secreted VLDL showed one predominant and several minor high molecular weight apoproteins. The predominant apoprotein was characterized as having a molecular weight of approximately 300 000 (Tarlow et al., 1977). At present, it is not possible to tell whether this 300 000 mol wt apoprotein corresponds to apo-VLDL-B or to the major 290 000 mol wt cleavage product observed in serum VLDL (Figure 6, band 2).

A point of interest in relation to the proteolytic cleavage of rooster apo-VLDL-B concerns recent evidence that VLDL may play a role in blood coagulation. Human and rabbit VLDL (Bajaj et al., 1976; Ploplis et al., 1977), for example, appear to serve as lipid sources in the activation of prothrombin by factor Xa under some in vitro reaction conditions. Similarly, human VLDL decreases the partial thromboplastin clotting time in recalcified platelet free human plasma (Vijayagopal & Ardlie, 1978). It has been proposed that some of the coagulation reactions may occur on the surface of VLDL particles under these conditions (Bajaj et al., 1977). If a similar phenomenon occurs in rooster blood, coagulation proteases localized to the VLDL particle could explain the marked cleavage of apo-VLDL-B when prepared from serum.

Acknowledgments

The excellent technical assistance of Hanna Klett is gratefully acknowledged.

References

Bajaj, S. P., Harmony, J. A. K., Martinez-Carrion, M., & Castellino, F. J. (1976) *J. Biol. Chem. 251*, 5233.

Chan, L., Jackson, R. L., O'Malley, B. W., & Means, A. R. (1976) *J. Clin. Invest. 58*, 368.

Chapman, M. J., Goldstein, S., & Laudat, M.-H. (1977) *Biochemistry 16*, 3006.

Davies, G. E., & Stark, G. R. (1970) *Proc. Natl. Acad. Sci. U.S.A. 66*, 651.

Fairbanks, G., Steck, T. L., & Wallach, D. F. H. (1971) *Biochemistry 10*, 2606.

Goldstein, J. L., & Brown, M. S. (1977) *Annu. Rev. Biochem. 46*, 897.

Hearn, V., & Bensadoun, A. (1975) *Int. J. Biochem. 6*, 295.

Hillyard, L. A., Entenman, C., & Chaikoff, I. L. (1956) *J. Biol. Chem. 223*, 359.

Hillyard, L. A., White, H. M., & Pangburn, S. A. (1972) *Biochemistry 11*, 511.

Holdsworth, G., Michell, R. H., & Finean, J. B. (1974) *FEBS Lett. 39*, 275.

Jackson, R. L., Lin, H.-Y., Chan, L., & Means, A. R. (1977) *J. Biol. Chem. 252*, 250.

Keck, K., Grossberg, A. L., & Pressman, D. (1973) *Eur. J. Immunol. 3*, 99.

Laemmli, U. K. (1970) *Nature (London) 227*, 680.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265.

Luskey, K. L., Brown, M. S., & Goldstein, J. L. (1974) *J. Biol. Chem. 249*, 5939.

Means, G. E., & Feeney, R. E. (1971) *Chemical Modification of Proteins*, p 219, Holden-Day, San Francisco, CA.

Ploplis, V., Bajaj, S. P., & Castellino, F. J. (1977) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 36*, 306.

Schjeide, O. A. (1954) *J. Biol. Chem. 211*, 355.

Tarlow, D. M., Watkins, P. A., Reed, R. E., Miller, R. S., Zwergel, E. E., & Lane, M. D. (1977) *J. Cell Biol. 73*, 332.

Vijayagopal, P., & Ardlie, N. G. (1978) *Thromb. Res. 12*, 721.

Williams, D. L., Tseng, M. T., & Rottmann, W. (1978) *Life Sci. 23*, 195.

Lac UV5 Transcription in Vitro. Rate Limitation Subsequent to Formation of an RNA Polymerase–DNA Complex†

James E. Stefano* and Jay Gralla

ABSTRACT: The kinetics of transcription of *lac* UV5 mRNA using purified DNA restriction fragment as template has been studied. This template, which contains only 203 base pairs, directs the formation of a 67-base *lac* mRNA with high specificity. The half-time for formation of a DNA–RNA polymerase complex is approximately 0.2 min. However, upon addition of 200 μM nucleoside triphosphates to this complex, RNA production proceeds with a half-time of approximately 1 min. Therefore, it is suggested that the rate-limiting step for *lac* UV5 mRNA production, under typical in vitro conditions, occurs subsequent to the formation of a promoter-specific complex.

