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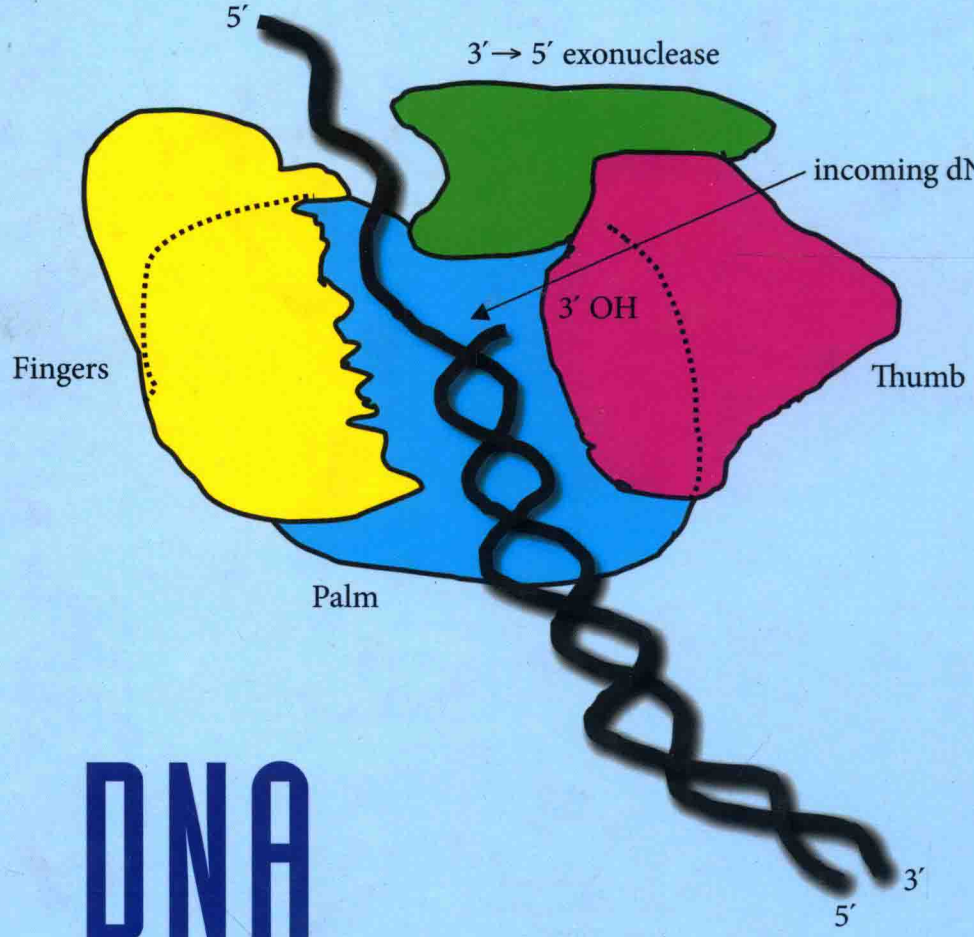

DNA POLYMERASES

Discovery, Characterization and Functions in Cellular DNA Transactions

World Scientific

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NEW JERSEY · LONDON · SINGAPORE · BEIJING · SHANGHAI · HONG KONG · TAIPEI · CHENNAI

Published by

World Scientific Publishing Co. Pte. Ltd.

5 Toh Tuck Link, Singapore 596224

USA office: 27 Warren Street, Suite 401-402, Hackensack, NJ 07601

UK office: 57 Shelton Street, Covent Garden, London WC2H 9HE

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

DNA POLYMERASES
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ISBN-13 978-981-4299-16-9
ISBN-10 981-4299-16-2

Typeset by Stallion Press
Email: enquiries@stallionpress.com

Printed by FuIsland Offset Printing (S) Pte Ltd. Singapore

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Preface

Maintenance of the information embedded in the genomic DNA sequence is crucial for the survival of any living species. DNA polymerases play pivotal roles in this complex process since they are involved in all DNA synthesis events occurring in nature. Besides their essential tasks *in vivo*, DNA polymerases are now the workhorses in numerous important molecular biological and medical core technologies such as the widely applied polymerase chain reaction (PCR), cDNA cloning, genome sequencing, nucleic acids–based diagnostics and in techniques to analyze ancient and otherwise damaged DNA.

The history of DNA polymerases goes back over 53 years to the mid-1950s. With the discovery of a DNA polymerase (now known as DNA polymerase I) in the bacterium *Escherichia coli* by Arthur Kornberg and his colleagues, it was for the first time possible to synthesize the genetic material, the DNA, in the test tube. A manuscript reporting the discovery submitted to the *Journal of Biological Chemistry* was rejected with comments such as "the researchers were incompetent" and DNA polymerase was "a poor name" for this enzyme. What a terrible judgment by an incompetent editor! The observation by Kornberg and collaborators that a DNA polymerase synthesizes DNA according to the Watson–Crick base pair rule (A–T and G–C), needs activated bases (dNTPs), a template, a primer and $MgCl_2$ is still true today for most DNA polymerases in all organisms tested.

