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Comparison of DNA Replication in Cells from Prokarya and Eukarya

Bruce Stillman
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

It is now supposed that utilization of DNA as genetic material emerged after an RNA world existed (Gesteland and Atkins 1993) and that all contemporary organisms evolved from a common ancestor related to primitive photosynthetic bacteria (Woese and Pace 1993). It is therefore not surprising that cells in the three major kingdoms, Bacteria, Archaea, and Eukarya, use roughly similar strategies and mechanisms for genome duplication. Nevertheless, the diversity of DNA replication is evident when the varied strategies used for replication of bacteriophage, plasmid, and virus genomes in both prokaryotes and eukaryotes are considered. This diversity becomes obvious when scanning the chapters that summarize the replication of virus genomes later in this book (Hassell and Brinton; Stenlund; Hay; Challberg; Yates; Traktman; Cotmore and Tattersall; Seeger and Mason; Bisaro; Ahrens et al.). I do not attempt to review these varied mechanisms here but rather focus on comparing the replication of cellular DNA in bacteria and eukaryotes. Unfortunately, except for some studies on DNA polymerases, little is known about the process of DNA replication in Archaea, and therefore inclusion of this kingdom adds little to the discussion.

In organisms from bacteria to the multicellular eukaryotes, DNA replication is intimately coupled to the physiology of the individual cell and to global growth controls imposed on a population of cells, whether in a colony of bacteria or in a subset of cells within a developing organism. These regulatory pathways are different in bacteria and eukaryotes and also vary between different cell types within a single organism. Nevertheless, the actual process of DNA replication has striking parallels between the two groups of organisms. A universal challenge for the replication machinery is that the process must be precise and tightly controlled to ensure faithful transmission of the genetic heritage to the next generation of cells. If this goal is not achieved, disastrous consequences await the organism or its descendants.

DNA Replication in Eukaryotic Cells

In general, the replication of chromosomes in both groups can be separated into multiple stages. First is the recognition of specific DNA sequences within the genome by initiator proteins. This interaction is the primary determinant of the location of the origin of DNA replication. At this location, a pre-replication complex of proteins is established that renders the genome competent for replication. The two important enzyme activities that must be attracted to the origin are a helicase that will eventually unwind the template DNA and a primase that will provide the necessary primer for initiation of DNA synthesis by DNA polymerases. Activation of the pre-replicative complex is a key step that eventually leads to the unwinding of the DNA and DNA synthesis. The establishment of a multiprotein complex called a replisome at the replication fork involves the assembly of the DNA polymerases and accessory proteins, followed by fork movement. Finally, termination of DNA replication occurs prior to separation of the daughter chromosomes. It is in each of these areas that the processes in bacteria and eukaryotes will be compared. This discussion relies heavily on the reviews presented in this volume on the replication mechanisms in eukaryotes for specific examples, and much of the primary literature is cited in these other chapters.

CHROMOSOME STRUCTURE AND REPLICATION

Perhaps the most obvious difference between the two classes of cells is how the genetic material is organized into chromosomes. Bacteria have a circular chromosome that contains one principal origin of DNA replication located at the *OriC* locus in *Escherichia coli*. The rate at which the DNA polymerases move along the *E. coli* chromosome is approximately 100 kb per minute, and thus the entire chromosome can be replicated from a single origin in about 40 minutes (Kornberg and Baker 1992). The genomes of eukaryotes are much larger than the bacterial chromosomes, and the rate of replication fork movement averages about 2 kb per minute, a rate that is prohibitive for even the smallest genome to replicate from a single origin (Fangman and Brewer 1992). Moreover, DNA in eukaryotes is invariably sequestered into multiple chromosomes, which demands more than one origin per genome. In certain artificial chromosomes in *Saccharomyces cerevisiae*, one origin is sufficient to replicate a chromosome of about 100 kb in size, but if the chromosome is larger than this limit, it becomes very unstable with only one origin (Wellinger and Zakian 1989; Dershowitz and Newlon 1993; Newlon, this volume).

It has been known for more than three decades that different regions

of individual eukaryotic chromosomes replicate at distinct times during S phase, demonstrating that multiple origins of DNA replication must exist (Hand 1978; Fangman and Brewer 1992; DePamphilis, this volume). Unlike bacteria, eukaryotes initiate chromosome replication in a temporally regulated manner that can change during development (Simon and Cedar, this volume). The existence of multiple origins affords considerable flexibility in the timing of replication.

In addition to the relative timing of replication of different regions of the genome, the total time taken to replicate the entire genome can vary considerably during development of an organism (Blumenthal et al. 1974; Callan 1974). This appears to be due to a change in the frequency of active origins of DNA replication distributed along the chromosome, rather than a change in the rate of replication fork movement. For example, in the rapid replication cycles of nuclei in the early *Drosophila* embryo, origins of DNA replication are located on average 3–7 kb apart, but later during embryogenesis, the frequency of replication origins changes to about 40–100 kb as cells begin to differentiate (Blumenthal et al. 1974). Interestingly, if mammalian cells are arrested for a long period of time at the G_1 to S phase, the frequency along the genome of active origins of DNA replication increases following removal of the block, and the total time taken to pass through S phase is much shorter than the time taken in the absence of the inhibitor (Taylor 1977). This suggests that a factor may accumulate in the nucleus of cells that determines the frequency of active origins of DNA replication. A good candidate protein is the mammalian homolog of the $p65^{cdc18}$ protein from *Schizosaccharomyces pombe* and the related protein Cdc6p from *S. cerevisiae* (Kelly et al. 1993; Liang et al. 1995).

A major difference between the two groups of cells emerges from these observations. The existence of a single origin in the bacterial chromosome fixes the relative order of gene duplication, except perhaps for occasional selective gene amplification. In contrast, eukaryotes have enormous flexibility in the relative time during S phase when specific genes replicate. This permits the replication of specific regions of the genome to be linked to gene expression, a topic discussed in an accompanying chapter (Simon and Cedar, this volume). This flexibility may be an important component of developmental patterning in cells of metazoan origin. Even though the number of active origins in a single bacterial chromosome does not vary, as described below in the section on control of DNA replication, the frequency of active origins within a cell can vary considerably in bacteria due to reinitiation in a single cell-division cycle.

