Arch. Biol. Med. Exp. *13*, 213-232, 1980

The Enzymology of DNA Replication

La enzimología de la replicación de DNA

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(Recibido el 26 de marzo de 1980)

1. INTRODUCTION

Progress made during the last years in our knowledge of DNA replication has shown that it is a somewhat complex process (for recent reviews see refs 1-6). Attempts to elucidate the mechanism of DNA synthesis have been undertaken using simple templates, such as bacterial and animal viral chromosomes and plasmids. All these systems have shown that several proteins are required to initiate and elongate DNA chains, as well as to bring about the topological changes the DNA molecule must undergo during its duplication. The requirement for these proteins can be directly demonstrated with the utilization of thermosensitive mutants, by using specific inhibitors and by reconstitution of an *in vitro* replication system with the purified components. Although in some cases

[1]The abbreviations used are: ss: single stranded; ds: double stranded; NEM: N-ethylmaleimide; DNA pol: DNA polymerase; M. W.: molecular weight; ddTTP: 2'3' dideoxythymidine triphosphate; HDP: helix destabilising protein; RF: replicative form; EF: elongation factor; SDS: sodium dodecyl sulfate.

direct implication of certains proteins has not been possible, the catalytic activity of some proteins (e.g. topoisomerases, helicases) justify the assumption that they play an essential role in DNA replication.

This article does not intend to be a critical nor a comprehensive review on DNA synthesis. It represents rather a survey of some of the proteins that are involved in this process, both in prokaryotic and eukaryotic systems.

2. THE INITIATION REACTION

None of the viral, bacterial or animal DNA polymerases isolated to date are able to start DNA chains *de novo*, suggesting that other proteins are involved in chain initiation. These enzymes, called DNA primases, show a high degree of site specificity when they act at the origin of chromosomal replication. They must also copy the small oligonucleotides that serve as primers for the synthesis of Okazaky fragments.

2.1. *E. Coli* PRIMING PROTEINS

— dna *G gene product*

Using the ss[1] DNA of phage G4 as template, *Bouché et al.* (7) were the first to show that the dna G gene product of *E. coli*, in the presence of binding protein, catalyzes the synthesis of a short polynucleotide primer at the origin of replication. Later, S. Wickner (8) demonstrated that dna G polymerizes rNTPs and dNTPs interchangeably, to yield ribo-, deoxyribo- and mixed ribo-deoxyribonucleotide primers.

The isolation of dna G primase has been approached in three different ways (9, 10). One method consists in an *in vitro* complementation assay, in which dna G protein present in an extract prepared from wild type cells restores the activity of an extract prepared from a dna G ts mutant. A second method has been to follow directly the enzymatic activity of dna G protein, that is, the synthesis of a primer using DNA binding protein plus G4, α 3 or ST-1 DNAs as templates. A third method involves the coupling of the priming reaction with the DNA elongation system. In this assay dNMP incorporation is measured, since it is proportional to the extent of priming.

The dna G protein is a single polypeptide, (M.W. 60,000); it has an isoelectric point of 5.9 at 8°C and it is resistant to NEM and rifampicin (9, 10), but it is sensitive to the nucleotide analogue 2'-deoxy-2'-azido-cytidine triphosphate (11).

dna G primase recognizes a unique region at the origin of minus strand replication of phages G4, α 3, ST-1 and ØK. These various initiation regions have been located with the aid of restriction enzymes and have been defined at the nucleotide level by comparing the sequence of these origin sites with the sequence of an RNA primer synthesized by dna G (12, 13). DNA sequencing studies show that all these origins are very similar, as compared to adjacent coding regions. It is possible to arrange them in similar patterns of two or three hairpin loops in the regions of negative strand initiation suggesting their importance as recognition signals for dna G protein.

Several lines of evidence show that the maintenance of the structure at the origin site rather than the sequence itself is essential for initiation: a) inactive phage DNA can be activated by heating and slow cooling in the presence of *E. coli* binding protein (9); b) *E. coli* dna topoisomerase I, an enzyme known to form knotts with ss DNA (14), when present, inhibits the preming reaction dependent on dna G (9); c) three well separated groups of nucleotides within the negative strand origin of phage ØK are protected by dna G protein agains nuclease digestion, suggesting some degree of folding of the DNA in a tertiary structure (J. Sims, E. Benz, personal communication); d) the priming reaction with ØX174 ss DNA, in addition to dna G and binding protein, requires the products of dna B and dna C, and the replication factors X, Y and Z (also called i, n and n') (15, 16). The role of these additional proteins, which form a pre-priming complex with ØX174 DNA, is perhaps to create a site on the template that can be recognized by dna G primase. However, the origin of ØX174 complementary strand synthesis seems not be unique: if the priming reaction is uncoupled from DNA synthesis, it gives rise to many

primers in each circle (17). Kornberg has proposed (18) that the dna B protein (M.W. 280,000), aided by its own rNTPase action (19, 20), moves processively on the template strand, acting as a mobil replicating promoter. dna B protein interacts specifically with dna C protein (M.W. 30,000) in the presence of ATP (21) and it is thus transferred to the pre-priming complex with the help of protein X (M.W. 45,000). (3). Protein Y (M.W. 70,000), like dna B, is a DNA-dependent ATPase or dATPase, but works best with DNAs that require this protein for initiation (22). This multienzyme system of the host used by ∅X174 to prime DNA synthesis is probably used by the cell to replicate its own chromosome.

The length of the primer synthesized by dna G in the presence of G4 or α3 DNA as templates and the four ribonucleotides is of 28 nucleotides (9, 23). However, the presence of deoxyribonucleotide residues in the chain limits primer chain length (9, 24). This effect occurs even when the ribonucleotides are present in a ten-fold excess in the priming reaction. Only after prolonged incubation periods is the full lenght primer obtained under these conditions (9). When only the four dNTPs are present in the reaction, the rate of primer formation is ten-fold slower compared to the rate with the four rNTPs alone, and the polydeoxynucleotide chains obtained are heterogeneous in size. Primer synthesis is initiated with ATP or dATP at the 5' end. (24). If ADP is used in place of any of these nucleoside triphosphates, it is incorporated both internally and at the 5' end (9).

To date, several genomes have been shown to require dna G for replication. These include the ss DNA phages, the *E. coli.* chromosome, plasmid col El and phage lambda.

— *RNA polymerase.*

Initiation of DNA replication by RNA polymerase both *in vivo* and *in vitro* is well documented for the Col El type plasmids and the filamentous ss DNA phages fd and M13. In both cases, RNA polymerase initiates DNA synthesis at unique origin sites and it is not involved in the priming of Okazaki fragments.

