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Protein machines mediate the basic genetic processes

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Multienzyme complexes powered by nucleoside triphosphate hydrolysis play central roles in many biological processes. The DNA replication apparatus is a particularly well-understood example.

As an experienced worker in the DNA synthesis field, it is quite sobering to look back at how we molecular geneticists have stumbled our way to the current understanding of DNA replication. Thus, for example, the first discovery of discontinuous DNA synthesis was a real surprise[1], as was the later finding of RNA primers[2]. Only in retrospect did we come to realize that these features reflect the fact that the asymetric replication fork is elegantly designed to ensure that all the DNA on both strands is synthesized with very high fidelity[2,3]. Likewise, only in recent years have we become aware of the fact that the replication fork is moved by a large multienzyme complex that can quite reasonably be viewed as a true 'replication machine': a sophisticated DNA-handling apparatus, whose numerous protein parts move relative to each other, without disassembling as the replication fork moves.

In retrospect, it seems obvious that complex living systems could not exist without the 'high technology' of such multicomponent protein machines. In these machines, ordered conformational changes in protein molecules are caused by the hydrolysis of bound nucleotide triphosphate molecules, enabling groups of interacting proteins to move coordinately. As a result, the biological process involved need not rely on numerous random collisions. It now seems likely that all the major processes occuring in cells make use of such highly sophisticated protein machines. As I discuss in detail elsewhere[4], and briefly outline here, acceptance of this view has important implications for the way that one approaches any research problem in molecular genetics. The DNA replication apparatus of T4 bacteriophage will serve as the model for the discussion that follows.

The DNA replication apparatus of T4

By purifying the proteins corresponding to seven T4 replication genes (genes *32, 41, 43, 44, 45, 61* and *62*) and then mixing various combinations of these proteins with different types of DNA templates, it has been possible to reconstruct the general mechanism of T4 replication fork movement (reviewed in Ref. 5). A two-dimensional view of the T4 replication fork is shown in Fig. 1. Only its most salient features will be outlined here. These are as follows:

(1) There are two DNA polymerase molecules working at any time, one on the leading strand and one on the lagging strand. In a process that requires periodic ATP hydrolysis, a complex of three polymerase-accessory proteins (the products of T4 genes *44, 62* and *45*) acts to clamp down each DNA polymerase molecule, thereby converting it into an enzyme that can remain associated with the same DNA template for a long time. These three proteins are thought to form a complex with the DNA polymerase that creates a unit analogous to the more complicated *E. coli* DNA polymerase III holoenzyme, with its seven different subunits[7].

(2) In front of the T4 DNA polymerase unit on the leading strand, the DNA double-helix is rapidly unwound into its two single strands by the combined actions of the DNA polymerase (encoded by T4 gene *43*), a helix-destabilizing protein that binds tightly and cooperatively to all single-stranded DNA (encoded by gene *32*) and a DNA helicase on the lagging strand that uses GTP hydrolysis energy to force its way into the template helix (encoded by gene *41*). Unwinding normally proceeds at a rate of about 500 nucleotides per second, which is over a million times faster than the estimated rate of spontaneous helix opening under physiological conditions[8–10].

Once positioned on the lagging strand, each functional unit of the 41 protein (an oligomer) remains with the same fork for a very long time; inside the cell, this unit of 41 protein probably enters each fork only when the fork forms at a replication origin, persisting until the fork finishes its DNA synthesis.

(3) The lagging strand template is a DNA single strand covered with the 32 protein. This strand provides sites at which a new, RNA-primed Okazaki DNA fragment is initiated about once every four seconds. Each RNA primer is a pentaribonucleotide (of sequence pppApCpNpNpN) that is synthesized by a complex of the 61 and 41 proteins on the lagging strand and then elongated by the DNA polymerase unit on this strand[11–13]. Thus, in addition to acting as a DNA helicase, the 41 protein plays a second key role at the fork: it forms a mobile site at which the 61 protein binds to help synthesize RNA primers. Since the 41 protein is only bound to the DNA at a replication fork, this mechanism presumably prevents the synthesis of RNA primers elsewhere in the cell, thus allowing other regions of single-stranded DNA to remain single-stranded.

