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Mechanisms for Completing DNA Replication

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DNA replication consists of three steps: initiation, ongoing replication, and termination. The termination of replication is important because the synchrony of the termination process with subsequent cell division should be a significant factor in the equal and orderly distribution of genetic material to the daughter cells of both prokaryotes and eukaryotes.

Termination in a circular chromosome in prokaryotes involves arrest of replication forks at specific sequences called replication arrest sites (for reviews of this process, see Hill 1992; Baker 1995). There are some exceptions to the phenomenon of sequence-specific replication arrest in both prokaryotic and eukaryotic chromosomes; e.g., the early stage of bacteriophage λ DNA replication (Valenzuela et al. 1976) and SV40 DNA replication (Lai and Nathans 1975). Replication fork arrest is the first step in the termination process. Termination also involves, in *Escherichia coli*, decatenation of the arrested, catenated daughter molecules of DNA by a special topoisomerase called topo IV (Kato et al. 1988, 1990; Schmid 1990; Adams et al. 1992; Hiasa and Marians 1994b). Mutants that are defective in topo IV, a heterodimeric enzyme, are also defective in nucleoid segregation (Kato et al. 1988; Schmid 1990; Adams et al. 1992). More information on topoisomerases can be found in the chapter by Hangaard Andersen et al. (this volume). A third step in termination and proper segregation of the newly completed daughter molecules involves a site-specific recombination system. The system resolves multimers generated by an odd number of recombinations which may occur between the two separating daughter, circular DNA molecules (Blakely et al. 1991; Kuempel et al. 1991).

Although a great deal is known about the termination of replication in prokaryotes, very little is known about this process in eukaryotes. In eukaryotic chromosomes, the linear DNA molecule faces two major problems for completing a round of replication. First, the newly synthesized strand at its 5′ end would have a gap created by the removal of

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the last primer RNA. The filling-in of the gap to complete a round of replication requires special structures at the ends of the DNA molecules, such as terminal redundancy, inverted repeats, or telomeres (for a discussion of the problem, see Kornberg and Baker 1992). Telomeres have been reviewed in a separate chapter in this volume (see Greider et al.). Second, since initiation of replication occurs at many origins in linear eukaryotic DNA, each replication unit must terminate or merge with the preceding and the succeeding units at internal termini. Whether these internal termini are sequence-specific remains unknown.

Since the movement of the replication fork is driven by two key enzymes, namely DNA helicase and DNA polymerase, interference with the activities of either of the two enzymes would cause pausing or arrest of replication forks. Interference with the movement of the replication fork may be due to specific DNA sequences, lesions on DNA, or specific DNA/protein complexes; interference may be transient (pausing) or may lead to arrest of the replisome complex for a longer period.

We first discuss pausing or arrest of replication forks at certain DNA sequences and the nature of these sequences. These pause or arrest sites do not necessarily define authentic replication termination sites but have interesting biological implications and, therefore, are discussed. A major part of this chapter focuses on our present knowledge of sequence-specific replication arrest systems in the prokaryotic organisms *E. coli* and *Bacillus subtilis*. Replication arrest at these sequences to which specific proteins bind has been referred to as termination process in the past. In fact, the fork arrest constitutes only the first step of replication termination. The few examples of known sequence-specific internal arrest sites in linear eukaryotic chromosomes are discussed at the end of the chapter.

PAUSING OF REPLICATION FORKS CAUSED BY DNA SEQUENCE/DNA POLYMERASE INTERACTION

Replication Pause Sites

Both prokaryotic and eukaryotic DNA polymerases pause or are arrested at certain DNA sequences (LaDuca et al. 1983; for review, see Bierne and Michel 1994). The arresting sequences are hairpin loops, polypurine stretches, or sequences that could adopt triple helix structures when subjected to negative supercoiling (Wells et al. 1988; Lindsey and Leach 1989; Dayn et al. 1992).

Weaver and DePamphilis (1984) have systematically analyzed the role of palindromic and nonpalindromic sequences in arresting DNA

synthesis in vivo and in vitro. They observed that DNA polymerase-α was arrested before entering a palindromic sequence (class I sites). The arrest was nonpolar. There were also nonpalindromic (class II) sites that arrested DNA polymerase-α, and the sequences in front of and behind the point of arrest were necessary for the arrest to occur. Interestingly, the complementary sequence did not arrest polymerase-α-catalyzed DNA synthesis. The physiological role of these pause sites is mostly unknown at this time. An interesting sequence that causes fork arrest was discovered by the investigation of mitomycin-C-induced "onion skin" replication of mammalian chromosomal DNA initiated from the origin of replication of an integrated polyomavirus DNA. The pause-inducing site present in the adjacent host DNA contained the sequence $(dG\text{-}dA)_{27}$-$(dT\text{-}dC)_{27}$ (Baran et al. 1983, 1987). This sequence, when cloned into SV40 DNA, caused altered plaque morphology and arrested SV40 replication in vivo for approximately a minute. It is conceivable that such sequences are positioned to limit onion skin replication to certain regions of the chromosome (Rao et al. 1988).

Tapper and DePamphilis (1980) examined the pausing of the two replication forks approaching the replication terminus of SV40 and discovered that replication pause sites were spread over several hundred base pairs. In the absence of a specific termination site in SV40 (Lai and Nathans 1975), it is conceivable that the pause sites promote confluence of the two forks within a given region of SV40 DNA.

G-rich polypurine tracts have been shown to arrest DNA synthesis in vitro, and the arrest of DNA polymerase at the homopurine tracts was not relieved by the addition of single-stranded DNA-binding proteins or other accessory proteins (d'Ambrosio and Furano 1987). Near the left end of the linear chromosome of bacteriophage ϕ29, a strong pause site has been observed in vitro (M. Salas, pers. comm.) and, interestingly, this pause site arrests the fork moving left to right to prevent it from moving in a direction opposite to the direction of transcription of strongly transcribed early genes. Thus, the pause site could serve to prevent DNA replication from entering actively transcribed regions.

The mechanistic aspects of DNA polymerase pausing caused by DNA sequences are not entirely clear and thus remain as a potentially interesting topic for future investigation.

Possible Biological Roles of Pause Sites

Analysis of the available data provides very few clues to the possible physiological roles of replication pause sites. The pausing of replication

forks in the regions near the replication termini could be a mechanism to terminate replication preferentially in that segment of the chromosome, in the absence of a sequence-specific replication terminus. The early and late transcription of SV40 DNA is controlled by promoters located near the origin, and the early and late transcription proceeds counterclockwise and clockwise, respectively, toward the terminus. It is conceivable that the pause sites fine-tune the bidirectional fork movement to proceed in the same directions as that of early and late transcripts and, thus, prevent the replication forks from entering actively transcribed regions from the opposite direction and from colliding with the transcriptional apparatus.

In summary, several types of DNA sequences can cause pausing of the movement of DNA polymerases in vivo and in vitro. In some cases, the pausing could serve physiological functions such as limiting DNA amplification by onion skin replication to a certain region of the chromosome or preventing replication forks from entering actively transcribed regions from a direction opposite to that of the transcribing RNA polymerase (Brewer 1988). The polypurine pause site that was discovered at the termini of adjacent chromosomal DNA that was replicated by polyomavirus-initiated onion skin replication of transformed rat cells could be acting to limit gene amplification to a limited region of the chromosome. However, since polyoma DNA can integrate into many chromosomal sites, it is likely that the replication is arrested wherever the forks find a polypurine stretch by chance. It is not known what function the polypurine stretches perform in host replication or recombination. Since pause sites are potentially recombinogenic, these sites could have evolved to make certain DNA regions available for the initiation of the recombinogenic process.

REPLICATION FORK ARREST AT THE TERMINI OF PROKARYOTES

Methods for Detection of Replication Termini

Most prokaryotic chromosomes initiate replication from one origin at a time, and the replication forks usually move bidirectionally until they meet each other at the terminus to generate two daughter molecules. The terminus region in most of the bacterial chromosomes and plasmids contains sequence-specific replication arrest sites that limit the end of the replication cycle to this region by blocking the fork progression (for review, see Hill 1992). To locate the origin and the terminus and to determine the direction of the fork movement, the frequencies of genetic markers located near the origin, termini, and the region in between are measured, during either exponential or synchronized growth. More than

three decades ago, the idea of marker frequency analysis was elegantly used by Yoshikawa and Sueoka (1963) to localize the replication origin and the terminus and to determine the direction of fork progression in *B. subtilis*. The principle behind the work is shown in Figure 1A. In a linear chromosome of unit length, when replication is proceeding unidirectionally from an origin located at 0 toward the terminus located at 1, the frequency $g(x)$ of a marker x is obtained by solving the integral shown in Figure 1A. The solution of the integral yields 2^{1-x}. When $x = 0$, $g(x) = 2$; when $x = 1$, $g(x) = 1$. Thus, the frequency of markers located close to the origin will be two times that of the markers located at or near the terminus. The frequencies of the markers located between the *ori* and the terminus follow a gradient of values between 1 and 2 shown in the curve of $g(x)$ plotted as a function of the map location. In practice, Yoshikawa and Sueoka (1963) determined the marker frequencies by extracting the sheared DNA from an exponentially growing prototrophic strain of *B. subtilis*, transforming a multiply marked auxotroph and measuring the number of transformants for each marker. The marker frequencies were normalized using a marker located in the middle of the chromosome, such as leucine (Fig. 1A). The same idea can be extended to investigate the movement of a bidirectionally replicating chromosome.

If the replication terminus is located at a position that is 180° from the origin, the symmetry of the replication fork movement will normally make it difficult to determine if the replication fork arrest is sequence-specific. If the movement of the forks is synchronous, the two forks meet each other at a location diametrically opposite to the origin even in the absence of an arresting sequence at the terminus. Thus, an asymmetry has to be generated in the fork progression to detect a sequence-specific terminus. The asymmetry can be created by moving the origin with respect to the terminus, either by introducing a replication origin at a new location or by introducing deletions or duplications into one arm of the chromosome between the *ori* and the terminus. A replication terminus that arrests forks can be detected by the arrest of one fork before the arrival of the second fork at an asymmetric location with respect to *ori*.

In *B. subtilis*, asymmetry was generated by making one arm from the *ori* to the terminus longer (than the other arm) by 25% by a chromosomal duplication (see Schnieder et al. 1983). Using this strain, O'Sullivan and Anagnostopoulus (1982) performed marker frequency analysis to localize an arresting terminus. Similarly, asymmetry in the *E. coli* chromosome was created by initiating replication from an integrated copy of P2 phage origin and by introducing deletions (Kuempel et al. 1977; Louarn et al. 1977; Kuempel and Duerr 1979; Francois et al. 1989, 1990). His-

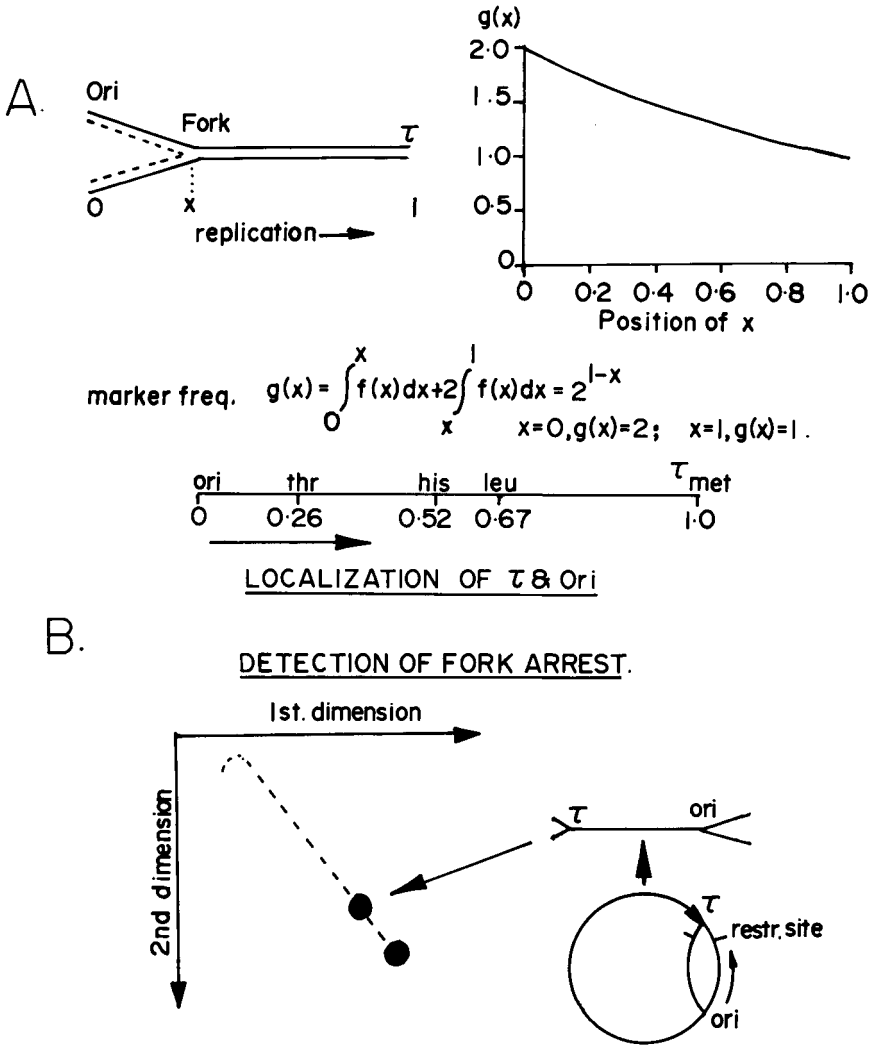

Figure 1 Methods for detection of replication origins and termini. (*A*) Marker frequency analysis developed by Yoshikawa and Sueoka (1963). The chromosome is linear with a replication origin at 0 and the terminus at 1.0. The fork movement is unidirectional. The equation for marker frequency $g(x)$, the plot of $g(x)$ as a function of map location x, and the chromosomal map of a segment of *B. subtilis* chromosome are shown. The equation predicts that the frequency of the markers at *ori*, in a log-phase cell population, would be twice that of the markers at the terminus. (*B*) Two-dimensional Brewer-Fangman gel electrophoresis of a unidirectional replicon. The *ori* and terminus (τ) are shown. The circular replicon is linearized at the unique restriction site, and the DNA is fractionated in the first dimension by molecular mass. The second dimension includes ethidium bromide, resulting in shape discrimination.

torically, the discovery of the specific terminus in *E. coli* was made by Kuempel et al. (1977) and Louarn et al. (1977). The replication terminus was identified in plasmid R6K by electron microscopy of the forks impeded by an asymmetrically located terminus (Lovett et al. 1975; Crosa et al. 1976). The bacterial termini were localized initially by measuring marker frequency by DNA-DNA hybridizations and comparing the frequency of markers located immediately before and after the terminus.