Using fd DNA as template, and in the presence of binding protein, RNase H (an enzyme that degrades RNA when hybridized to DNA) and discriminatory factors α and β, RNA polymerase recognizes a specific hairpin structure of the viral genome and transcribes from it a short RNA fragment (25-28). This primer is subsequently elongated by *E. coli* DNA polymerase III plus the elongation factors. The requirement of additional proteins besides RNA polymerase to initiate the priming reaction is related to the specificity of the system, i.e. to start at a unique origin and also to avoid unspecific priming by RNA polymerase on other templates.

To initiate replication of pBR 345, a Col El-type plasmid, RNA polymerase synthesizes a 100 nucleotide long transcript in a region which is about 450 bases pairs appart from the replication origin (29). Apparently, this RNA segment is processed by RNase III and RNase H (30, 31) and thereafter it hybridizes to the origin region where it serves as a primer for DNA synthesis. Tomizawa *et al.* (32) have mapped the origin of Col E1 DNA replication at the nucleotide level. They have also found that early DNA fragments (6 S) contain ribonucleotide-DNA linkages, most of them having very few ribonucleotides at their 5' ends (33).

RNA polymerase is also required to initiate new rounds of replication of *E. coli.* chromosome (34, 35). There is no evidence, however, that the RNA transcribed at the origin (36) functions as a primer for DNA synthesis. Alternatively, transcription may be a mechanism by which RNA polymerase activates the origin by separating both DNA strands, in order to help dna G to prime replication in the presence of the gene products of dna A, dna B, dna C, dna I, dna J, dna K and dna P (37).

Transcription by RNA polymerase at the initiation region of phage lambda chromosome has also been shown to be essential for DNA replication, not only to provide the messenger of genes O and P, but also to activate the origin (38). Alson in this case, there is no evidence that the transcript is elongated by DNA polymerase.

2.2. BACTERIOPHAGE-CODED DNA PRIMASES

— *T4 DNA primase.*

Two phage T4-coded proteins are required to initiate replication on a ss DNA template. They are the product of gene 41 (39-40) and another protein tentatively identified as the product of the DNA-delay gene 61 (40). Originally, the requirement of the latter protein had been missed because it was present as a minor contaminant in gene 32 protein preparations (41). Gene 41 protein has been purified to near homogeneity (42, 43). It is a single polypeptide chain with a M.W. of 58,000 and catalyzes a ss DNA-dependent hydrolysis of purinic nucleoside triphosphates. Synthesis of the primers, which are 6 to 8 nucleotides long, has an absolute requirement for ATP and CTP. In contrast to dna G and RNA polymerase, but similar to T7 primase, initiation by T4 proteins does not require binding protein.

The reason for the involvement of two proteins in the priming reaction of bacteriophage T4 DNA replication is not known. It could be related to mechanistic problems inherent to initiating synthesis in the lagging strand (39). It may also have to do with the maintenance of the duplex structure between the RNA primer and the DNA template, in order to facilitate elongation by the multienzyme complex formed by proteins coded by genes 32, 43, 44/62 and 45 (39, 44).

—*T7 gene 4 protein*

Gene 4 protein of bacteriophage T7 has been identified as a DNA primase (45-48). After purification with the use of an *in vitro* complementation assay, the enzyme has a M.W. of 58,000. (45,49). Gene 4 protein, in the presence of any natural ss DNA, ATP and CTP, or a mixture of all four rNTPs, catalyzes the synthesis of short oligonucleotides, predominantly pppACCA (45-48). The primary structure of these primers has been confirmed by T. Okazaki (50) by studies done *in vivo*. T7 DNA pol can utilize pppACCA or synthetic tri, tetra or pentanucleotides as chain initiators in the presence of T7 gene 4 protein. Apparently, in this case the primase plays an active role in chain elongation by stabilizing the short primer segments in a duplex state with the DNA template (51). The interaction between both T7-coded enzymes is specific, since other DNA polymerases cannot replace T7 DNA pol in the extension of these oligonucleotides. Unlike other primases, T7 gene 4 protein is activated five fold when dNTPs are present, although there is no evidence that they are incorporated (45). An explanation for this stimulatory effect could be that dNTPs protect rNTPs against hydrolysis by T7 primase, an activity that is intrinsic to the enzyme. (See also section 5.8.).

2.3. OTHER PRIMASES

E. Lanka *et al.* (52) have recently isolated a DNA primase specified by the I-like plasmids (R64, Col I). The enzyme has a sedimentation coefficient of 3.6 s and utilizes all four ribonucleoside triphosphates. In reactions carried out with crude extracts prepared from thermosensitive dna mutans, this primase can substitute for the host functions of dna B-dna C-dna G, RNA polymerase and dna G, in the conversion of the ss DNA of phage ∅X174, fd and G4 to the duplex form, respectively. So far, information about this enzyme is rather preliminary. Data regarding direct measurements of RNA synthesis, requirements of the reconstituted reaction and site specificity of initiation must be awaited.

Polyoma nascent DNA strands synthesized *in vitro* contain decaribonucleotides attached at their 5' end (53). These primers, called iRNA, begin always with ATP or GTP and are transcribed randomly on the genome. iRNA synthesized under conditions of limiting rNTP contains dNTPs. The initiating enzyme has been termed primase, in analogy to the dna G protein of *E. coli*.

2.4. INITIATION BY A SITE-SPECIFIC ENDONUCLEOLYTIC CUT

— *∅174 gene A and fd gene II products*

Circular ds genomes which replicate through the rolling circle mechanism must suffer an

endonucleolytic cleavage in one of their strands in order to unwind the duplex and initiate DNA synthesis. Some of the best known examples of this type of replication are those represented by phages ØX174 and fd. During the life cycle of these phages, their ss circular chromosome becomes a duplex ring which multiplies itself several fold utilizing the rolling circle model. (56-58).

The ØX174 gene A codes for a site-specific endonuclease called A protein (M.W. 58.000) which introduces a ss discontinuity in the viral strand of supercoiled ØXRFI DNA (59-65). The location of this nick is between nucleotides 4297 and 4298 in the A cistron (66). The enzyme is highly specific: it will not cleave relaxed ØXRFIV, Col El, PM2, fd or M13 DNAs (60,61). After cleavage, the A protein remains covalently attached to the 5' end (60,61), while the 3' hydroxyl generated serves as a primer for the synthesis of viral strands. The ØXRFII DNA·A protein complex can be isolated and it is able to support DNA synthesis by itself when supplemented with crude extracts of uninfected *E. coli* (60).