(4) Like the DNA polymerase molecule on the leading strand, the molecule of DNA polymerase on the lagging strand remains with its replication fork a long time, recycling every four seconds or so to start a new Okazaki fragment on the lagging strand. Therefore, the lagging strand must be folded to position the 3′OH end of a completed Okazaki fragment adjacent to the start site for the next Okazaki fragment, as in the example shown in Figs 2 and 3. Since nearly every RNA primer that is synthesized at a fork *in vitro* starts a new DNA chain[11], the synthesis of a new RNA primer is delayed until the preceding Okazaki fragment has been completed. The model for replication fork movement in Fig. 3 therefore not only explains why the pattern of *in vitro* DNA synthesis on the lagging strand is unchanged by extreme polymerase dilutions[9], but also why very few Okazaki fragments of less than 500 nucleotides are made in this system, despite the fact that potential start sites for primers are present about once every 50 nucleotides.

The lagging strand DNA polymerase molecule moves ahead rapidly with the fork (Fig. 3), and, in *in vitro* reactions, the T4 replication apparatus lays down a series of unsealed Okazaki fragments on the lagging

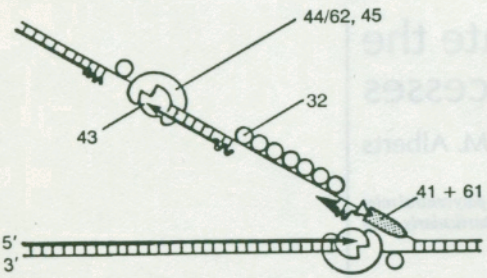

Fig. 1. *A two-dimensional view of the DNA replication fork of bacteriophage T4. The T4 DNA polymerase is the product of gene* 43, *and the proteins encoded by genes* 44/62 *and* 45 *that function with it are designated 'polymerase accessory proteins'. The 32 protein binds to all of the single-stranded DNA at the fork; it is a helix-destabilizing protein, sometimes described as a 'single-strand binding protein'. The 41 protein is both a DNA helicase and a protein that is required, together with the 61 protein, for RNA primer synthesis on the lagging strand. The average length of the Okazaki fragments formed on the lagging strand is 1500 nucleotides, both* in vivo *and* in vitro.

strand, which still retain intact RNA primers at their 5′ ends. It therefore seems likely that, inside the cell, these RNA primers are removed and replaced by DNA in a separate DNA-repair reaction, which is uncoupled from the replication fork itself and involves additional proteins.

In summary, the basic T4 replication apparatus consists of a moving complex of seven polypeptide chains, in which the entire DNA replication fork is embedded. The proteins have a total mass of at least 900 000 daltons, not including about 5×10^6 daltons of 32 protein that are bound to the regions of single-stranded DNA at a fork. As a group, the proteins at the fork proceed unidirectionally along the DNA at a rate of about 500 nucleotides per second, faithfully replicating both strands of the template DNA helix as they go. In spirit, the T4 DNA replication apparatus is like a tiny sewing machine, composed of protein parts and powered by several different types of nucleoside triphosphate hydrolyses.

The same protein in different complexes

Inside the cell infected with T4 bacteriophage, both DNA replication and DNA repair use the same DNA polymerase molecule, the product of gene *43* of T4 (reviewed in Ref. 14). Yet DNA replication and repair are very different processes. Much of the DNA synthesis in DNA repair is thought to involve simple gap-filling by the DNA polymerase, where it is important to avoid the type of DNA strand-displacement reaction that can lead to lagging strand DNA synthesis and thereby generate a complete fork. This fact becomes most obvious when considering the most frequent DNA repair event in the cell, the resealing of adjacent Okazaki fragments on the lagging strand. If even a small fraction of these repair reactions continued too far and created a replication fork, many new forks would form on each lagging strand, making the DNA replication process hopelessly complex.

Figure 4 illustrates how different types of complexes containing T4 DNA polymerase are likely to form at different sites on the same DNA molecule. In this view, only those special DNA sequences that direct the assembly of the 41 protein onto a DNA strand are replication origins (Fig. 4a). In principle, the different replication origins on the T4 chromosome[15,16] could assemble different types of replication forks, depending on which additional proteins are co-assembled with the DNA polymerase and the 41 protein onto the DNA. Other special sites on the DNA might allow the DNA polymerase to become associated not with the 41 protein, but with a different DNA

Table 1. Major protein species bound to 32 protein-agarose columns

Protein	Function
T4 32 protein	Helix-destabilizing protein
T4 43 protein	T4 DNA polymerase
T4 45 protein	DNA polymerase accessory protein
T4 *uvs*X protein	T4 recombination: recA analogue[a]
T4 *uvs*Y protein	T4 recombination
T4 dda protein	DNA-dependent ATPase:helicase
T4 46/47 protein	T4 recombination:exonuclease
T4 RNase H	Removes RNA from RNA:DNA hybrids
BP-1	Unknown, 30 000 daltons, T4-encoded
BP-2	Unknown, 32 000 daltons, host-encoded

[a] T. Minagawa, personal communication and T. Formosa, unpublished results.

