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Mechanism of DNA Replication Catalyzed by Purified T4 Replication Proteins

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Our long-range goal has been to obtain a complete set of highly purified T4 bacteriophage DNA replication proteins that can be mixed back together to reconstruct a functional replication apparatus in vitro. Ideally, this apparatus should synthesize DNA on double-helical DNA templates with the same fork geometry, fork rate, and base-pairing fidelity that is observed for T4 replication forks within the cell. In addition, it should initiate replication forks at replication origins by the correct biological mechanism.

The marriage of genetics and biochemistry has been a fruitful one in these studies of T4 DNA replication. Studies of the 47 complementation groups, or genes, in the original set of conditionally lethal T4 mutations (Epstein et al., 1964) revealed 10 genes that cause a noticeable defect in DNA replication. Subsequent work has enlarged the list of mutations, and now about 30 genes that severely affect T4 DNA synthesis are known (Mathews, 1977; Wood and Revel, 1976). Fortunately, not all of these genes directly affect replication fork mechanisms; for example, a sizable number are known to encode enzymes needed for nucleotide precursor biosynthesis (Cohen, 1968; Mathews, 1977; Mathews and Allen, this volume).

Genetically identified components of the T4 DNA replication apparatus have been purified by using in vitro complementation assays (Barry and Alberts, 1972; Morris, Hama-Inaba, Mace, et al., 1979; Morris, Moran, and Alberts, 1979; Nossal, 1979). In deciding which gene products to purify, we initially chose those whose alteration by mutation causes the most severe defects in DNA replication, without depleting the normal deoxyribonucleoside triphosphate precursor pools (a subclass of the "DO" [no DNA synthesis] mutants of Epstein et al. [1964], as defined by the experiments of Mathews [1972]). In this way, the proteins corresponding to genes 44/62, 45, and 41 were purified. Other components, such as the DNA polymerase (gene 43 product), the gene 32 DNA helix-destabilizing protein, the gene 61 priming protein, the *dda* DNA helicase, and the DNA topoisomerase, have been purified by more standard procedures, in which a direct property or activity of the protein was monitored. But in all cases, the phenotype of a bacteriophage carrying a mutant version of the protein in question has been crucial in establishing that the protein has an important role in DNA replication.

To date, T4 replication proteins corresponding to the products of 11 T4 genes have been purified to near homogeneity and extensively characterized (Table 1). Our studies have established definite roles for 8 of these 11 gene products in the process of replication fork movement, as is discussed in detail in this chapter (reviewed in Table 1). When these proteins are mixed together, replication forks form in vitro that in all respects closely resemble the replication forks found in vivo. However, the forks formed in the in vitro system are initiated by a non-biological mechanism, since they start by strand displacement synthesis from a random nick on a double-helical template, rather than from a replication bubble that forms at a specific origin DNA sequence.

Our present knowledge of the mechanism of replication fork initiation at a T4 replication origin has been derived from studies carried out on whole cells. Such in vivo studies suggest that both the T4 DNA topoisomerase and the host *Escherichia coli* RNA polymerase are likely to be involved in the true fork initiation process (see the chapters by Mosig, Kozinski, and Kreuzer and Huang in this volume). Attempts to reconstitute this biological fork initiation process in vitro have thus far been unsuccessful (unpublished data). We therefore confine our attention in this review to the details of the mechanism of replication fork movement.

T4 DNA POLYMERASE

The first of the T4 replication enzymes to be isolated was the DNA polymerase (Table 1). The analogous T2 enzyme was identified in extracts from infected cells as a polymerase differing from *E. coli* DNA polymerase I in its chromatographic properties and in its strong preference for single-stranded DNA templates (Aposhian and Kornberg, 1962). T4 DNA polymerase was subsequently identified and shown to be the product of gene 43 (de Waard et al., 1965; Warner and Barnes, 1966).

The purified T4 enzyme, a monomer of about 110 kilodaltons, by itself catalyzes three reactions: (i) 5′-to-3′ polymerization on a primed single-stranded DNA template; (ii) 3′-to-5′ exonucleolytic hydrolysis of single-stranded DNA and, at a slower rate, of a 3′-OH end on duplex DNA; and (iii) primer-template-dependent turnover of deoxyribonucleoside triphosphates to the corresponding monophosphates. (Englund, 1971; Goulian et al., 1968; Hershfield and Nossal, 1972). This turnover appears to result from hydrolysis of newly incorporated nucleotide at the 3′ terminus; thus, in addition to requiring a primer-template, turnover is enhanced when further polymerization is delayed by pause sites in the template (regions of special secondary structure or sequence) or when the polymerase is blocked at the end of a template or by the lack of the next required complementary nucleotide (Englund, 1971; Nossal and Hershfield, 1973; Roth et al., 1982).

TABLE 1. T4 DNA replication proteins[a]

Designation	T4 gene	Activities of protein alone[b]	Role in DNA replication		
			RNA primer synthesis	Synthesis on single-stranded templates	Strand displacement synthesis
DNA polymerase	43	5′-to-3′ polymerase on ssDNA templates only; 3′-to-5′ exonuclease	—[c]	Required	Required
Helix-destabilizing protein (gene 32 protein)	32	Cooperative binding to ssDNA; helix destabilization	—	Stimulates	Required
Polymerase accessory proteins					
Gene 44/62 protein	44/62	ssDNA termini-dependent ATPase (dATPase)	—	Stimulates	Required
Gene 45 protein	45	Stimulates gene 44/62 protein ATPase	—	Stimulates	Required
Priming proteins					
Gene 61 protein	61	Binds ssDNA	Required	—	—
Gene 41 protein	41	Long ssDNA-dependent GTPase and ATPase; DNA unwinding (5′-to-3′ helicase)[d]	Required	—	Stimulates
T4 DNA helicase	*dda*	ssDNA-dependent ATPase (dATPase); DNA unwinding (5′-to-3′ helicase)[d]	—	—	Stimulates
T4 DNA topoisomerase	39/52/60	dsDNA-dependent ATPase; type II topoisomerase (dsDNA strand passage); dsDNA cleavage when inhibitors added	—	—	—

[a] References and further details are given in the text.
[b] Abbreviations: ss, single stranded; ds, double stranded.
[c] —, No role has been demonstrated.
[d] Helicase direction is indicated as the direction of movement on the strand that is not displaced.

The conclusion that turnover is a two-step process of polymerization followed by excision is supported by recent studies with α-thio analogs of deoxyribonucleoside triphosphates; these show inversion of the configuration at the α-phosphorus in the nucleotidyl transfer reaction during polymerization (Romaniuk and Eckstein, 1982) and retention of this configuration in the deoxyribonucleoside monophosphate produced by the net turnover reaction (Gupta et al., 1982). The same studies argue against the formation of a covalent deoxyribonucleotide-polymerase complex as an intermediate in the polymerization process; in this case, a retention of configuration during polymerization would be expected due to the two-step nature of the polymerization event.

