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Productive and Abortive Initiation of Transcription in Vitro at the *lac* UV5 Promoter†

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ABSTRACT: The rates of productive and abortive initiation of transcription in vitro at the *lac* UV5 promoter have been determined and compared to values determined for phage λ and T7 promoters. The rate constants for productive initiation of *lac* transcript are consistently lower over a range of low to moderate concentration of initiating nucleoside triphosphate (ATP). Abortive initiation of *lac* dinucleoside tetraphosphate is also slower at low to moderate concentrations of ATP. These data demonstrate the existence of significant differences in initiation rate among promoters. We suggest that these differences may be a consequence of the initial mRNA sequences and extents of RNA polymerase cycling during initiation of promoter-specific transcription.

The detailed mechanism by which promoter-bound RNA polymerase binds incoming nucleoside triphosphates and initiates productive transcription is still uncertain [for reviews, see Krakow et al. (1976) and Chamberlain (1976)]. Many tentative conclusions concerning the rate and mechanism of initiation must now be reevaluated as a consequence of recent studies. For example, much information was derived from use of the rifampicin challenge assay for initiation (Mangel & Chamberlin, 1974; Rhodes & Chamberlin, 1975) which has now been shown to be based on an over-simplified mechanism of rifampicin action (McClure & Cech, 1978; Carpousis & Gralla, 1980). As a part of this reevaluation, certain conclusions based on this assay have been confirmed by a direct assay (Nierman & Chamberlin, 1979, 1980), but other conclusions remain uncertain. Moreover, it has been shown that initiation may be more complex than previously assumed since steps subsequent to formation of the first phosphodiester bond may be important to the mechanism. Specifically, McClure et al. (1978) showed that formation of the first phosphodiester does not necessarily lead to formation of a full-length transcript on the λ P_R' promoter. Subsequently, in a detailed study of the *lac*[1] UV5 promoter, Carpousis & Gralla (1980) showed initiation to be very complex; RNA polymerase catalyzes several cycles of synthesis accompanied by premature chain termination before escaping to form a fully committed elongation complex.

The *lac* UV5 promoter has been inferred to be a much slower initiator than phage T7 promoters (Stefano & Gralla, 1979). This inference, however, was based on comparing a rate determined by direct assay to one nucleoside triphosphate concentration with rate constants determined by rifampicin challenge assay. Since the T7 rate has now been studied by using a direct assay (Nierman & Chamberlin, 1979, 1980), a direct comparison with *lac* UV5 becomes possible. Below we show that the *lac* UV5 promoter has an initiation rate constant 2 orders of magnitude lower than that reported for T7 over a range of ATP concentration.

An important contributing factor to the initiation rate is assumed to be the rate of formation of the first phosphodiester bond. This rate has been estimated by using an "abortive initiation" assay which measures formation of dinucleoside tetraphosphate under conditions where chain elongation is disallowed (Johnston & McClure, 1976). In order to investigate the potential contribution of this rate to the slow overall rate of productive initiation of the *lac* UV5 promoter, we have determined the abortive initiation rate as a function of ATP

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[1] Abbreviations used: *lac*, lactose operon; Tris, tris(hydroxymethyl)-aminomethane; PPO, 2,5-diphenyloxazole.

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concentration. This rate at low to moderate concentration of ATP turns out to be very much slower than that reported for T7 and λ promoters (McClure et al., 1978; Nierman & Chamberlin, 1979). These comparative rates of productive and abortive initiation, coupled with our study of rates of oligonucleotide formation during initiation (Carpousis & Gralla, 1980), lead us to propose a general model to account for DNA sequence dependent variations in initiation rate.

Materials and Methods

Preparation of Materials. The DNA template used was a purified fragment, 203 base pairs in length, containing the *lac* UV5 promoter and coding for the initial 67 nucleotides of *lac* messenger RNA. The DNA was purified by endonuclease RI digestion of plasmid DNA, followed by a combination of centrifugation and chromatographic steps as described previously (Carpousis & Gralla, 1980). The purification of *Escherichia coli* RNA polymerase, according to the procedure of Burgess and Jendrisak, of nucleoside triphosphates and of [α-^{32}P]ATP was also as described. The enzyme was judged 50% active as measured by transcription titration of the *lac* restriction fragment. [α-^{32}P]CTP was prepared by a modification of the procedure of Reeve & Huang (1979).