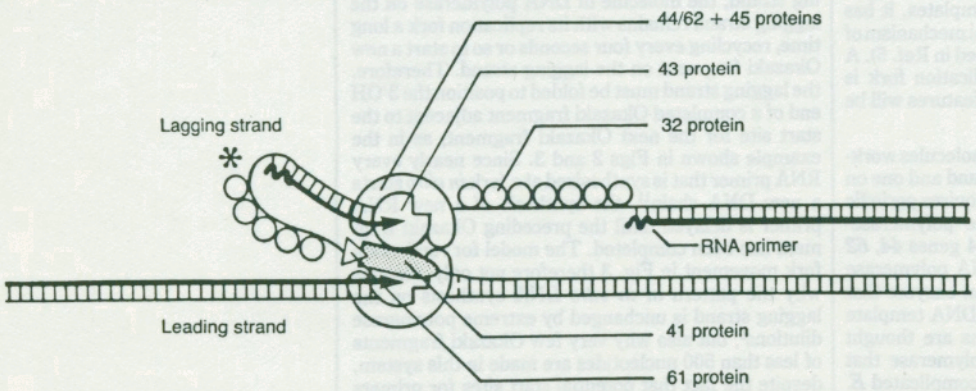

Fig. 2. *The seven proteins of the T4 DNA replication machine as they are thought to exist in an actual replication fork. The two-dimensional replication fork has been converted into the structure shown by folding the DNA on the lagging strand in such a way as to bring the DNA polymerase molecule on the lagging strand close to the DNA polymerase molecule on the leading strand. The lagging strand DNA polymerase molecule is thereby held to the rest of the replication proteins, allowing it to be retained for many successive cycles of Okazaki fragment synthesis, as shown in Fig. 3.*

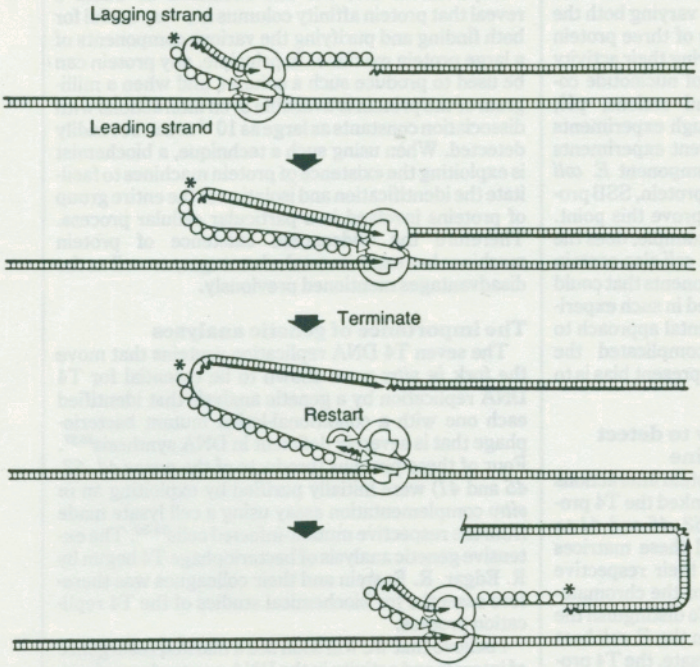

Fig. 3. *A model for DNA synthesis at a replication fork that allows the same DNA polymerase molecule to be repeatedly recycled to synthesize all the Okazaki fragments on the lagging strand. The crucial step here occurs when the synthesis of each Okazaki fragment is completed. Rather than falling off the entire fork assembly, the DNA polymerase molecule on the lagging strand remains bound to the assembly of replication proteins, letting go only of the DNA on the lagging strand. For the different symbols used to represent the T4 replication proteins, see Figs. 1 and 2.*

helicase that is unable to participate in RNA primer synthesis (for example, the T4 dda protein[17,18]). DNA synthesis at these sites would generate 'whiskers' of single-stranded DNA (Fig. 4b) that could either participate in the initiation of genetic recombination processes or be degraded in association with a 'long-patch' type of DNA repair. Finally, other types of sites on the DNA, including each junction between a pair of unsealed Okazaki fragments, will bind a DNA polymerase complex that is prevented from using any type of DNA helicase (Fig. 4c). This DNA polymerase complex is postulated to be unable to carry out any strand-displacement DNA synthesis inside the cells and will fall off the DNA after completing 'short-patch' DNA repair.