The ØXA protein does not act catalytically in the cleavage of ØXRFI DNA. Under conditions leading to the quantitative cleavage of ØXRFI DNA, the molar ratio of ØXRFI DNA to added ØXA protein is approximately 1:10, although the actual stoichiometry of the reaction is not known (61).

Eisenberg *et al.* (64)have reported that after the synthesis of a Ø174 unit lenght viral strand is completed, the A protein bound at the 5' end ligates the two ends of the viral DNA to form covalently closed circles.

Bacteriophage fd gene II protein is also a site-specific endonuclease which cleaves the viral strand of fd RFI DNA between nucleotides 5763 and 5764 (67-71). This site is only 24 nucleotides away from the origin of complementary strand synthesis, in the integenic region between genes II and IV (71). Gene II protein has a M.W. of 45,000, and in contrast to ØX174 A protein, it does not remain bound to the 5' end of the viral strand. Instead, it leaves 3' hydroxyl and 5' phosphate termini (71). Gene II protein is conceivably involved in the unwinding of the strands during replication and the closing of the viral strand which is displaced from the rolling circle (68).

It can be predicted that replication of phages lambda (72) and PM2 (73), which also proceed utilizing the rolling-circle system, will require initiation by site-specific viral-coded nucleases, as yet unidentified.

3. THE ELONGATION REACTION

DNA polymerases are the enzymes that actually synthesize the new polynucleotide chains. To accomplish this reaction, they function as one of the components of the "replisome" complex, of which primases, binding proteins, elongation factors, topoisomerases and helicases are also constituents. DNA polymerases are widely distributed in nature, being present in bacteria, plant and animal cells. They are also coded by some bacterial and animal viral genomes.

Genetic and biochemical evidence have demonstrated the presence of three different DNA polymerases in the bacteria *E. coli* (74, 75), *B. subtilis* (76, 77)and *A. calcoaceticus* (78), some of which have been purified to homogeneity (79-82). On the other hand, only one DNA polymerase species has been identified and characterized from *M. Luteus* (83), *T. aquaticus* (84) and the marine *Pseudomonas BAL-31* (85).

Eukaryotic cells contain three DNA polymerases, usually referred to as DNA polymerases α, β and γ (reviewed in refs. 86-88). When they are extracted using non aqueous solvents, they can be found mainly in the nuclei, although DNA pol γ is also present in the mitochondria. They all share the common property of being devoid of associated exonuclease activities.

Selected features of some DNA polymerases are presented below.

3.1. E. COLI DNA POLYMERASES

— *E. coli DNA polymerase I*

This enzyme is a single polypeptide of M.W. 109,000 and it is resistant to NEM. It represents the most extensively studied of all DNA polymerases. DNA pol I participates

actively in the repair of lessions suffered by the chromosome. However, its role in DNA synthesis as an elongation enzyme is very restricted. Only the Col El-type plasmids require DNA pol I for replication, although in conjuction with DNA pol III (89, 29). Nevertheless, the activity of nick translation which is intrinsic to DNA pol I (90) has been shown to be essential for cell viability (91). The evidence available (91) suggests that this activity is in charge of the processing of the primers of the Okazaki fragments, reaction after which DNA pol I leaves a nick that is thereafter sealed by DNA ligase.

— *E. coli DNA polymerase III*

This enzyme is coded by the gene dna E (92), being the M.W. of the native enzyme 180,000. It is composed of subunits of 140,000, 25,000 and 10,000 (62, 81). In addition of being the enzyme that replicates the host chromosome, DNA pol III also is required for the replication of the icosahedral and filamentous ss DNA phages, phage lambda and some plasmids. As DNA pol I, the enzyme contains both a 5' to 3' and a 3' to 5' (proofreading) exonuclease activities (62, 81, 93).

DNA pol III is unable to copy by itself a long primed ss DNA template. To accomplish this reaction, it requires additional elongation factors (EF) and ATP or dATP (3). These proteins are: EFI (M.W. 40,000), EFIII (M.W. 63,000) and the product of the gene dna Z (M.W. 110,000). The mechanism of the elongation reaction has been solved (94): a complex formed spontaneously by dna Z protein and EFIII catalyzes the transfer of EFI to a primed-template in an ATP or dATP-dependent reaction. DNA pol III binds then to the EFIDNA template complex and catalyzes the incorporation of dNTPs into DNA. The fate of ATP in the reaction is not clear, since hydrolysis of this molecule has not been detected. It might be required to induce a conformational change in one of these elongation proteins.

Kornberg's group has isolated a different form of DNA pol III, termed DNA pol III holoenzyme (95, 96). This form appears to be a complex of the three polymerase subunits plus the E F. The identity of the holoenzyme as such is not yet clear, since the E F can be separated from DNA pol III by conventional chromatographic procedures. In addition, these E F can also activate *E. coli* DNA pol II (97) and *B. subtilis* DNA pol III (82).

Lately, three new proteins have been implicated in the elongation reaction. One of them has been called protein u (96). The other two, which have been identified independently (96, 98), copurify with DNA pol III and weigh about 80,000, indicating that they may be the same entity.

The participation of at least six and possibly nine polypeptide chains in the elongation of a pre-primed template, makes this reaction far more complicated than first imagined.

3.2. BACTERIOPHAGE-CODED DNA POLYMERASES

— *T4 gene 43 protein*

T4 induced DNA pol is a single polypeptide of M.W. 110,000. Coded by gene 43, T4 DNA pol has a 3' to 5' exonuclease activity and it is inhibited by sulfhydryl blocking reagents (99-100). The preferred template for the enzyme is primed-ss DNA. The elongation reaction can be activated specifically by T4 gene 32 binding protein, which forms a complex with the polymerase (101). Incorporation of dNTPs is also activated synergistically by the products of T4 genes 45 and 44/62, in a reaction that requires ATP (102, 103). The 44/62 protein complex is an ATPase and the mechanism by which it increases the efficiency of the DNA pol reaction is not known.

— *T7 gene 5 protein*

T7 DNA pol is dimer composed of T7 gene 5 protein (M.W. 84,000) and thioredoxin (M.W. 12,000), a protein specified by the host gene Tsn C (104). The precise role of thioredoxin in T7 DNA replication is not known, although it could catalyze ribonucleotide reduction at the polymerization site. T7 DNA pol, as other DNA polymerases of prokaryotic origin contains an intrinsic 3' to 5' exonuclease activity.

