22

SV40 and Polyomavirus DNA Replication

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The papovavirus family comprises two virus subgroups: the polyomaviruses and the papillomaviruses. The polyomavirus subgroup consists of a dozen members and includes simian virus 40 (SV40), the murine polyomavirus (PyV), and two human isolates, JCV and BKV (for review, see Tooze 1980). The polyomaviruses display strict species specificity for their replication, but otherwise share a common architecture, genome organization, and replication cycle. SV40 replicates in simian cells and to a lesser extent in human cells, whereas PyV replicates in cells of murine origin. The circular double-stranded DNA genomes of SV40 and PyV comprise about 5300 bp. The viral DNA is assembled into chromatin both in virions and in infected cells, and apart from the absence of histone H1, it is structurally indistinguishable from that of the cell.

This review focuses principally on the replication of SV40 DNA and draws on the PyV literature to highlight differences between these two papovaviruses where appropriate. Several excellent reviews on this subject have appeared recently (DePamphilis and Bradley 1986; Challberg and Kelly 1989; Stillman 1989; Hurwitz et al. 1990; Melendy and Stillman 1992). We refer the reader to these and to other chapters in this volume for more in-depth discussion of topics we are unable to describe here and for more comprehensive citations to the primary literature.

Since their discovery in the late 1950s, SV40 and PyV have served as paradigms to understand cellular processes as diverse as DNA replication, transcription, and oncogenic transformation. The rationale for employing these viruses as models for DNA replication is mainly that their simple genomes are replicated by mechanisms similar to those of the cell, principally through the action of cellular enzymes. Only a single viral protein, large tumor (T) antigen, is required for viral DNA replica-

DNA Replication in Eukaryotic Cells

tion. Furthermore, like cellular DNA, the viral genome is packaged into chromatin, and its replication occurs only during the S phase of the cell cycle.

Large T antigen, the viral initiator protein for DNA replication, is a sequence-specific DNA-binding protein that also possesses intrinsic DNA helicase and ATPase activity. These activities of large T antigen are required directly for its ability to effect viral DNA replication. Large T antigen also functions indirectly to effect viral DNA replication by stimulating the synthesis of cellular enzymes whose activity is needed for viral DNA replication. For example, large T antigen binds to $p105^{Rb}$/E2F complexes, resulting in the release and activation of the transcription activator E2F, whose target genes encode enzymes such as thymidine kinase, dihydrofolate reductase, and DNA polymerase-α:primase.

Initiation of viral DNA replication takes place within the origin (*ori*), proceeds bidirectionally, and terminates when the two replication forks meet approximately 180° from where they started. Replication of the leading strand occurs by a continuous mechanism, whereas that of the lagging strand proceeds discontinuously (see Brush and Kelly, this volume). Hence, both SV40 and PyV DNA replication are semidiscontinuous. Large T antigen binds to repeats of its recognition element within *ori* and unwinds this region to create a replication bubble. Large T antigen also interacts with cellular replication proteins to assemble the primosome at *ori* and functions as a helicase at replication forks.

Replication of the viral genome requires the interplay among *cis*-acting sequences defining the functional *ori*, large T antigen, and a number of cellular proteins, including transcription activators and replication proteins. In the sections that follow, we summarize what is known about these three major components of the replication apparatus and suggest a model by which their interaction results in the replication of the viral genome.

VIRAL SEQUENCES AND PROTEINS REQUIRED FOR DNA REPLICATION

Origin for DNA Replication

The genetically defined *ori* for SV40 or PyV DNA replication is located in the noncoding region of the viral genome intermingled with early and late promoter elements (for review, see DePamphilis 1988). It comprises two functional components: a core region, referred to as *ori*-core (the minimal origin), and auxiliary regions (Fig. 1). *Ori*-core is required for replication under all conditions and encompasses the sites where DNA replication initiates (origin for bidirectional DNA replication [OBR]).

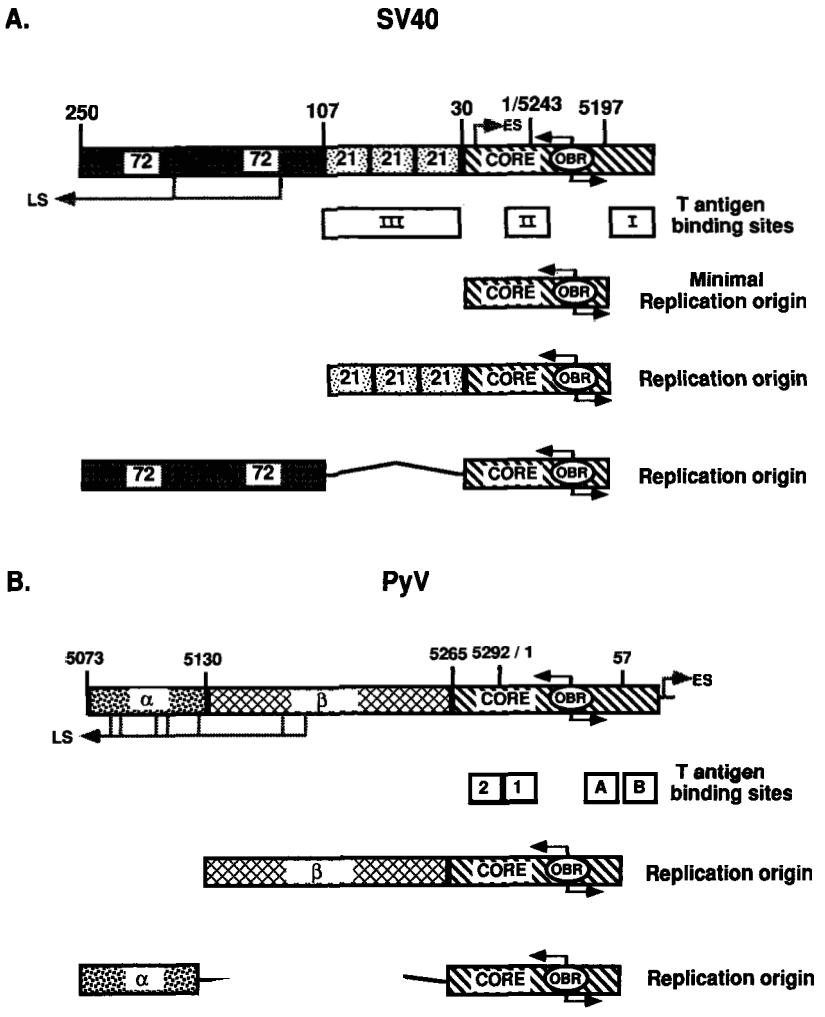

Figure 1 (*A*) Structure of the SV40 *ori* for DNA replication. The locations of the 72-bp repeat enhancer and the 21-bp repeat upstream promoter elements are shown. The *ori*-core contains the *ori* of bidirectional DNA replication (OBR). Stippled arrowheads represent the principal late (LS) and early (ES) start sites for transcription. The locations of the three large-T-antigen-binding sites are displayed below the origin region. The SV40 *ori*-core is sufficient to constitute a minimal *ori* for DNA replication in vivo; efficient replication *ori*s are composed of the *ori*-core and either the 21-bp repeats or the 72-bp repeats. (*B*) Structure of the PyV *ori* for DNA replication. The positions of the α and β elements of the PyV enhancer are shown. A functional PyV replication *ori* requires the *ori*-core and either the α or the β element located at the late border of *ori*-core.

The auxiliary regions increase the efficiency and cell-type specificity of viral DNA replication. The extent to which auxiliary elements enhance DNA replication differs between SV40 and PyV: SV40 DNA replication is stimulated 5- to 25-fold by these elements, whereas PyV DNA replication is enhanced between 100- and 1000-fold (Hassell et al. 1986).

The *ori*-core of each virus is about 65 bp and comprises three sets of sequences: an A/T-rich stretch at the late border; a central G/C-rich palindrome, and a region referred to as the early palindrome at the early border (Fig. 2) (Parsons et al. 1990 and references therein). All polyomaviruses, including distantly related members like SV40 and PyV, display significant sequence similarity in their *ori*-core. The A/T-rich stretch flanks the late border of the core and comprises 20 bp including a string of 8 consecutive A and corresponding T residues on each strand. The number of these residues is critical for *ori* function; deletion of only a few base pairs from this region compromises replication in vivo and in vitro (Gerard and Gluzman 1986). In SV40, the early TATA transcriptional element is located within the A/T-rich region. However, the PyV early TATA box is not found within its *ori*-core, suggesting that this transcriptional motif per se is not required for *ori* function. The A/T-rich region is a natural site of DNA bending (Deb et al. 1986); the binding of large T antigen to the central palindrome in the *ori*-core further distorts the structure of this region (Parsons et al. 1990).

The central 30-bp palindrome contains the four pentanucleotide (5′-GAGGC-3′) large-T-antigen-binding sites (DeLucia et al. 1983; Pomerantz and Hassell 1984). The sequence, arrangement, and spacing of these pentanucleotides are critical for *ori* function (Dean et al. 1987; Deb et al. 1987). This is consistent with the preservation of these features in the *ori*-cores of all polyomavirus subgroup members. The large-T-antigen-binding sites within the central palindrome are arranged differently than are those outside this region: The pentanucleotide-binding sites outside the *ori*-core are oriented in the same direction, whereas those within *ori*-core are arranged as two inverted pairs (Fig. 2). The arrangement of these pentanucleotide motifs may determine whether interaction of large T antigen with these sequences results in initiation of DNA replication or repression of early transcription.

The approximately 20-bp early palindrome comprises the remainder of *ori*-core. This sequence too is conserved among those polyomaviruses that replicate in cells from the same or similar species (i.e., monkey viruses SV40 and SA17, and the human papovaviruses BK and JC). Primer mapping experiments suggest that the early palindrome lies near the center of the replication bubble (Hay and DePamphilis 1982;

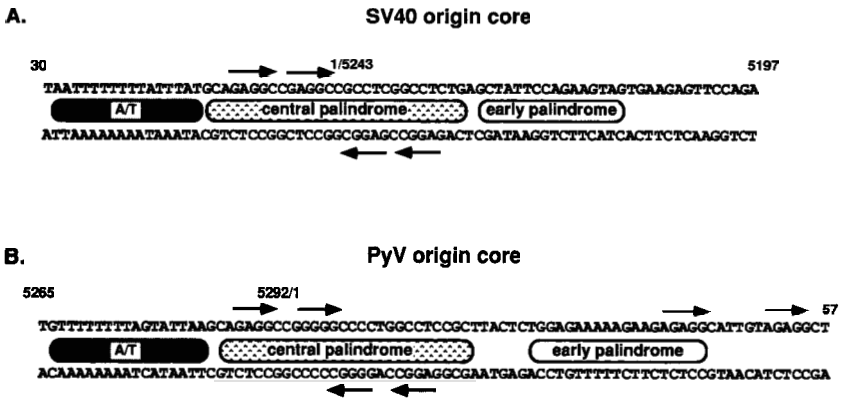

Figure 2 Sequence of the SV40 (*A*) and PyV (*B*) *ori*-cores for DNA replication. The three principal sequence elements of the *ori*-core are shown: the A/T-rich region at the late border, the central G/C-rich palindrome, and the early palindrome at the early border. Arrowheads depict the position and orientation of the 5′-GAGGC-3′ large-T-antigen-binding motifs.

Hendrickson et al. 1987). This region may be functionally analogous to the DNA unwinding element (DUE) first recognized in yeast *ori*s (Lin and Kowalski 1994). Surprisingly, at high concentrations, large T antigen is capable of binding with specificity to the early palindrome despite the absence of a recognizable pentanucleotide element (Parsons et al. 1990).

Two classes of auxiliary elements constitute the replication *ori*s of SV40 and PyV. One class comprises large-T-antigen-binding sites. In SV40 these sites flank both borders of *ori*-core (sites I and III); in PyV this class of auxiliary elements maps the early border of the PyV *ori*-core (sites A and B) (Fig. 1). The affinity of large T antigen for these sites in vitro exceeds or parallels that for those binding sites within *ori*-core. Hence, this class of auxiliary elements may stimulate DNA replication by recruiting large T antigen to the vicinity of *ori*-core and thereby facilitate large-T-antigen-mediated *ori* unwinding (Gutierrez et al. 1990). Deletion of site I in SV40 reduces *ori* activity but does not affect bidirectionality of DNA replication (Guo et al. 1991).

The other category of auxiliary elements flanks the late border of each *ori*-core; these elements are coincident with the transcriptional motifs that make up the viral enhancer and promoters (Hassell et al. 1986; Muller et al. 1988). Auxiliary elements of this type augment the replicative potential of the *ori*-core to a much greater extent than do those composed of large-T-antigen-binding sites (for review, see DePamphilis and Bradley 1986; DePamphilis 1988, 1993). There are several functionally

redundant sets of sequences in both the SV40 and PyV regulatory regions that can act as auxiliary elements (Fig. 1). For example, either one of two regions, termed alpha or beta, functions as an auxiliary element for PyV DNA replication (Muller et al. 1988). Similarly, the 21-bp or the 72-bp repeat of SV40 can functionally substitute for each other in the SV40 *ori*. Furthermore, the SV40 auxiliary elements can substitute for those of PyV. Indeed, repeats of many different types of transcriptional elements can replace the PyV auxiliary regions. Notably, binding sites for GAL4, a transcriptional activator from budding yeasts, can act as auxiliary elements for PyV DNA replication as long as GAL4 is provided in *trans* (Bennett-Cook and Hassell 1991; Guo and DePamphilis 1992). Both the DNA-binding domain of GAL4 and its transcription activation domains are required to enhance viral DNA replication (Bennett-Cook and Hassell 1991). GAL4 chimeras bearing a diversity of activation domains from other transcription factors are also capable of activating PyV DNA replication, albeit to differing extents, suggesting that this property is not unique to particular classes of activation domains (Bennett-Cook and Hassell 1991; Guo and DePamphilis 1992; B.T. Brinton et al., in prep.). In this regard, it is of interest that the activation domains of three different transcription activators bind to replication protein A (RP-A), a critical component of the preinitiation complex (He et al. 1993; Li and Botchan 1993).