The 3′-to-5′ exonuclease of T4 polymerase is more active than that of other procaryotic DNA polymerases. This makes it the enzyme of choice for labeling DNA fragments by hydrolysis and repair at the 3′ terminus (O'Farrell et al., 1980), but results in a considerable wasting of deoxyribonucleoside triphosphates due to turnover to the monophosphates even under optimal in vitro conditions for DNA synthesis (Hershfield and Nossal, 1972). The preference of the nuclease for non-base-paired nucleotides at the 3′ terminus of duplex DNA led to the proposal that it acts as a proofreader to remove most incorrect nucleotides inserted during polymerization (Brutlag and Kornberg, 1972; Muzyczka et al., 1972). This proposal is supported by the finding that the ratio of incorrect nucleotides turned over to those stably incorporated is decreased for the polymerase produced by some gene 43 mutants with a mutator phenotype and increased for the polymerase produced by some gene 43 mutants with an antimutator phenotype (Bessman et al., 1974; Gillin and Nossal, 1976a; Muzyczka et al., 1972). Replication fidelity can also be decreased by a reduction in the ability of mutant T4 DNA polymerases to select the correct nucleotide for insertion (Gillin and Nossal, 1976b; Hershfield, 1973; Reha-Krantz and Bessman, 1981; Sinha and Goodman, this volume.)

Purified T4 DNA polymerase by itself cannot carry out several of the reactions needed to copy duplex DNA: (i) the enzyme repairs single-stranded gaps in duplex DNA and copies primed single-stranded templates (Goulian et al., 1968; Masamune et al., 1971), but the processivity (nucleotides added per enzyme-binding event) is less than 10 at salt concentrations that approach physiological values (Das and Fujimura, 1979; Newport et al., 1980); (ii) unlike *E. coli* DNA polymerase I and bacteriophage T5 DNA polymerase, the T4 enzyme does not catalyze strand displacement synthesis on a duplex DNA, where polymerization is initiated by covalent addition to the 3′-OH end at a nick and is accompanied by unwinding of the template helix (Masamune and Richardson, 1971; Nossal, 1974); and (iii) like other DNA polymerases, T4 polymerase is unable to initiate the synthesis of a new DNA chain, as required to start each Okazaki fragment on the lagging strand. These characteristics of the purified T4 DNA polymerase, as well as the genetic evidence for the existence of other replication genes, made it clear that other T4 enzymes would be needed to reconstruct the reactions at a DNA replication fork.

GENE 32 HELIX-DESTABILIZING PROTEIN AND GENE 44/62 AND GENE 45 POLYMERASE ACCESSORY PROTEINS

The T4 gene 32 helix-destabilizing protein (single-stranded-DNA-binding protein) was isolated on the basis of its strong affinity for single-stranded DNA cellulose and identified by the fact that the protein is missing when *E. coli* is infected by any one of several phage mutants with mutations in gene 32 (Alberts et al., 1969). The gene 32 protein binds cooperatively to single-stranded DNA and destabilizes but does not melt natural duplex DNA (Alberts and Frey, 1970). These binding reactions have subsequently been studied in detail, as described in the chapters by von Hippel et al. and Williams and Konigsberg in this volume.

The products of T4 genes 44, 62, and 45 were purified by in vitro complementation assays that measure DNA synthesis with endogenous DNA templates in crude extracts made from *E. coli* infected with the corresponding mutant phage (Barry and Alberts, 1972; Morris, Hama-Inaba, Mace, et al., 1979; Nossal, 1979). The products of genes 44 and 62 purify as a complex that complements either 44^- or 62^- extracts (Table 1). This complex has a single-stranded-DNA-dependent ATPase (or dATPase) activity that is stimulated by the purified gene 45 protein (D. Mace, Ph.D. thesis, Princeton University, Princeton, N.J., 1975; Piperno et al., 1978).

Stimulation of DNA Synthesis on a Single-Stranded DNA Template

The gene 32 protein, on the one hand, and the combination of the three polymerase accessory proteins, on the other, act independently to stimulate the copying of primed single-stranded DNA templates by T4 DNA polymerase, while not stimulating other *E. coli* DNA polymerases (Huberman et al., 1971; Mace, thesis; Piperno and Alberts, 1978). The gene 32 protein and the accessory proteins act by different mechanisms. The gene 44/62 and gene 45 proteins are jointly required to increase the rate and processivity of DNA synthesis by a mechanism that requires ATP hydrolysis. Studies with oligonucleotide-primed fd DNA and restriction fragment-primed ϕX174 DNA templates have shown that regions of secondary structure such as palindromic hairpins act as strong kinetic barriers that cause the polymerase to pause when copying single-stranded templates (Challberg and Englund, 1979; Huang et al., 1981; Roth et al., 1982). The gene 44/62 and gene 45 proteins do not appear to change the structure of these template regions, but nevertheless facilitate synthesis up to and through the pause sites. These accessory proteins appear to act by decreasing the rate of dissociation of the polymerase from the primer-template end. Thus, their effect is greatest at low polymerase concentrations where the diffusion-mediated process of polymerase reassociation with primer-template ends is rate limiting in their absence (Huang et al., 1981). The rate of nucleotide turnover by a polymerase molecule detained at a pause site in the presence of the accessory proteins and ATP is much higher than that observed with the polymerase alone on the same template-primer, presumably reflecting repeated attempts at polymeriza-

tion through a pause site by a polymerase molecule that is tied down on the template in a complex with the accessory proteins (Roth et al., 1982). The addition of gene 32 protein to the mixture of the polymerase and accessory proteins tends to remove the regions of secondary structure in the template, thereby reducing the pauses, increasing the rate of synthesis, and reducing nucleotide turnover.

ATP hydrolysis, not just ATP binding, is required for the stimulation by the accessory proteins, since the stimulation is prevented by the addition of excess riboadenosine 5′-*O*-(3-thiotriphosphate) (ATP-γ-S). However, less than 1 ATP molecule is hydrolyzed for every 10 deoxyribonucleotides incorporated on single-stranded templates (Piperno and Alberts, 1978). It has been proposed that ATP hydrolysis is required for the assembly of a complex of the accessory proteins and the polymerase, but not for the movement of this complex along a single-stranded template (Alberts et al., 1980; Newport et al., 1980). This proposal is based on two findings. First, at short times (less than 45 s), the number of oligo(dT) primers extended on poly(dA) in the presence of the products of genes 43, 44/62, 45, and 32 increases with increasing ATP concentration, with no change in the average product length (Newport et al., 1980). Second, the effect of the accessory proteins on oligonucleotide primer extension on a single-stranded fd DNA template is partially resistant to the addition of excess ATP-γ-S once the reaction has been started, with the effect being reported to persist for at least 10 min (Huang et al., 1981). However, the degree of resistance of the accessory protein-stimulated synthesis to the addition of ATP-γ-S varies with the template-primer. Thus, after the addition of excess ATP-γ-S, the amount of elongation of a restriction fragment annealed to single-stranded ϕX174 DNA is equivalent to less than 30 s of synthesis (Nossal, unpublished data), and synthesis on a nicked duplex template that requires the accessory proteins stops in less than 10 s (Alberts et al., 1980).