The extent of specific operon expression in bacteria is largely determined by control at the level of initiation of transcription. Changing metabolic conditions may lead to the altered availability of regulatory factors which enhance or diminish the rate of production of specific mRNAs. These factors modulate the intrinsic efficiency of operon promoters which depends in an unknown manner on DNA sequence. Therefore, the mechanism by which DNA sequence directs RNA polymerase to initiate specific transcripts has received much attention [for a review, see Chamberlin (1976)].

Most current models (Pribnow, 1975; Seeburg et al., 1977; Chamberlin, 1976) suggest that regulation occurs primarily during the formation of a specific complex between RNA polymerase and promoter DNA. The evidence for this is as follows. Observed rates of complex formation for T7 (Hinkle & Chamberlin, 1972) and *lac*[1] DNA (Majors, 1977) are

† From the Biochemistry Division of the Chemistry Department and Molecular Biology Institute, University of California, Los Angeles, Los Angeles, California 90024. *Received July 19, 1978.* Supported by Grant No. CA 19941 from the National Cancer Institute. James E. Stefano was supported as a trainee on Grant No. GM 07185.

0006-2960/79/0418-1063$01.00/0

slower than expected from simple bimolecular association with rates governed by diffusion control. DNA sequences have been identified which may partially direct complex formation (Pribnow, 1975; Schaller et al., 1975). Seeburg et al. (1977) have shown that the rate of complex formation varies among fd promoters, and the half-time can be as slow as 3 min. The existence of intermediate complexes has been inferred from extensive kinetic studies on T7 DNA (Mangel & Chamberlin, 1974b,c). Once the final complex (termed rapid-starting or RS) has been formed on T7 DNA, it rapidly initiates a mRNA chain when nucleoside triphosphates are added (Mangel & Chamberlin, 1974a). Therefore, it is assumed that the rate of promoter utilization is determined by a slow promoter-specific association of RNA polymerase with DNA, which is then followed by rapid initiation of an RNA transcript.

One uncertainty associated with this model is that it assumes generalized characteristics of individual systems; for example, the existence of rapid-starting complexes has been inferred from rifamycin-challenge experiments on whole T7 phage DNA. There is no information available concerning the rate of initiation of complexes at *Escherichia coli* promoters. Therefore, we have begun a series of studies designed to test various models of transcriptional regulation in a highly purified system which uses as template a short, sequenced (Gilbert & Maxam, 1973; Dickson et al., 1975) DNA fragment containing the UV5 *lac* promoter.

The UV5 *lac* promoter is well characterized both in vivo and in vitro and functions without the assistance of catabolite activator protein. The UV5 mutation, a second site revertant of a *lac* promoter-minus strain, is a two-base alteration (Gralla, unpublished observations) in the region of partial promoter homology (Pribnow, 1975; Schaller et al., 1975). Extensive in vitro binding (Gralla, unpublished observations; Reznikoff, 1976) and transcription (Maizels, 1973; Majors, 1977) data are available, and RNA polymerase–UV5 DNA complexes have been probed by DNase I protection (Gralla, unpublished observations) and dimethyl sulfate reactivity (Johnsrud, unpublished observations). The intact promoter may be obtained on unique DNA fragments by use of restriction endonucleases (Gilbert et al., 1975).

In this paper we report the results of several simple experiments bearing on the steps involved in promoter utilization in this well-characterized system. The experimental results are not consistent with the formation of a complex which rapidly initiates *lac* mRNA upon addition of nucleoside triphosphates. They suggest that the overall rate of transcription, under typical in vitro conditions, initiated from the UV5 promoter is not determined by the rate of complex formation, but is rate limited at a subsequent step. This contrasts with the observed characteristics of T7 DNA transcription (cited above). Therefore, the possibility must be considered that transcription may in some instances be regulated by promoter-specific initiation rates.