3.3 DNA POLYMERASES α, β AND γ FROM EUKARYOTIC CELLS

The asignement of precise M.W. for DNA pol α has been difficult because it exhibits some heterogeneity. Multiple forms could represent proteolytic degradation (105), contamination of the cells used to isolate the enzyme (106) or the formation of complexes between DNA pol α and other proteins (107, 108). Most reports estimate the M.W. for DNA pol α ranging from 130,000 to 160,000, with variable subunit compositions. The enzyme is inhibited by NEM and by NaCl concentrations above 25 mM. It exhibits optimal activity with gapped DNA as template and shows little activity with primed-ribohomopolymer templates such as poly A-oligo dT.

DNA pol β has a M.W. of about 40,000 and it is resistant to NEM. It is stimulated by 100-200 mM NaCl and utilizes equally well activated DNA and primed-polyribo-or polydeoxyribonucleotides as templates.

DNA pol γ comprises about 1% of the total cellular polymerase activity. The M.W. of this enzyme ranges between 120,000 and 300,000. Its preferred template is poly A·oligo dT, it is stimulated by 200 mM KCl and it is inhibited by NEM.

Since conditional mutations in the genes specifying for the various DNA polymerase species are not available in eukaryotic cells, different approaches have been used to assign a role for each of them: a) the levels of DNA pol α have been found to increase in rapidly growing cells, such as those from regenerating rat liver (109) and neoplasic liver (110), and also during the S period of the cell cycle (111). No changes in the level of DNA pol β have been observed under these conditions, although DNA pol γ levels have also experimented a rise; b) the effect of the inhibitors 2'3' dideoxy TTP (ddTTP) and araCTP on DNA replication has been examined. DNA synthesis in isolated S-phase HeLa nuclei (112) and in permeabilized baby hamster kidney cells (113) is very sensitive to araCTP, while purified DNA pol β is resistant to this inhibitor. On the other hand, ddTTP, which inhibits DNA pols β and γ but not DNA pol α, does not inhibit either *in vitro* SV40 (114, 115) of HeLa cell (112, 116) DNA synthesis; c) a third approach has been to identify the different DNA polymerase species present in replication complexes of some animal viruses. Thus, DNA pols α and γ, but not DNA pol β, are present in adenovirus replication complexes (117-118), while DNA pol α is found in SV40 replication complexes (114, 115, 119). Addition of ddTTP to isolated nuclei which synthesize adenovirus DNA *in vitro* has a strong inhibitory effect, indicating that DNA pol γ is required for viral replication (120); d) nuclei from adult non-dividing brain neurons contain only DNA pol β (121). By stimulating repair-type synthesis with UV light, a seven- to ten-fold stimulation of DNA repair attributable to DNA pol β can be observed (121, 122). Also in these cells, evidence has been obtained for a role of DNA pol γ in mitochondrial DNA replication, since this is the only DNA polymerase species present in this organelle (106, 123); e) Spadari and Weissbach (124) have found that only DNA pol α can extend a natural RNA primer hybridized to a DNA template. Primers *in vivo* consist mainly of short RNA transcripts (53, 125-127).

The conclusions emerging from these studies seem to indicate the following: DNA pol α is the replicating enzyme in the nuclei. DNA pol β is a repair enzyme and DNA pol γ replicates mitochondrial DNA. However, the possibility that DNA pol α and DNA pol β have minor roles in repair and replication respectively, cannot be ruled out.

4. SEALING OF DNA FRAGMENTS

Cells have an enzyme capable of joining single-stranded interruptions which arise in DNA during the processes of replication, recombination and repair. This enzyme, called DNA ligase, is essential for viability of the cell (128). It was first found en *E. coli* using as an assay the enzymatic conversion of linear lambda DNA containing sticky ends to a covalently closed circular form (129, 130). DNA ligase plays an active role in discontinuous DNA replication, linking the short Okazaki fragments to the growing chromosome.

4.1. E. COLI DNA LIGASE

The enzyme has been purified to homogeneity; it is a single polypeptide with a M.W. of 75,000 (reviewed in ref. 131). The sealing reaction requires Mg^{2+} and also NAD as a cofactor, to form a ligase-AMP intermediate complex. The adenyl group of the coenzyme binds to an ε-amino group of a lysine residue of the enzyme, with the release of NMN. In a second step, the AMP moiety is transferred to the 5' phosphate terminus of DNA, in a reaction that requires the free 3' hydroxyl end. Thirdly, a phosphodiester bond is formed by a nucleophilic attack of the 3' hydroxyl terminus to the activated 5' end, with the concomitant release of AMP. Each step of this reaction can be reversed by the corresponding products. Ammonium ions activate the enzyme. *E. coli* DNA ligase can join adjacent DNA fragments and also 3'RNA to 5'DNA, only when they are hybridized to a DNA strand.

4.2. BACTERIOPHAGE T4 GENE 30 PROTEIN

Phage T4 induces (132), as does T7 (133), a DNA ligase which is the product of gene 30. T4 DNA synthesis is impaired in gene 30 mutants, therefore the phage-induced ligase cannot be replaced by the host enzyme (134, 135). Although the same three-step mechanism outlined for the *E. coli* enzyme is also followed by T4 ligase, both enzymes differ in several aspects: a) ATP is the cofactor of the phage enzyme, being AMP and pyrophosphate the products of the reaction instead of AMP and NMN; b) T4 DNA ligase can perform end-to-end joining of flush ended DNA molecules, converting ds phage P22 DNA into oligomers (136); c) in contrast to the host enzyme, T4 DNA ligase can join DNA or RNA segments in all combinations, either hybridized to DNA or RNA (137, 140).

4.3. MAMMALIAN DNA LIGASES I AND II

The major DNA ligase activity present in extracts prepared from mouse embryo fibroblasts (141), calf thymus (142), rat liver (143) and a human cell line (144) is called DNA ligase I. It is an ATP- requiring enzyme with a M.W. ranging from 175,000 to 200,000. The mechanism of the ligation reaction appears to be similar to that observed with DNA ligases of prokaryotic origin, since the formation of ligase-AMP and DNA-AMP intermediates has been detected (145, 146).

Mammalian cells also contain a second enzyme with an ATP-dependent DNA ligase activity, called DNA ligase II (142). Immunological tests indicate that this enzyme is not related to DNA ligase I (147). Both enzymes are present in the nuclei, although only DNA ligase I levels appear to be regulated during the cell cycle (147).

5. CONFORMATIONAL CHANGES OF DNA DURING REPLICATION

Strand separation at the replication fork, as well as the elimination of the topological constraints arising during the overall process of duplication, are problems inherent to DNA synthesis.

Among the proteins that alter DNA conformation are the ss DNA binding proteins, also called helix destabilizing proteins (HDP). They have been isolated from a variety of prokaryotic and eukaryotic sources, and they are thought to play several roles in the cell: a) HDPs are required for the priming reaction of the ss DNA phages and most probably of the *E. coli* chromosome; b) they activate the elongation reaction catalyzed by DNA polymerases; c) HDPs facilitate unwinding catalyzed by DNA helicases by binding to ss DNA at the replication fork.