Several mechanisms have been suggested to account for the ability of transcriptional activators to stimulate SV40 and PyV DNA replication (for review, see DePamphilis; van der Vliet; both this volume). These include relieving the repressive effects of nucleosomes (Cheng and Kelly 1989; Cheng et al. 1992), stimulating the binding and/or the activity of large T antigen at *ori* (Guo et al. 1989; Gutierrez et al. 1990), and recruiting RP-A and/or enhancing its function at *ori* (He et al. 1993; Li and Botchan 1993). These mechanisms are not mutually exclusive; the recruitment of RP-A to *ori* by transcription activators could enhance large T antigen's ability to unwind the *ori*-core.

Large T Antigen

The large T antigens of SV40 and PyV are multifunctional, nuclear phosphoproteins (for review, see Prives 1990; Fanning 1992; Fanning and Knippers 1992; Pipas 1992; Manfredi and Prives 1994). SV40 and PyV large T antigen are composed of 708 and 785 amino acids, respectively. These two proteins are related in primary structure (~36% sequence identity) and share the same biochemical activities (Fig. 3). These

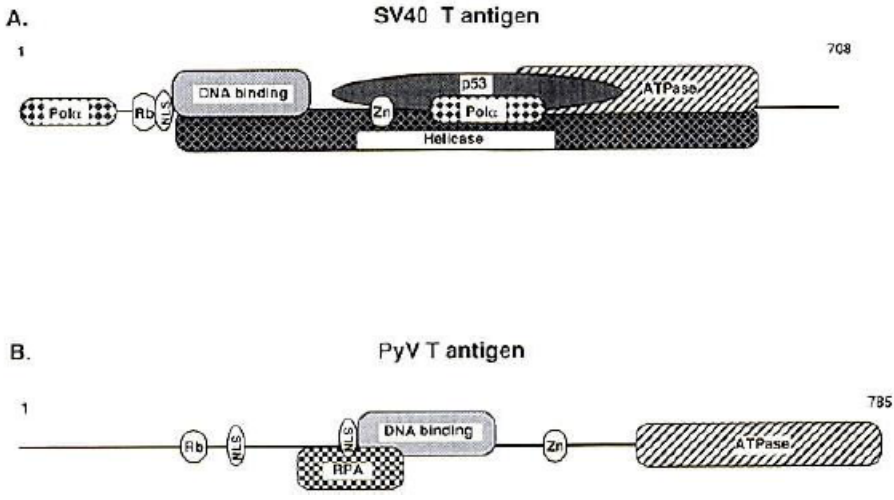

Figure 3 Schematic representation of the location of protein-binding sites, sequence motifs, and functional domains on a linear representation of SV40 (*A*) and PyV (*B*) large T antigen. Two regions in SV40 T antigen are required for interaction with DNA polymerase-α:primase (polα). Regions required for large T antigen binding to $p105^{Rb}$ (Rb), RP-A, and p53 are also shown. The nuclear localization signals (NLS) and putative Zn finger (Zn) are illustrated. The minimal functional domains of large T antigen required for DNA binding, and ATPase and helicase activity are shown. The regions in PyV large T antigen required for binding to the various subunits of DNA polymerase-α:primase have not been identified, nor has its helicase domain been localized. Similarly, the domain in SV40 large T antigen required for interaction with RP-A has not been reported. The first and last amino acids in each large T antigen are numbered.

include sequence-specific double-stranded and single-stranded DNA binding, and ATPase and helicase activity. Discrete functional domains located in similar regions of the two proteins are required to mediate these various functions. In SV40 large T antigen, the sequence-specific DNA-binding domain occupies 115 amino acids between residues 135 and 249 (for review, see Pipas 1992), whereas the corresponding domain in PyV large T antigen maps to a 116-amino-acid stretch between residues 282 and 398 (Sundstrom et al. 1991). Interestingly, both large T antigens recognize and bind to the same pentanucleotide sequence motif, 5′-G(A/G)GGC-3′(Pomerantz and Hassell 1984). This is consistent with the fact that their DNA-binding domains display considerable sequence similarity (Sundstrom et al. 1991). A solitary pentanucleotide is not sufficient for large T antigen binding; rather, repeats of this motif, as occur in the various large-T-antigen-binding sites in the viral genome,

are required for efficient sequence-specific DNA binding (Pomerantz and Hassell 1984). The binding of large T antigen to its recognition sites is also influenced by the nature and composition of the spacer region between pentanucleotide repeats (DeLucia et al. 1983; Dean et al. 1987; Deb et al. 1987). Large T antigen also binds to single-stranded DNA; this activity may be integral to its ability to unwind DNA at the replication fork (for review, see Fanning and Knippers 1992). Large T antigen hydrolyzes ATP, an activity that is required to act as a DNA helicase. The ATP-binding/ATPase domain of large T antigen occupies a region within the carboxyl terminus of the protein. Unlike many other helicases, large T antigen unwinds double-stranded *ori* DNA by translocating in a 3′to 5′direction along the leading-strand template to advance the replication fork (Goetz et al. 1988; Wiekowski et al. 1988).

A number of cellular proteins interact with SV40 and PyV large T antigens (Fig. 3). These include the DNA replication proteins, DNA polymerase-α:primase and single-stranded DNA-binding protein RP-A, and transcription regulatory proteins $p105^{Rb}$ and p53. Large T antigen also contains one (SV40) or more (PyV) nuclear localization signals (NLS) and several conserved sequence motifs associated with its replication functions (Fig. 3). One highly conserved sequence element, HPDKGG, in the amino-terminal region, is present in 11 of the 12 polyomavirus subgroup large T antigens. This sequence is known to be required for SV40 DNA replication (Peden and Pipas 1992; Pipas 1992); it may bridge the association of large T antigen with heat shock proteins (T. Roberts, pers. comm.). Another sequence element conserved among the large T antigens is a putative Zn-finger region (Zn) located between the DNA-binding and ATPase domains. This region is thought to be required for the formation of the double hexamer of large T antigen that assembles at the *ori*-core prior to the initiation of viral DNA replication (Deb and Tegtmeyer 1987; Mastrangelo et al. 1989; Loeber et al. 1991; Parsons et al. 1991).

The activity of SV40 large T antigen is regulated both positively and negatively by phosphorylation (for review, see Fanning 1994; see Weisshart and Fanning, this volume). Whereas large T antigen is phosphorylated in vivo at a total of nine serine and threonine residues clustered at its amino and carboxyl termini (Scheidtmann et al. 1991b), genetic (Schneider and Fanning 1988) and biochemical analyses suggest that the modification of only three of these sites, S120, S123, and T124, plays a critical role in regulating its activity (McVey et al. 1989, 1993; Cegielska et al. 1994). Phosphorylation of T124 positively influences the activity of large T antigen by increasing its ability to assemble into double

hexamers at *ori* and to bidirectionally unwind DNA (McVey et al. 1993; Moarefi et al. 1993). The cyclin-dependent kinase, cdc2, in association with cyclin B, phosphorylates T124 in vitro (McVey et al. 1989, 1993). Interestingly, the S-phase cyclin-dependent kinase, cyclin A/cdk2, also phosphorylates large T antigen at T124. Cyclin A/cdk2 is physically associated with large T antigen in SV40-infected cells (Adamczewski et al. 1993). This and the fact that SV40 and PyV DNA replication takes place only during S phase when cyclin A/cdk2 is active suggest that this cyclin-dependent kinase may be one of the physiologically relevant kinases that regulates large T antigen activity (Adamczewski et al. 1993).

Phosphorylation of S120 and S123 negatively regulates the replicative capacity of large T antigen in vitro (Scheidtmann et al. 1991b; Virshup et al. 1992; Cegielska et al. 1994). Paradoxically, genetic analyses imply that phosphorylation of these residues is required for large T antigen function because their mutation to alanine results in an inactive large T antigen (Schneider and Fanning 1988). These findings can be reconciled if phosphorylation-dephosphorylation of these sites plays a regulatory role during the virus life cycle, and if this is not manifest in the in vitro DNA replication system. An isoform of casein kinase I (Cegielska and Virshup 1993) phosphorylates these sites in vitro, and the catalytic subunit of protein phosphatase 2A dephosphorylates them (Sheidtmann et al. 1991a). It is still unclear whether these enzymes regulate the activity of large T antigen in vivo; neither is known to be regulated during the cell cycle, and one of them (phosphatase 2A) is located principally in the cytoplasm.

The capacity of large T antigen to assemble into double hexamers and to unwind *ori* DNA is regulated by phosphorylation, but the helicase activity of large T antigen is unaffected by this modification (Virshup et al. 1992; Moarefi et al. 1993; McVey et al. 1993). Helicase activity measures the ability of a single hexamer of large T antigen to unwind a partially single-stranded template, whereas *ori* unwinding requires that a double hexamer of large T antigen unwinds double-stranded *ori* DNA bidirectionally. Electron microscopic studies suggest that DNA unwinding is accomplished by reeling the DNA through the double-hexamer complex rather than by independent progress of each hexamer bidirectionally from the center of *ori*-core (Wessel et al. 1992; Fanning 1994).

CELLULAR PROTEINS REQUIRED FOR VIRAL DNA REPLICATION

The development by Li and Kelly (1984) of an in vitro system capable of faithfully replicating SV40 DNA represented a significant breakthrough.

This system has been used to identify numerous cellular replication proteins, thus providing insight into the mechanism and regulation of viral and cellular DNA replication. To date, a minimum of ten cellular proteins have been identified that are required together with large T antigen and *ori*-bearing plasmids to replicate SV40 DNA in vitro (Table 1). These proteins form complexes that act cooperatively to accomplish the replication of the viral genome. Examples of such macromolecular assemblies include the preinitiation complex or primosome comprising large T antigen, RP-A and DNA polymerase-α:primase, an elongation complex composed of replication factor C (RF-C), proliferating cell nuclear antigen (PCNA), and DNA polymerase-δ and a maturation complex constituting maturation factor I, ribonuclease H1, and DNA ligase I. Elucidation of the structure and function of these proteins has led to the realization that distantly related organisms employ structurally related proteins to achieve replication of their genomes by similar mechanisms (see Stillman, this volume).

Replication Protein A

RP-A, which is also known as replication factor A (RFA) and human single-stranded DNA-binding protein (HSSB), was originally identified as an essential single-stranded DNA-binding protein required for SV40 DNA replication in vitro (Wobbe et al. 1987; Wold and Kelly 1988; Fairman and Stillman 1988). RP-A functions in concert with large T antigen and DNA polymerase-α:primase to initiate DNA replication at *ori*. RP-A is also needed during the elongation phase, acting to stimulate the activity of both DNA polymerase-α:primase and DNA polymerase-δ at replication forks (for review, see Melendy and Stillman 1992). The subunit composition of RP-A and the sequence of each of its subunits are highly conserved among diverse species. Gene disruption experiments in the budding yeast *Saccharomyces cerevisiae* demonstrate that each subunit is required for viability (Heyer et al. 1990; Brill and Stillman 1991). In this organism, and likely all eukaryotes, RP-A participates not only in DNA replication, but also in recombination and repair (Longhese et al. 1994; Firmenich et al. 1995; Smith and Rothstein 1995).

Human RP-A is a heterotrimer of 70-kD, 32-kD, and 14-kD subunits (Fairman and Stillman 1988; Wold and Kelly 1988). The 70-kD subunit is independently capable of binding to single-stranded DNA (Brill and Stillman 1989; Wold et al. 1989; Kenny et al. 1990), to DNA polymerase-α:primase (Dornreiter et al. 1992), and to the activation domains of several transcription activators (He et al. 1993; Li and

Table 1 Proteins required for SV40 DNA replication in vitro

Factor	Molecular mass (kD)	Function
Large T antigen	90	origin recognition protein; unwinds DNA; helicase; primosome loading protein
RP-A	70 32 14	binds single-stranded DNA; promotes origin unwinding; stimulates DNA polymerase-α:primase; cooperates with RF-C and PCNA to stimulate DNA polymerase-δ
DNA polymerase-α:primase	180 70 58 48	initiates leading- and lagging-strand synthesis
DNA polymerase-δ	125 48	completes leading- and lagging-strand synthesis
DNA polymerase-ε	255 55	potential DNA polymerase for completing lagging-strand synthesis
RF-C	140 40 38 37 36.5	auxiliary factor for DNA polymerase-δ and ε; loads PCNA onto DNA; DNA-dependent ATPase
PCNA	36	auxiliary factor for DNA polymerase-δ and ε, increases processivity
Topoisomerase I	110	relieves torsional strain in front of replication forks
Topoisomerase IIa Topoisomerase IIb	170 180	segregates progeny strands by removing catenated intertwines behind forks
MF-I	45	5′→3′ exonuclease removes terminal monoribonucleotide from Okazaki fragments
RNase H1	89	endonuclease; cleaves RNA primer
DNA ligase I	125	ligates Okazaki fragments

Botchan 1993). No activity or function has yet been ascribed to the other two subunits, but the 70-kD subunit is incapable of substituting for the trimeric protein in SV40 DNA replication, implying that one or both of them are important in this process (Erdile et al. 1991). Antibodies specific for each of the subunits inhibit SV40 DNA replication in vitro, suggesting that all the subunits are required (Erdile et al. 1990, 1991; Kenny et al. 1990; Umbricht et al. 1993). The 32-kD and 14-kD subunits form a complex, which may serve to recruit the larger subunit (Hendrickson et al. 1994).