The termini of R6K were cloned into a unidirectionally replicating vector, and the replication was visualized by electron microscopy of replication intermediates (Kolter and Helinski 1978). The results showed that the replication termini transiently arrested the replication fork, and then the forks were released to finish replication near the *ori*. Using smaller and smaller pieces of the terminal DNA, the termini were localized to a 216-bp piece of DNA (Bastia et al. 1981a). Subsequent work revealed that the DNA fragment contained two sites of opposite polarity (Horiuchi and Hidaka 1988), each of which could block the replication fork approaching from one direction.

Another technique developed to map an arrested fork in larger bacterial chromosomes is one-dimensional gel electrophoresis designed to detect a Y-shaped stalled fork (Weiss and Wake 1984; Horiuchi and Hidaka 1988; Pelletier et al. 1988).

A technique that has greatly aided the investigation of replication origins, fork movement analysis, localization of replication arrest sites, and the termini is the two-dimensional neutral agarose gel electrophoresis developed by Brewer and Fangman (1987). A variant procedure using electrophoresis in the first dimension in a neutral agarose gel followed by a second dimension in alkaline condition has been developed by Huberman and colleagues (Linskens and Huberman 1988). For detailed discussion of the procedures, the reader is referred to the original papers. The Brewer-Fangman technique, as it applies to detecting replication termini in a unidirectionally replicating plasmid, is shown in Figure 1B. A replication terminus (τ) was cloned into a pUC19 vector approximately 450 bp away from the unidirectional replication origin. The replication intermediates were isolated from cells growing in the exponential phase, and the plasmid DNA was restricted at a unique restriction site between the *ori* and the terminus. Note that the arrested intermediate should have a double Y structure. The DNA was resolved by electrophoresis in the first dimension in an agarose gel at neutral pH and was run on the second dimension at the same pH in a gel containing ethidium bromide. The dye intercalates between DNA base pairs to different extents, depending on the shape of the DNA, and helps dis-

criminate between linear, Y-shaped, or X-shaped DNA molecules. The expected distribution is a hook-shaped pattern of DNA that is visualized by Southern blotting and hybridization by a labeled DNA probe. The monomeric linear DNA forms the prominent lower spot, whereas the termination intermediate is marked by the spot just above and to the left of the monomer spot. By cutting the DNA at other unique restriction sites, one can localize the terminator site (in this case already predetermined) on the plasmid DNA (Fig. 1B). The neutral-alkaline two-dimensional gel procedure is equally effective in detecting and localizing termination sites (Linskens and Huberman 1988).

Structure and Chromosomal Location of Replication Arrest Sequences of Prokaryotes

Definitive information on replication fork arrest sequences has come mostly from the studies of prokaryotic chromosomes. The relatively small amount of available information from eukaryotes such as ribosomal DNA of yeast, human, and plants, and from mammalian mitochondrial DNA, is discussed in a later section.

The replication arrest sites of chromosomes of *E. coli* and *B. subtilis* and of plasmid R6K are shown in Figure 2,A–C. The information on the replication fork arrest in *E. coli* comes from the work of Hill, Kuempel, and their associates (Hill et al. 1988) and from the work of Horiuchi and his associates (Hidaka et al. 1988, 1991). Most of the information on the structure and in vivo analysis of the replication arrest system of *B. subtilis* has come from the work done in the laboratory of Wake and coworkers (for review, see Lewis and Wake 1991 and many other papers by Wake and colleagues cited in this chapter). The replication arrest sites of plasmids of *E. coli* are almost indentical to those of the *E. coli* host chromosomes (Bastia et al. 1981b; Hidaka et al. 1988; Sista et al. 1989).

There are six known replication arrest sites in both *E. coli* (Hidaka et al. 1991; Hill 1992) and *B. subtilis* (Franks et al. 1995). In both *E. coli* and *B. subtilis* the sites are arranged in two sets of three, and in each set all three sites have the same polarity (Fig. 2). The polarity is defined as the orientation of the site for fork arrest with respect to the origin of replication (Hidaka et al. 1988; Hill et al. 1988; Sista et al. 1989; Smith and Wake 1992; Sahoo et al. 1995a). The location and orientation of the arrest sites are such that a replication fork approaching from left to right passes through the first set of three sites in both *E. coli* and *B. subtilis* and through one of the two sites of R6K and is arrested at the first site of

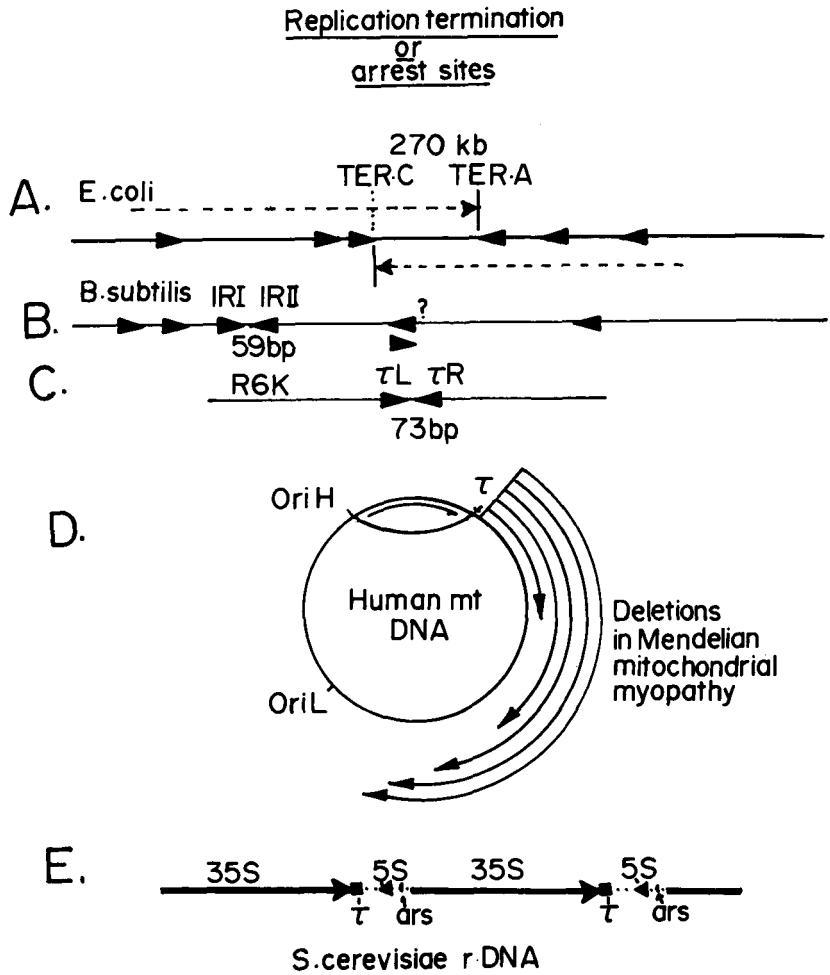

Figure 2 Replication termini in various systems. (*A*) Replication termini of *E. coli*. The dotted lines with arrows show the points of arrest of the two replication forks of the bidirectional replicon. (*B, C*) Replication termini of *B. subtilis* and the plasmid R6K. (*D*) D-loop of the human mitochondrial DNA. τ indicates the point of arrest of the ~600-nt-long newly synthesized H strand. In Mendelian mitochondrial myopathy, deletions extend from near τ, clockwise toward the origin of light-strand synthesis *oriL*. (*E*) Replication fork arrest sites (called replication fork barrier or RFB) τ in yeast rDNA. Replication forks moving in a direction opposite to the transcription of 35S rRNA are arrested at τ (RFB).

the second set that the fork encounters. The converse is true for the fork approaching from right to left. Once the first fork is arrested at a given site, the second fork approaching from the opposite direction stops at the

first site, thus preventing the reduplication of the region between two sets of arrest sites. The sites TerA, TerB, and TerC (also called τ1, τ2, and τ3) have been reported to be the most frequently used sites in *E. coli* under laboratory conditions (Pelletier et al. 1988; Louarn et al. 1991). TerI (also called IRI) of *B. subtilis* is reported to be the site that arrests the replication fork moving right to left, which usually arrives first at the terminus (Carrigan et al. 1991; Smith and Wake 1992; Franks et al. 1995).

The replication arrest sites of *B. subtilis* are related to one another by a consensus sequence, as are the sites of *E. coli* and plasmid R6K (Table 1). There is no sequence homology between the sites of gram-positive *B. subtilis* and gram-negative *E. coli*. Unlike the sites of *E. coli* and R6K, the sites of *B. subtilis* have two overlapping sequences called the core (or B site) and the auxiliary sequence (or A site) (Lewis et al. 1990; Sahoo et al. 1995a). The sequences of the fork arrest sites are binding sites for the replication terminator proteins, Ter (Tus) and RTP, encoded by *E. coli* and *B. subtilis,* respectively.

Replication Terminator Protein of *E. coli*

The existence of a terminator protein that causes replication fork arrest was first suggested by in vitro replication experiments carried out by Germino and Bastia (1981). A hybrid replicon containing the plasmid ColE1 origin of replication and the replication terminus of R6K was replicated in cell extracts of an *E. coli* strain that did not harbor a resident R6K plasmid. Replication fork initiated from the ColE1 origin in vitro was arrested at the R6K terminus. Considering that the plasmid did not encode a terminator protein and that the terminus sequence did not have features (e.g., hairpins, polypurine stretches) that are known to impede polymerase movement, the work suggested that the host cell extract probably contained a terminator protein that recognized the R6K terminus.

The terminator protein was first purified from cell extracts of *E. coli*, and the protein bound to two sites present in a 216-bp DNA fragment of R6K (Hidaka et al. 1989; Sista et al. 1989). The sites corresponded to the left and the right arrest sites of R6K (Horiuchi and Hidaka 1988). The *tus* gene encoding the Ter (Tus) protein was discovered by Hill et al. (1989) and Hidaka et al. (1989) and the protein, by direct analysis and as deduced from the DNA sequence, was found to be 36 kD in molecular mass. Cross-linking studies (Sista et al. 1991) and sedimentation equilibrium studies (Gottlieb et al. 1992) both showed a monomeric protein in solution.

