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Eukaryotic DNA Replication

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ABSTRACT: The past decade has witnessed an exciting evolution in our understanding of eukaryotic DNA replication at the molecular level. Progress has been particularly rapid within the last few years due to the convergence of research on a variety of cell types, from yeast to human, encompassing disciplines ranging from clinical immunology to the molecular biology of viruses. New eukaryotic DNA replicases and accessory proteins have been purified and characterized, and some have been cloned and sequenced. *In vitro* systems for the replication of viral DNA have been developed, allowing the identification and purification of several mammalian replication proteins. In this review we focus on DNA polymerases alpha and delta and the polymerase accessory proteins, their physical and functional properties, as well as their roles in eukaryotic DNA replication.

KEY WORDS: eukaryotic DNA replicases, accessory proteins.

I. INTRODUCTION

During the past decade, our understanding of eukaryotic DNA replication at the molecular level and the nature of the proteins that function at the replication fork have increased exponentially as a result of the convergence of research in a variety of disciplines and the successful application of knowledge gained from the elucidation of the basic mechanisms of DNA replication in prokaryotes. Highlights include the development of a cell-free system capable of replicating simian virus 40 (SV40) DNA, which has led to the identification and purification of several mammalian replication proteins, and the cloning and sequencing of replication proteins from organisms as phylogenetically distant as yeast, *Drosophila*, and mammals, which has elucidated the remarkable conservation of structure and function in the components of the eukaryotic replication apparatus. New eukaryotic DNA polymerases and their accessory proteins have been purified and characterized, and genetic approaches have been used to define the functional roles of these polymerases in yeast.

Of necessity, this review is not comprehensive but does focus primarily on the molecular mechanism of DNA replication, with particular emphasis on the proteins that function at the replication fork.

Just as the mechanisms by which phage DNAs are replicated were initially investigated as a strategy to elucidate the mechanism by which *E. coli* replicates its chromosome, the use of SV40 DNA as a model replicon has resulted in important insights into the mechanism of eukaryotic DNA replication. SV40 is a particularly useful model because, except for a single virally encoded protein, the large tumor antigen (T antigen), replication of the viral genome utilizes host replication proteins. A major breakthrough in the area of eukaryotic DNA replication was the development of cellular extracts capable of replicating plasmids containing the SV40 origin of replication (ori) when supplemented with T antigen. Replication in this reconstituted system is apparently identical to that of SV40 DNA replication *in vivo*, i.e., replication is bidirectional from the origin and semidiscontinuous. Fractionation of these cellular extracts and purification

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of the components required for replication resulted in the confirmation of the replicative function of several previously identified proteins, as well as in the identification and characterization of new replication proteins. The power of genetic manipulation in delineating the functions of prokaryotic replication functions had not been available previously in studying mammalian replication. However, the striking conservation of both the structure and function of replication proteins in phylogenetically diverse eukaryotic species has allowed the rapid establishment of suspected replication function of mammalian proteins via genetic manipulation of their counterparts in yeast.

This review has benefitted greatly from recent reviews on both prokaryotic DNA replication, notably those of Alberts,[1] Kornberg,[2] and McHenry,[3] as well as on eukaryotic DNA replication, i.e., those of Lehman and Kaguni,[4] Burgers,[5] Challberg and Kelly,[6] Hay and Russell,[7] Sitney and Campbell,[8] Stillman,[9] Bambara et al.,[10] Thommes and Hubscher,[11] and Wang.[12]

II. THE DNA POLYMERASES

A. The DNA Polymerase Alpha-Primase Complex

DNA polymerase alpha (pol alpha) was first isolated from calf thymus tissue over 30 years ago.[13] It has long been postulated to be the eukaryotic DNA replicase because of its sensitivity to a variety of inhibitors of DNA replication, e.g., aphidicolin, and its increased activity in rapidly proliferating cells.[12] In recent years substantial evidence has been accumulated that supports a replicative role for pol alpha and its homolog in yeast, pol I. Neutralizing monoclonal antibodies to pol alpha inhibit DNA replication in permeabilized cells or when microinjected into nuclei,[14,15] and a temperature-sensitive replication mutant of mouse cells has been identified as a pol alpha mutant.[16,17] Pol-alpha gene expression has been found to correlate positively with the activation of cell proliferation and cell transformation at both the transcriptional and post-transcriptional levels.[18,19] Furthermore, pol alpha is required for replication of SV40 DNA in *in vitro* systems from human or monkey cells.[20-23] The yeast homolog of pol alpha, pol I, is encoded by a single copy gene *POL1* (formerly called *CDC17*), which is essential for both mitotic and premeiotic DNA replication.[24-26] Conditional lethal mutants in *pol1* undergo S-phase arrest when grown at the nonpermissive temperature.

1. Structure and Properties of Pol Alpha-Primase

In spite of intensive biochemical research on pol alpha, the low abundance of the protein and its unusual sensitivity to proteolysis confounded efforts to define the subunit structure of the protein for many years, and it was not until recently that the structure of the homogeneous enzyme was definitively established.[4,12,27,28] The use of various protease inhibitors during purification by conventional methods and the introduction of immunoaffinity chromatography have minimized but not eliminated the problem of proteolysis. Pol-alpha species purified by immunoaffinity chromatography from calf thymus,[29-31] human KB cells,[32] and yeast[33] have been found to be similar in structure to those purified by conventional procedures from the same sources,[34-37] as well as from human HeLa cells,[38] monkey CV-1 cells,[39] rabbit bone marrow,[40] rat liver,[41,42] *Drosophila melanogaster*,[43,44] and cultured mouse cells.[45,46] It is now generally accepted that pol alpha is composed of four subunits: a catalytic subunit of approximately 160–180 kDa; a polypeptide of approximately 70 kDa whose function is unclear; and polypeptides of 60 and 50 kDa, which are associated with primase activity.[33,44] The catalytic polypeptide is frequently obtained as a cluster of polypeptides of 125–180 kDa, many of which are catalytically active. However, partial peptide mapping of the isolated polypeptides and the use of a panel of monoclonal antibodies to pol alpha have demonstrated that, whereas the 70-kDa, 60-kDa, and 50-kDa polypeptides are unrelated to the 180-kDa polypeptide and to each other, the other high-molecular-weight polypeptides are most likely proteolytic fragments of the 180-kDa catalytic polypeptide.[32]

a. The Catalytic Subunit

A full-length cDNA clone of the catalytic subunit of human KB cell DNA polymerase alpha was isolated by screening a cDNA library with oligonucleotide probes corresponding to the amino acid sequences of several pol alpha peptides.[18] A molecular weight of 165,000 was deduced from the single open reading frame. Antipeptide antibodies raised to both C- and N-terminal peptides have been used to demonstrate that the polypeptide encoded by the full-length cDNA has a molecular weight on SDS-polyacrylamide gel electrophoresis of 180,000.[47] This was the only species detected by immunoblotting when antibodies to an N-terminal peptide were used. The active proteolytic fragments were found to result from endoproteolytic cleavage, particularly at a susceptible lys-lys bond, which resulted in the generation of the 165-kDa major degradation product. The higher than expected molecular size of the catalytic polypeptide is due, at least in part, to posttranslational modification of the 165-kDa primary translation product. In addition to being a phosphoprotein, the catalytic subunit of pol alpha reacts with the lectins concanavalin A and *Ricinus communis* agglutinin, suggesting that it is a glycoprotein.[47] The functions of these covalent modifications of the primary structure are unclear; however, it has been suggested that they may play a role in nuclear localization of the enzyme and/or serve as recognition sites for other replication proteins.

A genomic clone of the catalytic subunit of *S. cerevisiae* DNA polymerase I was isolated from a lambda gt11 library using polyclonal antibodies to yeast pol I.[24,25] A molecular weight of 167,000 was deduced from the gene sequence. Comparison of the primary structures of pol I and pol alpha, as well as replicative DNA polymerases of bacterial and viral origin, revealed six regions of striking similarity, thought to represent functional domains.[18,48] Two of the regions of homology have been identified as potential deoxynucleoside triphosphate binding domains, and a third region, which contains cysteine-rich sequences, is a potential metal binding domain that could be involved in DNA binding. A DNA primase interaction domain has been postulated to be localized in the amino-terminal half of the polypeptide, a region that is not involved in catalysis.[12]

b. The 70-kDa Polypeptide

The function of the 70-kDa polypeptide of pol alpha has not been elucidated as yet. It has been postulated that the 70-kDa polypeptide might serve as an anchor, connecting polymerase with primase subunits and may possibly play a regulatory role.[33,50] Consistent with the proposed regulatory role is the recent demonstration that the 70-kDa polypeptide is a phosphoprotein, as is the 180-kDa catalytic subunit.[32] The amino acids phosphorylated are serine and threonine. However, dephosphorylation of the 180-kDa and the 70-kDa polypeptides by acid phosphatase did not affect either the polymerase or primase activities. In addition, a monoclonal antibody to the 70-kDa subunit of pol alpha from human and mouse cells inhibits the primase but not the DNA polymerase activity.[51]

c. DNA Primase

Pol alpha is usually isolated as a tight complex with DNA primase, an oligoribonucleotide polymerase that synthesizes short ribonucleotide primers, which can be extended by the DNA polymerase.[4] Its role in the initiation of Okazaki fragments at replication forks is well documented, and it may also function to initiate leading-strand synthesis at replication origins.[6,7,9,52] The isolation of the bifunctional pol alpha-primase complex from a variety of eukaryotic cells and tissues, e.g., *Drosophila*,[44,53,54] yeast,[33,55] human HeLa and KB cells,[38,56,57] cultured mouse cells,[46,51] and calf thymus,[30,58-60] implies a functional association of these two activities. Dissociation of enzymatically active primase from the pol alpha-primase complex has been accomplished by the use of mild denaturing agents, and it is found to be associated with polypeptides of approximately 60 and 50 kDa.[43,61] Complete dissociation of the 60- and 50-kDa subunits has not resulted in enzymatically active primase, and it is not clear at present whether both polypeptides are required for catalytic activity. However, polyclonal antibodies raised against the separated 49-kDa calf thymus polypeptide were found to markedly inhibit primase activity, while antibodies raised against the isolatd 59-kDa polypeptide

were much less inhibitory, suggesting that the active site of the enzyme resides on the 49-kDa polypeptide.[58] Furthermore, photocross-linking studies showed that only the 49-kDa polypeptide of calf thymus primase had a binding site for GTP. Similarly, the primase activity of yeast pol I is associated with two subunits of 58 and 48 kDa. The primase activity is inhibited by polyclonal antibodies raised against the 48-kDa subunit,[62] which was also found to contain an ATP binding site, indicating that the active site is located in this subunit.[63] The precise role of the 58-kDa polypeptide is not known at this time.

The yeast gene encoding the 48-kDa subunit has been cloned and sequenced and the molecular weight of 47,623 deduced from the gene sequence[62] is in good agreement with the molecular mass determined by SDS-polyacrylamide gel electrophoresis.[33] Similarly, the cDNA for the 49-kDa polypeptide of mouse primase has been cloned and sequenced.[64] The sequence predicts a molecular weight of 49,295. Comparison of the amino acid sequences of mouse p49 and yeast p48 revealed extensive homology in the amino-terminal halves of the proteins, although the carboxyl-terminal halves were divergent. The N-terminal halves of mouse and yeast p50 are even more highly conserved than the catalytic subunits of human pol alpha and yeast pol I.[64] This region also contains a metal-binding motif, suggesting a role in DNA binding.

2. Role of Pol Alpha in Replication

Pol alpha-primase was among the first replication proteins identified by the use of the *in vitro* SV40 DNA replication system.[65-68] Cell extracts immunodepleted of pol alpha-primase were unable to replicate SV40 DNA, and activity was restored by the addition of purified pol alpha-primase.[20] Interestingly, the source of the pol alpha-primase was found to be species specific, i.e., only pol alpha-primase from permissive cells (human or monkey) could function in the SV40 system,[69] and a specific interaction of T antigen with pol alpha-primase was suggested.[70,71] Recently, it was demonstrated that T antigen binds specifically to the catalytic subunit of pol alpha-primase.[72] Although both primate and nonprimate pol alpha species were capable of interacting with T antigen, the affinity of the human enzyme for T antigen was an order of magnitude greater than that of a calf-thymus enzyme.

Pol alpha, by virtue of its tightly associated DNA primase, is unique among DNA polymerases in its ability to initiate DNA synthesis in the absence of a primer.[4,27] The pol alpha-primase complex is ideally suited to act as a lagging-strand replicase, since the primase activity is capable of the frequent initiations required for the synthesis of Okazaki fragments.[73] Experimental evidence supporting a role for pol alpha-primase in lagging-strand synthesis has come from recent studies in which plasmids containing the SV40 origin of replication were replicated with purified mammalian proteins, supplemented with SV40 large T antigen.[74,75] These studies demonstrated that both DNA polymerases alpha and delta are required for the replication of the leading and lagging strands at the replication fork. When pol alpha-primase was the only DNA polymerase present, only short lagging-strand fragments were synthesized. It is likely that pol alpha-primase is also responsible for the initiation of bidirectional replication at the origin, since in the absence of pol alpha-primase no replication takes place.[74,75] In the SV40 system, the interaction between T antigen, which binds to origin sequences, and pol alpha-primase would result in the localization of pol alpha-primase at the origin. The identity of the protein(s) that fulfills this function of T antigen in cellular DNA replication is unknown.

B. DNA Polymerase Delta

DNA polymerase delta (pol delta) was first identified in 1976 as a distinct mammalian DNA polymerase that differed from other known species of mammalian DNA polymerase (alpha, beta, and gamma) in having an intrinsic 3′-5′ exonuclease activity.[76] The discovery of a mammalian DNA polymerase having an associated 3′-5′ exonuclease implied that this DNA polymerase would be able to proofread errors made during DNA synthesis and that the mechanisms whereby the fidelity of DNA replication is maintained in eukaryotes may be similar to those of prokaryotes.[77]

1. Structure and Properties of Pol Delta

DNA polymerase delta was purified to homogeneity from calf thymus. A native molecular weight of 173,000 was calculated, based on a sedimentation coefficient of 7.9 S and a Stokes radius of 53 Å. The homogeneous enzyme comprises two polypeptides of 125 and 48 kDa, as estimated from SDS-polyacrylamide gel electrophoresis.[78] The catalytic polypeptide has been identified as the 125-kDa subunit by immunoblotting with neutralizing polyclonal antibodies to pol delta.[79]

In spite of evidence that strongly suggested that the 3′-5′ exonuclease activity is an integral part of pol delta, e.g., the constant association of both polymerase and exonuclease activities throughout various purification and analytical procedures and identical rates of heat inactivation of the two activities, we, as well as others, were concerned that pol delta might be a form of pol alpha contaminated with an unrelated 3′-5′ exonuclease activity. Both pol alpha and pol delta were found to be high molecular weight, acidic proteins with very similar sensitivities to a variety of replication inhibitors, i.e., both polymerases are sensitive to inhibition by *N*-ethylmaleimide, arabinosyl nucleotides, and aphidicolin but are relatively resistant to dideoxynucleotides. However, a variety of studies with homogeneous pol alpha and pol delta have established unequivocally that they are distinct DNA polymerases with different biochemical properties.[80] For example, DNA polymerases alpha and delta have distinctive chromatographic properties and can be separated from one another by chromatography on DEAE-cellulose and hydroxylapatite. The enzymes have very different template preferences, i.e., pol alpha prefers DNase-activated DNA, whereas the most active template for pol delta is poly(dA-dT). The nucleotide analogs butylphenyl-dGTP and butylanilino-dATP also discriminate between the two polymerases, i.e., pol alpha is very sensitive to the analogs (Ki = 1 μ*M*), while pol delta is relatively resistant (Ki = 100 μ*M*). The subunit structures of the enzymes are also different; pol alpha is composed of four subunits of 165, 70, 65, and 55 kDa,[4,12] whereas pol delta is composed of two subunits of 125 and 48 kDa.[5,78,81,82] Furthermore, pol delta has an intrinsic 3′-5′ exonuclease activity but is devoid of primase activity, whereas pol alpha has a tightly associated primase activity but lacks 3′-5′ exonuclease activity. With the exceptions of pol alpha purified as a multiprotein complex from calf thymus[83] or HeLa cells,[84] the homogeneous DNA polymerase alpha from *Drosophila,* which has a cryptic 3′-5′ exonuclease activity,[85] and human DNA polymerase alpha purified by immunoaffinity chromatography,[86] homogeneous DNA polymerase alpha from all other species thus far examined has been found to lack any exonuclease activity.