The above example provides one illustration of how a single protein (the T4 DNA polymerase) can participate in several different types of discrete protein machines. Perhaps the best studied case is that of the T4 gene *32* protein, which plays major roles in DNA replication, DNA repair and genetic recombination[19]. This helix-destabilizing protein interacts with a large number of different T4 proteins by both genetic[20] and biochemical[21] methods. These interactions are thought to reflect different associations of the 32 protein in a variety of different multiprotein complexes.

Isolating the components of a replication complex

The T4 replication machine is constructed from proteins that are only loosely associated with each other. For example, most of the known protein–protein interactions are too weak to be detected by simple co-sedimentation experiments on sucrose gradients. Consequently, six of the proteins shown in Fig. 1 must be individually isolated from the crude lysate (the exception being the 44/62 protein, which is isolated as a tight complex of two different protein chains). Not all protein machines fall apart in cell lysates in this way (the ribosome being a notable example of an especially stable complex). However, there is no reason to suspect that the T4 replication apparatus is unusual: intracellular protein concentrations are sufficiently high that even quite weak associations between proteins can be effective[22]. Thus, one will generally need to isolate intracellular protein complexes piecewise, eventually reconstructing each of them by mixing together a number of different highly purified protein subunits. Some of these purified proteins will have no detectable activity by themselves; for example, this is true for the T4 gene *45* protein. Other proteins will display only a partial activity that reveals almost nothing about their biological function. For example, the T4 44/62 protein is a very weak DNA-dependent ATPase[23]; only when it is stimulated by the 45 protein does this ATPase activity 'come alive', facilitating DNA synthesis by the T4 DNA polymerase (43 protein).

A more subtle example relates to our initial discovery that the 41 protein plays an important role in leading-strand DNA synthesis, in addition to being required for the synthesis of RNA primers on the lagging strand[24]. This realization was made possible by the development of a method for measuring the rate of movement of an individual DNA polymerase molecule on a double-helical template, which demonstrated that fork rates *in vitro* are much slower than the fork rate *in vivo* unless the 41 protein is added. If one can extrapolate to other situations, our experience suggests that it is important to reproduce intracellular reaction rates in *in vitro* systems, as well as the correct reaction pathway.

It is often difficult to know when a protein machine has been reconstructed well enough to warrant an extensive study of its *in vitro* properties. This is a major problem for biochemists who are attempting to work out the detailed mechanism of any genetic process from studies *in vitro*. The situation is complicated by the fact that the number of different experiments that can be done in any *in vitro* system containing three

or more components is enormously greater than one's intuitive expectations. For example, varying both the relative and absolute concentrations of three protein components in a mixture and measuring their activity as a function of the concentrations of nucleotide co-factors, Mg^{2+}, specific anions and cations, pH, temperature etc., could provide enough experiments for a lifetime. The number of different experiments already published with the three-component *E. coli* genetic recombination system: recA protein, SSB protein and DNA, should conclusively prove this point. But, to continue with this arbitrary example, does the genetic recombination machine of *E. coli* also contain other, as yet unisolated protein components that could drastically change the results obtained in such experiments? In part, the correct experimental approach to this problem depends on how complicated the recombination apparatus is; my own present bias is to suspect a greater complexity.

Using affinity chromatography to detect components of a protein machine

To identify some of the protein–protein interactions in the T4 replication apparatus, we linked the T4 proteins encoded by genes *32, 43, 44/62, 45* and *41* to separate agarose matrices and used these matrices (each containing about 1 mg/ml of their respective bound protein) as affinity columns for the chromatography of T4-infected cell extracts. To distinguish the bacteriophage-encoded proteins from the *E. coli* host proteins that are also present in the lysate, the T4 proteins were selectively-labelled by adding radioactive amino acids to the infected cells. We found that each T4 replication–protein column selected a different subset of proteins from the extract (these proteins were not bound by a control albumin column)[9]. The most striking results were obtained with the column that contained the immobilized 32 protein. As shown in Table 1, more than ten different T4-induced proteins are bound specifically by the 32 protein; most of them could be identified as specific T4 gene products important in DNA replication or genetic recombination[21]. In addition, one major host protein also bound to the column. This protein is unusual in that it also binds to the T4 uvsX protein (the T4 analogue of the *E. coli* recA protein) and to DNA; its function in *E. coli* is unknown[25].

