# On the Processivity of Polymerases[a]

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## INTRODUCTION

Many enzymes are processive,[d] in that they can attach to polymeric substrates or templates and carry out a sequence of polymerization or degradation steps without intervening dissociation. Enzymes that display processivity in this sense include nucleic acid polymerases, a number of nucleases, and similar enzymes that synthesize, degrade, or modify other long-chain biopolymers.

Processivity is also a central attribute of the function of the DNA (and RNA) polymerases with which this *Annal* is largely concerned. In this article we focus on the processivity properties of these enzymes, emphasizing both the central role of processivity in permitting these enzymes to carry out their biological functions and how processivity is defined and measured. The latter topic, in particular, has engendered much confusion in the literature, and we hope that this presentation and review might help to move the field toward a more generally accepted definition and set of measurement protocols for the processivity parameter. In this article we focus on DNA polymerases and, in particular, on the bacteriophage T4–coded DNA-dependent DNA polymerase (gene 43 protein) that has been studied extensively in our laboratory, though examples will be drawn from other systems as well.

## FUNCTIONAL ROLES AND ADVANTAGES OF POLYMERASE PROCESSIVITY

A DNA (or RNA) polymerase engaged in the template-directed synthesis (or editing) of a complementary nucleic acid chain has effectively three choices at each template position I, as shown schematically in FIGURE 1. (i) It can add another nucleotide residue to the 3′-OH terminus of the elongating (primer) strand, with an

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[d]A totally processive (e.g.) DNA or RNA synthesis event is one in which (e.g.) an entire DNA molecule is replicated, or an entire gene is transcribed into RNA, as a consequence of one binding event of the relevant polymerase to the DNA template. DNA synthesis (or degradation) may be moderately processive if several (more than one) nucleotide residues are incorporated or excised per polymerase binding event. Dispersive synthesis or degradation means that the polymerase dissociates from the chain being extended or degraded after each single step of nucleotide residue addition or excision.

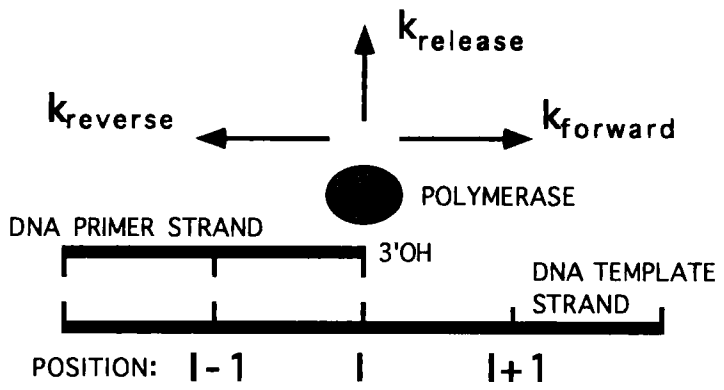


**FIGURE 1.** Alternative (and kinetically competitive) pathways for a template-dependent polymerase located at template position **I** (see text).

apparent overall first order rate constant of $\mathbf{k_{forward}}$. As a consequence the active site of the polymerase is translocated to template position **I+1**. (ii) It can remove a nucleotide residue from the primer strand through either exonuclease or pyrophosphorolysis action (see below) with an apparent overall first order rate constant of $\mathbf{k_{reverse}}$, resulting in a translocation of the active site of the enzyme to template position **I–1**. (iii) It can dissociate from the primer-template junction with an apparent overall first order rate constant of $\mathbf{k_{release}}$, leaving (for dispersive or partially processive synthesis) the 3′-OH of the nascent primer located at template position **I**. We note that the actual rates of the synthesis and pyrophosphorolysis pathways will also depend on the concentrations of NTP and $PP_i$, respectively, if these entities are present at subsaturating amounts.

There is considerable evidence in the literature that suggests that the template-directed synthesis (and degradation) of nucleic acid chains can be viewed as rate processes that are effectively in kinetic competition at each template position with (one or more) alternative pathways leading to polymerase release.[e] A quantitative formalism for this competition in transcription termination has been put forward elsewhere;[1] we note that this formalism (as schematized in FIG. 1) can be applied equally well to the processes of synthesis, editing, and polymerase release in DNA replication, recombination, and repair.

