

Processivity of DNA polymerases: two mechanisms, one goal

Zvi Kelman^{1*}, Jerard Hurwitz¹ and Mike O'Donnell²

Replicative DNA polymerases are highly processive enzymes that polymerize thousands of nucleotides without dissociating from the DNA template. The recently determined structure of the *Escherichia coli* bacteriophage T7 DNA polymerase suggests a unique mechanism that underlies processivity, and this mechanism may generalize to other replicative polymerases.

Addresses: ¹Department of Molecular Biology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA and ²Laboratory of DNA Replication, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.

*Corresponding author.

E-mail: z-kelman@ski.mskcc.org

Structure 15 February 1998, 6:121–125
<http://biomednet.com/elecref/0969212600600121>

© Current Biology Ltd ISSN 0969-2126

DNA polymerases are a group of enzymes that use single-stranded DNA as a template for the synthesis of the complementary DNA strand. This family of enzymes plays an essential role in nucleic acid metabolism, including the processes of DNA replication, repair and recombination. DNA polymerases are ubiquitous in nature, having been identified in all cellular organisms from bacteria to humans as well as in many eukaryotic viruses and bacteriophages [1]. Furthermore, the proteins from different organisms share amino acid sequence similarities as well as a similar three-dimensional appearance (for examples see [2]).

Replicative DNA polymerases, or replicases, form a subset of the DNA polymerase family. These polymerases are generally multisubunit complexes that replicate the chromosomes of cellular organisms and viruses. Another general feature of replicases is their high processivity of DNA synthesis; they are capable of polymerizing thousands of nucleotides without dissociating from the DNA template [1]. During replication of cellular chromosomes, DNA synthesis is continuous on the leading strand and discontinuous on the lagging strand. The discontinuous strand is synthesized as a series of short fragments (Okazaki fragments) that are joined later. Due to the intracellular scarcity of the replicase, the enzyme must be recycled and some specific mechanism must exist to accomplish this phenomenon. Thus, the polymerase has two apparently contradictory activities: it tightly associates with the DNA during elongation, but rapidly dissociates from DNA upon the completion of each Okazaki fragment.

Processive DNA synthesis by cellular replicases and the bacteriophage T4 replicase

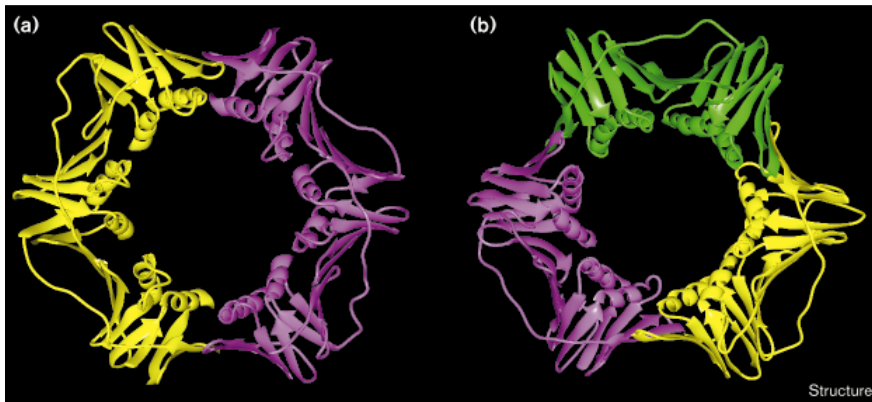
Until recently, the only mechanism for high processivity that was understood in detail was that utilized by cellular replicases and the replicase of bacteriophage T4. This mechanism involves a ring-shaped protein called a 'DNA sliding clamp' that encircles the DNA and tethers the polymerase catalytic unit to the DNA [3,4]. The three-dimensional structures of several sliding clamps have been determined: the eukaryotic proliferating cell nuclear antigen (PCNA) [5,6]; the β subunit of the prokaryotic DNA polymerase III [7]; and the bacteriophage T4 gene 45 protein (gp45) (J Kuriyan, personal communication) (Figure 1). The overall structure of these clamps is very similar; the PCNA, β subunit and gp45 rings are superimposable [8]. Each ring has similar dimensions and a central cavity large enough to accommodate duplex DNA (Figure 1).