Experiments like the one described in Table 1 reveal that protein affinity columns are very useful for both finding and purifying the various components of a large protein machine. In principle, any protein can be used to produce such a column, and when a milligram of the protein is available even interactions with dissociation constants as large as 10^{-5} M can be readily detected. When using such a technique, a biochemist is exploiting the existence of protein machines to facilitate the identification and isolation of the entire group of proteins involved in a particular cellular process. Therefore the widespread existence of protein machines has experimental advantages, as well as the disadvantages mentioned previously.

The importance of genetic analyses

The seven T4 DNA replication proteins that move the fork *in vitro* were shown to be essential for T4 DNA replication by a genetic analysis that identified each one with a conditional-lethal mutant bacteriophage that is severely deficient in DNA synthesis[26,27]. Four of these proteins (products of the genes *44, 62, 45* and *41*) were initially purified by exploiting an *in vitro* complementation assay using a cell lysate made from the respective mutant-infected cells[28–30]. The extensive genetic analysis of bacteriophage T4 begun by R. Edgar, R. Epstein and their colleagues was therefore essential for biochemical studies of the T4 replication process.

I believe that we will soon see a marked resurgence of interest and activity in the DNA enzymology of prokaryotic organisms, including *E. coli* and its bacteriophages. I hold this belief despite the fact that we have just been through an era in which both prokaryotes and enzymology have been generally ignored, with students seemingly eager to clone any eukaryotic gene in preference to spending a day in the cold room

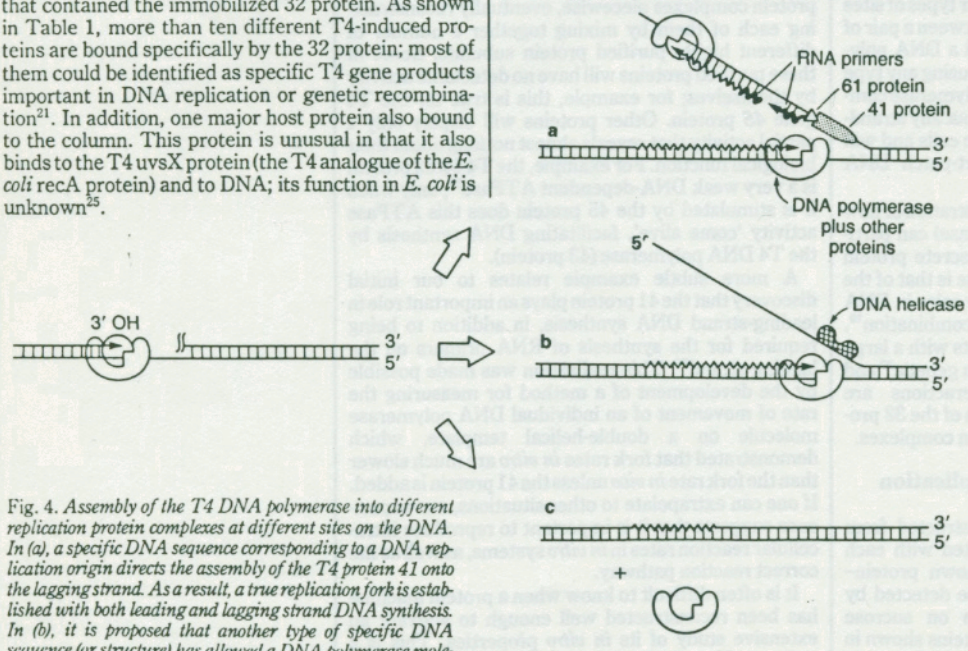

Fig. 4. *Assembly of the T4 DNA polymerase into different replication protein complexes at different sites on the DNA. In (a), a specific DNA sequence corresponding to a DNA replication origin directs the assembly of the T4 protein 41 onto the lagging strand. As a result, a true replication fork is established with both leading and lagging strand DNA synthesis. In (b), it is proposed that another type of specific DNA sequence (or structure) has allowed a DNA polymerase molecule to co-assemble into a protein complex that contains a DNA helicase different from the 41 protein. Because this DNA helicase is unable to participate in RNA primer synthesis, the DNA on the lagging strand remains single-stranded (see text). In (c), a typical DNA repair reaction is shown in which no DNA helicase is involved.*