In bacteria that have distinct cell types, developmentally imposed control of the initiation of DNA replication can occur. In *Caulobacter crescentus,* cell division can produce two morphologically different cell types that have different replicative fates (Marczynski et al. 1995). One cell type, called the stalked cell, replicates the entire chromosome after cell division, whereas the other cell type, the swarmer cell, only replicates its DNA after a delay. In the stalked cell, a single chromosomal origin of DNA replication is utilized. The replicator element, *Cori,* has recently been characterized, and in addition to the initiator protein-binding sites that are analogous to the DnaA initiator protein-binding sites, within the *E. coli OriC* replicator, *Cori* contains essential transcriptional promoter elements that are only active in the stalked cell (Marczynski et al. 1995). Thus, cell-type-specific transcription can influence replication.

It has been known for some years that transcription can facilitate initiation of DNA replication from the bacteriophage λ origin as well as the *E. coli OriC* (Kornberg and Baker 1992). The *C. crescentus* case is an example in bacteria where transcription may effect cell-type-specific initiation of DNA replication. Transcription factors in eukaryotic cells can also be intimately involved in the initiation of DNA replication and influence origin firing in a cell-type- and temporal-specific manner (van der Vliet, this volume; for review, see DePamphilis 1993). A recent example in mammalian cells is the locus controlling region (LCR) that regulates the developmentally controlled expression of the human β-globin gene. The LCR is required for the initiation of DNA replication from an origin located near the β-globin gene (Aladjem et al. 1995). These selected examples demonstrate that despite the quite different organization of the chromosomes, and the number of replication origins, bacteria and eukaryotes can utilize similar strategies to control whether or not initiation of DNA replication will occur.

Consideration of the influence of chromosome structure on DNA replication in bacteria and eukaryotes must also take into account the different organization of DNA in the cell. The bacterial chromosome is associated with the cell membrane but otherwise is exposed to the entire intracellular environment. In contrast, eukaryotic DNA replication occurs in a distinct compartment in the cell, affording the separation of proteins that may influence the initiation of DNA replication. Within the nucleus, initiation of eukaryotic DNA replication occurs at pre-replicative complexes that are established prior to the beginning of S phase, and these presumably contain the initiator and other replication proteins (Adachi and Laemmli 1994; Diffley et al. 1994). A similar initiation complex

may exist at the membrane-bound bacterial replicator, since DnaA protein from *E. coli* is a lipid-binding protein and is associated with the membrane (Sekimizu and Kornberg 1988; Sekimizu et al. 1988a,b). Thus, in both cell types, initiation may actually occur on a solid-state support, albeit that the supports and environment may be quite different.

REPLICATORS

The mechanism of initiation was first proposed for replication of the bacterial chromosome in the early 1960s (Jacob et al. 1964). In this classic paper, a *cis*-acting DNA element called the replicator was proposed to be a primary determinant of the location in the genome where initiation of DNA replication was to occur. Furthermore, it was posited that initiation of replication of the bacterial genome also required a *trans*-acting factor called the initiator that interacted with the replicator. The replicator can be defined by genetic means, and, indeed, the *E. coli* replicator *OriC* was characterized by the isolation of fragments from the *E. coli* genome that could support autonomous replication of recombinant plasmids (Hirota et al. 1979; Messer et al. 1979; von Meyenburg et al. 1979). These plasmids behaved in a manner similar to the complete chromosome.

Fine-structure analysis by mutagenesis and evolutionary comparison of replicators from five enteric bacteria revealed that the bacterial replicator is highly conserved between species and is composed of multiple essential DNA sequence elements (Fig. 1A). The *E. coli* replicator has four recognition sites for the initiator protein DnaA that are flanked on one side by an A•T-rich sequence and on the other by a series of repeats that can be easily unwound in negatively supercoiled DNA (Kornberg and Baker 1992). These three repeats, or 13-mers, are locally unwound by DnaA once it is bound to *OriC* (Bramhill and Kornberg 1988). This general structure for bacterial replicators is also found in the origins of DNA replication from the lambdoid phages. Interspersed within the *E. coli* replicator are ten recognition sites for the DNA adenine methyltransferase (dam methylase), and methylation of these sites plays an important role in the control of initiation (see below).

The replicators present in eukaryotic viruses have been characterized by mutational analysis. One of the best understood of these is the simian virus 40 (SV40) replicator called the SV40 *ori* (Stillman 1989; Hassell and Brinton, this volume). Like the bacterial replicators, the SV40 *ori* contains multiple binding sites for the SV40 initiator protein, T antigen, and these binding sites are flanked by an A•T-rich sequence on one side and a partial palindromic repeated sequence (Fig. 1B) (Parsons et al.

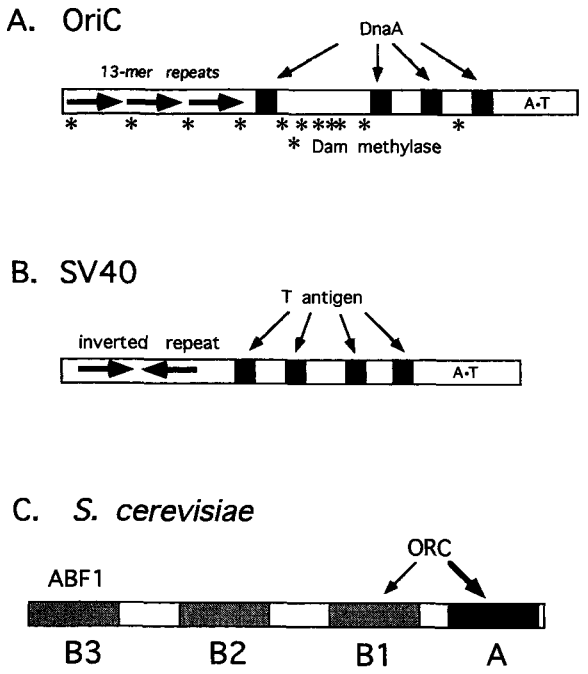


Figure 1 Comparison of the replicators from *E. coli*, SV40, and *S. cerevisiae*. (*A*) *E. coli OriC*. (*B*) The SV40 *ori*. (*C*) *S. cerevisiae ARS1*.