Experimental Procedures

Heparin and nucleoside triphosphates were purchased from Sigma (unlabeled), ICN (tritium label, sp act. ca. 25 Ci/mmol), and New England Nuclear (^{32}P labeled, sp act. ca. >100 Ci/mmol). *E. coli* RNA polymerase was obtained from Boehringer-Mannheim. We believe our transcription is due to holoenzyme rather than core for the following reasons. (1) We performed $NaDodSO_4$–gel electrophoresis which showed qualitatively high σ content. (2) Our transcripts are promoter specific and complexes are stable to heparin attack (Zillig et al., 1971). (3) A modification of the rifamycin-challenge assay showed an intercept characteristic of holoenzyme (Mangel & Chamberlin, 1974c).

Lac-containing plasmid constructed by F. Fuller (pMB9-UV5) was prepared by the method of Clewel (1972) with amplification of plasmid replication by treatment with 150 μg/mL of chloramphenicol. Briefly, cells were harvested and lysates prepared according to the method of Guerry et al. (1973). The lysates were stored overnight at 4 °C and centrifuged for 30 min at 17 000 rpm in an SS-34 rotor. The resulting supernatant was incubated for 1 h at 37 °C in the presence of 50 μg/mL of RNase and subsequently with 100 μg/mL of Pronase for 30 min at 37 °C. Protein was removed by chloroform/isoamyl alcohol (24:1) extraction. The plasmid DNA was ethanol precipitated and further purified by preparative CsCl buoyant density centrifugation for 48 h at 38 000 rpm in a 50 Ti fixed angle rotor.

pMB9-UV5 contains 203-base-pair *E. coli* control region DNA inserted in the Eco RI cleavage site in vector pMB9. To excise the *lac* insert DNA, the *lac*-containing plasmid was digested with Eco RI for 1 h at 37 °C in 10 mM Tris, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, pH 7.5, and loaded on an 8% polyacrylamide gel containing 0.27% bis(acrylamide), 50 mM Tris borate, pH 8.3, 1 mM EDTA. The gel was stained with 1 μg/mL of ethidium bromide, and the fragment was excised, crushed, and soaked in gel elution buffer containing 0.5 M NH_4OAc, 10 mM $Mg(OAc)_2$, 0.1% $NaDodSO_4$, 0.1 mM EDTA overnight at 37 °C. After ethanol precipitation, the extracted restriction fragment was cleaned from contaminating acrylamide polymer by DEAE-cellulose chromatography according to Hirsch & Schlief (1976). The column eluate was dialyzed against 1 M NH_4OAc and 10 mM MgOAc and then precipitated by addition of 2 volumes of ethanol as noted above. The dried pellet was dissolved in 100 μL of TE and the spectrum was determined on a Beckman Model 25 spectrophotometer, using a 0.2-mm cell width cuvette previously treated with dichlorodimethylsilane. The concentration was determined using 50 μg/mL per A_{260} unit, which is within 3% of the extinction coefficient calculated by the method of Gray & Tinoco (1970).

Transcription was in the following buffer with exceptions as stated in the figure legends: 30 mM Tris-HCl, pH 8.0, 100 mM KCl, 3 mM $MgCl_2$, 0.1 mM EDTA, 0.2 mM dithiothrietol, 0.5 mg/mL of iodoacetate-treated bovine serum albumin. Reactions were typically 25 or 50 μL with 200 μM CTP, ATP, GTP and 10 μM labeled UTP except as noted in the figure legends. Incorporation was followed by adding aliquots (usually 4 μL) to 2 mL of cold 7.5% trichloroacetic acid and 0.1% pyrophosphate and collection of the precipitate on glass fiber filters. Samples were prepared by gel analysis by the procedure of Majors (1977) except the second precipitation was omitted. Denaturing polyacrylamide–urea gels were constructed according to Maniatis et al. (1975). Autoradiography involved Cronex 4 X-ray film with image intensification by Du Pont Quanta II screen at −70 °C. After autoradiography, radioactive bands were excised from the gel and counted by Cerenkov radiation.