Initiation Rate Assay. All solutions contained standard reaction salts of 30 mM Tris-HCl, pH 8.0, 100 mM KCl, 3 mM $MgCl_2$, 0.1 mM ethylenediaminetetraacetic acid, and 0.2 mM dithiothreitol. In a typical reaction, DNA (3 pmol) was preincubated with RNA polymerase (nominally 7 pmol) at 37 °C for 5 min in 75 μL. At zero time the reaction was initiated by addition of 75 μL of the prewarmed initiation mixture containing 200 μg/mL heparin and sufficient amounts of the four nucleoside triphosphates to reach the specified concentrations. Unless otherwise specified, this concentration was 140 μM and the only radioactive species was [α-^{32}P]CTP at 10 Ci/mmol and 2.5 μM. Since CTP is the ninth nucleotide incorporated in *lac* mRNA, the low concentration should not alter the initiation process. At various times 10-μL aliquots were withdrawn and added to 10 μL of the termination mixture which contained identical concentrations of nucleoside triphosphates and 400 μM rifampicin. Elongation of RNA then proceeded for a minimum of 15 min at 37 °C. Cold 7.5% trichloroacetic acid plus 0.25% pyrophosphate was then added to precipitate radioactive RNA, and the solution was filtered through glass fiber filters (Whatman GF/C) and rinsed 3 times with acid and twice with ethanol. The dried filters were counted in PPO–toluene. Background radioactivity was determined by performing a mock reaction where the termination mixture was added prior to initiation mix.

The experiment involving gel electrophoresis of radioactive RNA synthesis was identical except that the termination mixture was not added. Instead, after a 15-min incubation the reaction was terminated by addition of an equal volume of saturated urea containing 0.05% xylene cyanol and bromophenol blue. Electrophoresis and autoradiography were as described previously (Stefano & Gralla, 1979).

Abortive Initiation Assay. RNA polymerase–DNA complexes were formed by a 10-min incubation at 37 °C with 1.6 pmol of *lac* UV5 DNA and 4.0 pmol of RNA polymerase in 6.0 μL of standard salts plus 0.1 mg/mL bovine serum albumin acetylated according to the method of Gonzalez et al. (1977). The reaction was started by the addition of [α-^{32}P]ATP (final specific activity was ~0.1–1.0 Ci/mmol) in 6.0 μL of standard salts. ATP was added as an equimolar mixture of $MgCl_2$ and ATP, thus raising the final $MgCl_2$ concentration and assuring that sufficient $MgCl_2$ was available at high ATP concentrations. After 10 min at 37 °C, the reaction was stopped by the addition of 3.0 μL of 0.1 M EDTA (pH 8)–0.1% xylene cyanol. 3.0 μL of the reaction was spotted on Whatmann 3MM filter paper, and a chromatogram was developed as described by McClure et al. (1978) with the following modification. Descending chromatography, which permitted better separation of the species of interest, was used.

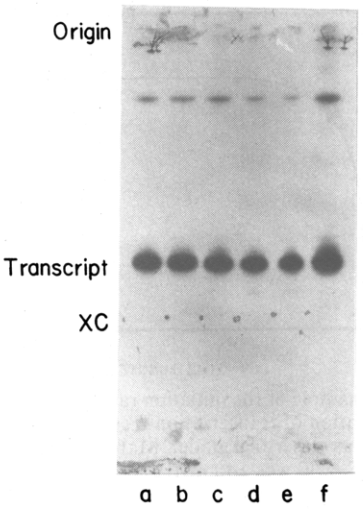

FIGURE 1: *lac* RNA synthesized in initiation reactions at various concentrations of ATP. *lac* RNA was synthesized with [α-^{32}P]CTP as described under Materials and Methods and was subjected to electrophoresis on a 12% polyacrylamide–urea denaturing gel. A radioautograph, exhibiting the *lac*-specific runoff RNA (labeled "transcript") is shown. XC refers to the xylene cyanol marker dye. The concentrations of ATP used in synthesis were as follows: lane a, 35 μM; lane b, 70 μM; lane c, 105 μM; lane d, 140 μM; lane e, 175 μM; lane f, 210 μM.