From these results, we conclude that a periodic event, dependent on ATP hydrolysis, is required to maintain the stimulation by the accessory proteins. Since synthesis on both single-stranded and nicked duplex templates is extremely processive in the presence of the accessory proteins, it is clear that the complex cannot completely dissociate from the primer terminus between each ATP hydrolysis event. As a working hypothesis, it has therefore been proposed that the accessory proteins assemble into a "sliding clamp" composed of two "half-clamps" around the polymerase. Each half-clamp can keep the polymerase on the template during periodic ATP-dependent resetting of the other half-clamp (Alberts et al., 1980). In this view, the accessory protein-polymerase complex is especially labilized on certain templates and under certain conditions. In these cases relatively frequent ATP hydrolysis will be required to maintain the accessory protein effect on the polymerase (Bedinger and Alberts, J. Biol. Chem., in press).

Stimulation of Polymerase Exonuclease Hydrolysis of Duplex DNA

In addition to stimulating DNA polymerase movement in the forward direction, the accessory proteins and the gene 32 protein also stimulate the reverse reaction: hydrolysis of duplex DNA by the 3′-to-5′ exonuclease of the polymerase (Alberts et al., 1980; Bedinger and Alberts, in press, Venkatesan and Nossal, 1982). The stimulation requires a single molecule of the gene 44/62 complex per DNA end (Bedinger and Alberts, in press). Maximum stimulation by the accessory proteins requires that there is a single-stranded extension of the complementary strand that protrudes more than three nucleotides beyond the 3′-OH end of the duplex region to be hydrolyzed. This single-stranded DNA extension may serve as an important part of the binding site for one or more of the accessory proteins (Venkatesan and Nossal, 1982). Nucleotides behind the 3′-OH terminus of the strand being degraded are also required for the accessory protein action, since the accessory proteins do not stimulate hydrolysis by the polymerase exonuclease once the strand being degraded has been reduced to a chain length of 7 to 11. In contrast, the polymerase exonuclease alone yields a limit product of mono-, di-, and trinucleotides (Venkatesan and Nossal, 1982).

All of the stimulatory effects of the gene 44/62, gene 45, and gene 32 proteins seem to require specific protein-protein interactions. Neither the gene 32 protein nor the accessory proteins increase the 3′-to-5′ exonuclease activity (Bedinger and Alberts, in press) or the polymerase activity (Kolodner and Nossal, unpublished data) of bacteriophage T7 DNA polymerase. The accessory proteins also fail to stimulate the exonuclease activity of the amber fragment produced by the *am*B22 mutant in T4 gene 43. This suggests that the carboxyl terminal 20% of the wild-type polymerase, which is the region missing in the *am*B22 amber fragment (Nossal and Hershfield, 1971), may be involved in the polymerase interaction with the accessory proteins (Venkatesan and Nossal, 1982).

RNA PRIMER SYNTHESIS ON THE LAGGING STRAND

Because DNA polymerases only synthesize DNA in the 5′-to-3′ chain direction, all of the DNA synthesized on the lagging strand of a replication fork must be made discontinuously, as a series of short Okazaki fragments that are subsequently joined together to create an intact daughter strand (Fig. 1) (reviewed in Kornberg, 1980). In T4 bacteriophage-infected cells, an average Okazaki fragment is about 2,000 nucleotides long, and, at the observed polymerization rate of roughly 500 nucleotides per s, it should take about 4 s to complete. Furthermore, since the DNA polymerases

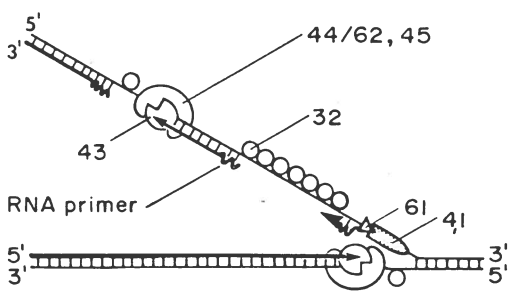

FIG. 1. Model of a replication fork showing the roles of bacteriophage T4 proteins in the continuous synthesis on the leading strand and in the discontinuous synthesis on the lagging strand. See text for details.

studied so far are unable to start a new polynucleotide chain, special mechanisms involving either terminal proteins or RNA primers to which deoxyribonucleotides can be added are required to begin new DNA chains (reviewed in Kornberg, 1980, and Kornberg, 1982). In the T4 DNA replication system, each Okazaki fragment begins with a short base-paired RNA primer that is synthesized from ribonucleoside triphosphates (rNTPs) by the T4 gene 41 and gene 61 proteins.

The process of T4 RNA primer synthesis has been characterized by studying rNTP-dependent DNA synthesis on single-stranded DNA templates. Any natural single-stranded DNA, as well as the synthetic polymer poly(dI,dT), can be used as a substitute for the lagging-strand template. This single-stranded DNA, the gene 41 and gene 61 proteins, ATP, and CTP are the minimum requirements for RNA primer synthesis (Liu and Alberts, 1980; Liu and Alberts, 1981b; Liu et al., 1979; Nossal, 1980; Silver and Nossal, 1979). In the presence of the other replication proteins, more than 90% of the RNA oligonucleotides synthesized by the gene 41 and gene 61 proteins become covalently attached to the 5′ end of newly initiated DNA chains, from which they can be recovered by nuclease digestion (Liu and Alberts, 1980; Liu and Alberts, 1981b; Nossal, 1980).

Virtually all of the primers made in vitro in the presence of four rNTPs are pentaribonucleotides. Sequence analysis of 5′-terminally labeled primers reveals that the major products have the sequence pppApCpNpNpN, where (at least in positions 3 and 4) N can be any one of the four nucleotides (Liu and Alberts, 1980; Liu and Alberts, 1981b; Nossal, 1980). Whereas only pppA starts primers on single-stranded hydroxymethyldeoxycytidine-containing T4 DNA, additional primers that begin with pppG are also found when single-stranded deoxycytosine-containing T4 DNA or bacteriophage fd DNA (Liu and Alberts, 1981) or bacteriophage ϕX174 DNA (Nossal, 1980) is used as the template. The size and sequence of the RNA primers made in vitro on hydroxymethyldeoxycytidine-containing T4 DNA agree completely with those isolated from T4-infected cells (Kurosawa and Okazaki, 1979).