A major biological advantage of a processive polymerization process featuring alternate pathways in kinetic competition for the polymerase at each template position is that if, for example, a noncomplementary nucleotide is misincorporated into the nascent DNA or RNA chain, the overall rate of synthesis is slowed,[2–4] and fidelity correction mechanisms, operating through either the "reverse" or the "release" pathways of FIGURE 1, are relatively favored. On the other hand, the achievement of significant overall rates of DNA (and RNA) synthesis is critical within the multiple

[e]In contrast to DNA replication, for RNA synthesis in transcription, where the length of the nascent RNA that is actually base-paired with the DNA template is confined to a short length at the 3′-OH end of the RNA within the unpaired DNA region that comprises the moving transcription bubble, dissociation of the RNA polymerase from the template is generally accompanied by release of the nascent transcript from the template as well. This release process corresponds to the termination phase of transcription (see Ref. 1).

processes that comprise cell function and cell division. Processive synthesis is central to achieving such rates. Thus, in processive synthesis, the end of the nascent nucleic acid chain that is to be extended (or edited) needs to be diffusionally located, recognized, and correctly bound only once by the polymerase for each series of synthesis (or editing) events. Since diffusion at *in vitro* (or *in vivo*) polymerase and template concentrations is often the rate-limiting event in a polymerase reaction, processive synthesis (or editing) makes possible substantial increases in the overall rate of such processes.

For example, we estimate that (at physiological salt, enzyme, and dNTP concentrations) the extension by T4 DNA polymerase of a DNA strand at a primer-template junction by dispersive synthesis will proceed at <1 nucleotide residue per second per active polymerase molecule. Actual synthesis rates driven by DNA replication complexes *in vivo* and *in vitro* can run as high as 500 nucleotide residues incorporated per second per active polymerase molecule. Clearly, to achieve such rates (i.e., to avoid the slowing effects of diffusion) synthesis must be processive, and thousands of nucleotide residues must be added to the growing DNA chain for each primer-template location and binding event of the polymerase.

## DNA REPLICATION COMPLEXES

We note that the functional *in vivo* T4 DNA replication system contains a number of T4-coded proteins in addition to the polymerase itself. The polymerase accessory proteins (the genes 44/62 and 45 proteins of the T4 system) serve primarily to make the polymerase function in a processive manner at physiological salt conditions (see below). These accessory proteins assemble, with polymerase, into a five-protein elongation complex that includes also the single-stranded DNA binding (gene 32) protein, to achieve leading-strand DNA synthesis at physiological rates, fidelities, and processivities (for a review see Ref. 5). The full seven-protein complex (including gene 41 and 61 proteins; the helicase and primase of the T4 system) is required to reach physiological rates of leading- and lagging-strand synthesis at replication forks.[6] Comparable complexes, containing a number of analogous protein subunits, are involved in the functional DNA replication (elongation) systems of most other prokaryotic and eukaryotic organisms.[7] Clearly if these entire complexes were required to disassemble and reassemble after each single-nucleotide addition or excision event, the (dispersive) rate of DNA synthesis (or editing) would be impossibly slow.

## POLYMERASE REACTIONS

As shown schematically in FIGURE 1, the T4 DNA polymerase (and most other polymerases as well), functioning either alone or as parts of replication, recombination, repair, or transcription complexes, can engage in three types of single-step nucleotide addition (or excision) reactions.

### *DNA Synthesis*

In the presence of deoxyribonucleotide triphosphates, $Mg^{++}$, and an appropriate DNA template, the enzyme can bring about template-directed single-nucleotide extension of the 3′ end of an annealed DNA primer as follows:

$$\text{Primer-3'OH} + \text{dNTP} \Leftrightarrow \text{Primer-dNMP-3'OH} + \text{PP}_i. \quad (1)$$

Under conditions of active replication (or transcription) the above reaction proceeds largely to the right. However, the reaction is reversible, and at low concentrations of dNTPs (or at high concentrations of $PP_i$) DNA (and RNA) polymerases can also catalyze the degradation of the nascent primer by sequential steps of pyrophosphorolysis.

### *DNA "Editing"*

Most DNA polymerases, including the T4 enzyme, carry a 3′ to 5′ exonuclease activity that can degrade the growing DNA primer in a series of one-step reactions as follows:

$$\text{Primer-dNMP-3'OH} \rightarrow \text{Primer-3'OH} + \text{dNMP}. \quad (2)$$

This exonuclease activity can work with either single-stranded DNA or primer-template junctions as substrate. The reaction is considerably faster with single-stranded DNA, and we have shown that the T4 enzyme can degrade both single-stranded DNA and annealed primers processively from the 3′-OH end under processive low-salt reaction conditions.[8,9] This exonuclease activity results in effective "editing" (i.e., increases the fidelity of the replication process) because synthesis is much slower (and thus degradation much more probable) at an unpaired (presumably misincorporated) nucleotide residue at the 3′-end of the primer than at a properly base-paired terminus.[2,3,9]

We and others have shown[2,3,8,9] that polymerase alone, under processive conditions, can first shorten and then extend a base-paired primer without intervening dissociation of the polymerase from the primer-template complex. Thus the reactions shown in equations (1) and (2) can occur successively within the context of a single processive polymerase binding reaction.

