## Contents

Preface		v
About the	Authors	ix
Chapter 1	History of DNA Polymerases	1
	1.1 Imaging an enzyme that assembles the nucleotides into DNA	١
	1.1.1 DNA polymerase activity in extracts of Escherichia coli	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	
	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

Contents
----------

1.2	Late 1	1960s to early 1970s DNA replication	
		s 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	Concl	uding remarks, parts 1.1–1.2	18
1.4	Multi	ple 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
		polymerases $\delta$ and $\varepsilon$	36
1.7	Yeast	DNA polymerases	38
	1.7.1	Revised nomenclature for eukaryotic DNA	
		polymerases	40

xiv

	Contents	XV
	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
Chapter 2	DNA Polymerases: General Aspects	63
	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	70
	functions: An overview	70
	2.5 DNA polymerase holoenzymes	78
	2.6 DNA polymerases, ring-like clamps and	<b>.</b>
	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
Chapter 3	Human DNA Polymerases: From Structure to Function	99
	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

xvi

	Contents	xvii
	3.9 Chapter summary	161
	References	162
Chapter 4	Human DNA Polymerases in Different DNA Transactions	191
	<ul><li>4.1 Sixteen DNA polymerases and telomerase: share of workload and redundancies</li><li>4.2 DNA replication in living organisms requires three</li></ul>	191
	DNA polymerase molecules at the replication fork	197
	4.2.1 Prokaryotes	197
	4.2.1.1 The Escherichia coli 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	210 216
	<ul><li>4.5 Expression of DNA polymerases</li><li>4.6 DNA polymerases switch between different</li></ul>	210
	DNA polymenases swhen between unreferr	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
Chapter 5	DNA Polymerases and Human Diseases	249
	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
Chapter 6	Human DNA Polymerases and Chemotherapy	281
	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	200
	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
Chapter 7	Polymerase Chain Reaction and Heat-Stable DNA Polymerases: The History and the Potential of	
	Evolved DNA Polymerases	303
	References	307

			Contents	xix
Chapter 8	-	nthetic opertic	e Evolution of DNA Polymerases for Novel es	309
		-	design enzymes with novel properties? polymerases have a tight active site to	309
		whicl	h the substrates fit	310
	8.3		ods to evolve DNA polymerases with properties	314
		8.3.1	Detection and characterization of DNA polymerases and mutants thereof by functional complementation in	214
		0.0.0	Escherichia coli	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	217
		071	self-tagging (CST)	317
		0.3.4	DNA polymerase evolution by phage display	318
		8.3.5	DNA polymerase evolution by oligonucleotide addressed enzyme assay	510
			(OAEA)	318
	8.4	Appli prope	cations of DNA polymerases with novel rties	321
	8.5	DNA	polymerases with novel and/or improved	
		prope	rties	323
		8.5.1	DNA polymerases with improved	
			properties for PCR	323
			DNA polymerases resistant to inhibitors	326
			DNA polymerases handling altered DNA	327
			DNA polymerase hybrids and dimers	330
		8.5.5	DNA polymerase with RNA polymerase properties	331

	8.5.6 Improved reverse transcriptase PCR	332
	enzymes 8.5.7 DNA polymerases and expansion of the	
	genetic code 8.5.8 DNA polymerases and next-generation	334
	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
		541
Chapter 9	Market for Evolved DNA Polymerases in Routine and Medical Applications	349
Chapter 9	Market for Evolved DNA Polymerases in Routine	
Chapter 9	Market for Evolved DNA Polymerases in Routine and Medical Applications	349
Chapter 9	Market for Evolved DNA Polymerases in Routine and Medical Applications 9.1 Introduction: The market	349
Chapter 9	<ul> <li>Market for Evolved DNA Polymerases in Routine and Medical Applications</li> <li>9.1 Introduction: The market</li> <li>9.2 Next-generation sequencing (NGS): Prices drop while speed increases</li> <li>9.3 Single-cell sequencing</li> </ul>	<b>349</b> 349
Chapter 9	<ul> <li>Market for Evolved DNA Polymerases in Routine and Medical Applications</li> <li>9.1 Introduction: The market</li> <li>9.2 Next-generation sequencing (NGS): Prices drop while speed increases</li> </ul>	<b>349</b> 349 350
Chapter 9	<ul> <li>Market for Evolved DNA Polymerases in Routine and Medical Applications</li> <li>9.1 Introduction: The market</li> <li>9.2 Next-generation sequencing (NGS): Prices drop while speed increases</li> <li>9.3 Single-cell sequencing</li> </ul>	<b>349</b> 349 350 352
Chapter 9	<ul> <li>Market for Evolved DNA Polymerases in Routine and Medical Applications</li> <li>9.1 Introduction: The market</li> <li>9.2 Next-generation sequencing (NGS): Prices drop while speed increases</li> <li>9.3 Single-cell sequencing</li> <li>9.4 Synthetic biology</li> </ul>	<b>349</b> 349 350 352 353 354
Chapter 9	<ul> <li>Market for Evolved DNA Polymerases in Routine and Medical Applications</li> <li>9.1 Introduction: The market</li> <li>9.2 Next-generation sequencing (NGS): Prices drop while speed increases</li> <li>9.3 Single-cell sequencing</li> <li>9.4 Synthetic biology</li> <li>9.5 Direct and digital PCR</li> <li>9.6 Microbiomics, metagenomics and personal genomics</li> </ul>	<b>349</b> 349 350 352 353 354 355
Chapter 9	<ul> <li>Market for Evolved DNA Polymerases in Routine and Medical Applications</li> <li>9.1 Introduction: The market</li> <li>9.2 Next-generation sequencing (NGS): Prices drop while speed increases</li> <li>9.3 Single-cell sequencing</li> <li>9.4 Synthetic biology</li> <li>9.5 Direct and digital PCR</li> <li>9.6 Microbiomics, metagenomics and personal</li> </ul>	<b>349</b> 349 350 352 353 354
Chapter 9	<ul> <li>Market for Evolved DNA Polymerases in Routine and Medical Applications</li> <li>9.1 Introduction: The market</li> <li>9.2 Next-generation sequencing (NGS): Prices drop while speed increases</li> <li>9.3 Single-cell sequencing</li> <li>9.4 Synthetic biology</li> <li>9.5 Direct and digital PCR</li> <li>9.6 Microbiomics, metagenomics and personal genomics</li> </ul>	<b>349</b> 349 350 352 353 354 355
Chapter 9	<ul> <li>Market for Evolved DNA Polymerases in Routine and Medical Applications</li> <li>9.1 Introduction: The market</li> <li>9.2 Next-generation sequencing (NGS): Prices drop while speed increases</li> <li>9.3 Single-cell sequencing</li> <li>9.4 Synthetic biology</li> <li>9.5 Direct and digital PCR</li> <li>9.6 Microbiomics, metagenomics and personal genomics</li> <li>9.7 Additional amplification techniques</li> </ul>	<b>349</b> 349 350 352 353 354 355 356

Contents	xxi
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