Enzymes that alter the degree of supercoiling of DNA are called topoisomerases. Among them, nicking-closing enzymes (swivelases) remove superhelical turns from circular duplex DNA to yield a relaxed and covalently closed molecule. The reaction involves the introduction of a transient nick into the helix, which allows the strand to swivel in order to relieve the torque created during the unwinding of the duplex. As nicking-closing enzymes operate without an energy source, it has been proposed that a covalent enzyme-DNA complex conserves the energy of the phosphodiester bond during the

relaxation reaction. Swivelases lack DNA ligase-type activity. Their participation in DNA replication is only assumed, since no mutans have been isolated to date. On the contrary, it has been clearly established that *E. coli* DNA gyrase, an enzyme that introduces superhelical turns into DNA in an ATP-dependent reaction, plays an active role in DNA synthesis (as well as in transcription and recombination). There is now good evidence showing that DNA must be supertwisted in order to be replicated (31, 148-151). In addition, DNA gyrase is the target enzyme of nalidixic acid and novobiocin, two potent inhibitors of DNA synthesis (152).

Topoisomerases can be assayed by agarose-gel electrophoresis, where supercoiled circular duplex DNA can be separated from relaxed DNA. (153). Since the reaction catalyzed by both nicking-closing enzymes and DNA gyrase does not follow a single hit mechanism, the separation of topoisomers with an intermediate degree of superhelicity can be achieved.

Actual strand separation is carried out by DNA helicases. These enzymes catalyze the hydrolysis of ATP to ADP and Pi in a reaction coupled to strand separation. Some of them, like *E. coli* rep protein and the recBC nuclease, require in addition the presence of a HDP to avoid reassociation of the DNA strands. The substrate utilized to measure the activity of DNA helicases consist in a labelled polynucleotide (e.g. a restriction fragment) hybridized to non-labelled ss DNA. The degree of unwinding can be quantitated as the amount of radioactive DNA which is rendered acid soluble after treatment of the product with a ss specific deoxyribonuclease. Helicases have been isolated from *E. coli*, bacteriophage-infected cells and mammalian cells.

5.1. *E. COLI* HDP

E. coli DNA binding protein is a heat stable tetramer of 18,500 M.W. subunits (154) which binds cooperatively to ss DNA. In the absence of Mg^{2+} and at low ionic strength, it lowers the melting temperature of the duplex by nearly 40°C (155). Each tetramer of the native protein interacts with 32 nucleotides, causing a 40 percent shortening in the length of ss DNA (155). Binding of *E. coli* HDP to DNA reduces the activity of most ss DNases, except micrococcal nuclease, *E. coli* exonuclease I and the nucleases associated with *E. coli* DNA pol II and T7-induced DNA pol (156). Molineux *et al.* (156) have shown that, except for micococcal nuclease, these enzymes form stable complexes with the binding protein. Moreover, the DNA pol II-HDP complex is able to copy a long gap in duplex DNA, a reaction the free polymerase cannot carry out (157).

In vitro, *E. coli* HDP is required for the conversion of ss DNA of icosahedral and filamentous bacteriophages to the ds replicative form. (3). Its role in DNA replication *in vivo* became evident only recently, when Meyer *et al.* (158) identified a mutant with a temperature-sensitive defect in *E. coli* HDP.

5.2. BACTERIOPHAGE-CODED HDPs

— *T4 gene 32 protein*

The product of T4 gene 32 (M.W. 35,000) was the first HDP isolated (159). When gene 32 protein binds to ss DNA at saturating levels,it covers about eight nucleotides, extending the DNA chain 50 percent (160, 161). In this ss DNA-protein complex, T4 HDP forms linear aggregates in which the DNA binding sites are thought to be aligned. These protein-protein interactions could be the basis for cooperative binding to DNA. Gene 32 protein stimulates specifically T4 DNA pol when assayed with a long ss DNA template (101) and also with nicked ds DNA (162); in the later case, it does it presumably by allowing the displacement of the non-template strand. T4 HDP plays a prominent role in DNA metabolism, since it is required for replication (163), repair (164), and genetic recombination (165).

— *T7 HDP*

T7 DNA binding protein (M.W. 30,000) stimulates specifically T7 DNA pol (166, 167). It has not been defined genetically, possibly because it can be replaced by *E. coli* HDP. T7 HDP lowers the Tm of poly dAT by 40°C, and

aggregates itself in the presence of Mg^{2+} (168).

— *fd gene V protein*

Bacteriophage fd-coded binding protein is absolutely required to direct the synthesis of progeny ss viral DNA (169, 170). The native protein consists of a dimer of 20,000, each monomer covering approximately four nucleotides (170, 171). Cooperative binding is so strong, that fd HDP lowers the Tm of ds DNA by nearly 40°C (169). The terciary structure of gene V product of bacteriophage fd and its complexes with DNA have been studied (172). The enzyme binds to circular single strands in such a way that brings together non adjacent regions of the DNA, giving to the complex a rod-like shape (173).

5.3. EUKARYOTIC HDPs

Proteins have been isolated from plant (174), mammalian (175-180) and viral infected cells (181, 182) by virtue of their preferential binding to ss DNA. This property, common to their prokaryotic counterparts, besides facilitating melting of a duplex, promotes also renaturation of single strands, a reaction required during recombination and repair. HDPs isolated from the various eukaryotic sources activate specifically homologous DNA polymerases (108, 178, 183), indicating a possible involvement of these proteins in the replicative process *in vivo*.

Nuclei isolated from meiotic cells of lilies and some mammals contain an HDP (M.W. 32,000) which is only active during the interval following the S-phase until termination of chromosome pairing (174, 184). This enzyme, as well as HDP isolated from mouse ascites cells, changes its binding capacity to ss and ds DNA upon phosphorylation; which may constitute a regulatory mechanism (178, 185).

Human adenovirus induces an HDP (its M.W. varies with the type of virus) which has several functions: a) it is involved in the initiation and elongation steps of viral DNA replication (186, 187); b) it modulates the levels of several early viral transcripts, including autoregulation of its own levels (188, 189); c) temperature-sensitive mutants in adeno HDP transform rat cells at non permissive temperature at higher frequencies than wild type virus (190); d) the enzyme is also required for in vitro viral replication. When this reaction contains endogenous viral DNA as template, adeno HDP can be replaced by *E. coli* HDP (191, 192). Adeno-induced HDP is present as a phosphoprotein that exhibits different degrees of phosphorylation (192-195). This modification, however, does not affect the binding of this protein to DNA.

