

I

Prokaryotic DNA Replication

WILLIAM FIRSHEIN

Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown,
 Connecticut 06459

I. Introduction	3
II. General Concepts of DNA Replication	4
A. Semiconservative Synthesis	4
B. The Replicon Model	5
III. Replication Operations	6
A. Initiation	6
B. Elongation	7
1. Fine Details of Elongation	8
C. Termination	12
D. Precursors in DNA Replication	16
1. Introduction	16
2. Types of Metabolic Pathways	16
3. Multienzyme Complexes	18
IV. The Replicon Membrane Interaction	18
A. Introduction	18
B. Specific Organisms	19
1. <i>E. coli</i>	19
2. <i>B. subtilis</i>	20
3. Plasmid RK2	21
V. General Conclusions	22

I. INTRODUCTION

Ultimately DNA structure must be understood in terms of its function just as function requires knowledge of structure. Each function must be resolved and reconstituted in complete detail in order to connect it to a structure in the cell. In the case of DNA, three hierarchical functions—storage of genetic information, replication of this information from generation to generation, and ultimate control of the functions of cellular activities—have been elucidated in exquisite detail, although our understanding of those details is far from complete. Much of

the success was made possible after Watson and Crick (1953) proposed that the structure of DNA existed as a double helix of sugar-phosphates held together by two purine and pyrimidine base pairs, adenine-thymine and guanine-cytosine, respectively. It was the sequence of these base pairs that determined the exact composition of the DNA molecule and the molecular structure of the gene (storage of genetic information).

Replication of the double helix was proposed by Watson and Crick to be based upon the separation of two helices which acted as templates for the precise copying of complementary strands to form two progeny double helices according to the sequence of the base pairs (termed *semi-conservative replication*). However, in attempting to identify the components (enzymes, control factors) responsible for this precise duplication, it became obvious that the process was interdependent with other related phenomena such as repair and recombination of DNA. Some of the enzymes could be used for all of the processes. In fact there is a growing body of knowledge that not only are the pathways intimately related, but many of the proteins may be part of a "superfamily" in which all of them share a highly conserved DNA-binding motif as determined by X-ray crystallography or electron microscopy (Engelman, 2000).

The difficulty (and complexity) of elucidating these interactions is further underscored by two additional characteristics of the replicative process. First, unlike RNA and protein synthesis, DNA replication occurs at discrete times during the cell cycle. The many components involved must be assembled and disassembled after each round of replication. Second, unlike the organelle involved in protein synthesis (the ribosome) which is held together with strong forces, those that maintain the DNA replisome (the components involved in DNA replication) involve weak electrostatic forces which can be dissociated under mild salt conditions. Thus *in vitro* studies that have formed the bases for understanding many of the intricacies of replication are subject to artifacts because extraction of the replisome from cells may be disruptive and not represent the *in vivo* condition as fully as possible.

Nevertheless, much has been revealed by classic *in vitro* studies of prokaryotes using single stranded DNA viruses that infect *Escherichia coli* and sequester many of the host's components (Kornberg and Baker, 1992) and recombinant plasmids containing

the beginning (origin) of replication (*oriC*) for this and other organisms such as *Bacillus subtilis* (Kornberg and Baker, 1992; Moriya et al., 1994).

II. GENERAL CONCEPTS OF DNA REPLICATION

A. Semiconservative Synthesis

How could Watson and Crick's model be proven that replication occurred in a semi-conservative manner? In fact two additional possibilities existed besides such a mechanism. These included conservative (both strands replicated simultaneously) or dispersive (each strand was fragmented, copied and joined to form a completely new parental and progeny strand).

The most important and definitive experiments that proved that DNA was replicated semiconservatively were carried out by Meselson and Stahl (1958). They adapted *E. coli* to a growth medium containing $N^{15}H_4Cl$ ensuring that every molecule in the cell containing nitrogen (including DNA) would have the N^{15} heavy density label. When these cells were shifted to a medium containing the normal light density $N^{14}H_4Cl$, the resulting progeny double helices after one generation consisted of a hybrid density DNA species containing presumably one strand of N^{15} -DNA and one strand of N^{14} -DNA. After a second generation in light density medium, the double helices consisted equally of both the hybrid density species and a complete light density species. This is seen in Figure 1 where the various DNA species are separated by centrifugation in a neutral cesium chloride equilibrium density gradient.

The other hypotheses could not be supported by these results. Further proof of the mechanism was obtained by separating the hybrid density species in an alkaline cesium chloride density gradient which denatured the DNA into two single stranded forms on the gradient, one consisting of N^{15} -DNA, the other of N^{14} -DNA (Meselson and Stahl, 1958).