Table 1 Sequences of replication fork arrest sites of different organisms

E. coli chromosome	
TerA	5′-AATTAGTATGTTGTAACTAAAGT-3′
TerB	5′-AATAAGTATGTTGTAACTAAAGT-3′
TerC	5′-ATATAGGATGTTGTAACTAATAT-3′
TerD	5′-CATTAGTATGTTGTAACTAAATG-3′
TerE	5′-TTAAAGTATGTTGTAACTAAGNN-3′
TerF	5′-CCTTCGTATGTTGTAACGACGAT-3′
R6K plasmid	
TerR1	5′-CTCTTGTGTGTTGTAACTAAATC-3′
TerR2	5′-CTATTGAGTGTTGTAACTACTAG-3′
Consensus	AAA AA A
	5′-NN G TGTTGTAACTA NNN-3′
	TTT TG C
B. subtilis chromosome (strain 168)	
TerI (IRI)	5′-ACTAAGAAAACTATGTACCAAATGTTCAGT-3′
TerII (IRII)	5′-ACTGACAACACTAGTTACTAAATATTCAAT-3′
TerIII	5′-ACTAATTGATCTATGTACTAAATATTCATA-3′
TerIV	5′-ACTAACTAAACTATGTACTAAATATTCACT-3′
TerV	5′-ACTAAATAAATAATGTACTAAATATTCAAC-3′
TerVI	5′-ACTAAATAATCTATGTACCAAATGTTCAAT-3′
B. subtilis chromosome (strain W23)	
IRI	5′-ACTAAGTGAACTGTGAACCAAATGTTCACT-3′
IRII	5′-ACTGAGAACACTATGTACTAAATATTCAAT-3′
Consensus	A TAAACTA T T A
	5′-ACT AN TG AC AAAT TTCANN-3′
	G AGCTTAG A C G
Saccharomyces cerevisiae rRNA	
5′-TTGCCCGGACAGTTTGCTTCATGGAGCAGTTTTTTCCGCACCATC	
AGAGCGGCAAACATGAGTGCTTGTATAAGTTTAGAGAATTGAGA-3′	
Mitochondrial D-loop sequence that may be a termination sequence	
	5′-TTGACTGTACATAGTACATTATGTCAAATTC-3′

The contact points of Ter protein with the replication arrest sites of R6K (Sista et al. 1991) and of *E. coli* (Gottlieb et al. 1992) have been analyzed showing a pattern of asymmetric contacts involving both strands

of the cognate sites. The equilibrium dissociation constant varied between the Ter sites. Sista et al. (1991) have found the K_d for the R6K Ter site to be 5 x 10^{-9} moles/liter, whereas Gottlieb et al. (1992) found the K_d for the *E. coli* Ter site to be 10^{-11} moles/liter. For the more efficient TerB sites of *E. coli*, Gottlieb et al. (1992) found the K_d value to be 3.4 x 10^{-13} moles/liter. The differences between two different Ter sites may be due to differences in their contact points, and the differences in the values for the same site in different observations may be due to buffer conditions and methods used in the different experiments. Table 2 shows characteristics of different Ter sites. Gottlieb et al. (1992) have compared K_d, dissociation rate constant, and half-life values of Ter-Tus complex with that of the *lac* repressor-operator complex. Although the K_d values for both the complexes were found to be similar, the half-life of the Ter-Tus complex was very high in comparison to that of *lac* repressor-operator complex.

The Ter protein, added either to cell extracts of *tus*$^-$ *E. coli* (Khatri et al. 1989; MacAllister et al. 1990) or to a defined replication system for ColE1 type origins (Hill and Marians 1990) or for the *oriC* system of *E. coli* (Lee et al. 1989; Lee and Kornberg 1992), caused orientation-specific arrest of replication forks. Although both the leading and the lagging strands were arrested at the terminus, a transient intermediate, consistent with a D-loop structure, could be detected in the in vitro reaction (MacAllister et al. 1990). Both Hill and Marians (1990) and Lee and Kornberg (1992) mapped the point of arrest in vitro of the newly synthesized DNA at the terminator sites. The site was located just within the region protected in hydroxyradical and DNase I footprinting experiments.

A significant develoment in understanding the biochemistry of fork arrest emerged from the discovery by Khatri et al. (1989) and Lee et al. (1989) that the Ter (Tus) protein of *E. coli* was able to impede, in one orientation of the terminus, the activity of the main replicative helicase, DnaB. The DnaB helicase translocates in the 5′→3′ direction (LeBowitz and McMacken 1986), whereas the helicase PriA (factor Y) of *E. coli* translocates in the 3′→5′ direction (Lee and Marians 1987). The Ter (Tus) protein of *E. coli* was also able to impede PriA helicase in an orientation-specific manner (Hiasa and Marians 1992; Lee and Kornberg 1992).

Interestingly, the Ter protein was able to impede the helicase activity of SV40 large T antigen in an orientation-dependent mode in vitro (Bedrosion and Bastia 1991; Amin and Hurwitz 1992; Hidaka et al. 1992). A hybrid replicon with SV40 origin and the R6K terminus, when

Table 2 Characteristics of replication fork arrest sites and the terminator proteins

Sequence	Name	Source	Protein	Mol. mass (kD)	Gene	K_d (moles/l)
5′-TGAGTGTTGTAACTACTA-3′ 3′-ACTCACAACATTGATGAT-5′ →|	TerR	R6K	Ter (Tus)	36	*Tus*	1×10^{-11} 5×10^{-9}
5′-AATAAGTATGTTGTAACTAAAGT-3′ 3′-TTATTCATACAACATTGATTTCA-5′ →|	TerB	*E. coli*	Ter (Tus)	36	*Tus*	3.4×10^{-13}
5′-ACTAAGAAAACTATGTACCAAATGTTCAGT-3′ 3′-TGATTCTTTTGATACATGGTTTACAAGTCA-5′ |←	TerI (IRI)	*B. subtilis*	RTP	14	*rtp*	1.2×10^{-11} 6×10^{-11}

Closed circles show methylation protection of G residues. Arrows and vertical lines indicate the site and polarity of replication blockage.

replicated in HeLa cell extract containing the SV40 T antigen and Ter protein, showed polar fork arrest at the R6K terminus (Bedrosian and Bastia 1991; Amin and Hurwitz 1992).

What is the mechanism of orientation-specific fork arrest? Does it involve strictly Ter protein/terminus DNA interaction that imposes a polar roadblock to most helicases? Alternatively, are both terminator protein/DNA interaction and helicase/terminator protein interaction involved in polar fork arrest?

The issue of helicase specificity of the Ter protein has been debated (Khatri et al. 1989; Lee et al. 1989; Hiasa and Marians 1992; Lee and Kornberg 1992). Some laboratories have reported that Ter protein blocks all helicases, including DnaB and SV40 Tag, that promote Cairns-type replication to generate θ-shaped replication intermediates and also the helicases involved in rolling-circle replication, e.g., Rep helicase; in DNA repair, e.g., helicase II; and in conjugative DNA transfer, e.g., helicase I (Lee et al. 1989; Hidaka et al. 1992; Lee and Kornberg 1992).

Khatri et al. (1989) and Hiasa and Marians (1992) have reported that Ter protein does not block helicase II or Rep helicase. The inhibition of helicase II reported by Bedrosian and Bastia (1991) was due to high concentrations of Ter protein used in the work. In a detailed study of the helicase specificity of the contrahelicase (antihelicase) activity of Ter protein, Sahoo et al. (1995b) discovered that the Ter protein did not impede helicase I or Rep helicase over a wide range of enzyme (helicase)-to-substrate and terminator protein-to-substrate ratios. Under the same conditions, the Ter protein readily impeded activities of both DnaB and PriA helicases in a polar fashion.

Lee and Kornberg (1992) have reported that Ter protein impedes the activities of the Klenow fragment of DNA polymerase I and the DNA polymerases of T7 and T5 phages. The blocks showed minimal orientation dependence of the terminus sequence (a twofold difference). It is likely that the polymerase block is mainly due to a nonspecific roadblock created on DNA by Ter protein and is not considered to be of any major physiological significance.

Recently, Mohanty et al. (1996) have discovered that the terminator proteins of both *E. coli* and *B. subtilis* block the elongation of RNA chains by several prokaryotic RNA polymerases in a completely polar fashion. The antihelicase activity and the RNA polymerase anti-elongation activities were isopolar. Mohanty et al. (1996) have investigated the possible biological significance of the RNA polymerase impedance by the terminator proteins. Using model substrates, they discovered that passage of an RNA transcript through the non-blocking end

of the terminus abrogates the contrahelicase activity of both Ter and RTP. Transcriptional passage also releases the replication forks arrested at the τ2 (TerB) terminus of *E. coli*. The in vivo significance of the results can be understood by considering the finding along with the observations of Roecklein and Kuempel (1992) that transcription elongation initiated from an upstream promoter (P1) and directed toward the TerB (τ2) sequence in vivo in *tus*$^+$ cells was arrested at the τ2 site but invaded the τ2 terminus in *tus*$^-$ cells. Thus, the anti-transcriptase activity of the Ter protein and RTP most likely has evolved to protect the functional integrity of the replication arrest sites from transcriptional invasion.

Consistent with this notion, Hidaka et al. (1988) had observed that the non-blocking ends of each of the three frequently used replication termini τ1, τ2, and τ3 (Fig. 2A) were flanked by sequences with a GC-rich hairpin loop and an AT-rich tail characteristic of ρ-independent terminator sites. Considered together, these results support the idea that the ability of the terminator protein to block transcriptional elongation is a mechanism to protect the replication termini from functional inactivation by invading transcripts.

Replication Terminator Protein of *B. subtilis*

Unlike the Ter protein of *E. coli*, RTP of *B. subtilis* is a dimer with subunit molecular mass of 14.5 kD (Lewis et al. 1989, 1990). Two dimers of RTP bind to each arrest site of *B. subtilis* in a stepwise manner. A single dimer first binds to the core site (B site) and then, by apparent cooperative protein-protein interaction, promotes the binding of a second dimer to the auxiliary (A) site (Fig. 3). The core site by itself, binding to a single monomer, is incapable of arresting replication forks (Smith and Wake 1992; Sahoo et al. 1995a).

The mode of interaction of RTP with the cognate binding site has been studied both by missing nucleoside hydroxyradical footprinting (Langley et al. 1993) and by methylation protection and interference studies (Sahoo et al. 1995a). The results show that the protein dimer contacts the core site (B site) more frequently than the auxiliary site (A site), and also that the contacts at the core site are on both strands of DNA whereas the contacts at the auxiliary site were mostly one-stranded. Sahoo et al. (1995a) reported that RTP showed purine contacts on both strands of the core but with only one strand of the auxiliary site (Table 2).

There have been two major breakthroughs in the biochemical and structure-function analysis of RTP. Although there is very little primary

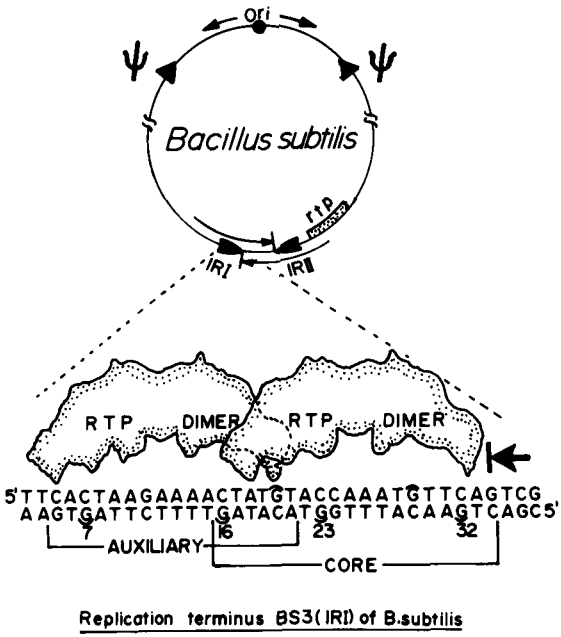

Figure 3 Replication of a *B. subtilis* chromosome showing the termini ψ that are active during stringent response. The IRI and IRII termini contain overlapping core and auxiliary sequences. A single dimer of RTP binds to the core and, by cooperative protein-protein interaction, promotes the binding of a second dimer to the auxiliary site. RTP is encoded by the *rtp* gene. (Reprinted, with permission, from Manna et al. 1996.)

structural homology between Ter of *E. coli* and RTP of *B. subtilis*, Kaul et al. (1994) discovered that RTP functions both in vivo and in vitro in *E. coli*. It blocks the activity of both DnaB and PriA helicases of *E. coli* (Kaul et al. 1994; Sahoo et al. 1995b). Independently, Wake and colleagues have also reported that RTP functions in vivo in *E. coli* (Young and Wake 1994). Since there is no currently available in vitro replication system for *B. subtilis*, it is now possible to analyze the biochemical functions of RTP using the well-defined surrogate *E. coli* in vitro replication system. A second major breakthrough has been the solving of the crystal structure of RTP apoprotein at 2.6 Å (Bussiere et al. 1995).

Crystal Structure of RTP

The crystal structure of the RTP apoprotein has the following features: (1) an amino-terminal disordered region; (2) four α helices with the carboxy-terminal, longest α helix (α4) forming an antiparallel coiled-coil

dimerization domain; (3) one short and two long β sheets, the β2 and β3 being connected by an extended loop (Fig. 4).

On the basis of the known activities of RTP, one would expect the protein to have the following domains: (1) a DNA-binding domain, (2) a dimerization domain, (3) a dimer-dimer interaction surface, (4) a helicase-blocking surface, and (5) a region that blocks elongation of RNA chains by prokaryotic RNA polymerases unless, of course, the postulated helicase-blocking domain is also involved in blocking chain extension by RNA polymerase. An interesting question to consider is how a symmetric dimer of RTP is able to act in an asymmetric fashion by blocking helicase activity in a polar mode.