The 32-kD species from budding yeast and humans is phosphorylated in a cell-cycle-dependent manner (Din et al. 1990). Phosphorylation occurs at the G_1 to S-phase transition, and dephosphorylation occurs during mitosis. The kinase(s) responsible for these phosphorylation events in vivo is not known, but both cyclin B/cdc2 (Dutta and Stillman 1992) and cyclin A/cdk2 phosphorylate RP-A in vitro (Elledge et al. 1992; Pan et al. 1993). Exposure of human cells to ionizing radiation (Liu and Weaver 1993) or UV light also results in phosphorylation of the 32-kD subunit (Carty et al.1994). During SV40 replication in vitro, RP-A is phosphorylated by a DNA-activated kinase (Fotedar and Roberts 1992; Brush et al. 1994; Hendrickson et al. 1994). However, none of these phosphorylations demonstrably affects the activity of RP-A as assessed in vitro. Indeed, mutation of the sites in the 32-kD subunit that are targets for the cyclin-dependent kinases does not alter the capacity of RP-A to participate in SV40 DNA replication in vitro (Hendrickson and Wold 1994). Hence, phosphorylation of RP-A may affect a function that has yet to be determined or cannot be observed in the cell-free replication system.

RP-A binds to large T antigen in a partially species-specific manner. For example, SV40 large T antigen binds to vertebrate sources of RP-A, but not to yeast RP-A (Dornreiter et al. 1992; Schneider et al. 1992; Melendy and Stillman 1993). This likely accounts for the fact that all mammalian sources of RP-A tested functionally substitute for human RP-A in SV40 DNA replication, albeit to differing extents (Schneider et al. 1992). In general, the greater the relatedness between the species of origin of RP-A and that in which the virus replicates, the greater the affinity of large T antigen for that particular source of RP-A (Schneider et al. 1992). Hence, yeast and trypanosome RP-A cannot functionally substitute for human, simian, or bovine sources of RP-A to replicate SV40 DNA in vitro (Brill and Stillman 1989; Brown et al. 1992; Schneider et al. 1992; Melendy and Stillman 1993). The RP-A subunit that contacts large T antigen has not been identified. However, the large subunit alone is insufficient to mediate this interaction (Dornreiter et al. 1992). The region in SV40 large T antigen required for binding RP-A also has not been determined; a region in PyV large T antigen that overlaps with its DNA-binding domain interacts with human and mouse RP-A in vitro (Fig. 3) (P. Desjardins and J.A. Hassell, unpubl.).

RP-A binds in vitro to the activation domains of at least three transcription activators, including that of GAL4, VP16, and p53 (He et al. 1993; Li and Botchan 1993). The 70-kD subunit is sufficient for direct binding to the activation domains of these transcription factors. The physiological relevance of these findings is suggested by the observation

that transcription activators are required for efficient replication of SV40 and PyV DNA in vivo (see DePamphilis; van der Vliet; both this volume). Thus, the binding of transcription activators to the *ori* auxiliary elements may recruit and/or stabilize the interaction of RP-A with the *ori* through protein:protein interactions, thereby facilitating assembly of the primosome. Alternatively, the function of RP-A at this site may be stimulated by direct contact with transcription activators. RP-A also interacts physically with DNA polymerase-α:primase, which is required to initiate SV40 and PyV DNA replication. These various protein:protein interactions are consistent with a role for RP-A in the initiation of DNA replication.

DNA Polymerase-α:Primase

DNA polymerase-α:primase is the only DNA polymerase in mammalian cells capable of initiating DNA synthesis de novo through the synthesis of RNA primers (for review, see Wang 1991 and this volume). Studies with inhibitors and inactivating antibodies to the protein reveal that it is required to replicate SV40 and PyV DNA (Melendy and Stillman 1992 and references therein). Currently, it is thought that the role of DNA polymerase-α:primase is to initiate both leading-strand synthesis at the *ori* and Okazaki fragment synthesis on the lagging-strand template at replication forks (Waga and Stillman 1994a; Waga et al. 1994 and references therein).

Human DNA polymerase-α:primase is composed of four subunits of molecular mass 180, 70, 58, and 48 kD; the subunits of the mouse protein are similar in size (180, 68, 54, and 46 kD) (Wang 1991; Miyazawa et al. 1993). DNA polymerase activity is intrinsic to the 180-kD subunit, whereas primase activity resides in the 48-kD subunit (Copeland and Wang 1993). The 58-kD subunit binds to the 48-kD subunit and appears to stabilize the primase activity of the smaller subunit. The role of the 70-kD subunit is not clear. It associates with the 180-kD subunit (Collins et al. 1993) and is phosphorylated in a cell-cycle-dependent manner, suggesting that it may play a regulatory role (Nasheuer et al. 1991). These attributes of the human DNA polymerase-α:primase subunits are conserved in their murine counterparts.

The replication of SV40 and PyV DNA is highly species-specific (Bennett et al. 1989 and references therein). SV40 DNA replication takes place in monkey or human cells, but not in mouse cells. In contrast, mouse cells, but not primate cells, support the replication of PyV DNA. Species-specific DNA replication in vivo requires large T antigen, the

cognate virus *ori*, and factors from permissive cells (Bennett et al. 1989). Cell-free replication systems recapitulate this species specificity and have proven invaluable in identifying permissive factors. The addition of purified mouse DNA polymerase-α:primase to human cell extracts renders them permissive for PyV DNA replication, and correspondingly, the addition of the human enzyme to mouse cell extracts allows the mouse extracts to support SV40 DNA replication (Murakami et al. 1986). These experiments suggest that DNA polymerase-α:primase is the primary host determinant for species-specific viral DNA replication. Further fractionation of the DNA polymerase revealed that the primase subunits (a heterodimer of the 54-kD and 46-kD subunits) from mouse DNA polymerase-α:primase enabled human extracts to replicate PyV DNA (Eki et al. 1991). Extension of these experiments with DNA polymerase-α:primase, reconstituted in insect cells after infection with recombinant baculoviruses encoding individual subunits of human or mouse origin, showed that the small mouse 46-kD primase subunit is sufficient to mediate species-specific replication of PyV DNA in vitro in human cell extracts (Bruckner et al. 1995). Surprisingly, similar experiments with these reconstituted DNA polymerase-α:primase enzymes of mixed human-mouse origin showed that SV40 DNA replication in vitro required a different subunit, namely, the p180 catalytic subunit of the DNA polymerase from human cells (Stadlbauer et al. 1996). Presumably, this reflects a difference in the functional interaction between the DNA polymerase and the large T antigen of each virus.

The large T antigens of both SV40 and PyV physically interact with several of the subunits of DNA polymerase-α:primase in vitro (Smale and Tjian 1986; Gannon and Lane 1987; Dornreiter et al. 1990; Moses and Prives 1994: Schneider et al. 1994; Bruckner et al. 1995). SV40 large T antigen binds independently to both the 180-kD and the 70-kD subunits of human DNA polymerase-α:primase (Dornreiter et al. 1992; Collins et al. 1993), although the principal interaction appears to be with the 70-kD subunit (Collins et al. 1993). Amino acids 195–313 in the 180-kD subunit (Dornreiter et al. 1993) and residues 1–239 of the 70-kD subunit are sufficient for interaction with SV40 large T antigen (Collins et al. 1993). Interestingly, two discontinuous regions in SV40 large T antigen are required to bind to the human holoenzyme (Fig. 3), but it is not known whether each region binds to a different subunit (Smale and Tjian 1986; Gannon and Lane 1987; Dornreiter et al. 1990). PyV large T antigen binds to the 180-kD subunit of mouse DNA polymerase-α:primase, to a complex containing the murine 180-kD and 68-kD subunits, and to the 46-kD primase subunit (Bruckner et al. 1995). It is not

known whether PyV large T antigen binds independently to the 68-kD subunit (Bruckner et al. 1995). Hence, the large T antigens of SV40 and PyV make contact with multiple subunits of DNA polymerase-α:primase, and at least one of these represents a functional species-specific interaction.

The binding of SV40 large T antigen to human DNA polymerase-α:primase increases both the primase and DNA polymerase activity of the holoenzyme (Collins and Kelly 1991; Erdile et al. 1991). This stimulatory effect requires the 70-kD subunit (Collins and Kelly 1991). Large T antigen is thought to bridge the interaction between DNA polymerase-α:primase and the DNA template, thereby facilitating DNA replication (Collins et al. 1993).

DNA polymerase-α:primase also binds to RP-A (Dornreiter et al. 1992; Melendy and Stillman 1993). This interaction occurs between the dimeric primase component of DNA polymerase-α:primase (the 48- to 58-kD complex) and the largest (70-kD) subunit of RP-A (Dornreiter et al. 1992). The association appears to be species-specific; single-stranded DNA-binding proteins encoded by prokaryotes and their viruses do not associate with mammalian sources of DNA polymerase-α:primase (Dornreiter et al. 1992). Furthermore, DNA polymerase-α:primase from human sources does not functionally interact with *Drosophila melanogaster* RP-A to effect SV40 DNA replication in a cell-free system (Kamakaka et al. 1994). These findings are consistent with reports demonstrating that only mammalian sources of RP-A function in the cell-free systems to replicate SV40 DNA. One consequence of the interaction of these proteins is the stimulation of the DNA polymerase and primase activity of DNA polymerase-α:primase on artificial DNA templates in vitro (Erdile et al. 1991; Melendy and Stillman 1993). Hence, multiple and species-specific protein:protein interactions occur among the three major protein components (large T antigen, RP-A, and DNA polymerase-α:primase) of the primosome, resulting in the stimulation of those enzymatic activities required for the initiation of DNA replication at *ori*.

Proliferating Cell Nuclear Antigen

The simultaneous demonstration that a chromatographic fraction (named replication factor B) required for SV40 DNA replication in vitro corresponded by many criteria, including sequence analysis, to a 36-kD protein known as proliferating cell nuclear antigen (PCNA) (Prelich et al. 1987), and that this protein is identical to a polymerase-δ auxiliary factor (Bravo et al. 1987; Prelich et al. 1987), led to the realization that DNA

polymerase-α:primase is not the only DNA polymerase required to replicate SV40 DNA. PCNA colocalizes with sites of active DNA replication, suggesting that it participates in cellular DNA replication. Although PCNA is synthesized shortly before the onset of cellular DNA replication and its abundance in the nucleus is highest during S phase, it is a stable protein with a half-life of about 20 hours that is present throughout the cell cycle. PCNA has been found in all eukaryotes that have been examined; its primary structure has been highly conserved among diverse species including yeasts, plants, and mammals. In *S. cerevisiae*, PCNA is essential for viability (Bauer and Burgers 1988).

Analyses of SV40 replication intermediates synthesized in vitro in the presence and absence of PCNA showed that it is not required for the synthesis of nascent DNA fragments at *ori*, but acts at the elongation phase to stimulate leading-strand synthesis (Prelich and Stillman 1988). It is thought that DNA polymerase-α:primase initiates the synthesis of nascent DNA in a reaction that does not require PCNA. As discussed below, RF-C recognizes the primer-template junction and loads PCNA onto the DNA, which in conjunction with DNA polymerase-δ effects the complete synthesis of the leading strand (Tsurimoto and Stillman 1990; Lee et al. 1991). Lagging-strand synthesis requires PCNA as well; here too, DNA polymerase-α:primase initiates synthesis that is completed by either DNA polymerase-δ or perhaps DNA polymerase-ε (Waga and Stillman 1994a). Hence, PCNA together with RF-C plays a critical role in coordinating leading- and lagging-strand synthesis (for review, see Stillman 1994).

PCNA is the first cellular protein component of the eukaryotic replicative machinery whose crystal structure has been determined (Kong et al. 1992; Krishna et al. 1994). It is a trimer, in keeping with the prediction of its molecular mass in solution (Bauer and Burgers 1988), and forms a donut-shaped closed circular ring that is thought to surround duplex DNA (Krishna et al. 1994). This structure fits nicely with its role as a sliding clamp that tracks along duplex DNA in conjunction with DNA polymerase-δ (for review, see Kelman and O'Donnell 1994; Stillman 1994). In this regard, it is noteworthy that there are striking structural and functional similarities between PCNA and the *Escherichia coli* β protein and bacteriophage T4 45 protein, which also function as sliding clamps during DNA replication in prokaryotes (see Stillman, this volume).

PCNA is required not only for DNA replication, but also for recombination (Jessberger et al. 1993) and repair (Shivji et al. 1989). DNA repair is mediated in part by DNA polymerase-ε (Nishida et al. 1988). A

functional interaction occurs between PCNA and DNA polymerase-ε (Burgers 1991; Lee et al. 1991; Podust et al. 1992). PCNA stimulates the binding of DNA polymerase-ε to DNA primer/template junctions and augments the rate of DNA synthesis on these templates (Maga and Hübscher 1995). Hence, PCNA appears to be an auxiliary protein for both DNA polymerase-δ and ε.