The sliding clamp cannot assemble around DNA by itself, but must be loaded onto the DNA by a protein complex referred to as a 'clamp loader' (Figure 2a) [9]. In prokaryotes and eukaryotes the clamp loaders are five-subunit complexes called the γ complex and replication factor C (RFC), respectively. The clamp loader recognizes the 3' end of the single-strand–duplex (primer–template) junction and utilizes ATP hydrolysis to assemble the clamp around the DNA primed site (Figure 2a; step I). The sliding clamp, encircling the DNA, then interacts with the polymerase for rapid and processive DNA synthesis (Figure 2a; steps II–IV). Upon completion of an Okazaki fragment, the polymerase is rapidly ejected from the clamp (Figure 2a; step V), thus freeing the polymerase to associate with another clamp for the synthesis of the next Okazaki fragment. The sliding clamp is left behind assembled around the duplex DNA. During lagging strand synthesis, a new sliding clamp is needed for the synthesis of each Okazaki fragment. Ten times more Okazaki fragments are formed during replication than the number of sliding clamps present within the cell, therefore, clamps must also be recycled. In prokaryotes and eukaryotes the clamp loader has a dual function: besides its role as a clamp loader it also functions as a clamp unloader to remove used clamps from the DNA [10] (Figure 2a; step VII).

Processive DNA synthesis by the bacteriophage T7 replicase

The replicases of several viruses and bacteriophages utilize a different mechanism to achieve high processivity. The T7 DNA polymerase (also called gene 5 protein [gp5]) is an 80 kDa protein which has low processivity and

Figure 1

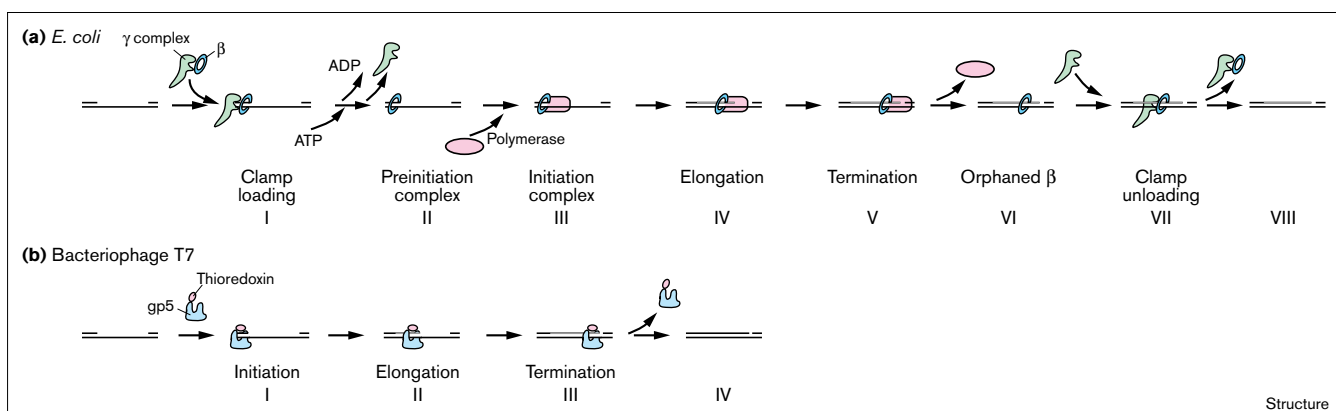


Computer generated images of two DNA sliding clamps. The figure shows ribbon representations of the polypeptide backbone of (a) a dimer of the *E. coli* DNA polymerase III holoenzyme β subunit and (b) a trimer of human PCNA. The strands of β sheets are shown as flat ribbons and α helices are shown as spirals. The subunits within each ring are distinguished by different colors.

dissociates from the DNA after incorporation of only a few nucleotides. To become processive, T7 DNA polymerase recruits a host-encoded protein, thioredoxin (12 kDa) [11,12] (Figure 2b). The T7 DNA replicase is a 1:1 heterodimer of these two subunits. Thioredoxin is not a ring-shaped protein but nevertheless its association with T7 polymerase results in an ~ 80 -fold increase in the affinity of the polymerase for the primer terminus. The resulting polymerase–thioredoxin complex acts processively over several thousands of nucleotides [12]. The T7 replicase, therefore, utilizes neither a symmetric ring-shaped sliding clamp (although the T7 polymerase–thioredoxin complex may function like a sliding clamp) nor a clamp loader, yet is highly processive.