5.4. BACTERIAL DNA TOPOISOMERASES

—*Swivelases*

E. coli omega protein was the first nicking-closing enzyme to be discovered (196). Since then, they have been isolated from *M. luteus* (197-198), *Pseudomonas* BAL-31 (199) and *A. tumefaciens* (200). Bacterial swivelases (M.W. about 110,000) are designated type I topoisomerases, since they can readily remove negative superhelical turns but remove positive turns inefficiently. They all require only Mg^{2+} ion for activity, except the enzyme from *Pseudomonas* BAL-31, which requires in addition a monovalent cation salt.

In the absence of Mg^{2+}, *E. coli* omega protein forms stable complexes with ss DNA, which dissociate when minute amounts of Mg^{2+} are added (201). Treatment of the complex with alkali or pronase leads to the cleavage of the DNA chain, after which the protein remains covalently linked to the 5' terminus of the DNA.

It has been demonstrated that the ss character of a duplex DNA molecule is proportional to its degree of negative supercoiling (202). Therefore, the removal of only negative superhelical turns, the dependency on the degree of supercoiling for catalysis and the inhibition of the reaction by ss DNA, are consistent with the initial binding of bacterial swivelases to ss regions of the substrate in order to catalyze the relaxation reaction.

E. coli nicking-closing enzyme also forms salt stable complexes with ds DNA, although they are neither dissociated by the addition of Mg^{2+}, nor does DNA scission occur when protein denaturants are added (203).

Two novel reactions are catalyzed by the *E. coli* and *M. luteus* enzymes with circular ss DNA: a) in the presence of monovalent cations they form knotted rings which can be identified by sedimentation and electron microscopy (197, 204), and b) they promote the interwinning of circles of complementary sequences into a covalently closed duplex ring (205).

— *DNA gyrase*

DNA gyrase is an enzyme that induces negative supercoiling into closed circular ds DNA at the expense of ATP hydrolysis (206). It has been isolated from *E. coli* (206) and *M. luteus* (207), although most of the studies have been performed with the former enzyme. DNA gyrase is an essential enzyme in *E. coli* (208) and it is involved in: a) replication of ØX174 (148), some plasmids (31, 209), T7 (201, 211) and host DNAs (149); b) integrative recombination of phage lambda (212); c) DNA repair (213) and, d) transcription (214-218). Replication of phage T4 mutants in DNA-delay genes 39, 52 and 60 under non permissive conditions, also requires *E. coli* DNA gyrase (219). These genes code for the components of a phage ATP-dependent topoisomerase (see below). In addition to ATP, gyrase requires Mg^{2+} and it is activated by spermidine (206).

The purified enzyme can catalyze five different reactions: a) the introduction of negative superhelical turns in the presence of ATP; b) relaxation of supercoils in the absence of ATP; c) DNA-dependent hydrolysis of ATP to ATP and Pi; d) enzyme binding to DNA, e) site-specific cleavage of DNA.

DNA gyrase is composed of two subunits, A and B, which can be purified separately and recombined to reconstitute active DNA gyrase (220-222). Apparently, both subunits are present independently in the cell, as well as forming a complex. Subunit A is coded by nal A gene, which governs sensitivity to nalixidic and oxolinic acids (218, 221, 220, 223). When purified separately to homogeneity, nal A gene product is a dimer of two identical subunits, each with a M.W. of 110,000. Nal A gene product catalyzes, as does native DNA gyrase, the ATP-independent and nalidixic acid sensitive relaxation of positive and negative superhelical turns of DNA, therefore it is a type II topoisomerase (220, 223). Several criteria indicate that in spite of their similar M.W. and activities, the nal A protein is not related to the omega protein (220). Subunit B of DNA gyrase is coded by the cou gene, which governs sensitivity to coumermicin and novobiocin (224). Gellert *et al.* have found that the cou gene product (M.W. 95,000) is involved in the energy transduction aspect of the supercoiling reaction and that this protein has a specific binding site for ATP which is blocked by novobiocin (225-227). Native DNA gyrase is able to induce negative superhelical turns in the presence of nonhydrolyzable ATP analogs (226). Therefore, Cozzarelli *et al.* have proposed that ATP acts as an allosteric effector which is hydrolyzed to ADP and Pi in order to make the enzyme work catalically (226).

When oxolinic acid and SDS are added to the reaction mixture, DNA gyrase cleaves ds DNA site-specifically (220, 223, 225, 226, 228). It makes a four nucleotide staggered cut, creating DNA termini with a 3' hydroxyl end and a 5' extension which contains the enzyme covalently bonded (228). DNA gyrase follows then the pattern of type I and type II topoisomerases, storing the energy released by scission of the DNA backbone in a covalent enzyme-DNA intermediate.

5.5 BACTERIOPHAGE-CODED DNA TOPOISOMERASES

— *T4 DNA topoisomerase*

A distinct ATP-dependent DNA topoisomerase has been isolated from T4 infected cells (229, 230). The purified enzyme preparation exhibits three protein components: the product of the DNA-delay gene 39 (M.W. 64,000), the product of the DNA-delay gene 52 (M.W. 51,000) and a third protein (M.W. 110,000) the identify of which is not known. T4 topoisomerase hydrolyzes ATP during the relaxation of both positive and negative superhelical turns. The enzyme is not inhibited by the antibiotics oxolinic acid and novobiocin, which are known antagonists

of *E. coli* DNA gyrase. T4 DNA topoisomerase is not essential for viral replication, since it can be replaced by *E. coli* DNA gyrase (219). Under these conditions, however, phage DNA synthesis is depressed.

Liu *et al.* (230) have proposed that the enzyme may act at the origin site in conjunction with the products of phage genes 41 and 61 (primase) in order to initiate DNA synthesis. It remains to be seen if this ATP-dependent T4 topoisomerase is able to induce superhelical turns on ds DNA, as well as to establish the role of ATP in the relaxation reaction.

—Lambda int gene product

This phage-coded enzyme, which is required for integrative recombination, contains swivelase activity (231). The int gene product is a type II topoisomerase, since it relaxes positive and negative supercoils. The nicking-closing activity of int protein shows no sequence specificity and functions in the absence of a divalent cation. It is inhibited by Mg^{2+} ions, spermidine and ss DNA.

—ØX174 gene A and fd gene II proteins

These enzymes, which are site-specific endonucleases required to start replication of the ds replicative forms ØX174 and fd phages, (59, 67) also display nicking-closing activity.