In mammalian cells, PCNA is found in a quaternary complex with components of the cell-cycle machinery. These complexes include a cyclin (A, B, D1, or D3), its cognate cyclin-dependent kinase (cdc2, cdk2, cdk4, or cdk5), and the 21-kD (p21) inhibitor of these kinases, also known as Cip1 or Waf 1 (Xiong et al. 1992, 1993; Zhang et al. 1993). PCNA interacts directly and independently with the carboxy-terminal region of p21 (Waga et al. 1994; Chen et al. 1995). In the SV40 in vitro system this interaction between PCNA and p21 is sufficient to inhibit DNA replication (Flores-Rozas al. 1994; Waga et al. 1994). p21 is a p53 target gene and, hence, under conditions of p53-mediated growth arrest, p21 may block cellular DNA replication by targeting PCNA for inactivation. In contrast to its ability to inhibit SV40 DNA replication in vitro, p21 does not inhibit DNA repair (Li et al. 1994). The repair synthesis of short gaps in duplex DNA by DNA polymerase-ε is insensitive to inhibition by p21, perhaps because under these circumstances, PCNA is needed principally to load the DNA polymerase onto the primer/template junction and not to stimulate DNA synthesis (Li et al. 1994). In this regard, it is noteworthy that PCNA also interacts directly with another p53 target gene product, GADD45, whose synthesis, like that of p21, increases in response to DNA damage (Smith et al. 1994). GADD45 may play a positive role in DNA repair. It has been suggested that GADD45 releases PCNA from p21/cdk complexes and facilitates its interaction with components of the DNA repair machinery of the cell (Smith et al. 1994). PCNA is not known to be modified posttranslationally, and hence its activity in DNA replication and repair appears to be regulated principally by interaction with other proteins such as p21 and GADD45. The physical interaction between PCNA and the p21-cyclin-dependent kinase inhibitor illustrates a novel means of regulating the activity of elements of the DNA replication machinery and of coordinating DNA replication and repair with cell-cycle progression.

Replication Factor C

Human RF-C (also called activator 1 or A1) is a multisubunit DNA polymerase accessory protein composed of five independent subunits of

molecular mass 140, 40, 38, 37, and 36.5 kD. RF-C functions in conjunction with PCNA to increase the DNA binding and processivity of DNA polymerases δ and ε (Tsurimoto and Stillman 1989; Lee et al. 1991). It binds to the 3′ terminus of DNA primers annealed to single-stranded DNA templates and, in a reaction requiring ATP, loads PCNA onto the DNA. This complex is recognized by DNA polymerase-δ or ε, which then elongates the primer (Lee and Hurwitz 1990; Tsurimoto and Stillman 1990, 1991b; Fien and Stillman 1992). Whereas PCNA can independently bind to the ends of linear DNA and clamp onto the template, its loading onto circular DNA requires RF-C (Podust et al. 1995). Interestingly, RF-C is similar in subunit composition, primary structure, and function to the 5-subunit "clamp loader" proteins of *E. coli* (γ complex) and phage T4 (gene 44/62 complex), suggesting that eukaryotic RF-C functions as a clamp loader or brace protein and that PCNA acts as a sliding clamp during DNA synthesis (O'Donnell et al. 1993; Kelman and O'Donnell 1994; Stillman 1994 and this volume).

RF-C possesses DNA-dependent ATPase activity, which is stimulated by PCNA and RP-A, and is required for its function in SV40 DNA replication (Tsurimoto and Stillman 1990, 1991a,b; Lee et al. 1991). ATPase activity is not required for translocation of the DNA polymerase but is needed to load PCNA onto DNA at replication forks (O'Donnell et al. 1993). The 40-kD subunit in the holoenzyme can be cross-linked to ATP, suggesting that this subunit possesses ATPase activity (Tsurimoto and Stillman 1991a). RF-C also recognizes and binds to primer/template junctions; this activity is stimulated by ATP and further stabilized by PCNA (Tsurimoto and Stillman 1990; Lee et al. 1991). The DNA-binding activity of the RF-C holoenzyme has been localized by UV cross-linking experiments to the 140-kD subunit (Tsurimoto and Stillman 1991a).

cDNAs encoding all the subunits of mammalian RF-C have been isolated and sequenced (Chen et al. 1992a,b; Bunz et al. 1993; Burbelo et al. 1993; O'Donnell, et al. 1993), and most recently, genes encoding all five subunits of *S. cerevisiae* RF-C have also been identified and analyzed (Howell et al. 1994; Li and Burgers 1994a,b; Noskov et al. 1994; Cullman et al. 1995). Gene disruption experiments in yeast reveal that each of the five subunits is essential for viability (Cullman et al. 1995 and references therein).

Biochemical analyses of the activities of the mammalian and yeast RF-C subunits expressed in *E. coli* have begun to shed light on their individual roles in DNA replication. Complete cDNAs have been isolated and the encoded proteins characterized for three of the mammalian sub-

units (140, 40, and 37 kD); only partial cDNAs are available for the 38-kD and 36.5-kD species. The 140-kD subunit binds to double-stranded oligonucleotides likely by recognizing a structure that mimics a primer/template junction (Tsurimoto and Stillman 1991a; Burbelo et al. 1993; Lu et al. 1993). The 37-kD subunit also is capable of binding to primer/template junctions, but its specific DNA-binding activity is only 0.1% that of the RF-C holoenzyme, and hence its role in primer binding is unclear (Pan et al. 1993; Noskov et al. 1994). Whereas all of the RF-C subunits bear the putative ATP-binding motif, only the 40-kD subunit is known to bind to ATP (Pan et al. 1993).

To accomplish its role in DNA replication, the RF-C subunits must interact with each other, with PCNA, and with DNA polymerase-δ and ε. To date, two interactions among the various RF-C subunits have been described: The 40-kD subunit associates with the 37-kD subunit in vitro (Pan et al. 1993), and the yeast Rfc4p subunit (equivalent to 40-kD mammalian subunit) binds to the Rfc3p subunit (the homolog of the mammalian 36.5-kD subunit) during coexpression in *E. coli*. Interaction of RF-C with PCNA is accomplished by direct binding of the latter to the 40-kD subunit (Pan et al. 1993). Genetic evidence in yeasts suggests that the Rfc1p subunit (homologous to the mammalian 140-kD subunit) interacts with PCNA (Howell et al. 1994). There is also indirect evidence supporting interactions between RF-C and DNA polymerase-δ and ε. For example, the isolated 37-kD subunit stimulates DNA polymerase-ε activity in vitro under high-salt conditions, and the 40-kD subunit inhibits DNA polymerase-δ activity (Pan et al. 1993). The molecular cloning of complete cDNAs encoding all the mammalian subunits, coupled with more complete analyses of their interaction with each other, with PCNA, and with the various subunits of DNA polymerase-δ and ε, should lend insight into the mechanism of action of this very important multisubunit enzyme.

DNA Polymerases δ and ε

The demonstration that PCNA stimulates SV40 DNA replication in vitro and that it acts as a processivity factor for DNA polymerase-δ strongly suggested that this DNA polymerase is required to replicate SV40 DNA. This prediction has been borne out by biochemical experiments demonstrating that DNA polymerase-δ effects leading-strand synthesis in vitro (Waga and Stillman 1994a and references therein). DNA polymerase-δ is a heterodimer comprising a 125-kD catalytic subunit and a 55-kD sub-

unit of unknown function (for review, see Linn 1991). Unlike DNA polymerase-α:primase, DNA polymerase-δ has 3′→5′ proofreading exonuclease activity. The gene encoding the catalytic subunits of *S. cerevisiae* and *Schizosaccharomyces pombe* DNA polymerase-δ has been cloned and shown to be required for viability (for review, see Campbell 1993; see also Wang, this volume). Human cDNAs encoding the catalytic subunit also have been isolated (Yang et al. 1992).

DNA polymerase-δ binds poorly to DNA, and hence PCNA is required to mediate its interaction with the DNA template. Deletion of the first 220 amino acids of the DNA polymerase-δ catalytic subunit from yeast renders it insensitive to PCNA stimulation, suggesting that this region is required for association with PCNA (Brown and Campbell 1993). Zhang et al. (1995) have provided direct evidence for an association between human DNA polymerase-δ and PCNA that is mediated by the amino-terminal 182 amino acids of the 125-kD DNA polymerase catalytic subunit.

The potential that DNA polymerase-ε might be required for SV40 and PyV DNA replication has begun to receive increased attention for a number of reasons. First, the gene encoding the catalytic subunit of DNA polymerase-ε is essential, and its protein product is required for chromosomal DNA replication in yeast (Morrison et al. 1990). Second, in vivo (Nethanel et al. 1988) and in vitro in unfractionated mammalian cell lysates with SV40 DNA as template, DNA polymerase-α:primase synthesizes DNA fragments less than 40 nucleotides in length, which is considerably shorter than Okazaki fragments (~200 nucleotides), implying that another DNA polymerase synthesizes these fragments (Bullock et al. 1991). These findings suggest that DNA polymerase-ε may be required for lagging-strand replication.

DNA polymerase-ε was first isolated from budding yeast (known as DNA polymerase II) and later from mammalian sources. Highly purified mammalian DNA polymerase-ε is composed of two subunits of molecular mass 215 and 55 kD, whereas the budding yeast enzyme comprises subunits of 255, 80, 34, 30, and 29 kD (for review, see Linn 1991; Hübscher and Spadari 1994). The mammalian enzyme may possess additional subunits that dissociate from the core DNA polymerase during purification. cDNAs encoding the large catalytic subunit of human DNA polymerase-ε have been cloned (Kesti et al. 1993), as have the genes encoding the 255-, 80-, 34-, and 30-kD subunits of the *S. cerevisiae* enzyme (for review, see Campbell 1993). The largest subunit possesses DNA polymerase and 3′→5′ exonuclease activity; the function of the smaller subunits is not known. Given the conservation of subunit com-

position among the various eukaryotic DNA polymerases, it seems likely that additional mammalian subunits of DNA polymerase-ε exist. It will be interesting to learn whether this is so and to determine what their role might be in DNA replication. One possibility is that these subunits are involved in coordinating leading- and lagging-strand synthesis at replication forks.

DNA polymerase-ε is similar to DNA polymerase-δ in possessing proofreading 3′→5′ exonuclease activity, but unlike the latter, it is processive in the absence of PCNA. Despite its processivity, DNA polymerase-ε interacts with PCNA and RF-C to form a stable complex at primer/template junctions (Burgers 1991); PCNA increases the rate of DNA synthesis by DNA polymerase-ε on synthetic primer templates in vitro (Maga and Hübscher 1995). Furthermore, DNA polymerase-ε activity is inhibited by physiological salt concentrations, but its activity is restored upon addition of RP-A, PCNA, and RF-C (Yoder and Burgers 1991). Hence, like DNA polymerase-δ, DNA polymerase-ε likely requires these auxiliary proteins for its proper functioning in vivo.

Topoisomerases I and II

DNA topoisomerases are enzymes that alter the topology of DNA (for review, see Watt and Hickson 1994; see Hangaard Andersen et al., this volume). They have been classified as types I and II according to their catalytic mechanism of action. Type I topoisomerases are nicking-closing enzymes that transiently cleave one strand of the DNA duplex, allowing the other strand to go through the nick before ligating the nicked strand. These enzymes do not require ATP for their action, but their activity is stimulated by Mg^{++} ions in vitro. Type II topoisomerases also transiently cleave DNA, but they make double-stranded breaks so that a double-stranded segment of DNA can go through the break before it is sealed. Type II enzymes require ATP for their activity. During SV40 DNA replication, topoisomerase I or II is required to unwind the positive supercoils that accumulate ahead of the replication forks (Yang et al. 1987), whereas topoisomerase II is needed for the decatenation (segregation) of the progeny DNA molecules after replication is completed (Yang et al. 1987; Ishimi et al. 1991).

Type I topoisomerases are single-chain enzymes that function as monomers. *S. cerevisiae* possesses two genes that encode type I topoisomerase; human cells have only a single gene. Neither of the yeast genes is essential for viability. The human enzyme has a measured

molecular mass of 100 kD and comprises 765 amino acids. Topoisomerase I functions not only in DNA replication and recombination, but also in transcription, where it may relax positive supercoils ahead of the transcribing RNA polymerases.

Topoisomerase II is encoded by a single essential gene in *S. cerevisiae* and two genes in mammals (topoisomerase IIa and IIb). Human topoisomerase IIa comprises 1530 amino acids (molecular mass 170 kD), whereas IIb is made up of 1621 amino acids (180 kD). Topoisomerase IIa is likely involved in chromosome condensation and separation of daughter chromosomes during mitosis; the function of topoisomerase IIb is not known, but this protein is localized primarily to the nucleolus.

Maturation Factor I, Ribonuclease H1, and DNA Ligase I

The complete synthesis of covalently closed circular SV40 DNA requires, in addition to the various cellular proteins described earlier, RNase H1, MF-I (also known as FEN-1), and DNA ligase I. These enzymes function coordinately to remove ribonucleotide primers from Okazaki fragments and to ligate these to form completed lagging strands (Turchi et al. 1994; Waga and Stillman 1994b; Waga et al. 1994).

RNase H specifically degrades the RNA strand of an RNA:DNA heteroduplex (for review, see Crouch 1990). In both prokaryotes and eukaryotes, RNase H is thought to remove RNA primers from Okazaki fragments prior to their ligation. This activity is also encoded by retroviruses, where it constitutes a domain in a dual domain protein that possesses reverse transcriptase activity. Two mammalian RNase H activities have been characterized; RNase H1 (~60–100 kD) and RNase H2 (~30–40 kD), but cDNAs encoding these enzymes have not yet been isolated (Busen 1980). A role for RNase H1 in DNA replication is suggested by the observation that its activity increases in accordance with mammalian cell DNA synthesis, and by the finding that RNase H1 purified from *D. melanogaster* stimulates DNA synthesis by DNA polymerase-α:primase and forms a complex with the DNA polymerase (DiFrancesco and Lehman 1985). Most recently, Huang et al. (1994) have provided compelling evidence that RNase H1 removes RNA primers from Okazaki fragments during DNA replication through an endonucleolytic cleavage of the RNA, resulting in its release intact from the DNA fragment. This leaves a monoribonucleotide at the RNA/DNA junction, which is removed by the exonuclease activity of MF-I.