A further distinguishing property of pol delta is that the core enzyme alone is not very processive and requires the presence of an auxiliary protein, the proliferating cell nuclear antigen (PCNA), to become highly processive, whereas pol alpha is moderately processive and is unaffected by PCNA.[87-89]

Immunological studies have further established that pol alpha and pol delta are distinct enzymes.[73,79] Neutralizing polyclonal antibodies to calf thymus pol delta failed to inhibit or immunoprecipitate pol alpha from either calf thymus of human KB cells or to immunoblot any polypeptides in pol alpha preparations. Conversely, neutralizing monoclonal antibodies to pol alpha from KB cells inhibit both human and calf pol alpha but not calf pol delta[73,79] nor human pol delta.[81]

The structural relationship between the catalytic subunits of pol alpha and pol delta have also been examined by both two-dimensional tryptic peptide mapping[79] and by partial peptide mapping using *N*-chlorosuccinimide.[81] These studies demonstrated that the two enzymes have unique and unrelated peptide structures.

Recently, pol delta was purified from HeLa cells and was shown to consist of subunits of 130 and 47 kDa.[81] A 178-kDa species of pol delta has been purified from mouse cells consisting of subunits of 125 and 50 kDa.[82] A distinct form of pol delta consisting of a single polypeptide of 125 kDa was also purified from mouse cells.[82] It would appear that the 125-kDa polypeptide is the dissociated catalytic subunit of pol delta; however, the identity of the polypeptide has not yet been established, either by peptide mapping or immunological studies. In contrast to pol delta,

p125 is not stimulated by PCNA, and it has been suggested that p50 is required for the interaction of pol delta with PCNA.[82]

An analog of pol delta called *DNA polymerase III* has been discovered in yeast.[90] This enzyme was successfully purified from a protease-deficient strain of *Saccharomyces cerevisiae* in the presence of protease inhibitors. Similar to pol delta, pol III is tightly associated with a 3′-5′ exonuclease activity, is devoid of primase activity, is extremely sensitive to aphidicolin, and is relatively resistant to butylphenyl-dGTP. The polypeptide composition of pol III is also identical to that of mammalian pol delta, i.e., both enzymes are heterodimers with subunits of approximately 125 and 50 kDa.

Like pol delta, pol III is relatively nonprocessive in the absence of yeast PCNA but is highly processive in its presence. Unexpectedly, calf thymus PCNA was able to stimulate the activity and processivity of pol III and, conversely, yeast PCNA was able to partially substitute for mammalian PCNA in increasing the processivity of calf thymus pol delta.[89,91] This suggests that the sites of protein-protein interaction between pol delta and its auxiliary protein have been conserved during evolution.

It has been demonstrated that pol I and pol III are two distinct enzymes and are the products of separate genes. Pol I has been identified as the product of the *S. cerevisiae* cell-cycle division gene *CDC17 (POL1)*,[24,25] while pol III has been identified as the product of *CDC2 (POL3)*.[92,93] The levels of pol III are normal in *cdc17* mutants that are deficient in pol I and, conversely, *cdc2* mutants lack pol III but have normal levels of pol I.[92,93]

It was recently proposed to utilize the Greek letter nomenclature for all eukaryotic DNA polymerases and to assign a different Greek letter to each genetically distinct DNA polymerase.[94] Consequently, pol I is now called *yeast pol alpha (ypol alpha)* and pol III is now called *ypol delta*.

2. Role of Pol Delta in Replication

Although DNA polymerase delta was first described over a decade ago,[76] it is only recently that its essential role in eukaryotic DNA replication was firmly established. A replicative function for pol delta was initially inferred from studies that showed the proliferating cell nuclear antigen (PCNA), an auxiliary protein specific for pol delta,[87,88] is essential for *in vitro* SV40 DNA replication.[95] Additional support for a replicative role for pol delta has come from studies of DNA synthesis in permeabilized mammalian cells. Inhibitors that preferentially affect pol alpha and not pol delta, i.e., monoclonal antibodies to pol alpha and butylphenyl-dGTP, at concentrations that completely inhibited pol alpha activity, resulted in only partial inhibition of DNA replication.[96,97] However, an inhibitor that is equally effective in inhibiting both pol alpha and pol delta, i.e., aphidicolin, led to complete inhibition of DNA replication.[98] More direct evidence for an essential role for pol delta in eukaryotic DNA replication has come from studies with *in vitro* SV40 DNA replication systems.[21-23] In these studies pol delta, in addition to pol alpha, was found to be required for efficient replication of DNA containing the SV40 origin of replication. Further evidence supporting the essential role of pol delta in the replication of eukaryotic chromosomes has come from genetic analysis of the budding yeast *Saccharomyces cerevisiae,* which demonstrated that the catalytic subunit of yeast pol delta is encoded by the *CDC2* gene,[92,93] now called *POL3,* an essential gene required for DNA replication.[99] Temperature-sensitive mutants of *pol3* arrest in the S phase of the cell cycle at nonpermissive temperatures.[100]

A specific role for pol delta as the leading-strand replicase was postulated based on the functional properties of the enzyme.[73,80,101,102] Evidence in support of this hypothesis has come from more recent studies with the SV40 replication system reconstituted with highly purified proteins, which demonstrated that in the absence of pol delta or any of its accessory proteins, synthesis occurred preferentially on the lagging-strand template.[74,75]

C. DNA Polymerase Epsilon

Recently, another high-molecular-weight DNA polymerase was discovered in mammalian tissues and was also designated pol delta because

of a tightly associated 3′-5′ exonuclease activity.[103,104] As the second mammalian DNA polymerase found to have an associated 3′-5′ exonuclease, it has also been called pol delta-2, or, since it differs from pol delta in being highly processive in the absence of PCNA, it has been called PCNA-independent pol delta.[105-107] Recent evidence strongly suggests that pol delta and pol delta-2 are distinct enzymes (reviewed in Syvaoja[108]), and, to avoid confusion, pol delta-2 has been renamed DNA polymerase epsilon (pol epsilon).[94]

1. Structure and Properties of Pol Epsilon

Pol epsilon, similar to pol delta, is sensitive to aphidicolin and is resistant to butylphenyl-dGTP. However, in contrast to pol delta, pol epsilon is relatively insensitive to inhibition by carbonyldiphosphonate, a triphosphate analog.[81] At a concentration of 15 μM carbonyldiphosphonate, pol alpha was inhibited 5%, pol epsilon 20%, and pol delta 93%. Comparative studies between calf thymus pol alpha and pol epsilon have shown that carbonyldiphosphonate is approximately 20 times more potent in inhibiting pol epsilon than pol alpha.[109]

A multisubunit form of pol epsilon purified from calf thymus was found to have a native molecular weight of 290,000, whereas a second form had a native molecular weight of 240,000.[103] The active-site subunits of these species of pol epsilon were identified by photoaffinity labeling and were found to be 220 and 145 kDa, respectively.[110] Another form of calf thymus pol epsilon has been purified to near homogeneity and has been found to consist of four subunits of 140, 125, 48, and 40 kDa, with the 140- and 125-kDa polypeptides identified as catalytically active by activity gel analysis.[107]

Pol epsilon isolated from HeLa cells appears to be a single polypeptide of 215 kDa.[106] Immunological and structural studies suggest that pol delta and pol epsilon are distinct but they may share some homology.[79] Polyclonal antibodies to calf-thymus pol delta were able to neutralize the activity of HeLa pol epsilon, albeit at higher antibody concentrations. However, the same antibody preparation that immunoblotted the 125-kDa catalytic subunit of pol delta did not immunoblot any polypeptides in the pol epsilon preparation.[79] Furthermore, partial peptide mapping of the catalytic subunits of pol delta and pol epsilon from HeLa cells suggested that these two peptides do not share any primary sequence.[81]

The DNA polymerase isolated from human placenta, which has an associated 3′-5′ exonuclease activity and consists of a single polypeptide of 170 kDa,[111] has now been identified as pol epsilon.[112] However, it is not clear whether the rabbit bone marrow enzyme isolated by Byrnes,[113] which is a single polypeptide of 122 kDa and is highly processive in the absence of PCNA, should be classified as pol epsilon or pol delta, in light of the recent report that the isolated 125-kDa catalytic subunit of pol delta is unresponsive to PCNA and can be highly processive under certain assay conditions.[82] Recently, both pol delta and pol epsilon have been found to be present in rabbit bone marrow[114] and human placenta,[112] as well as in HeLa cells.[81]

Mammalian pol epsilon appears to be the counterpart of yeast pol II, which also has a tightly associated 3′-5′ exonuclease activity.[115-117] Genetic analysis and immunological studies suggest that pol II, now called yeast pol epsilon, is distinct from both ypol alpha and ypol delta.[117,118] Yeast pol epsilon activity is not inhibited by either monoclonal antibodies to ypol alpha or by polyclonal antibodies to ypol delta.[118] Furthermore, the levels of ypol epsilon are normal in mutants that entirely lack ypol alpha or ypol delta.[117] Activity gel analysis of ypol epsilon suggested that the catalytic polypeptide is 170 kDa, although active proteolytic fragments of 132, 120, and 105 kDa were also observed in the preparation.[117] As with mammalian pol epsilon, ypol epsilon is sensitive to inhibition by aphidicolin but is resistant to butylphenyl-dGTP. It is devoid of detectable primase activity but has an associated 3′-5′ exonuclease activity and is highly processive in the absence of yPCNA.

Recently, two forms of pol epsilon were isolated from yeast. One form is a single polypeptide of 145 kDa and the other, a more complex form, designated pol II* or ypol epsilon*, consists of 5 polypeptides of 200, 80, 34, 30, and 29 kDa.[119] Activity gel analysis demonstrated that the 145-

kDa polypeptide of ypol epsilon and the 200-kDa polypeptide of ypol epsilon* were catalytically active. Immunological studies that demonstrated p145 is immunoblotted by polyclonal antibodies raised against ypol epsilon* and partial peptide mapping with *Staphylococcus aureus* V8 protease suggested that the 145-kDa polypeptide of ypol epsilon is derived from the 200-kDa polypeptide of ypol epsilon*. It was proposed that ypol epsilon* is a holoenzyme form of ypol epsilon, although no differences in the activity or processivity of these two forms of the enzyme were detected. Similar to mammalian pol epsilon, ypol epsilon is highly processive in the absence of PCNA; however, in contrast to mammalian pol epsilon, the processivity of ypol epsilon and ypol epsilon* can be somewhat stimulated by calf thymus PCNA.

Very recently the *POL2* gene-encoding yeast pol epsilon was cloned and analyzed.[120] The gene expressed a transcript of 7.5 kb containing a reading frame that encoded a protein of M_r 255,649, suggesting that the various isolated forms of yeast pol epsilon have undergone proteolytic degradation.

2. Role of Pol Epsilon in Replication or Repair

The *in vivo* role of pol epsilon is unclear at present. It has been suggested that the mammalian enzyme is involved in DNA repair, since pol epsilon has been shown to be required for DNA repair synthesis in permeabilized, UV-irradiated human diploid fibroblasts.[104,121] A similar role in DNA repair has been suggested for ypol epsilon; however, gene disruption studies have shown that the gene for ypol epsilon *(POL2)* encodes an essential protein.[120] Deletion of the *POL2* gene resulted in inviability and a terminal morphology typical of S-phase arrest, suggesting that pol epsilon may be required for chromosomal DNA replication, in addition to pol alpha and pol delta. However, the establishment of a replicative role for pol epsilon will require the characterization of conditional lethal mutants in the *POL2* gene. Pol epsilon has not been found to be required for SV40 DNA replication, suggesting that it may not be essential for cellular DNA replication. It is clear that further studies are necessary to completely define the *in vivo* function of this enzyme.

III. POLYMERASE ACCESSORY PROTEINS

A. Proliferating Cell Nuclear Antigen

The proliferating cell nuclear antigen (PCNA) is a protein that has been rediscovered a number of times (for reviews see Celis et al.,[122] Mathews,[123] and Tan[124]). It was initially identified in 1978 as a nuclear protein reactive with sera from a subset of patients with the autoimmune disorder systemic lupus erythematosus (SLE) and was found only in actively proliferating cells.[125,126] In 1980 an acidic nuclear protein of 36 kDa that correlated with the proliferative state of cells was identified by Bravo and Celis and was named *cyclin*.[127] This protein was subsequently demonstrated to be identical to PCNA by Mathews and his colleagues.[128] To avoid confusion with other cell-cycle-regulated proteins involved in the control of mitosis that have also been called cyclins, the term *PCNA* is used to designate this nuclear protein.

The synthesis of PCNA is induced when quiescent cells are stimulated to divide by serum or mitogens, and its synthesis immediately precedes that of DNA.[123,129-132] The increase is reflected by a corresponding increase in the PCNA mRNA levels.[133,134] Indirect immunofluorescence using human autoantibodies to PCNA detected the protein only in S-phase nuclei and demonstrated that the nuclear distribution of PCNA mimicked the topographical pattern of DNA synthesis, suggesting that PCNA may be a cell-cycle-regulated protein involved in the regulation of DNA synthesis.[122,130,135-137] However, recent studies with continuously cycling HeLa cells have shown that the level of PCNA, as detected by immunoblotting[138] or immunoprecipitation,[139] does not fluctuate significantly during the cell cycle, arguing against a cyclin-like role for this protein in the regulation of entry into S phase. Although there is an increase in the rate of synthesis of both PCNA and PCNA mRNA beginning in late G1 and peaking during the S phase of the cell cycle, the magnitude of the increase

is only two- to threefold. Thus, like several other proteins required for DNA replication, PCNA is synthesized in a cell-cycle-regulated manner, but it is not degraded at the end of the S phase.

The discrepancy between the results obtained by indirect immunofluorescence and the results obtained by quantitating PCNA in continuously cycling cells has been clarified recently with the discovery of two populations of PCNA in the nucleus.[140] Approximately 65% of the nuclear PCNA is not bound at sites of DNA replication and is lost when methanol is used as a fixative. The fraction of PCNA that is tightly bound to DNA (approximately 35%) is resistant to extraction by methanol and is detected by immunofluorescence. It is this fraction that increases at the G1-S interface and reaches a maximum in mid-S phase. Thus, during S phase PCNA localizes in the nucleus at sites of active DNA synthesis.

DNA synthesis is not required for PCNA synthesis, since inhibitors of DNA synthesis, such as hydroxyurea and aphidicolin, have no effect on PCNA synthesis following the stimulation of quiescent cells.[141] However, both DNA synthesis and cell-cycle progression were inhibited in Balb c/3T3 and Chinese hamster ovary cells when they were exposed to antisense oligonucleotides to the PCNA codon sequences.[142,143] Furthermore, when human autoantibodies to PCNA were injected into *Xenopus* eggs, both chromosomal and plasmid DNA replication were inhibited.[144] These results are consistent with the involvement of PCNA in DNA replication.