1990). The latter repeat also interacts with T antigen, resulting in the unwinding of 8 bp of DNA. Thus, the SV40 *ori* is remarkably similar in organization to the *E. coli* replicator.

Understanding the nature of replicators in eukaryotic cell chromosomes has been more problematic, particularly in multicellular eukaryotes. Only in the yeasts have replicators been isolated by selection of fragments that permit autonomous replication of plasmids (Campbell and Newlon 1991; DePamphilis; Newlon; both this volume). Attempts to isolate such autonomous replicating sequences (ARSs) in other eukaryotes have not been successful, and thus the replicators from metazoan cells remain poorly defined. In *S. cerevisiae,* a number of replicators have been characterized extensively, and the general picture that has emerged is that the replicators have little similarity to the bacterial and viral replicators (Fig. 1C). The best-characterized replicator is ARS1 from chromosome IV, and it consists of four DNA sequence elements, only one of which (A) is essential (Marahrens and Stillman 1992). The initiator protein for eukaryotes is the origin recognition complex (ORC), and this six-subunit protein binds to the A and B1 elements in an

ATP-dependent manner (Bell and Stillman 1992; Rao and Stillman 1995; Rowley et al. 1995). Another element, which is not essential and is only present in some replicators in the *S. cerevisiae* chromosomes, is a binding site for the transcription factor ABF1 (Marahrens and Stillman 1992). Interestingly, other transcription factors can substitute for ABF1 and activate initiation of DNA replication, similar to the stimulation of initiation of replication at virus origins by transcription factors (DePamphilis 1993; Hassell and Brinton; van der Vliet; both this volume).

In general, the yeast replicators contain DNA sequences that are easily unwound when a plasmid in which they are cloned is in a highly negatively supercoiled state (Natale et al. 1993). A similar phenomenon has been observed with the *E. coli OriC* and the SV40 *ori* (Kowalski and Eddy 1989; Lin and Kowalski 1994), and in these cases, the region that unwinds first corresponds to the region that is unwound by the binding of DnaA or SV40 T antigen, respectively. This suggests that all origins of DNA replication have a tendency to unwind, and the energy for separation of the double helix either comes from torsional strain in the DNA, binding of the initiator protein, or both. For replication of plasmid DNAs containing the phage λ or *OriC* replicators in vitro, torsional strain provided by a balance of gyrase, topoisomerase I, and the HU protein greatly facilitates origin unwinding and initiation of DNA replication (Sekimizu et al. 1988a,b; Mensa-Wilmot et al. 1989; Hwang and Kornberg 1992). Furthermore, transcription of the template DNA in the vicinity of the origin is necessary for the earliest stages of duplex strand unwinding during the initiation of DNA replication (Baker and Kornberg 1988; Learn et al. 1993). Whether transcription per se or the action of transcriptional activation domains present on sequence-specific DNA-binding proteins activates initiation at eukaryotic virus and cell origins remains to be determined.

INITIATORS

As indicated above, initiator proteins bind in a sequence-specific manner to the replicator, and together they function as the primary determinants of the location of the origin of DNA replication. The primary function of the initiator-replicator complex is as a landing pad for other replication proteins such as the helicase and the primase. Initiators can come in many different versions. All function as DNA-binding proteins, and all interact with other proteins that are required for the initiation of DNA replication. Some, such as the phage λ O protein, function only in this capacity (Dodson et al. 1985), whereas others have additional activities.

For example, the DnaA protein from *E. coli* is an ATP/ADP-binding protein with the ability to slowly hydrolyze the ATP to ADP, whereas the SV40 T antigen, the papillomavirus E1 protein, and the herpes simplex virus (HSV) UL9 initiators bind ATP, have a potent intrinsic ATPase activity, and in addition, contain DNA helicase function (Stahl et al. 1986; Sekimizu et al. 1988a,b; Bruckner et al. 1991; see Boroweic; Challberg; Hassell and Brinton; Stenlund; all this volume). One intriguing initiator from bacteriophage P4 is a sequence DNA-binding protein, an ATPase, a DNA helicase, and a DNA primase, and thus fulfills many of the functions required for initiation of DNA replication (Ziegelin et al. 1993).

A common characteristic of initiator proteins is that they often oligomerize upon binding to the DNA. The DnaA and λ O initiator proteins form complexes of 8 or about 20 proteins bound to *OriC* or *oriλ*, respectively, even though there are only four DNA recognition sequences in the replicator (Echols 1986; Kelman and O'Donnell 1994). Similarly, the SV40 T antigen has four recognition sites in the replicator, yet two hexamers of T antigen bind to the SV40 *ori* in an ATP-dependent manner (Dean et al. 1987). The oligomerization probably acts to stabilize the initiator-replicator interactions and to allow efficient interaction with other proteins. In the case of SV40 T antigen, the functional helicase unit is a hexamer. In many respects, the T-antigen hexamer resembles the RuvB ATPase that is involved in branch migration of DNA at a recombination Holliday junction (Parsons et al. 1995).

Oligomerization of an initiator protein can prevent DNA replication. The bacteriophage P1 genome can exist in a lysogenic state in its host bacteria as a plasmid, and initiation of DNA replication requires the *OriP1* replicator and the plasmid-encoded RepA initiator (Baker and Wickner 1992). The RepA protein exists as a stable dimer when not bound to the DNA. The *E. coli* chaperone proteins DnaJ, DnaK, and GrpE combine to break apart the RepA dimer into RepA monomers, thereby allowing RepA to bind to *OriP1* in cooperation with the DnaA protein to form a productive replicator-initiator complex (Baker and Wickner 1992).