MF-I is a single-subunit, double-strand specific, 5′→3′exonuclease that degrades either double-stranded DNA or the RNA moiety of an DNA/RNA heteroduplex from its 5′terminus (Ishimi et al. 1988; Goulian et al. 1990; Siegal et al. 1992; Turchi and Bambara 1993; Turchi et al. 1994; Waga et al. 1994). MF-I was described as a 44-kD activity from human HeLa cells required to achieve synthesis of form I SV40 DNA in vitro using solely DNA polymerase-α:primase as the replicative DNA polymerase (Ishimi et al. 1988). This enzyme is the same as three other exonucleases that have been described, including DNase IV (Lindahl 1970), AF-II (Goulian et al. 1987, 1990), pL (Guggenheimer et al. 1984; Kenny et al. 1988), and a 56-kD 5′→3′exonuclease found in association with DNA polymerase-ε from calf thymus (Siegal et al. 1992).). MF-I is required to synthesize covalently closed circular DNA using single-stranded circular DNA as template in a system with purified DNA polymerase-α:primase that emulates lagging-strand synthesis. Using synthetic templates, Turchi et al. (1994) have provided compelling evidence that MF-I functions in conjunction with RNase H1 to remove RNA primers from Okazaki fragments. Furthermore, Murante et al. (1994) have shown that MF-I activity is stimulated by DNA polymerase-α:primase, DNA polymerase-δ, and DNA polymerase-ε. The model that has emerged from these experiments is that RNase H1 makes an endonucleolytic cut within the RNA primer one nucleotide from the DNA/RNA junction of Okazaki fragments, the RNA primer dissociates as an intact species from the DNA template, MF-I removes the remaining monoribonucleotide, DNA polymerase-δ or ε fills in the gap using an adjacent Okazaki fragment as primer, and DNA ligase I seals the nick (Turchi et al. 1994). Hence, the combined action of these enzymes leads to the completion of lagging-strand synthesis and ultimately to the circularization of newly replicated SV40 DNA.

Four DNA ligases (DNA ligase I, II, III, and IV) have been identified in mammalian cells that can be discriminated principally on the basis of their biochemical and physical properties (Wei et al. 1995; see Nash and Lindahl, this volume). During SV40 DNA replication, DNA ligase I covalently joins Okazaki fragments arising from discontinuous lagging-strand synthesis (Ishimi et al. 1988; Waga et al. 1994). Neither mammalian DNA ligase III nor bacteriophage T4 DNA ligase can substitute for human DNA ligase I during SV40 DNA replication in vitro, suggesting that the mammalian enzyme makes specific protein:protein contacts with other replication factors (Waga et al. 1994). RF-C inhibits the activity of DNA ligase I in vitro, and hence these proteins may functionally interact during lagging-strand synthesis (Waga et al. 1994).

MODEL FOR SV40 AND PYV DNA REPLICATION

Knowledge of the structure of SV40 and PyV DNA replication intermediates occurring in vivo, and of the mechanism of action of the principal viral and cellular proteins that participate in DNA replication in vitro, suggests a model for replication of these viral genomes. The temporal sequence of events can be broadly defined as large T antigen binding to and unwinding of *ori*, assembly of the primosome, initiation of leading- and lagging-strand synthesis, DNA polymerase switching and elongation of leading and lagging strands, maturation of lagging DNA strands, and replication termination and segregation of the daughter DNA molecules.

The first presynthesis event leading to the initiation of DNA replication is the binding of large T antigen to the *ori*-core (for review, see Borowiec et al. 1990; see also SenGupta and Borowiec 1994 and references therein). Large T antigen binds as a monomer to each of the four pentanucleotide repeats within *ori*-core. Additional large T antigen binds to this complex and assembles into two hexamers that straddle each side of *ori*-core. Each hexameric complex contacts the sugar-phosphate DNA backbone on different strands. ATP stimulates the binding of large T antigen to *ori*-core and is required for the assembly of hexameric large T antigen, but hydrolysis of ATP is not needed for either process. Large T antigen then effects structural alterations in the *ori*-core, including untwisting of the A/T-rich region and melting of the early palindrome. RP-A joins the large-T-antigen/*ori* complex facilitating more extensive bidirectional unwinding of the *ori* through the intrinsic $3' \rightarrow 5'$ helicase activity of large T antigen. This process requires ATP hydrolysis and leads to the establishment of a replication bubble within the *ori*. It is noteworthy that whereas many different single-stranded DNA-binding proteins can facilitate these early reactions, subsequent events at *ori* require mammalian RP-A. RP-A may be recruited to *ori* through interaction with transcription factors bound to the auxiliary regions, by direct interaction with large T antigen bound to the *ori*-core, or by both mechanisms. The RP-A/large-T-antigen complex in turn recruits DNA polymerase-α:primase to form a preinitiation complex or primosome. As recounted previously, protein:protein interactions among all the constituents of this complex serve to stabilize the primosome. Hence, during the initiation of DNA replication, large T antigen plays a number of distinct roles; it alters the structure of *ori* DNA, unwinds DNA ahead of the replication fork, and nucleates the assembly of the primosome at *ori*.

The steps leading to the initiation of SV40 and PyV DNA replication are thought to be very similar, with one notable difference. PyV large T

antigen binds poorly to its *ori*-core in comparison to the binding of SV40 large T antigen to its cognate *ori*. Therefore, it is possible that assembly of the primosome at the PyV *ori* is more dependent on transcription activators than is assembly of the SV40 primosome. This may account for the greater requirement for transcription activators for PyV DNA replication than for SV40 DNA replication.

Initiation of leading-strand synthesis at *ori* is carried out by DNA polymerase-α:primase. RNA-primed DNA synthesis occurs at one of several possible sites within an initiation zone and proceeds in the direction of the early region, using the early mRNA strand as a template (Hay and DePamphilis 1982; Hendrickson et al. 1987). RNA primers are extended by DNA polymerase-α:primase, yielding RNA/DNA primers for the leading-strand DNA polymerase, DNA polymerase-δ. The RNA/DNA primers synthesized by DNA polymerase-α:primase are about 35 nucleotides long. Elongation of these to about 200 nucleotides by DNA polymerase-δ exposes a single-stranded region on the retrograde template (the second initiation zone). It is thought that the average distance between nucleosomes arranged on the viral genome dictates the size of the initiation zones. This allows the priming of leading-strand DNA synthesis by DNA polymerase-α:primase in the opposite direction toward the late region, thus establishing another replication fork moving in the opposite direction. Following the switch from DNA polymerase-α:primase to DNA polymerase-δ, DNA synthesis proceeds continuously in the direction of fork movement.

Initiation of lagging-strand synthesis at each replication fork is also effected by DNA polymerase-α:primase and appears to be mechanistically indistinguishable from the initiation of leading-strand synthesis. Lagging-strand synthesis proceeds discontinuously away from the overall direction of fork movement, resulting in the synthesis of Okazaki fragments that are about 200 nucleotides long. Like the size of the initiation zones at *ori*, the length of Okazaki fragments likely is determined by the distance between nucleosomes. Analyses of Okazaki fragments synthesized in vivo have revealed that the RNA moiety attached to these fragments averages 9 bases, although this value can vary from as few as 3 to as many as 12 nucleotides (Hay and DePamphilis 1982; Hay et al. 1984). The 5′ end of the RNA moiety of these fragments begins with a purine (generally A), followed by an A and, less frequently, a G at the second position, and an A or T at the third position. Examination of the DNA templates complementary to these RNA primers reveals a preference in the DNA template for a purine at position −1 preceding the RNA start site (defined as +1). These authors suggest that 3′-PuTT-5′ in the

DNA template constitutes an initiation signal for DNA polymerase-α:primase.

In part, these findings contrast with those of Bullock et al. (1994), who analyzed RNA/DNA primers synthesized in a 5-second pulse in an in vitro system containing SV40 DNA, large T antigen, and a HeLa cell nuclear extract. The majority of RNA/DNA primers synthesized under these conditions are between 24 and 34 nucleotides long, are derived largely from the lagging-strand template, and are composed of an RNA moiety of 8 or 9 nucleotides. These particular observations are in good agreement with those made by analyzing Okazaki fragments synthesized in vivo. However, examination of the DNA template giving rise to these RNA/DNA primers in vitro led to the inference that initiation occurs with any one of the four ribonucleotide triphosphates. There was a strong preference for A in the second and third positions, in agreement with the results from the in vivo analyses. Although the general features of the RNA moiety of the primers synthesized in vivo and in vitro are similar, there is a clear discrepancy in assignment of the initiating nucleotide. It is not immediately obvious what accounts for this difference. It is of interest to note that if one assumes that the in vitro data suffer from a consistent error of one nucleotide, then the in vitro assignment of the initiating nucleotide is compatible with all the in vivo findings. Whatever the explanation, Bullock et al. (1994) suggest a model for the initiation of DNA synthesis whereby DNA polymerase-α:primase translocates along the DNA template until it recognizes an initiation signal (3′-NTT-5′) and binds an ATP molecule opposite the T residue at position +2 in the DNA template. Then a second ribonucleotide triphosphate complementary to the +1 position is bound by the primase, a dinucleotide is formed, and the RNA chain is extended. Therefore, it would seem that the sole role of DNA polymerase-α:primase is to synthesize RNA/DNA primers for leading- and lagging-strand synthesis (Waga and Stillman 1994a).

Extension of leading- and lagging-strand primers can be accomplished by DNA polymerase-δ in vitro (for review, see Stillman 1994; see also Waga and Stillman 1994a). DNA polymerase switching is achieved when the RF-C/PCNA complex, which is capable of tracking around the circular viral genome, recognizes the 3′end of a primer/template junction. DNA polymerase-α:primase is displaced from the template, and DNA polymerase-δ is recruited in its place. Leading-strand synthesis proceeds continuously in a processive manner after the switch of DNA polymerases, whereas lagging-strand synthesis occurs in a discontinuous fashion. RF-C and PCNA are required for both leading-

and lagging-strand synthesis. As discussed earlier, the synthesis of Okazaki fragments from retrograde templates may require DNA polymerase-ε, but its precise role, if any, in this process remains to be established. Hence, at least two, and perhaps as many as three, DNA polymerases are required for SV40 and PyV DNA replication.

These events lead to the establishment of two replication forks that move in opposite directions at about the same rate around the circular viral genome. Propagation of both replication forks requires the helicase function of large T antigen, which translocates on the forward arm of the parental DNA template. This is consistent with the observation that SV40 large T antigen is located at replication forks in vivo, and with the finding that no other helicase is known to be required for SV40 DNA replication in vitro. Topoisomerase I or topoisomerase II is required to unwind positive supercoils in the DNA template that accumulate ahead of the replication forks.

The maturation of Okazaki fragments requires removal of the RNA primer by RNase H1 and the 5′→3′ exonuclease activity of MF-I, gap-filling by one of the DNA polymerases, and ligation of Okazaki fragments by DNA ligase I (Turchi et al. 1994; Waga et al. 1994). By extrapolation from in vitro studies with model lagging-strand templates, it would seem that the role of RNase H1 in SV40 and PyV DNA replication is to make an endonucleolytic cut one nucleotide 5′ of the RNA/DNA junction in the RNA primer (Turchi et al. 1994). MF-I then eliminates the remaining monoribonucleotide from the initiator RNA. Hence, both RNase HI and MF-I are required and act cooperatively to remove RNA primers. In vitro gap-filling can be accomplished by any one of the replicative DNA polymerases (α, δ, or ε). However, it seems unlikely that DNA polymerase-α:primase fulfills this role because RP-A, PCNA, and RF-C block maturation of Okazaki fragments by DNA polymerase-α:primase in vitro (Waga and Stillman 1994b). There is a specific requirement for DNA ligase I for the completion of lagging-strand synthesis; neither mammalian DNA ligase III nor bacteriophage T4 DNA ligase is capable of substituting for DNA ligase I (Waga et al. 1994). Therefore, the completion of lagging-strand synthesis and joining of Okazaki fragments appears to require specific contacts among the proteins of the lagging-strand machinery.

The mechanism accounting for the coordinated synthesis of both leading and lagging strands at replication forks is unknown. Recently, Waga and Stillman (1994a) proposed a model whose central thesis posits a dimer between the leading- and lagging-strand DNA polymerases analogous to the dimeric DNA polymerase III at the *E. coli* replication

fork. These authors provide evidence that DNA polymerase-δ is the principal replicative enzyme required for both leading- and lagging-strand synthesis in vitro, and suggest that this DNA polymerase dimerizes at replication forks. However appealing this hypothesis may be, there is currently no evidence to support the occurrence of DNA polymerase-δ homodimers in solution. However, the possibility remains that a heretofore undiscovered cellular protein mediates dimerization of the DNA polymerases at the replication fork. It will be interesting to learn whether any of these or some other protein mediates dimerization of the DNA polymerases at replication forks.

Termination of SV40 and PyV DNA replication occurs on average 180° from the *ori* when the two replication forks converge; the small genomes of these viruses do not contain replication termination signals (for review, see DePamphilis and Bradley 1986; see Bastia and Mohanty, this volume). When replication is about 90% completed, fork movement is impeded and replication intermediates accumulate, indicating that the completion of replication and the segregation of progeny molecules comprise a slow step in the DNA replication cycle. If DNA replication and unwinding mediated by topoisomerase II continue concurrently, then the progeny molecules separate, yielding double-stranded circular DNAs with a small gap of about 50 nucleotides. However, should DNA replication be completed without concomitant unwinding, then catenated dimers result whose resolution also requires topoisomerase II. Examination of the structure of SV40 and PyV replication intermediates suggests that both pathways can be used to complete replication of these viral genomes.