Recently, direct biochemical links between PCNA and DNA replication were established by the finding that PCNA is required for efficient replication of SV40 DNA in cell-free extracts from human 293[95] or HeLa cells[138] and that PCNA is identical to an auxiliary protein for DNA polymerase delta.[87,88,145]

A factor (auxiliary protein) was discovered that markedly stimulates the activity of pol delta on template/primers containing long stretches of single-stranded DNA, e.g., poly(dA)/oligo(dT).[87] In the absence of the auxiliary protein, pol delta core enzyme has a very low processivity and extends a primer only 10–20 nucleotides before dissociating from the primer terminus. However, in the presence of the auxiliary protein pol delta becomes highly processive, essentially replicating the entire length of the template at each enzyme binding event.[73,88] This factor has no effect on the activity or processivity of pol alpha.

Using the following criteria, the auxiliary protein for pol delta was demonstrated to be physically, immunologically, and functionally identical to PCNA: (1) both proteins comigrate on SDS-polyacrylamide gel electrophoresis and on two-dimensional gel electrophoresis;[145] (2) autoantibodies to PCNA neutralize the activity of the auxiliary protein and monoclonal antibodies to authentic PCNA detect the auxiliary protein in immunoblots;[146] and (3) the calf thymus auxiliary protein for pol delta can substitute for human PCNA in SV40 DNA replication *in vitro* and, conversely, human PCNA can effectively substitute for calf-thymus pol delta auxiliary protein in stimulating the activity and processivity of calf-thymus pol delta with primed homopolymer templates.[88]

The molecular mechanism by which PCNA increases the activity and processivity of pol delta has recently been investigated kinetically.[147] PCNA decreases the Km for the template/primer poly(dA)/oligo(dT), consistent with the suggestion that PCNA increases the processivity of pol delta by increasing the residence time of the enzyme on the template/primer.

PCNA is a highly conserved protein that has been found in species ranging from mammals to insects to yeast to higher plants.[148] Furthermore, a gene with significant homology to rat PCNA has been found in the genome of the baculovirus *Autographa californica*.[149] The cDNAs for several mammalian PCNAs have been cloned and sequenced, and have been shown to differ by only a few amino acids.[133,134] Calf thymus and yeast PCNA are both acidic proteins with very similar chromatographic properties.[89] PCNA from mammalian species is a homodimer of 36-kDa subunits by SDS-polyacrylamide gel electrophoresis, although the deduced amino-acid sequence gives a M_r of 29,000, whereas the yeast PCNA is a trimer or tetramer of 26-kDa subunits. Similar to calf-thymus PCNA, yeast PCNA stimulates the activity of ypol delta by increasing its processivity.[89] Most striking is the ability of calf-thymus PCNA to stimulate the activity and processivity of ypol delta and the ability of yeast PCNA to partially substitute for mammalian

PCNA in stimulating the processivity of calf-thymus pol delta. This suggests that the sites of protein-protein interaction between pol delta and its auxiliary protein have been conserved during evolution.[89,91] However, it has been shown that yeast PCNA is not recognized by two monoclonal antibodies that were raised against SDS-denatured rabbit PCNA.[89,150] These antibodies can immunoblot PCNAs from other mammalian species, but they do not immunoprecipitate mammalian PCNA or block PCNA-dependent pol delta activity.[146] More recently, monoclonal antibodies that were raised against recombinant rat PCNA have been shown to react with a 36-kDa band in insect cells.[151] In contrast to monoclonal antibodies raised against denatured PCNA, these antibodies were able to immunoprecipitate native mammalian PCNA.

The structure of the human PCNA gene has been determined.[152] It is a unique copy gene that consists of six exons. Analysis of the 5′-flanking region identified several functional promoters, and full expression of a linked reporter gene required 395 base pairs of 5′-flanking sequence. Interestingly, the PCNA promoter is bidirectional and directs the transcription of reporter genes in both orientations. The gene has recently been localized to human chromosome 20.[153]

The role of PCNA in SV40 DNA replication has been investigated by analyzing the products synthesized in the presence and absence of PCNA.[102,154] In the presence of PCNA, the synthesis of both leading and lagging strands occurs, whereas in the absence of PCNA, only early replication intermediates and short lagging-strand fragments are synthesized. This suggests that PCNA, like pol delta, is required for leading-strand synthesis, although it is not required for the initiation of DNA synthesis at the SV40 origin of replication.

B. Replication Factor C

Replication factor C (RFC) is the most recently identified replication factor required for *in vitro* replication of SV40 DNA.[155-158] An independently discovered factor, named *activator 1 protein* (A1), may be the same as RFC. Both are multisubunit complexes with polypeptide molecular weights reported as 140, 41, and 37 kDa (RFC)[155-158] and 145, 40, 38, 37, and 36.5 kDa (A1).[159,160] Purified RFC (A1) has DNA-dependent ATPase activity and, like PCNA, is believed to be an accessory protein for DNA polymerase delta, since its absence from a reconstituted SV40 DNA replication reaction results in the loss of leading-strand synthesis.[75,158,160]

Studies on the interaction of RFC (A1) with other components of the replication complex have supported its designation as a pol delta accessory protein.[155-160] Although pol delta/PCNA can replicate poly(dA)/oligo(dT) template/primers with high processivity in the absence of RFC,[73,88] coating of the template/primer with RPA, a single-stranded DNA binding protein, results in the requirement for RFC for utilization of the template.[158] Similarly, singly primed phage DNA, e.g., M13 (+) strand DNA, is not a template for pol delta/PCNA, except in the presence of RFC and single-stranded DNA binding protein.[23] RFC has also been reported to slightly stimulate the activity of pol alpha, either in the presence or absence of RPA.[23] Whether this is physiologically significant is not clear as yet. A1 apparently has no effect on pol alpha activity.[159,160]

RFC has been postulated to be a primer binding protein, binding specifically to template/primer. Primed single-stranded DNA, but not single-stranded DNA or double-stranded DNA, competes for binding of labeled poly(dA)/oligo(dT) to RFC.[158] Similarly, primed templates were found to be the most effective in stimulating the ATPase activity of RFC, whereas single-stranded and double-stranded DNA were less active.

Studies on the interactions of RFC (A1), pol delta, and PCNA with template/primers coated with RPA demonstrated that pol delta and RFC form an isolatable complex.[159,160] In these studies it was observed that RFC is the first protein to bind to the RPA-coated template/primer, followed by PCNA, and finally by pol delta. It was also found that ATP is required for the binding of PCNA to the RFC-template/primer complex, although ATP-gamma-S can substitute for ATP in this reaction. Binding of pol delta to the complex absolutely requires ATP, and nonhydrolyzable ATP analogs cannot substitute.

The functional properties of the accessory

proteins PCNA and RFC (A1) are very similar to those of the auxiliary subunits (beta, gamma, delta) of *E. coli* DNA polymerase III[161,162] and of the accessory proteins (products of genes 44/62 and gene 45) of T4 DNA polymerase.[163-167] These accessory proteins are required by their respective core polymerases to constitute a fully functional replicative DNA polymerase holoenzyme for the replication of the leading strand at the replication fork.[1-3] The prokaryotic accessory proteins endow the cognate core polymerase with the ability to replicate singly primed single-stranded DNA with high processivity and to replicate through kinetic barriers generated by the single-stranded DNA template.[168] It is believed that the accessory proteins increase the affinity of DNA polymerases for the template-primer junction by forming a "sliding clamp",[169] thus preventing the DNA polymerase from dissociating from the 3′ end of the growing DNA chain upon encountering a kinetic block. This hypothesis is supported by the observation that DNA polymerases form stable complexes with template/primer in the presence of the accessory proteins and ATP.[162,170,171]

The function of RFC in eukaryotic DNA replication has been likened to that of the protein products of T4 genes 44/62, in T4 DNA replication, and to that of the gamma and delta subunits of *E. coli* pol III. Similarly, PCNA is thought to be analogous to T4 gene 45 protein and the beta subunit of pol III.[158-160] Similar to the stimulation of the DNA-dependent ATPase activity of the gene 44/62 proteins by the gene 45 protein, the DNA-dependent ATPase of RFC is markedly stimulated by PCNA.[158-160] Furthermore, some amino-acid sequence similarity has been reported between human PCNA and T4 gene 45 protein.[158]

C. Other Polymerase Accessory Proteins

There are several reports in the literature of accessory protein for pol alpha, so named because they affect the activity or template specificity of pol alpha in *in vitro* assays of the purified enzyme. It is not clear as yet whether any of these proteins function as pol alpha accessory proteins *in vivo*.

1. Primer Recognition Proteins

Two proteins, originally called *C1* and *C2*,[172,173] and more recently *PRP1* and *PRP2*,[174] which function as a complex to affect the ability of pol alpha to utilize template/primers with high template to primer ratios, have been isolated from both human and monkey cells. The complex did not alter the processivity of pol alpha, nor did it affect the Km values for dNTP substrates. It did lower the Km value for the primer terminus when DNA templates with high template to primer ratios were used. Thus, it was proposed that the complex increased the ability of pol alpha to find the primer terminus by reducing nonproductive binding of the enzyme to single-stranded DNA.

PRP1 has a molecular weight of 36 kDa and PRP2 has a weight of 41 kDa, although the ratio of the two polypeptides in the active complex is unclear. It has been reported recently that PRP1 is physically and functionally identical to phosphoglycerate kinase.[175]

2. Alpha Accessory Factor

A protein that stimulates pol alpha/primase activity with unprimed DNA templates has been purified from cultured mouse cells.[176] The protein, alpha accessory factor (AAF), is a heterodimer with subunits of 132 and 44 kDa. AAF has been characterized as a template affinity protein, since it appears to both increase the processivity of pol alpha/primase on unprimed templates and to cause pol alpha/primase to remain associated with a single-stranded DNA template while priming and extending multiple fragments. This results in the serial replication of a population of DNA molecules.[177]

IV. OTHER REPLICATION PROTEINS

A. Replication Protein A

Replication protein A (RPA),[178] also known as replication factor A (RFA)[179] or human single-stranded DNA binding protein (hSSB),[180] was identified as a cell-derived replication protein in the *in vitro* SV40 DNA replication system. Ho-

mogeneous RPA is a multisubunit protein consisting of three polypeptides of approximately 70, 32, and 14 kDa.[178-181] This complex is tightly associated, as evidenced by the cosedimentation of all three subunits as a single species in glycerol gradients containing 0.5 *M* KCl and 1.7 *M* urea[178] or after treatment with 6 *M* urea.[179] RPA has high affinity for single-stranded vs. double-stranded DNA[154,180] but has no detectable enzymatic activity, e.g., exonuclease, endonuclease, ATPase, topoisomerase, or helicase.[154]

RPA is absolutely required in the reconstituted SV40 replication system.[23,154,181] It is needed for the T-antigen catalyzed unwinding of duplex DNA at the origin of replication and, thus, is involved at an early stage of initiation.[182-184] T antigen, in the presence of ATP, RPA, and topoisomerase I or II, catalyzes extensive unwinding of SV40-origin-containing DNA, forming a highly underwound plasmid DNA (form U),[178,182,185-187] which is similar to form I unwound DNA in *E. coli*.[188] In the unwinding reaction, it is believed that RPA acts to facilitate the generation of a single-stranded region and to stabilize the single-stranded DNA as it is being generated by T-antigen helicase activity.[154] The requirement for RPA for origin unwinding is relatively nonspecific, since other single-stranded DNA binding proteins, e.g., those from *E. coli*, adenovirus, and herpes simplex virus, can substitute for RPA in this reaction.[182,184] However, heterologous single-stranded DNA binding proteins are unable to substitute for RPA in the complete SV40 DNA replication reaction.[182,184] This suggests that, in addition to the initial DNA unwinding, RPA is involved in DNA chain elongation, most likely through protein-protein interactions with other replication proteins. Although the requirement for RPA to unwind SV40 origin-containing DNA seems not to be very specific, it appears that some specificity is involved in this reaction, since T4-gene 32 protein and calf-thymus UP1 cannot substitute for RPA.[187] The UP1 protein was long thought to be a eukaryotic single-stranded DNA binding protein, based on its high affinity for single-stranded DNA and its ability to stimulate pol alpha;[189] however, later studies have shown it to be a proteolytic product of a heterogeneous ribonucleoprotein.[190] The inability of UP1 to substitute for human RPA in the initial DNA unwinding further argues against this protein as an eukaryotic SSB.

Single-stranded DNA binding protein is absolutely required for chromosomal DNA replication in prokaryotes,[1,191,192] and these proteins have been shown to stimulate DNA replication through protein-protein interactions. The T4 gene 32 protein has been shown to stimulate DNA synthesis, in part through a direct interaction with the DNA polymerase holoenzyme.[169,193] Analogously, human RPA is absolutely required for eukaryotic DNA replication and has been shown to stimulate both of the DNA polymerases believed to be involved in DNA replication at the fork, pol alpha and pol delta.[23,154,187]

Recent studies have shown that RPA stimulates DNA synthesis catalyzed by pol alpha on both poly(dA)/oligo(dT) and primed single-stranded M13 DNA templates, and appears to act by increasing the processivity of pol alpha. The products of the reaction are in the range of Okazaki fragments (100–200 nucleotides).[23] Similar results were obtained by Kenny et al.,[187] who demonstrated that with $(dA)_{4000}/(dT)_{12-18}$, RPA stimulated pol alpha activity five- to tenfold. Several single-stranded DNA binding proteins, e.g., those of *E. coli*, adenovirus, and herpes simplex virus, which were able to substitute for RPA in the origin unwinding reaction, were unable to stimulate pol alpha activity.[23,187] However, *E. coli* SSB was found to stimulate the processivity of the 182-kDa catalytic subunit of *Drosophila* pol alpha but not the intact four-subunit enzyme.[194] Calf thymus UP1, which is not active in the unwinding of SV40 origin sequences, does stimulate pol alpha activity.[187,190]

RPA also stimulates pol delta activity;[23,160,187] however, the mechanism of stimulation differs from that of pol alpha. RPA has no effect on the processivity of pol delta on poly(dA)/oligo(dT). The stimulation of pol delta activity by RPA appears to be relatively nonspecific, since it can be substituted by other single-stranded DNA binding proteins, e.g., those of *E. coli*, adenovirus, and phage T4 (gene 32 protein). However, calf thymus UP1 had no effect on pol delta activity.[187] The stimulation of pol delta activity by RPA was observed only in the presence of PCNA and RFC.[23,160,180]

Single-stranded DNA binding activity has

been localized to the 70-kDa subunit of RPA.[154,195] The functions of the two smaller subunits (32 kDa and 14 kDa) are unknown, although monoclonal antibody to the 32-kDa subunit has been found to inhibit SV40 DNA replication *in vitro*.[196] Recently four monoclonal antibodies raised against RPA were shown to inhibit SV40 DNA replication using crude extracts from HeLa cells.[195] Three of the antibodies were specific for the 70-kDa subunit, and one was specific for the 32-kDa subunit. None of these antibodies inhibited the binding of RPA to single-stranded DNA. One of the antibodies to the 70-kDa subunit was found to inhibit both the stimulation of pol delta by RPA and the origin unwinding reaction, but it had no effect on the stimulation of pol alpha by RPA. In contrast, the other two monoclonal antibodies to the 70-kDa subunit and the monoclonal antibody to the 32-kDa subunit inhibited the stimulation of pol alpha by RPA but had no effect on DNA unwinding or pol delta stimulation. It was suggested that there exist at least three domains in the RPA protein. In addition to a domain required for DNA binding, a separate domain is required for DNA unwinding and pol delta stimulation, and a third domain is involved in the stimulation of pol alpha.

