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PROTEINS INVOLVED IN DNA REPLICATION

Edited by Ulrich Hübscher and Silvio Spadari

PROTEINS INVOLVED IN DNA REPLICATION

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PREFACE

This book collects the Proceedings of a workshop sponsored by the European Molecular Biology Organization (EMBO) entitled "Proteins Involved in DNA Replication" which was held September 19 to 23,1983 at Vitznau, near Lucerne, in Switzerland.

The aim of this workshop was to review and discuss the status of our knowledge on the intricate array of enzymes and proteins that allow the replication of the DNA. Since the first discovery of a DNA polymerase in Escherichia coli by Arthur Kornberg twenty eight years ago, a great number of enzymes and other proteins were described that are essential for this process: different DNA polymerases, DNA primases, DNA dependent ATPases, helicases, DNA ligases, DNA topoisomerases, exo- and endonucleases, DNA binding proteins and others. They are required for the initiation of a round of synthesis at each replication origin, for the progress of the growing fork, for the disentanglement of the replication product, or for assuring the fidelity of the replication process.

The number, variety and ways in which these proteins interact with DNA and with each other to the achievement of replication and to the maintenance of the physiological structure of the chromosomes is the subject of the contributions collected in this volume. The presentations and discussions during this workshop reinforced the view that DNA replication in vivo can only be achieved through the cooperation of a high number of enzymes, proteins and other cofactors. The need for clean and refined enzymological work, coupled to the contribution of the genetic analysis and molecular cloning, is as pressing as ever in order to obtain a satisfactory picture of the processes at the molecular level.

The authors thank all participants for contributing to a friendly and scientifically fruitful meeting. Fifty eight papers were selected to cover some of the most relevant recent approaches and efforts in molecular biology of DNA replication.

We want to express our gratitude to all those who helped to organize this meeting and to the European Molecular Biology

Organization for its generous financial support. Furthermore we are indebted to Ursula Hübscher-Faé for her secretarial assistance before, during and after the meeting and for carefully typing all the manuscripts.

March 1984

Ulrich Hübscher
Silvio Spadari

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I
In Vitro Prokaryotic DNA Replication Systems

ENZYME STUDIES OF REPLICATION OF THE ESCHERICHIA COLI CHROMOSOME

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I want at the outset to express my gratitude to the organizers, Ulrich Hübscher and Silvio Spadari, for their wisdom and initiative in convening a conference on a subject that is important, timely and not adequately appreciated: The Proteins Involved in DNA Replication.

During the very days of this Workshop, a more highly publicized symposium is being held in Cambridge, Massachusetts. It is organized by Nature magazine to celebrate 30 years of DNA. The subjects include DNA structure, gene expression, developmental biology and biomedical applications, but none of the twenty-one contributions to the program deals with proteins in DNA replication, repair or recombination. What better way is there to chronicle and glorify the recent history of DNA than to celebrate the enzymes that create and maintain it! It is often forgotten that these enzymes are the reagents that gave rise to the recombinant DNA technology that made studies on DNA structure, gene expression and biomedical applications possible. Not only do the proteins involved in DNA replication, repair, recombination and transposition have an important place in the recent history of biologic science, but the elucidation of chromosome structure and function in the future will depend far more on understanding these and related proteins than it will on the sequence and organization of DNA itself.

After more than twenty years of studying the proteins of DNA replication and their mechanisms, a number of basic facts have become clear. These verities are: (i) dNTPs $\rightarrow$ $(dNMP)_n$ + nPP_i, (ii) 5' $\rightarrow$ 3' elongation, (iii) Watson-Crick base pairing, (iv) auxiliary subunits for processivity and fidelity, and (v) generally, but not universally, chain initiation by RNA priming.

I will dwell here on predominant patterns, knowing that there are exceptions or multiple variations, even within a single cell. It is disadvantageous or even lethal for a cell to lack metabolic alternatives; the metabolism of DNA is no exception. As we learn more about DNA metabolism of a single cell, we discover auxiliary and alternative enzymes and arrangements, cryptic origins of replication and many possibilities for suppressing otherwise lethal mutations. Among the variations known, or likely, in DNA replication are: (i) sizes and subunits of polymerases, (ii) processivity and fidelity of polymerases, (iii) primases and their mechanisms, (iv) chromosome initiation, and (v) chromosome termination and segregation. The stages and principal actors in DNA replication to be reviewed here will include: (i) elongation of a DNA chain by DNA polymerase III holoenzyme, (ii) initiation of a chain by RNA priming dependent on a primase or a primosome, (iii) organization of chain elongation and initiations at the replication fork in a complex assembly (the putative "replisome"), and (iv) initiation of a cycle of chromosome replication at its unique origin, called oriC. Not included in this review is the biochemistry of termination of replication and segregation of the daughter chromosome. Too little is yet known about this subject. It will surely be enriched by future studies of the partition functions of plasmids.

Studies of duplex DNA replication in Escherichia coli, including that of its viruses and plasmids, impinge on many features of DNA repair, recombination and transposition. In all these aspects, Escherichia coli DNA replication continues to be an experimentally attractive subject and is proving to be prototypical for basic mechanisms in replication throughout nature.

The strategy of my experimental approach to understanding DNA replication has been to resolve and reconstitute the responsible proteins. Mutants, when available, are invaluable as the source of assays for isolation of proteins by functional complementation and as touchstones to verify the pathway under study. Even without mutants as pillars and guides, fractionation and purification of the entities needed for properly defined replication events can proceed. Proteins purified without adequate functional criteria are likely to remain chronically unemployed.

Elongation of a DNA chain: Escherichia coli DNA polymerase III holoenzyme

Replication of DNA duplexes is generally semidiscontinous. One strand (leading) is synthesized continuously; the other strand (lagging) is synthesized in small pieces (Okazaki fragments), that is, discontinuously (1, 2). The principal synthetic enzyme for both strands in Escherichia coli is the DNA pol III holoenzyme. Holoenzyme has a core of 3 subunits with a polypeptide of 140 kdal (α subunit) responsible for polymerization and proofreading (Table 1)(3). Four