When ØXRFI DNA is incubated with low levels of A protein, a small proportion of relaxed DNA is detected (60). Treatment of relaxed ØXRFIV DNA with sarkosyl or SDS has no effect in its structure. In contrast, treatment with phenol and/or proteinase K quantitatively yields nicked RFII DNA (60). Incubation of ØXRFI with large amounts of A protein yields RFII with large detectable relaxed structures.

Upon treatment of fd supercoiled DNA with gene II endonuclease, two thirds of this substrate are coverted to the nicked form and one third is transformed to relaxed covalently closed circles. This additional activity of gene II protein might be important for the circularization of the linear viral strands during phage DNA synthesis (68).

5.6 EUKARYOTIC DNA TOPOISOMERASES

The eukaryotic enzymes have been termed type II topoisomerases since they can catalyze relaxation of both positive and negative superhelical turns. They have been found in mouse (232), drosophila eggs (233), KB cells (234), HeLa cells (235), rat liver nuclei (236), vaccinia virions (237), yeast (238), etc. In addition to their possible role at the replication fork, they participate in the association of histones to the chromosomes (239). Eukaryotic swivelases exhibit M.W. ranging from 60.000 to 80.000 and posses several features that distinguish them from swivelases of bacterial origin: a) they require between 0.15 M and 0.20 M monovalent cation salt; b) they can be assayed in the presence of excess EDTA, and c) their activity does not depend on the degree of superhelicity of the substrate. On the other hand, like type I topoisomerases, they do not require an energy source and catalyze the stepwise relaxation of the supercoiled substrate.

Working with the rat liver enzyme, Champoux (240-242) has been able to trap a nicked protein-DNA intermediate complex which contains the enzyme covalently attached to the 3' end. The chemical nature of this linkage is not known. With respect to specificity, the enzyme can act at any site on ds DNA. However, studies carried out with the SV40 chromosome, show that rat liver nicking-closing enzyme has a preference for six discrete sites (243). Champoux *et al.* have also demonstrated that in contrast to other enzymes involved in DNA replication, rat liver topoisomerase levels are not regulated during the cell cycle (243).

Recently, a DNA topoisomerase has been purified from rat liver mitochondria (244). The enzyme sensitivity to ethidium bromide and to the trypanocidal drug berenil is different to that of its nuclear counterpart, suggesting that they may be different enzymes.

5.7. E. COLI DNA HELICASES

— DNA helicase I

E. coli helicase I is a long, fibrous single polypeptide (M.W. 180,000) which is present

at about 600 copies per cell (245-247). The enzyme has a ss DNA-dependent NPTase activity: ATP and dATP are the preferred substrates, although the other rNTPs are hydrolyzed to some extent. To initiate unwinding of ds DNA, helicase I binds first to protruding 5' ss ends which have to be at least 200 nucleotides long. It then advances in the 5' to 3' direction of the DNA chain to which it is bound, in a reaction coupled to ATP hydrolysis. A DNA molecule with protruding 3' ss ends is not a substrate for helicase I (248, 249). About 70 molecules of enzyme are present continously at the separation fork. Since the protein has a strong tendency to form aggregates in solution, this type of protein-protein interaction may be the basis for cooperativity and translocation of the enzyme at the replication fork.

ATPase activity of helicase I is optimal under conditions where the enzyme is known to form aggregates and it is abolished when ds DNA is used as a cofactor.

DNA helicase I works in a processive fashion: if ss DNA is added once the reaction has started, there is no inhibition of the unwinding reaction (248, 249).

— *DNA helicase II*

E. coli helicase II(M.W. 75,000) (250, 251) is probably identical to DNA-dependent ATPase I isolated by Kohiyama's group (252, 253). The enzyme shows specificity for ATP or dATP hydrolysis and it is inactive with other nucleotides (250). As helicase I, it starts unwinding ds DNA sequentially in the 5' to 3' direction, although it requires ss ends only 12 nucleotides long (248, 249). In contrast to helicase I, helicase II requires the continous absortion of enzyme molecules to DNA at the separation fork. This stoichiometric requirement of enzyme is consistent with the inhibition observed when competing ss DNA is added to the assay.

— *rep protein*

A third *E. coli* DNA helicase is coded by the rep gene. This protein (M.W. 70,000) is required for the replication both *in vito* and *in vitro* of certain bacteriophage genomes (ØX174, fd, P2) and for the normal replication of the host chromosome (63, 254-257). This requirement has provided an assay for the purification of rep protein and the subsequent study of its physiological role (62-64, 258-261).

Replication *in vitro* of ds ØX174 RFI requires the phage coded A protein, rep protein, *E. coli* HDP and DNA pol III plus elongation factors (62, 64). First, the A protein cleaves site-specifically the viral strand of ØX174 RFI and remains covalently bound to the 5' end of the nick (60). The duplex DNA is then unwound by the rep protein in the presence of HDP with the simultaneous hydrolysis of two ATP (or dATP) molecules per base pair broken (258-262). Nicked ØX174 RF lacking A protein is not a substrate nor a cofactor for rep ATPase, suggesting that the A protein present at the nick interacts with rep to start unwinding (62, 261). In the absence of A protein, but in the presence of HDP, rep enzyme unwinds ds DNA when it has ss extending regions. As DNA helicases I and II, rep protein unwinds in the 5' to 3' direction (261). This is important in the reaction described above, because the 3' end of the A protein-induced nick is left in a duplex structure, to be used as a primer for viral replication via rolling circle mechanism.

ATPase activity of rep protein requires ss DNA as a cofactor; ds DNA or HDP-coated ss DNA are inactive as cofactors. However, since the rep catalyzed unwinding reaction requires HDP, it appears that the true effector for the ATPase is the separating fork (261, 262).

— *rec BC nuclease*

E. coli rec BC nuclease, also called exonuclease V, plays a central role in genetic recombination and recombinational repair (263). The native enzyme is a composite protein of M.W. 270,000 which exhibits several activities: a) it is an ATP-dependent 5' to 3' and 3' to 5' exonuclease, which degrades both ds and ss DNA; b) it is also an ATP-stimulated ss endonuclease and c) it is a DNA dependent ATPase, although simultaneous DNA degradation is not necessary for ATP hydrolysis (264, 265).

S. Linn has proposed a model for the degradation of ds DNA by rec BC nuclease (266): the enzyme binds to both strands at the end of the duplex. It then unwinds the strands from one terminus, cleaving fragments at 500 nucleotide intervals while it remains bound to the end of the undegraded strand. After unwinding about 5,000 nucleotides, the enzyme switches strands and degrades the ss tail from its terminus, yielding a shortened wholly duplex molecule. The 500 nucleotides long fragments are then degraded by the enzyme to acid soluble oligonucleotides. Presumably, ATP hydrolysis is coupled to the unwinding of the DNA helix and it is not related to the actual nucleolytic activity, since several ATP molecules are hydrolyzed per each phosphodiester bond broken (264, 267).