A yeast analog of RPA has been identified in *S. cerevisiae* based on its ability to substitute for human RPA in the origin unwinding reaction.[197] As with other heterologous single-stranded DNA-binding proteins, yeast RPA could not substitute for human RPA in the complete SV40 DNA replication reaction. Similar to human RPA, yeast RPA is composed of three subunits of 69, 36, and 13 kDa, and the largest subunit contains the single-stranded DNA binding activity.

Recently, a 34-kDa single-stranded DNA binding protein that functions as an accessory protein for strand exchange protein (SEP1), a protein required for recombination, was purified from *S. cerevisiae*.[198] Cloning and sequencing of the gene encoding the 34-kDa protein revealed an open reading frame encoding a 70-kDa protein, suggesting that the 34-kDa protein is a proteolytic product of the primary translation product. Comparison of the sequence of the 70-kDa yeast protein with the 70-kDa subunit of human RPA showed extensive similarity. Furthermore, the 70-kDa subunit of yeast RPA, isolated on the basis of its ability to substitute for human RPA in the origin unwinding assay, was 100% identical to the product of this yeast gene, which has been called *RPA1*. Gene disruption experiments have shown that the *S. cerevisiae* gene (RPA1) codes for an essential function, and the terminal phenotype of spores containing a disrupted copy of RPA1 is consistent with a defect in an early stage of DNA replication.[199] Thus, as is the case with the prokaryotic single-stranded DNA binding proteins, e.g., *E. coli* SSB and T4 gene 32 protein, which are required for both replication and recombination, the eukaryotic single-stranded DNA-binding protein RPA may also have more than one function.

The 32-kDa subunit of RPA from both yeast and human cells undergoes phosphorylation/dephosphorylation in a cell-cycle-dependent manner.[197,200] This subunit, whose function is unclear, is phosphorylated on serine residues at the G1 to S-phase transition and is dephosphorylated during mitosis, suggesting that the replication activity of this protein may be regulated by phosphorylation. However, the effects of phosphorylation of the 32-kDa subunit on RPA activity are not clear, as both phosphorylated and dephosphorylated forms of RPA were fully active in a reconstituted SV40 DNA replication reaction. The cDNA for the 32-kDa subunit of RPA has been cloned and sequenced, and the protein has been overexpressed in *E. coli*.[196] The delineation of the functions of the 32-kDa subunit, as well as the 70- and 14-kDa subunits, of RPA will be facilitated by the ability to overexpress these proteins and to carry out site-directed mutagenesis.

B. DNA Helicases

DNA helicases enzymatically unwind duplex DNA in a reaction that is coupled with the hydrolysis of nucleoside 5′-triphosphates.[201] In the prokaryotic and eukaryotic DNA replication systems studied thus far, one or more DNA helicases have been found to be essential components of the replication machinery. These enzymes translocate unidirectionally along one DNA strand, thus displacing the other strand, to allow the separated strands to serve as templates for the lead-

ing-strand and lagging-strand replicases at the fork.

In the replication of bacterial and phage genomes, the DNA helicases that are responsible for moving the replication fork, e.g., *E. coli* dnaB helicase,[202] T4 gene 41 helicase,[203] and T7 gene 4 helicase,[204] have several common properties: they all translocate in the 5′ to 3′ direction along the lagging-strand template, they all prefer a replication-fork-like DNA substrate, and they all are tightly associated with a DNA primase. These properties are also shared by the herpes virus replicative helicase.[205]

On the other hand, the DNA helicase that unwinds the parental DNA duplex in the replication of SV40 DNA appears to be T antigen, a virally encoded protein. T antigen is clearly required for the initiation of DNA replication in plasmids containing the SV40 origin of replication.[6,7,9,52] T antigen binds to origin sequences and, with the help of RPA, unwinds the origin to allow the entry of pol alpha-primase to initiate both leading- and lagging-strand synthesis. Studies with neutralizing monoclonal antibodies to T-antigen helicase suggest that T-antigen helicase must continue to function during the elongation phase of replication.[206-208] In contrast to the prokaryotic DNA helicases, T-antigen helicase translocates in the 3′ to 5′ direction, presumably along the leading-strand template.[209,210]

The cellular counterpart(s) of T antigen, i.e., origin binding proteins and replicative DNA helicases, are still unknown. Over the past few years several eukaryotic DNA helicases have been purified and characterized, at least in part.[201,211] It is not yet clear whether any of these helicases is involved in replication. Further purification and characterization of these enzymes, as well as the preparation of immunological reagents, will be required to elucidate their cellular roles.

1. Yeast DNA Helicases

a. RAD3 Protein

The *RAD3* gene of *S. cerevisiae* encodes an 89-kDa polypeptide with both DNA-dependent ATPase and DNA helicase activities.[212,213] The helicase requires either ATP or dATP, translocates in the 5′ to 3′ direction, and is capable of unwinding long stretches of duplex DNA (up to 850 bp). A single-stranded tail on the fragment to be displaced is not required for duplex unwinding, but it has not been determined whether a fork-like substrate structure is preferred. The Km for ATP is 67 μ*M*. *RAD3* mutants are defective in the excision of pyrimidine dimers and other bulky adducts, suggesting that the *RAD3* protein is involved in excision repair of damaged DNA. Site-directed mutagenesis was employed to produce a mutant protein that lacked ATPase and helicase activity. Although the *RAD3* gene is essential for cell viability, the *rad3* mutant that was devoid of helicase activity was viable, suggesting that the protein has another, as yet unidentified, activity that is essential for viability.[213]

b. ATPase III

ATPase III is a 63-kDa polypeptide from *S. cerevisiae* that has both DNA-dependent ATPase and DNA helicase activities.[214] The direction of translocation of ATPase III has not been determined, nor has the activity of the enzyme with a fork-like substrate. ATP is the preferred substrate for hydrolysis (Km 700 μ*M*), and other ribonucleoside and deoxyribonucleoside triphosphates are poor substrates, with Km values in excess of 10 m*M*. ATPase III stimulates the activity of yeast pol alpha on a variety of template/primers. The stimulation appears to be specific, since the activities of yeast pol epsilon, calf-thymus pol alpha, and *E. coli* pol III are not affected by the helicase. Interestingly, a *rad3* mutant was found to have markedly reduced levels of ATPase III activity. Since these two helicases are encoded by different genes, it was suggested that the activity of ATPase III might be regulated by the *RAD3* protein.[214]

2. Xenopus DNA Helicase

A DNA helicase has been partially purified from *Xenopus laevis* ovaries.[215] A native molecular weight of 140,000–170,000 has been calculated from the Stokes radius and sedimentation coefficient. Analysis by SDS-PAGE suggests that

75- and 62-kDa polypeptides copurify with the helicase activity. ATP and dATP were the only nucleoside triphosphates that supported DNA unwinding. The Km for ATP in the hydrolysis reaction was found to be 1 m*M*. The direction of unwinding has not been determined; however, the *Xenopus* helicase efficiently unwinds both tailed and fully hybridized DNA substrates, suggesting that there is no preference for a fork-like substrate.

3. Mouse DNA Helicase

ATPase B, isolated from mouse FM3A cells, has been found to be a DNA helicase.[216-218] The protein has only been partially purified; however, a polypeptide of 58 kDa has been identified as having an ATP binding site by photoaffinity labeling. The enzyme has a native molecular weight of approximately 100,000, based on glycerol gradient sedimentation. ATPase B unwinds duplex DNA in a 5′ to 3′ direction and is capable of unwinding duplexes up to 140 base pairs in length. The unwinding reaction requires a hydrolyzable NTP as a cofactor. ATPase B is unusual in that all NTPs and dNTPs can serve as substrates for hydrolysis, although ATP is slightly preferred. The Km for ATP in the hydrolysis reaction was found to be 750 μ*M*. During the early stages of purification, ATPase B was found to be associated with pol alpha, although it did not stimulate pol alpha activity on a variety of template/primers. ATPase B is equally efficient at unwinding tailed or untailed partial duplexes.

4. HeLa Cell DNA Helicases

a. DNA Helicase I

DNA helicase I, isolated from HeLa cell nuclei, is a 65-kDa polypeptide that unwinds DNA in the 3′ to 5′ direction.[219] It also unwinds RNA/DNA hybrids. The enzyme does not require a replication-fork-like substrate. The nucleoside triphosphate requirement can only be satisfied by ATP or dATP.

b. RIP100

RIP100 is a 100-kDa helicase that has been purified from HeLa cell nuclei.[220] It copurifies with a 60-kDa protein (RIP60) that binds to a putative replication origin. ATP or dATP is required for activity, and the enzyme translocates in the 3′ to 5′ direction.

5. Calf Thymus DNA Helicases

a. Pol Alpha Associated Helicase

A DNA helicase that partially copurifies with pol alpha has been purified to homogeneity from calf thymus.[221] The 47-kDa helicase translocates in the 3′ to 5′ direction and can utilize ATP, dATP, CTP, or dCTP as a cofactor. The Km for ATP is approximately 200 μ*M*. A fork-like substrate is not required for unwinding, although it has not been determined whether it may be preferred over other substrates.

b. Pol Delta Associated Helicase

A DNA helicase that partially copurifies with pol delta has been purified from fetal calf thymus.[222,223] The helicase (delta helicase) has a molecular weight by gel filtration of approximately 56,000, and a 56-kDa polypeptide containing an ATP binding site has been identified by photoaffinity labeling. The direction of translocation is 5′ to 3′, and a fork-like substrate is required for activity. The nucleoside triphosphate requirement is satisfied by ATP or dATP, and other nucleotides do not support unwinding to any significant extent. The Km for ATP for hydrolysis is approximately 40 μ*M*.

Delta helicase was identified as a component of a protein fraction that allowed pol delta to carry out strand-displacement synthesis, suggesting that the helicase is required to displace the nontemplate strand in this reaction. However, in reconstitution experiments with purified pol delta, PCNA, and delta helicase, strand displacement synthesis was not observed, suggesting that one or more components of the protein fraction that are required for strand-displacement synthesis had been lost on purification of the helicase. Since RFC functions as an accessory protein for pol delta,[155-160] it is likely that RFC is required for strand-displacement synthesis by pol delta/PCNA.

V. FIDELITY OF DNA REPLICATION

In order to maintain viability and species identity, DNA must be replicated with great fidelity, whereas evolution is only possible if genetic variability is generated. These opposing demands have resulted in DNA replication processes with low but finite error frequencies. Measurements of spontaneous mutation rates in prokaryotes suggest that the average frequency of substitution mutation is of the order of 10^{-7} to 10^{-10} misincorporations per base pair per generation.[224] This level of accuracy is the result of a multistep process: (1) base selection in the initial incorporation of a complementary nucleotide at the 3′ end of a growing DNA chain, (2) exonucleolytic excision of a newly added mismatched nucleotide at the primer terminus, i.e., proofreading, and (3) postreplication repair.[225-227] The first two steps in ensuring the fidelity of replication, i.e., base selection and proofreading, are integral properties of prokaryotic DNA polymerases, and the relative contributions of these processes to the fidelity of prokaryotic DNA polymerases and multicomponent replication complexes have been extensively studied (reviewed in Kunkel,[226] Kunkel and Bebenek,[228] and Loeb and Perrino[229]).

In eukaryotes the fidelity of DNA replication is at least as great as that of prokaryotes; spontaneous mutation rates of 10^{-10} to 10^{-12} have been estimated.[230] However, the mechanism by which fidelity is achieved in eukaryotes is less clear. Pol alpha, which is generally found to be devoid of 3′-5′ exonuclease activity, was the only known eukaryotic DNA replicase for many years. Consequently, proofreading by the DNA polymerase was thought not to be a significant factor in maintaining replication fidelity in eukaryotes. The findings that nuclear DNA polymerases that are capable of proofreading are present in both higher and lower eukaryotes, i.e., DNA polymerases delta and epsilon,[76,92,93,103,106,115,117,231,232] and that proofreading by a 3′-5′ exonuclease activity can reduce the frequency of some base-substitution errors as much as 100-fold,[85,232] suggested that proofreading during DNA synthesis may be a significant factor in maintaining the fidelity of eukaryotic DNA replication, as is the case in prokaryotes. Recent *in vivo* studies on the effects of imbalances in intracellular pools of dNTPs on the spectrum of mutations at the *aprt* locus in Chinese hamster ovary cells suggests a "next nucleotide" effect, characteristic of exonucleolytic proofreading,[233] further supporting the importance of proofreading in eukaryotic DNA replication.

In vitro measurements of error frequency are dependent upon the method used, the type of mispair being measured, and the sequence context. However, by most measures of error frequency, pol alpha, which is generally found to be devoid of 3′-5′ exonuclease activity, is relatively unfaithful as compared to DNA polymerases delta and epsilon, both of which have an associated 3′-5′ exonuclease activity. Since pol alpha-primase is thought to be the lagging-strand replicase, the low fidelity of this enzyme is problematic in that it is unlikely that lagging-strand synthesis is less faithful than leading-strand synthesis.

Several models have been proposed to account for the *in vivo* fidelity of pol alpha.[234] Exonuclease activity has been measured in multiprotein pol alpha preparations from calf-thymus and HeLa cells,[83,84] suggesting that a loosely associated 3′-5′ exonuclease, which is removed during purification of the enzyme, might proofread errors of incorporation by pol alpha. Furthermore, it was recently reported that purified calf-thymus pol alpha, which lacked 3′-5′ exonuclease activity, was unable to extend a mismatched primer terminus efficiently.[235] Purification of an exonuclease activity from calf-thymus extracts that was capable of removing terminal mispairs, thereby allowing primer extension by pol alpha, was identified as pol delta, and it was suggested that the 3′-5′ exonuclease of pol delta may be responsible for proofreading errors of incorporation by both pol alpha and pol delta at the replication fork.[236]

Consistent with the observation that putative 3′-5′ exonuclease domains, first identified in *E. coli* pol I[237] and present in a wide variety of prokaryotic and eukaryotic DNA polymerases, are also present in the catalytic subunit of both human and yeast pol alpha,[238] several immunoaffinity purified preparations of pol alpha have been found to have 3′-5′ exonuclease activity. Pol alpha from *Drosophila melanogaster* has been found to have a "cryptic" proofreading exonu-

clease activity, i.e., no exonuclease activity was detectable in the four-subunit holoenzyme; however, dissociation of the 73-kDa subunit resulted in the appearance of exonuclease activity and an accompanying 100-fold increase in fidelity.[85] Human lymphocyte pol alpha-primase, purified by immunoaffinity chromatography, has also been found to have a proofreading 3′-5′ exonuclease activity, but, unlike the *Drosophila* enzyme, the presence of the 68-kDa subunit did not mask the expression of the exonuclease activity of the human enzyme.[86] The fidelity of the immunoaffinity-purified human pol alpha was found to be one to two orders of magnitude higher than that of the conventionally purified enzyme and to show a "next nucleotide" effect, suggesting that the increased fidelity of the immunoaffinity-purified enzyme was the result of exonucleolytic proofreading. Similarly, the 180-kDa catalytic subunit of yeast pol alpha has recently been found to have a weak 3′-5′ exonuclease activity.[239] However, the fidelity of the isolated catalytic subunit was low and was not significantly different from that of the intact four-subunit yeast pol alpha,[231] suggesting that the 3′-5′ exonuclease does not serve an editing function. The association of 3′-5′ exonuclease activity with the human lymphocyte pol alpha has been attributed to the rapid isolation of the enzyme with an intact, undegraded catalytic subunit.[86] However, immunoaffinity-purified calf thymus pol alpha was found to be totally devoid of 3′-5′ exonuclease activity, and no exonuclease activity was detected in the dissociated catalytic subunit.[235,236] Thus, it is still not clear whether pol alpha has any endogenous 3′-5′ exonuclease activity that can serve a proofreading function.

