DNA Polymerase I: Essential Replication Enzyme

Coordination of polymerization and 5′ → 3′ exonuclease is an essential feature of discontinuous DNA replication.

I. R. Lehman and Dennis G. Uyemura

The discovery of DNA polymerase in the bacterium *Escherichia coli* in 1956 revealed for the first time the existence of an enzyme that can catalyze the polymerization of nucleotides at the direction of a nucleic acid template (*1*). It is apparent, some 20 years later, that what is now termed DNA polymerase I is only one of a large family of very similar enzymes that can copy DNA or RNA templates or both. Despite the fact that DNA polymerase I was the first of these enzymes to be discovered, its function has yet to be completely clarified.

The purpose of this article is to summarize our attempts to define the role of DNA polymerase I in the replication of the chromosome of *E. coli*. The approach that we have taken has been to correlate the consequences in vivo of a mutation in the gene (*pol*A) that codes for DNA polymerase I with the effect of that mutation on the physical and catalytic properties of the mutationally altered enzyme. We discuss (i) the nature of the enzymatic defect produced by two nonlethal mutations in the *pol*A gene, *pol*A1 and *pol*A12; and (ii) the defect resulting from the conditionally lethal mutation, *pol*Aex1.

Mechanism of Polymerase Action

The detailed analysis of the structure and mechanism of *E. coli* DNA polymerase I by Arthur Kornberg and his colleagues spanned a period of approximately 15 years (*2*, *3*) and provided the conceptual and experimental framework for much of present-day research on nucleic acid biosynthesis. The results of these studies can be briefly summarized as follows: (i) DNA polymerase I consists of a single polypeptide chain of molecular weight 110,000, which can catalyze both the synthesis and hydrolysis of phosphodiester bonds. There are two different hydrolytic activities; one degrades DNA from the 3′ end (3′ → 5′ exonuclease) and a second degrades DNA from the 5′ end (5′ → 3′ exonuclease). (ii) Cleavage of the enzyme with proteases yields a "large fragment" containing the polymerase and 3′ → 5′ exonuclease and a "small fragment" containing only the 5′ → 3′ exonuclease. DNA polymerase thus contains two distinct enzymes within a single polypeptide chain; the two may function coordinately. (iii) The polymerization reaction proceeds exclusively in the direction 5′ → 3′ by addition of mononucleotide units from deoxynucleoside 5′-triphosphates to the 3′-hydroxyl terminus of a primer chain. (iv) A template is absolutely required and directs the enzyme in its selection of the specific triphosphate according to the Watson-Crick base pairing rules.

Inadequacy of DNA Polymerase I in Accounting for DNA Replication

In addition to providing much information regarding the range of its enzymatic capabilities, Kornberg's studies (*2*) of the structure and mechanism of DNA polymerase I also pointed up certain inadequacies, at least in terms of its capacity to catalyze the semiconservative replication of the *E. coli* chromosome. First of all, DNA polymerase I was unable to initiate the synthesis of a polynucleotide chain de novo; the requirement for a 3′-hydroxyl terminated primer chain was absolute (*4*). Second, its turnover number under the best conditions in vitro was two orders of magnitude below that necessary to maintain the rate of DNA chain propagation deduced from studies in vivo (*5*). Third, the direction of polymerization was uniquely 5′ → 3′, hence there was no mechanism to account for synthesis of the complementary DNA strand whose overall direction of synthesis must be 3′ → 5′; this dilemma was provisionally resolved by the finding that DNA replication may occur by the synthesis of small fragments joined together by DNA ligase (*6*); the 5′ → 3′ synthesis of short fragments could therefore proceed in the overall 3′ → 5′ direction (Fig. 1).

There were clear indications from studies of mutants of bacteriophage T4 that no less than six different proteins were required for the replication of the T4 chromosome (*7*); DNA polymerase and ligase could account for only two of these. An equally complex picture emerged from a genetic analysis of *E. coli* DNA replication (*8*). A reasonable point of view that evolved from these studies was that, while DNA polymerase I might be responsible for DNA chain propagation in vivo, additional enzymes and factors were necessary for the semiconservative replication of a duplex DNA molecule.

Discovery of the "Cairns Mutant" and Reassessed Role of DNA Polymerase I

Reliance on DNA polymerase I as the sole polymerizing enzyme received a severe setback in 1969 when De Lucia and Cairns reported the isolation of a completely viable mutant of *E. coli*, extracts of which contained only 1 percent or less of the normal DNA polymerase activity (*9*). Although the "Cairns mutant" was fully viable, it showed some defect in the repair of damage to DNA resulting from ultraviolet irradiation or treatment with alkylating agents such as methylmethane sulfonate. The general interpretation put on this discovery was that DNA polymerase is a dispensable enzyme that occupies a position in one of several DNA repair pathways known to exist in *E. coli* (*10*). In fact, the rather extraordinary suggestion was made that the deoxynucleoside triphosphates were substrates only for "repair synthesis," and that some yet to be identified nucleotide derivatives were the substrates for true "replicative DNA synthesis" (*10*). This proposal was surprising since it had been shown some 5 years earlier that the DNA polymerases induced by phages T4 and T5, which are basically very similar to *E. coli* DNA polymerase I, were absolutely essential for the replication of these viral DNA's (*11*).

Dr. Lehman is professor and chairman and Mr. Uyemura is a graduate student in the Department of Biochemistry at the Stanford University School of Medicine, Stanford, California 94305.

The discovery of the Cairns mutant was instrumental in leading to a reassessment of the role of DNA polymerase I in DNA replication. Of even greater consequence was the impetus to the discovery of DNA polymerases II (*12*) and III (*13*), and the subsequent realization that DNA polymerase III is the product of a gene (*dna*E) whose proper functioning is essential for DNA replication in *E. coli* (*14*). Polymerases II and III are present in low molar concentrations relative to DNA polymerase I, and their detection was possible only when extracts of mutant cells containing very low levels of polymerase I activity became available.