The structure of the large fragment of *Escherichia coli* DNA polymerase I (Klenow fragment), determined several years ago [13,14], contains grooves to accommodate the DNA and its structure resembles that of a right hand with ‘thumb’, ‘palm’ and ‘fingers’ subdomains. T7 DNA polymerase is homologous to the Klenow fragment [15] and has been hypothesized to have a similar structure. Amino acid sequence alignment of T7 DNA polymerase with the Klenow fragment suggests that the T7 enzyme contains an extra 71 amino acids in the thumb region located between α helices H and H1 of the Klenow fragment structure. It was shown biochemically that this extension interacts with thioredoxin and it was suggested that thioredoxin forms a cap over the DNA groove thus

Figure 2



Two mechanisms to assemble a processive polymerase. (a) The diagram illustrates the stages of DNA replication on the lagging strand by the *E. coli* DNA polymerase III holoenzyme. The γ complex recognizes a primer template (step I) and couples ATP hydrolysis to the assembly of the β clamp around the DNA (step II). The polymerase assembles with the β clamp (step III) to form a processive polymerase (step IV). Upon completion of an Okazaki fragment (step V) the polymerase dissociates from the clamp (step VI) leaving the β clamp

around the DNA. The γ complex assembles with the clamp (step VII) and removes it from the DNA (step VIII). (b) A putative mode of action for bacteriophage T7 DNA polymerase. T7 polymerase (gp5) in complex with thioredoxin recognizes and assembles around the primer template (step I) to form a processive polymerase (step II). Upon completion of an Okazaki fragment (step III) the polymerase dissociates from the DNA (step IV).

locking the DNA inside the polymerase for processive DNA synthesis [16] (Figure 2b). Indeed mutations in the gene encoding T7 DNA polymerase (gene 5), that suppress mutations in thioredoxin, lie on the other side of the groove and could be indicative of thioredoxin binding across the groove [17].

These hypotheses, based on biochemical results, were recently examined with the determination of the three-dimensional structure of T7 DNA polymerase in complex with thioredoxin, a primer template and a deoxynucleoside triphosphate [18]. The overall architecture of the complex is the same as that of the Klenow fragment, but the structure also shows that the palm ligates two metal ions in the polymerase active site and the incoming nucleotide is sandwiched between the fingers and the 3' end of the primer. The thumb presses on the DNA product as it exits the polymerase, creating an S-shaped bend in the DNA and helping to secure it in the active site. The structure also shows that thioredoxin, as anticipated, is associated with the extended loop of the thumb between α helices H and H1, located near the duplex DNA within the polymerase (Figure 3). Although the three-dimensional structure of the T7 polymerase (Figure 3) does not show it encircling the DNA, the structure suggests that the enzyme may be capable of opening and closing around the DNA as the biochemical results indicated. The thioredoxin is rotated away from the primer template in the crystal and thus may be in the open conformation, a prerequisite to assembling onto DNA.