Fidelity studies with the *in vitro* SV40 replication system, using crude cell extracts rather than purified components, has shown that, although DNA replication with the cell extract is more faithful than that with isolated pol alpha-primase, the error frequency was found to be significantly higher than the *in vivo* error frequency for SV40 replication.[240,241] Whether this is due to the fact that postreplication repair processes contribute significantly to the overall error rate *in vivo* or to the lack of replication factors in the cell extract that are necessary for fidelity is not clear at present.

VI. SUMMARY AND PERSPECTIVES

It is becoming increasingly apparent that the fundamental mechanisms of DNA replication have been highly conserved during evolution. Recent studies suggest that, similar to DNA replication in prokaryotes, proofreading by a DNA polymerase-associated 3′-5′ exonuclease is a major mechanism by which eukaryotes maintain replication fidelity. An ever-increasing number of eukaryotic DNA polymerases are found to have associated 3′-5′ exonuclease activity, e.g., DNA polymerase epsilon, DNA polymerase gamma,[242-244] and some preparations of DNA polymerase alpha, in addition to DNA polymerase delta. Analogous to prokaryotes, the replication fork in eukaryotes is asymmetric, i.e., leading-strand synthesis is continuous, whereas synthesis of the lagging strand is discontinuous, and accessory proteins modify the properties of the DNA polymerases that carry out leading- and lagging-strand DNA synthesis. However, significant differences are also evident. In contrast to prokaryotic DNA replication, where a single core DNA polymerase catalyzes both leading- and lagging-strand DNA synthesis, e.g., Pol III of *E. coli*, two distinct DNA polymerases may catalyze leading- and lagging-strand synthesis in eukaryotes, i.e., pol delta for replication of the leading strand and pol alpha for the lagging strand. Furthermore, in prokaryotes DNA primase is tightly associated with the replicative DNA helicase, whereas in eukaryotes DNA primase is tightly associated with the lagging-strand replicase, pol alpha.

Within eukaryotes, replication proteins appear to be highly conserved. There is a striking similarity in both the structural and functional properties of mammalian and yeast DNA polymerases. For example, mammalian and yeast pol alpha have identical subunit structures. Both are heterotetramers with subunits of nearly identical size. Both enzymes have DNA primase activity and both are moderately processive. Similarly, mammalian and yeast pol delta are both heterodimers with catalytic subunits of 125 kDa. Both enzymes have endogenous 3′-5′ exonuclease activity, and both have very low intrinsic processivities but become highly processive in the presence of PCNA. Remarkably, calf PCNA can

increase the processivity of yeast pol delta and vice versa. The deduced primary structures of the catalytic subunits of human and yeast pol alpha are 31% identical, whereas those of calf and yeast pol delta are 44% identical.[245] In each case, regions of even higher similarity have been identified that may be important in substrate binding and catalysis or may be necessary for protein-protein or protein-DNA interactions.

The recent success in the reconstitution of SV40 DNA replication with purified proteins has led to the identification and characterization of at least seven mammalian replication proteins and offers approaches and opportunities for further elucidation of molecular mechanisms involved in the replication of chromosomal DNA. It appears that additional protein(s) may be required for a fully reconstituted SV40 DNA replication apparatus, since the rate of replication-fork movement in the reconstituted system is only one tenth of that found *in vivo,* and the Okazaki fragments are unusually long.[246] In addition, the cellular proteins whose functions are fulfilled by large T antigen in the SV40 DNA replication system, i.e., origin binding and DNA helicase activities, are yet to be identified. It is also not known whether a cellular helicase is required for efficient replication of the SV40 chromosome, in addition to T antigen. The delta helicase, which has several properties characteristic of a replicative helicase, may be a candidate for a replicative eukaryotic DNA helicase.

A schematic representation of a eukaryotic replication fork, based on our current understanding and adopted from a model previously proposed for prokaryotic DNA replication,[247] is shown in Figure 1. In this model the replicative DNA helicase unwinds parental DNA strands, thus providing templates for both leading- and lagging-strand synthesis. Pol delta, PCNA, and RFC organize as a leading-strand replicase, whereas pol alpha-primase replicates the lagging-strand template. RPA binds to and stabilizes the single-stranded DNA exposed by the helicase to protect it from nuclease attack and, possibly, to facilitate the organization of a more efficient replication complex. Although this model summarizes our knowledge to date, many events at the fork are still unknown: How are leading- and lagging-strand synthesis coordinated? Are pol alpha and pol delta components of a replisome? What is the mechanism of recycling pol alpha from a completed Okazaki fragment to a newly exposed lagging-strand template at the advancing fork? These are only a few of the questions that are currently being addressed, with the ultimate goal of understanding how eukaryotic chromosomal DNA replication is regulated and cellular proliferation is controlled.

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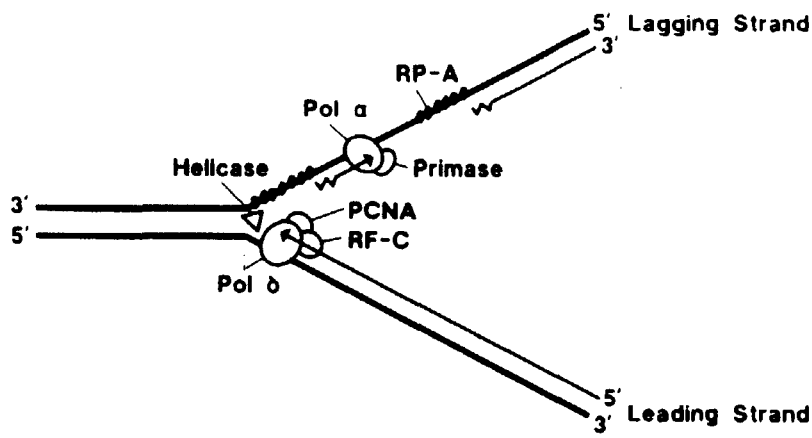

FIGURE 1. Model of a eukaryotic DNA replication fork. Details are given in the text.

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REFERENCES

1. **Alberts, B. M.,** Prokaryotic DNA replication mechanisms, *Phil. Trans. R. Soc. Lond. B,* 317, 395, 1987.
2. **Kornberg, A.,** DNA replication, *J. Biol. Chem.,* 263, 1, 1988.
3. **McHenry, C. S.,** DNA polymerase III holoenzyme of *Escherichia coli, Annu. Rev. Biochem.,* 57, 519, 1988.
4. **Lehman, I. R. and Kaguni, L. S.,** DNA polymerase alpha, *J. Biol. Chem.,* 264, 4265, 1988.
5. **Burgers, P. M. J.,** Eukaryotic DNA polymerases alpha and delta: conserved properties and interactions from yeast to mammalian cells, *Prog. Nucleic Acid Res.,* 37, 325, 1989.
6. **Challberg, M. D. and Kelly, T. J.,** Animal virus DNA replication, *Annu. Rev. Biochem.,* 58, 671, 1989.
7. **Hay, R. T. and Russell, W. C.,** Recognition mechanisms in the synthesis of animal virus DNA, *Biochem. J.,* 258, 3, 1989.
8. **Sitney, K. C. and Campbell, J. L.,** The yeast DNA replication apparatus: genetic and biochemical analysis, in *The Eukaryotic Nucleus,* Vol. 1, Strauss, P. R. and Wilson, S. H., Eds., Telford Press, Caldwell, NJ, 1990, 125.
9. **Stillman, B.,** Initiation of eukaryotic DNA replication *in vitro, Annu. Rev. Cell Biol.,* 5, 197, 1989.
10. **Bambara, R. A., Meyers, T. W., and Sabatino, R. D.,** DNA polymerase delta, in *The Eukaryotic Nucleus,* Vol. 1, Strauss, P. R. and Wilson, S. H., Eds., Telford Press, Caldwell, NJ, 1990, 69.
11. **Thommes, P. and Hubscher, U.,** Eukaryotic DNA replication, *Eur. J. Biochem.,* 194, 699, 1990.
12. **Wang, T. S.-F.,** Human DNA polymerase alpha: a retrospective and prospective, in *The Eukaryotic Nucleus,* Vol. 1, Strauss, P. R. and Wilson, S. H., Eds., Telford Press, Caldwell, NJ, 1990, 147.
13. **Bollum, F. J.,** Calf thymus polymerase, *J. Biol. Chem.,* 235, 2399, 1960.
14. **Miller, M. R., Ulrich, R. G., Wang, T. S.-F., and Korn, D.,** Monoclonal antibodies against human DNA polymerase alpha inhibit DNA replication in permeabilized human cells, *J. Biol. Chem.,* 260, 134, 1985.
15. **Kaczmarek, L., Miller, M. R., Hammond, R. A., and Mercer, W. E.,** A microinjected monoclonal antibody against human DNA polymerase alpha inhibits DNA replication in human, hamster and mouse cell lines, *J. Biol. Chem.,* 261, 10802, 1986.
16. **Murakami, Y., Yasuda, H., Miyazawa, H., Hanaoka, G., and Yamada, M.-A.,** Characterization of a temperature-sensitive mutant of mouse FM3A cells defective in DNA replication, *Proc. Natl. Acad. Sci. U.S.A.,* 82, 1716, 1985.
17. **Eki, T., Murakami, Y., Enomoto, T., Hanaoka, F., and Yamada, M.,** Characterization of DNA replication at a restrictive temperature in a mouse DNA temperature sensitive mutant ts FT20 strain, containing heat-labile DNA polymerase alpha activity, *J. Biol. Chem.,* 261, 8888, 1986.
18. **Wong, S. W., Wahl, A. F., Yuan, P.-M., Arai, N., Pearson, B. E., Arai, K., Korn, D., Hunkapillar, M. W., and Wang, T. S.-F.,** Human DNA polymerase alpha gene expression is cell proliferation dependent and its primary structure is similar to both prokaryotic and eukaryotic replicative DNA polymerases, *EMBO J.,* 7, 37, 1988.
19. **Wahl, A. F., Geis, A. M., Spain, B. H., Wong, S. W., Korn, D., and Wong, T. S.-F.,** Gene expression of human DNA polymerase alpha during cell proliferation and cell cycle, *Mol. Cell. Biol.,* 8, 5016, 1988.
20. **Murakami, Y., Wobbe, C. R., Weissbach, L., Dean, F. B., and Hurwitz, J.,** Role of DNA polymerase alpha and DNA primase in simian virus 40 DNA replication *in vitro, Proc. Natl. Acad. Sci. U.S.A.,* 83, 2869, 1986.
21. **Weinberg, D. H. and Kelly, T. J.,** Requirement for two DNA polymerases in the replication of simian virus 40 DNA *in vitro, Proc. Natl. Acad. Sci. U.S.A.,* 86, 9742, 1989.
22. **Lee, S.-H., Eki, T., and Hurwitz, J.,** Synthesis of DNA containing the simian virus 40 origin of replication by the combined action of DNA polymerases alpha and delta, *Proc. Natl. Acad. Sci. U.S.A.,* 86, 7361, 1989.
23. **Tsurimoto, T. and Stillman, B.,** Multiple replication factors augment DNA synthesis by the two eukaryotic DNA polymerases alpha and delta, *EMBO J.,* 8, 3883, 1989.
24. **Johnson, L. M., Snyder, M., Chang, L. M. S., Davis, R. W., and Campbell, J. L.,** Isolation of the gene encoding yeast DNA polymerase I, *Cell,* 43, 369, 1985.
25. **Lucchini, G., Maxxa, C., Scocheri, E., and Plevani, P.,** Genetic mapping of the *Saccharomyces cerevisiae* DNA polymerase I gene and characterization of a *pol1* temperature-sensitive mutant altered in DNA primase-polymerase complex stability, *Mol. Gen. Genet.,* 212, 459, 1988.
26. **Budd, M. and Campbell, J. L.,** Temperature sensitive mutations in the yeast DNA polymerase gene, *Proc. Natl. Acad. Sci. U.S.A.,* 84, 2838, 1987.
27. **Fry, M. and Loeb, L. A.,** *Animal Cell DNA Polymerases,* CRC Press, Boca Raton, FL, 1986.
28. **Kaguni, L. S. and Lehman, I. R.,** Eukaryotic DNA polymerase-primase structure, mechanism and function, *Biochim. Biophys. Acta,* 950, 87, 1988.

