 Herpes virus DNA polymerase                                         | 43        |
| 1.9.2 Vaccinia virus DNA polymerase                                       | 44        |
| 1.9.3 DNA polymerase activity in Hepatitis B particle                     | 44        |
| 1.9.4 Retroviruses reverse transcriptase                                  | 45        |
| 1.10 1999–2000: Appearance of many novel specialized DNA polymerases      | 45        |
| 1.10.1 DNA polymerase $\zeta$ , the lesion extender                       | 46        |
| 1.10.2 DNA polymerase $\theta$                                            | 46        |
| 1.10.3 DNA polymerases $\lambda$ and $\mu$ , two family X DNA polymerases | 47        |
| 1.10.4 The complex Y family of DNA polymerases                            | 47        |
| 1.10.5 PrimPol                                                            | 48        |
| 1.10.6 Present nomenclature for eukaryotic DNA polymerases                | 48        |
| 1.11 Concluding remarks, parts 1.4–1.10                                   | 48        |
| References                                                                | 51        |
| <b>Chapter 2 DNA Polymerases: General Aspects</b>                         | <b>63</b> |
| 2.1 Synthesis and maintenance of DNA in nature need DNA polymerases       | 63        |
| 2.2 The DNA polymerase reaction                                           | 65        |
| 2.3 The universal right-hand structure of a DNA polymerase                | 68        |
| 2.4 The eight DNA polymerase families and their functions: An overview    | 70        |
| 2.5 DNA polymerase holoenzymes                                            | 78        |
| 2.6 DNA polymerases, ring-like clamps and clamp loaders                   | 81        |

|                  |                                                                                                                         |           |
|------------------|-------------------------------------------------------------------------------------------------------------------------|-----------|
| 2.7              | DNA polymerases, alternative clamps and clamp loaders                                                                   | 86        |
| 2.8              | Replicative DNA polymerases and interacting proteins                                                                    | 88        |
| 2.9              | DNA polymerases and the single-stranded DNA binding replication protein A                                               | 89        |
| 2.10             | Chapter summary                                                                                                         | 91        |
|                  | References                                                                                                              | 93        |
| <b>Chapter 3</b> | <b>Human DNA Polymerases: From Structure to Function</b>                                                                | <b>99</b> |
| 3.1              | The high number of specialized pathways in eukaryotic cells requires a plethora of specialized DNA synthesizing enzymes | 99        |
| 3.2              | Eukaryotic DNA polymerase structure: The "right hand" of the cell                                                       | 104       |
| 3.2.1            | Common features                                                                                                         | 104       |
| 3.2.2            | Specific features of the different families                                                                             | 106       |
| 3.3              | Eukaryotic DNA polymerases accessory subunits                                                                           | 115       |
| 3.4              | Eukaryotic DNA polymerase fidelity: Structural and functional aspects                                                   | 120       |
| 3.5              | Biochemical and functional properties of the different eukaryotic DNA polymerases                                       | 124       |
| 3.5.1            | Family A DNA polymerases                                                                                                | 124       |
| 3.5.2            | Family B DNA polymerases                                                                                                | 130       |
| 3.5.3            | Family X DNA polymerases                                                                                                | 139       |
| 3.5.4            | Family Y DNA polymerases                                                                                                | 146       |
| 3.5.5            | PrimPol                                                                                                                 | 151       |
| 3.6              | Interaction with auxiliary factors                                                                                      | 152       |
| 3.7              | Incorporation of ribonucleotides in DNA                                                                                 | 156       |
| 3.8              | Eukaryotic DNA polymerases are tightly regulated in the cell cycle                                                      | 157       |

|                                                                                                                           |            |
|---------------------------------------------------------------------------------------------------------------------------|------------|
| 3.9 Chapter summary                                                                                                       | 161        |
| References                                                                                                                | 162        |
| <b>Chapter 4 Human DNA Polymerases in Different DNA Transactions</b>                                                      | <b>191</b> |
| 4.1 Sixteen DNA polymerases and telomerase: share of workload and redundancies                                            | 191        |
| 4.2 DNA replication in living organisms requires three DNA polymerase molecules at the replication fork                   | 197        |
| 4.2.1 Prokaryotes                                                                                                         | 197        |
| 4.2.1.1 The <i>Escherichia coli</i> replisome                                                                             | 197        |
| 4.2.2 Eukaryotes                                                                                                          | 201        |
| 4.2.3 Proofreader versus non-proofreader DNA polymerases                                                                  | 203        |
| 4.3 Different DNA repair pathways have their own DNA polymerases, but can also borrow them from the replication machinery | 204        |
| 4.4 Translesion DNA synthesis in eukaryotes generally requires two DNA polymerases: An inserter and an extender           | 210        |
| 4.5 Expression of DNA polymerases                                                                                         | 216        |
| 4.6 DNA polymerases switch between different DNA transactions                                                             | 217        |
| 4.6.1 Prokaryotes                                                                                                         | 217        |
| 4.6.2 Eukaryotes                                                                                                          | 220        |
| 4.7 Functions of DNA polymerases in checkpoint control                                                                    | 229        |
| 4.8 Chapter summary                                                                                                       | 232        |
| References                                                                                                                | 233        |
| <b>Chapter 5 DNA Polymerases and Human Diseases</b>                                                                       | <b>249</b> |
| 5.1 Introduction                                                                                                          | 249        |
| 5.2. DNA polymerases and genetic stability                                                                                | 253        |