Results

Specificity of Transcription. The specific RNA transcripts produced with various assay conditions using restriction fragment as template are displayed in Figure 1. Notice that

[1] Abbreviations used: DEAE, diethylaminoethyl; DNase, deoxyribonuclease; DTT, dithiothrietol; EDTA, ethylenediaminetetraacetic acid; *lac*, lactose operon; RNase, ribonuclease; $NaDodSO_4$, sodium dodecyl sulfate; TE, 10 mM Tris-HCl, 1 mM EDTA, pH 7.4; Tris, tris(hydroxymethyl)aminomethane.

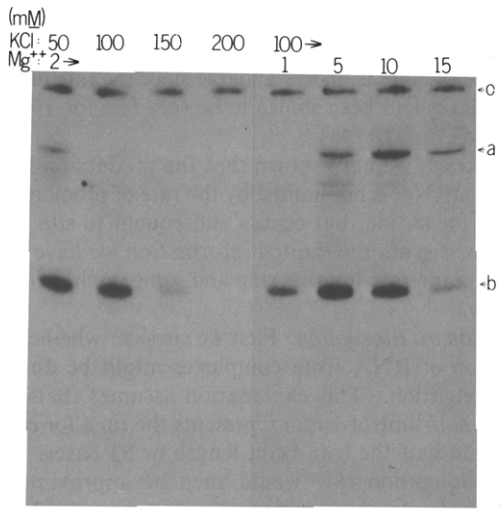

FIGURE 1: Urea–acrylamide gel electrophoresis patterns of transcription products. Ten-minute reactions were run under conditions given in the text except as indicated. The major product (b) and the minor product (a) used for comparison are approximately 67 and 185 nucleotides in length, respectively. The major product corresponds to properly initiated *lac* mRNA.

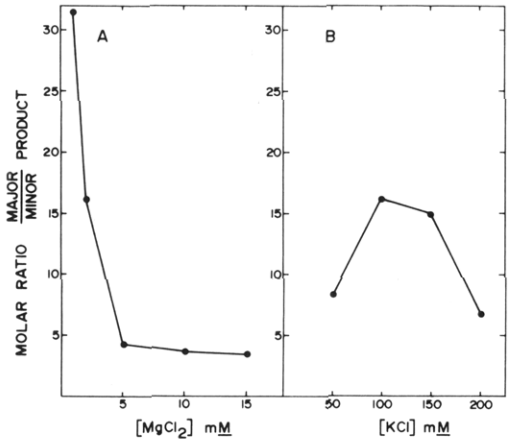

FIGURE 2: Molar ratios of major to minor products shown in Figure 1. Gel bands were cut out and counted by Cerenkov radiation. (A) Transcription at 100 mM KCl. (B) Transcription at 2 mM $MgCl_2$.

both the specificity and quantity of transcripts vary with solution conditions. In each case the major species produced is approximately 67 nucleotides in length, which represents properly initiated UV5 *lac* mRNA terminated near the end of the restriction fragment [see Majors (1977), for example]. Higher $MgCl_2$ and lower KCl concentrations enhance transcription of minor species; the most prominent of these appears to be an improperly initiated nearly end-to-end transcript of the restriction fragment. Production of this latter species at higher $MgCl_2$ concentrations may be due in part to the presence of the four unpaired bases at each 5′ DNA terminus.

We have chosen to perform our experiments using solution conditions which select for transcript specificity over quantity. The data in Figure 2 require that we use low $MgCl_2$ and intermediate KCl concentrations, similar to conditions used by Majors (1977). Under these conditions acid-precipitable RNA production reflects specific transcription. Fewer than one in ten RNA chains produced under these conditions are abnormal.

Rate of Complex Formation. We determined the half-time for formation of a stable RNA polymerase–UV5 DNA complex to be approximately 0.2 min. In this experiment (Figure 3), DNA and RNA polymerase are mixed at zero

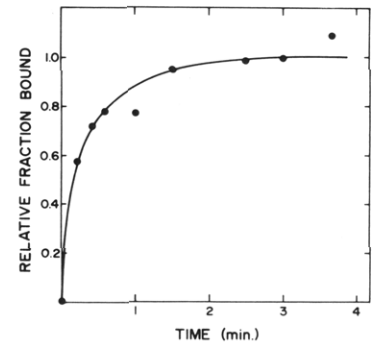

FIGURE 3: Kinetics of association of RNA polymerase with the promoter fragment. RNA polymerase (2.5 pmol) was added to a reaction mixture containing ^{32}P end-labeled restriction fragment (1.25 pmol). Aliquots were removed at the times indicated and added to 1 mL of prewarmed transcription buffer (except containing 20 μg/mL of BSA) plus 100 μg/mL of heparin. These solutions were passed through 1-cm diameter BA-85 nitrocellulose filters which were then rinsed with 100 μL of buffer, dried, and counted. Total reaction volume was 25 μL. The extent of the reaction at each time did not vary when the volume was increased to 100 μL (data not shown).