FUTURE PROSPECTS

The last decade has witnessed a dramatic increase in our understanding of SV40 and PyV DNA replication, which can be attributed primarily to the development of a cell-free system for the replication of these viral genomes. Biochemical fractionation and complementation assays have led to the isolation and characterization of many cellular replication proteins from several mammalian sources, and to the molecular cloning of cDNAs and genes that encode these proteins from diverse organisms. Genetic studies in yeasts have validated the role of these proteins in cellular DNA replication. This progress notwithstanding, much remains to be learned. For example, we are only now beginning to appreciate the link between cell-cycle control and the regulation of viral DNA replication. Both large T antigen and several of the cellular replication proteins

are regulated by cyclins, cyclin-dependent kinases, and their protein inhibitors. Regulation occurs at the level of phosphorylation of replication proteins and by direct interactions between cell-cycle components and those of the replication machinery. Similarly, the mechanism whereby transcription activators function to stimulate DNA replication is incompletely understood. Our understanding of the mechanism of replication of SV40 and PyV DNA is based largely on the use of in vitro systems with naked DNA. Little is known concerning the replication of chromatin, the natural state of the viral genome in cells. All the replicative DNA polymerases have not been unambiguously defined, and the mechanism that accounts for coordinated synthesis of the leading and lagging strands at replication forks remains to be elucidated. Finally, there are very likely additional cellular replication proteins that remain to be identified. These are but a few of the more obvious areas for future research.

The primary rationale for studying papovavirus DNA replication is to gain greater insight into cellular DNA replication. These viruses have served us well in this regard. Now we appear to stand on the threshold of discovering what is for some the "holy grail" in this field; namely, the identity of functional mammalian replication origins and the initiator proteins that regulate their utilization. The knowledge we have gained from studies of papovavirus DNA replication will serve as a solid foundation for these endeavors.

REFERENCES

Adamczewski, J.P., J.V. Gannon, and T. Hunt. 1993. Simian virus large T antigen associates with cyclin A and $p33^{cdk2}$. *J. Virol.* **67:** 6551–6557.

Bauer, G.A. and P.M.J. Burgers. 1988. The yeast analog of mammalian cyclin/proliferating-cell nuclear antigen interacts with mammalian DNA polymerase δ. *Proc. Natl. Acad. Sci.* **85:** 7506–7510.

Bennett, E.R., M. Naujokas, and J.A. Hassell. 1989. Requirements for species-specific papovavirus DNA replication. *J. Virol.* **63:** 5371–5385.

Bennett-Cook, E.R. and J.A. Hassell. 1991. Activation of polyomavirus DNA replication by yeast GAL4 is dependent on its transcriptional activation domains. *EMBO J.* **10:** 959–969.

Borowiec, J.A., F.B. Dean, P.A. Bullock, and J. Hurwitz. 1990. Binding and unwinding-how T antigen engages the SV40 origin of DNA replication. *Cell* **60:** 959–969.

Bravo, R., R. Frank, P.A. Blundell, and H. MacDonald-Bravo. 1987. Cyclin/PCNA is the auxiliary protein of polymerase-δ. *Nature* **326:** 515–517.

Brill, S. and B. Stillman. 1989. Yeast replication factor-A functions in the unwinding of the SV40 origin of DNA replication. *Nature* **342:** 92–95.

———. 1991. Replication factor-A from *Saccharomyces cerevisiae* is encoded by three essential genes coordinately expressed at S phase. *Genes Dev.* **5:** 1589–1600.

Brown, G.W., T. Melendy, and D.S. Ray. 1992. Conservation of structure and function of DNA replication protein A in trypanasomitid *Crithidia fasciculata*. *Proc. Natl. Acad. Sci.* **89:** 10227–10231.

Brown, W.C. and J.L. Campbell. 1993. Interaction of proliferating cell nuclear antigen with yeast DNA polymerase δ. *J. Biol. Chem.* **268:** 21706–21710.

Bruckner, A., F. Stadlbauer, L.A. Guarino, A. Brunahl, C. Schneider, C. Rehfuess, C. Prives, E. Fanning, and H.P. Nasheuer. 1995. The mouse DNA polymerase α-primase subunit p48 mediates species-specific replication of polyomavirus DNA in vitro. *Mol. Cell. Biol.* **15:** 1716–1724.

Brush, G.S., C.W. Anderson, and T.J. Kelly. 1994. The DNA-activated protein kinase is required for the phosphorylation of replication protein A during simian virus 40 DNA replication. *Proc. Natl. Acad. Sci.* **91:** 12520–12524.

Bunz, F., R. Kobayashi, and B. Stillman. 1993. cDNAs encoding the large subunit of human replication factor C. *Proc. Natl. Acad. Sci.* **90:** 11014–11018.

Bullock, P.A., Y.S. Seo, and J. Hurwitz. 1991. Initiation of simian virus 40 DNA synthesis *in vitro*. *Mol. Cell. Biol.* **11:** 2350–2361.

Bullock, P.A., S. Tevosian, C. Jones, and D. Denis. 1994. Mapping initiation sites for simian virus 40 DNA synthesis events *in vitro*. *Mol. Cell. Biol.* **14:** 5043–5055.

Burbelo, P.D., A. Utani, Z.-Q. Pan, and Y. Yamada. 1993. Cloning of the large subunit of activator 1 (replication factor C) reveals homology with bacterial DNA ligases. *Proc. Natl. Acad. Sci.* **90:** 11543–11547.

Burgers, P.M.J. 1991. *Saccharomyces cerevisiae* replication factor C. *J. Biol. Chem.* **266:** 22698–22706.

Busen, W. 1980. Purification, subunit structure, and serological analysis of calf thymus ribonuclease H I. *J. Biol. Chem.* **255:** 9434–9443.

Campbell, J.L. 1993. Yeast DNA replication. *J. Biol. Chem.* **268:** 25261–25264.

Carty, M.P., M. Zernick-Kobak, S. McGrath, and K. Dixon. 1994. UV light-induced DNA synthesis arrest in HeLa cells is associated with changes in phosphorylation of human single-stranded DNA-binding protein. *EMBO J.* **13:** 2114–2123.

Cegielska, A. and D.M. Virshup. 1993. Control of simian virus 40 replication by the HeLa cell nuclear kinase I. *Mol. Cell. Biol.* **13:** 1202–1211.

Cegielska, A., I. Moarefi, E. Fanning, and D.M. Virshup. 1994. T-antigen inhibits simian virus 40 DNA replication by phosphorylaton of intact T antigen on serines 120 and 123. *J. Virol.* **68:** 269–275.

Challberg, M.D. and T.J. Kelly. 1989. Animal virus DNA replication. *Annu. Rev. Biochem.* **58:** 671–717.

Chen, J., P.K. Jackson, M.W. Kischner, and A. Dutta. 1995. Separate domains of p21 involved in the inhibition of Cdk kinase and PCNA. *Nature* **374:** 386–388.

Chen, M., Z.-Q. Pan, and J. Hurwitz. 1992a. Sequence and expression in *Escherichia coli* of the 40-kDa subunit of activator 1 (replication factor C) of HeLa cells. *Proc. Natl. Acad. Sci.* **89:** 2516–2520.

———. 1992b. Studies of the cloned 37 kDa subunit of activator 1 (replication factor C) of HeLa cells. *Proc. Natl. Acad. Sci.* **89:** 5211–5215.

Cheng, L. and T.J. Kelly. 1989. Transcriptional activator nuclear factor I stimulates the replication of SV40 minichromosomes *in vivo* and *in vitro*. *Cell* **89:** 541–551.

Cheng, L., J.L. Workman, R.E. Kingston, and T.J. Kelly. 1992. Regulation of DNA replication *in vitro* by the transcriptional activator GAL4-VP16. *Proc. Natl. Acad. Sci.* **89:** 589–593.

Collins, K.L. and T.J. Kelly. 1991. Effects of T antigen and replication protein A on the initiation of DNA synthesis by DNA polymerase α-primase. *Mol. Cell. Biol.* **11:** 2108–2115.

Collins, K.L., A.A.R. Russo, B.Y. Tseng, and T.J. Kelly. 1993. The role of the 70 kd subunit of human DNA polymerase α in DNA replication. *EMBO J.* **12:** 4555–4566.

Copeland, W.C. and T.S.F. Wang. 1993. Enzymatic characterization of the individual mammalian primase subunits reveals a biphasic mechanism for initiation of DNA replication. *J. Biol. Chem.* **268:** 26179–26189.

Crouch, R.J. 1990. Ribinonuclease H: From discovery to 3D structure. *New Biol.* **2:** 771–777.

Cullman, G., K. Fien, R. Kobayashi, and B. Stillman. 1995. Characterization of five replication factor C genes of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **15:** 4661–4671.

Dean, F.B., J.A. Borowiec, Y. Ishim, S. Deb, P. Tegtmeyer, and J. Hurwitz. 1987. Simian virus 40 large tumor antigen requires three core replication domains for DNA unwinding and replication *in vitro*. *Proc. Natl. Acad. Sci.* **84:** 8267–8271.

Deb, S.P. and P. Tegtmeyer. 1987. ATP enhances the binding of simian virus 40 large T antigen to the origin of replication. *J. Virol.* **61:** 3649–3654.

Deb, S.P., A.L. DeLucia, C. Baur, A. Koff, and P. Tegtmeyer. 1986. Domain and structure of the simian virus 40 core origin of replication. *Mol. Cell. Biol.* **6:** 1663–1670.

Deb, S.P., S. Tsui, A. Koff, A.L. DeLucia, R. Parsons, and P. Tegtmeyer. 1987. The T-antigen-binding protein domain of the simian virus 40 core origin of replication. *J. Virol.* **61:** 2143–2149.

DeLucia, A.L., B.A. Lewton, R. Tjian, and P. Tegtmeyer. 1983. Topography of simian virus 40 A protein-DNA complexes: Arrangement of pentanucleotide interaction sites at the origin of replication. *J. Virol.* **46:** 143–150.

DePamphilis, M.L. 1988. Transcriptional elements as components of eukaryotic origins of DNA replication. *Cell* **52:** 635–638.

———. 1993. How transcription factors regulate origins of DNA replication in eukaryotic cells. *Trends Cell Biol.* **3:** 161–167.

DePamphilis, M.L. and M. Bradley. 1986. Replication of SV40 and polyomavirus chromosomes. In *The papovaviridae* (ed. N. Salzman), vol. 1, pp. 99–246. Plenum Press, New York.

DiFrancesco, R.A. and I.R. Lehman. 1985. Interaction of ribonuclease H from *Drosophilia melanogaster* embryos with DNA polymerase-primase. *J. Biol. Chem.* **260:** 14764–14770.

Din, S., S.J. Brill, M.P. Fairman, and B. Stillman. 1990. Cell-cycle-regulated phosphorylation of DNA replication factor A from human and yeast cells. *Genes Dev.* **4:** 968–977.

Dornreiter, I., W.C. Copeland, and T.S.F. Wang. 1993. Initiation of simian virus 40 DNA replication requires the interaction of a specific domain of human DNA polymerase α with large T antigen. *Mol. Cell. Biol.* **13:** 809–820.

Dornreiter, I., A. Hoss, A.K. Arthur, and E. Fanning. 1990. SV40 T antigen binds directly to the large subunit of purified DNA polymerase α. *EMBO J.* **9:** 3329–3336.

Dornreiter, I., L.F. Erdile, I.U. Gilbert, D. von Winkler, T.J. Kelly, and E. Fanning. 1992. Interaction of DNA polymerase α-primase with cellular replication protein A and SV40 T antigen. *EMBO J.* **11:** 769–776.

Dutta, A. and B. Stillman. 1992. Cdc2 family kinases phosphorylate a human cell DNA

replication factor, RPA, and activate DNA replication. *EMBO J.* **11:** 2189–2199.

Eki, T., T. Enomoto, C. Masutani, A. Miyajima, R. Takada, Y. Murikami, T. Ohno, F. Hanaoka, and M. Ui. 1991. Mouse DNA primase plays a principal role in determination of permissiveness for polyomavirus DNA replication. *J. Virol.* **65:** 4874–4881.

Elledge, S.J., R. Richman, F.L. Hall, R.T. Williams, N. Lodgson, and J.W. Harper. 1992. CDK2 encodes a 33-kDa cyclin A-associated protein kinase and is expressed before CDC2 n the cell cycle. *Proc. Natl. Acad. Sci.* **89:** 2907–2911.

Erdile, L.F., M.S. Wood, and T.J. Kelly. 1990. The primary structure of the 32 kDa subunit of human replication protein A. *J. Biol. Chem.* **265:** 3177–3182.

Erdile, L.F., W.D. Heyer, R. Kolodner, and T.J. Kelly. 1991. Characterization of a cDNA encoding the 70 kDa single stranded DNA-binding subunit of human replication protein A and the role of the protein in DNA replication. *J. Biol. Chem.* **266:** 12090–12098.

Fairman, M.P. and B. Stillman. 1988. Cellular factors required for multiple stages of SV40 DNA replication *in vitro*. *EMBO J.* **7:** 1211–1218.

Fanning, E. 1990. Minireview—Simian virus 40 large T antigen: The puzzle, the pieces, and the emerging picture. *J. Virol.* **66:** 1289–1293.

———. 1994. Control of SV40 DNA replication by protein phosphorylation: A model for cellular DNA replication. *Trends Cell Biol.* **4:** 250–254.

Fanning, E. and R. Knippers. 1992. Structure and function of simian virus 40 large tumor antigen. *Annu. Rev. Biochem.* **61:** 55–85.

Fien, K. and B. Stillman. 1992. Identification of replication factor C from *Saccharomyces cerevisiae:* A component of the leading strand DNA replication complex. *Mol. Cell. Biol.* **12:** 155–163.