|                                                                                                                                      |            |
|--------------------------------------------------------------------------------------------------------------------------------------|------------|
| 5.3 DNA polymerases and resistance to chemotherapy                                                                                   | 265        |
| 5.4 DNA polymerase $\gamma$ and human diseases                                                                                       | 266        |
| 5.5 Chapter summary                                                                                                                  | 270        |
| References                                                                                                                           | 271        |
| <b>Chapter 6 Human DNA Polymerases and Chemotherapy</b>                                                                              | <b>281</b> |
| 6.1 DNA polymerases are important chemotherapeutic targets                                                                           | 281        |
| 6.2 Strategies and problems for the design of inhibitors of DNA polymerases                                                          | 282        |
| 6.2.1 Substrate analogs                                                                                                              | 282        |
| 6.2.2 Non-substrate analogs                                                                                                          | 284        |
| 6.2.3 Novel <i>in silico</i> technologies for designing inhibitors of DNA polymerases                                                | 285        |
| 6.3 The rationale behind the inhibition of DNA polymerases                                                                           | 288        |
| 6.3.1 The synthetic lethality approach                                                                                               | 288        |
| 6.3.2 DNA polymerases in cancer progression and chemoresistance                                                                      | 290        |
| 6.4 Inhibitors of family X DNA polymerases                                                                                           | 291        |
| 6.4.1 Terminal deoxynucleotidyl transferase inhibitors                                                                               | 291        |
| 6.4.2 DNA polymerase $\lambda$ and $\beta$ inhibitors                                                                                | 293        |
| 6.5 Inhibitors of family Y DNA polymerases                                                                                           | 294        |
| 6.6 Natural inhibitors of DNA polymerases                                                                                            | 295        |
| 6.7 Chapter summary                                                                                                                  | 296        |
| References                                                                                                                           | 297        |
| <b>Chapter 7 Polymerase Chain Reaction and Heat-Stable DNA Polymerases: The History and the Potential of Evolved DNA Polymerases</b> | <b>303</b> |
| References                                                                                                                           | 307        |

|                                                                                                                                      |            |
|--------------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>Chapter 8 Synthetic Evolution of DNA Polymerases for Novel Properties</b>                                                         | <b>309</b> |
| 8.1 Why design enzymes with novel properties?                                                                                        | 309        |
| 8.2 DNA polymerases have a tight active site to which the substrates fit                                                             | 310        |
| 8.3 Methods to evolve DNA polymerases with novel properties                                                                          | 314        |
| 8.3.1 Detection and characterization of DNA polymerases and mutants thereof by functional complementation in <i>Escherichia coli</i> | 314        |
| 8.3.2 DNA polymerase evolution by random point mutagenesis                                                                           | 315        |
| 8.3.3 DNA polymerase evolution by compartmentalized self-replication (CSR) and by compartmentalized self-tagging (CST)               | 317        |
| 8.3.4 DNA polymerase evolution by phage display                                                                                      | 318        |
| 8.3.5 DNA polymerase evolution by oligonucleotide addressed enzyme assay (OAEA)                                                      | 318        |
| 8.4 Applications of DNA polymerases with novel properties                                                                            | 321        |
| 8.5 DNA polymerases with novel and/or improved properties                                                                            | 323        |
| 8.5.1 DNA polymerases with improved properties for PCR                                                                               | 323        |
| 8.5.2 DNA polymerases resistant to inhibitors                                                                                        | 326        |
| 8.5.3 DNA polymerases handling altered DNA                                                                                           | 327        |
| 8.5.4 DNA polymerase hybrids and dimers                                                                                              | 330        |
| 8.5.5 DNA polymerase with RNA polymerase properties                                                                                  | 331        |

|                                                                                             |                |
|---------------------------------------------------------------------------------------------|----------------|
| 8.5.6 Improved reverse transcriptase PCR enzymes                                            | 332            |
| 8.5.7 DNA polymerases and expansion of the genetic code                                     | 334            |
| 8.5.8 DNA polymerases and next-generation sequencing (NGS)                                  | 336            |
| 8.5.9 DNA polymerases and processing of modified dNTPs and rNTPs                            | 337            |
| 8.5.10 DNA polymerases active on surfaces                                                   | 338            |
| 8.6 DNA polymerases and recent applications                                                 | 338            |
| 8.6.1 DNA polymerases and loop-mediated isothermal amplification of DNA                     | 338            |
| 8.6.2 DNA polymerases and recombinase polymerase amplification                              | 339            |
| 8.6.3 DNA polymerases and ultrafast PCR                                                     | 340            |
| 8.7 Chapter summary                                                                         | 340            |
| References                                                                                  | 341            |
| <br><b>Chapter 9 Market for Evolved DNA Polymerases in Routine and Medical Applications</b> | <br><b>349</b> |
| 9.1 Introduction: The market                                                                | 349            |
| 9.2 Next-generation sequencing (NGS): Prices drop while speed increases                     | 350            |
| 9.3 Single-cell sequencing                                                                  | 352            |
| 9.4 Synthetic biology                                                                       | 353            |
| 9.5 Direct and digital PCR                                                                  | 354            |
| 9.6 Microbiomics, metagenomics and personal genomics                                        | 355            |
| 9.7 Additional amplification techniques                                                     | 356            |
| 9.7.1 Loop-mediated isothermal amplification                                                | 356            |
| 9.7.2 Rolling circle amplification                                                          | 357            |
| 9.7.3 Recombinase polymerase amplification                                                  | 357            |



|                                             |     |
|---------------------------------------------|-----|
| 9.7.4 Nicking enzyme amplification reaction | 358 |
| 9.7.5 Helicase-dependent amplification      | 358 |
| 9.8 Chapter summary                         | 358 |
| References                                  | 359 |

|               |     |
|---------------|-----|
| Subject Index | 363 |
| Author Index  | 373 |