29. **Wahl, A. F., Kowalski, S. P., Harwell, L. W., Lord, E. M., and Bambara, R. A.,** Immunoaffinity purification and properties of a high molecular weight calf thymus DNA alpha-polymerase, *Biochemistry*, 23, 1894, 1984.
30. **Chang, L. M. S., Ratler, E., Augl, C., and Bollum, F. J.,** Purification of a DNA polymerase-DNA primase complex from calf thymus glands, *J. Biol. Chem.*, 259, 14679, 1984.
31. **Nasheuer, H.-P. and Grosse, F.,** Immunoaffinity-purified DNA polymerase alpha displays novel properties, *Biochemistry*, 26, 8458, 1897.
32. **Wong, S. W., Paborsky, L. R., Fisher, P. A., Wang, T. S.-F., and Korn, D.,** Structural and enzymological characterization of immunoaffinity-purified DNA polymerase alpha-DNA primase complex from KB cells, *J. Biol. Chem.*, 261, 7958, 1986.
33. **Plevani, P., Foiani, M., Valsasnini, P., Badaracco, G., Cheriathundam, E., and Chang, L. M. S.,** Polypeptide structure of DNA primase from a yeast DNA polymerase alpha-primase complex, *J. Biol. Chem.*, 260, 7102, 1985.
34. **Holmes, A. M., Hasslewood, I. P., Wichremasinghe, R. F., and Johnston, I. R.,** The heterogeneity of deoxyribonucleic acid polymerase-alpha, *Biochem. Soc. Symp.*, 42, 17, 1977.
35. **Grosse, F. and Krauss, G.,** Purification of a 9S DNA polymerase-alpha species from calf thymus, *Biochemistry*, 20, 5470, 1981.
36. **Masaki, S., Koiwai, O., and Yoshida, S.,** 10 S DNA polymerase alpha of calf thymus shows a microheterogeneity in its large polypeptide component, *J. Biol. Chem.*, 257, 7172, 1982.
37. **Badaracco, G., Capucci, L., Plevani, P., and Chang, L. M. S.,** Polypeptide structure of DNA polymerase I from *Saccharomyces cerevisiae*, *J. Biol. Chem.*, 258, 10720, 1983.
38. **Gronostajski, R. M., Field, J., and Hurwitz, J.,** Purification of a primase activity associated with DNA polymerase-alpha from HeLa cells, *J. Biol. Chem.*, 259, 9479, 1984.
39. **Yamaguchi, M., Hendrickson, E. A., and DePamphilis, M. L.,** DNA primase-DNA polymerase alpha from simian cells. Modulation of RNA primer synthesis by ribonucleoside triphosphate, *J. Biol. Chem.*, 260, 6254, 1985.
40. **Goscin, L. P. and Byrnes, J. J.,** Isolation of the catalytic core of DNA polymerase-alpha from rabbit bone marrow, *Nucleic Acids Res.*, 10, 6023, 1982.
41. **Philippe, M., Rossignol, J. M., and Recondo, A. M.,** DNA polymerase alpha from rat liver: physiological variations, *Biochemistry*, 25, 1611, 1986.
42. **Mechali, M., Abadidebat, J., and deRecondo, A. M.,** Eukaryotic DNA polymerase alpha. Structural analysis of the enzyme from regenerating rat liver, *J. Biol. Chem.*, 255, 2114, 1980.
43. **Kaguni, L. S., Rossignol, J.-M., Conaway, R. C., Banks, G. R., and Lehman, I. R.,** Association of DNA primase with the beta/gamma subunits of DNA polymerase alpha from *Drosophila melanogaster* embryos, *J. Biol. Chem.*, 258, 9037, 1983.
44. **Kaguni, L. S., Rossignol, J.-M., Conaway, R. C., and Lehman, I. R.,** Isolation of an intact DNA polymerase-primase from embryos of *Drosophila melanogaster*, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 2221, 1983.
45. **Faust, E. A., Gloor, G., Macintyre, M.-F., and Nagy, R.,** ATP(CTP)-dependent conversion of MVM parvovirus single-stranded DNA to its replicative form by a purified 10 S species of mouse DNA polymerase alpha, *Biochim. Biophys. Acta*, 781, 216, 1884.
46. **Goulian, M. and Heard, C. J.,** Intact DNA polymerase alpha/primase from mouse cells, *J. Biol. Chem.*, 264, 19407, 1989.
47. **Hsi, K.-L., Copeland, W. C., and Wang, T. S.-F.,** Human DNA polymerase alpha catalytic polypeptide binds ConA and RCA and contains a specific labile site in the N-terminus, *Nucleic Acids Res.*, 18, 6231, 1990.
48. **Wang, T. S.-F., Wong, S. W., and Korn, D.,** Human DNA polymerase alpha. Predicted functional domains and relationship with viral DNA polymerases, *FASEB J.*, 3, 14, 1989.
49. **Pizzagalli, A., Valsasnini, P., Plevani, P., and Lucchini, G.,** DNA polymerase I gene of *Saccharomyces cerevisiae:* nucleotide sequence, mapping of a temperature-sensitive mutation, and protein homology with other DNA polymerases, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 3772, 1988.
50. **Holmes, A. M., Cheriathundam, E., Bollum, F. J., and Chang, L. M. S.,** Immunological analysis of the polypeptide structure of calf thymus DNA polymerase-primase complex, *J. Biol. Chem.*, 261, 1924, 1986.
51. **Yagura, T., Kozu, T., Seno, T., and Tanaka, S.,** Immunochemical detection of a primase activity related subunit of DNA polymerase alpha from human and mouse cells using the monoclonal antibody, *Biochemistry*, 26, 7749, 1987.
52. **Borowiec, J. A., Dean, F. B., Bullock, P. A., and Hurwitz, J.,** Binding and unwinding — how T antigen engages the SV40 origin of DNA replication, *Cell*, 60, 181, 1990.
53. **Conaway, R. C. and Lehman, I. R.,** A DNA primase activity associated with DNA polymerase-alpha from *Drosophila melanogaster* embryos, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 2523, 1982.
54. **Conaway, R. C. and Lehman, I. R.,** Synthesis by the DNA primase of *Drosophila melanogaster* of a primer with a unique chain length, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 4585, 1982.
55. **Singh, H. and Dumas, L. B.,** A DNA primase that copurifies with the major DNA polymerase from the yeast *Saccharomyces cerevisiae*, *J. Biol. Chem.*, 259, 7936, 1984.
56. **Wang, T. S.-F., Hu, S.-Z., and Korn, D.,** DNA primase from KB cells. Characterization of a primase activity tightly associated with immunoaffinity-pur-

ified polymerase-alpha, *J. Biol. Chem.*, 259, 1854, 1984.
57. **Hu, S. J., Wang, T. S.-F., and Korn, D.,** DNA primase from KB cells. Evidence for a novel model of primase catalysis by a highly purified primase/polymerase-alpha complex, *J. Biol. Chem.*, 259, 2612, 1984.
58. **Nasheuer, H.-P. and Grosse, F.,** DNA polymerase alpha-primase from calf thymus. Determination of the polypeptide responsible for primase activity, *J. Biol. Chem.*, 263, 8981, 1988.
59. **Masaki, S., Tanabe, K., and Yoshida, S.,** Large polypeptides of 10 S DNA polymerase alpha from calf thymus, rapid isolation using monoclonal antibody and tryptic peptide mapping analysis, *Nucleic Acids Res.*, 12, 4455, 1984.
60. **Yoshida, R., Suzuki, S., Masaka, S., and Koiwai, O.,** DNA primase associated with 10 S DNA polymerase alpha of calf thymus, *J. Biochem.*, (Tokyo), 98, 427, 1985.
61. **Cotterill, S. M., Chui, G., and Lehman, I. R.,** DNA polymerase-primase from embryos of *Drosophila melanogaster*. DNA primase subunits, *J. Biol. Chem.*, 262, 16105, 1987.
62. **Lucchini, G., Francesconi, S., Foiani, M., Badaracco, G., and Plevani, P.,** Yeast DNA polymerase-DNA primase complex; cloning of *PRI1*, a single essential gene related to DNA primase activity, *EMBO J.*, 6, 737, 1987.
63. **Foiani, M., Lindner, A. J., Hartmann, G. R., Lucchini, G., and Plevani, P.,** Affinity labeling of active center and ribonucleoside triphosphate binding site of yeast DNA primase, *J. Biol. Chem.*, 264, 2189, 1989.
64. **Prussak, C. E., Slinazan, M. T., and Tseng, B. Y.,** Mouse primase p49 subunit molecular cloning indicates conserved and divergent regions, *J. Biol. Chem.*, 264, 4957, 1989.
65. **Li, J. J. and Kelly, T. J.,** Simian virus 40 DNA replication *in vitro*, *Proc. Natl. Acad. Sci. U.S.A.*, 81, 6973, 1984.
66. **Li, J. J. and Kelly, T. J.,** Simian virus 40 DNA replication *in vitro*. Specificity of initiation and evidence for bidirectional replication, *Mol. Cell. Biol.*, 5, 1238, 1985.
67. **Stillman, B. W. and Gluzman, Y.,** Replication and supercoiling of simian virus 40 DNA in cell extracts from human cells, *Mol. Cell. Biol.*, 5, 2051, 1985.
68. **Wobbe, C. R., Dean, F., Weissbach, L., and Hurwitz, J.,** *In vitro* replication of duplex circular DNA containing the simian virus 40 DNA origin site, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 5710, 1985.
69. **Murakami, Y., Eki, T., Yamada, M., Prives, C., and Hurwitz, J.,** Species-specific *in vitro* synthesis of DNA containing the polyoma virus origin of replication, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 6347, 1986.
70. **Smale, S. T. and Tjian, R.,** T-antigen-DNA polymerase alpha complex implicated in simian virus 40 replication, *Mol. Cell. Biol.*, 6, 4077, 1986.
71. **Gannon, J. V. and Lane, D. P.,** p53 and DNA polymerase alpha compete for binding to SV40 T antigen, *Nature*, 329, 456.
72. **Dornreiter, I., Hoss, A., Arthur, A. K., and Fanning, E.,** SV40 T antigen binds directly to the large subunit of purified DNA polymerase alpha, *EMBO J.*, 9, 3329, 1990.
73. **Downey, K. M., Tan, C.-K., Andrews, D. A., Li, X., and So, A. G.,** Proposed roles for DNA polymerases alpha and delta at the replication fork, *Cancer Cells*, 6, 413, 1988.
74. **Tsurimoto, T., Melendy, T., and Stillman, B.,** Sequential initiation of lagging and leading strand synthesis by two different polymerase complexes at the SV40 DNA replication origin, *Nature*, 346, 543, 1990.
75. **Weinberg, D. H., Collins, K. L., Simancek, P., Russo, A., Wold, M. S., Virship, D. M., and Kelly, T. J.,** Reconstitution of SV40 DNA replication with purified proteins, *Proc. Natl. Acad. Sci. U.S.A.*, 87, 8692, 1990.
76. **Byrnes, J. J., Downey, K. M., Black, V. L., and So, A. G.,** A new mammalian DNA polymerase with 3′ to 5′ exonuclease activity: DNA polymerase delta, *Biochemistry*, 15, 2817, 1976.
77. **Lee, M. Y. W. T., Tan, C.-K., Downey, K. M., and So, A. G.,** Structural and functional properties of calf thymus DNA polymerase delta, *Prog. Nucleic Acids Res. Mol. Biol.*, 26, 83, 1981.
78. **Lee, M. Y. W. T., Tan, C.-K., Downey, K. M., and So, A. G.,** Further studies on calf thymus DNA polymerase delta purified to homogeneity by a new procedure, *Biochemistry*, 23, 1906, 1984.
79. **Wong, S. W., Syvaoja, J., Tan, C.-K., Downey, K. M., So, A. G., Linn, S., and Wang, T. S.-F.,** DNA polymerases alpha and delta are immunologically and structurally distinct, *J. Biol. Chem.*, 264, 5924, 1989.
80. **So, A. G. and Downey, K. M.,** Mammalian DNA polymerases alpha and delta: current status in DNA replication, *Biochemistry*, 27, 4591, 1988.
81. **Syvaoja, J., Svomensaari, S., Nishida, C., Goldsmith, J. S., Chui, G. S. J., Jain, S., and Linn, S.,** DNA polymerases alpha, delta and epsilon: three distinct enzymes from HeLa cells, *Proc. Natl. Acad. Sci. U.S.A.*, 87, 6664, 1990.
82. **Goulian, M., Hermann, S. M., Sackett, J. W., and Grimm, S. L.,** Two forms of DNA polymerase delta from mouse cells. Purification and properties, *J. Biol. Chem.*, 265, 16402, 1990.
83. **Ottiger, H. P. and Hubscher, U.,** Mammalian DNA polymerase alpha holoenzyme with possible functions at the leading and lagging strand of the replication fork, *Proc. Natl. Acad. Sci. U.S.A.*, 81, 3993, 1984.
84. **Vishwanatha, J. K., Coughlin, S. A., Wesolowski-Owen, M., and Baril, E. F.,** A multiprotein form of DNA polymerase alpha from HeLa cells. Resolution of its catalytic activities, *J. Biol. Chem.*, 261, 6619, 1986.

85. **Cotterill, S. M., Reyland, M. E., Loeb, L. A., and Lehman, I. R.,** A cryptic proofreading 3'-5' exonuclease associated with the polymerase subunit of the DNA polymerase-primase from *Drosophila melanogaster*, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 5635, 1987.
86. **Bialek, G., Nasheuer, H.-P., Goetz, H., and Grosse, F.,** Exonucleolytic proofreading increases the accuracy of DNA synthesis by human lymphocytic DNA polymerase alpha-DNA primase, *EMBO J.*, 8, 1833, 1989.
87. **Tan, C.-K., Castillo, C., So, A. G., and Downey, K. M.,** An auxiliary protein for DNA polymerase delta from fetal calf thymus, *J. Biol. Chem.*, 261, 12316, 1986.
88. **Prelich, G., Tan, C.-K., Kostura, M., Mathews, M. B., So, A. G., Downey, K. M., and Stillman, B.,** Functional identity of proliferating cell nuclear antigen and a DNA polymerase delta auxiliary protein, *Nature*, 326, 517, 1987.
89. **Bauer, G. A. and Burgers, P. M. J.,** The yeast analog of mammalian cyclin/proliferating cell nuclear antigen interacts with mammalian DNA polymerase delta, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 7506, 1988.
90. **Bauer, G. A., Heller, H. M., and Burgers, P. M. J.,** DNA polymerase III from *Saccharomyces cerevisiae*. I. Purification and characterization, *J. Biol. Chem.*, 263, 917, 1988.
91. **Burgers, P. M. J.,** Mammalian cyclin/PCNA (DNA polymerase delta auxiliary protein) stimulates processive DNA synthesis by yeast DNA polymerase III, *Nucleic Acids Res.*, 16, 6297, 1988.
92. **Boulet, A., Simon, M., Faye, G., Bauer, G. A., and Burgers, P. M. J.,** Structure and function of *Saccharomyces cerevisiae CDC2* gene encoding the large subunit of DNA polymerase III, *EMBO J.*, 8, 1849, 1989.
93. **Sitney, K. C., Budd, M. E., and Campbell, J. L.,** DNA polymerase III, a second essential DNA polymerase, is encoded by the *S. cerevisiae CDC2* gene, *Cell*, 56, 599, 1989.
94. **Burgers, P. M. J., Bambara, R. A., Campbell, J. L., Chang, L. M. S., Downey, K. M., Hubscher, U., Lee, M. Y. W. T., Linn, S. M., So, A. G., and Spadari, S.,** Revised nomenclature for eukaryotic DNA polymerases, *Eur. J. Biochem.*, 191, 617, 1990.
95. **Prelich, G., Kostura, M., Marshak, D. R., Mathews, M. B., and Stillman, B.,** The cell-cycle regulated proliferating cell nuclear antigen is required for SV40 DNA replication *in vitro*, *Nature*, 326, 471, 1987.
96. **Dresler, S. L. and Frattini, M. G.,** DNA replication and UV-induced repair synthesis in human fibroblasts are much less sensitive than DNA polymerase alpha to inhibition by butylphenyl-deoxyguanosine triphosphate, *Nucleic Acids Res.*, 14, 7093, 1986.
97. **Hammond, R. A., Brynes, J. J., and Miller, M. R.,** Identification of a DNA polymerase delta in CV-1 cells: studies implicating both DNA polymerase delta and DNA polymerase alpha in DNA replication, *Biochemistry*, 26, 6817, 1987.
98. **Decker, R. S., Yamaguchi, M., Possenti, R., Bradley, M. K., and DePamphilis, M. L.,** *In vitro* initiation of DNA replication in simian virus 40 chromosomes, *J. Biol. Chem.*, 262, 10863, 1987.
99. **Hartwell, L. H.,** Sequential function of gene products relative to DNA synthesis in the yeast cell cycle, *J. Mol. Biol.*, 104, 803, 1976.
100. **Conrad, M. N. and Newlon, C. S.,** *cdc2* mutants of yeast fail to replicate approximately one third of their nuclear genome, *Mol. Cell. Biol.*, 3, 1000, 1983.
101. **Blow, J.,** Eukaryotic chromosome replication requires both alpha and delta DNA polymerases, *Trends Genet.*, 5, 134, 1989.
102. **Prelich, G. and Stillman, B.,** Coordinated leading and lagging strand synthesis during DNA replication *in vitro* requires PCNA, *Cell*, 53, 117, 1988.
103. **Crute, J. J., Wahl, A. F., and Bambara, R. A.,** Purification and characterization of two new high molecular weight forms of DNA polymerase delta, *Biochemistry*, 25, 26, 1986.
104. **Nishida, C., Reinhard, P., and Linn, S.,** DNA repair synthesis in human fibroblasts requires DNA polymerase delta, *J. Biol. Chem.*, 263, 501, 1988.
105. **Sabatino, R. D., Myers, T. W., Bambara, R. A., Kwon-Shin, O., Marracino, R. L., and Frickey, P. H.,** Calf thymus DNA polymerases alpha and delta are capable of highly processive DNA synthesis, *Biochemistry*, 27, 2998, 1988.
106. **Syvaoja, J. and Linn, S.,** Characterization of a large form of DNA polymerase delta from HeLa cells that is insensitive to proliferating cell nuclear antigen, *J. Biol. Chem.*, 264, 5924, 1989.
107. **Focher, F., Gassman, M., Hafkemeyer, P., Merrari, E., Spadari, S., and Hubscher, U.,** Calf thymus DNA polymerase delta independent of proliferating cell nuclear antigen (PCNA), *Nucleic Acids Res.*, 17, 1805, 1989.
108. **Syvaoja, J. E.,** DNA polymerase epsilon: the latest member in the family of mammalian DNA polymerases, *BioEssays*, 12, 533, 1990.
109. **Talamian, R. V., Brown, N. C., McKenna, C. E., Ye, T.-G., Levy, J. N., and Wright, G.,** Carbonyldiphosphonate, a selective inhibitor of mammalian DNA polymerase delta, *Biochemistry*, 28, 8270, 1989.
110. **Bambara, R. A. and Jessee, C. B.,** Properties of DNA polymerases delta and epsilon and their roles in eukaryotic DNA replication, *Biochim. Biophys. Acta*, in press, 1991.
111. **Lee, M. Y. W. T. and Toomey, N. L.,** Human placental DNA polymerase delta: identification of a 170-kilodalton polypeptide by activity staining and immunoblotting, *Biochemistry*, 26, 1076, 1987.
112. **Lee, M. Y. W. T., Jiang, Y., Zhang, J. S., and Toomey, N. L.,** Characterization of human DNA polymerase delta and its immunochemical relationship with DNA polymerases alpha and epsilon, *J. Biol. Chem.*, 266, 2423, 1991.