That DNA polymerase I might play some role in DNA replication in vivo became apparent with the reports by Kuempel and Veomett (*15*), and by Okazaki and his co-workers (*16*) who observed that the joining of nascent DNA fragments was retarded in *pol*A mutants. This finding suggested that DNA polymerase I does indeed serve some function in the discontinuous replication of the *E. coli* chromosome, possibly in the joining of "Okazaki fragments." This view was greatly strengthened by the isolation in two different laboratories of temperature-sensitive, conditionally lethal *pol*A mutants. In both cases, the joining of Okazaki fragments was severely retarded (*17, 18*).

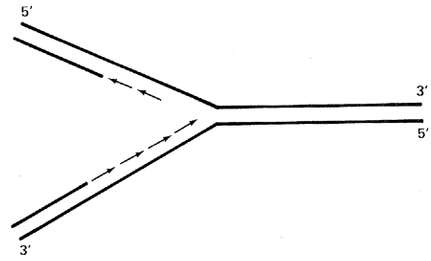


Fig. 1. Discontinuous DNA replication at the replication fork.

Nonlethal DNA Polymerase I Mutants

The pol*A1 mutant*. In the course of inspecting extracts of the Cairns mutant (strain P3478) for DNA polymerase, we noted that a portion of the residual polymerase activity could be inhibited by antiserum prepared against pure DNA polymerase I, suggesting that there was some DNA polymerase I in these extracts (*19*). Similarly, some polymerase activity persisted after addition of *N*-ethylmaleimide, a potent inhibitor of the sulfhydryl-sensitive DNA polymerases II and III, but not of DNA polymerase I. In fact, the DNA polymerase I of strain P3478 could be separated physically from polymerases II and III by ammonium sulfate fractionation (Table 1). In ten such preparations, the level of DNA polymerase I ranged from 0.5 to 2 percent of that obtained from the parental wild-type strain W3110, fractionated in the same way. An obvious concern in dealing with such low levels of activity is that they might represent 0.5 to 2 percent of wild-type (*pol*A$^+$) revertants in the cultures from which the extracts were prepared. This, however, was not the case since these cultures contained only about one *pol*A$^+$ revertant in 10^6 cells as judged by growth in the presence of methylmethane sulfonate.

What is the origin of the residual DNA polymerase I activity in extracts of strain P3478? Inasmuch as *E. coli pol*A1 bears a chain-terminating or *amber* mutation, it could be the result of some misreading of the *amber* codon (*20*), or it might represent an intrinsically low activity of the prematurely terminated polypeptide chain. One way of distinguishing between these two possibilities is to determine whether the polymerase I activity extracted from P3478 is associated with a smaller polypeptide than that from the *pol*A$^+$ strain, and hence sediments more slowly. As shown in Fig. 2, the two sedimentation coefficients (5.4*S*) were identical, an indication that the mutant polymerase does not differ greatly in size from the wild-type enzyme.

We also examined strain JG112, an unmutagenized strain (W3110) into which the *pol*A1 *amber* mutation had been transferred by transduction from the heavily mutagenized P3478 (*20*). Here again, the sedimentation coefficient (5.4*S*) of the residual DNA polymerase I activity was indistinguishable from that of the wild-type enzyme. However, the total amount of activity sedimenting at 5.4*S* in JG112 was only about one-fifth of that found in P3478, possibly because the *amber* mutation is more efficiently suppressed in strain P3478 than in JG112. The DNA polymerase I activity of JG112 is also more labile than that of P3478, so that the difference might reflect an intrinsic instability of the JG112 enzyme in extracts.

Table 1. Separation of DNA polymerase I from DNA polymerases II and III by ammonium sulfate fractionation of mutant (*pol*A1) extracts. Extracts were prepared and treated with ammonium sulfate to yield fraction I (0 to 40 percent saturation) and fraction II (40 to 60 percent saturation). Assays of DNA polymerase activity were performed with nicked calf thymus DNA as template primer (*19*).

Additions	[^{32}P]dTMP incorporated (pmole)
Ammonium sulfate fraction I	
None	12.9
Antiserum to polymerase I	14.2
N-ethylmaleimide	0.8
Ammonium sulfate fraction II	
None	8.8
Antiserum to polymerase I	0.8
N-ethylmaleimide	9.2

In contrast to the low polymerase activity in P3478, the 5′ → 3′ exonuclease activity of DNA polymerase I was present in nearly normal amounts. However, unlike the wild-type enzyme in which the 5′ → 3′ exonuclease and polymerase cosedimented at 5.4*S*, the P3478 polymerase peak showed no detectable 5′ → 3′ exonuclease activity (< 5 percent of wild type). Instead, the 5′ → 3′ exonuclease activity sedimented more slowly than the polymerase, at 2.8*S* (Fig. 2). Like the 5′ → 3′ exonuclease activity of the intact polymerase, it was unaffected by *N*-ethylmaleimide and specifically inhibited by polymerase I antibody. An identical result was obtained with the other *pol*A1 mutant, JG112.

Quite unlike the *pol*A1 *amber* mutant, the 5′ → 3′ exonuclease activity of similar preparations of two other *pol*A mutants, *pol*A12, a temperature-sensitive mutant (see below), and *pol*A5, a nonsuppressible mutant, cosedimented with the polymerase activity at 5.0 and 5.4*S*, respectively (*19*). Thus, only the *amber* mutant showed the peak of slowly sedimenting 5′ → 3′ exonuclease activity. A reasonable interpretation of these findings is that the low polymerase activity of *pol*A1 is due to misreading (or read through) of the *amber* codon (Fig. 3). The nearly normal level of 5′ → 3′ exonuclease activity with a sedimentation coefficient of 2.8*S* is the result of rapid proteolytic cleavage of the *amber* fragment to generate a polypeptide identical with the polymerase I small fragment, whose sedimentation coefficient is also 2.8*S* (*21*). Thus, the site at which the wild-type polymerase I is particularly susceptible to proteolysis may be even more vulnerable in the case of the incomplete *amber* polypeptide. An alternative, but perhaps less likely interpretation, is that the 5′ → 3′ exonuclease represents an intrinsic activity of the *amber* peptide whose sedimentation coefficient happens to be the same as that of the polymerase I small fragment (Fig. 3).