In many cases, but not all, the binding of the initiator protein to the replicator causes local unwinding of a region of DNA at the origin of DNA replication. The DnaA protein induces unwinding of the 13-mer elements at *OriC*, and SV40 T antigen causes unwinding of 8 bp in the partial "early" palindrome within the SV40 *ori* (Bramhill and Kornberg 1988; Borowiec et al. 1990; Hassell and Brinton; Brush and Kelly; both this volume). Unwinding of the DNA at the origin of DNA replication is a key step in the pathway leading to initiation of DNA synthesis at the

origin. This is facilitated by regions within the origin of DNA replication that have a low helical stability and thus have a tendency to unwind when placed under torsional strain (Kowalski and Eddy 1989; Natale et al. 1993; Lin and Kowalski 1994).

The assembly and disassembly of the multiprotein complex on the replicator and the ability of the complex to function as a landing pad are highly regulated processes. The function of the *E. coli* DnaA complex is regulated by membrane-bound acidic phospholipids (Crooke et al. 1992). The ATP form, but not the ADP form, of DnaA is able to unwind the 13-mers in *OriC*, and membrane-bound acidic phospholipids greatly stimulate the exchange of ADP to ATP bound to DnaA. Exchange to the active ATP•DnaA complex requires the DnaA to be bound to *OriC* because the acidic phospholipids inactivate DNA-free DnaA protein so that it cannot bind *OriC*. These results suggest a link between DnaA activity and the membrane localization of *OriC*.

Phosphorylation of proteins is not known to be a direct mechanism for control of DNA replication in bacteria, but it is a major mechanism for regulation of some eukaryotic virus initiator proteins. For example, the ability of SV40 T antigen to unwind the origin of DNA replication is positively regulated by phosphorylation of a single threonine by a cyclin-dependent protein kinase (Ismail et al. 1993; McVey et al. 1993). On the other hand, the DNA-binding activity is negatively regulated by phosphorylation of two serine residues by casein kinase I, which can be reversed by the action of phosphatase 2A (Cegielska et al. 1994a,b). In eukaryotes, phosphorylation can also control the cellular localization of proteins. For example, phosphorylation by casein kinase II of serine residues that lie adjacent to the nuclear localization signal in SV40 T antigen greatly facilitates the transport of the protein from the cytoplasm to the nucleus. It is easy to imagine that this may play a regulatory role for the initiation of DNA replication. Thus, in eukaryotes, unlike bacteria, control of the intracellular locale of proteins may affect their function. A discussion of the role of the nuclear envelope in the initiation of cellular DNA replication can be found in Laskey and Madine (this volume).

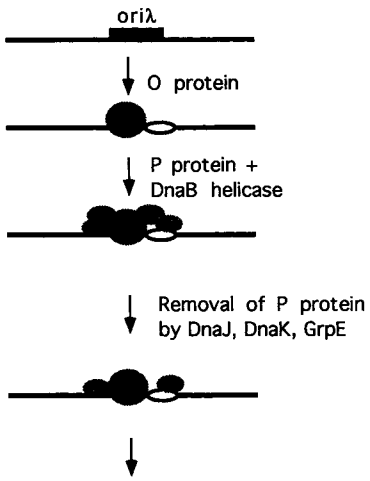
UNWINDING AT THE ORIGIN

Following DNA sequence recognition and establishment of the initiator-protein complex at the origin of DNA replication, the next important events are unwinding of the template DNA and priming DNA replica-

tion. Initiator proteins such as DnaA protein (Bramhill and Kornberg 1988) and SV40 T antigen (Borowiec et al. 1990) can locally unwind a region of the origin of DNA replication, and this region of single-stranded DNA is presumed to be the entry site for a DNA helicase and single-stranded DNA-binding proteins such as the *E. coli* DnaB helicase and SSB, respectively. A DNA helicase has to be loaded onto the initiator-DNA complex for more extensive unwinding to occur. SV40 T antigen is an unusual case because it is a DNA helicase as well as an initiator protein; thus, the helicase is automatically located in a prime site for extensive unwinding. Indeed, T antigen unwinds almost the entire SV40 genome in cooperation with the eukaryotic cell single-stranded DNA-binding protein RP-A and a topoisomerase (Borowiec et al. 1990; see Borowiec; Hassell and Brinton; both this volume). In most cases, however, the helicase is a separate polypeptide from the initiator protein and needs to be loaded onto the DNA.

Loading of the DNA helicase is a complicated affair. The DnaB helicase from *E. coli* is used to initiate DNA replication from *OriC* and is also required for replication of the bacteriophage λ genome (Baker and Wickner 1992; Stillman 1994a). In neither case can the DnaB helicase recognize the initiator protein (DnaA or λ O protein) by itself, but needs to be chaperoned by a specialized protein. The *E. coli* DnaC and the phage λ P proteins function as helicase-loading proteins because they both bind to the DnaB helicase and also bind either to the DnaA or λ O proteins, respectively (Fig. 2). These helicase-loading proteins can inhibit the DNA helicase activity, and it is necessary to remove them before the helicase can be activated to unwind the template DNA (Stillman 1994a). For initiation at the phage λ origin (*oriλ*), the *E. coli* heat shock chaperone proteins DnaJ, DnaK, and GrpE cooperate to remove λ P protein and thus activate the DnaB helicase (Baker and Wickner 1992; Stillman 1994a). Here is another example from the prokaryotic world where chaperone proteins pull apart protein complexes that are involved in the initiation of DNA replication. To date, chaperones have not been shown to play a role in disassembling protein complexes in eukaryotes, but little is known about the eukaryotic pre-replicative complex. It is likely that similar assembly and disassembly of multiprotein complexes occur at eukaryotic replicators.

The helicase-loading proteins can also facilitate the assembly of a DNA helicase onto the template DNA in the absence of an origin of DNA replication. For example, the bacteriophage T4 gene 59 protein (gp59) binds to both single-stranded DNA and the phage single-strand-binding protein, gp32 (Barry and Alberts 1994; Morrical et al. 1994).



PRIMING AND INITIATION OF DNA REPLICATION

Figure 2 Loading of a DNA helicase. The bacteriophage λ P protein loads the DNA helicase onto the DNA. The λ P protein must be removed before the helicase (DnaB) can function to unwind the DNA prior to DNA replication. This is facilitated by the chaperone proteins DnaJ, DnaK, and GrpE. (Reprinted, with permission, from Stillman 1994a.)