The chromatogram was eluted until the ATP was near the end of the paper, dried, cut into strips, and counted by Cerenkov radiation. The major product of the reaction, previously identified as pppApA (Carpousis & Gralla, 1980), ran about half as far as the ATP. The rate of synthesis was calculated from the percentage of ATP incorporated into product, the total amount of ATP in the reaction mixture, and the concentration of promoter in the reaction. In all cases, the amount of ATP incorporated during the 10-min reaction was less than 20% of the total ATP present, and thus the measured rates approximate initial velocities of reaction.

Results

Rate of Productive Initiation of Transcript. Previously, we have shown that in a highly purified transcription system, a restriction fragment containing the *lac* UV5 promoter supports specific *lac* transcription (Stefano & Gralla, 1979). The UV5 promoter is a very well characterized template (Schmitz & Galas, 1979; Johnsrud, 1978; Simpson, 1979) and allows *lac* expression without the assistance of catabolite activator protein in vivo (Silverstone et al., 1970) and in vitro (Maizels, 1973). In anticipation of experiments involving various ATP concentrations, we wished to demonstrate the specificity of transcription at these particular nucleotide concentrations.

Figure 1 shows that the specificity of transcription is maintained as the concentration of ATP is varied from 35 to 210 μM. The predominant product is represented by a band of RNA which originates at the *lac* promoter and terminates near the end of the restriction fragment template. Therefore, the specificity of transcription is not altered, and we are able to apply a modification of our previous assay (Stefano & Gralla, 1979) to determine the initiation rate at the various ATP concentrations.

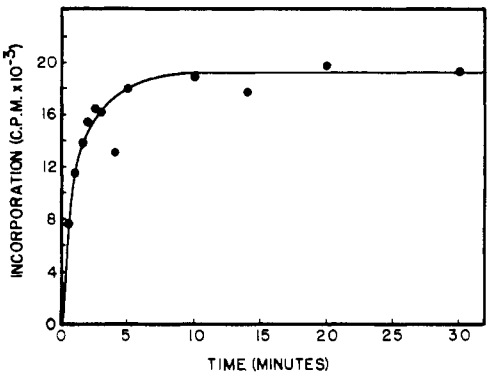

FIGURE 2: An example of the initiation rate assay. The assay, which follows the initiation of transcription from preinitiation complexes, was performed as described under Materials and Methods. The concentration of ATP was 140 μM, and [α-^{32}P]CTP had a specific activity of $\sim$10 Ci/mmol.

This assay involves following the rate of formation of RNA during a single round of transcription begun by the addition of substrate nucleoside triphosphates to promoter-bound RNA polymerase. First, preinitiation complexes are formed by the preincubation of RNA polymerase with DNA. Next, at time zero heparin and nucleoside triphosphates are added. The initiation reaction begins with this addition of substrate; heparin is added to ensure that synthesis is restricted to a single round by inactivating any free RNA polymerase molecules. At various intervals thereafter rifampicin is added to inactivate complexes which have not yet initiated. Those initiated complexes which survive are allowed to elongate a single, full-length RNA. The rate of formation of productively initiated complexes may be followed by quantitating the amount of RNA produced. This assay differs slightly from our previous assay in that the rate of initiation may be estimated directly by following the time course of RNA production with the variable of chain elongation essentially eliminated. This advantage is offset slightly by the use of rifampicin to distinguish between those complexes which have or have not initiated. Figure 2 shows an example of this assay.