The association of the 5′ → 3′ exonuclease component of DNA polymerase I with the *amber* fragment or its proteolytic cleavage product suggests that it is positioned within the amino-terminal por-

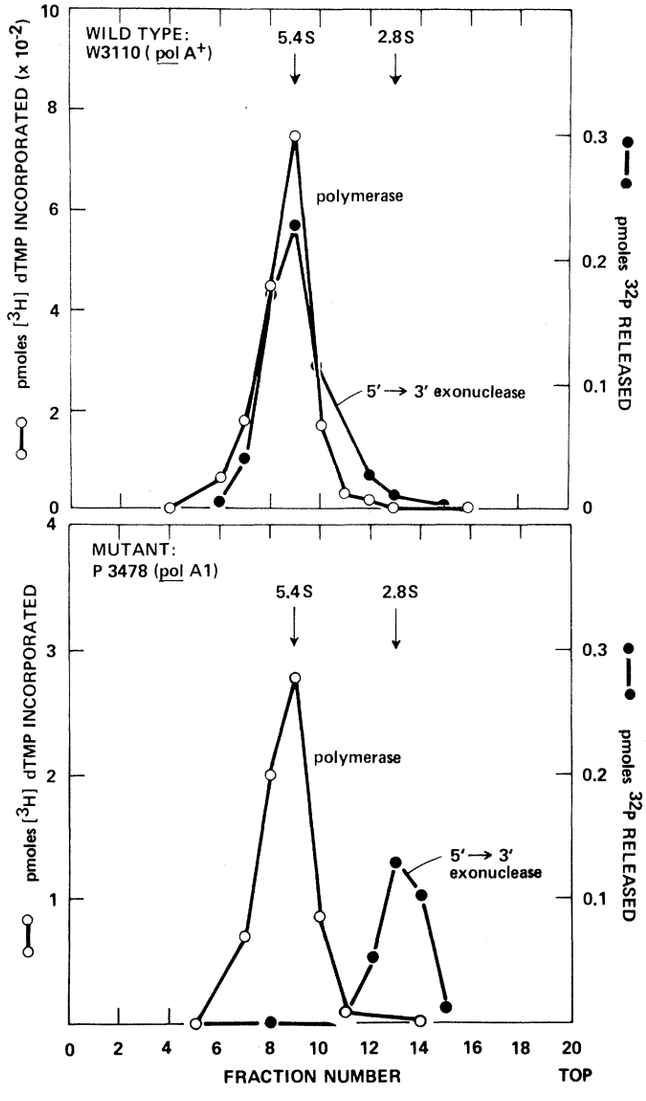
tion of the polymerase I molecule (Fig. 3). In fact, by analyzing the amino-terminal amino acid sequences of intact polymerase I, and the large and small fragments, Jacobson *et al.* (*22*) have shown unambiguously that the 5′ → 3′ exonuclease occupies a position at the amino-terminal end of the native enzyme.

We have explored further the relationship of the small fragment obtained from the *pol*A1 mutant to that formed by proteolytic cleavage of the wild-type enzyme. Can the small fragment from the mutant interact with the polymerase I large fragment that contains the polymerase and 3′ → 5′ exonuclease activities of the intact enzyme? Although isolated small and large fragments from the wild-type enzyme show no direct affinity for one another, either in the presence or absence of DNA, they can bind next to one another at a phosphodiester bond break (a nick) in a DNA molecule; this adjacent binding permits concomitant action of the polymerase and 5′ → 3′ exonuclease activities, as judged by the stimulation of 5′ → 3′exonuclease by concurrent DNA synthesis (*21*). Thus, we have asked whether the 5′ → 3′ exonuclease activity of the 2.8*S* peptide from the *pol*A1 mutant can be enhanced by interaction with the polymerase I large fragment and the four deoxynucleoside triphosphates. For these studies, we used as an assay for 5′ → 3′ exonuclease activity the 5′ → 3′ excision of thymine dimers from ultraviolet irradiated DNA (*23*). Addition of the large fragment and the four deoxynucleoside triphosphates to the 2.8*S* peptide isolated from *pol*A1 cells stimulated the rate of thymine dimer excision (Fig. 4). Its behavior was indistinguishable from that observed with the small fragment produced by proteolytic digestion of wild-type DNA polymerase I.

Although these experiments provide strong support for the model shown in Fig. 3, conclusive proof still requires the isolation in pure form of the 2.8*S* peptide and the demonstration that its amino-terminal amino acid sequence is the same as that of the authentic small fragment. Thus far, we have been frustrated in our attempts to purify the fragment because of its extreme lability.

The PolA12 Mutant

The temperature-sensitive *pol*A mutant, *E. coli pol*A12, was isolated by Monk and Kinross by screening mutagenized cells for their sensitivity to methylmethane sulfonate at the elevated temperature of 42°C (*24*). They found only barely detectable DNA polymerase I activity in *pol*A12 extracts even when assayed at 30°C. It is now clear that their failure to find DNA polymerase I was due to inactivation of the mutant protein by the conditions used in preparing the extract. The *pol*A12 polymerase is extremely thermosensitive. It is also rapidly denatured by even brief exposure to ionic strengths less than 0.1. Once this