With only ATP and CTP, di-, tri-, and tetranucleotides beginning with pppApC are found in addition to pentanucleotides. The shorter products apparently result when synthesis is prematurely terminated due to the absence of the next nucleotide required by the template. Primers containing only A and C are efficiently elongated by T4 DNA polymerase and the accessory proteins (Liu et al., 1979; Silver and Nossal, 1979). Although there are more than 40 sites on fd DNA which could serve as a template for pentanucleotides pppApCpNpNpN, where N is A or C, only about 10 start sites for new DNA chains were observed on this template with ATP and CTP (Liu and Alberts, 1980). This finding suggests that there may be some requirement for a start site beyond the sequence complementary to the primer.

The catalytic roles of the gene 41 and gene 61 proteins are not yet clear. There is no primer synthesis in the absence of either protein (Alberts et al., 1980; Burke and Alberts, manuscript in preparation; Liu et al., 1979; Nossal, 1980; Silver and Nossal, 1982). The gene 61 protein was first identified by Liu et al. (1979) as a protein present in some preparations of gene 32 protein that was required for rNTP-dependent DNA synthesis with the T4 gene 43, 44/62, 45, and 41 proteins. It was subsequently shown to be a very basic protein with a molecular weight of about 44,000 that is missing in extracts made from *E. coli* infected with T4 gene 61 mutants (Alberts et al., 1980; Liu et al., 1979; Silver and Nossal, 1979; Silver and Nossal, 1982). The phenotype of gene 61 mutants is a delayed synthesis of DNA (Yegian et al., 1971). In contrast, gene 41 mutants were originally classified as DO (no DNA synthesis) (Epstein et al., 1964), although there is some accumulation of single-stranded DNA (Oishi, 1968). The more severe defect in gene 41 mutants may be due to the additional role of gene 41 protein in stimulating leading-strand DNA synthesis (see below).

The gene 41 protein is a single-stranded-DNA-dependent nucleotidase that hydrolyzes rATP, rGTP, dATP, and dGTP (Liu and Alberts, 1981a; Morris, Moran, and Alberts, 1979; Nossal, 1979; Venkatesan et al., 1982). Liu and Alberts (1981a) found that long DNA chains or single-stranded DNA circles are the best cofactors for the gene 41 protein nucleotidase activity and that there is only limited RNA primer synthesis when excess GTP-γ-S is added to inhibit this nucleotidase. On the basis of these results, they suggested that the gene 41 protein moves along a DNA single strand, driven by conformational changes induced by GTP hydrolysis. At the replication fork, the gene 41 protein was proposed to act as a "mobile promoter" which marks the locations where RNA primers are to be synthesized on the lagging strand. Liu and Alberts (1981a) also showed that incubation of a high concentration of gene 41 protein with GTP or GTP-γ-S at 30°C increases its ability to catalyze rNTP-dependent DNA synthesis and that incubation with GTP-γ-S increases the sedimentation coefficient of the protein from 4.9 to 6.1S, consistent with the formation of an active dimer (or higher oligomer). RNA primer synthesis and rNTP-dependent DNA synthesis normally have a linear dependence on gene 61 protein concentration, but a sigmoidal dependence on gene 41 protein concentration. Silver and Nossal (1982) showed that the response to gene 41 protein concentration becomes linear when gene 41 protein is first activated by incubation with GTP and that there is then a stoichiometric relationship between the gene 41 and gene 61 proteins. They have therefore proposed that a large oligomer of gene 41 protein interacts with a monomer of gene 61 protein to form a complex that is active in primer synthesis.

A model consistent with these observations is shown in Fig. 1. A complex of the gene 41 and gene 61 proteins is envisioned to move along the lagging strand in the 5′-to-3′ direction, in a reaction dependent on nucleotide hydrolysis. At intervals of about 2,000 nucleotides, the complex stops at appropriate sites to make pentaribonucleotide primers. These primers probably remain attached to the priming proteins until elongated by the DNA polymerase, since added pentaribonucleotides by themselves are not efficiently used as primers. Further evidence for the proposed movement of the priming proteins, and the effect of this movement on leading strand DNA synthesis, is described below.

To produce a continuous DNA chain from the many

Okazaki fragments made on the lagging strand, a special DNA repair system is needed behind the fork. The minimal requirements are for an RNase H enzyme to degrade the RNA primer, a DNA polymerase molecule to replace the ribonucleotides removed, and a DNA ligase to form a phosphodiester linkage between the 3′ end of each fragment and the 5′ end of the preceding one. The T4 DNA polymerase (gene 43 protein), T4 DNA ligase (gene 30 protein), and a T4-induced RNase H of 44,000 daltons whose gene has yet to be identified are all thought to be involved (V. Chandler and B. M. Alberts, and R. Crouch and N. G. Nossal, unpublished data). However, this Okazaki fragment repair process has not yet been studied in detail in the T4 system.

LEADING-STRAND SYNTHESIS

The DNA synthesis on the leading strand of a replication fork requires rapid unwinding of the DNA duplex ahead of the growing chain (Fig. 1). Strand displacement synthesis that starts from a preexisting nick in duplex DNA has been used as a model for this type of synthesis. Five T4 proteins are the minimum required for this type of synthesis: DNA polymerase, gene 32 protein, and the three gene products that act as polymerase accessory proteins (Table 1) (Liu et al., 1979; Nossal and Peterlin, 1979). The products of this five-protein reaction are duplex DNA molecules from which long single-stranded branches protrude. Distinct pause sites are revealed by analysis of the products that begin at a single nick at a defined location on duplex circular ϕX174 DNA or fd DNA (Alberts et al., 1983; Venkatesan et al., 1982).

Gene 32 protein appears to play an active role in destabilizing the duplex, since the rate of fork movement increases steadily with increasing free gene 32 protein concentration, reaching a rate of about 180 nucleotides per s at a gene 32 protein concentration of 200 μg/ml (Alberts et al., 1980). The proteolytic product called 32* I protein, which is missing 8,000 daltons from the carboxy-terminal end of gene 32 protein, can replace intact gene 32 protein in this reaction (Burke et al., 1980). Although T4 DNA polymerase cannot use a duplex template by itself, it also must contribute to destabilizing the helix. Thus, the polymerase made by an antimutator phage, T4 tsCB120, copies single-stranded homopolymers at rates comparable to those of the wild-type enzyme, but is more inhibited by hairpin pause sites in single-stranded natural DNA and poly(dA-dT) templates (Gillin and Nossal, 1976a). This tsCB120 mutant polymerase is stimulated by gene 32 protein and the accessory proteins on single-stranded DNA templates, but it is unable to catalyze strand displacement synthesis on double-helical DNA templates with the gene 32, gene 44/62, and gene 45 proteins present. Further addition of the gene 41 protein, which stimulates strand displacement synthesis with the wild-type DNA polymerase present (see below), is still insufficient for strand displacement synthesis with the mutant polymerase (Nossal, unpublished data).