The "Winged Helix" DNA-binding Domain of RTP

The DNA-binding domain of RTP is of interest for at least two reasons: First, the DNA/protein interaction at the terminus presents the terminator protein to the approaching helicase; second, the DNA/protein interaction is likely to generate functional asymmetry from two interacting, symmetric dimers, since DNA/protein contacts are different at the core (B site) and the auxiliary site (A site). Figure 3 shows diagrammatically the interaction between two dimers of RTP on the IRI (BS3) terminus. IRI stands for inverted repeat I and is synonymous with binding site 3, BS3 (see Sahoo et al. 1995a).

Pai et al. (1996) have localized the DNA-binding domain by systematic saturation mutagenesis of DNA encoding RTP and then by screening of the mutants for any defect in DNA binding by employing the genetic selection scheme of Elledge et al. (1990). The mutants that showed defects in DNA binding by the in vivo genetic assay were further characterized by biochemical analysis. By site-directed mutagenesis, mutants of RTP were isolated with cysteine residues substituted at selected sites, derivatized with azido-phenacyl bromide, and cross-linked to the DNA. The cross-linking experiments provided direct evidence for either the contact of α3, β2, and the amino-terminal arm with DNA or the very close proximity of these regions to the DNA. One mutant protein showing a severe DNA-binding defect was crystallized, and the crystal structure was solved and compared with the wild-type RTP structure. The structures were almost identical, thereby showing no misfolding caused by the mutation. The combination of these approaches showed that the amino-terminal unstructured arm, the β2 sheet, and the α3 helix make contacts with the terminus DNA (see Fig. 5). The structure has been referred to as a "winged helix." The β2-β3 sheets and the extended loops

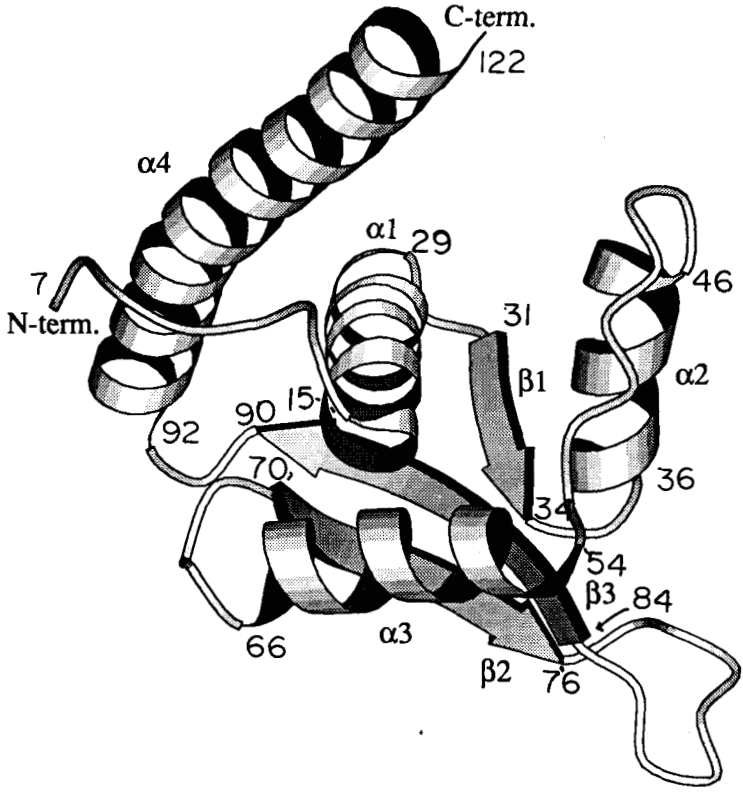

Figure 4 Ribbon diagram of an RTP monomer. The protein contains an unstructured amino-terminal region, four α helices, three β strands, and an extended loop connecting β2 with β3.

connecting the two form the wings (Figs. 4 and 5). Swindels (1995) has compared the structure of RTP with that of the eukaryotic fork head transcription factor, histone H5, and LexA, and has noted the remarkable similarity in the tertiary structures of these, otherwise unrelated, proteins.

Dimerization Domain

The carboxy-terminal α helices form the antiparallel, coiled-coil dimerization domain of RTP (Bussiere et al. 1995).

Dimer-dimer Interaction Domain

Wake and his colleagues (Lewis et al. 1990; Carrigan et al. 1991; Langley et al. 1993) have observed that in vivo, a single dimer of RTP

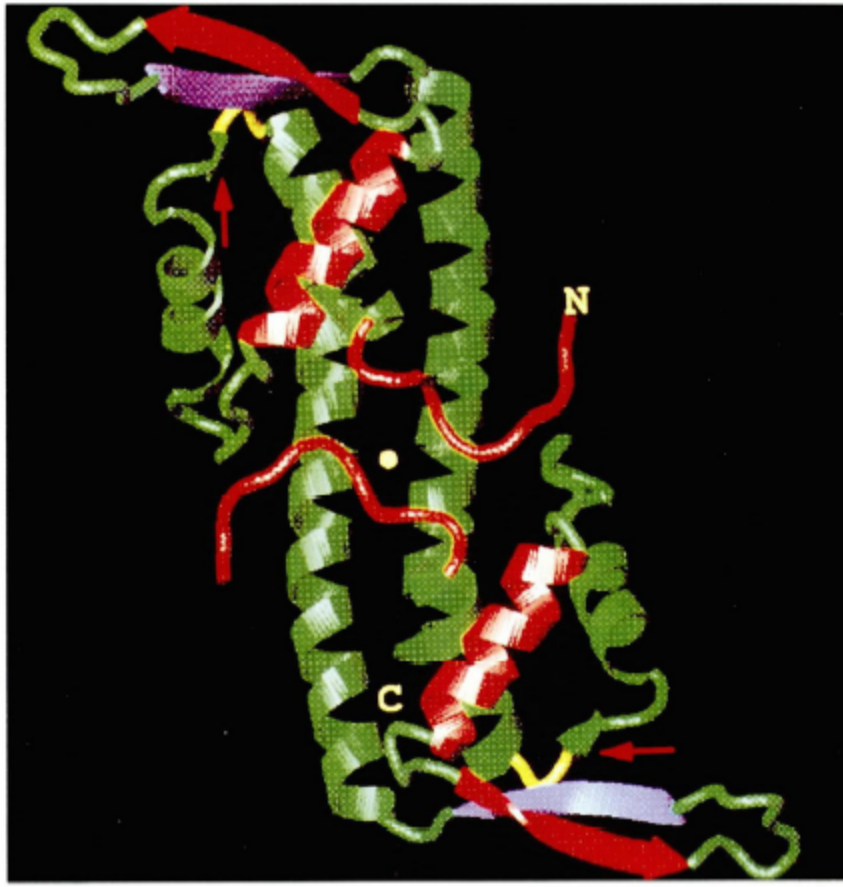

Figure 5 Ribbon diagram of an RTP dimer. (*Red*) DNA-binding regions; (*blue*) dimer-dimer interaction region; (*yellow*) helicase-blocking region. Mutations at the regions marked by red arrows result in loss of RNA polymerase block with no change in DNA binding. The carboxy-terminal α4 helices form an antiparallel, coiled-coil dimerization domain.

binding to a core site fails to impede replication forks. Sahoo et al. (1995a) have extended these results in an in vitro system and have shown that a single dimer of RTP, binding to a core site of a terminator DNA, cannot impede DnaB helicase or replication forks. Thus, two dimers of RTP binding to the core and the auxiliary sites (see Fig. 3) are needed to form a functional replication terminus.

The dimer-dimer interaction domain is therefore an important region of RTP structure. Since RTP crystallizes as a dimer, crystallography did not provide any clue as to the location of the dimer-dimer interaction domain. We resorted to mutagenesis and biochemical analysis of the

mutant forms of the protein to localize the dimer-dimer interaction site. The site seems to be located in the β3 strand of RTP (blue region, Fig. 5). A tyrosine residue, located at the coordinate 88 on the β3 strand, upon mutation to a phenylalanine, yielded a protein that binds to the core site but fails to bind to the auxiliary site. The tyrosine at the position 88 is within hydrogen-bond-forming distance of a glycine residue at position 34. Mutation of the glycine at 34 to an arginine also abolishes dimer-dimer interaction. It appears that β3-β3 stacking between two dimers may be the basis of dimer-dimer interaction (Manna et al. 1996). The mutant is completely defective in impeding helicases, replication forks, and RNA polymerases. In addition, a valine to glycine substitution at position 85 and leucine to serine substitution at 82, at the loop connecting β2 to β3, also abolishes dimer-dimer interaction.

Helicase-blocking Surface

Mechanistically, the helicase-blocking surface is of considerable importance, because the existence of such a surface would strongly support a mechanism of fork arrest that involves specific blocking of the helicase activity by protein-protein interaction with RTP. The following criteria were used to look for such a mutant. The mutations affecting the putative helicase-blocking surface of RTP should not affect DNA binding. If the impedance of RNA polymerase-catalyzed chain elongation is controlled by a separate region of RTP, the mutants impairing helicase block should not affect the ability to block RNA polymerase. Using the above criteria, we examined mutants of the region of an exposed hydrophobic patch with a few charged residues located between the α1 helix and the β1 sheet (Figs. 4 and 5). We discovered that a mutation in the region colored yellow in Figure 5 met the criteria stated above. The mutant bound to DNA almost normally, blocked RNA chain extension normally, but failed to block DnaB helicase, PriA helicase, and replication forks in vitro (A.C. Manna et al., in prep.). This result is significant in two ways. First, the mutant RTP marks the helicase-blocking domain of RTP and supports a mechanism of contrahelicase activity that involves RTP/helicase interaction. Second, the results indicate that different regions of the protein are probably involved in the antihelicase and RNA chain anti-elongation activities. A third mutation at the site marked by red arrows in Figure 5 greatly reduced RNA polymerase block without detectably affecting DNA binding. Thus, this region marked by the mutation identifies the RNA polymerase-blocking surface of RTP (Fig. 5). The mutant defective in RNA polymerase block was also defective in helicase block.

The structure-function analysis of RTP, guided by the crystal structure, has been very informative with regard to the mechanism of replication fork impedance. Future work will be directed toward mapping of the sites of DnaB helicase, of SV40 T antigen, and of T7 RNA polymerase that interact with RTP.

It should be noted that the replication terminator proteins of *E. coli* and *B. subtilis*, without the aid of any other protein, block not only the translocation of DnaB and PriA helicases, but also the authentic unwinding of DNA, regardless of the length of the duplex region in helicase substrates (Sahoo et al. 1995a,b). These findings disagree with an earlier report that the replication terminator protein of *E. coli* by itself can inhibit helicase translocation on DNA, but for blockage of DNA unwinding, needed the participation of other replisomal proteins (Hiasa and Marians 1992). It would be surprising, however, if the fork arrest is not more efficient in the presence of a full complement of proteins that drive the replication fork.

Replication Arrest Sites of *B. subtilis* Active under Stringent Conditions

Simone Seror and coworkers (Henckes et al. 1989) have shown that conditions that generate high levels of the alarmone ppGpp in the cell, either by treating *B. subtilis* with hydroxamate or by shifting a temperature-sensitive mutant of the DnaB gene to the nonpermissive temperature (note the DnaB of *B. subtilis*, unlike the protein with the same designation in *E. coli*, is not a helicase but is an initiator protein), cause the replication forks initiated at the *ori* to be arrested approximately 200 kb away on either side at the so-called stringent termini (marked ψ in Fig. 3). Seror's group has shown further that the arrest of replication forks at the stringent termini ψ requires RTP (Levine et al. 1995). Although the marker frequency analysis by DNA-DNA hybridization used to map the arrest site did not allow a precise localization of the ψ sites, the requirement for RTP might indicate the presence of arrest sites such as those present at the normal replication terminus (Fig. 2) that is used under relaxed conditions. How does RTP arrest forks at ψ sites under high ppGpp but not under relaxed conditions? It is known that many promoters such as that for rRNA are shut off during stringent conditions (Cashel and Rudd 1987). Combining this observation with the finding of Mohanty et al. (1996) that replication arrest sites of *B. subtilis* can be rendered ineffective in blocking replication forks by passage of an RNA transcript, a possible mechanism of conditional usage of a replication ar-

rest site can be hypothesized. It is tempting to suggest that the ψ sites are normally kept in a nonfunctional state by transcripts directed through the sequences by promoters that are sensitive to high ppGpp. Under stringent conditions, we suggest that these promoters are turned off, thus allowing RTP bound to the ψ sites to block replication forks. Under low ppGpp, we suggest that the promoters are turned on, and the passage of RNA transcript through the ψ sequences keeps them inactive. In this scheme, ψ sites may be simply regular terminator sequences that bind RTP. Alternative models would involve modification of RTP under stringent conditions. The scheme proposed can be experimentally tested once the ψ sites have been more precisely localized. Levine et al. (1995) have pointed out that the ψ sites serve as checkpoints of replication under stringent conditions. Interestingly, in *E. coli*, ppGpp causes replication arrest at the origin of the *E. coli* chromosome and not at other sites (Levine et al. 1991).