Bruce Alberts in a feature article in *Nature* stated in 2003[1]: "Knowledge of the structure of DNA enabled scientists to undertake the difficult task of deciphering the detailed molecular mechanisms of two dynamic processes that are central to life: the copying of the genetic information by DNA replication, and its reassortment and repair by DNA recombination. Despite dramatic advances towards this goal over the past five decades, many challenges remain for the next generation of molecular biologists."

A comprehensive book focusing on DNA polymerases appeared in 1986 and covered exclusively animal DNA polymerases.[2] At that time the animal DNA polymerase family was still small. Only three DNA polymerases, α, β and γ were

known in detail and the scientific community just started to believe in a fourth DNA polymerase, called DNA polymerase δ. In the last 23 years, due to the analysis of various genomes, including the one from humans, we have witnessed the discovery of an abundance of novel DNA polymerases in the three kingdoms of life (bacteria, archaea and eukaryotes) with specialized properties whose physiological functions are only beginning to be understood. For several decades the dogma was widely accepted that very accurate DNA polymerases guarantee the faithful duplication of DNA, while their limited capacity of making mistakes might be one of the drivers for evolution and a cause of disease. When in 1999 the human genome was sequenced, nine newly discovered DNA polymerases appeared within three years in the literature (1999–2002). Many of these DNA polymerases appear to have distinct functions in translesion synthesis, in different DNA repair events or in immunoglobulin V(D)J recombination.

This book starts by presenting the history of the discovery of DNA and DNA polymerases, including the polymerase chain reaction (PCR) (Chapter 1), followed by the presentation of DNA polymerases from the three kingdoms of life: bacteria, archaea and eukaryotes (Chapter 2). Next, the structural and functional aspects of the different DNA polymerase families are described in prokaryotes (Chapter 3) and in eukaryotes (Chapter 4). Preventing genetic instability is of great importance in life. Temporal and spatial regulation of DNA polymerases is of paramount interest for the organism. This might occur via regulation of their expression, their stability and their localization. Many posttranslational modifications contribute to these properties. DNA polymerases are not autistic enzymes but rather work in a broader context within a cell and they can replace one another under certain physiological and even pathological situations (Chapter 5: Global functions of DNA polymerases). Many fundamental mechanistic properties have been elucidated, thanks to the study of DNA polymerases from bacterial and animal cells viruses. The most relevant ones will be described in Chapter 6. A recent field of Chemical Biology has developed techniques that allow the evolution of DNA polymerases in the test tube and thus a variety of novel applications may be ahead of us in the near future (Chapter 7). Many diseases have been correlated with malregulations and malfunctions of DNA polymerases (Chapter 8) and DNA polymerase inhibitors are used as chemotherapeutic agents (Chapter 9). The chapters are written so that they can be read and understood in their own and this will necessarily bring a certain redundancy. We have also made cross-references where appropriate. In summary, this book provides the arguments and evidence that characterize DNA polymerases as enzymes essential to life and also place them among the most important tools in modern chemistry, biology and medicine.

We would like to thank Ursula Hübscher, Ralph Imhof, Nicolas Tanguy Le Gac and Fabio Cobianchi for artwork and the following colleagues who revised chapters of this book: Paul Boehmer, Bill Copeland, Myron Goodman, Ghyslaine Henneke, Jean-Sébastien Hoffmann, Neil Johnson, Enni Markkanen, Andreas Marx, Kristijan Ramadan, Arthur Weissbach and George Wright.

Zurich (Switzerland), November 2009

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Contents

CHAPTER 1

History and Discovery of DNA Polymerases

1.1 Discovering DNA: A First Step Towards Understanding the Basis of Life

In retrospect, serendipity is often invoked as a major component of scientific discoveries and inventions although scientists and inventors might conceivably be reluctant to admit it. The accidental nature of some or many scientific discoveries does not in our opinion apply to DNA polymerases (pols), discovered in 1955–1957 by Arthur Kornberg (***1918–2007***) and co-workers. As a highly well-prepared and open-minded scientist, he was certainly more able than others to detect the importance of previous information that led to the discovery of DNA, to the demonstration that it carries genetic information, and finally to the elucidation of its structure and function in 1953.

But in his discovery of DNA pols Arthur Kornberg was undoubtedly guided by "*his unremitting fascination with enzymes, molecules which give the cell its life and personality and make things happen*". He learned his love for enzymes from some of the best enzymologists of his time such as Severo Ochoa (***1905–1993***), Carl F. (***1896–1984***), and Gerty Cori (***1896–1957***). As we shall see later, prior to the discovery of *E. coli* DNA pol, he did in fact fundamental work on the elucidation of coenzymes (NAD, NADP, FAD) and nucleotide biosynthesis in a way that "*one thing had always led to the next one*". This previous work, his deep belief that enzymes were the key to understanding biochemical processes, and the proposed structure of DNA by Watson and Crick, led him to discover DNA pols and to devote most of his scientific career to the enzymes that assemble DNA.