The assay was applied to determine the rate of initiation at various concentrations of the initiating nucleotide ATP. Each productive initiation event represents the conversion of a preinitiation complex to a rifampicin-resistant elongation complex as a consequence of binding and condensing the nucleoside triphosphates which are in vast excess over complex. Since the reaction is rendered irreversible by the presence of heparin, preinitiation complexes are consumed in the process and pseudo-first-order kinetics are expected. The linearity of the replots for these reactions confirms this expectation (Figure 3). The slope of these lines changes systematically with increasing ATP concentration, demonstrating that the initiation rate increases with the concentration of the initiating nucleotide.

Figure 4 shows the variation in initiation rate constant (k'), calculated from the data of Figure 3, as a function of ATP concentration. The shape of the curve can be construed as either a straight line or as slightly curvilinear. Since these data were obtained by using a direct assay of initiation rate, they may be compared to rate constants determined for initiation on a T7 DNA template, derived from direct assays (Nierman & Chamberlin, 1979, 1980). Such a comparison reveals both similarities and differences between *lac* UV5 and phage T7 initiation kinetics. The two systems behave similarly in that the initiation rate increases with the concentration of the initiating nucleotide and in that this dependence extrapolates smoothly to zero rate at zero concentration.

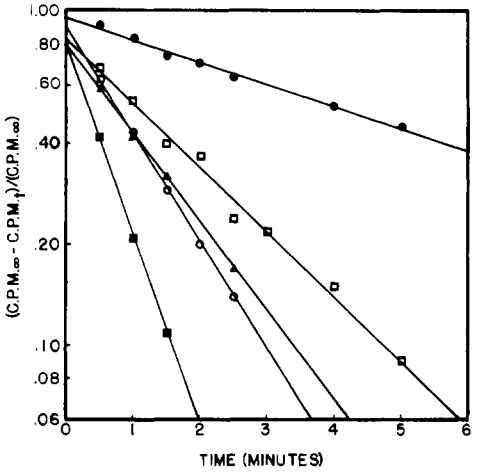

FIGURE 3: Semilogarithmic analysis of initiation rate data at various ATP concentrations. The fraction of complexes not yet initiated (fraction of radioactivity not yet incorporated) is plotted as a function of time after beginning the initiation reaction (C.P.M.$_t$ is incorporation at time t; C.P.M.$_\infty$ is maximal incorporation). ATP concentrations displayed are as follows: 210 (■), 175 (○), 140 (▲), 70 (□), and 35 μM (●). Intercepts of slightly less than 1.00 represent either a minor component of the reaction which initiates rapidly or a small systematic error of unknown origin in the measurements.

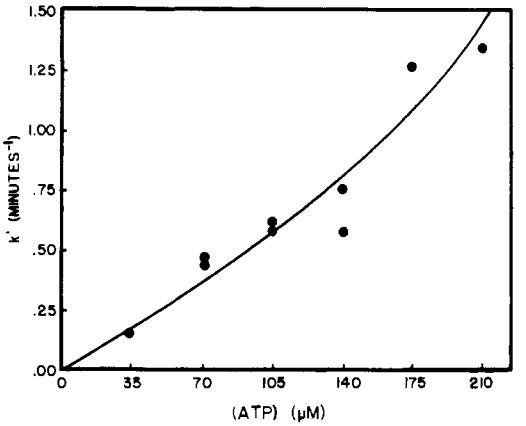

FIGURE 4: Pseudo-first-order rate constant for productive initiation as a function of the concentration of ATP. Initiation rate assays were performed as in Figure 2, and the data plotted as in Figure 3. Pseudo-first-order rate constants (k') were calculated from the half-times of the resulting linear semilog plots.

However, if the rate constants are calculated at comparable concentrations of all nucleotides, the *lac* UV5 promoter is seen to have an initiation rate constant $\sim$2 orders of magnitude lower than that for the T7 promoters. For example, at 35 μM ATP (and saturating concentrations of other nucleotides), the k' for *lac* UV5 initiation is 0.003 s^{-1}, while the estimated value for T7A$_1$ is $\sim$0.6 s^{-1}. The estimate for T7A$_1$ involves a short extrapolation to equivalent penultimate nucleoside triphosphate concentrations. This difference persists over the accessible range of ATP concentration (below 100 to 200 μM); above this range the rates of initiation become too fast to measure accurately by direct assay.