If HDP is added to this reaction, rec BC works as a DNA helicase (268, 269). Binding of HDP to the extending ss tails results in protection against exonucleolytic degradation. Therefore, the products are long ss pieces derived from limited nuclease cutting after helix unwinding.

Rec BC enzyme is regulated by Ca^{2+} ions. When both Ca^{2+} and Mg^{2+} are present simultaneously in the reaction, a marked inhibition of the nuclease activity is observed, while the ATPase is not affected (269, 270). Under these conditions, the enzyme binds initially to the terminus of duplex DNA and then tracks along the DNA molecule, producing local and transient unwinding of the strads as it moves. As a result, ds DNA remains as a duplex structure, in spite of the fact that it contains a few nicks. The combined action of Mg^{2+}, Ca^{2+} and HDP produces complete denaturation of ds DNA and solely ss fragments are found (269, 270).

5.8. BACTERIOPHAGE-CODED DNA HELICASES

— *T4 dda protein*

The purified product of T4 gene dda is DNA-dependent ATPase (M.W. 56,000) which is able to unwind ds DNA in the 5' to 3' direction (248, 249, 271). The enzyme action is non processive as indicated by the following criteria: a) the number of enzyme molecules required to unwind DNA is a function of the lenght of the DNA; b) the reaction is inhibited when ss DNA is added to trap free enzyme molecules. As most DNA helicases, T4-dda protein binds first to a ss region and chain separation is brought about by the continued adsortion of protein molecules to DNA at the separating fork. This reaction is driven by ATP (or dATP) hydrolysis. Mutational loss of this enzyme is not lethal to the virus (272).

— *T7 gene 4 protein*

This enzyme does not only fuction as a DNA primase for T7 replication (45-48), but also as a nucleoside triphosphate-dependent DNA unwinding catalyst (45, 49, 273-275). T7 DNA pol is unable to use intact or nicked duplex DNA as template. However, in the presence of gene 4 protein and the four dNTPs, T7 DNA pol catalyzes the incorporation of dNTPs, which is started at a nick in ds DNA (49, 273-275). This activation of the polymerase by gene 4 protein is specific. Polymerization occurs simultaneously with the displacement of one of the parental strands, with the concomitant hydrolysis of 4.6 dNTPs to dNDPs and Pi for each dNTP polymerized (273). At later times in the reaction, the polymerase jumps across the fork and copies the displaced strand, therefore all the newly synthesized DNA is covalently attached to the template (274, 275).

If the reaction mixture with nicked DNA contains both dNTPs and rNTPs, T7 gene 4 unwinding protein synthesizes oligoribonucleotides on the displaced ss tail which function as primers for T7 DNA pol. As a result, DNA chains copied in the lagging strand are not covalently attached to the DNA template (47, 48, 274). Under these conditions, the total number of dNTPs and rNTPs hydrolyzed per dNTP incorporated are 3.0 and 1.2, respectively (273). Both primer and DNA synthesis are stimulated about 5-fold by either *E. coli* or T7 binding proteins (47).

T7 gene 4 protein is a ss DNA-dependent rNTPase or dNTPase (45, 273). Duplex DNA is a cofactor only when hydrolysis of nucleoside triphosphates is coupled to DNA synthesis; ddTTP, an inhibitor of T7 DNA pol, stops NTPase activity. Moreover, $\beta - \gamma$

methylene dTTP, which cannot be hydrolyzed to TDP and Pi, inhibits also DNA synthesis (273).

5.9. EUKARYOTIC DNA HELICASES

Cobianchi *et al.* (276) have isolated from human cells a ss DNA-dependent ATPase (M.W. 110,000). The enzyme binds first to ss DNA and moves along in the 5' to 3' direction unwinding duplex DNA. Human ATPase can activate DNA pol β from HeLa cells possibly by providing it with a template upon denaturation of the duplex. dNTP incorporation is enhanced even futher by calf thymus HDP.

In contrast to *E. coli* helicase I, human helicase is unable to unwind RNA fragments hybridized to DNA.

6. CONCLUSIONS

A brief outline of some of the proteins involved in DNA replication has been presented. As stated before, there is direct proof for the participation of most of them in this process. Other proteins, like lambda int gene product and rec BC nuclease are not involved in DNA synthesis, but they have been included due to their similarity with replicating proteins.

Although all these enzymes have been discussed independently, one has to imagine all of them working as a replisome complex at the replication fork.

DNA synthesis starts at a specific origin and proceeds almost continously in the leading strand. At the same time, helicases with the help of HDPs unwind the duplex, and discontinuous synthesis proceeds in the lagging strand. Priming of Okazaki fragments may require several proteins and elongation proteins. Later, Okazaki fragments are processed and sealed by DNA ligase to form a continuous polynucleotide chain.

Some aspects of DNA synthesis remain still obscure. Not much is known about its regulation and the mechanism of termination of chromosome replication. The answer of these questions presents certain difficulties, since the various systems which have been investigated, although sharing a common general pattern, present also several different features. For example, a circular duplex molecule may replicate through a rolling circle or a Cairns (tetha) type of intermediate, or both. We still have to learn the role of adenovirus terminal protein, the lambda genes O and P and the T antigen of SV 40 in the initiation reaction, etc. The picture complicates even further when considering the involvement of enzymes that display more than a single catalytic activity, such as DNA gyrase, ØX174 A protein, fd gene II protein and T7 gene 4 protein.

Finally, it is interesting to point out how actively ATP participates in DNA replication: a) primases prefer to start with ATP at the 5' end; b) ATP is required for the formation of specific complexes among proteins, i.e. dna B-dna C, primed-template-elongation factor I, etc.; c) ATP es a cofactor for DNA ligases and d) ATP is hydrolyzed by helicases, gyrase, dna B, replication factor Y, T4 coded topoisomerase, T4 gene 41 protein, etc.

ACKNOWLEDGEMENTS

I am grateful to E. Cardemil, J. Eyzaguirre and A. Yudelevich for their help in the preparation of this manuscript.

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8. APPENDIX

The following translations into spanish for the names of the enzymes discussed in this paper are proposed:

primase: enzima partidora.
DNA binding protein: proteína DNA-ligante.
HDP: proteína hélice-inestabilizadora.
nicking-closing enzyme: enzima de corte y sellado.
gyrase: girasa.
helicase: helicasa.
swivelase: enzima DNA-relajante.