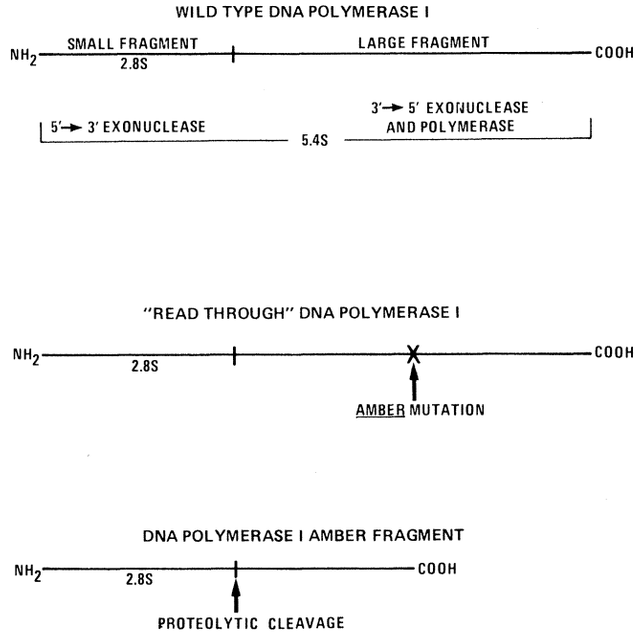


Fig. 2 (left). Sedimentation of polymerase and 5′ → 3′ exonuclease activities in preparations from *E. coli pol*A+ and *pol*A1 strains. Sucrose density gradient centrifugation and assays of polymerase and 5′ → 3′ exonuclease activities were performed as described (*19*). dTMP, deoxythymidine monophosphate. Fig. 3 (above). Model to account for the presence in *pol*A1 *amber* mutants of a low level of DNA polymerase I activity and a normal level of 5′ → 3′ exonuclease activity, the latter in association with a polypeptide smaller than the intact native enzyme.

property of the mutant enzyme was recognized, a purification procedure was developed which avoided exposure to solutions of low ionic strength and produced a homogeneous (> 90 percent pure) protein (*25*).

The temperature sensitivity and instability at low ionic strength of the *pol*A12 DNA polymerase appear to result from a significant alteration in the tertiary structure of the enzyme. The *pol*A12 polymerase comigrates with wild-type enzyme in polyacrylamide gels containing either sodium dodecyl sulfate or urea, showing that there is no detectable change in molecular weight or charge density due to the mutation. However, the mutant protein has a significantly lower mobility than the wild-type enzyme in discontinuous polyacrylamide gel electrophoresis of the two native proteins. Furthermore, the *pol*A12 enzyme sediments at a lower rate than the wild type in a sucrose velocity gradient. The decrease in electrophoretic mobility taken together with the lower sedimentation coefficient suggests that the *pol*A12 mutation has produced a misfolding of the mutant protein so that it is less compact than the wild-type enzyme, possibly the cause of its thermal instability and rapid inactivation in low salt solutions.

All three activities associated with the mutant enzyme are very labile at elevated temperature (Table 2). Thus, the lability of the polymerase activity at 43°C must reflect a general inactivation of the *pol*A12 DNA polymerase I. An earlier report that the 5′ → 3′ exonuclease activity was more thermostable than the polymerase was due to the much shorter incubation period used in assaying 5′ → 3′ exonuclease (5 minutes as compared to 20 minutes) (*19*). Indeed, when 1-minute assays were performed, the mutant enzyme was indistinguishable from the wild type in its polymerase and 5′ → 3′ exonuclease activities. At longer times, the rate at 43°C fell rapidly to zero. Thus, the apparently low activity of the *pol*A12 DNA polymerase I is due to a time dependent inactivation in the course of the reaction rather than to low intrinsic activity at this temperature.

Although the mutant enzyme is rapidly inactivated at 43°C in vitro, it is difficult to know to what extent this lability will be expressed in vivo. When cells with the *pol*A12 mutation were grown at 43°C, they had about one-fourth as much DNA polymerase I activity (assayed in extracts at 30°C) as those grown at 30°C. Furthermore, the purified enzyme is stabilized to a significant extent at 43°C by salt concentrations (0.2*M*) comparable to those that may exist in vivo (*25*).

A striking feature of the *pol*A12 enzyme, even at permissive temperature, is its decreased ability to polymerize at a nick in duplex DNA. This point is illustrated in Figs. 5 and 6. Closed circular duplex DNA from bacteriophage PM2 was treated with pancreatic deoxyribonuclease to introduce about 10 to 50 nicks per circle. Controlled treatment of the nicked DNA with exonuclease then converted the nicks to gaps (Fig. 5). In the presence of nicked PM2 DNA, wild-type DNA polymerase I catalyzed the incorporation of nucleotides at the 3′-hydroxyl end and the release of nucleotides from the 5′ end of the nick in equimolar amounts at both 30° and 43°C (Fig. 6). The net effect of this concerted 3′ → 5′ polymerase and 5′ → 3′ exonuclease action is to propagate the nick along the circular duplex (nick translation) (Fig. 5) (*4*). As was expected, the rate of 5′ → 3′ exonucleolytic removal of nucleotides was substantially enhanced by concurrent polymerization (Table 2; see also Fig. 4). In the case of gapped PM2 DNA, the gaps were rapidly filled in, regenerating the nicks, which then became sites for nick translation (Fig. 7).

The incorporation and release of nucleotides at a nick catalyzed by the *pol*A12 enzyme were also equivalent. However, the rate at which nick translation proceeded was tenfold lower than that seen with the wild-type enzyme at 30°C, and was even further reduced at 43°C (Fig.

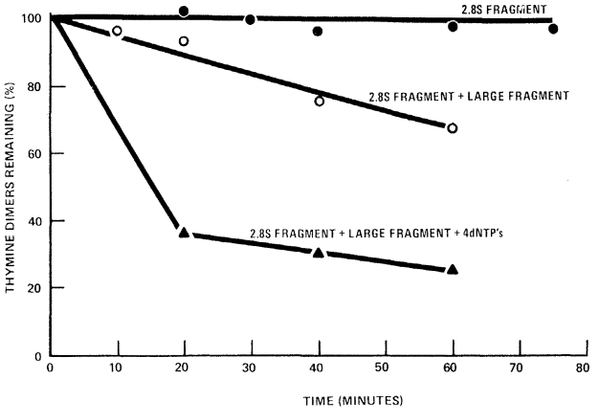


Fig. 4. Thymine dimer excision by 2.8*S* peptide from *E. coli pol*A1. Measurements of thymine dimer excision were performed as described (*23*). An amount of 2.8*S* peptide equivalent in 5′ → 3′ exonuclease activity to 6.9 pmole of polymerase I was added to 8.0 pmole of polymerase I large fragment.