Rate of Abortive Initiation of Dinucleotide. In order to understand this difference in initiation kinetics, we must consider the factors which contribute to the rate of initiation. The rate of long RNA formation cannot, of course, exceed the rate of first phosphodiester bond formation. This latter rate should depend on the concentration of initiating nucleotide(s). McClure et al. (1978) have demonstrated such a nucleotide dependence using an "abortive initiation" assay (see below), which measures the rate of promoter-specific di-

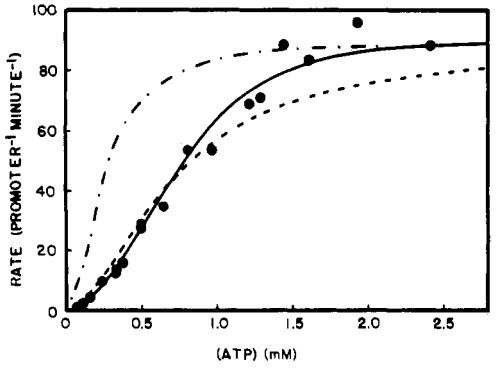

FIGURE 5: Rate of abortive initiation of dinucleoside tetraphosphate as a function of ATP concentration. The amount of pppApA produced was determined as described under Materials and Methods. Individual experiments are marked by circles (●), and the solid line (—) represents the observed dependence on ATP concentration. The dashed–dotted line (–·–·) represents the dependence of dinucleoside tetraphosphate formation on the nucleoside triphosphate concentration for λ P_L + P_R DNA template abortive initiation. This curve was calculated from the data of McClure et al. (1978) and represents the predicted rate of formation of pppApU at the ATP (and UTP) concentration specified on the ordinate. The dashed (- - -) line is a theoretical curve fit to the *lac* UV5 data based on the equilibrium-ordered mechanism deduced by McClure et al. for λ abortive initiation. The calculation assumes the $K_{i,A}$ for interacting with ATP is 1.8 mM, the value deduced by McClure et al. for λ DNA. Since the penultimate nucleotide (B) is UTP for λ but ATP for *lac*, K_B was then varied to obtained the best fit to the observed data in the sensitive region below 1 mM ATP. The equation used was $v = V_{max}[A][B]/(K_{i,A}K_B + K_B[A] + [A][B])$, and V_{max} was taken as 90 min^{-1} from the observed value for both *lac* and λ.

nucleotide synthesis. Therefore, one possible source of the slow productive initiation rate at the *lac* UV5 promoter would be unfavorable parameters governing the binding and condensation of the initiating nucleotides to form the first phosphodiester bond. The ATP dependence of the productive initiation rate is then most simply explained as a consequence of the ATP dependence of the rate of initial bond formation.

We have investigated this possibility by assaying the rate of production of pppApA, the predominant dinucleotide complementary to the *lac* UV5 initial RNA sequence (Maizels, 1973). We have used the abortive initiation assay (Johnston & McClure, 1976), in which specific nucleotides are excluded from the reaction in order to disallow extension of the RNA beyond the complementary dinucleoside tetraphosphate. ATP is simply added to preinitiation complexes formed as in the initiation rate assay, and the accumulation of pppApA is followed by paper chromatography (see Materials and Methods). The relative accumulation of pppApA was measured in this manner over a very wide range of ATP concentration. The results (Figure 5) show that, as expected, the rate of dinucleotide formation increases with increasing ATP concentration.

Strikingly, at low or moderate concentrations of nucleoside triphosphate, the rate of dinucleotide formation is much slower than the rates reported for the $T7A_1$ (Nierman & Chamberlin, 1979) and λ P_L and P_R (McClure et al., 1978) promoters. Only for the λ promoters was the rate studied over a wide concentration range; for the sake of comparison we show a calculated curve for the rate of dinucleotide formation from the λ promoters (Figure 5). These curves cover a much wider concentration range than can be used in a direct assay of productive initiation rate of long RNA (Figure 4), which is restricted to low to moderate concentrations of nucleotide. At these latter concentrations, the rate of *lac* dinucleotide formation averages 1 order of magnitude slower than the rate of λ dinucleotide formation. The limited information available concerning the $T7A_1$ promoter suggests that it may exhibit rates faster than both *lac* and λ.