113. **Goscin, L. P. and Byrnes, J. J.,** DNA polymerase delta: one polypeptide, two activities, *Biochemistry*, 21, 2513, 1982.
114. **Byrnes, J. J.,** personal communication.
115. **Wintersberger, E.,** Deoxyribonucleic acid polymerase from yeast: further purification and characterization of DNA-dependent DNA polymerases A and B, *Eur. J. Biochem.*, 50, 41, 1974.
116. **Chang, L. M. S.,** DNA polymerases from baker's yeast, *J. Biol. Chem.*, 252, 1873, 1977.
117. **Budd, M. E., Sitney, K. C., and Campbell, J. L.,** Purification of DNA polymerase II, a distinct DNA polymerase from *Saccharomyces cerevisiae*, *J. Biol. Chem.*, 264, 6557, 1989.
118. **Burgers, P. M. J. and Bauer, G. A.,** DNA polymerase III from *Saccharomyces cerevisiae*. II. Inhibitor studies and comparison with DNA polymerases I and II, *J. Biol. Chem.*, 263, 925, 1988.
119. **Hamatake, R. K., Hasegawa, H., Clark, A. B., Babenek, K., Kunkel, T. A., and Sugino, A.,** Purification and characterization of DNA polymerase II from the yeast *Saccharomyces cerevisiae*, *J. Biol. Chem.*, 265, 4072, 1990.
120. **Morrison, A., Araki, H., Clark, A. B., Hamatake, R. K., and Sugino, A.,** A third essential DNA polymerase in *S. cerevisiae*, *Cell*, 62, 1143, 1990.
121. **Nishida, C. and Linn, S.,** DNA repair synthesis in permeabilized human fibroblasts mediated by DNA polymerase delta and application for purification of xeroderma pigmentosum factors, *Cancer Cells*, 6, 411, 1988.
122. **Celis, J. E., Madsen, P., Celis, A., Nielsen, H. V., and Gesser, B.,** Cyclin (PCNA, auxiliary protein of DNA polymerase delta) is a central component of the pathway(s) leading to DNA replication and cell division, *FEBS Lett.*, 220, 1, 1987.
123. **Mathews, M. B.,** The proliferating cell nuclear antigen, PCNA, a cell cycle regulated DNA replication factor, in *Growth Control During Cell Aging*, Warner, H. R. and Wang, E., Eds., CRC Press, Boca Raton, in press.
124. **Tan, E. M.,** Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology, *Adv. Immunol.*, 33, 167, 1989.
125. **Miyachi, K., Fritzler, M. J., and Tan, E. M.,** Autoantibody to a nuclear antigen in proliferating cells, *J. Immunol.*, 121, 2228, 1978.
126. **Tahasaki, Y., Deng, Y.-S., and Tan, E. M.,** A nuclear antigen associated with cell proliferation and blast transformation: its distribution in synchronized cells, *J. Exp. Med.*, 154, 1899, 1981.
127. **Bravo, R. and Celis, J. E.,** A search for differential polypeptide synthesis throughout the cell cycle of HeLa cells, *J. Cell Biol.*, 84, 795, 1980.
128. **Mathews, M. B., Bernstein, R. M., Franza, B. R., Jr., and Garrels, J. I.,** Identity of the proliferating cell nuclear antigen and cyclin, *Nature*, 309, 374, 1984.
129. **Bravo, R.,** Coordinated synthesis of the nuclear protein cyclin and DNA in serum-stimulated quiescent 3T3 cells, *FEBS Lett.*, 169, 185, 1984.
130. **Bravo, R.,** Synthesis of nuclear protein cyclin (PCNA) and its relationship with DNA replication, *Exp. Cell Res.*, 163, 287, 1986.
131. **Bravo, R. and Macdonald-Bravo, H.,** Induction of the nuclear protein "cyclin" in quiescent mouse 3T3 cells stimulated by serum and growth factors. Correlation with DNA synthesis, *EMBO J.*, 13, 3177, 1984.
132. **Celis, J. E. and Celis, A.,** Cell cycle-dependent variations in the distribution of the nuclear protein cyclin (proliferating cell nuclear antigen) in cultured cells: subdivision of S-phase, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 3262, 1985.
133. **Almendral, J. M., Huebsch, D., Blundell, P. A., Macdonald-Bravo, H., and Bravo, R.,** Cloning and sequence of human nuclear protein cyclin: homology with DNA binding proteins, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 1575, 1987.
134. **Matsumoto, K., Moriuchi, T., Koji, T., and Nakane, P. K.,** Molecular cloning of cDNA coding for rat proliferating cell nuclear antigen (PCNA/cyclin), *EMBO J.*, 6, 637, 1987.
135. **Bravo, R. and Macdonald-Bravo, H.,** Changes in the nuclear distribution of cyclin (PCNA) but not its synthesis depend on DNA replication, *EMBO J.*, 4, 655, 1985.
136. **Madsen, P. and Celis, J. E.,** S-phase patterns of cyclin (PCNA) antigen staining resemble topographical patterns of DNA synthesis. A role for cyclin in DNA replication?, *FEBS Lett.*, 193, 5, 1985.
137. **Sadie, M. K. and Mathews, M. B.,** Immunochemical and biochemical analysis of the proliferating cell nuclear antigen (PCNA) in HeLa cells, *Exp. Cell Res.*, 163, 423, 1986.
138. **Wold, M. S., Li, J., Weinberg, D. H., Vershup, D. M., Verkeyer, E., and Kelly, T.,** Cellular proteins required for SV40 DNA replication *in vitro*, *Cancer Cells*, 6, 133, 1988.
139. **Morris, G. F. and Mathews, M. B.,** Regulation of proliferating cell nuclear antigen during cell cycle, *J. Biol. Chem.*, 264, 13856, 1989.
140. **Bravo, R. and Macdonald-Bravo, H.,** Existence of two population of cyclin/proliferating cell nuclear antigen during the cell cycle: association with DNA replication sites, *J. Cell Biol.*, 105, 1549, 1987.
141. **Macdonald-Bravo, H. and Bravo, R.,** Induction of the nuclear protein cyclin in serum-stimulated quiescent 3T3 cells is independent of DNA synthesis, *Exp. Cell Res.*, 156, 455, 1985.
142. **Jaskulski, D., deRiel, J. K., Merier, W. E., Calabretta, B., and Baserga, R.,** Inhibition of cellular proliferation by antisense oligonucleotides to PCNA cyclin, *Science*, 240, 1544, 1988.
143. **Liu, Y. C., Marracino, R. L., Keng, P. C., Bambara, R. A., Lord, E. M., Chow, W. G., and Zain, S. B.,** Requirement for proliferating cell nuclear antigen expression during stages of the Chinese hamster ovary cell, *Biochemistry*, 28, 2967, 1989.

144. **Zuber, M., Tan, E. M., and Ryoji, M.,** Involvement of proliferating cell nuclear antigen (cyclin) in DNA replication in living cells, *Mol. Cell. Biol.*, 9, 57, 1989.
145. **Bravo, R., Frank, R., Blundell, P. A., and Macdonald-Bravo, H.,** Cyclin/PCNA is the auxiliary protein of DNA polymerase delta, *Nature*, 326, 515, 1987.
146. **Tan, C.-K., Sullivan, K., Li, X., Tan, E. M., Downey, K. M., and So, A. G.,** Autoantibody to the proliferating cell nuclear antigen neutralizes the activity of the auxiliary protein for DNA polymerase delta, *Nucleic Acids Res.*, 15, 9299, 1987.
147. **Ng, L., Tan, C.-K., Downey, K. M., and Fisher, P. A.,** Enzymologic mechanism of calf thymus DNA polymerase delta, *J. Biol. Chem.*, 266, 11699, 1991.
148. **Suzuka, I., Daidoji, H., Matsuoka, M., Kadowski, K.-I., Takasaki, Y., Nakane, P. K., and Moriuchi, T.,** Gene for proliferating cell nuclear antigen (DNA polymerase delta auxiliary protein) is present in both mammalian and higher plant genomes, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 3189, 1989.
149. **O'Reilly, D. R., Crawford, A. M., and Miller, L. K.,** Viral proliferating cell nuclear antigen, *Nature*, 337, 606, 1989.
150. **Ogata, K., Kurki, P., Celis, J. E., Nakamura, R., and Tan, E. M.,** Monoclonal antibodies to a nuclear protein (PCNA/cyclin) associated with DNA replication, *Exp. Cell Res.*, 168, 475, 1987.
151. **Waseem, N. H. and Lane, D. P.,** Monoclonal antibody analysis of the proliferating cell nuclear antigen, *J. Cell Sci.*, 96, 121, 1990.
152. **Travali, S., Ku, D.-H., Rizzo, M. G., Oltavio, L., Baserga, R., and Calabretta, B.,** Structure of the human gene for the proliferating cell nuclear antigen, *J. Biol. Chem.*, 264, 7466, 1989.
153. **Ku, D. H., Travali, S., Calabretta, B., Huebna, K., and Baserga, R.,** Human gene for proliferating cell nuclear antigen has pseudogenes and localizes to chromosome 20, *Somatic Cell Mol. Genet.*, 15, 297, 1989.
154. **Wold, M. S., Weinberg, D. H., Virshup, D. M., Li, J. J., and Kelly, T. J.,** Identification of cellular proteins required for simian virus 40 DNA replication, *J. Biol. Chem.*, 264, 2801, 1989.
155. **Tsurimoto, T. and Stillman, B.,** Purification of a cellular replication factor, RFC, that is required for coordinated synthesis of leading and lagging strands during SV40 DNA replication *in vitro*, *Mol. Cell. Biol.*, 9, 609, 1989.
156. **Tsurimoto, T. and Stillman, B.,** Replication factors required for SV40 DNA replication *in vitro*. I. DNA structure-specific recognition of a primer-template junction by eukaryotic DNA polymerases and their accessory proteins, *J. Biol. Chem.*, 266, 1950, 1991.
157. **Tsurimoto, T. and Stillman, B.,** Replication factors required for SV40 DNA replication *in vitro*. II. Switching of DNA polymerase alpha and delta during initiation of leading and lagging strand synthesis, *J. Biol. Chem.*, 266, 1961, 1991.
158. **Tsurimoto, T. and Stillman, B.,** Functions of replication factor C and proliferating cell nuclear antigen: functional similarity of DNA polymerase accessory proteins from human cells and bacteriophage T4, *Proc. Natl. Acad. Sci. U.S.A.*, 87, 1023, 1990.
159. **Lee, S.-H. and Hurwitz, J.,** Mechanism of elongation of primed DNA by DNA polymerase delta, proliferating cell nuclear antigen and activator I, *Proc. Natl. Acad. Sci. U.S.A.*, 87, 5672, 1990.
160. **Lee, S.-H., Kwong, A. D., Pan, Z.-Q., and Hurwitz, J.,** Studies on the activator 1 protein complex, an accessory factor for proliferating cell nuclear antigen-dependent DNA polymerase delta, *J. Biol. Chem.*, 266, 594, 1991.
161. **Maki, S. and Kornberg, A.,** DNA polymerase III holoenzyme of *Escherichia coli*. III. Distinctive processive polymerases reconstituted from purified subunits, *J. Biol. Chem.*, 263, 6561, 1988.
162. **O'Donnell, M. and Studwell, P. S.,** Total reconstitution of DNA polymerase III holoenzyme reveals dual accessory protein clamps, *J. Biol. Chem.*, 265, 1179, 1990.
163. **Mace, D. C. and Alberts, B. M.,** The complex of T4 bacteriophage gene 44 and 62 replication proteins forms an ATPase that is stimulated by DNA and by T4 gene 45 protein, *J. Mol. Biol.*, 177, 279, 1984.
164. **Mace, D. C. and Alberts, B. M.,** Characterization of the stimulatory effect of T4 gene 45 protein and gene 44/62 protein complex on DNA synthesis by T4 DNA polymerase, *J. Mol. Biol.*, 177, 313, 1984.
165. **Piperno, J. R. and Alberts, B. M.,** An ATP stimulation of T4 DNA polymerase mediated via T4 gene 44/62 and 45 proteins: the requirement of ATP hydrolysis, *J. Biol. Chem.*, 253, 5174, 1978.
166. **Jarvis, T. C., Paul, L. S., Hockensmith, J. W., and von Hippel, P. H.,** Structural studies of the T4 DNA replication system. II. ATPase properties of the polymerase accessory protein complex, *J. Biol. Chem.*, 264, 12717, 1989.
167. **Cha, T.-A. and Alberts, B. M.,** *In vitro* studies of the T4 bacteriophage DNA replicative system, *Cancer Cells*, 6, 1, 1988.
168. **Huang, C.-C., Hearst, J. E., and Alberts, B. M.,** Two types of replication proteins increase the rate at which T4 DNA polymerase traverses the helical regions in a single-stranded DNA template, *J. Biol. Chem.*, 256, 4087, 1981.
169. **Alberts, B. M., Barry, J., Bedinger, P., Formosa, T., Jongenell, C. V., and Dreuzer, K. N.,** Studies on DNA replication in the T4 bacteriophage *in vitro* system, *Cold Spring Harbor Symp. Quant. Biol.*, 47, 655, 1983.
170. **Burgers, P. M. J. and Kornberg, A.,** ATP activation of DNA polymerase III holoenzyme of *Escherichia coli:* ATP dependent formation of an initiation complex with a primed template, *J. Biol. Chem.*, 257, 11468, 1982.
171. **Richardson, R. W., Ellis, R. L., and Nossal, N. G.,** Protein-protein interactions within the bacteriophage T4 DNA replication system, in *Molecular Mecha-*