Table 2. Thermolability of polymerase and exonuclease activities of DNA polymerase I isolated from *E. coli pol*A12. Polymerase and exonuclease assays were performed as described (*25*). dNTP, deoxynucleoside triphosphates.

Enzyme	Temperature (°C)	Enzyme activity (μmole/mg protein)			
		Polymerase	5′ → 3′ exonuclease		3′ → 5′ exonuclease
			−dNTP	+dNTP	
*pol*A⁺ (wild type)	30°	28.1	1.8	6.6	2.1
	43°	85.3	7.5	18.3	5.8
*pol*A12	30°	12.7	0.7	0.7	2.1
	43°	(3.6)*	(0.5)*	(0.1)*	(1.0)*

*The values for the *pol*A12 enzyme at 43°C represent extents of reaction rather than initial rates, due to inactivation of the mutant enzyme during assays at this temperature.

Table 3. Defective 5′ → 3′ exonuclease activity of DNA polymerase I isolated from *E. coli pol*Aex1. Polymerase and exonuclease assays were performed as described (*25*). dNTP, deoxynucleoside triphosphates.

Enzyme	Temperature (°C)	Enzyme activity (μmole/mg protein)			
		Polymerase	5′ → 3′ exonuclease		3′ → 5′ exonuclease
			−dNTP	+dNTP	
*pol*A⁺	30°	14.8	1.1	6.1	1.6
	43°	41.9	5.4	11.8	3.4
*pol*Aex1	30°	23.8	0.07	0.33	2.5
	43°	66.0	(0.34)*	(0.19)*	3.1

*A portion (25 to 50 percent) of the apparent 5′ → 3′ exonuclease activity at 43°C may be atrributable to 3′ → 5′ exonuclease action. This estimate is based on the extent of hydrolysis observed on incubation of the nicked PM2 DNA with T4 DNA polymerase which has 3′ → 5′ but no 5′ → 3′ exonuclease activity (*33*).

6). With DNA containing gaps, the mutant enzyme rapidly filled in the gaps to the same extent as the wild-type enzyme, then catalyzed very little further synthesis or release of nucleotides (Fig. 7). Thus, even at permissive temperature, the *pol*A12 polymerase was competent at gap filling, but was defective in nick translation. The reduced ability of the *pol*A12 enzyme to polymerize at a nick is also evident from the lack of stimulation of 5′ → 3′ exonuclease activity by adding the four deoxynucleoside triphosphates (Table 2).

Inasmuch as the activities of the polymerase and 5′ → 3′ exonuclease associated with the *pol*A12 enzyme are nearly normal at 30°C, the abnormally low rate of nick translation suggests that there is a substantial defect in the coordination of polymerization and 5′ → 3′ exonuclease action. Since such coordination presumably demands a rigid spatial arrangement of the two active sites, this novel defect may be a consequence of the structural perturbation caused by the *pol*A12 mutation.

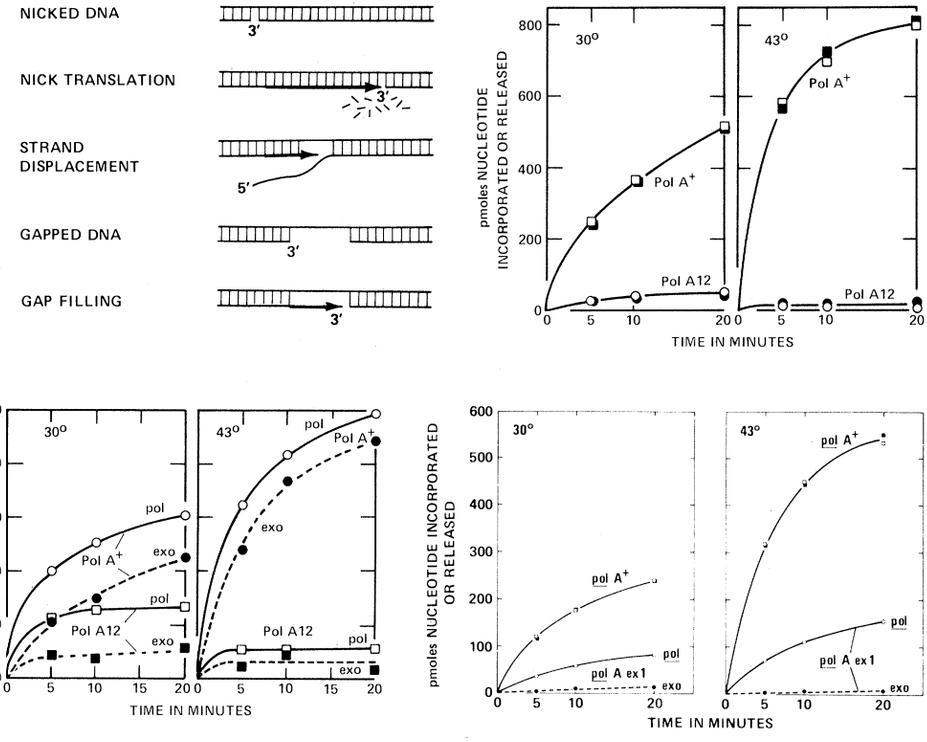


Fig. 5 (top left). DNA polymerase I action at a nick or gap in duplex DNA. Nicked DNA is subject to nick translation or strand displacement; gapped DNA can be filled in to generate a nick, which can then support nick translation or strand displacement. Fig. 6 (top right). Action of wild-type and *pol*A12 polymerases on nicked PM2 DNA. The procedures used to measure nucleotide incorporation and release are given in (*25*). Fig. 7 (bottom left). Action of wild-type and *pol*A12 polymerases on gapped PM2 DNA (*25*). Fig. 8 (bottom right). Action of wild-type and *pol*Aex1 polymerases on nicked PM2 DNA (*17*).