During synthesis on a double-helical template, the gene 44/62 and gene 45 proteins presumably act to keep the polymerase continuously on the growing 3′-OH chain end where it can take advantage of gene 32 protein-catalyzed breathing in the template helix ahead. Polymerase dilution experiments reveal that the DNA synthesis obtained is highly processive, with the same DNA polymerase molecule synthesizing stretches of DNA that are tens of thousands of nucleotides long (Alberts et al., 1980; Barry, unpublished data). As noted above, very frequent ATP hydrolysis by the accessory proteins is required for synthesis on duplex templates, although, with duplex templates as well as single-stranded templates, less than 1 molecule of ATP is hydrolyzed for every 10 deoxyribonucleotides polymerized (Liu et al., 1979).

Even at high gene 32 protein concentrations, the rate of fork movement in the five-protein reaction described above is significantly lower than the rate at which the fork moves in vivo (Alberts et al., 1980; Liu et al., 1979). The in vitro rate is enhanced by the addition of either of two T4-induced DNA helicases: the gene 41 protein or the product of the *dda* gene (Table 1). As discussed above, the gene 41 protein is a single-stranded-DNA-dependent nucleotidase that is required together with the gene 61 protein for RNA primer synthesis. The addition of gene 41 protein greatly increases the rate of fork movement above that obtained in the five-protein reaction, with the largest stimulation being observed when limiting concentrations of gene 32 protein are present (Alberts et al., 1980; Liu et al., 1979; Venkatesan et al., 1982). However, gene 41 protein does not remove the requirement for each of the five other replication proteins. The gene 41 protein is also a DNA helicase that can unwind short DNA fragments (50 to 400 base pairs) that are base paired to a complementary DNA strand; as expected, this reaction is dependent on GTP or ATP hydrolysis (Venkatesan et al., 1982). Helicase activity requires a single-stranded DNA extension adjacent to the duplex region to be unwound, and the unwinding begins at the 3′ end of the fragment. This direction of helix unwinding is consistent with the proposal (Alberts et al., 1980) that the gene 41 protein destabilizes the helix ahead of the replication fork as it moves 5′ to 3′ on the lagging strand of the fork toward the next site for primer synthesis (Fig. 1). The helicase activity of gene 41 protein is stimulated by both gene 61 protein and the combination of the three polymerase accessory proteins, suggesting that all of these proteins may be closely associated with each other at the replication fork (Venkatesan et al., 1982).

The T4 *dda* protein was initially identified as a single-stranded-DNA-dependent ATPase (Purkey and Ebisuzaki, 1977) and later shown to be an ATP-dependent DNA helicase (Krell et al., 1979). The helicase activity of the *dda* protein, like that of the gene 41 protein, requires a short single-stranded extension beyond the 3′ OH of the duplex region to be unwound, supporting the conclusion that the *dda* protein unwinds DNA in the same direction as does the gene 41 protein (Krell et al., 1979). Unlike the gene 41 protein, the *dda* helicase can unwind very long DNA helices. However, in this reaction it acts stoichiometrically with approximately one protein molecule required for each two base pairs unwound. At a low concentration, the *dda* helicase increases the rate of fork movement of the five-protein replication reaction on a nicked DNA template, mimicking the action of the gene 41 protein in this regard. The *dda* protein neither further stimulates the fork rate when gene 41

protein is present nor substitutes for the gene 41 protein in RNA priming reactions.

A clue to the biological role of the *dda* protein was obtained in experiments that examined the effect of template-bound RNA polymerase molecules on the movement of T4 replication forks in vitro. Replication forks formed in the absence of the *dda* protein, whether or not they contain the gene 41 protein, are stopped by template-bound RNA polymerase molecules. The addition of small amounts of the *dda* helicase completely removes this inhibition, allowing the replication fork to move rapidly past RNA polymerase molecules that are bound to the DNA template (Alberts et al., 1983; Bedinger et al., submitted for publication). RNA polymerase molecules that are not transcribing are displaced by the DNA replication enzymes, but the fate of transcribing RNA polymerase molecules has not been determined (Bedinger et al., submitted for publication). It seems likely that the removal of barriers to DNA replication caused by RNA polymerase and other DNA-bound proteins is an important function of the type of helicase encoded by the T4 *dda* gene. This might explain why more than one 5′-to-3′ DNA helicase seems to be required at a T4 replication fork.

There is genetic evidence to support a role for the *dda* helicase in T4 DNA replication. Although T4 bacteriophage that have the *dda* gene deleted grow normally in most *E. coli* hosts (Behme and Ebisuzaki, 1975), they are unable to grow in an *E. coli optA* mutant (Gauss et al., 1983). The *optA*1 mutant was originally selected as a nonpermissive host for the growth of bacteriophage T7 mutants in gene 1.2, a gene of unknown function that is located close to the T7 replication origin (Saito and Richardson, 1981; Saito et al., 1980). The *optA*1 mutant also fails to grow certain bacteriophage T4 temperature-sensitive mutations in the polymerase gene, including the antimutator tsCB120, even at low temperatures (Gauss et al., 1983). As noted above, the tsCB120 polymerase is defective in strand displacement synthesis in vitro. On the basis of these studies, Gauss and his colleagues have proposed that the *E. coli optA* gene encodes a host DNA helicase that interacts with the T4 DNA replication proteins and is required unless both the T4 *dda* helicase and the full helix-destabilizing activity of the wild-type T4 DNA polymerase are present.

COUPLING OF LEADING-STRAND AND LAGGING-STRAND DNA SYNTHESIS

The simple replication fork model illustrated in Fig. 1 implies that a completely separate complexe of the polymerase and the accessory proteins elongates the leading and lagging strands. In this model, the complex of the polymerase and accessory proteins on the leading strand is assumed to remain permanently bound to the template as the fork moves. Thus, synthesis on the leading strand is continuous and highly processive, accounting for the finding of a minor fraction of template DNA molecules on which there has been extensive synthesis (>50,000 nucleotides added) under conditions where there has been little or no synthesis on the majority of other template molecules (Nossal and Peterlin, 1979; Sinha et al., 1980). In contrast, the model in Fig. 1 predicts that the polymerase complex on the lagging strand dissociates from its template each time that it finishes the 3′ end of an Okazaki fragment and encounters the 5′ end of the previous fragment. If this model is correct, decreasing the polymerase concentration to very low levels should delay the start of each Okazaki fragment by making the collision of a free DNA polymerase molecule with an RNA primer unlikely, while having little effect on further synthesis on the leading strand. In fact, this model is contradicted by recent studies in which the concentration of T4 DNA polymerase was varied over a wide range, with each of the other six replication proteins present in excess. In these reactions, DNA synthesis on the lagging strand was unaffected by extreme polymerase dilution, which is only explicable if the DNA polymerase molecule on the lagging strand is recycled for multiple rounds of Okazaki fragment synthesis and thus remains bound to the fork at all times (Alberts et al., 1983; Barry and Alberts, manuscript in preparation). Similar experiments suggest that the gene 41 protein and gene 61 protein also remain bound to the fork for prolonged periods (Barry, unpublished data).