### *Chain Termination*

The polymerase (or the entire replication or transcription complex) can also dissociate from the primer-template junction. The overall reaction can be written:

$$\text{Primer-Template-Polymerase} \Leftrightarrow \text{Primer-Template} + \text{Polymerase}. \quad (3)$$

This process is reversible for DNA polymerases (though not generally for RNA polymerases) under many conditions. Thus a nascent DNA chain can be substantially extended (though usually quite slowly) under dispersive or partially processive conditions as a consequence of multiple dissociation and rebinding events of the polymerase at the primer-template junction. This occurs with DNA polymerase under "multi-hit" kinetic conditions, that is, when the ratio of polymerase molecules to primer-template junctions is low and numerous rebinding and extension events can take place at a single primer-template junction during a single synthesis reaction.[f]

[f]We note that the reaction that is equivalent to equation (3) for transcription catalyzed by RNA polymerase is generally not reversible; that is, a nascent transcript cannot be further extended by rebinding the polymerase to an RNA chain from which it has dissociated (though see Ref. 10). Thus transcript elongation is fully processive by definition, and chain termination

The presence of multi-hit kinetics can significantly confuse the analysis of polymerase processivity.[g] We return to a consideration of multi-hit kinetics below.

## MECHANISMS OF PROCESSIVITY

Obviously the processive extension of a DNA primer involves a number of sequential steps. A schematic model of the major reactions involved in this process, cast into a form that we have called the "processivity triangle,"[12] is shown in FIGURE 2.[h] Each single-nucleotide addition (or excision) step involves cycling by the polymerase through at least two different enzyme conformations that occur in the course of binding the next NTP, phosphodiester bond formation and $PP_i$ release, and translocation of the enzyme to the next template position (see, for example, References 13–15). In one conformation the polymerase recognizes explicitly the primer-template (P-T) junction (and, of course, the 3′-OH terminus of the primer). Binding the polymerase to the P-T junction appears to be quite salt concentration–insensitive (F. R. Fairfield, unpublished experiments). While in this conformation we assume that the polymerase "pairs" the next required NTP with the complementary template residue and incorporates it (as NMP) at the 3′-end of the nascent DNA strand. After this (chemical) step is completed (perhaps simultaneously with the ejection of the $PP_i$ product?), the polymerase appears to switch to a largely electrostatically bound conformation, in which binding is very salt concentration–sensitive and *not* sensitive to the details of DNA sequence around the P-T junction (F. R. Fairfield, unpublished experiments). In this "translocating" form, we speculate that the polymerase may then "slide" via one-dimensional diffusion (see Ref. 16) to the next template position, at which point it can again be "locked-down" in the salt-insensitive form by the incoming next-required NTP, and the whole process can begin again. This model suggests that the polymerase cycles through such salt-sensitive and salt-insensitive steps in the course of processive synthesis, and that dissociation of the polymerase (leading ultimately to dispersive synthesis with the translocation step of the processivity triangle being replaced by dissociation) is readily induced by high concentrations of salt (presumably in the salt concentration–dependent translocation phase of the one-step nucleotide addition or editing cycle).

This hypothesis is consistent with the fact that the processivity of polymerase acting alone at a P-T junction is very salt concentration dependent. We have shown[17] that DNA synthesis is fully dispersive at physiological salt concentrations (~160 mM KCl and 5–10 mM $Mg^{++}$; see Ref. 18) for T4 polymerase acting alone, while at low

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occurs only at specific termination signals located at the ends of genes. As a consequence, for *in vitro* transcription reactions one is generally not concerned about the extension of RNA chains by multihit kinetic processes, and all chains can be considered to be synthesized in a "one-hit" process from specific promoter sites.

[g]A good example, involving *E. coli* DNA polymerase I, of such a shift from single-hit to multi-hit kinetics with decreasing primer-template to polymerase ratio, can be seen in FIGURE 5 of Ref. 11. Clearly the very long products synthesized at the lowest template concentrations displayed in this figure result from multiple rebindings of the polymerase to each nascent primer, and the apparent processivity measured in such an experiment will be artifactually large.