Therefore, at substrate concentrations where the rate of *lac* UV5 productive initiation is slow, the rate of *lac* UV5 initial dinucleotide synthesis is also slow. Thus, in the simplest view, the *lac* UV5 promoter must initiate RNA production more slowly since the promoter-specific preinitiation complex can only slowly produce the initial dinucleotide of the message. We note, however, that the difference in the rate of dinucleotide synthesis disappears at saturating concentrations of ATP (Figure 5). In fact, although *lac* and λ rates differ by 1 order of magnitude at low to moderate concentrations of nucleotide, the two templates exhibit similar V_{max} values. Although the precise kinetic mechanism is not known, the coincidence of V_{max} values at saturating concentrations suggests that the *differences* in rates below saturation may be due to different parameters governing the binding of nucleoside triphosphates.

Whether or not this explanation is strictly correct, these experiments identify a reasonable source of the slow observed initiation rate, that is, the slow observed rate of initial dinucleotide formation. Therefore, we wished to explore the mechanism of dinucleotide formation. In a detailed study of a λ template, McClure et al. (1978) deduced a mechanism which was equilibrium ordered with respect to the binding of the two substrates, the ultimate (A) and penultimate (B) nucleoside triphosphates. The reaction velocity is thus a function of both substrate interaction constants ($K_{i,A}$ and K_B), substrate concentrations, and V_{max} (see the legend to Figure 5). In the case of the *lac* UV5 promoter, *both* species A and B are ATP (for λ, A is ATP and B is UTP), and it is therefore impossible to test the proposed mechanism directly by varying the concentration of each substrate independently in rate experiments.

Therefore, we used curve fitting to decide whether the mechanism deduced for λ also provides a reasonable description of the observed kinetics of *lac* dinucleotide formation. Since species A is ATP for both *lac* and λ, but species B is UTP for λ and ATP for *lac*, as the simplest case we assumed $K_{i,A}$ to be the same for both promoters, but K_B to be different. K_B was then varied in search of the best fit for the data in Figure 5 using the rate equation (see the legend). The dashed curve in Figure 5 shows that a reasonable fit to our data occurs when K_B = 250 μM and $K_{i,A}$ = 1.8 mM. Thus, the simple assumption that the *lac* and λ promoters are mechanistically similar but differ in rate of dinucleotide synthesis as a consequence of binding different penultimate nucleoside triphosphates leads to a reasonable and self-consistent description of the system. Thus, a simple but unproven explanation for the slower rate of *lac* dinucleotide formation is that the interaction of the penultimate nucleoside triphosphate with the preinitiation complex is highly disfavored (K_B = 250 μM compared to 30 μM for UTP in λ).

Comparison of Productive and Abortive Initiation. A comparative analysis of our data indicates that the slow rate of initial dinucleotide formation cannot account *entirely* for the slow rate of initiation of *lac* UV5 transcript. The results of two different direct assays allow independent estimations of the rates at which preinitiation complexes form both *lac* RNA and *lac* dinucleotides. Both rates increase with ATP concentration below 250 μM, which is the only range for which comparative data are available. However, the rate of RNA production is ~4–5-fold slower than the rate of dinucleotide production. For example, at 120 μM ATP, RNA is made at

the rate of 0.7/min while pppApA is made at the rate of 3/min; at 170 μM ATP, the respective rates are 1 and 5 per min. Thus, although the rate of initial dinucleotide synthesis is indeed slow, the rate of RNA formation is substantially slower. The elongation rate of the 67 nucleotide long transcript cannot contribute to this difference since our assay protocol eliminates any such contribution by allowing a lengthy elongation time in the presence of rifampicin *after* terminating the initiation reaction.

The 4–5 times slower rate of productive compared to abortive initiation should probably be considered a minimum estimate of the rate difference. For example, if the abortive initiation rate was limited in part by product dissociation, then the true rate of initial dinucleotide formation would be even faster than measured. Thus, the 4–5-fold difference quoted above would actually be accentuated. Below, we discuss a potential source of this difference.