Regulation of Synthesis of Ter Protein

Both in vitro studies by Natarajan et al. (1991) and in vivo studies by Roecklein et al. (1991) showed that Ter protein is a transcriptional repressor of its own synthesis and prevents RNA polymerase from binding to a promoter that is present immediately upstream of the sequence encoding the Ter (Tus) protein. Thus, Ter (Tus) autoregulates its synthesis by binding to the τ2 site that acts as an operator of the autoregulated promoter. Interestingly, Roecklein and Kuempel (1992) also discovered that transcription from an upstream promoter is impeded at the elongation stage by the τ2 sequence in a *tus*$^+$ but not in a *tus*$^-$ host. Natarajan et al. (1993) have reported that a partially purified protein fraction of *E. coli* was able to abrogate both the contrahelicase activity and the fork arrest by the Ter protein in vitro. This opens the possibility of regulation of replication arrest function at a different level by protein-protein interaction.

Possible Physiological Roles of Sequence-specific Replication Arrest System

Since the *tus* gene of *E. coli* and the *rtp* gene of *B. subtilis* can be deleted without causing lethality, specific fork arrest and termination of replication do not appear to be essential for cell viability. Yet the fact that multiple replication arrest sites have been maintained against mutational drift suggests that there is a physiological need for the sites. At this time, there are no definitive clues as to why specific arrest sites have been

maintained in the chromosomes. Several suggestions have been made regarding the possible physiological role of these sites. Brewer (1988) has suggested that the sites prevent replication forks from entering the chromosome from a direction opposite to that of transcription, in order to avoid collision between RNA polymerase and the replisomal machinery. Liu and Alberts (1995) have shown that such collision can cause pausing of replication forks. The pausing may invite recombination involving the free DNA ends exposed by falling off of the replication proteins, thus exposing free DNA ends that may lead to genomic instability. Lee et al. (1989) have suggested that the replication arrest sites prevent the θ-type replication from turning into a σ-type or rolling-circle replication. Lewis and Wake (1991) have speculated that the arrest sites might provide the proper structure for more efficient decatenation of daughter molecules. Dasgupta et al. (1991) integrated a copy of the R1 plasmid into the *E. coli* chromosome and caused chromosomal replication to be initiated from the R1 origin in the *tus*$^+$ cell of *E. coli*. The location of the R1 *ori* was such that the almost unidirectional initiation caused the replication fork to travel over a much greater length of DNA to reach the terminus. Under this condition, cell division was perturbed, causing a lack of proper septation and filament formation. Deletion of the *tus* gene greatly reduced filament formation by eliminating fork arrest at the terminus, allowing the forks to pass through and finish replication. Dasgupta et al. (1991) have speculated that the Ter sites restrict each fork to travel one-half the length of the chromosome before meeting each other at a site located diametrically opposite to the *ori* and thus maintain symmetry of replication. Perhaps this also maintains proper gene dosage of markers on both arms of the chromosome. Thus, the Ter system ensures the symmetry of replication. Do termination events signal subsequent cell division at the end of a replication cycle? Although the existence of such a mechanism has been proposed, recent work using synchronized initiation of a single cycle of replication and a DNA synthesis inhibitor shows that septa can form on partially replicated chromosomes that have not reached the terminus. Thus, under these experimental conditions, no linkage of cell septation to completion of the termination of replication was observed. Nordstrom et al. (1991) have also argued for separate mechanisms controlling termination from that of cell division. Recently Hiasa and Marians (1994a), using minichromosome templates containing *oriC* and Ter-binding sites, have shown that the Tus-Ter (Ter-τ) complex prevents overreplication of bidirectionally replicating template. In summary, information on the physiological role of the replication arrest system remains largely obscure.

Decatenation of Circular Daughter Chromosomes

The separation of the daughter molecules after replication requires decatenation; i.e., separation of the two intertwined DNA molecules. The separation at the termini could occur in two steps: In the first, melting of the hydrogen bonds between the remaining parental duplex by a helix-destabilizing protein or a helicase is followed by repair of the gaps of the two catenated rings; in the second step, a topoisomerase decatenates the two daughter molecules (Adams et al. 1992). Topo IV has been identified as the enzyme responsible for decatenation (Kato et al. 1988, 1990; Peng and Marians 1993). Topo IV mutants are defective in partition and accumulate nucleoids in the middle of the cell (Kato et al. 1988; Schmid 1990). Inhibition of topo IV in temperature-sensitive mutants of the two structural genes *par*C and *par*E of *Salmonella* causes the accumulation of catenated dimers. The catenanes were right-handed and parallel, consistent with a melting step followed by a decatenation step of chromosome separation at the terminus (see Fig. 6) (Adams et al. 1992; Zechiedrich and Cozzarelli 1995). A more detailed discussion of topoisomerases can be found in the chapter by Hangaard Andersen et al. (this volume).

The movement of the fork generates positive superhelical stress that can be removed by gyrase, which is uniquely capable of putting in negative superhelicity. However, gyrase can not substitute for topo IV in decatenation (Zechiedrich and Cozzarelli 1995). It is conceivable that topo IV is compartmentalized in a termination complex that gyrase cannot penetrate (Adams et al. 1992). Under certain conditions, topo IV can carry out *oriC* replication in vitro, replacing the need for gyrase, but only under substoichiometric ratios of enzymes to substrate (Hiasa and Marians 1994b). At stoichiometric ratios, the enzyme has been found to relax the substrate.

Pulse-chase experiments with bacterial plasmids under conditions where topo IV is inhibited yielded only about 10% accumulation of catenated dimers, raising questions as to whether topo IV was the true decatenating enzyme at the terminal stages of replication. Recent work in which such pulse-chase experiments were carried out using topo IV ts-gyrA^R (quinoline resistant) and topo IV ts-gyrA$^+$ double mutants showed that substantial amounts of catenated dimers accumulated when gyrase activity was blocked by quinolines under conditions that also blocked topo IV. Thus, catenated dimers are authentic and major kinetic intermediates of termination of replication. These intermediates are subject to decay by gyrase at a rate that is one one-hundredth of that of the decatenating activity of topo IV (Zechiedrich and Cozzarelli 1995).

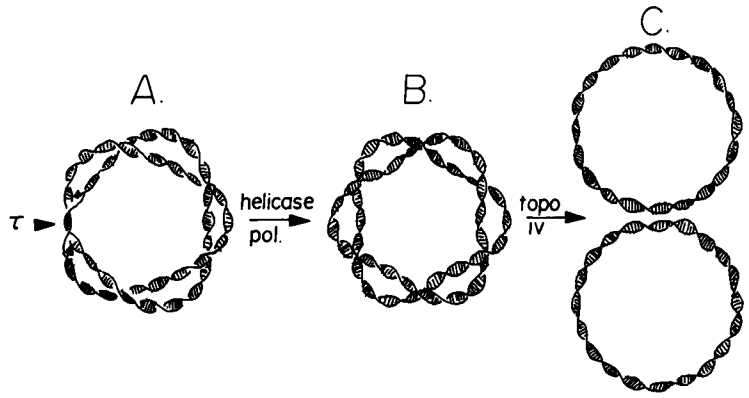

Figure 6 Schematic representation of the decatenation and segregation of two terminated daughter molecules. The scheme is redrawn from Adams et al. (1992) and Zechiedrich and Cozzarelli (1995). (*A*) Two daughter molecules with the replication forks arrested at the terminus τ. The molecules are still held together by base-pairing of a single turn of the unreplicated parental DNA. (*B*) Unreplicated parental DNA is separated by melting of the base pairs by a helicase, followed by synthesis to fill in the gap, thus generating two fully replicated catenated daughter molecules. (*C*) Topo IV acts on the catenated daughter molecules, thus separating the two. The two-step process of decatenation is supported by topological evidence (Adams et al. 1992).

In plasmid DNA replication in eukaryotic cells (driven by the SV40 origin of replication), the sequence at the termination site strongly affects the fraction of catenated dimers that form (Weaver et al. 1985; Fields-Berry and DePamphilis 1989). For example, the normal termination site for SV40 DNA replication and the yeast CEN3 sequence both promote formation of catenated intertwines when replication terminates in these sequences, but not when these sequences were placed elsewhere on the plasmid and termination occurred outside of them. Therefore, topo II must act behind the replication forks as they enter the termination region, and these two sequences must impede the ability of topo II to resolve catenated intertwines. If the action of topo II did not occur until replication was completed and catenated dimers were formed, then the ability of topo II to resolve catenation should not be affected by the sequence at the termination region, because the catenated intertwines would be distributed throughout the two interlocked sibling molecules.

Multimer Resolution

Because of an odd number of random crossovers between the catenated daughter molecules, multimers are generated. The terminal region of the *E. coli* chromosome has a 33-bp-long site called *dif* (*d*eletion *i*nduced *f*ilamentation) that interacts with the host-encoded recombinases *XerC* and *XerD* to resolve the multimers into monomers. Deletions of the *dif* site or mutations in *XerC* or *XerD* cause filamentation of cells (Blakely et al. 1991; Kuempel et al. 1991; Leslie and Sherratt 1995). The 33-bp *dif* site can substitute for the natural *dif* region, but only in a relatively location-specific manner (Tecklenberg et al. 1995).

The Replication Terminus Is a Recombinogenic Hot Spot

Several groups of workers have reported that the replication terminus of *E. coli* is a hot spot for recombination (Bierne et al. 1991; Louarn et al. 1991; Horiuchi et al. 1994; Horiuchi and Fujimura 1995). Using a λ cI 857 temperature-sensitive repressor-encoding prophage integrated at several sites on the *E. coli* chromosome, Louarn et al. (1991) discovered that the prophage inserted at the terminus was excised at a significantly higher frequency than phage insertions elsewhere on the chromosome. The excision was compatible with recombination events occurring at each replication cycle. The authors have proposed a model which postulates that RecA-mediated recombination plays a critical role in the resolution of catenated dimers. The model is an interesting one but does not seem to take into account the known requirement for topo IV in catenane resolution (Kato et al. 1990; Adams et al. 1992). A hybrid replicon of pBR322 and M13 origins containing the replication arrest site with blocking end toward both origins yielded, in a *tus*$^+$ cell, deletions of which the majority mapped to within 5–6 nucleotides of the replication arrest site of leading-strand synthesis. The authors pointed out the recombinogenic potential of the replication arrest sites and the consequent propensity to cause genome instability (Bierne et al. 1991).

Horiuchi and Fujimura (1995) constructed *E. coli* strains with two replication arrest sites that blocked both forks of the bidirectionally expanding replication bubble. These strains were hyperrecombinogenic and were dependent on *recA*$^+$ and *recB*$^+$(*C*$^+$) genes for growth and induced SOS response constitutively. The authors have postulated a recombinogenic event caused by a double strand break at the stalled fork and reconstruction of the fork by recBCD-dependent recombination events. In summary, stalled recombination forks at the replication arrest sites generate a hyperrecombinogenic state that would cause genome instability and genome rearrangement.

REPLICATION ARREST SITES IN EUKARYOTES

Arrest of DNA Replication Forks at Specific Sites

Replication forks do not travel at a continuous rate along the genome, but pause at various sequences (Tapper and DePamphilis 1980). Some sequences have been identified at specific sites in the genomes of animal viruses, eukaryotic cells, and mitochondria that can arrest progress of DNA replication forks. Brewer (1988) has suggested that the primary function of arrest sites for DNA replication forks in the rRNA gene repeats is to prevent collision between replication forks and the RNA polymerase I transcription apparatus. Whether or not this hypothesis can be generally applied to other genes awaits further investigation.

The Epstein-Barr virus (EBV) genome contains a tandem repeat of 20 copies of a 30-bp sequence that binds tightly the virally encoded EBNA1 protein and functions both as an enhancer for viral promoters and as a component of the EBV origin of DNA replication (*oriP*). Replication forks originating at *oriP* do not traverse this EBV enhancer region (Gahn and Schildkraut 1989). Termination of DNA replication in circular plasmids driven by the EBV *oriP* and EBNA1 terminate replication within the enhancer. The EBV enhancer, in the presence of EBNA1 protein, also can arrest replication forks originating from the SV40 replication origin (Dhar and Schildkraut 1991). Arrest of DNA replication forks required at least two, but not more than six, tandem repeats. More recent studies show that this EBNA1-binding sequence can prevent SV40 T-antigen helicase from unwinding DNA (C. Schildkraut, pers. comm.). Whether or not the ability of EBNA1 DNA-binding sites to arrest replication forks exhibits polarity remains to be determined.