Although most exciting and fundamental discoveries in DNA research occurred over a period of 20 years in the middle of the twentieth century, research into DNA had begun some 80 years earlier. But the work of many DNA pioneers was completely obscured by the subsequent discovery by Watson and Crick of the double

helix structure of DNA and that work is now underappreciated by or even unknown to many young students and researchers. The structure of DNA proposed by Watson and Crick immediately appeared to everybody so naturally logical and unquestionable that there was no time for astonishment and for looking back at the times when the genetic secret of our life was unknown.

Thus, before discussing the discovery of DNA pols, we would like to mention and acknowledge the work of many scientists that led to the discovery of DNA, of its structure and function, and, as a consequence, to the search and discovery of DNA pols by Arthur Kornberg. For the first part of this chapter, references will be limited to few historical books.[1–3]

Following the discovery of the first practical Microscope in late 17th century by A. van Leeuwenhoek (***1632–1723***), research was dominated for several decades by cytologists doing observations of all kinds of material under the microscope. The English Robert Hooke (***1635–1703***) in his *Micrographia*, published in London in 1665, named *cells* the smallest living biological structures surrounded by walls observed in cork. A name derived by the latin *cella*, meaning a "small room" as those where the monks were living in.

Regarding the initial content of this chapter, the discovery of DNA, the way to the knowledge of *variation of individuals in certain characters, of the heredity of parental characters and finally of natural selection that allowed some individuals to survive and reproduce better than others*, was opened many years later when, in 1858, Charles R. Darwin (***1809–1882***) and Alfred Russel Wallace (***1823–1913***) published their joined communication "*On the tendency of species to form varieties and on the perpetuation of varieties and species by natural means of selection*",[4] thus suggesting the conception of the *struggle for existence and of the survival of the fittest*. This communication was followed in November 1859 by Darwin's book *On the Origin of Species by Means of Natural Selection* published in London. Then a few years later (1865–1869), Gregor Mendel (***1822–1884***) published in a local journal the results of his experiments with pea plants, made in the monastery garden of the convent at Brünn in Moravia, on the *nature of genetic inheritance of particular traits carried by units*. Almost unnoticed for some decades by the scientific world because his communications were to a local journal, Mendel's work defined the basic rules of heredity. In 1905 the Danish scientist W. L. Johannsen (***1857–1927***) called these *units*, residing in the chromosomes, *genes* and also introduced the terms *genotype*, *phenotype* and *biotype* that are now part of the common language of genetics. Historically, however, the terms "*genos*" (family or clan) and "*gonos*" (sperm or seed), forefathers of "*gene*", appeared for the first time in a text of *Corpus Hippocraticum* where Hippocrates stated that the so-called Sacred Disease (Epilepsy) was not a divine but a hereditary disease.

According to C.D. Darlington (***1903–1981***), an English biologist and geneticist, who significantly contributed to understand chromosomal crossing-over and its role in inheritance and evolution, it was the German botanist and biologist Wilhelm Friedrich Benedikt Hofmeister (***1824–1877***) who first observed, in 1848, small *rodlike bodies* in plant cells nuclei during mitosis, at least 30 years in advance of another German biologist, Walther Flemming (***1843–1905***). Thanks to developments in microscopy, Flemming, in 1879, observed and described within the nucleus *tiny thread-like structures,* which strongly absorbed basophilic dyes, and he called them *chromatin* from the Greek word for "color". Later, in 1888, they were called *chromosomes* — colored bodies — by the German anatomist Wilhelm von Waldeyer-Hartz (***1836–1910***) for their ability to absorb certain dyes. Flemming further investigated the process of cell division and the distribution of such chromosomes to the daughter nuclei, a process he called *mitosis* (from the Greek word *mitos* meaning "thread"). Flemming hypothesized that all cell nuclei came from another predecessor nucleus. He coined the phrase "*omnis nucleus e nucleo*" ("every nucleus from nucleus") after Virchow's (***1821–1902***) "*omnis cellula e cellula*" ("every cell from a cell"), but he did not realize the splitting of chromosomes into identical halves, the *daughter* chromatids. Unaware, as other men of science of his days, of Gregor Mendel's work on heredity, published, as we mentioned, in a local journal and left unnoticed for a long time, Flemming did not make the connection between his observations and genetic inheritance.

1.1.1 *Nuclein*

It was in 1869, almost a century before the Nobel Prize awarded to Watson, Crick and Wilkins for elucidating the structure of DNA, that a young Swiss physician, Friedrich Miescher (***1844–1895***) isolated a substance that he called *nuclein*, now known as *DNA*, as reviewed in Refs. 5 and 6. Just after receiving his M.D., encouraged by one of his Professors Ernst Felix Immanuel Hoppe-Seyler (***1825–1895***), Miescher chose scientific research rather than Medicine as a career.

Only few years earlier Gregor Mendel had finished a series of experiments with peas and made observations that turned out to be closely connected to the finding of *nuclein*. Mendel was in fact able to show that certain traits in the peas, such as their shape or color, were inherited in different units (later called *genes*), but his work went unnoticed for many years.

Thus at a time when scientists were still debating the concept of the *cell* and of its origin from another cell, Hoppe-Seyler, one of the pioneers in physiological chemistry, and his lab at the Faculty of Natural Sciences in Tübingen, were trying to isolate and study the molecules that made up cells and cellular organelles such