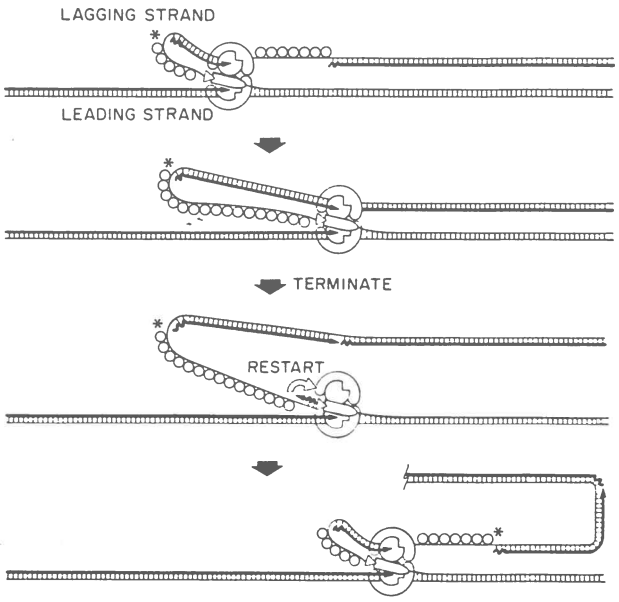

FIG. 2. Model of T4 proteins at a replication fork that accounts for the recycling of the polymerase on the lagging strand and the stimulation of leading-strand synthesis by the gene 41 protein primase-helicase. T4 DNA replication proteins are represented by the symbols used in Fig. 1. In this model the polymerase-accessory protein complex on the lagging strand is bound to the corresponding complex on the leading strand, and it is assumed that the two strands are replicated at the same rate (adapted from Alberts et al., 1983).

Two closely related models of the replication fork which account for the recycling of the polymerase and priming proteins on the lagging strand, as well as the coupling of leading- and lagging-strand synthesis by the gene 41 protein helicase, are illustrated in Fig. 2 and 3. In the "trombone" model (Fig. 2) proposed by Alberts et al. (1983), which is based in part on protein-protein interactions determined by protein affinity column chromatography (Alberts et al., 1983; Formosa et al., Proc. Natl. Acad. Sci. U.S.A., in press), the

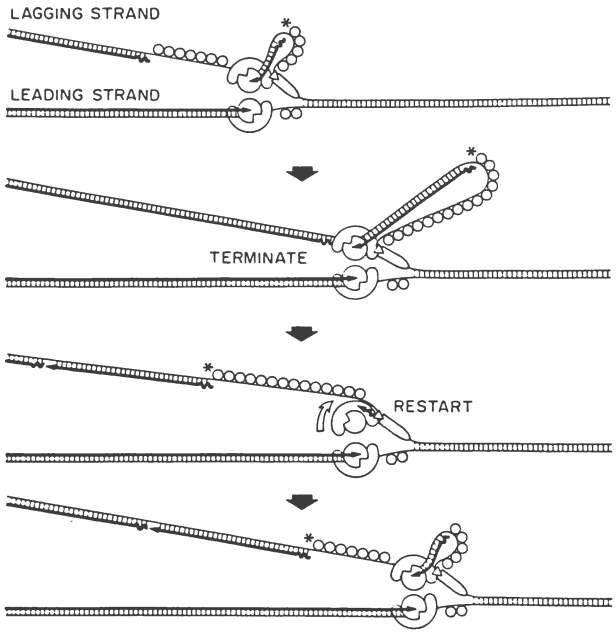

FIG. 3. A second possible model of T4 proteins at a replication fork that, like the model in Fig. 2, accounts for recycling of the polymerase-accessory protein complex on the lagging strand and the stimulation of leading-strand synthesis by the gene 41 protein primase-helicase. T4 proteins are represented by the symbols used in Fig. 1. In this model the polymerase-accessory protein complex is bound by the gene 41-gene 61 protein primase-helicase complex that remains on the lagging strand.

polymerase-accessory protein complex on the lagging strand is tied to the corresponding polymerase complex on the leading strand. The synthesis of each Okazaki fragment involves the enlargement of a large loop of DNA, half of which is single stranded and half of which is double stranded. The crucial points in the proposed cycle (Fig. 2) are the termination and restart steps, which involve movements of the DNA on the lagging strand of the fork around a fixed lagging-strand DNA polymerase molecule, as indicated. Primer synthesis will take place at an appropriate sequence reached after termination of the preceding fragment.

In the trombone model, it was assumed that the two polymerase molecules joined in the complex must elongate the two growing DNA strands at the same rate so that there is always a constant amount of single-stranded DNA on the lagging strand. This was proposed to make it possible for the gene 32 protein molecules bound to the loop to be continuously recycled on the lagging strand. In this case, the length of the single-stranded DNA that accumulates in the loop (Fig. 2)—which will determine the size of the next Okazaki fragment—will always be equal to the length of the previously synthesized fragment. Therefore, each Okazaki fragment on an individual lagging strand should be about the same size as the preceding fragment (Alberts et al., 1983). The proposition that the Okazaki fragments are of constant size and the idea that the gene 32 protein molecules recycle remain to be tested.

In the model in Fig. 3, the polymerase-accessory protein complex on the lagging strand is tied to the fork by being bound to the gene 41 protein-gene 61 protein primase-helicase complex that moves 5′ to 3′ on the lagging strand of the fork. Synthesis on the two strands is coupled because the gene 41 protein helicase on the lagging strand helps to destabilize the helix ahead of the new leading strand. The rate of elongation of the polymerase-accessory protein complexes on the leading and lagging strands would not in general be precisely the same; the relative rates would be expected to vary due to the different distribution of pause sites on the two template strands. Since T4 Okazaki fragments are about 2,000 nucleotides long, the gene 41 protein-gene 61 protein complex must pass by many potential priming sites. Nossal has proposed (unpublished) that the release of the polymerase-accessory protein complex from the 3′-OH end of a completed Okazaki fragment alters the conformation of the attached primase-helicase complex and that this conformational change acts as a signal to the priming proteins to make a new primer at the next appropriate sequence. This proposition, which also remains to be tested, predicts that the length of each Okazaki fragment will be determined by the particular distance covered by the gene 41 protein helicase during the time necessary for the polymerase to complete the previous fragment.

It is important to realize that the models in Fig. 2 and 3 are closely related. The proposed conformational change in the priming proteins when the polymerase completes an Okazaki fragment could also occur if the polymerase complexes on the leading and lagging strands were physically linked to each other. Furthermore, the polymerase molecules could be linked as shown in Fig. 2 and still copy the two strands at different rates. In that case, the remaining difference between the two models is that in Fig. 3 the proteins are arranged so that the 3′-OH termini of the two new strands enter the polymerase complex from opposite sides, whereas in Fig. 2 it is proposed that the two growing chains enter the tightly coupled protein complex from the same side.