Conditionally Lethal DNA Polymerase I Mutants

The pol*Aex1 mutant*. The isolation of the temperature-sensitive conditionally lethal mutant, *E. coli pol*Aex1, established that DNA polymerase I is essential for the viability of *E. coli* (*26*). Except for its conditional lethality, *pol*Aex1 resembles other *pol*A mutants in its retarded sealing of nascent DNA fragments and in its sensitivity to methylmethane sulfonate and to ultraviolet irradiation. Unlike the other *pol*A mutants discussed thus far, the defect is in the 5′ → 3′ exonuclease rather than in the polymerase activity of DNA polymerase I.

In contrast to the *pol*A12 enzyme, the *pol*Aex1 polymerase is not particularly labile in low salt solutions. The *pol*Aex1 protein comigrates with wild-type DNA polymerase I in polyacrylamide gels containing sodium dodecyl sulfate as well as in native, discontinuous, polyacrylamide gels. Thus, there is no obvious structural alteration comparable to that observed with the *pol*A12 polymerase (*17*).

The polymerase and 3′ → 5′ exonuclease activities of the *pol*Aex1 enzyme do not differ significantly from those of the wild-type DNA polymerase I at either 30° or 43°C. In contrast, the 5′ → 3′ exonuclease activity is substantially reduced at both temperatures (Table 3). Furthermore, there is a significant difference in the 5′ → 3′ exonuclease activity at 30°C as compared to that at 43°C. Addition of the four deoxynucleoside triphosphates produced a marked stimulation in 5′ → 3′ exonuclease activity at 30°C, just as is seen with the wild-type enzyme. However, there was no such stimulation at 43°C. In fact, the 5′ → 3′ exonuclease was actually inhibited by addition of the deoxynucleoside triphosphates, so that the rate of removal of nucleotides at 43°C was significantly lower than at 30°C. Whereas the 5′ → 3′ exonuclease is not temperature sensitive in the absence of deoxynucleoside triphosphates, it is clearly temperature sensitive in their presence.

With nicked PM2 DNA (Fig. 5) as template primer, the *pol*Aex1 enzyme catalyzed the incorporation of nucleotides at a rate far in excess of the rate of hydrolysis of nucleotides from preexisting DNA. This contrasts sharply with the action of the wild-type polymerase, which maintains an almost perfect correspondence between nucleotide release and incorporation (Fig. 8). The low rate of nucleotide release by the *pol*Aex1 enzyme is presumably due to some nick translation that occurs at 30°C. The increment of nucleotide incorporation beyond the amount released must therefore be due to strand displacement; that is, polymerization in the 5′ → 3′ direction accompanied by unwinding of the strand preceding the enzyme molecule (Fig. 5).

The discrepancy between the rates of nucleotide incorporation and release was even greater at 43°C than that at 30°C, probably as a result of an increase in the rate of polymerization coupled with the decrease in 5′ → 3′ exonuclease activity at the elevated temperature. The polymerase activity of the mutant is lower than that of the wild-type enzyme in this experiment (compare Fig. 8 and Table 3). This may be a consequence of the 5′ → 3′ exonuclease defect of the mutant. When 5′ → 3′ exonuclease activity is reduced, the 5′ terminated strand must be displaced ahead of the enzyme molecule for polymerization to proceed (Fig. 5), and this constraint might be expected to lower the polymerization rate. It therefore appears that, at 30°C, the mutant enzyme can catalyze nick translation (at a low rate) and polymerization accompanied by strand displacement. At 43°C, nick translation is abolished and polymerization proceeds only with strand displacement.

Table 4. Summary of *Escherichia coli* DNA polymerase I mutants.

Mutation	Type of mutation	Conditionally lethal	Defective repair of DNA	Defective joining of nascent DNA fragments	Enzymatic defect	Reference
*pol*A1	*amber*	No	Yes	Yes	Polymerase	(*19*)
*pol*A12	Temperature sensitive	No	Yes	Yes	Nick translation	(*25*)
*pol*Aex1	Temperature sensitive	Yes	Yes	Yes	5′ → 3′ exonuclease; nick translation at restrictive temperature	(*17*)
BT4113	Temperature sensitive	Yes	Yes	Yes	Polymerase and 5′ → 3′ exonuclease	(*18*)
*pol*A′107	Nonsuppressible	No	Yes	?	5′ → 3′ exonuclease	(*27*)

Two additional *pol*A mutations are known which affect the 5′ → 3′ exonuclease activity of DNA polymerase I. One of these, *pol*A′107 isolated by Glickman and co-workers (*27*), resembles other *pol*A mutants in its reduced capacity to repair damage to DNA resulting from exposure to ultraviolet irradiation and methylmethane sulfonate. However, it is not conditionally lethal. The extent of the enzymatic defect in *pol*A′107 is not completely clear, and a direct comparison with the lesion in *pol*Aex1 has not yet been made. The second, BT4113, which is a temperature-sensitive conditionally lethal mutant, was isolated by Olivera and Bonhoeffer by an elegant replica plating selection procedure in which microcolonies growing on membranes were tested after lysis in situ for their capacity to synthesize and degrade DNA (*18*). Partially purified preparations of DNA polymerase I from BT4112 show some reduction in polymerase and 5′ → 3′ exonuclease activity at 30°C, and both activities are markedly depressed at 45°C.

Conclusions

The conditional lethality of two mutant strains (*pol*Aex1 and BT4113) establishes that DNA polymerase I is essential for viability in *E. coli*. The finding that the joining of nascent DNA fragments is greatly retarded in these and other polymerase mutants indicates that DNA polymerase I is required for the discontinuous replication of the *E. coli* chromosome. It is therefore reasonable to suppose that the loss of viability in these strains at restrictive temperatures is a consequence of a severe defect in discontinuous DNA replication. In fact, the rate of joining of nascent DNA fragments in the conditionally lethal mutants is significantly more retarded than in *pol*A1 strains (*17, 18*).