When gp32 coats a single-stranded DNA template, the phage helicase gp41 cannot bind to the DNA. The gp59 protein loads the gp41 helicase onto the gp32-coated DNA, and the helicase can now move rapidly along the template in search of duplex DNA to unwind. This mechanism of helicase loading is almost certainly used during the elongation stages of DNA replication. In a similar manner, SV40 T antigen can bind the RP-A protein, the eukaryotic SSB, facilitating loading of a helicase onto the RP-A-coated single-stranded DNA (Collins and Kelly 1991; Melendy and Stillman 1993).

Although many DNA helicases have been identified in eukaryotes, it is not clear which are required for the initiation of DNA replication (Borowiec, this volume). A good candidate is the helicase B from a murine cell line that harbors a temperature-sensitive helicase which causes a defect in DNA replication at the nonpermissive temperature (Seki et al. 1995). Moreover, although proteins such as Cdc6p that interact with the eukaryotic initiator protein ORC are beginning to be identified (Liang et al. 1995), it is not yet clear whether the same mechanism of helicase loading occurs in eukaryotic cells as occurs in bacteria.

PRIMING DNA SYNTHESIS

Following unwinding of the template DNA surrounding the replicator, priming of DNA synthesis occurs, usually by a DNA primase. As discussed by others in this volume (Brush and Kelly; Salas et al.), priming can occur by a number of different mechanisms, including priming by a nucleotide linked to a protein such as occurs during Φ29 phage and adenovirus DNA replication. For initiation of chromosomal DNA replication in bacteria and almost certainly in eukaryotes, priming involves the assembly of a primase on the DNA. In *E. coli*, the primase exists as a free enzyme that is not always bound to the DNA polymerase (Baker and Wickner 1992; Kornberg and Baker 1992), whereas in eukaryotes, the primase enzyme is always in a complex with DNA polymerase-α (Wang, this volume).

Herein lies a significant difference between prokaryotes and eukaryotes: Bacteria contain only one principal replicative DNA polymerase (DNA polymerase III in *E. coli*) that must function on both the leading and lagging strands at a replication fork, but in eukaryotes, the DNA polymerizing activity is shared by three DNA polymerases, pol-α, pol-δ, and pol-ε (Kornberg and Baker 1992; Wang, this volume). Thus, the pol III enzyme in *E. coli* needs to be free of the primase when replicating the continuously synthesized leading strand, but must cyclically interact with the primase during lagging-strand replication (Hacker and Alberts 1994a,b; Stillman 1994b; Stukenberg et al. 1994). Eukaryotes have devised a DNA polymerase switching mechanism that involves the pol-α:primase complex in priming DNA replication at the origin of DNA replication and for every Okazaki fragment, and then a second DNA polymerase that is not associated with the primase (pol-δ or ε) completing the synthesis (Figs. 3 and 4) (Nethanel and Kaufmann 1990; Tsurimoto et al. 1990; Waga and Stillman 1994; Stillman 1994b).

A key question is how the primase finds either the origin of DNA replication or the site at which Okazaki fragments must begin. Bacterial and phage primases can exist in two different classes: those that are part of the helicase polypeptide and others that exist as proteins separate from the helicase polypeptide (Ilyina et al. 1992). If the primase activity coexists in the same polypeptide as the helicase, then the primase is associated with the replication fork by default. For bacterial chromosome replication and for the replication of some eukaryote virus genomes such as SV40 and herpesvirus, the primase-loading problem has been solved by the primase having an affinity either for the helicase that has already been loaded onto the template DNA or, alternatively, for a structure in the DNA created by the helicase (Baker and Wickner 1992; Marians

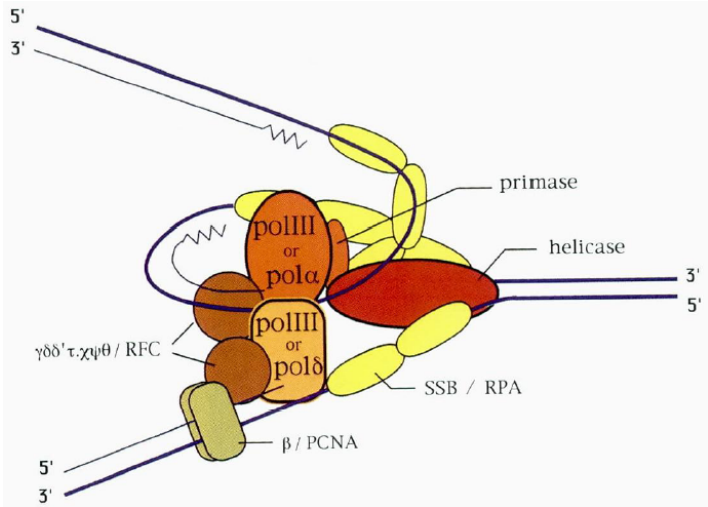


Figure 3 Replication fork proteins. Model for a dimeric DNA polymerase at the replication fork. (Reprinted, with permission, from Stillman 1994b [copyright Cell Press].)

1992; Kelman and O'Donnell 1994; Stillman 1994a; Hassell and Brinton; Challberg; both this volume). For example, the SV40 T antigen binds directly to the pol-α:primase complex and can load the primase onto an RP-A-coated template DNA by direct interaction with RP-A (Collins and Kelly 1991; Melendy and Stillman 1993; Dornreiter et al. 1993). In *E. coli,* the DnaB helicase activates the DnaG primase (Baker and Wickner 1992; Marians 1992). Once located at the priming site, the primase in both prokaryotes and eukaryotes synthesizes a short (8–12 nucleosides) RNA primer that is utilized by the DNA polymerase for synthesis of DNA.