Discussion

We have used direct assays for productive initiation of transcript and abortive synthesis of initiating dinucleotide to characterize the process of initiation of transcription at the *lac* UV5 promoter. These assays show that both processes are slower than reported at other promoters, and both rates increase with the concentration of the initiating nucleotide ATP. Since the rate of productive initiation (of long RNA) cannot exceed the rate of first bond formation, it is likely that the slow rate of productive initiation reflects in part the slow rate of initial bond formation. However, the rate of productive initiation is actually slower than predicted quantitatively from the dinucleotide production rate (see above). Below we discuss a potential molecular reason for this difference and then discuss some possible implications of promoter-specific variations in the rate of initiation.

Contributions to the Initiation Rate. We showed above that the rate of productive initiation is at least 5-fold slower than the rate of initial bond formation. However, we have shown elsewhere that the pathway to productive initiation involves synthesis of promoter-specific oligonucleotides (Carpousis & Gralla, 1980). From each preinitiation complex formed, on the average, several oligonucleotides are produced before the RNA polymerase escapes to produce long transcript. The enzyme does not dissociate from the template during this process and thus actually cycles during initiation. However, the important point is that this process requires that RNA polymerase form an initial phosphodiester bond several times (as part of oligonucleotides) for each RNA transcript ultimately produced. Therefore, the cycling of the enzyme should lead to a slowing of the productive initiation rate since the slow step of initial bond formation must be performed repeatedly. This emphasizes that both the extent of cycling and the rate of initial bond formation are potential contributing factors to an observed "initiation" rate. Quantitatively, our data are not sufficiently accurate to assess the relative contributions of these processes to the productive initiation rate in this system.

Thus, our view of the initiation process is as follows. RNA polymerase and DNA form a preinitiation complex which can lead to formation of the first phosphodiester bond. The rate of this process depends on the parameters governing interactions with the ultimate and penultimate nucleoside triphosphates and their concentrations. This step leads to the formation of ternary complexes, which allow premature chain termination and recycling of the RNA polymerase. However, since the probability of premature termination is less than 1, RNA polymerase eventually escapes from the cyclical production of oligonucleotides. The frequency of this cycling therefore determines the average number of times a first phosphodiester bond must be slowly formed prior to escape of the RNA polymerase to produce a full-length RNA transcript. Thus, the rate of RNA production from preinitiation complexes is dually regulated.

Our demonstration that the *lac* UV5 promoter is a slower initiator than the $T7A_1$ and A_2 promoters is easily understood in terms of this model. We have shown that the rate of initial bond formation is slower, possibly due to the disfavored binding of the penultimate nucleoside triphosphate. Cycling is frequent at the *lac* UV5 promoter (Carpousis & Gralla, 1980) but apparently infrequent at the $T7A_1$ (Nierman & Chamberlin, 1979) and $T7A_2$ (Nierman & Chamberlin, 1980) promoters. Thus, the combination of these two factors likely leads to the observed promoter-specific difference in initiation rate, which was inferred previously from less direct experiments (Stefano & Gralla 1979).

Contributions to Overall Transcription Rate. lac mRNA is synthesized in vivo at a rate of $\sim$20/min (Kennel & Riezman, 1977). Is this rate limited by the rate at which polymerase bound at the promoter initiates transcription? Or is it the rate at which polymerase binds the promoter which is limiting? Our results raise the possibility that there may be a delicate balance between the effects of these limitations.

If the initiation rate argument we have developed is valid, then one can extrapolate to predict a maximal initiation rate of RNA polymerase bound at this promoter in vitro of 18/min. This number is derived by assuming that the 5-fold difference between the productive and abortive initiation rates is maintained at saturating nucleotide concentrations (due to the effect of cycling). Thus, the initiation rate would be $^1/_5$ the V_{max} for initial bond formation (90/5 min^{-1}). Since we consider this type of quantitation to be very uncertain, this calculation only shows that the simplest assumptions lead to a maximal initiation rate in vitro which is similar to the estimated rate of RNA synthesis in vivo.