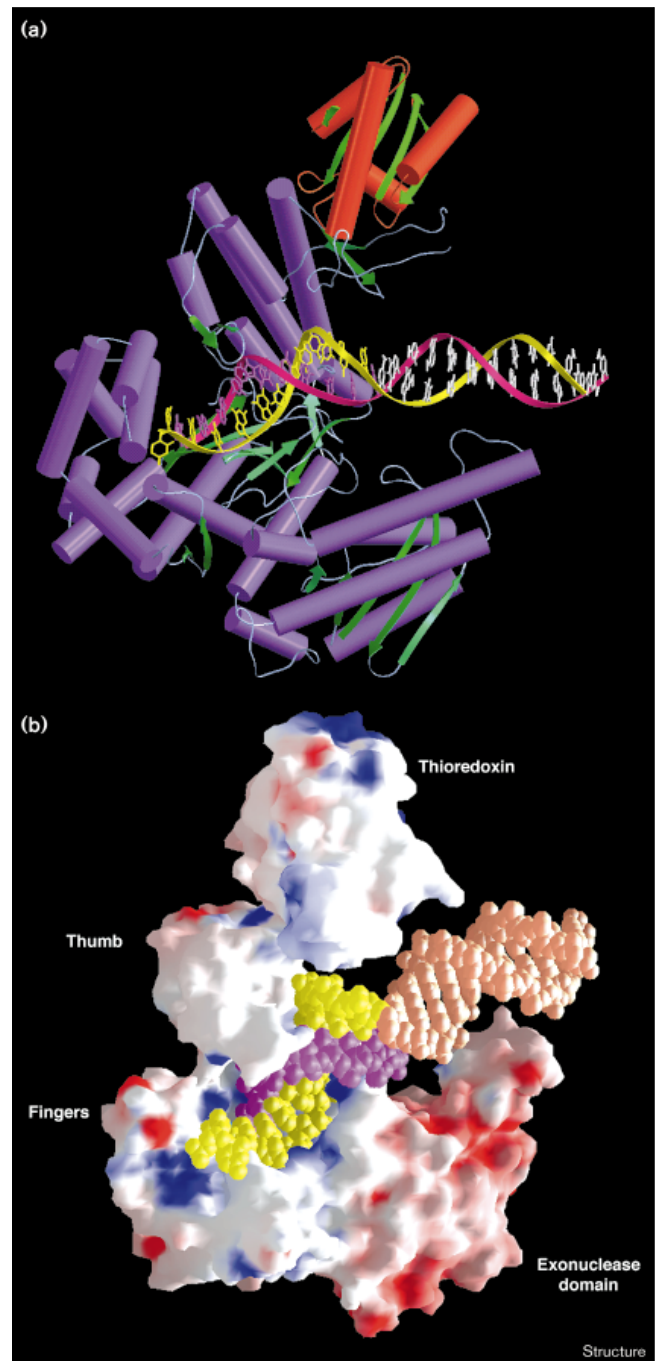
The thioredoxin and the loop of T7 polymerase to which it binds appear to be flexibly attached to the thumb and could swing across the DNA-binding groove to encircle the DNA. Further studies will be required to test these ideas.

Advantages and disadvantages of the mechanism adopted by T7 replicase to achieve processivity

The mechanism adopted by bacteriophage T7 to replicate its DNA is more economic than that used by cellular organisms. In T7 only three proteins are needed for processive DNA elongation: the polymerase (gp5), *E. coli* thioredoxin, and a single-stranded DNA-binding protein (SSB; gene 2.5 protein). In T7 there is no need for a sliding clamp protein or a clamp loader. However, there may be several advantages for an organism to utilize a sliding clamp which is not part of the catalytic unit. While replication is in progress on the lagging strand the clamp loader may assemble a new clamp on the primer of the next Okazaki fragment. When replication of an Okazaki fragment is completed the polymerase can rapidly recycle upon dissociating from the DNA by reassociating with the new clamp that was preassembled on the next primer.

A precise regulation of DNA replication with other cellular events is essential. In eukaryotes, several cell-cycle

Figure 3



Structure of the bacteriophage T7 DNA replicase. (a) Schematic representation in which α helices are shown as cylinders and β strands as arrows; the polymerase is shown in purple and thioredoxin is in orange. (b) Molecular surface representation of T7 polymerase in complex with thioredoxin and a primer-template DNA. Proteins are colored according to electrostatic potential with regions of intense positive charge appearing blue and electronegative regions in red. The primer strand is shown in purple and the template strand is shown in yellow. In orange are 11 base pairs that are present in the crystal but are disordered. The fingers, thumb and exonuclease domains are labeled. (Figure adapted from [18] with permission.)

regulators (e.g. p21/Cip1 and Gadd45) have been shown to interact with the PCNA clamp and they affect its function during the cell cycle and DNA repair [8]. PCNA also interacts with the enzymes needed for Okazaki fragment maturation (e.g. Fen-1 and DNA ligase I) [19] and post-replication events (e.g. DNA methyltransferase) [20]. In bacteriophage T4, the gp45 sliding clamp was shown to be important for late gene transcription. In association with two other virus-encoded proteins, the gene 33 product (gp33) and gene 55 product (gp55), gp45 binds to the *E. coli* RNA polymerase and directs it to the promoters of the bacteriophage genes needed to be expressed in the late stages of infection [21].