[h]The scheme shown in FIGURE 2 is, of course, just intended to show the major chemical and physical steps of the single-nucleotide addition (or excision) process. Pre-steady state kinetic measurements[2,3,15] have shown that the actual chemical steps involved in single-nucleotide addition or excision are considerably more numerous and that the complete processivity triangle should actually be a polygon with many more "sides."

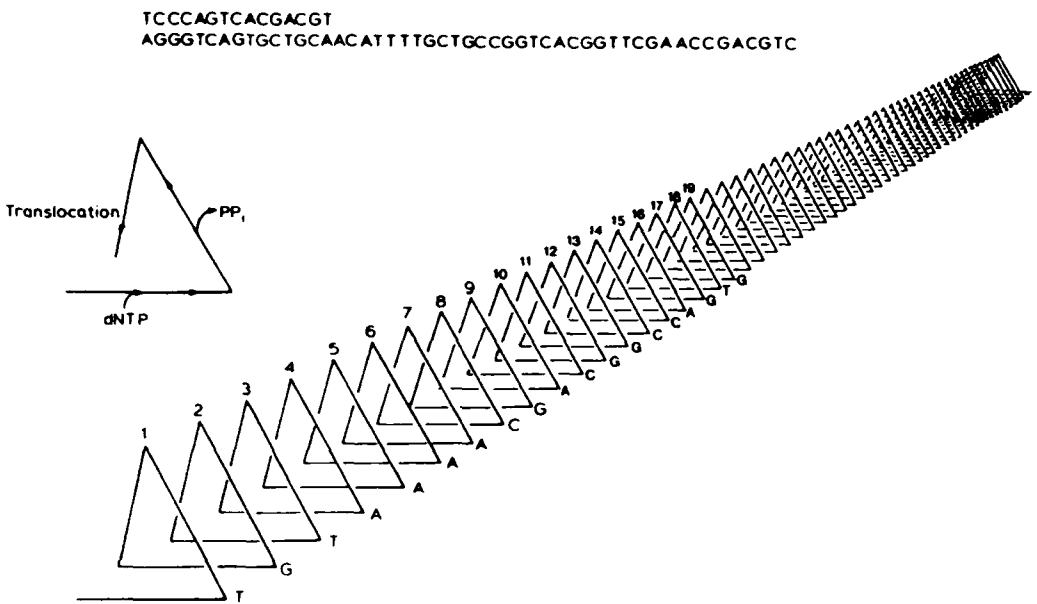


**FIGURE 2.** The kinetic "processivity triangle." Along the **top** of the figure is displayed the base sequence of a short region of M13mp8 viral (+) DNA: from a 3′ end at base 6300 (on the **left**) to a 5′ end at base 6251 (on the **right**). The *triangular drawing* on the **left** of the figure represents an overall single-nucleotide addition (or excision) process. (Modified, with permission, from Ref. 12.)

salt concentrations the polymerase can become significantly processive on short DNA templates. (Using long templates we have shown that this high processivity is still less than that seen with the full five-protein T4 DNA replication complex; see below.)

We have further shown that DNA synthesis catalyzed by the T4 polymerase can be made fully processive at physiological salt concentrations by the addition of stoichiometric concentrations of the T4 polymerase accessory proteins (gene 44/62 and 45 proteins), plus gene 32 protein and 300 μM ATP.[17] It thus appears that one of the major functions of the polymerase accessory proteins may be to protect the polymerase from dissociating from the template during the (salt concentration–sensitive) polymerase translocation step of the processivity triangle.[i]

## DEFINITIONS AND MEASUREMENT OF PROCESSIVITY

The processivity of a polymerase (whether acting alone or as a part of a DNA replication complex) can be defined in a number of ways. In earlier times (prior to the advent of gels) processivity was measured (presumably under one-hit conditions) in terms of the (number-average) degree of polymerization of 5′-end labeled extended DNA primer chains. Average extensions of 5 to 100 nucleotide residues per enzyme binding event were routinely found for various polymerases, though little attention was paid to the concentrations of salt or the particular DNA template sequences at which these measurements were made.

More recently the availability of polyacrylamide and other types of DNA sequencing gels has made it possible to define processivity more precisely. Now DNA primers (usually 10 to 20 nucleotide residues in length and 5′-end labeled with $^{32}P$) are annealed to specific templates (e.g., single-stranded M13 DNA circles) and elongated in reactions carried out under single-hit conditions. The entire distribution of extended primers is then subjected to electrophoresis on gels at various levels of cross-linking to permit the resolution by size of the nascent primer strands. The resulting ladders of DNA bands can then be quantitated by autoradiography and densitometry, or analyzed in real time on a radioactivity gel scanner.