Regardless of the details, an important feature of the type of models shown in Fig. 2 and 3 is that the replication apparatus lays down unsealed fragments that must then be ligated. Assuming that the polymerase is released before the RNA primer is removed from the preceding fragment, a second polymerase molecule will be needed to repair the gap.

The different possibilities discussed above will need to be resolved by further experiments. However, the major focus of both models is the coupling of leading- and lagging-strand synthesis and the recycling of the replication proteins that are characteristic of the T4 DNA replication system. The picture that emerges is one of a large "replication machine" whose component parts move relative to each other during different stages of DNA replication without disassembling from the complex. The enzymes that synthesize nucleotide precursors also seem to be part of the replication complex (Chiu et al., 1982; Mathews and Allen, this volume). The precise assembly of these large replication protein aggregates may be a rate-limiting step in the process of replication fork initiation at T4 replication origins.

SIMILARITIES BETWEEN T4 DNA REPLICATION AND OTHER DNA REPLICATION SYSTEMS

The goal of the enzymatic studies that have been carried out on the T4 replication proteins has been to understand in detail the types of reactions that are required to replicate duplex DNA. It is therefore satisfying that mechanisms similar to those described for the T4 proteins have also been demonstrated in the bacteriophage T7 and *E. coli* DNA replication systems (reviewed in Nossal, Annu. Rev. Biochem., in press). Analogous reactions are beginning to be found in eucaryotic systems as well.

DNA Polymerases and Accessory Proteins

Like the T4 replication system, the T7 bacteriophage and *E. coli* DNA replication systems utilize a single type of DNA polymerase to elongate both the leading and lagging strands (Table 2). The T7 DNA polymerase is relatively simple and does not require accessory proteins analogous to the T4 gene 44/62 and gene 45 proteins. In contrast, *E. coli* DNA polymerase III (PolIII) is quite complex. PolIII core, composed of three subunits (α, ε, and θ), can be purified as a complex with at least four other subunits (β, γ, δ, and τ). This whole complex has been designated "PolIII holoenzyme." This holoenzyme can be converted to smaller complexes by standard protein purification procedures (reviewed in McHenry and Kornberg, 1981). Alternatively, an activity equivalent to the PolIII holoenzyme can be reconstructed from PolIII core by the addition of three separate factors (EFI, EFIII, and *dnaZ* protein) that have been purified on the basis of their ability to allow PolIII core to use primed single-stranded DNA templates (reviewed in Wickner, 1978). The factors or subunits have functions analogous to the T4 polymerase accessory proteins: factors γ and δ (*dnaZ* and EFIII) increase the processivity of PolIII core and are required, together with β (EFI), for the binding of the polymerase core to primed single-stranded templates.

The accessory proteins that function in concert with the T4 and *E. coli* DNA polymerases seem to be important for keeping the polymerase tightly bound to the template, and they presumably reduce the frequency with which major "geometrical errors" occur in replication. Such errors include both "template slippage" errors that can cause groups of nucleotides to be either added or deleted in the new DNA chain and "template switching" errors. The latter arise in a reaction first discovered with *E. coli* DNA polymerase I, in which the DNA polymerase turns around to copy a previously replicated region, thereby creating a region of self-complementary sequence detected as "reversibly denaturable" DNA (reviewed in Kornberg, 1980). Reversibly denaturable DNA is a major product of the limited synthesis on nicked duplex templates catalyzed by T4 DNA polymerase in the presence of only the gene 32 protein (Nossal, 1974), but it can not be detected in the products made in the T4 five-protein reaction that includes the polymerase accessory proteins (Nossal and Peterlin, 1979; Sinha et al., 1980).

Why should bacteriophages T4 and T7, with progressively smaller genomes and larger copy numbers, require simpler DNA polymerase complexes than *E. coli* for their replication? It is tempting to speculate that the replication of longer single-copy genomes requires an increasingly complex array of accessory polypeptide chains to reduce the frequency of polymerase errors, since it is crucial that the essential regions of the single copy be replicated without a geometrical mistake. In this view, the genome of bacteriophage T7 is so small (40,000 base pairs) and present at such a high copy number that it can tolerate the errors made by a relatively simple form of DNA polymerase.

Strand Displacement Synthesis from a Nick on a Double-Helical Template

One component that is apparently quite similar in all three of the DNA replication systems described above is that designated as a helix-destabilizing protein (HD protein) by some workers (Alberts and Sternglanz, 1977) and as a single-stranded-DNA-binding protein (SSB protein) by others (Kornberg, 1980). The *E. coli* SSB protein closely resembles the T7 gene 2.5 protein and the T4 gene 32 protein in its properties, and, like the T4 protein, it increases the rate and processivity of DNA synthesis on single-stranded templates catalyzed by its homologous DNA polymerase (the PolIII holoenzyme).

The demonstrated requirements in the *E. coli* system for strand displacement synthesis on a nicked duplex template are considerably more complex than in the T4 system; they include the PolIII holoenzyme, the *E. coli* SSB protein, and a 3′-to-5′ helicase (*rep* protein); in addition, at least one other protein is involved at the start of synthesis (for example, the bacteriophage fd gene 2 protein [Geider et al., 1982]) (Table 2). Moreover, at least three other *E. coli* DNA helicases (helicases I, II, and III) exist which unwind DNA in the same direction as the T4 gene 41 and *dda* proteins; however, the role of these helicases in replication is still not clear (reviewed in Geider and Hoffmann-Berling, 1981). The 3′-to-5′ DNA helicases are thought to move on the leading strand of the fork, 5′-to-3′ DNA helicases, like the T4 gene 41 protein, move on the lagging strand (Fig. 4).

In contrast to the complex *E. coli* system, the T7 DNA polymerase catalyzes efficient strand displacement synthesis on nicked duplex templates with only the T7 gene 4 protein present. This gene 4 protein is a multifunctional protein with primase, DNA-dependent nucleotidase, and helicase activities (reviewed in Richardson, *in* Y. Becker, ed., *Replication of Viral and Cellular Genomes,* in press). While a helix-destabilizing protein is not required, DNA synthesis is stimulated by either the *E. coli* SSB protein or the T7 gene 2.5 protein, and it would appear that one or another of these two proteins is required for T7 DNA replication in vivo.