Firmenich, A., M. Elias-Arnanz, and P. Berg. 1995. A novel allele of *Saccharomyces cerevisiae* that is deficient in recombination and repair and suppressible by *RAD52*. *Mol. Cell. Biol.* **15:** 1620–1631.

Flores-Rozas, H., Z. Kelman, F.B. Dean, Z.-Q. Pan, J.W. Harper, S.J. Elledge, M. O'Donnell, and J. Hurwitz. 1994. Cdk-interacting protein 1 directly binds with proliferating cell nuclear antigen and inhibits DNA replication catalyzed by the DNA polymerase δ holoenzyme. *Proc. Natl. Acad. Sci.* **91:** 8655–8659.

Fotedar, R. and J.M. Roberts. 1992. Cell cycle regulated phosphorylation of RPA-32 occurs within the replication initiation complex. *EMBO J.* **11:** 2177–2187.

Gannon. J.V. and D.P. Lane. 1987. p53 and DNA polymerase α compete for binding to SV40 T antigen. *Nature* **329:** 456–458.

Gerard, R. and Y. Gluzman. 1986. Functional analysis of the role of the A + T rich region and upstream flanking sequences in simian virus 40 DNA replication. *Mol. Cell. Biol.* **6:** 4570–4577.

Goetz, G.S., F.B. Dean, J. Hurwitz, and S.W. Matson. 1988. The unwinding of duplex regions in DNA by the simian virus 40 large tumor antigen-associated DNA helicase activity. *J. Biol. Chem.* **263:** 383–392.

Goulian, M., C. Carton, L. deGrandpre, B. Olinger, and S. Richard. 1987. Processive discontinuous DNA synthesis by mammalian DNA polymerase α-primase. In *DNA replication and recombination* (ed. T. Kelly and R. McMacken), pp. 101–109. A.R. Liss, New York.

Goulian, M., S.H. Richards, C.J. Heard, and B.M. Bigsby. 1990. Discontinous DNA synthesis by purified mammalian proteins. *J. Biol. Chem.* **265:** 18461–18471.

Guggenheimer, R.A., K. Nagata, M. Kenny, and J. Hurwitz. 1984. Protein-primed

replication of plasmids containing the terminus of the adenovirus genome. *J. Biol. Chem.* **259:** 7815–7825.

Guo, Z.-S. and M.L. DePamphilis. 1992. Specific transcription factors stimulate simian virus 40 and polyomavirus origins of DNA replication. *Mol. Cell. Biol.* **12:** 2514–2524.

Guo, Z-S., Y. Heine, and M.L. DePamphilis. 1991. T-antigen binding to site I facilitates initiation of SV40 DNA replication, but does not affect bidirectionality. *Nucleic Acids Res.***19:** 7081–7088.

Guo, Z.-S., C. Gutierrez, U. Heine, J.M. Sogo, and M.L. DePamphilis. 1989. Origin auxiliary sequences can facilitate initiation of simian virus 40 DNA replication *in vitro* as they do *in vivo*. *Mol. Cell. Biol.* **9:** 3593–3602.

Gutierrez, C., Z.S. Guo, J.Roberts, and M.L. DePamphilis. 1990. Simian virus 40 origin auxiliary sequences weakly facilitates T-antigen binding but strongly facilitate DNA unwinding. *Mol. Cell. Biol.* **10:** 1719–1728.

Hassell J.A., W.J. Muller, and C.R. Mueller. 1986. The dual role of the polyomavirus enhancer in transcription and DNA replication. *Cancer Cells* **6:** 561–569.

Hay, R.T. and M.L. DePamphilis. 1982. Initiation of SV40 DNA replication *in vivo*: Location and structure of 5′ ends of DNA synthesized in the *ori* region. *Cell* **28:** 767–779.

Hay, T.T., E.A. Hendrickson, and M.L. DePamphilis. 1984. Sequence specificity for the initiation of RNA-primed simian virus 40 DNA synthesis *in vivo*. *J. Mol. Biol.* **175:** 131–157.

He, Z., B.T. Brinton, J. Greenblatt, J.A. Hassell, and C.J. Ingles. 1993. The transcription proteins VP16 and GAL4 bind replication factor A. *Cell* **73:** 1223–1232.

Hendrickson, E.A., C.E. Fritze, W.R. Folk, and M.L. DePamphilis. 1987. The origin of bidirectional DNA replication in polyomavirus. *EMBO J.* **6:** 2011–2018.

Hendrickson, L.A. and M.S. Wold. 1994. Replication protein A mutants lacking phosphorylation sites for $p34^{cdc2}$ kinase support DNA replication. *J. Biol. Chem.* **269:** 24203–24208.

Hendrickson, L.A., C.B. Umbricht, and M.S. Wold. 1994. Recombinant replication protein A: Expression, complex formation, and functional characterization. *J. Biol. Chem.* **269:** 11121–11132.

Heyer, W.D., M.R.S. Rao, L.F. Erdile, T.J. Kelly, and R.D. Kolodner. 1990. An essential *Saccharomyces cerevisiae* single-stranded DNA binding protein is homologous to the large subunit of human RP-A. *EMBO J.* **9:** 2321–2329.

Howell, E.A., M.A. McAlear, D. Rose, and C. Holm. 1994. CDC44: A putative nucleotide-binding protein required for cell cycle progression that has homology to subunits of replication protein C. *Mol. Cell. Biol.* **14:** 255–267.

Huang, L., Y. Kim, J.J. Turchi, and R.A. Bambara. 1994. Structure-specific cleavage of the RNA primer from Okazaki fragments by calf thymus RNAse H1. *J. Biol. Chem.* **269:** 25922–25927.

Hübscher, U. and S. Spadari. 1994. DNA replication and chemotherapy. *Physiol. Rev.* **74:** 259–304.

Hurwitz, J., F.B. Dean, A.D. Kwong, and S.H. Lee. 1990. The *in vitro* replication of DNA containing the SV40 origin. *J. Biol. Chem.* **265:** 18043–18046.

Ishimi, Y., A. Claude, P. Bullock, and J. Hurwitz. 1988. Complete enzymatic synthesis containing the SV40 origin of replication. *J. Biol. Chem.* **263:** 19723–19733.

Ishimi, Y., K. Sugasawa, F. Hanaoka, and A. Kikuchi. 1991. Replication of the simian virus 40 chromosome with purified proteins. *J. Biol. Chem.* **266:** 16141–16148.

Jessberger, R., V. Podust, U. Hübscher, and P. Berg. 1993. A mammalian protein complex that repairs double-strand breaks and deletions by recombination. *J. Biol. Chem.* **268:** 15070–15079.

Kamakaka, R.T., P.D. Kaufman, B. Stillman, P.G. Mitsis, and J.T. Kadonaga. 1994. Simian virus 40 origin- and T-antigen-dependent DNA replication with *Drosophila* factors *in vitro*. *Mol. Cell. Biol.* **14:** 5114–5122.

Kelman, Z. and M. O'Donnell. 1994. DNA replication: Enzymology and mechanisms. *Curr. Opin. Genet. Dev.* **4:** 185–195.

Kenny, M.K., L. Balogh, and J. Hurwitz. 1988. Initiation of adenovirus DNA replication. *J. Biol. Chem.* **263:** 9801–9808.

Kenny, M.K., U. Schlegel, H. Furneaux, and J. Hurwitz. 1990. The role of human single-stranded-DNA binding protein and its individual subunits in simian virus 40 DNA replication. *J. Biol. Chem.* **265:** 7693–7700.

Kesti, T., H. Frantti, and J.E. Syvaoja. 1993. Molecular cloning of the cDNA for the catalytic subunit of human DNA polymerase ε. *J. Biol. Chem.* **268:** 10238–10245.

Krishna, T.S.R., X-P. Kong, S. Gary, P.M. Burgers, and J. Kuriyan. 1994. Crystal structure of the eukaryotic DNA polymerase processivity factor PCNA. *Cell* **79:** 1233–1243.

Kong, X.P., R. Ontrust, M. O'Donnell, and J. Kuriyan. 1992. Three-dimensional structure of the β subunit of *E. coli.* DNA polymerase III holoenzyme: A sliding DNA clamp. *Cell* **69:** 425–437.

Lee, S.H. and J. Hurwitz. 1990. Mechanism of elongation of primed DNA by DNA polymerase δ, proliferating cell nuclear antigen, and activator 1. *Proc. Natl. Acad. Sci.* **87:** 5672–5676.

Lee, S.H., A.D. Kwong, Z.H. Pan, and J. Hurwitz. 1991. Studies on the activator 1 protein complex, an accessory factor for proliferating cell nuclear antigen-dependent DNA polymerase δ. *J. Biol. Chem.* **266:** 594–602.

Li, J.J. and T.J. Kelly. 1984. Simian virus 40 DNA replication *in vitro*. *Proc. Natl. Acad. Sci.* **81:** 6973–6977.

Li, R. and M.R. Botchan. 1993. The acidic transcriptional activation domains of VP16 and p53 bind the cellular replication protein A and stimulate *in vitro* BPV-1 DNA replication. *Cell* **73:** 1207–1221.

Li, R., S. Waga, G.J. Hannon, D. Beach, and B. Stillman. 1994. Differential effects by the p21 CDK inhibitor on PCNA-dependent DNA replication and repair. *Nature* **371:** 534–537.

Li, X. and P. Burgers. 1994a. Molecular cloning and expression of the *Saccharomyces cerevisiae RFC3* gene, an essential component of replication factor C. *Proc. Natl. Acad. Sci.* **91:** 868–872.

———. 1994b. Cloning and characterization of the essential *Saccharomyces cerevisiae RFC4* gene encoding the 37 kDa subunit of replication factor C. *J. Biol. Chem.* **269:** 21880–21884.

Lin, S. and D. Kowalski. 1994. DNA instability facilitates initiation at the SV40 replication origin. *J. Mol. Biol.* **235:** 496–507.

Linn, S. 1991. How many pols does it take to replicate nuclear DNA? *Cell* **66:** 185–187.

Lindahl, T. 1970. An endonuclease specific for double-stranded DNA: Deoxyribonuclease IV from rabbit tissues. *Methods Enzymol.* **21:** 148–153.

Liu, V.F. and D.T. Weaver. 1993. The ionizing radiation-induced replication protein A phosphorylation response differs between ataxia telangiectasia and normal human cells.

Mol. Cell. Biol. **13:** 7222–7231.

Loeber, G., J.E. Stenger, S. Ray, R.E. Parsons, M.E. Anderson, and P. Tegtmeyer. 1991. The zinc finger region of simian virus 40 large T antigen is needed for hexamer assembly and origin melting. *J. Virol.* **65:** 3167–3174.

Longhese, M.P., P. Plevani, and G. Lucchini. 1994. Replication factor A is required *in vivo* for DNA replication, repair, and recombination. *Mol. Cell. Biol.* **14:** 7884–7890.

Lu, Y., A.S. Zeft, and A.T. Riegal. 1993. Cloning and expression of a novel human DNA binding protein, PO-GA. *Biochem. Biophys. Res. Commun.* **193:** 779–786.

Maga, G. and U. Hübscher. 1995. DNA polymerase ε interacts with proliferating cell nuclear antigen in primer recognition and elongation. *Biochemistry* **34:** 891–901.

Manfredi, J. and C. Prives. 1994. The transforming activity of simian virus 40 large tumor antigen. *Biochim. Biophys. Acta* **1198:** 65–83.

Mastrangelo, I.A., P.V.C. Hough, J.S. Wall, M. Dodson, F.B. Dean, and J. Hurwitz. 1989. ATP-dependent assembly of double hexamers of SV40 T antigen at the viral origin of DNA replication. *Nature* **338:** 658–662.

McVey, D., L. Brizuela, I. Mohr, D.R. Marshak, Y. Gluzman, and D. Beach. 1989. Phosphorylation of large tumour antigen by cdc2 stimulates SV40 DNA replication. *Nature* **341:** 503–507.

McVey, D., S. Ray, Y. Gluzman, L. Berger, A. Wildeman, D.R. Marshak, and P. Tegtmeyer. 1993. cdc2 phosphorylation of threonine 124 activates the origin-unwinding functions of simian virus 40 T antigen. *J. Virol.* **67:** 5206–5215.

Melendy, T. and B. Stillman. 1992. SV40 DNA replication. *Nucleic Acids Mol. Biol.* **6:** 129–158.

———. 1993. An interaction between replication protein A and SV40 T antigen appears essential for primosome assembly during SV40 DNA replication. *J. Biol. Chem.* **268:** 3389–3395.

Miyazawa, H., M. Izumi, S. Tada, R. Takada, M. Masutani, M. Ui, and F. Hanoaka. 1993. Molecular cloning of the cDNAs for the four subunits of mouse DNA polymerase α-primase complex and their gene expression during cell proliferation and the cell cycle. *J. Biol. Chem.* **268:** 8111–8122.

Moarefi, I.F., D. Small, I. Gilbert, M. Hopfner, S. K. Randall, C. Schneider, A.A.R. Russo, U. Ramsperger, A.K. Arthur, H. Stahl, T. Kelly, and E. Fanning. 1993. Mutation of the cyclin-dependent kinase phosphorylation site in simian virus 40 (SV40) large T antigen specifically blocks SV40 origin DNA unwinding. *J. Virol.* **67:** 4992–5002.

Morrison, A., H. Araki, A.B. Clark, R.K. Hamatake, and A. Sugino. 1990. A third essential DNA polymerase in *S. cerevisiae*. *Cell* **62:** 1143–1151.

Moses, K. and C. Prives. 1994. A unique subpopulation of murine DNA polymerase α-primase specifically interacts with polyomavirus T antigen and stimulates DNA replication. *Mol. Cell. Biol.* **14:** 2767–2776.