Processivity can then be defined (for a given salt concentration and template sequence) either directly in terms of the size distribution of the extended primers or by determining the fraction of the input radioactivity that occurs in bands exceeding a given number of nucleotide residues in length. Another definition of processivity (though one that is sometimes hard to apply at some template positions in natural DNA) can be framed in terms of probabilities, or of what we have called "microscopic processivity parameters."[12] This approach is particularly useful when one wishes to examine mechanisms of enzyme processivity—that is, to define the molecular, thermodynamic, and kinetic details involved in the various steps of the processivity triangle. For these purposes one needs to work under conditions of moderate processivity, so that both the probability of terminating and the probability of not terminating at a particular template position are of comparable magnitudes.

We define the probability of not terminating at a specific position **I** on a specific template as $\mathbf{P_I}$; this probability is defined as the microscopic processivity parameter for this position. (The probability of terminating at this position will then, of course, be $\mathbf{1-P_I}$.) FIGURE 3 shows schematically the (single-hit) processivity assay used in

The molecular mechanisms by which these polymerase accessory proteins bring about processive DNA synthesis have been described, in part, by O'Donnell.[19]

making such measurements. In these terms $P_I$ can be defined operationally as the fraction of extended primers that reach position **I**, but do not terminate there. If $n_I$ is the number of extended primers that terminate at position **I** and $n_T$ is the sum of the number of extended primers that terminate at position **I** and at all positions downstream of **I** (i.e., the total of all the radioactivity found at band positions **I** and beyond), then for any particular template position **I**:

$$P_I = (n_T - n_I)/n_T. \quad (4)$$

This is, in principle, a perfectly general definition of processivity and stresses the fact that this parameter may differ at each position on a natural template. $P_I$ is, of course, difficult to measure on natural templates at positions at which only small amounts of polymerase release occur, since the bands that must be analyzed to determine $n_I$ for such positions may be very faint. Determining $n_T$ may also be difficult, since this requires integration over many faint bands, for which background correction errors may be substantial.

Some of these problems are more easily handled by making measurements of $P_I$

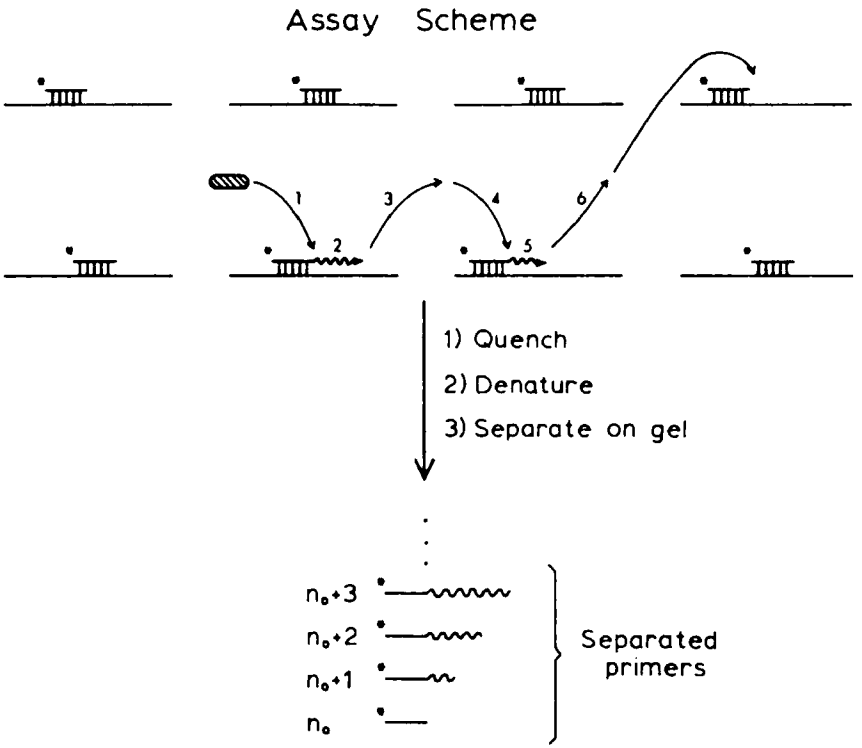


**FIGURE 3.** Assay for measuring processivity (for details, see text). The extended (5′-end labeled) primers are separated from one another by polyacrylamide gel electrophoresis at the end of the reaction, and the size distribution of elongated primers is determined by quantitative autoradiography (see FIG. 4). (Reprinted, with permission, from Ref. 17.)