The best-studied replication fork barriers in eukaryotes are found in the nontranscribed spacer regions of yeast rRNA genes (Brewer and Fangman 1988; Linskens and Huberman 1988; Brewer et al. 1992). Actively transcribed rRNA gene tandem repeats exhibit a barrier to replication forks traveling upstream (i.e., opposite to the direction of transcription), as diagrammed in Figure 2E. Replication forks seldom originate from origins of replication located immediately downstream from inactive genes (Lucchini and Sogo 1994). A site in the intergenic spacer sequence exhibits polarity by blocking forks coming from one direction but not from the other (Brewer et al. 1992; Kobayashi et al. 1992). Transcription by yeast RNA polymerase I of a DNA fragment containing a transcription terminator near the putative replication arrest site revealed three sites that could arrest transcription (Lang and Reeder 1993). The first site bound the yeast Reb1 protein and arrested RNA polymerase I arriving from one direction, but not the other. The second site did not ex-

hibit polarity. It may bind as-yet-unidentified proteins. Interestingly, the third site, which also lacked polarity, overlapped the putative replication arrest site. Recently, Horiuchi and Kobayashi (cited in Horiuchi and Fujimura 1995) have confirmed the earlier observations of Voelkel-Meimon et al. (1987) that the putative replication arrest site in yeast rRNA gene repeats is associated with a recombination hot spot (HOT 1 sequence). One of the mutants with reduced recombination activity at this HOT spot also showed reduced arrest of DNA replication forks. These results suggest that DNA replication forks in yeast may be subject to the same type of orientation-specific replication arrest sites that have been described in bacteria. DNA replication arrest sites analogous to those described in budding yeast have been mapped in the tandem repeats of rRNA genes in human cells (Little et al. 1993), plant cells (Hernandez et al. 1988, 1993), mouse cells and fission yeast (Lopez-Estrano et al. 1996), and the mouse rDNA replication fork arrest sites exhibit polarity. Thus, DNA replication fork arrest sites appear to be a common feature of rRNA gene repeats in nature.

Another example of a site-specific DNA replication arrest site is found in mitochondrial DNA. Mammalian (and other animal) mitochondrial DNA carry out replication in two stages. The parental light strand serves as the template for initiation from Ori_H, and the fork, after copying about 600 nucleotides, is arrested at a specific site forming a D-loop (Fig. 2E) (for reviews, see Clayton 1991a,b). Eventually, the daughter H strand elongates to expose Ori_L, which initiates the daughter light strand. The arrest of the heavy strand occurs near the terminus-associated sequences (TAS), which are evolutionarily conserved in vertebrates. A protein isolated from bovine cells binds to the mitochondrial TAS (Madsen et al. 1993). No further information is presently available on the physiological role of the putative protein in D-loop biogenesis.

The region of the D-loop, as in cases of replication termination sites, appears to be recombinogenic. In a Mendelian, autosomal dominant disorder called mitochondrial myopathy, the carriers develop an accumulation of deletions in the mitochondrial DNA, starting from a unique location to within a few nucleotides of the 3′ end of the arrested nascent H-strand DNA (Zeviani et al. 1989). In a more recent report, Zeviani (1992) has shown that some of the deletions in the mitochondrial DNA of myopathic patients are found downstream from the D-loop.

The origins of deletions in mitochondrial DNA triggered by a mutant nuclear gene are interesting to contemplate. First, the deletions could be caused by a mutant replication terminator protein, encoded by a nuclear gene, but acting to cause H-strand arrest. If the mutant protein has a

propensity to allow formation of more free ends or gaps or a hyper-recombinogenic substrate, deletions could be generated by non-homologous recombination. The lesions found downstream from the 3′ end of the arrested H strand (Zeviani 1992) could also be potentiated by the extension and downstream pausing of the daughter strand. The isolation and analysis of the probable human mitochondrial replication arrest protein and the nuclear gene encoding the protein are interesting problems to tackle in the future.

Sequence-specific arrest sites for DNA replication forks may also exist in regions where DNA amplification events occur. For example, cells in culture and cancer cells in animals undergo constant genomic rearrangements and gene amplification events that can be demonstrated by selecting for cells that continue to proliferate in the presence of various metabolic inhibitors (for review, see Schimke 1984; Stark et al. 1989; Di Leonardo et al. 1993; Stark 1993; Wintersberger 1994). Developmentally orchestrated gene amplification occurs in the chorion genes of *Drosophila* (Heck and Spradling 1990) and the polytene chromosomes of *Chironomous* and *Sciara* (Gerbi et al. 1993). A mechanism that can account for selective gene amplification is the "onion skin model" first proposed to explain the excision of integrated SV40 genomes from the chromosomes of virus-transformed cell lines (Botchan et al. 1979). Direct evidence for this model comes from analysis of chorion gene amplification in *Drosophila* embryos (Kafatos et al. 1985).

The onion skin mechanism for gene amplification requires that multiple initiation events occur at the same origin of replication before the completion of replication of the replication unit, and that replication forks emanating from this origin are arrested at sites on either side of the sequence that is amplified. Thus, the second set of forks resulting from reinitiation at the origin eventually catches up with the first set of forks, creating multiple, concentric replication bubbles (like the layers of an onion). At this time, there is no direct evidence for specific sequences that arrest replication forks flanking known amplification loci, but one could anticipate finding them. In addition, there may be specific terminator proteins produced by the specialized cells in which programmed gene amplification occurs. Such systems should provide fertile ground for future experiments.

Two Pathways for Separation of Sibling Molecules at Termination Sites

Termination of DNA replication occurs whenever two oncoming replication forks collide. Since DNA replication begins at thousands of sites dis-

tributed throughout the chromosomes of eukaryotic cells, termination of DNA replication must also occur at thousands of sites during each S phase. However, in contrast to initiation of DNA replication where specific sequences determine where replication begins in the genomes of eukaryotic cells, animal viruses, and mitochondria (see DePamphilis, this volume), termination of DNA replication can occur at virtually any sequence. However, it is possible, and perhaps likely, that some replication units (e.g., rDNA) terminate replication at specific sequences. In SV40, although termination of replication does not require specific sequences (Lai and Nathans 1975), the sequence at the termination site does strongly influence the pathway by which the two sibling molecules are separated (Weaver et al. 1985; Fields-Berry and DePamphilis 1989).

As replication forks advance, DNA unwinding introduces positive superhelical turns in front of the fork that are relaxed by topoisomerase I (Minden and Marians 1986). As two oncoming replication forks approach each other, the length of unreplicated DNA between them grows shorter, and therefore the target for topo I grows smaller. Eventually, topo I can no longer act in front of the forks. When this happens, DNA unwinding at the replication fork produces one catenated intertwine in the two sibling molecules behind the replication fork for each 10 bp of DNA unwound in front of the replication fork. In eukaryotes, topo II is required to remove these catenated intertwines. Mutations in yeast topo II that inactivate its decatenation activity result in interlocked DNA cellular chromosomes during S phase and in catenated dimers in plasmids that replicate in yeast cells (DiNardo et al. 1984; Uemura et al. 1987). Inhibition of topo II in mammalian cells either by indirect means (Sundin and Varshavsky 1981; Weaver et al. 1985; Fields-Berry and DePamphilis 1989) or by the use of specific inhibitors (Richter et al. 1987; Snapka et al. 1988; Ishimi et al. 1992) results in accumulation of late-replicating intermediates (θ structures that have completed >90% of their replication) of circular DNA molecules as well as catenated dimers containing multiple intertwines. Depending on experimental conditions, the primary product can be almost all late-replicating intermediates (Ishimi et al. 1992) or almost entirely catenated dimers (Sundin and Varshavsky 1981). The fact that late-replicating intermediates accumulate when topo II is inhibited demonstrates that catenated intertwines are normally removed prior to separation of sibling DNA molecules (Weaver et al. 1985).

Neither the SV40 termination region nor the yeast CEN3 sequence promotes formation of catenated intertwines under normal physiological conditions (Koshland and Hartwell 1987; Fields-Berry and DePamphilis

1989). Therefore, catenated intertwines are normally removed prior to or concomitant with termination of DNA replication when two replication forks collide. The presence of sequence-specific termination sites in bacterial chromosomes may ensure that this occurs in bacterial chromosomes prior to cell division.

CONCLUSION

The two known replication terminator proteins, namely Ter (Tus) of *E. coli* and RTP of *B. subtilis*, arrest replication forks by their polar contrahelicase activity. These proteins also block RNA chain elongation in a polar fashion. It is interesting to note that programmed cell death of bacterial cells that lose a resident plasmid also involves a plasmid-coded contrahelicase and an antidote protein. The contrahelicase is long-lived, but the antidote (contra-contrahelicase) protein is short-lived. Thus, in the plasmid-free segregants, the antidote decays and the longer-lived contrahelicase neutralizes the helicase, thereby causing replication arrest and cell death (Ruiz-Echevarria et al. 1995).

A polar contrahelicase from a eukaryote has not yet been discovered. We strongly suspect that such proteins exist in eukaryotes and are likely to be involved in fork arrest at specific sequences. The search for such protein(s) should prove to be interesting and rewarding.

It has been noted that fork arrest sites are very recombinogenic and therefore are regions of potential chromosome instability. That certain chromosome instabilities lead to the induction of cancer has been recognized (Hartwell 1992). This fact should provide additional incentive to search for eukaryotic replication terminator proteins.

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REFERENCES

Adams, D.E., E.M. Schekhtman, E.L. Zechiedrich, M.B. Schmid, and N. Cozzarelli. 1992. The role of topoisomerase IV in partitioning bacterial replicons and the structure

of catenated intermediates in DNA replication. *Cell* **71:** 277–288.

Amin, A.A. and J. Hurwitz. 1992. Polar arrest of the simian virus 40 tumor antigen-mediated replication fork movement *in vitro* by the tus protein-terB complex of *E. coli*. *J. Biol. Chem.* **267:** 18612–18622.

Baker, T. 1995. Replication arrest. *Cell* **80:** 521–524.

Baran, N., A. Lapidot, and H. Manor. 1983. Onion skin replication of integrated polyoma-transformed rat cells: Termination within a specific cellular DNA segment. *Proc. Natl. Acad. Sci.* **80:** 105–109.

———. 1987. Unusual sequence element found at the end of an amplicon. *Mol. Cell. Biol.* **7:** 2636–2640.

Bastia, D., J. Germino, J.H. Crossa, and P. Hale. 1981a. Molecular cloning of the replication terminus of the plasmid R6K. *Gene* **14:** 81–89.

Bastia, D., J. Germino, J.H. Crossa, and J. Ram. 1981b. The nucleotide sequence surrounding the replication terminus of R6K. *Proc. Natl. Acad. Sci.* **78:** 2095–2099.

Bedrosian, C.L. and D. Bastia. 1991. *E coli* replication terminator protein impedes simian virus 40 SV40 replication fork movement and SV40 large tumor antigen helicase activity *in vitro* at a prokaryotic terminus sequence. *Proc. Natl. Acad. Sci.* **88:** 2618–2622.

Bierne, H. and B. Michel. 1994. When replication forks stop. *Mol. Microbiol.* **13:** 17–23.

Bierne, H., S.D. Ehrlich, and B. Michel. 1991. The replication termination signal terB of the *Escherichia coli* chromosome is a deletion hot spot. *EMBO J.* **10:** 2699–2705.

Blakely, G., S.D. Colloms, G. May, M. Burk, and D.J.C. Sherratt. 1991. *E. coli XerC* recombinase is required for chromosomal segregation at cell division. *New Biol.* **3:** 789–798.

Botchan, M., W. Topp, and J. Sambrook. 1979. Studies on simian virus 40 excision from cellular chromosomes. *Cold Spring Harbor Symp. Quant. Biol.* **43:** 709–719.

Brewer, B. 1988. When polymerases collide: Replication and transcriptional organization of the *Escherichia coli* chromosome. *Cell* **53:** 679–686.

Brewer, B. and W.L. Fangman. 1987. The localization of replication origins on ARS plasmids in *Saccharomyces cerevisiae*. *Cell* **51:** 463–471.

———. 1988. A replication fork barrier at the 3′ end of yeast ribosomal RNA genes. *Cell* **55:** 637–743.

Brewer, B.J., D. Lockshon, and W.L. Fangman. 1992. The arrest of replication forks in the rDNA of yeast occurs independently of transcription. *Cell* **71:** 267–276.

Bussiere, D.E., D. Bastia, and S. White. 1995. Crystal structure of the replication terminator protein from *Bacillus subtilis* at 2.6 Å. *Cell* **80:** 651–660.

Carrigan, C.M., R.A. Peck, M.T. Smith, and R.G. Wake. 1991. Normal *ter*C region of the *Bacillus subtilis* chromosome acts in a polar manner to arrest the clockwise replication fork. *J. Mol. Biol.* **222:** 197–207.

Cashel, M. and K. Rudd. 1987. The stringent response. In Escherichia coli *and* Salmonella typhimurium: *Cellular and molecular biology* (ed. F.C. Neidhardt et al.), pp. 1410–1438. American Society for Microbiology, Washington, D.C.