with a DEAE-cellulose column. But, as some of the initial excitement wears off, it is becoming clear that to understand such fascinating processes as the control of eukaryotic gene expression will require the same kind of serious protein biochemistry that was required to work out DNA replication enzymology. There is no reason to think that any central genetic mechanism will be less complex than DNA replication, and every reason to believe that the same type of sophisticated multiprotein complex will be involved. If we think of molecular genetics in terms of 'protein machines', rather than sequential reactions carried out by individual proteins, it becomes abundantly clear why it is important to study the detailed enzymology of all these processes in organisms that are simpler than the higher eukaryotes – even if one is self-centred enough to care only about human biology. In a number of simpler organisms, including bacteria and yeast cells, the combined power of genetics and biochemistry should make it possible to find and study every piece of a multiprotein complex, as required to come to any real understanding of how these complexes work.

At present, most higher eukaryotic systems are not amenable to the type of genetic analysis that greatly facilitates a direct approach to the study of loosely associated multienzyme complexes. However, it seems likely that, once we have a solid understanding of the various protein machines that function on DNA in other organisms, we will be able to proceed by analogy to a successful study of the similar machines that are certain to exist in higher eukaryotic cells.

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References

1 Okazaki, R., Okazaki, T., Sakabe, K., Sugimoto, K., Kainuma, R., Sugino, A. and Iwatsuki, N. (1969) *In vivo* mechanism of DNA chain growth. *Cold Spring Harbor Symp. Quant. Biol.* 33, 129–143

2 Kornberg, A. (1980) *DNA Replication.* W. H. Freeman and Co., San Francisco

3 Alberts, B. and Sternglanz, R. (1977) Recent excitement in the DNA replication problem. *Nature* 269, 655–661

4 Alberts, B. M. (1984) The DNA enzymology of protein machines. *Cold Spring Harbor Symp. Quant. Biol.* 49, 1–12

5 Nossal, N. G. and Alberts, B. M. (1983) The mechanism of DNA replication catalyzed by purified bacteriophage T4 DNA replication proteins. In *Bacteriophage T4* (Mathews, C. K., Kutter, E. M., Mosig, G. and Berget, P. B., eds) pp. 71-81, American Society for Microbiology, Washington, DC

6 Huang, C.-C., Hearst, J. E. and Alberts, B. M. (1981) Two types of replication proteins increase the rate at which T4 DNA polymerase traverses the helical regions in a single-stranded DNA template. *J. Biol. Chem.* 256, 4087–4094

7 McHenry, C. and Kornberg, A. (1981) DNA polymerase III holoenzyme. In *The Enzymes* (Boyer, P. D., ed.), Third edn, Vol. 14, pp. 39–50, Academic Press

8 Alberts, B. M., Barry, J., Bedinger, P., Burke, R. L., Hibner, U., Liu, C.-C. and Sheridan, R. (1980) Studies of replication mechanisms with the T4 bacteriophage *in vitro* system. In *Mechanistic Studies of DNA Replication and Genetic Recombination* (Alberts, B. M., ed.), ICN-UCLA Symposium on Molecular and Cellular Biology, Vol. 19, pp. 449–473, Academic Press

9 Alberts, B. M., Barry, J., Bedinger, P., Formosa, T., Jongeneel, C. V. and Kreuzer, K. N. (1983) Studies on DNA replication in the T4 bacteriophage *in vitro* system. *Cold Spring Harbor Symp. Quant. Biol.* 47, 655–668

10 Venkatesan, M., Silver, L. L. and Nossal, N. G. (1982) Bacteriophage T4 gene 41 protein, required for the synthesis of RNA primers, is also a DNA helicase. *J. Biol. Chem.* 257, 12426–12434

11 Liu, C.-C. and Alberts, B. M. (1980) Pentaribonucleotides of mixed sequence are synthesized and efficiently prime *de novo* DNA chain starts in the T4 bacteriophage DNA replication system. *Proc. Natl Acad. Sci. USA* 77, 5698–5702