on homopolynucleotide templates (and homo-oligonucleotide primers), where $\mathbf{P_I}$ is expected to be the same at every position. FIGURE 4 shows such a measurement (gel and densitometry) for the extension of a 5′-end labeled oligonucleotide primer [oligo(dT)] against a homopolynucleotide template [poly(dA)], carried out under single-hit kinetic conditions and under salt conditions resulting in moderate levels of processivity.[j] Based on the definition of $\mathbf{P_I}$ presented above, the data of FIGURE 4 can

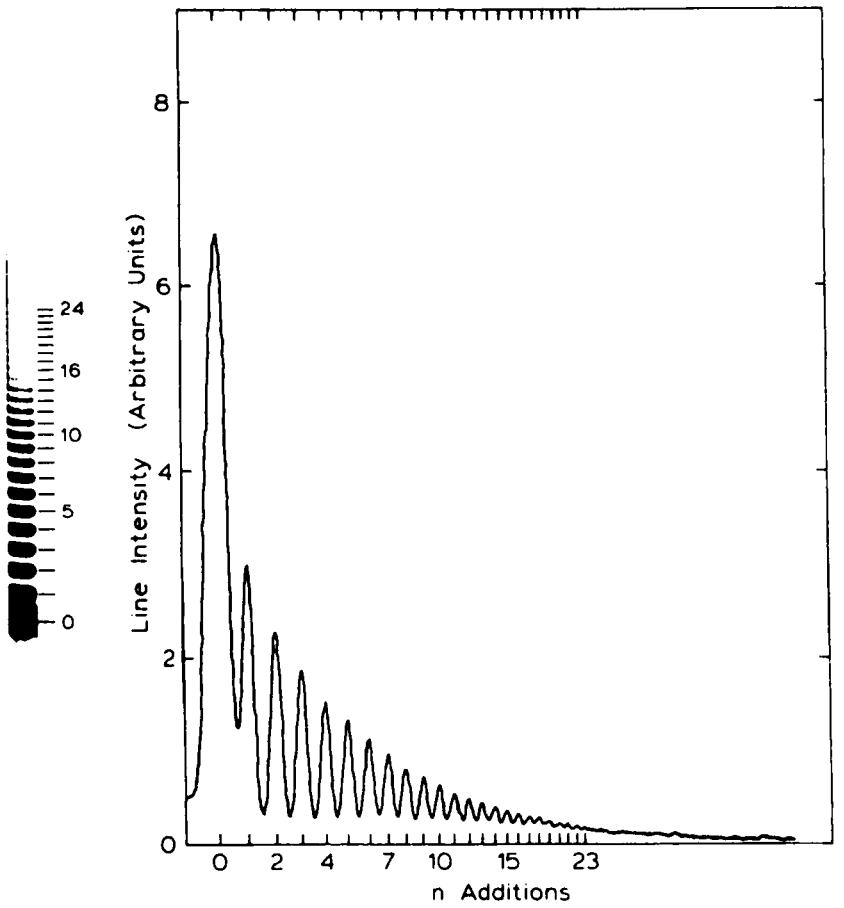


**FIGURE 4.** Processivity assay for the oligo(dT)-poly(dA) system (for reaction conditions see text). (Reprinted, with permission, from Ref. 17.)

[j]We note that under "faint band" conditions the resolution of such experiments can be increased by "body-labeling" the extended primers (e.g., with α-labeled $^{32}P$ nucleotide triphosphates). However, the analysis described below must be somewhat modified for such experiments since the bands will now be weighted in proportion to molecular weight rather than in proportion to the number of 5′-ends.

be plotted as $-\log(n_I/n_T)$ versus $n-1$ (FIG. 5), where $n_I$ and $n_T$ are as defined above, and $n$ is the number of nucleotide residues added to the primer—that is, the number of addition cycles completed prior to dissociation for each band at each position $I$.

Assuming, as is implicit in the definition of $P_I$, that dissociation is a first-order process, the fraction of primers that will have been extended by exactly $n$ nucleotide residues at the 3′-terminus will be:

$$n_I/n_T = \{P_I^{(n-1)}\}\{1-P_I)\} \tag{5}$$

and

$$\log(n_I/n_T) = (n-1)\log P_I + \log(1-P_I). \tag{6}$$

For systems where $P_I$ is expected to be the same at each position $I$ (as in the "homo-homo" primer-template system used in the experiment pictured and analyzed in FIGS. 4 and 5), the value of $P_I$ can be calculated from the straight line obtained in FIGURE 5. Here the slope of the line is equal to $\log P_I$, and the intercept of the straight line with the y-axis is $\log(1-P_I)$ [see equation (6)]. The data of FIGURE 5 (measured in 0.095 M NaCl, 10 mM Tris (pH 7.8), 0.5 mM DTT, 200 μg/ml BSA, 0.1 mM EDTA, 2.5 mM $MgCl_2$, 30 s incubation at 37°C) yield a straight line, confirming that $P_I$ is indeed constant for this system over the range tested. Here a value of $P_I = 0.84$ (± 0.1) is obtained.[k] This corresponds to an average primer extension of $(1/(1-P_I)) = 6.25$ nucleotide residues per polymerase binding event. At lower salt concentrations $P_I$ will, of course, be larger, and the average primer extension per binding event will also be larger.

We have shown that $P_I$ is a constant (for primers greater than ~12 nucleotide residues in length) for such "homo-homo" primer-template systems under any specific set of salt conditions.[17] On the other hand, we and others have shown that $P_I$ is not a constant for templates of natural sequence.[12,20] Rather this parameter turns out to be a function of the base-pair sequence of the primer and the template, and for T4 polymerase this sequence dependence extends approximately 10 base pairs into the double-stranded portion and 5 bases into the single-stranded portion from the primer-template junction.[12]

We have analyzed the sites at which $P_I$ is low (i.e., the probability of polymerase release is high) for a number of positions on the phage M13 DNA template, and have found that while some of these sites correspond to positions just upstream of hairpins (elements of stable secondary structure) in the single-stranded DNA template, many others do not. Thus there is clearly a sequence-dependent component, as well as a structure-dependent component, involved in defining template positions at which the probability of polymerase release is high. (Again, this type of analysis has been carried out mostly with the T4 polymerase.) It has been shown,[20] and we have confirmed (F. R. Fairfield, unpublished experiments), that positions characterized by low $P_I$ values retain this character (relative to other sites), even in the presence of added accessory protein complexes.

[k]This value may be used to calculate other measures of processivity, as follows. For example, for this system, with $P_I = 0.84$, the probability of adding at least one nucleotide residue to the 3′-terminus of such a primer-template will be 0.84, that of adding at least two nucleotide residues will be $P_I^2 = 0.71$, that of adding at least three nucleotides will be $P_I^3 = 0.59$, and so forth. The probability of adding exactly one nucleotide residue to the primer will be $P_I^1 - P_I^2 = 0.13$, that of adding exactly two nucleotide residues will be $P_I^2 - P_I^3 = 0.12$, and so on. (We note that these data, and the description of their analysis, are taken in part from Ref. 17.)

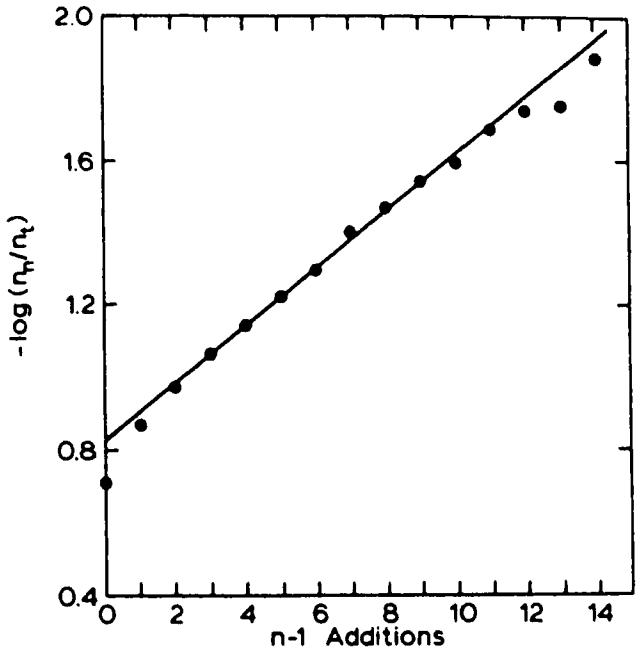


**FIGURE 5.** Data from FIGURE 4, plotted according to equation (6). A value of $P_I = 0.833$ is obtained from the slope, and a value of $P_I = 0.848$ is obtained from the y-intercept (see text). (Reprinted, with permission, from Ref. 17.)

## PROCESSIVITY MEASUREMENTS WITH ASSEMBLED REPLICATION COMPLEXES

However, in addition these complexes (and, to a lesser extent, gene 32 protein) can increase the apparent value of $P_I$ substantially. Thus Jarvis *et al.*[21] have shown that the value of $P_I$ at a template position just downstream of a stable hairpin (at position 6207 of the M13 template) changes from <0.02 for polymerase alone, under fairly high salt conditions, to ~0.75 for the five-protein T4 DNA replication complex in the presence of ATP under the same conditions.