Clayton, D. 1991a. Nuclear gadgets in mitochondrial DNA replication and transcription. *Trends Biochem. Sci.* **16:** 107–111.

———. 1991b. Replication and transcription of vertebrate mitochondrial DNA. *Annu. Rev. Cell Biol.* **7:** 453–478.

Crosa, J.H., L. Luttropp, and S. Falkow. 1976. Mode of replication of the conjugative R-plasmid RSF1040 in *Escherichia coli*. *J. Bacteriol.* **26:** 454–466.

d'Ambrosio, E. and A.V. Furano. 1987. DNA synthesis arrest sites at the right terminus of rat long interspersed repeated [LINE or L1Rn] DNA family members. *Nucleic Acids Res.* **15:** 3155–3175.

Dasgupta, S., R. Bernander, and K. Nordstrom. 1991. *In vivo* effect of the *tus* mutation on cell division in an *Escherichia coli* strain where chromosome replication is under control of plasmid R1. *Res. Microbiol.* **142:** 177–180.

Dayn, A., G.M. Samadashwily, and S. Mirkin. 1992. Intramolecular DNA triplexes: Unusual sequence requirements and influence on DNA polymerization. *Proc. Natl. Acad. Sci.* **89:** 11406–11410.

Dhar, V. and C.L. Schildkraut. 1991. Role of EBNA-1 in arresting replication forks at the Epstein-Barr virus oriP family of tandem repeats. *Mol. Cell. Biol.* **11:** 6268–6278.

Di Leonardo, A., S.P. Linke, Y. Yin, and G.M. Wahl. 1993. Cell cycle regulation of gene amplification. *Cold Spring Harbor Symp. Quant. Biol.* **58:** 655–667.

DiNardo, S., K. Voelkel, and R. Sternglanz. 1984. DNA topoisomerase mutant of *Saccharomyces cerevisiae:* Topoisomerase II is required for segregation of daughter molecules at the termination of DNA replication. *Proc. Natl. Acad. Sci.* **81:** 2616–2620.

Elledge, S.J., P. Sugino, L. Guarente, and R.W. Davis. 1990. Genetic selection for genes encoding sequence-specific DNA-binding proteins. *Proc. Natl. Acad. Sci.* **86:** 3689–3693.

Fields-Berry, S.C. and M.L. DePamphilis. 1989. Sequences that promote formation of catenated intertwines during termination of DNA replication. *Nucleic Acids Res.* **17:** 3261–3273.

Francois, V., J. Louarn, and J.-M. Louarn. 1989. The terminus region of *E. coli* chromosome is flanked by several polar replication pause sites. *Mol. Microbiol.* **3:** 995–1002.

Francois, V., J. Louarn, J.-E. Rebollo, and J.-M. Louarn. 1990. Replication termination, nondivisible zones and structure of the *Escherichia coli* chromosome. In *The bacterial chromosome* (ed. K. Drilca and M. Riley), pp. 351–359. American Society for Microbiology, Washington, D.C.

Franks, A.H., A.A. Griffith, and R.G. Wake. 1995. Identification and characterization of new DNA replication terminators in *Bacillus subtilis*. *Mol. Microbiol.* **17:** 13–23.

Gahn, T.A. and C.L. Schildkraut. 1989. The Epstein-Barr virus origin of replication, *ori*P, contains both the initiation and termination sites of DNA replication. *Cell* **58:** 527–535.

Gerbi, S.A., C. Liang, N. Wu, S.M. DiBartolomeis, B. Bienz-Tadmor, H.S. Smith, and F.D. Urnov. 1993. DNA amplification in DNA puff II/9A of *Sciaria coprophila*. *Cold Spring Harbor Symp. Quant. Biol.* **58:** 487–494.

Germino, J. and D. Bastia. 1981. Termination of DNA replication *in vitro* at a sequence-specific replication terminus. *Cell* **23:** 681–687.

Gottlieb, P.A., S. Wu, X. Zhang, M. Tecklenberg, P.L. Kuempel, and T.M. Hill. 1992. Equilibrium, kinetic, and footprinting studies of the Tus-Ter protein DNA interaction. *J. Biol. Chem.* **267:** 7434–7443.

Hartwell, L. 1992. Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. *Cell* **71:** 543–546.

Heck, M.M.S. and A.C. Spradling. 1990. Multiple replication origins are used during *Drosophila* chorion gene amplification. *J. Cell Biol.* **110:** 903–914.

Henckes, G., F. Harper, A. Levine, F. Vannier, and S.J. Seror. 1989. Overreplication of the origin region in the dnaB37 mutant of *Bacillus subtilis*: Postinitiation control of

chromosomal replication. *Proc. Natl. Acad. Sci.* **86:** 8660–8664.

Hernandez, P., S.S. Lamm, C.A. Bjerknes, and J. Van't Hof. 1988. Replication termini in the rDNA of the synchronized pea root cells (*Pisum sativum*). *EMBO J.* **7:** 303–308.

Hernandez, P., L. Martin-Parras, M.L. Martinez-Robles, and J.B. Schvartzman. 1993. Conserved features in the mode of replication of eukaryotic ribosomal RNA genes. *EMBO J.* **12:** 1475–1485.

Hiasa, H. and K.J. Marians. 1992. Differential inhibition of the DNA translocation and DNA unwinding activities of DNA helicases by the *E. coli* tus protein. *J. Biol. Chem.* **267:** 11379–11385.

———. 1994a. Tus prevents overreplication of *ori*C plasmid DNA. *J. Biol. Chem.* **269:** 26959–26968.

———. 1994b. Topoisomerase IV can support *ori*C DNA replication *in vitro*. *J. Biol. Chem.* **269:** 16371–16375.

Hidaka, M., M. Akiyama, and T. Horiuchi. 1988. A consensus sequence of three DNA replication terminus sites on the *E. coli* chromosome is highly homologous to the terR sites of the R6K plasmid. *Cell* **55:** 467–475.

Hidaka, M., T. Kobayashi, and T. Horiuchi. 1991. A newly identified DNA replication terminus site, TerE, on the *Escherichia coli* chromosome. *J. Bacteriol.* **173:** 391–393.

Hidaka, M., T. Kobayashi, S. Takenaka, H. Takeya, and T. Horiuchi. 1989. Purification of a DNA replication terminus (ter) site-binding protein in *Escherichia coli* and identification of the structural gene. *J. Biol. Chem.* **264:** 21031–21037.

Hidaka, M., T. Kobayashi, Y. Ishimi, M. Seki, T. Enomoto, M. Abdel-Monem, and T. Horiuchi. 1992. Termination complex in *Escherichia coli* inhibits SV40 DNA replication *in vitro* by impeding the action of T antigen helicase. *J. Biol. Chem.* **267:** 5361–5365.

Hill, T.M. 1992. Arrest of bacterial DNA replication. *Annu. Rev. Microbiol.* **46:** 603–633.

Hill, T.M. and K.J. Marians. 1990. *Escherichia coli* tus protein acts to arrest the progression of DNA replication forks *in vitro*. *Proc. Natl. Acad. Sci.* **87:** 2481–2485.

Hill, T.M., A.J. Pelletier, M.L. Tecklenberg, and P.L. Kuempel. 1988. Identification of DNA sequence from the *E. coli* terminus region that halts replication forks. *Cell* **55:** 459–466.

Hill, T.M., M. Tecklenberg, A.J. Pelletier, and P.L. Kuempel. 1989. *tus*, the *trans*-acting gene required for termination of DNA replication in *Escherichia coli*, encodes a DNA-binding protein. *Proc. Natl. Acad. Sci.* **86:** 1593–1597.

Horiuchi, T. and Y. Fujimura. 1995. Recombinational rescue of the stalled fork: A model based on analysis of an *Escherichia coli* strain with a chromosome region difficult to replicate. *J. Bacteriol.* **177:** 783–791.

Horiuchi, T. and M. Hidaka. 1988. Core sequence of two separable terminus sites of the R6K plasmid that exhibit polar inhibition of replication is a 20 bp inverted repeat. *Cell* **54:** 515–523.

Horiuchi, T., Y. Fujimura, H. Nishitani, T. Kobayashi, and M. Hidaka. 1994. The DNA replication fork blocked at the Ter site may be an entrance for the RecBCD enzyme into duplex DNA. *J. Bacteriol.* **176:** 4656–4663.

Ishimi, Y., R. Ishida, and T. Andoh. 1992. Effect of ICRF-193, a novel DNA topoisomerase II inhibitor, on simian virus 40 DNA and chromosome replication *in vitro*. *Mol. Cell. Biol.* **12:** 4007–4012.

Kafatos, F.C., W. Orr, and C. Delidakis. 1985. Developmentally regulated gene

amplification. *Trends Genet.* **1:** 301–306.

Kato, J., Y. Nishimura, M. Yamada, and H. Suzuki. 1990. A new topoisomerase essential for chromosome segregation in *E. coli*. *Cell* **63:** 393–404.

Kato, J., Y. Nishimura, R. Imamura, H. Niki, S. Hiraga, and H. Suzuki. 1988. Gene organization in a region containing a new gene involved in chromosome partitioning in *Escherichia coli*. *J. Bacteriol.* **170:** 3967–3977.

Kaul, S., B.K. Mohanty, T. Sahoo, I. Patel, S. Khan, and D. Bastia. 1994. The replication terminator protein of the gram-positive bacterium *B. subtilis* functions as a polar contrahelicase in gram-negative *E. coli*. *Proc. Natl. Acad. Sci.* **91:** 11143–11147.

Khatri, G.S., T. MacAllister, P.R. Sista, and D. Bastia. 1989. The replication termination protein of *E. coli* is a DNA sequence specific contra-helicase. *Cell* **59:** 667–674.

Kobayashi, T., M. Hidaka, M. Nishizawa, and T. Horiuchi. 1992. Identification of a site required for DNA replication fork blocking activity in the rRNA gene cluster in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **233:** 355–362.

Kolter, R. and D.R. Helinski. 1978. Activity of the replication terminus of plasmid in hybrid replicons in *Escherichia coli*. *J. Mol. Biol.* **124:** 425–441.

Kornberg, A. and T.A. Baker. 1992. *DNA replication*, 2nd edition. W.H. Freeman, New York.

Koshland, D. and L.H. Hartwell. 1987. The structure of sister minichromosome DNA before anaphase in *Saccharomyces cerevisiae*. *Science* **238:** 1713–1716.

Kuempel, P.L. and S.A. Duerr. 1979. Chromosome replication in *Escherichia coli* is inhibited in the terminus region near the *rac* locus. *Cold Spring Harbor Symp. Quant. Biol.* **43:** 563–567.

Kuempel, P.L., S.A. Duerr, and N.R. Seely. 1977. Terminus region of the chromosome of *Escherichia coli* inhibits replication forks. *Proc. Natl. Acad. Sci.* **74:** 3927–3931.

Kuempel, P.L., J.M. Henson, L. Dircks, M. Tecklenberg, and D.F. Lim. 1991. *dif*, a *rec*A-independent recombination site in the terminus region of the chromosome of *Escherichia coli*. *New Biol.* **3:** 799–811.

Lai, C.S. and D. Nathans. 1975. Non-specific termination of SV40 DNA replication. *J. Mol. Biol.* **97:** 113–118.

LaDuca, R.J., P.J. Fay, C. Chuang, C.S. McHenry, and R.A. Bambara. 1983. Site-specific pausing of deoxyribonucleic acid synthesis catalyzed by four forms of *Escherichia coli* DNA polymerase III. *Biochemistry* **22:** 5177–5188.

Lang, W.H. and R.H. Reeder. 1993. The Reb1 site is an essential component of a terminator for RNA polymerase I in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **13:** 649–658.

Langley, D.B., M.T. Smith, P.J. Lewis, and R.G. Wake. 1993. Protein-nucleoside contacts in the interaction between the replication terminator protein of *Bacillus subtilis* and the DNA terminator. *Mol. Microbiol.* **10:** 771–779.

LeBowitz, J.O. and R. McMacken. 1986. The *Escherichia coli* DnaB protein is a DNA helicase. *J. Biol. Chem.* **261:** 4738–4748.

Lee, E.H. and A. Kornberg. 1992. Features of replication fork blockage by the *Escherichia coli* terminus binding protein. *J. Biol. Chem.* **267:** 8778–8784.

Lee, E.H., A. Kornberg, M. Hidaka, T. Kobayashi, and T. Horiuchi. 1989. *Escherichia coli* replication termination protein impedes the action of helicases. *Proc. Natl. Acad. Sci.* **86:** 9104–9108.

Lee, M.S. and K.J. Marians. 1987. *Escherichia coli* replication factor Y, a component of the primosome, can act as a DNA helicase. *Proc. Natl. Acad. Sci.* **84:** 8345–8349.

Leslie, N.R. and D.J. Sherratt. 1995. Site-specific recombination in the replication terminus region of *Escherichia coli*: Functional replacement of *dif*. *EMBO J.* **14:** 1561–1570.