ASSEMBLY OF THE DNA POLYMERASE HOLOENZYME

A common feature of the DNA replication fork in bacteria and eukaryotes is that DNA is synthesized in a semidiscontinuous manner, with one strand, the leading strand, polymerized in a continuous manner and the other lagging strand formed in a discontinuous manner via the synthesis of Okazaki fragments (Brush and Kelly, this volume). The DNA polymerase is loaded onto the template DNA at the site of the primer by a mechanism that is remarkably conserved between prokaryotes and eukaryotes (Table 1) (Kelman and O'Donnell 1994; Stillman 1994b). The process is accomplished by polymerase accessory

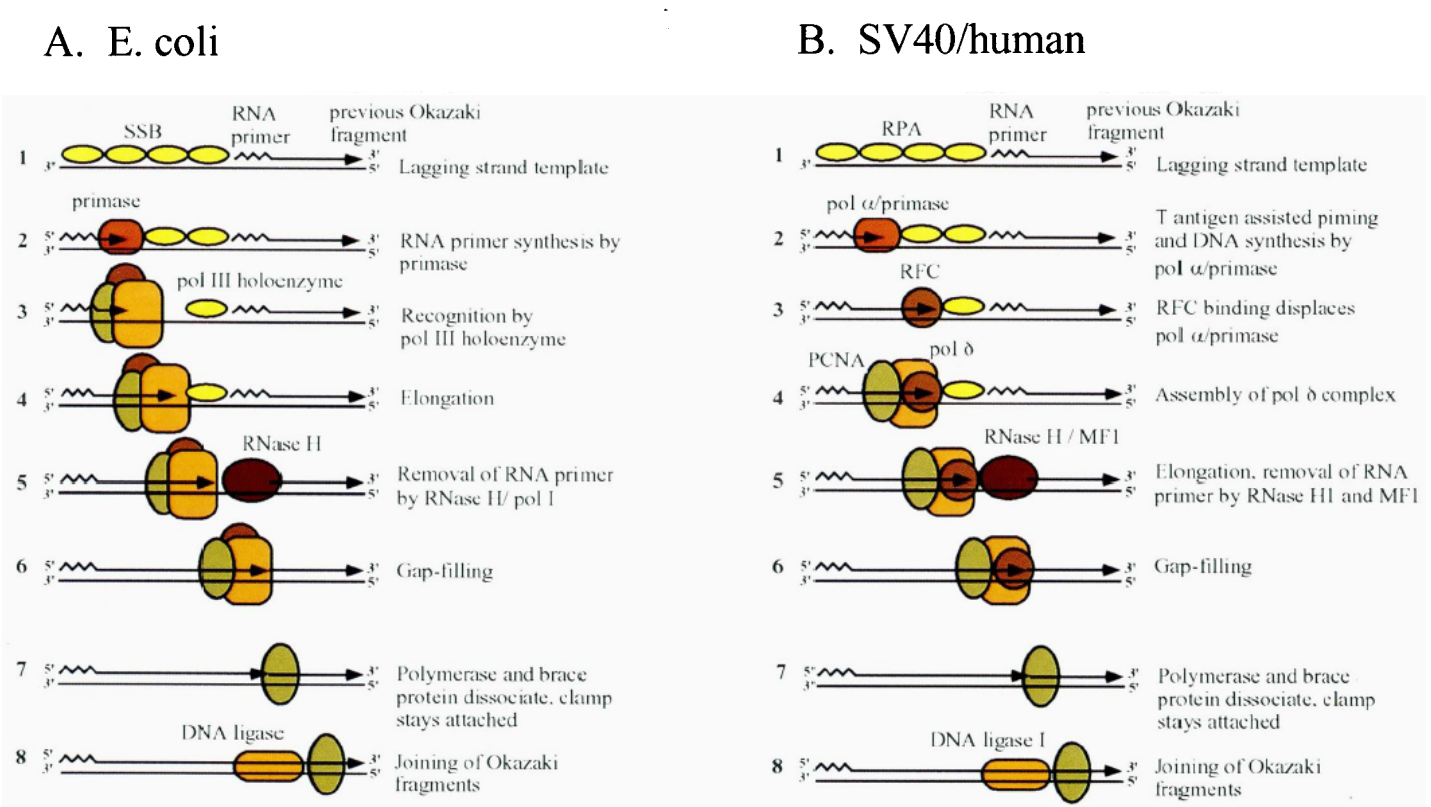


Figure 4 Replication fork proteins. Comparison between the synthesis of Okazaki fragments in bacteria and eukaryotes. (Reprinted, with permission, from Stillman 1994b [copyright Cell Press].)

Table 1 Functions of DNA replication fork proteins

E. coli	Phage T4	SV40/human	Functions
DnaB	41	T antigen	DNA helicase; stimulates priming on ssDNA
DnaC	59	T antigen	allows loading of helicase and primase onto SSB-coated DNA (primosome assembly)
SSB	32	RP-A	single-stranded DNA-binding; stimulates DNA polymerase; facilitates helicase loading
γ-Complex ($\gamma\delta.\delta'\chi\psi$)	44/62	RF-C	DNA-dependent ATPase; primer-template binding; stimulates DNA polymerase
τ	43	?	dimerization of holoenzyme
β	45	PCNA	stimulates DNA polymerase; stimulates DNA-dependent ATPase
pol III core ($\alpha\theta\varepsilon$)	43	pol-δ[a]	DNA polymerase 3′→5′ exonuclease
DnaG	61	primase pol-α[b]	primase
Ligase	T4 ligase	ligase I	ligation of DNA
DNA pol I	43	FEN-1/MF1	nuclease for removal of RNA primers
RNase H	RNase H	RNase H1	nuclease for removal of RNA primers

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[a]DNA polymerase-δ has been shown to function in SV40 DNA replication; an essential DNA polymerase, DNA polymerase-ε, has not yet been assigned a specific function in DNA replication.

[b]The human DNA polymerase-α and primase activities function as a multiprotein complex to synthesize RNA/DNA primers. (RP-A) Replication protein A; (RF-C) replication factor C; (PCNA) proliferating cell nuclear antigen; (SSB) single-strand DNA-binding protein.

proteins. The eukaryotic proteins are discussed by Hübscher et al. (this volume). Similar mechanisms and enzymatic activities in these eukaryote proteins and the bacteriophage T4 counterparts were recognized first (Tsurimoto and Stillman 1990; Tsurimoto et al. 1990), and later, some sequence similarities were observed (O'Donnell et al. 1993).