## SINGLE-HIT (OR SINGLE-TURNOVER) VERSUS MULTI-HIT KINETICS

Clearly the processivity of DNA polymerases needs to be measured under well-defined conditions in order to provide meaningful comparisons with other systems. Thus not only must one define salt concentrations, pH, and temperature, but all the dNTP substrates need to be present at concentrations above their respective $K_m$ values, and single-hit conditions must obtain. Single-hit kinetics conditions are defined

as those under which any given primer will have an effectively zero probability of being extended by more than a single polymerase molecule during a given reaction; that is, the probability that a particular primer-template will bind a second polymerase during the course of a synthesis experiment must be essentially nil.[l] Operationally single-hit kinetic conditions are attained for reasonably processive systems if one retains a vast excess of primer-template junctions over active polymerase molecules during the entire course of the reaction. If this condition breaks down in experiments such as that analyzed in FIGURE 5, the resulting line will be curved.

If one wishes to work at higher polymerase concentrations than this arrangement normally permits, one can use what are called "single-turnover" conditions (e.g., see Ref. 21). Here higher ratios of polymerase to primer-template sites are used, but the polymerase is trapped by unlabeled "quencher" DNA after initial dissociation from the primer to prevent further extension of the labeled primer by rebound polymerase molecules. Nicked or gapped calf thymus DNA is often used as a quenching agent. The quencher must be present in great excess and is added immediately after the reaction has been initiated. In order to obtain valid results from such single-turnover experiments—that is, to measure values of processivity parameters or dissociation rate constants that are comparable to those obtained in single-hit kinetic experiments, one must show that quenching is "passive," meaning that the quencher does not actively remove the polymerase from the original primer-template by some form of direct transfer mechanism. This is most easily shown by demonstrating that the measured processivity is independent of quencher concentration.[m]

## COMPARISONS OF PROCESSIVITIES FOR DIFFERENT POLYMERASES

It is sometimes difficult to carry out comparative studies of processivity mechanisms on polymerases of different types using natural templates. However, comparative measures of the extent of processivity of closely similar polymerases may be made on templates that are devoid of strong "stop" sites for DNA polymerase by determining how much of the input radioactivity ends up in DNA sequences that are too long to be resolved by the gel system being used. Polymerases of different types (with or without their homologous accessory proteins) may react differently to template "obstructions" (hairpins or "difficult" sequences) that are characterized by high $P_I$ values. Thus it is important to compare polymerases under identical conditions of salt and dNTP concentrations, pH, and temperature, and with the same template sequence. Experiments with "homo-homo" primer-templates, such as those illustrated FIGURES 4 and 5, are probably most useful in studies designed to compare mechanistic details of the processivity of different polymerases.[n]

[l]We assume that all primer-template sites have the same affinity for polymerase, regardless of whether or not the primer has been extended. For T4 polymerase we have shown that this is true if the primer is more than ~12 nucleotide residues in length.[8,9]

[m]This means that the apparent processivity or dissociation rate constant must be independent of quencher concentration. However, in striving to reach quencher concentrations for which this is true, one must not go to such low concentrations of quencher (relative to primer-template sites) that one inadvertently blunders back into the domain of multi-hit kinetics.

[n]Oligo(dT) primers tend to cluster on poly(dA) templates, rather than being distributed at random.[22] Thus to achieve perfectly defined "homo-homo" primer-templates, we use primer-templates with "sticky ends"; for example, a dG-dC-dG triplet is placed at the 5′-end of the primer oligo(dT) sequence, and a dC-dG-dC triplet at the 3′-end of the poly(dA) template (see Ref. 23).

## SUMMARY AND OVERVIEW

As indicated in the INTRODUCTION, processivity is a crucial property that permits DNA replication, recombination, repair, and transcription systems to function at physiological rates and fidelities. Structural and mechanistic aspects of processivity and its biological role have been considered extensively elsewhere (see references herein and other articles in this *Annal*). In this review we have focused on the definition and experimental characterization of the processivity parameter in order to provide a context for a more consistent and quantitative use of this concept in the rapidly expanding literature that deals with the processivities and fidelities of DNA and RNA polymerases.

## ACKNOWLEDGMENTS

The authors are grateful for many stimulating discussions with our laboratory colleagues who participated in studies relating to processivity in our laboratory over the years (see References). These studies and discussions led to many of the ideas and procedures set out in this article.

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