12 Liu, C.-C. and Alberts, B. M. (1981) Characterization of RNA primer synthesis in the T4 bacteriophage *in vitro* DNA replication system. *J. Biol. Chem.* 256, 2821–2829

13 Nossal, N. G. (1980) RNA priming of DNA replication by bacteriophage T4 proteins. *J. Biol. Chem.* 155, 2176–2182

14 Bernstein, C. and Wallace, S. S. (1983) DNA Repair. In *Bacteriophage T4* (Mathews, C. K., Kutter, E. M., Mosig G. and Berget, P. B., eds) pp. 138–151, American Society for Microbiology, Washington, DC

15 Kozinski, A. W. (1983) Origins of T4 DNA Replication. In *Bacteriophage T4* (Mathews, C. K., Kutter, E. M., Mosig G. and Berget, P. B., eds) pp. 111–119, American Society for Microbiology, Washington, DC

16 Mosig, G. (1983) Relationship of T4 DNA replication and recombination. In *Bacteriophage T4* (Mathews, C. K., Kutter, E. M., Mosig G. and Berget, P. B., eds) pp. 120–130, American Society for Microbiology, Washington, DC

17 Krell, H., Durwald, H. and Hoffmann-Berling, H. (1979) A DNA unwinding enzyme induced in bacteriophage T4-infected *E. coli* cells. *Eur. J. Biochem.* 93, 387–395

18 Jongeneel, C. V., Bedinger, P. and Alberts, B. M. (1984) Effects of the bacteriophage T4 dda protein on DNA synthesis catalyzed by purified T4 replication proteins. *J. Biol. Chem.*, 259, 12933–12938

19 Alberts, B. M. and Frey, L. (1970) T4 bacteriophage gene 32: a structural protein in the replication and recombination of DNA. *Nature* 227, 1313–1318

20 Mosig, G., Luder, A., Garcia, G., Dannenberg R. and Bock, S. (1979) *In vivo* interactions of genes and proteins in DNA replication and recombination of phage T4. *Cold Spring Harbor Symp. Quant. Biol.* 43, 501–515

21 Formosa, T., Burke, R. L. and Alberts, B. M. (1983) Affinity purification of T4 bacteriophage proteins essential for DNA replication and genetic recombination. *Proc. Natl Acad. Sci. USA* 80, 2442–2446

22 Fulton, A. B. (1982) How crowded is the cytoplasm? *Cell* 30, 345–347

23 Mace, D. C. and Alberts, B. M. (1984) The complex of T4 bacteriophage gene 44 and 62 replication proteins form an ATPase that is stimulated by DNA and by T4 gene 45 protein. *J. Mol. Biol.* 177, 279–293

24 Liu, C.-C., Burke, R. L., Hibner, U., Barry, J. and Alberts, B. M. (1979) Probing DNA replication mechanisms with the T4 bacteriophage *in vitro* system. *Cold Spring Harbor Symp. Quant. Biol.* 43, 469–487

25 Formosa, T. and Alberts, B. M. (1984) The use of affinity chromatography to study proteins involved in bacteriophage T4 genetic recombination. *Cold Spring Harbor Symp. Quant. Biol.* 49, 363–370

26 Epstein, R. H., Bolle, A., Steinberg, C., Kellenberger, E., Boy de la Tour, E., Chevalley, R., Edgar, R., Susman, M., Denhardt, C. and Lielausis, I. (1964) Physiological studies of conditional lethal mutants of bacteriophage T4D. *Cold Spring Harbor Symp. Quant. Biol.* 28, 375–392

27 Warner, H. R. and Hobbs, M. D. (1967) Incorporation of uracil-C^{14} into nucleic acids in *E. coli* infected with bacteriophage T4 and T4 amber mutants. *Virology* 33, 376–384

28 Barry, J. and Alberts, B. (1972) *In vitro* complementation as an assay for new proteins required for bacteriophage T4 DNA replication: purification of the complex specified by T4 genes 44 and 62. *Proc. Natl Acad. Sci. USA* 69, 2717–2721

29 Morris, C. F., Hama-Inaba, H., Mace, D., Sinha, N. K. and Alberts, B. M. (1979) Purification of the gene 43, 44, 45 and 62 proteins of the bacteriophage T4 DNA replication apparatus. *J. Biol. Chem.* 254, 6787–6796

30 Morris, C. F., Moran, L. A. and Alberts, B. M. (1979) Purification of gene 41 protein of bacteriophage T4. *J. Biol. Chem.* 254, 6797–6802