What might the precise function of DNA polymerase I be in the discontinuous replication of the *E. coli* chromosome? DNA polymerase I from *E. coli* *pol*Aex1 has a normal polymerase activity and is defective only in its 5′ → 3′ function. Thus, a simple gap-filling role for the enzyme can be eliminated. Clearly, the 5′ → 3′ exonuclease as well as the polymerase activity of polymerase I are essential for the normal joining of discontinuously synthesized, nascent DNA fragments. Both are certainly necessary for the efficient removal of thymine dimers in the repair in vitro of ultraviolet irradiated DNA. Inasmuch as the temperature-sensitive conditional lethality of the *pol*Aex1 strain correlates only with a defect in 5′ → 3′ exonuclease activity measured in the presence of deoxynucleoside triphosphates, we infer that the coordination of polymerization and 5′ → 3′ exonucleolytic cleavage (that is, nick translation) is the essential function provided by DNA polymerase I. Indeed, it seems unnecessary to consider the physiological significance of 5′ → 3′ exonuclease activity in the absence of deoxynucleoside triphosphates since such a depleted cellular state would itself be lethal.

Why should nick translation be essential for discontinuous DNA replication? One possibility is that it is required for the coordinated 5′ → 3′ exonucleolytic removal of an RNA primer and the filling in of the gap thus created, to permit ligation of the discontinuously synthesized nascent DNA fragments to the growing chromosome.

Claims that nascent DNA fragments isolated from *E. coli* contain a 100-nucleotide segment of RNA at their 5′ termini with a unique ribodeoxyribonucleotide juncture (*28*) have not yet been substantiated (*17, 29*). Nevertheless, there is compelling evidence that RNA does serve as a primer in discontinuous DNA synthesis, and that this primer is excised prior to incorporation of the nascent DNA fragment into the chromosome. Reichard and his colleagues have demonstrated the existence of a ten-residue long RNA at the 5′ terminus of the 4*S* nascent polyoma DNA fragments synthesized in nuclei from virus-infected mouse cells (*30*). Moreover, Kornberg and his co-workers have demonstrated that the *dna*G gene product, which is absolutely required for DNA synthesis in *E. coli*, is a rifampicin-insensitive RNA polymerase that synthesizes a 25- to 30-residue oligonucleotide primer that is required for the synthesis of the replicative form of phage G4 from the single-stranded parental circle (*31*). Clearly, the coordinated removal of the RNA primer of a nascent DNA fragment and extension of the 3′ terminus of the abutting fragment may be an essential function provided by DNA polymerase I in discontinuous replication of the *E. coli* chromosome.

Of the five *pol*A mutants that have thus far been thoroughly characterized, only two are conditionally lethal (Table 4). This is not too surprising. It may reflect the relative severity of the various defects in nick translation in vivo, and the fact that DNA polymerase I is present in large excess over that required to support DNA replication. Most of the 300 or so enzyme molecules in a cell may be employed in DNA repair reactions which are going on continuously throughout the life of the cell. A similar situation exists for DNA ligase, an enzyme that participates in the repair and recombination of DNA as well as in its replication (*32*).

References and Notes

1. A. Kornberg, I. R. Lehman, M. J. Bessman, E. S. Simms, *Biochim. Biophys. Acta* **21**, 197 (1956).
2. A. Kornberg, *Science* **163**, 1410 (1969).
3. _____, *DNA Synthesis* (Freeman, San Francisco, 1974), pp. 67–121.
4. R. B. Kelley, N. R. Cozzarelli, M. P Deutscher, I. R. Lehman, A. Kornberg, *J. Biol. Chem.* **245**, 39 (1970).
5. H. Manor, M. P Deutscher, U. Z. Littauer, *J. Mol. Biol.* **61**, 503 (1971).
6. R. Okazaki, T. Okazaki, K. Sakabe, K. Sugimoto, A. Sugino, *Proc. Natl. Acad. Sci. U.S.A.* **59**, 598 (1968); R. Okazaki, T. Okazaki, K. Sakabe, K. Sugimoto, R. Kainuma, A. Sugino, N. Iwatsuki, *Cold Spring Harbor Symp. Quant. Biol.* **33**, 129 (1968).
7. R. H. Epstein *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 375 (1963).
8. J. A. Wechsler and J. D. Gross, *Mol. Gen. Genet.* **113**, 273 (1971); F. Bonhoeffer, R. Hermann, L. Gloger, H. Schaller, *J. Bacteriol.* **113**, 1381 (1973).
9. P. De Lucia and J. Cairns, *Nature (London)* **224**, 1164 (1969).
10. R. Werner, *Nature (London) New Biol.* **233**, 99 (1971).
11. A. De Waard, A. V. Paul, I. R. Lehman, *Proc. Natl. Acad. Sci. U.S.A.* **54**, 1241 (1965); H. E. Warner and J. E. Barnes, *Virology* **28**, 100 (1966).
12. T. Kornberg and M. L. Gefter, *Biochem. Biophys. Res. Commun.* **40**, 1348 (1970); R. E. Moses and C. C. Richardson, *Proc. Natl. Acad. Sci. U.S.A.* **67**, 674 (1970); R. Knippers, *Nature (London)* **228**, 1050 (1970).