Alternatively, it might be the rate of promoter *binding* by RNA polymerase which determines the overall rate of RNA production. This rate has been estimated to be 3 or 4 per min at the *lac* UV5 promoter (Stefano & Gralla, 1979), somewhat slower than the in vivo estimate for RNA synthesis. The fact that the rate of promoter binding in vitro is slower than the maximal rate of initiation by bound polymerase in vitro could be construed as suggesting that binding is rate limiting in vivo. However, below saturating nucleotide concentration, initiation can be rate limiting in vitro [this paper and Stefano & Gralla (1979)]. Therefore, in vitro, the choice of conditions determines the rate-limiting step. Similarly, since either step alone could account for the in vivo rate, the possibility exists that depending on in vivo conditions, the rate-limiting step could be *either* binding or "initiation". Recently, we have determined that for various mutant *lac* promoters in a particular in vivo situation it is probably the binding rate which determines levels of gene expression (Stefano et al., 1980).

However, several considerations suggest that initiation rate is a potential point of control. Recall that the abortive initiation rate was slower at subsaturating ATP concentration for *lac* compared to λ. If the suggestion that *lac* UV5 is the slower initiator due to the disfavored binding of a penultimate purine nucleotide is correct, then promoters with penultimate purines such as *lac* would be expected to be slow initiators. Purine–purine starting sequences are relatively common and include lac i, lac z, trp, P_R', and ara C promoters [see Court & Rosenberg (1979) for references]. In general, promoters which deviate from purine–pyrimidine starts could be slower

initiators in vivo, especially if V_{max} is not achieved due to nucleotide inhibitors or submaximal nucleotide concentrations. This may be especially true of the *E. coli* ribosomal cistrons, all of which contain a promoter with CTP as the initiating nucleotide. A very high $K_{i,A}$ for this species could make the promoter subject to initiation control. The level of transcription in vitro does indeed depend on the concentration of CTP (Gilbert et al., 1979). Although initiation rate control of transcription is probably not typical, it may be important for certain promoters, especially under physiological conditions involving suboptimal activity of nucleoside triphosphates.

Acknowledgments

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Single-Stranded Poly(deoxyguanylic acid) Associates into Double- and Triple-Stranded Structures†

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ABSTRACT: Circular plasmid deoxyribonucleic acid (DNA), pBR322, was digested with the restriction endonuclease *PstI* to give full-length double-stranded DNA molecules, terminated by two self-complementary single-stranded sequences:

pG–C-T-G-C-A
A-C-G-T-C–Gp

The protruding 3′ termini were extended with dG by using calf thymus terminal deoxynucleotidyl transferase and dGTP, to form single-stranded tails of oligo(dG). At a length of about dG_{15}, such tails become resistant to single strand specific endonuclease S_1, and also cease to function as substrate (initiator) for the terminal deoxynucleotidyl transferase. This altered reactivity arises from association of the oligo(dG) tails into double- and triple-stranded structures, resulting in linear, circular, and branched polymers of the monomeric linear plasmid DNA. All these polymeric structures of the plasmid DNA are stable at room temperature, can be observed in the electron microscope, and can be separated from each other by agarose gel electrophoresis. At 60 °C or in 50% formamide, most of the oligo(dG) self-association can be reversed (melted), and the plasmid DNA is again found as the original linear monomer.

Among the principal nucleic acid components, it is notorously difficult to obtain experimental data on guanylic acid because of its low solubility and aggregation into viscous gels (Shapiro, 1968). An exact structure of its homopolymer remains uncertain. X-ray diffraction patterns for poly(rG) (Zimmerman et al., 1975) have been found to be virtually identical with those for poly(rI) (Rich, 1958; Arnott et al., 1974) and have been interpreted to represent four-stranded structures. Guanylic acid continues to reveal new and unpredicted properties, adding to our understanding of nucleic acids. For example, the structure of the self-complementary hexanucleotide d(CpGpCpGpCpG) has been unexpectantly established (Wang et al., 1979) to be an antiparallel left-

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