The T7 replicase is a stable heterodimer. Therefore, if the replicase had a closed structure in solution, it would be expected to have a slow rate of association with the DNA template (slow k_{on}), which would have the net effect of slowing DNA replication. The three-dimensional structure of T7 replicase (Figure 3) suggests that the enzyme may be stable in an open conformation. The replicase complex may, therefore, assume an open structure in solution which allows it to rapidly associate with the template DNA. On the lagging strand the polymerase has to recycle between Okazaki fragments. Thioredoxin increases the affinity of the polymerase for DNA and thus may be predicted to slow its dissociation from DNA during lagging strand synthesis. It has been demonstrated, however, that whereas thioredoxin increases the intrinsic affinity of T7 polymerase for the primer–template junction, it does not increase the intrinsic affinity of the polymerase for double-stranded DNA [22]. This may resolve the recycling dilemma by allowing the polymerase to dissociate from the DNA upon completing synthesis of an Okazaki fragment (C Richardson, personal communication). The nick in the DNA may also cause a structural change within the polymerase, resulting in the opening of the structure (e.g. lifting of the thioredoxin cap as depicted in Figure 2b; step III) and allowing it to transfer to a new primer. Alternatively, if the bacteriophage in infected *E. coli* cells synthesize sufficient numbers of polymerase molecules to satisfy the number of Okazaki fragments produced, then the polymerase need not recycle and could dissociate even very slowly from the DNA without a detrimental effect on replication. In addition, it has been proposed that the interaction between the T7 primase/helicase protein and the polymerase at the replication fork may facilitate the dissociation and recycling of the polymerase [23].

Do other polymerases achieve processivity by a mechanism similar to that of bacteriophage T7?

Can the mechanism used by bacteriophage T7 to achieve processive DNA synthesis be generalized to other polymerases? T7 DNA polymerase belongs to the *E. coli* DNA polymerase I family [15]. Two other members of this

family contain a putative domain in a location similar to the one in T7 polymerase between helices H and H1. The polymerase of the *E. coli* bacteriophage T3 contains a thioredoxin-binding domain and thus may use thioredoxin as a processivity factor in a similar manner to T7. Similarly, the DNA polymerase of the *Bacillus subtilis* bacteriophage Spo1 also contains an insertion of 45 amino acids between α helices H and H1 [15]. This region is shorter than the one found in T3 and T7 and does not have significant similarities to the thioredoxin-binding domain. The region may interact, however, with a yet unidentified protein with the same function as thioredoxin in the T7 replicase. Bacteriophage T5 is also a member of the DNA polymerase I family. In contrast to the polymerases mentioned above, the T5 enzyme is processive by itself. Interestingly, the T5 polymerase has an extension of 75 amino acids at its C terminus [15]. In the three-dimensional structures of other members of the DNA polymerase I family, the polymerase C terminus is located near the thumb domain. It is possible that the C-terminal amino acid extension in T5 polymerase forms a domain that functions to lock the polymerase around the DNA in a manner similar to that of thioredoxin.

Several eukaryotic viral DNA polymerases have also been shown to associate with a smaller protein to become processive enzymes. The 116 kDa vaccinia virus DNA polymerase was shown to be a non-processive enzyme, but it becomes processive when associated with a 48 kDa virally encoded protein [24]. The 136 kDa polymerase of the herpes simplex virus associates with the 51 kDa double-stranded DNA-binding protein UL42 for high processivity [25]. Similarly, the DNA-binding protein (DBP) of adenovirus (59 kDa protein) stimulates the processivity of the adenovirus DNA polymerase (140 kDa) [26]. The 125 kDa mitochondrial DNA polymerase γ is a processive enzyme and has been shown to be associated with a 35 kDa protein [27]. This small protein may serve as a processivity factor for the polymerase. In all these examples, the polymerase and its associated protein may achieve processivity by a mechanism similar to that of the T7 polymerase–thioredoxin complex.