The polymerase accessory proteins from *E. coli*, bacteriophage T4, and eukaryotes include the single-strand-binding protein (SSB, gp32, or RP-A), a polymerase clamp protein (β subunit of pol III, gp45, or PCNA), and a DNA-dependent ATPase that loads the polymerase clamp onto the DNA ($\gamma\delta\delta'\tau\chi\psi$, gp44/62, and RF-C). Only in the case of the

clamp-loading proteins have significant DNA sequence similarities been observed (O'Donnell et al. 1993), but there is remarkable structural similarity between the polymerase clamp proteins even though there is no primary amino acid sequence similarity (Fig. 5) (Krishna et al. 1994). The β subunit of the pol III enzyme from *E. coli* and the *S. cerevisiae* PCNA have three-dimensional structures that can be almost superimposed one on top of the other. Both form a donut or torus-like shape, and the DNA passes through the hole in the middle of the structure. The β subunit contains 366 amino acids, whereas the PCNA contains only 258 amino acids, approximately two-thirds the size of the bacterial counterpart. Two monomers of the β subunit form the torus structure, whereas three monomers of the smaller PCNA combine to form an identical structure. Each torus ring has an internal sixfold symmetry. The 228-amino acid phage T4 gp45 protein is presumed to also form a trimer.

Interestingly, the carrot plant has two highly related PCNA proteins that are similar in sequence to PCNA from human and yeast; one is 264 amino acids, similar to the "eukaryotic short" form and the other is 365 amino acids, similar to the "bacterial long" form (Kelman and O'Donnell 1995). It has been suggested that these plants have one dimer PCNA and one trimer PCNA that may function at different times during development when DNA replication rates vary. Alternatively, since PCNA also functions in DNA repair (Nichols and Sancar 1992; Shivji et al. 1992), it is possible that one PCNA is involved in DNA repair and the other in DNA replication.

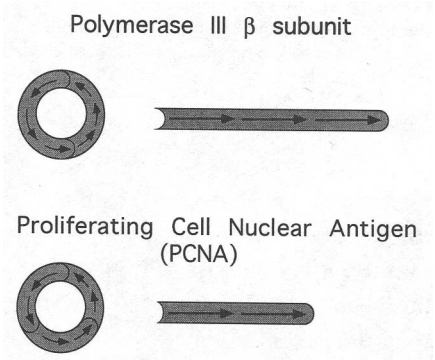

Figure 5 Comparison of the structures of the clamp proteins from bacteria and yeast. Both proteins form a torus or donut-shaped molecule with sixfold symmetry. The *E. coli* pol III β subunit forms a dimer and PCNA forms a trimer. The arrows represent a repeated structural motif in the proteins. The DNA passes through the center of the structure.

The mechanism of polymerase loading is very similar in bacteria and eukaryotes and is discussed in detail elsewhere in this volume (Hübscher et al.) and in other reviews (Kelman and O'Donnell 1994; Stillman 1994b). In both systems, an ATP-dependent clamp-loading protein recognizes a primer-template junction and assembles the polymerase clamp onto the DNA. The single-stranded DNA-binding protein that coats the single-stranded template DNA facilitates the recognition of the primer-template junction by preventing the clamp-loading protein from binding to single-stranded DNA. Once the clamp complex is assembled, a DNA polymerase is assembled onto the primer ready for DNA synthesis.

TOPOLOGY AND DNA REPLICATION

Topoisomerases in both prokaryotes and eukaryotes deal with the topological problems associated with unwinding the double helix during DNA replication and with separating the daughter chromosomes after DNA replication is completed. The different types of topoisomerase have been reviewed recently (Roca 1995), and a discussion of the eukaryotic topoisomerases appears in Hangaard Andersen et al. (this volume). The role of topoisomerases from bacteria and eukaryotes in DNA replication has been well discussed in a recent review (Ullsperger et al. 1995), and only some general points are noted here. Bacterial chromosome topology is controlled by a balance between topoisomerases that relieve torsional strain on the one hand and gyrases that induce negative supercoiling on the other. There is net negative supercoiling in the bacterial chromosome, and these negative supercoils are available to facilitate unwinding of the double helix for processes such as transcription and the initiation of DNA replication. In contrast, eukaryotic chromosomes have more complex topology. Eukaryotic chromosomal DNA has the same supercoil density as found in bacteria, but all the negative supercoils are believed to be constrained in nucleosomes (Ullsperger et al. 1995). Furthermore, the eukaryotic chromosome is folded into complex higher-order structures that the DNA replication apparatus must deal with. Thus, the DNA replication machineries in the two cell types must deal with the very different templates, although as indicated above, the basic replication proteins are quite similar.

Unwinding of the DNA helix during replication fork progression generates positive supercoils ahead of the replication fork in the unreplicated DNA. There are different mechanisms to remove these positive supercoils (Ullsperger et al. 1995). The first is to actively generate negative

Table 2 Topoisomerase activities

	Prokaryotes				Eukaryotes		
	topo I	topo II	topo III	topo IV	topo I	topo II	topo III
Name	omega	gyrase	–	–	–	–	–
Type	1	2	1	2	1	2	1
Genes	*topA*	*gyrA, gyrB*	*topB*	*parC, parE*	*top1*	*top2*	*top3*
(+) Supercoil removal in vivo	no	yes	no	yes?	yes	yes	no
Decatenation	no	yes	no	yes	no	yes	no
Relaxes only (–) supercoils	yes	no	yes	no	no	no	yes

(Adapted from Ullsperger et al. 1995 [copyright Springer-Verlag GmbH].)

supercoils in the unreplicated DNA before the positive supercoils accumulate, a role for the bacterial type II topoisomerase commonly referred to as DNA gyrase (Table 2). The bacterial gyrase can keep up with the generation of positive supercoils ahead of the replication fork by introducing negative supercoils. A second mechanism is to remove directly the positive supercoils in the unreplicated DNA; both the eukaryotic type I and type II topoisomerases can perform this function (Table 2). This mechanism is thought to occur in the early stages of replication from an origin when there is a relatively large amount of unreplicated DNA. In the later stages of DNA replication, it is more difficult to introduce positive supercoils into the unreplicated DNA, and the replicated DNA tends to become interwound or catenated (Ullsperger et al. 1995). Thus, a third mechanism for relieving the positive supercoils generated by replication fork progression is to remove catenated DNA, a job for different types of topoisomerases in bacteria and eukaryotes. The bacterial type I topoisomerase, topo III, binds to single-stranded DNA and thus can operate behind the replication fork to decatenate the replicated DNA. In contrast, the type II topoisomerases, topo II in eukaryotes and type IV in bacteria, can decatenate the replicated duplex DNA and thus segregate completely replicated daughter chromosomes (Ullsperger et al. 1995).