Levine, A., S. Autret, and S.J. Seror. 1995. A checkpoint involving RTP, the replication terminator protein, arrests replication downstream of the origin during the stringent response in *Bacillus subtilis*. *Mol. Microbiol.* **15:** 287–295.

Levine, A., F. Vannier, M. Dehbi, G. Henckes, and S.J. Seror. 1991. The stringent reponse blocks DNA replication outside the *ori* region in *Bacillus subtilis* and at the origin in *Escherichia coli*. *Mol. Microbiol.* **219:** 605–613.

Lewis, P.J. and R.G. Wake. 1991. Termination of chromosome replication in *Bacillus subtilis*. *Res. Microbiol.* **142:** 893–900.

Lewis, P.J., M.T. Smith, and R.G. Wake. 1989. A protein involved in termination of chromosome replication in *Bacillus subtilis* binds specifically to the *ter*C site. *J. Bacteriol.* **171:** 3564–3567.

Lewis, P.J., G.B. Ralston, R.I. Christopherson, and R.G. Wake. 1990. Identification of the replication terminator protein binding sites in the terminus region of the *Bacillus subtilis* chromosome and stoichiometry of the binding. *J. Mol. Biol.* **214:** 73–84.

Lindsey, J.C. and J.R.F.C. Leach. 1989. Slow replication of palindrome-containing DNA. *J. Mol. Biol.* **206:** 779–782.

Linskens, M.H.K. and J.A. Huberman. 1988. Organization of origin of replication of rDNA in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **8:** 4927–4935.

Little, R.D., T.H.K. Platt, and C.L. Schildkraut. 1993. Initiation and termination of DNA replication in human rRNA genes. *Mol. Cell. Biol.* **13:** 6600–6613.

Liu, B. and B. Alberts. 1995. Head-on collision between a DNA replication apparatus and RNA polymerase transcription complex. *Science* **267:** 1131–1137.

Lopez-Estrano, C., J. Schvartzman, and P. Hernandez. 1996. The replication of ribosomal RNA genes in eukaryotes. In *Chromosomes today* (ed. M. Puertas et al.). Chapman and Hall, London. (In press.)

Louarn, J., J. Patte, and J.-M. Louarn. 1977. Evidence for a fixed termination site of chromosome replication in *Escherichia coli* K12. *J. Mol. Biol.* **115:** 295–314.

Louarn, J.-M., J. Louarn, V. Francois, and J. Patte. 1991. Analysis and possible role of hyperrecombination in the termination region of the *Escherichia coli* chromosome. *J. Bacteriol.* **173:** 5097–5104.

Lovett, M.A., R.A. Sparks, and D.R. Helinski. 1975. Bidirectional replication of plasmid R6K DNA in *Escherichia coli*: Correspondence between origin of replication and a single strand break in relaxed complex. *Proc. Natl. Acad. Sci.* **72:** 2905–2909.

Lucchini, R. and J.M. Sogo. 1994. Chromatin structure and transcriptional activity around the replication forks at the 3′ end of the yeast rRNA genes. *Mol. Cell. Biol.* **14:** 318–326.

MacAllister, T., G.S. Khatri, and D. Bastia. 1990. Sequence-specific and polarized replication termination *in vitro*: Complementation of extracts of tus$^-$ *Escherichia coli* by purified Ter protein and analysis of termination intermediates. *Proc. Natl. Acad. Sci.* **87:** 2828–2832.

Madsen, C.S., S.C. Ghivizzani, and W.W. Hauswirth. 1993. Protein binding to a single termination-associated sequence in the mitochondrial DNA D-loop region. *Mol. Cell. Biol.* **13:** 2162–2171.

Manna, A.C., K.S. Pai, D.E. Bussiere, S.W. White, and D. Bastia. 1996. The dimer-dimer interaction surface of the replication terminator protein of *Bacillus subtilis* and termina-

tion of DNA replication. *Proc. Natl. Acad. Sci.* (in press).

Minden, J.S. and K.J. Marians. 1986. *Escherichia coli* topoisomerase I can segregate replicating pBR322 daughter DNA molecules *in vitro*. *J. Biol. Chem.* **261:** 11906–11917.

Mohanty, B.K., T. Sahoo, and D. Bastia. 1996. Relationship between sequence-specific termination of DNA replication and transcription. *EMBO J.* (in press).

Natarajan, S., W.I. Kelly, and D. Bastia. 1991. Replication terminator protein of *Escherichia coli* is a transcriptional repressor of its own synthesis. *Proc. Natl. Acad. Sci.* **88:** 3867–3871.

Natarajan, S., S. Kaul, A. Miron, and D. Bastia. 1993. A 27 kd protein of *E. coli* promotes antitermination of replication *in vitro* at a sequence-specific replication terminus. *Cell* **72:** 113–120.

Nordstrom, K., R. Bernander, and S. Dasgupta. 1991. Analysis of the bacterial cell cycle using strains in which chromosome replication is controlled by plasmid R1. *Res. Microbiol.* **142:** 181–188.

O'Sullivan, M.A. and C. Anagnostopoulos. 1982. Replication terminus of the *Bacillus subtilis* chromosome. *J. Bacteriol.* **151:** 135–143.

Pai, K.S., D.E. Bussiere, F. Wang, C. Hutchison, S. White, and D. Bastia. 1996. The structure and function of the replication terminator protein of *Bacillus subtilis:* Identification of the "winged-helix" DNA binding domain. *EMBO J.* (in press).

Pelletier, A.J., T.M. Hill, and P.L. Kuempel. 1988. Location of sites that inhibit progression of replication forks in the terminus region of *Escherichia coli*. *J. Bacteriol.* **170:** 4295–4298.

Peng, H. and K.J. Marians. 1993. Decatenation activity of topoisomerase IV during *oriC* and pBR322 DNA replication in vitro. *Proc. Natl. Acad. Sci.* **90:** 8571–8575.

Rao, P.S., H. Manor, and R.G. Martin. 1988. Pausing in SV40 DNA replication by a sequence containing $(dG\text{-}dA)_{27}(dT\text{-}dc)_{27}$. *Nucleic Acids Res.* **16:** 8077–8094.

Richter, A., U. Strausfeld, and R. Knippers. 1987. Effects of VM26 (teniposide), a specific inhibitor of type II DNA topoisomerase, on SV40 DNA replication *in vivo*. *Nucleic Acids Res.* **15:** 3455–3468.

Roecklein, B.A. and P.L. Kuempel. 1992. *In vivo* characterization of *tus* gene expression in *Escherichia coli*. *Mol. Microbiol.* **6:** 1655–1661.

Roecklein, B., A. Pelletier, and P.L. Kuempel. 1991. The *tus* gene of *Escherichia coli*: Autoregulation, analysis of flanking sequences and identification of a complementary system in *Salmonella typhimurium*. *Res. Microbiol.* **142:** 169–175.

Ruiz-Echevarria, M.J., G. Gimenez-Gallego, R. Sabariegos-Jareno, and R. Diaz-Orejas. 1995. Kid, a small protein of the parD stability system of plasmid R1, is an inhibitor of DNA replication acting at the initiation of DNA synthesis. *J. Mol. Biol.* **247:** 568–577.

Sahoo, T., B.K. Mohanty, I. Patel, and D. Bastia. 1995a. Termination of DNA replication *in vitro*: Requirement for stereospecific interaction between two dimers of the replication termination protein of *Bacillus subtilis* and with the terminator site to elicit polar contrahelicase and fork impedance. *EMBO J.* **14:** 619–628.

Sahoo, T., B.K. Mohanty, A. Manna, M. Lobert, and D. Bastia. 1995b. The contrahelicase activities of the replication terminator proteins of *Escherichia coli* and *Bacillus subtilis* are helicase-specific and impede both helicase translocation and authentic DNA unwinding. *J. Biol. Chem.* **270:** 29138–29144.

Schimke, R.T. 1984. Gene amplification in cultured cells. *Cell* **37:** 705–713.

Schmid, M.B. 1990. A locus affecting nucleoid segregation in *Salmonella typhimurium*.

J. Bacteriol. **172:** 5416–5424.

Schneider, A.M., M. Gaisne, and C. Anagnostopoulos. 1983. Genetic structure and internal rearrangements of stable merodiploids from *Bacillus subtilis* strains carrying the trpE26 mutation. *Genetics* **101:** 189–210.

Sista, P.R., C.A. Hutchison, and D. Bastia. 1991. DNA-protein interaction at the replication termini of plasmid R6K. *Genes Dev.* **5:** 74–82.

Sista, P.R., S. Mukherjee, P. Patel, G.S. Khatri, and D. Bastia. 1989. A host-encoded DNA-binding protein promotes termination of plasmid replication at a sequence-specific replication terminus. *Proc. Natl. Acad. Sci.* **86:** 3026–3030.

Smith, M.T. and R.G. Wake. 1992. Definition and polarity of action of DNA replication terminators in *Bacillus subtilis*. *J. Mol. Biol.* **227:** 648–657.

Snapka, R.M., M.A. Powelson, and J.M. Strayer. 1988. Swivelling and decatenation of replicating simian virus 40 genomes *in vivo*. *Mol. Cell. Biol.* **8:** 515–521.

Stark, G.R. 1993. Regulation and mechanisms of mammalian gene amplification. *Adv. Cancer Res.* **61:** 87–113.

Stark, G.R., M. Debatisse, E. Giulotto, and G.M. Wahl. 1989. Recent progress in understanding mechanisms of mammalian DNA amplification. *Cell* **57:** 901–908.

Sundin, O. and A. Varshavsky. 1981. Terminal stages of SV40 DNA replication proceed via multiply intertwined catenated dimers. *Cell* **21:** 103–114.

Swindels, M.B. 1995. Identification of a common fold in the replication terminator protein suggests a possible mode for DNA binding. *Trends Biochem. Sci.* **20:** 300–302.

Tapper, D.P. and M.L. DePamphilis. 1980. Preferred sites are involved in the arrest and initiation of DNA synthesis during the replication of SV40 DNA. *Cell* **22:** 97–108.

Tecklenberg, M., A. Naumer, O. Nagappan, and P. Kuempel. 1995. The *dif* resolvase locus of the *Escherichia coli* chromosome can be replaced by a 33-bp sequence, but function depends on location. *Proc. Natl. Acad. Sci.* **92:** 1352–1356.

Uemura, T., H. Ohkura, Y. Adachi, K. Morino, K. Shiozaki, and M. Yanagida. 1987. DNA topoisomerase II is required for condensation and separation of mitotic chromosomes in *S. pombe*. *Cell* **50:** 917–925.

Valenzuela, M.S., D. Freifelder, and R. Inman. 1976. Lack of a unique terminator site for the first round of bacteriophage DNA replication. *J. Mol. Biol.* **102:** 569–589.

Voelkel-Meimon, K., R.L. Keil, and G.S. Roeder. 1987. Recombination-stimulating sequences in yeast ribosomal DNA correspond to sequences regulating transcription by RNA polymerase I. *Cell* **48:** 1071–1079.

Weaver, D.T. and M.L. DePamphilis. 1984. The role of palindromic and non-palindromic sequences in arresting DNA synthesis *in vitro* and *in vivo*. *J. Mol. Biol.* **180:** 961–986.

Weaver, D.T., S.C. Fields-Berry, and M.L. DePamphilis. 1985. The termination region for SV40 DNA replication directs the mode of separation for the two sibling molecules. *Cell* **41:** 565–575.

Weiss, A.S. and R.G. Wake. 1984. A unique DNA intermediate associated with termination of chromosome replication in *Bacillus subtilis*. *Cell* **39:** 683–689.

Wells, R.D., D.A. Collier, J.C. Hanvey, M. Shimizu, and F. Wohlrab. 1988. The chemistry and biology of unusual DNA structures adopted by oligopurine.oligopyrimidine sequences. *FASEB J.* **2:** 2939–2949.

Wintersberger, E. 1994. DNA amplification: New insights into its mechanism. *Chromosoma* **103:** 73–81.

Yoshikawa, H. and N. Sueoka. 1963. Sequential replication of *Bacillus subtilis* chromosome I: Comparison of marker frequencies in exponential and stationary growth

phases. *Proc. Natl. Acad. Sci.* **49:** 559–566.

Young, P.A. and R.G. Wake. 1994. The *Bacillus subtilis* replication terminator system functions in *Escherichia coli*. *J. Mol. Biol.* **240:** 275–280.

Zechiedrich, E.L. and N.R. Cozzarelli. 1995. Roles of topoisomerase IV and DNA gyrase in DNA unlinking during replication in *Escherichia coli*. *Genes Dev.* **9:** 2859–2869.

Zeviani, M. 1992. Nucleus-driven mutations of human mitochondrial DNA. *J. Inherited Metab. Dis.* **15:** 456–471.

Zeviani, M., S. Servidei, C. Gellera, E. Bertini, S. DiMauro, and S. DiDonato. 1989. An autosomal dominant disorder with multiple deletions of mitochondrial DNA starting at the D-loop region. *Nature* **339:** 309–311.