DNA polymerases and sliding clamps are not the only proteins that encircle DNA. Like DNA polymerases, RNA polymerases are highly processive enzymes. Based on the structural analysis of RNA polymerases from *E. coli* and yeast, it was proposed that these enzymes encircle the DNA template to anchor the polymerase to the DNA [28]. Furthermore, several other enzymes involved in DNA metabolic processes (e.g. DNA helicases) have been shown to form ring-shaped structures that encircle DNA [29].

Concluding remarks

The two mechanisms known to date by which replicative DNA polymerases achieve their high processivity involve

encircling the DNA. The tools used in these mechanisms are different, however. Cellular replicases and bacteriophage T4 utilize a ring-shaped processivity factor that encircles the DNA but is not a part of the catalytic unit. Bacteriophage T7 polymerase, on the other hand, forms a structure that might surround the DNA like a sliding clamp. Although the general theme of surrounding the DNA may be common to all replicative DNA polymerases, it may be achieved by different means in different organisms. It will be of great interest to determine whether different structures are employed by other eukaryotic viruses and bacteriophages to anchor their polymerase to the DNA. Future structural studies of replicases and other proteins involved in chromosome replication are sure to bring to light new and exiting mechanisms for handling the DNA helix.

Acknowledgements

We are grateful to David Shechter for his contribution to Figure 1 and Sylvie Doublé and Tom Ellenberger for assistance in providing Figure 3. We wish to thank Tom Ellenberger, Daochun Kong, and Charles Richardson for their comments on the manuscript. We would like to apologize to colleagues whose primary work has not been cited due to space limitations. ZK is a postdoctoral fellow of the Helen Hay Whitney Foundation. JH is a Professor of the American Cancer Society. This work was supported by grants GM38839 (MO'D) and GM34559 (JH).