Finally, as pointed out by Ullsperger et al. (1995), if the replication fork contains a dimeric DNA polymerase with physically linked polymerase molecules of the type shown in Figure 3, this creates even more problems with DNA topology because the DNA polymerases are

not free to rotate about the template DNA. If the DNA polymerases are connected to each other, as seems likely in bacteria and eukaryotes, then enzymatic mechanisms must exist that deal with the passage of the double helix through the polymerase complex.

FIDELITY AND DNA REPAIR

Both bacteria and eukaryotic cells have mechanisms for repairing DNA (Friedberg et al. 1995), and it is beyond the scope of this discussion to review these varied mechanisms here (for eukaryotes, see Roberts and Kunkel; Friedberg and Wood; both this volume). As discussed by Nasmyth (this volume) and others elsewhere (Li and Deshaies 1993; Murray 1994; Nurse 1994), eukaryotic cells have a monitoring system that links DNA replication with cell-cycle progression, cell growth, and DNA repair. The existence of DNA damage-checkpoint mechanisms in bacteria that temporally delay DNA replication and cell-cycle progression in response to DNA damage is not as clear as has become apparent in eukaryotes, but inhibition of DNA synthesis and recovery are known to occur (Bridges 1995). Although the mechanisms of DNA repair are similar between bacteria and eukaryotes, the mechanisms that control the cell division cycle are very different. Thus, simple parallels between prokaryotes and eukaryotes have not emerged.

CONTROL OF INITIATION

Because the pathways that control the cell division cycle are very different between bacteria and eukaryotes, the mechanisms for controlling the frequency of initiation are also different. Methylation of the bacterial origin of DNA replication by the dam methylase causes the bacterial DNA to become hemi-methylated immediately after it has replicated (Baker 1994). In the *E. coli OriC* replicator, the Dam methylation sequences (5′-GATC-3′) are clustered in the 13-mer repeats that unwind in the presence of DnaA and ATP. *OriC* is negatively regulated by a process called sequestration. For some time after the initiation of DNA replication, the hemi-methylated DNA is unable to reinitiate, thereby limiting multiple rounds of DNA replication in a cell. The product of the SeqA gene is required for sequestration, and SeqA interacts genetically with DnaA (Campbell and Kleckner 1990; Baker 1994). Recently, it has been shown that the SeqA protein binds to hemi-methylated DNA from either the *E. coli OriC* 13-mer region or from the bacteriophage P1 origin, *P1oriR* (Brendler et al. 1995). Binding to these replication origins

is preferred because these sequences are somehow slow to be fully methylated and therefore remain in the hemi-methylated state for some time. These data suggest that the SeqA protein might prevent initiation of DNA replication by interacting with the newly hemi-methylated replicator, thereby preventing productive binding of the replicator by the initiator DnaA.

Methylation probably does not play a role in the regulation of initiation of DNA replication in eukaryotes. More likely, a multiprotein complex is formed on the DNA at the site of the replicator to produce a "competent" pre-replicative complex. This complex can then be acted on by other regulators of DNA replication that coordinate the initiation of DNA replication with progression through the cell division cycle (see Nasmyth, this volume). In many ways, this type of regulation may be similar to the assembly and disassembly of proteins at the phage λ replicator (Stillman 1994a). This type of regulation may also facilitate the coordination of replication from multiple origins of DNA replication that does not occur in bacteria.

TERMINATION OF DNA REPLICATION

The termination of DNA replication has been well studied in bacteria, including *E. coli* and *Bacillus subtilis* (Baker 1995). Replication of the bacterial chromosome terminates when the two replication forks that diverged from *OriC* converge in a region of genome that is 180° from the origin. There are multiple *cis*-acting sites in the DNA called Ter sites in *E. coli* and imperfect repeats (IR-I and IR-II) in *B. subtilis*. These DNA elements have directionality with respect to the advancing replication fork and interact with a protein called Tus in *E. coli* and RTP in *B. subtilis*. The Ter-Tus protein complex functions as an anti-helicase complex because it blocks the progression of DNA unwinding by a DNA helicase in the absence of DNA replication. The Tus-Ter complex has been shown to block the DnaB helicase (Khatri et al. 1989; Lee et al. 1989) and, interestingly, it can also inhibit the SV40 T-antigen helicase (Bedrosian and Bastia 1991). A more thorough description of termination is given by Bastia and Mohanty (this volume).

Replication termination in eukaryotes is not well studied, but it is clear that specific DNA sequences are not generally required. For example, the SV40 origin of DNA replication has been cloned into many plasmids, and these plasmids replicate very well when they are introduced into primate cells, yet the DNA sequences located in the region opposite the origin are very different (Lai and Nathans 1975). Similarly, replica-

tion forks that meet each other following initiation at adjacent origins in eukaryotic chromosomes are believed to terminate when the two forks meet each other, but in a sequence-independent manner.

There are, however, DNA sequences in eukaryotic genomes that cause DNA replication to arrest and occasionally terminate. For example, repeated DNA recognition sites for the Epstein-Barr virus (EBV) nuclear antigen 1 (EBNA1) cause replication forks to pause or arrest completely (Little and Schildkraut 1995). Furthermore, DNA replication fork barriers have been found in the ribosomal DNA repeats in *S. cerevisiae* (Brewer et al. 1992). Thus, it is possible that sequence-specific DNA-binding proteins may affect the passage of the eukaryotic DNA replication fork in much the same way as the Tus-Ter system in *E. coli* and that these sites may play some regulatory role that has yet to be appreciated.

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