RNA Primer Synthesis

The T7 bacteriophage also has the simplest priming system. The T7 gene 4 primase-helicase recognizes the template sequence N_2N_1GTC and synthesizes tetranucleotide primers beginning with pppApC on all natural single-stranded DNAs tested (Romano et al., 1979; Scherzinger et al., 1977; Tabor and Richardson, 1981). By comparing the relative frequency with which dif-

TABLE 2. Comparison of T4 DNA replication proteins with those of other procaryotic DNA replication systems

Source of proteins	Proteins					
	T4	T7[a]	*E. coli* and bacteriophage G4, φX174, and fd systems[b]			
			General priming	G4	φX174	fd, M13
Primer synthesis (ssDNA)[c]						
Primase	T4 gene 61[d] T4 gene 41[d]	T7 gene 4	*dnaG*	*dnaG*	*dnaG*	RNA polymerase
Other	None	None	*dnaB*	SSB	SSB; n′ (Y); n,n″ (Z); i (X); *dnaB*; *dnaC*	SSB
DNA chain elongation						
Polymerase	T4 DNA polymerase (gene 43)	T7 DNA polymerase (T7 gene 5 + *E. coli* thioredoxin)	PolIII core[e]	PolIII core	PolIII core	PolIII core
Polymerase accessory	T4 gene 44/62 T4 gene 45	None	PolIII holoenzyme subunits[f]	PolIII holoenzyme subunits	PolIII holoenzyme subunits	PolIII holoenzyme subunits
SSB (helix destabilizing)	T4 gene 32	T7 gene 2.5 or *E. coli* SSB	*E. coli* SSB	*E. coli* SSB	*E. coli* SSB	*E. coli* SSB
DNA helicases[g]	T4 gene 41 (5′ to 3′) T4 *dda* (5′ to 3′)	T7 gene 4 (5′ to 3′)		*rep* (3′ to 5′)	*rep* (3′ to 5′)	*rep* (3′ to 5′)
Site-specific nicking	None	None		G4 gene *A*	φX gene *A*	fd gene 2

[a] Reviewed in Richardson (1982).
[b] Reviewed in Kornberg (1982), Meyer and Geider (1982), Reinberg et al. (1981), and Wickner (1978). All are *E. coli* proteins, except the phage-encoded site-specific nicking enzymes.
[c] ssDNA, Single-stranded DNA.
[d] Both T4 gene 41 protein and T4 gene 61 protein are required to observe any primer synthesis. Gene 41 protein also has a single-stranded-DNA-dependent nucleotidase activity analogous to that of the T7 gene 4 and *E. coli* n′ (Y) and *dnaB* priming proteins.
[e] *E. coli* DNA PolIII core subunits are α (*dnaE*), ε (*mutD*, *dnaQ*), and θ.
[f] Subunits present in PolIII holoenzyme but not in PolIII core include γ (*dnaZ*), δ (EFIII), β (EFI, *dnaN*), and τ. These function like "accessory proteins" in increasing the processivity of the core polymerase.
[g] Helicase direction is indicated as the direction of movement on the strand that is not displaced.

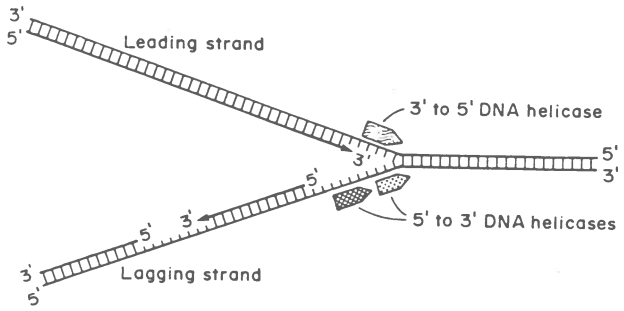

FIG. 4. General model of a replication fork, showing the proposed location of 5′-to-3′ DNA helicases on the lagging strand and 3′-to-5′ DNA helicases on the leading strand. By convention, DNA helicases are designated by their direction of movement on the strand that is not displaced.

ferent priming sites are used on ϕX174 DNA, it has been inferred that the gene 4 protein binds at random and travels 5′ to 3′ on the single-stranded DNA template strand (Tabor and Richardson, 1981).

In *E. coli*, primer synthesis has been studied by using the replication of a variety of single-stranded circular bacteriophage DNAs as model systems. Either *E. coli* RNA polymerase or the *dnaG* protein serves as the primase, depending on which bacteriophage DNA is the template (Table 2) (reviewed in Kornberg, 1982; Meyer and Geider, 1980; Reinberg et al., 1981; Wickner, 1978). In the general priming reaction, the *dnaG* primase requires the *dnaB* protein in order to make short ribooligonucleotide (or mixed ribo- and deoxyribooligonucleotide) primers on natural single-stranded DNA and on poly(dT). This priming reaction most resembles those found in the T4 and T7 bacteriophage systems.

Upon addition of *E. coli* SSB protein, the *dnaG* primase reaction becomes dependent on specific recognition structures or sequences on the DNA. On the DNA of G4 and related phages, *dnaG* protein (in the presence of only the SSB protein) makes a 26- to 29-nucleotide primer at one specific site. In contrast, on ϕX174 DNA, a complex of at least six additional proteins is required for primer synthesis. Two of these proteins, the n′ (Y) and the *dnaB* proteins, are DNA-dependent nucleotidases. The priming complex, which has been termed a "primosome," is initially assembled on the DNA at a specific site recognized by the n′ (Y) protein but then moves in the 5′ to 3′ direction on the template, as judged by the frequency of primers made at different locations (Arai and Kornberg, 1981; Arai et al., 1981).

The primosome complex moves in the same direction along a DNA strand as the T4 gene 41 and T7 gene 4 priming proteins and thus may also serve as a 5′-to-3′ helicase at the replication fork. While helicase activity has not been shown directly, the inhibition of leading-strand DNA synthesis on the ColE1 plasmid by antisera to the *dnaB*-primosome protein has led to the proposal that this protein helps to unwind the DNA duplex ahead of the leading strand (Staudenbauer et al., 1979).

CONCLUSION

Studies of DNA replication in the T4 and T7 bacteriophages and the *E. coli* systems have indicated that efficient and accurate replication requires an impressive array of interacting proteins. Helicases, helix-destabilizing (SSB) proteins, and polymerase complexes all contribute to helix destabilization. A dual role of the priming proteins as helicases helps to coordinate leading- and lagging-strand synthesis. A dimeric form of DNA polymerase has been proposed to copy the leading and lagging strands simultaneously in both the T4 (Alberts et al., 1983) and *E. coli* (Kornberg, Burgers, and Stayton, cited in Kornberg, 1982) DNA replication systems.

The manner in which the bacteriophage T4 DNA replication proteins are physically connected and the mechanisms by which the activities of the T4 replication proteins are coordinated with each other (and with the activity of the enzymes synthesizing nucleotide precursors) remain to be determined. Current progress in locating the bacteriophage T4 DNA replication origins (see the chapters by Mosig, Kozinski, and Kreuzer and Huang in this volume) should greatly facilitate studies on the mechanism of fork initiation by the T4 DNA replication proteins.

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