13. T. Kornberg and M. L. Gefter, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 761 (1971).
14. M. L. Gefter, Y. Hirota, T. Kornberg, J. A. Wechsler, C. Barnoux, *ibid.*, p. 3150; V. Nüsslein, B. Otto, F. Bonhoeffer, H. S. Schaller, *Nature (London) New Biol.* **234**, 285 (1971).
15. P. L. Kuempel and G. W. Veomett, *Biochem. Biophys. Res. Commun.* **41**, 973 (1970).
16. R. Okazaki, M. Arisawa, A. Sugino, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2954 (1971).
17. D. Uyemura, D. C. Eichler, I. R. Lehman, *J. Biol. Chem.* **251**, 4078 (1976).
18. B. M. Olivera and F. Bonhoeffer, *Nature (London)* **250**, 513 (1974).
19. I. R. Lehman and J. R. Chien, *J. Biol. Chem.* **248**, 7717 (1973).
20. J. Gross and M. Gross, *Nature (London)* **224**, 1166 (1969).
21. P. Setlow and A. Kornberg, *J. Biol. Chem.* **247**, 232 (1972).
22. H. Jacobson, H. Klenow, K. Overgaard-Hansen, *Eur. J. Biochem.* **45**, 623 (1974).
23. E. C. Friedberg and I. R. Lehman, *Biochem. Biophys. Res. Commun.* **58**, 132 (1974).
24. M. Monk and J. Kinross, *J. Bacteriol.* **109**, 971 (1972).
25. D. Uyemura and I. R. Lehman, *J. Biol. Chem.* **251**, 4085 (1976).
26. E. B. Konrad and I. R. Lehman, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2048 (1974).
27. B. W. Glickman, C. A. van Sluis, H. L. Heijneker, A. Rörsch, *Mol. Gen. Genet.* **124**, 69 (1973); H. L. Heijneker, D. J. Ellens, R. H. Tjeerde, B. W. Glickman, B. van Dorp, P. H. Pouwels, *ibid.*, p. 83.
28. A. Sugino, S. Hirose, R. Okazaki, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1863 (1972); S. Hirose, R. Okazaki, F. Tamanoi, *J. Mol. Biol.* **77**, 501 (1973).
29. R. Okazaki, S. Hirose, T. Okazaki, T. Ogawa, Y. Kurosawa, *Biochem. Biophys. Res. Commun.* **62**, 1018 (1975).
30. R. Eliasson, R. Martin, P. Reichard, *ibid.* **59**, 307 (1974).
31. J.-P. Bouche, K. Zechel, A. Kornberg, *J. Biol. Chem.* **250**, 5995 (1975); J.-P. Bouche, S. Rowen, A. Kornberg, personal communication.
32. I. R. Lehman, *Science* **186**, 790 (1974).
33. M. Goulian, Z. Lucas, A. Kornberg, *J. Biol. Chem.* **243**, 627 (1968).
34. Support for the research on DNA polymerase I mutants performed in my laboratory was provided by NIH research grant GM-06196 and NSF grant GB-41927.

Pattern Regulation in Epimorphic Fields

Cells may make use of a polar coordinate system for assessing their positions in developing organs.

Vernon French, Peter J. Bryant, Susan V. Bryant

Classical embryological analysis has led to the concept of a developmental unit, which Weiss (*1*) and others have called the field. It can be defined operationally as the domain within which changes in the presumptive fates of cells (regulation) can occur in response to surgical manipulation. In several organisms it has been shown that up to a certain stage (for example, up to the gastrula stage in the amphibian) the whole embryo can regulate in response to the removal of parts and it therefore constitutes a single field (the primary field). But later surgical interventions have more localized effects, restricted to developmentally autonomous parts of the embryo which we will call secondary fields (*2*). Examples of secondary fields are the developing limb buds and eye, ear, and heart primordia in amphibian embryos, and the appendages and imaginal disks of developing insects.

Following the removal of parts of a field, regulation of the presumptive pattern of differentiation may result in the regeneration of missing elements or in the duplication of elements already present in the fragment. It can occur by epimorphosis, in which pattern elements are added during growth with little change in the remaining part of the pattern, or by morphallaxis, in which regulation involves remodeling of the remaining part of the field to form a miniature but complete pattern (*3*). Most primary embryonic fields seem to regulate by morphallaxis, whereas secondary fields in general show epimorphic regulation.

Fields can also be given a rigorous theoretical definition in terms of Wolpert's positional information theory (*4*). Wolpert proposed that in studying the formation and regulation of spatial patterns of differentiation, we make a distinction between the events by which cells are assigned positional values (positional information) according to their physical locations in the coordinate system of a developing field, and the subsequent responses of the cells (interpretation of positional information) resulting in specific cytodifferentiation. Stern (*5*) had previously proposed a similar distinction between an underlying "prepattern" and the cellular competence to respond. Distinguishing positional information from the cells' response to it is justified on the grounds that genetic mutations can affect the two events separately, and that different patterns of cytodifferentiation can apparently result from the same underlying map of positional values because of differences in the interpretation event (*4*, *6*–*8*). In terms of positional information theory, the field can be defined as a set of cells which have their positions specified with respect to the same coordinate system (*4*).

In this article, we propose a model which accounts formally and in a simple and unified way for the kinds of developmental regulation seen in the secondary fields of both vertebrates and invertebrates. We will discuss in detail the regulative behavior of the limbs of amphibians and of hemimetabolous insects, and of the imaginal disks of *Drosophila*, systems which have been extensively investigated. We expect the model to be applicable to other secondary fields, but its applicability to situations where regulation does not occur [for example, the limbs of higher vertebrates during the later stages of outgrowth and in the mature animal (*9*)] or is limited [the early limb bud of chicks (*10*)] is at present difficult to test. We do not present a detailed molecular model for pattern formation; rather, we consider how the regulative behavior of tissues can be explained in terms of rules for the behavior of individual cells. The problem can subsequently be reduced to consideration of molecular mechanisms to explain cellular behavior.

The model we present here is restricted to two dimensions. This is justified in the case of imaginal disks and insect appendages since we are only considering the cuticular patterns secreted by epithelial sheets, and it is also not unrealistic for amphibian appendages, as we shall show later. In fact, it might be generally true that patterns are established in two dimensions rather than

Vernon French is a research scientist at the National Institute for Medical Research, Mill Hill, London, N.W.7, England. Peter J. Bryant and Susan V. Bryant are in the Department of Developmental and Cell Biology and the Center for Pathobiology, University of California, Irvine 92717.