Muller, W.J., D. Dufort, and J.A. Hassell. 1988. Multiple subelements within the polyomavirus enhancer function synergistically to activate DNA replication. *Mol. Cell. Biol.* **8:** 5000–5015.

Murakami, Y., C.R. Wobbe, L. Weissbach, F.B. Dean, and J. Hurwitz. 1986. Role of DNA polymerase α and DNA primase in simian virus 40 DNA replication *in vitro*. *Proc. Natl. Acad. Sci.* **83:** 2869–2873.

Murante, R.S., L. Huang, J.J. Turchi, and R. A. Bambara. 1994. The calf 5′- to 3′-exonuclease is also an endonuclease with both activities dependent on primers annealed

upstream of the point of cleavage. *J. Biol. Chem.* **269:** 1191–1196.

Nasheuer, H.P., A. Moore, A. Wahl, and T.S.F. Wang. 1991. Cell cycle-dependent phosphorylation of human DNA polymerase α. *J. Biol. Chem.* **266:** 7893–7903.

Nethanel, T.S., G. Reisfeld, G.D. Gottlieb, and G. Kaufmann. 1988. An Okazaki piece of simian virus 40 may be synthesized by ligation of shorter precursor chains. *J. Virol.* **62:** 2867–2873.

Noskov, V., S. Maki, Y. Kawasaki, S.-H. Leem, B.-I. Ono, H. Araki, Y. Pavlov, and A. Sugino. 1994. The *RFC2* gene encoding a subunit of replication factor C of *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **22:** 1527–1535.

Nishida, C., P. Reinhard, and S. Linn. 1988. DNA repair synthesis in human fibroblasts requires DNA polymerase. *J. Biol. Chem.* **263:** 501–510.

O'Donnell, M., R. Ontrust, F. Dean, M. Chen, and J. Hurwitz. 1993. Homology in accessory proteins of replicative polymerases—*E. coli* to humans. *Nucleic Acids Res.* **21:** 1–3.

Pan, Z.Q., M. Chen, and J. Hurwitz. 1993. The subunits of activator 1 (replication factor C) carry out multiple functions essential for proliferating-cell nuclear antigen-dependent DNA synthesis. *Proc. Natl. Acad. Sci.* **90:** 6–10.

Parsons, R.E., M. Anderson, and P. Tegtmeyer. 1990. Three domains in the simian virus 40 core origin orchestrate the binding, melting, and helicase activities of T antigen. *J. Virol.* **64:** 509–518.

Parsons, R.E., J.E. Stenger, S. Ray, R. Welker, M.E. Anderson, and P. Tegtmeyer. 1991. Cooperative assembly of simian virus 40 T-antigen hexamers on functional halves of the replication origin. *J. Virol.* **65:** 2798–2806.

Peden, K.W.C. and J.M. Pipas. 1992. Simian virus 40 mutants with amino-acid substitutions near the amino terminus of large T antigen. *Virus Genes* **6:** 107–118.

Pipas, J.M. 1992. Common and unique features of T antigens encoded by the polyomavirus group. *J. Virol.* **66:** 3979–3985.

Podust, L.M., V.N. Podust, C. Floth, and U. Hübscher. 1992. Assembly of DNA polymerase δ and ε holoenzymes depends on the geometry of the DNA template. *Nucleic Acids Res.* **22:** 2970–2975.

Podust, L.M., V.N. Podust, J.M. Sogo, and U. Hübscher. 1995. Mammalian DNA polymerase auxiliary proteins: Analysis of replication factor C-catalyzed proliferating cell nuclear antigen loading onto circular double-stranded DNA. *Mol. Cell. Biol.* **15:** 3072–3081.

Pomerantz, B.J. and J.A. Hassell. 1984. Polyomavirus and simian virus 40 large T antigens bind to common DNA sequences. *J. Virol.* **49:** 925–937.

Prelich, G. and B. Stillman. 1988. Coordinated leading and lagging strand synthesis during SV40 DNA replication *in vitro* requires PCNA. *Cell.* **53:** 117–126.

Prelich, G., M. Kostra, D.R. Marshak, M.B. Mathews, and B. Stillman. 1987. The cell-cycle regulated proliferating cell nuclear antigen is required for SV40 DNA replication *in vitro*. *Nature* **326:** 471–475.

Prives, C. 1990. The replication functions of SV40 T antigen are regulated by phosphorylation. *Cell* **61:** 725–738.

Scheidtmann, K.H., D.M. Virshup, and T.J. Kelly. 1991a. Protein phosphatase 2A dephosphorylates simian virus 40 large T antigen specifically at residues involved in regulation of DNA-binding activity. *J. Virol.* **65:** 2098–2101.

Scheidtmann, K.H., M. Buck, J. Schneider, D. Kalderon, E. Fanning, and A.E. Smith. 1991b. Biochemical characterization of phosphorylation site mutants of simian virus 40

large T antigen: Evidence for interaction between amino- and carboxy-terminal domains. *J. Virol.* **65:** 1479–1490.

Schneider, C., K. Weisshart, L.A. Guarino, I. Dornreiter, and E. Fanning. 1994. Species specific functional interactions of DNA polymerase α-primase with simian virus 40 (SV40) T antigen require SV40 origin DNA. *Mol. Cell. Biol.* **14:** 3176–3185.

Schneider, C., D. von Winkler, L. Dornreiter, H.-P. Nasheuer, I. Gilbert, S. Dehda, A.K. Arthur, and E. Fanning. 1992. Bovine RP-A functions in SV40 DNA replication *in vitro*, but bovine polymerase α-primase inhibits replication competitively. In *DNA replication: The regulatory mechanism* (ed. P. Hughes et al.), pp. 385–397. Springer Verlag, Berlin.

Schneider, J. and E. Fanning. 1988. Mutations in the phosphorylation sites of simian virus 40 (SV40) T antigen alter its origin DNA-binding specificity for sites I or II and affect SV40 DNA replication activity. *J. Virol.* **65:** 1598–1605.

SenGupta, D. and J.A. Borowiec. 1994. Strand and face: The topography of interactions between SV40 origin of replication and T-antigen during the initiation of replication. *EMBO J.* **13:** 982–992.

Shivji, M.K.K., S.J. Grey, U.P. Strausfeld, R.D. Wood, and J.J. Blow. 1989. Cip1 inhibit DNA replication but not PCNA-dependent nucleotide excision-repair. *Curr. Biol.* **4:** 1062–1068.

Siegal, G., J.J. Turchi, T.W. Myers, and R.A. Bambara. 1992. A 5′ to 3′ exonuclease functionally interacts with calf DNA polymerase ε. *Proc. Natl. Acad. Sci.* **89:** 9377–9381.

Smale, S.T. and R. Tjian. 1986. T-antigen-DNA polymerase α complex implicated in simian virus 40 DNA replication. *Mol. Cell. Biol.* **6:** 4077–4087.

Smith, M.L., I.-T. Chen, Q. Zhan, I. Bae, C.-Y. Chen., T.M. Gilmer, M.B. Kastan, P.M. O'Conner, and A.J. Fornace, Jr. 1994. Interaction of the p53-regulated protein GADD45 with proliferating cell nuclear antigen. *Science* **266:** 1376–1379.

Smith, J. and R. Rothstein. 1995. A mutation in the gene encoding the *Saccharomyces cerevisiae* single-stranded DNA-binding protein Rfa1 stimulates a *RAD*52-independent pathway for direct-repeat recombination. *Mol. Cell. Biol.* **15:** 1632–1641.

Stadlbauer, F., C. Voitenleitner, A. Bruckner, E. Fanning, and H.-P. Nasheuer. 1996. Species-specific replication of simian virus 40 DNA *in vitro* requires the p180 subunit of human DNA polymerase α-primase. *Mol. Cell. Biol.* **16:** 94–104.

Stillman, B. 1989. Initiation of eukaryotic DNA replication *in vitro*. *Annu. Rev. Cell Biol.* **5:** 197–245.

———. 1994. Smart machines at the DNA replication fork. *Cell* **78:** 725–728.

Sundstrom, N.A., N.H. Acheson, and J.A. Hassell. 1991. Determination of the origin-specific DNA-binding domain of polyomavirus large T antigen. *J. Virol.* **65:** 6998–7003.

Tooze, J., ed. 1980. *Molecular biology of tumor viruses,* 2nd edition: *DNA tumor viruses.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Tsurimoto, T. and B. Stillman. 1989. Purification of a cellular replication factor, RF-C, that is required for coordinated synthesis of leading and lagging strands during simian virus 40 DNA replication *in vitro*. *Mol. Cell. Biol.* **9:** 609–619.

———. 1990. 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.* **87:** 1023–1027.

———. 1991a. Replication factors required for SV40 DNA replication *in vitro*. *J. Biol.*

Chem. **266:** 1950–1960.

———. 1991b. Replication factors required for SV40 DNA replication *in vitro*. *J. Biol. Chem.* **266:** 1961–1968.

Turchi, J.J. and R.A. Bambara. 1993. Completion of mammalian lagging strand DNA replication using purified proteins. *J. Biol. Chem.* **268:** 15136–15141.

Turchi, J.J., L. Huang, R.S. Murante, Y. Kim, and R.A. Bambara. 1994. Enzymatic completion of mammalian lagging strand DNA replication. *Proc. Natl. Acad. Sci.* **91:** 9803–9807.

Umbricht, C.B., L.F. Erdile, E.W. Jabs, and T.J. Kelly. 1993. Cloning, overexpression, and genomic mapping of the 14-kDa subunit of human replication protein A. *J. Biol. Chem.* **268:** 6131–6138.

Virshup, D.M., A. Russo, and T.J. Kelly. 1992. Mechanism of activation of simian virus 40 DNA replication by protein phosphatase 2A. *Mol. Cell. Biol.* **12:** 4883–4895.

Waga, S. and B. Stillman. 1994a. Anatomy of a DNA replication fork revealed by reconstitution of SV40 DNA replication *in vitro*. *Nature* **369:** 207–212.

———. 1994b. Reconstitution of complete SV40 DNA replication with purified replication factors. *J. Biol. Chem.* **269:** 10923–10934.

Waga, S., G.J. Hannon, D. Beach, and B. Stillman. 1994. The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. *Nature* **369:** 574–578.

Wang, T.S.F. 1991. Eukaryotic DNA polymerases. *Annu. Rev. Biochem.* **60:** 513–552.

Watt P.M. and I.D. Hickson. 1994. Structure and function of type II DNA topoisomerases. *Biochem. J.* **303:** 681–695.

Wei, Y.-F., P. Robins, K. Carter, K. Caldecott, D. Pappin, G.-L. Yu, R.-P. Wang, B. Shell, R. Nash, P. Schar, D. Barnes, W. Haseltine, and T. Lindahl. 1995. Molecular cloning and expression of human cDNAs encoding a novel DNA ligase IV and DNA ligase III, an enzyme active in DNA repair and recombination. *Mol. Cell. Biol.* **15:** 3206–3216.

Wiekowski, M., M.W. Schwarz, and H. Stahl. 1988. Simian virus 40 large T antigen DNA helicase. *J. Biol. Chem.* **263:** 436–442.

Wessel, R., J. Schweizer, and H. Stahl. 1992. Simian virus 40-T antigen DNA helicase is a hexamer which forms a binary complex during bidirectional unwinding from the viral origin of DNA replication. *J. Virol.* **66:** 804–815.

Wobbe, C.R., L. Weissbach, J.A. Borowiec, F. Dean, Y. Murakami, P. Bullock, and J. Hurwitz. 1987. Replication of simian virus 40 origin-containing DNA *in vitro* with purified proteins. *Proc. Natl. Acad. Sci.* **84:** 1834–1838.

Wold, M.S. and T.J. Kelly. 1988. Purification and characterization of replication protein A, a cellular protein required for *in vitro* replication of simian virus 40 DNA. *Proc. Natl. Acad. Sci.* **85:** 2523–2527.

Wold, M.S., D.H. Weinberg, D.M. Virshup, J.J. Li, and T.J. Kelly. 1989. Identification of cellular proteins required for simian virus 40 DNA replication. *J. Biol. Chem.* **264:** 2801–2809.

Xiong, Y., H. Zhang, and D. Beach. 1992. D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. *Cell* **71:** 505–514.

Xiong, Y., G.J. Hannon, H. Zhang, D. Casso, R. Kobayashi, and D. Beach. 1993. p21 is a universal inhibitor of cyclin kinases. *Nature* **366:** 701–704.

Yang, C.-L., M.S. Wold, J.J. Li, T.J. Kelly, and L.F. Liu. 1987. Roles of DNA topoisomerases in simian virus 40 DNA replication *in vitro*. *Proc. Natl. Acad. Sci.* **84:**

950–954.

Yang, C.-L. L.-S. Chang, P. Zhang, H. Hao, Z. Lingyn, N.L. Toomey, and M.Y.W.T. Lee. 1992. Molecular cloning of the cDNA for the catalytic subunit of human DNA polymerase δ. *Nucleic Acids Res.* **20:** 735–745.

Yoder, B.L. and P.M.J. Burgers. 1991. *Saccharomyces cerevisiae* replication factor C. *J. Biol. Chem.* **266:** 22689–22697.

Zhang, H., Y. Xiong, and D. Beach. 1993. Proliferating cell nuclear antigen and p21 are components of multiple cell cycle kinase complexes. *Mol. Biol. Cell* **4:** 897–906.

Zhang, S.J., X.R. Zeng, P. Zang, N.L. Toomey, R.Y. Chuang, L.S. Chang, and M.Y.W.T. Lee. 1995. A conserved region in the amino terminus of DNA polymerase δ is involved in proliferating cell nuclear antigen binding. *J. Biol. Chem.* **270:** 7988–7992.