nisms in DNA Replication and Recombination, UCLA Symp. Mol. Cell Biol., New Series, Vol. 127, Richardson, C. C. and Lehman, I. R., Eds., Alan R. Liss, New York, 1990, 247.
172. **Pritchard, C. G. and DePamphilis, M. L.,** Preparation of DNA polymerase alpha-C1C2, *J. Biol. Chem.*, 258, 9801, 1983.
173. **Pritchard, C. G., Weaver, D. T., Baril, E. F., and DePamphilis, M. L.,** DNA polymerase alpha cofactors C1C2 function as primer recognition proteins, *J. Biol. Chem.*, 258, 9810, 1983.
174. **Jindal, H. K. and Vishwanatha, J. K.,** Purification and characterization of primer recognition proteins from HeLa cells, *Biochemistry,* 29, 4767, 1990.
175. **Jindal, H. K. and Vishwanatha, J. K.,** Functional identity of a primer recognition protein as phosphoglycerate kinase, *J. Biol. Chem.*, 265, 6540, 1990.
176. **Goulian, M., Heard, C. J., and Grimm, S. L.,** Purification and properties of an accessory protein for DNA polymerase alpha/primase, *J. Biol. Chem.*, 265, 13221, 1990.
177. **Goulian, M. and Heard, C. J.,** The mechanism of action of an accessory protein for DNA polymerase alpha/primase, *J. Biol. Chem.*, 265, 13231, 1990.
178. **Wold, M. S. and Kelly, T. J.,** Purification and characterization of replication protein A, a cellular protein required for *in vitro* replication of simian virus 40 DNA, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 2523, 1988.
179. **Fairman, M. P. and Silman, B.,** Cellular factors required for multiple stages of SV40 DNA replication *in vitro, EMBO J.*, 7, 1211, 1988.
180. **Ishimi, Y., Claude, A., Bullock, P., and Hurwitz, J.,** Complete enzymatic synthesis of DNA containing the SV40 origin of replication, *J. Biol. Chem.*, 263, 19723, 1988.
181. **Wobbe, C. R., Weissbach, L., Borowiec, J. A., Dean, F. B., Murakami, Y., Bullock, P., and Hurwitz, J.,** Replication of simian virus 40 origin-containing DNA *in vitro* with purified proteins, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 1834, 1987.
182. **Dean, F. B., Bullock, P., Murakami, Y., Wobbe, C. R., Weissbach, L., and Hurwitz, J.,** Simian virus 40 (SV40) DNA replication: SV40 large T antigen unwinds DNA containing the SV40 origin of replication, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 16, 1987.
183. **Dodson, M., Dean, F. B., Bullock, P., Echols, H., and Hurwitz, J.,** Unwinding of duplex DNA from the SV40 origin of replication by T antigen, *Science,* 238, 964, 1987.
184. **Wold, M. S., Li, J. J., and Kelly, T. J.,** Initiation of simian virus 40 DNA replication *in vitro:* large-tumor antigen and origin-dependent unwinding of the template, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 3643, 1987.
185. **Borowiec, J. A. and Hurwitz, J.,** Localized melting and structural changes in the SV40 origin of replication induced by T antigen, *EMBO J.*, 7, 3149, 1987.
186. **Tsurimoto, T., Fairman, M. P., and Stillman, B.,** Simian virus 40 replication *in vitro:* identification of multiple stages of initiation, *J. Cell Biol.*, 9, 3839, 1989.
187. **Kenny, M. K., Lee, S.-H., and Hurwitz, J.,** Multiple functions of human single-stranded-DNA binding protein in simian virus 40 DNA replication: single-strand stabilization and stimulation of DNA polymerases alpha and delta, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 9757, 1989.
188. **Baker, T. A., Sekimezu, K., Furnell, B. E., and Kornberg, A.,** Extensive unwinding of the plasmid template during staged enzymatic initiation of DNA replication from the origin of the *Escherichia coli* chromosome, *Cell,* 45, 53, 1986.
189. **Herrick, G., Delius, H., and Alberts, B.,** Single stranded DNA structure and DNA polymerase activity in the presence of nucleic acid helix-unwinding proteins from calf thymus, *J. Biol. Chem.*, 251, 2142, 1976.
190. **Chase, J. W. and Williams, K. R.,** Single-stranded DNA binding protein required for DNA replication, *Annu. Rev. Biochem.*, 55, 103, 1986.
191. **Kornberg, A.,** *DNA Replication,* W. H. Freeman and Co., San Francisco, 1980.
192. **Kornberg, A.,** *Supplement to DNA Replication,* W. H. Freeman and Co., San Francisco, 1982.
193. **Formosa, T., Burke, R. L., and Alberts, B. M.,** Affinity purification of T4 bacteriophage proteins essential for DNA replication and genetic recombination, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 2442, 1983.
194. **Cotterill, S., Chui, G., and Lehman, I. R.,** DNA polymerase-primase from embryos of *Drosophila melanogaster.* The DNA polymerase subunit, *J. Biol. Chem.*, 262, 16100, 1987.
195. **Kenny, M. K., Schlegel, U., Furneaux, H., and Hurwitz, J.,** The role of human single-stranded DNA binding protein and its individual subunits in simian virus 40 DNA replication, *J. Biol. Chem.*, 265, 7693, 1990.
196. **Erdile, L. F., Wold, M. S., and Kelly, T. J.,** The primary structure of the 32-kDa subunit of human replication protein A, *J. Biol. Chem.*, 265, 3177, 1990.
197. **Brill, S. J. and Stillman, B.,** Yeast replication factor-A functions in the unwinding of the SV40 origin of DNA replication, *Nature,* 324, 92, 1989.
198. **Heyer, W.-D., Rao, M. R. S., Erdile, L. F., Kelly, T. J., and Kolodner, R. D.,** An essential *Saccharomyces cerevisiae* single-stranded DNA binding protein is homologous to the large subunit of human RP-A, *EMBO J.*, 9, 2321, 1990.
199. **Pringle, R. and Hartwell, H.,** The *Saccharomyces cerevisiae* cell cycle, in *The Molecular Biology of the Yeast Saccharomyces. Life Cycle and Inheritance,* Stratherm, J. N., Jones, E. W., and Broach, J. E., Eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1981, 97.
200. **Din, S., Brill, S. J., Fairman, M. P., and Stillman, B.,** Cell-cycle-regulated phosphorylation of DNA

replication factor A from human and yeast cells, *Genes Dev.*, 4, 968, 1990.
201. **Matson, S. W. and Kaiser-Rogers, K. A.,** DNA helicases, *Annu. Rev. Biochem.*, 59, 289, 1990.
202. **LeBowitz, J. H. and McMacken, R.,** The *Escherichia coli* dnaB replication protein is a DNA helicase, *J. Biol. Chem.*, 261, 4738, 1986.
203. **Richardson, R. W. and Nossal, N. G.,** Characterization of bacteriophage T4 gene 41 DNA helicase, *J. Biol. Chem.*, 264, 4725, 1989.
204. **Matson, S. W., Tabor, S., and Richardson, C. C.,** The gene 4 protein of bacteriophage T7. Characterization of helicase activity, *J. Biol. Chem.*, 258, 14017, 1983.
205. **Crute, J. J., Mocarski, E. S., and Lehman, I. R.,** A DNA helicase induced by herpes simplex virus Type I, *Nucleic Acids Res.*, 16, 6585, 1988.
206. **Stahl, H., Droge, P., Zentgraf, H., and Knippers, R.,** A large-tumor-antigen-specific monoclonal antibody inhibits DNA replication of simian virus 40 mini-chromosomes in an *in vitro* elongation system, *J. Virol.*, 54, 473, 1985.
207. **Stahl, H., Droge, P., and Knippers, R.,** DNA helicase activity of SV40 large tumor antigen, *EMBO J.*, 5, 1939, 1986.
208. **Wiekowski, M., Droge, P., and Stahl, H.,** Monoclonal antibodies as probes for a function of large T antigen during the elongation process of simian virus 40 DNA replication, *J. Virol.*, 61, 411, 1987.
209. **Goetz, G. S., Dean, F. B., Hurwitz, J., and Matson, S. W.,** The unwinding of duplex regions in DNA by the simian virus 40 large tumor antigen-associated DNA helicase activity, *J. Biol. Chem.*, 263, 383, 1988.
210. **Wiekowski, M., Schwarz, M., and Stahl, H.,** Simian virus 40 large T antigen DNA helicase. Characterization of the ATPase-dependent DNA unwinding activity and its substrate requirements, *J. Biol. Chem.*, 263, 436, 1988.
211. **Thommes, P. and Hubscher, U.,** Eukaryotic DNA helicases, *FEBS Lett.*, 268, 325, 1990.
212. **Sung, P., Prakash, L., Matson, S. W., and Prakash, S.,** *RAD3* protein of *Saccharomyces cerevisiae* is a DNA helicase, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 8951, 1987.
213. **Harosh, I., Naumovski, L., and Friedberg, E. C.,** Purification and characterization of *RAD3* ATPase/DNA helicase from *Saccharomyces cerevisiae*, *J. Biol. Chem.*, 264, 20532, 1989.
214. **Sugino, A., Ryu, B. H., Sugino, T., Naumovski, I., and Freidberg, E. C.,** A new DNA-dependent ATPase which stimulates yeast DNA polymerase I and has DNA-unwinding activity, *J. Biol. Chem.*, 261, 11744, 1986.
215. **Poll, E. H. A. and Benbow, R. M.,** A DNA helicase from *Xenopus laevis* ovaries, *Biochemistry*, 27, 8701, 1988.
216. **Seki, M., Economoto, T., Watanabe, Y., Tawaragi, Y., Kawasaki, K., Hanaoka, F., and Yamada, M.,** Purification and characterization of a deoxyribonucleic acid dependent adenosinetriphosphatase from mouse FM3A cells: effects of ribonucleoside triphosphates on the interaction of the enzyme with single-stranded DNA, *Biochemistry*, 25, 3239, 1986.
217. **Seki, M., Enomoto, T., Hanaoka, F., and Yamada, M.,** DNA-dependent adenosine-triphosphatase B from mouse FM3A cells has DNA helicase activity, *Biochemistry*, 26, 2924, 1987.
218. **Seki, M., Enomoto, T., Yanagisawa, J., Hanaoka, F., and Ui, M.,** Further characterization of DNA helicase activity of mouse DNA-dependent adenosinetriphosphatase B (DNB helicase B), *Biochemistry*, 27, 1766, 1988.
219. **Tuteja, N., Tuteja, R., Rahman, K., Kana, L. Y., and Falaschi, A.,** A DNA helicase from human cells, *Nucleic Acids Res.*, 18, 6785, 1990.
220. **Daily, L., Caddle, M. S., Heintz, N., and Heintz, N. H.,** Purification of RIP60 and RIP100, mammalian proteins with origin specific DNA-binding and ATP-dependent DNA helicase activities, *Mol. Cell. Biol.*, 10, 6225, 1990.
221. **Thommes, P. and Hubscher, U.,** DNA helicase from calf thymus. Purification to apparent homogeneity and biochemical characterization of the enzyme, *J. Biol. Chem.*, 265, 14347, 1990.
222. **Li, X., Tan, C.-K., Castillo, C., So, A. G., and Downey, K. M.,** A DNA polymerase delta-associated DNA helicase: proposed role in eukaryotic DNA replication, in *Eukaryotic DNA Polymerases*, Engler, J. and Wang, T. S.-F., Eds., Springer-Verlag, New York, in press.
223. **Li, X., Tan, C.-K., So, A. G., and Downey, K. M.,** Purification and characterization of delta helicase from fetal calf thymus, in press.
224. **Drake, J. W.,** Comparative rates of spontaneous mutation, *Nature*, 221, 1132, 1969.
225. **Loeb, L. A. and Kunkel, T. A.,** Fidelity of DNA synthesis, *Annu. Rev. Biochem.*, 52, 429, 1982.
226. **Kunkel, T. A.,** Exonucleolytic proofreading, *Cell*, 53, 837, 1988.
227. **Modrich, P.,** DNA mismatch correction, *Annu. Rev. Biochem.*, 56, 435, 1987.
228. **Kunkel, T. A. and Bebenek, K.,** Recent studies of the fidelity of DNA synthesis, *Biochim. Biophys. Acta*, 951, 1, 1988.
229. **Loeb, L. A. and Perrino, F. W.,** Fidelity of animal cell DNA polymerases, in *The Eukaryotic Nucleus*, Vol. 1, Strauss, P. R. and Wilson, S. H., Eds., Telford Press, Caldwell, NJ, 1990, 315.
230. **Wabl, M., Burrows, P. D., Von Gabain, A., and Steinberg, O.,** Hypermutation at the immunoglobulin heavy chain locus in a pre-beta-cell line, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 479, 1985.
231. **Kunkel, T. A., Hamatake, R. K., Motto-Fox, J., Fitzgerald, M. P., and Sugino, A.,** Fidelity of DNA polymerase I and the DNA polymerase I-DNA primase complex from *Saccharomyces cerevisiae*, *Mol. Cell. Biol.*, 9, 4447, 1989.

232. **Kunkel, T. A., Sabatino, R. D., and Bambara, R. A.,** Exonucleolytic proofreading by calf thymus DNA polymerase delta, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 4865, 1987.
233. **Phear, G., Nalbantoglu, J., and Meuth, M.,** Next-nucleotide effects in mutations driven by DNA precursor pool imbalance at the *aprt* locus of Chinese hamster ovary cells, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 4450, 1987.
234. **Loeb, L. A., Liu, P. K., and Fry, M.,** DNA polymerase-alpha: enzymology, function, fidelity and mutagenesis, *Prog. Nucleic Acid Res.*, 33, 57, 1986.
235. **Perrino, F. W. and Loeb, L. A.,** Differential extension of 3′-mispairs is a major contribution to the high fidelity of calf thymus DNA polymerase-alpha, *J. Biol. Chem.*, 264, 2898, 1989.
236. **Perrino, F. W. and Loeb, L. A.,** Hydrolysis of 3′-terminal mispairs *in vitro* by the 3′-5′ exonuclease of DNA polymerase delta permits subsequent extension by DNA polymerase alpha, *Biochemistry*, 29, 5227, 1990.
237. **Derbyshire, V., Freemont, P. S., Sanderson, M. R., Beese, L., Friedman, J. M., Joyce, C. M., and Steitz, T. A.,** Genetic and crystallographic studies of the 3′-5′ exonucleolytic site of DNA polymerase I, *Science*, 240, 199, 1988.
238. **Bernad, A., Blanco, L., Lazaro, J. M., Martin, G., and Salas, M.,** A conserved 3′-5′ exonuclease active site in prokaryotic and eukaryotic DNA polymerases, *Cell*, 59, 219, 1989.
239. **Brooke, R. G., Singhal, R., Hinckle, D. C., and Dumas, L. B.,** Purification and characterization of the 180- and 86-kilodalton subunits of the *Saccharomyces cerevisiae* DNA primase-DNA polymerase protein complex, *J. Biol. Chem.*, 266, 3005, 1991.
240. **Roberts, J. D. and Kunkel, T. A.,** Fidelity of a human cell DNA replication complex, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 7064, 1988.
241. **Hauser, J., Levine, A. S., and Dixon, K.,** Fidelity of DNA synthesis in a mammalian *in vitro* replication system, *Mol. Cell. Biol.*, 8, 3267, 1988.
242. **Kunkel, T. A. and Soni, A.,** Exonucleolytic proofreading enhances the fidelity of DNA synthesis by chick embryo DNA polymerase gamma, *J. Biol. Chem.*, 263, 4450, 1988.
243. **Kaguni, L. S. and Olson, M. W.,** Mismatch-specific 3′-5′ exonuclease associated with mitochondrial DNA polymerase from *Drosophila* embryos, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 6469, 1989.
244. **Insdorf, N. F. and Bogenhagen, D. G.,** DNA polymerase gamma from *Xenopus laevis*. II. A 3′ to 5′ exonuclease is tightly associated with the DNA polymerase activity, *J. Biol. Chem.*, 264, 21498, 1989.
245. **Zhang, J., Chung, D., Tan, C.-K., Downey, K. M., Davie, E. W., and So, A. G.,** Primary structure of the catalytic subunit of calf thymus DNA polymerase delta: sequence similarities with other DNA polymerases, in press.
246. **Alberts, B. M.,** Recipes for replication, *Nature*, 346, 514, 1990.
247. **Sinha, N. K., Morris, C. F., and Alberts, B. M.,** Efficient replication of double-stranded DNA templates by a purified T4 bacteriophage replication system, *J. Biol. Chem.*, 255, 4290, 1980.