References

- Kornberg, A. & Baker, T.A. (1991). *DNA Replication*. WH Freeman, New York, NY.
- Wang, J., Sattar, A.K.M.A., Wang, C.C., Karam, J.D., Konigsberg, W.H. & Steitz, T.A. (1997). Crystal structure of a pol α family replication DNA polymerase from bacteriophage RB69. *Cell* **89**, 1087-1099.
- Stukenberg, P.T., Studwell-Vaughan, P.S. & O'Donnell, M. (1991). Mechanism of the β -clamp of DNA polymerase III holoenzyme. *J. Biol. Chem.* **266**, 11328-11334.
- Kuriyan, J. & O'Donnell, M. (1993). Sliding clamps of DNA polymerases. *J. Mol. Biol.* **234**, 915-925.
- Krishna, T.S.R., Kong, X.-P., Gary, S., Burgers, P.M. & Kuriyan, J. (1994). Crystal structure of the eukaryotic DNA polymerase processivity factor PCNA. *Cell* **79**, 1233-1243.
- Gulbis, J.M., Kelman, Z., Hurwitz, J., O'Donnell, M. & Kuriyan, J. (1996). Structure of the C-terminal region of p21^{WAF1/CIP1} complexed with human PCNA. *Cell* **87**, 297-306.
- Kong, X.-P., Onrust, R., O'Donnell, M. & Kuriyan, J. (1992). Three dimensional structure of the β subunit of *Escherichia coli* DNA polymerase III holoenzyme: a sliding DNA clamp. *Cell* **69**, 425-437.
- Kelman, Z. & O'Donnell, M. (1995). Structural and functional similarities of prokaryotic and eukaryotic sliding clamps. *Nucleic Acids Res.* **23**, 3613-3620.
- Kelman, Z. & O'Donnell, M. (1994). DNA replication – enzymology and mechanisms. *Curr. Opin. Genet. Dev.* **4**, 185-195.
- Yao, N., *et al.*, & O'Donnell, M. (1996). Clamp loading, unloading and intrinsic stability of the PCNA, β and gp45 sliding clamps of human, *E. coli* and T4 replicases. *Genes to Cell* **1**, 101-113.
- Richardson, C.C. (1983). Bacteriophage T7: minimal requirements for the replication of a duplex DNA molecule. *Cell* **33**, 315-317.
- Nakai, H., Beauchamp, B.B., Bernstein, J., Huber, H.E., Tabor, S. & Richardson, C.C. (1988). Formation and propagation of the bacteriophage T7 replication fork. In *DNA Replication and Mutagenesis*. (Moses, R.E. & Summers, W.C., eds), pp. 85-97, American Society for Microbiology, Washington, DC.
- Ollis, D.L., Hamlin, B.R., Xuong, N.G. & Steitz, T.A. (1985). Structure of large fragment of *Escherichia coli* DNA polymerase I complexed with dTMP. *Nature* **313**, 762-766.
- Beese, L.S., Derbyshire, V. & Steitz, T.A. (1993). Structure of DNA polymerase I Klenow fragment bound to duplex DNA. *Science* **260**, 352-355.
- Braithwaite, D.K. & Ito, J. (1993). Compilation, alignment, and phylogenetic relationships of DNA polymerases. *Nucleic Acids Res.* **21**, 787-802.
- Bedford, E., Tabor, S. & Richardson, C.C. (1997). The thioredoxin binding domain of bacteriophage T7 DNA polymerase confers processivity on *Escherichia coli* DNA polymerase I. *Proc. Natl. Acad. Sci. USA* **94**, 479-484.
- Himawan, J.S. & Richardson, R.R. (1996). Amino acid residues critical for the interaction between bacteriophage T7 DNA polymerase and *Escherichia coli* thioredoxin. *J. Biol. Chem.* **271**, 19999-20008.
- Doublé, S., Tabor, S., Long, A.M., Richardson, C.C. & Ellenberger, T. (1998). Crystal structure of bacteriophage T7 DNA polymerase complexed to a primer-template, a nucleoside triphosphate, and its processivity factor thioredoxin. *Nature* **391**, 251-258.
- Levin, D.S., Bai, W., Yao, N., O'Donnell, M. & Tomkinson, A.E. (1997). An interaction between DNA ligase I and proliferating cell nuclear antigen: implication for Okazaki fragment synthesis and joining. *Proc. Natl. Acad. Sci. USA* **94**, 12863-12868.
- Chuang, L.S.-H., Ian, H.-I., Koh, T.-W., Ng, H.-H., Xu, G. & Li, B.F.L. (1997). Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21^{waf1}. *Science* **277**, 1996-2000.
- Geiduschek, E.P. (1995). Connecting a viral DNA replication apparatus with gene expression. *Semin. Virol.* **6**, 25-33.
- Huber, H.E., Tabor, S. & Richardson, C.C. (1987). *Escherichia coli* thioredoxin stabilizes complexes of bacteriophage T7 DNA polymerase and primed templates. *J. Biol. Chem.* **262**, 16223-16232.
- Debyser, Z., Tabor, S. & Richardson, C.C. (1994). Coordination of leading and lagging strand DNA synthesis at the replication fork of bacteriophage T7. *Cell* **77**, 157-166.
- McDonald, W.F., Klemperer, N. & Traktman, P. (1997). Characterization of a processive form of the vaccinia virus DNA polymerase. *Virology* **234**, 168-175.
- Boehmer, P.E. & Lehman, I.R. (1997). Herpes simplex virus DNA replication. *Annu. Rev. Biochem.* **66**, 347-384.
- Hay, R.T. (1996). Adenovirus DNA replication. In *DNA Replication in Eukaryotic Cells*. (DePamphilis, M.L., ed), pp. 699-719, CSH Laboratory Press, Cold Spring Harbor.
- Olson, M.W., Wang, Y., Elder, R.H. & Kaguni, L.S. (1995). Subunit structure of mitochondrial DNA polymerase from *Drosophila* embryos. Physical and immunological studies. *J. Biol. Chem.* **270**, 28932-28937.
- Polyakov, A., Severinova, E. & Darst, S.A. (1995). Three-dimensional structure of *E. coli* core RNA polymerase: promoter binding and elongation conformations of the enzyme. *Cell* **83**, 365-373.
- Hingorani, M.M. & O'Donnell, M. (1998). Toroidal proteins: running rings around DNA. *Curr. Biol.* **8**, R83-R86.