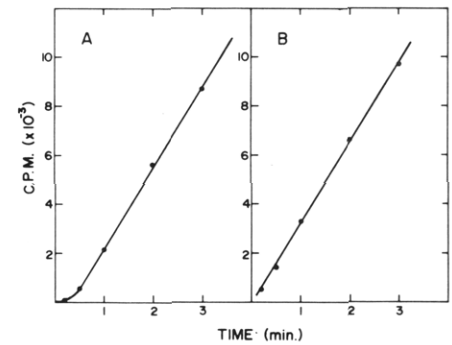

FIGURE 4: Kinetics of incorporation into acid-insoluble product. (A) Reaction initiated by addition of polymerase. (B) Reaction initiated by addition of NTP's, after preincubation of fragment and RNA polymerase for 10 min.

time, and aliquots are subsequently added to a heparin solution to inactivate enzyme which has not yet bound DNA. The mixture is then passed through a nitrocellulose filter which retains DNA–protein complexes. Thus a promoter-specific complex is formed as evidenced by resistance to attack by heparin and ability to bind filters. The observed half-time for association is orders of magnitude faster than the dissociation half-time (Gralla, unpublished observations). Majors (1977) reaches the same conclusion, using a different assay for complex formation.

Free Start vs. Prebinding Transcription. We have measured the rate of production of transcript by two methods. Figure 4A shows that when RNA polymerase is added to a solution containing DNA and nucleoside triphosphates (free start conditions), there is a short lag, followed by slow production of acid-precipitable RNA product. This lag time is unexpected since we know that complexes have formed during this time (cf. Figure 3). If one assumes that complexes once formed initiate and elongate rapidly, acid-precipitable product should be appearing. Since no product appears, these assumptions may not be justified.

In order to further examine this issue we performed a prebinding experiment to test whether *preformed* complexes would rapidly form acid-precipitable product upon addition of nucleoside triphosphates. The above-mentioned assumptions predict an initial "burst" of incorporation by preformed complexes, followed by a slower "steady-state" rate of incorporation determined by the rate of formation of new complexes. That is, if complex formation is rate limiting,

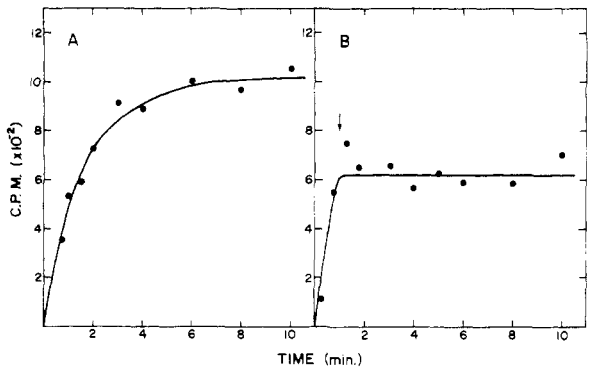

FIGURE 5: Kinetics of incorporation from a single round of transcription. Complexes were allowed to form during a 10-min preincubation. Heparin was then added to 100 μg/mL, followed 30 s later by addition of nucleoside triphosphates. (A) GTP, ATP, UTP added to 200 μM, CTP (6.2 Ci/mmol) added to 100 μM. (B) An experiment identical with (A) except rifamycin was added 1 min after addition of nucleoside triphosphates to a final concentration of 100 μM.

preformed complexes should synthesize rapidly amounts of product which are made more slowly under steady-state conditions.

We observed no immediate burst of transcription from preformed complexes (Figure 4B). Instead the rate of incorporation was linear for several minutes, extrapolating to zero incorporation at zero time. The simplest explanation is that the rate of transcription is determined by a step *subsequent* to complex formation, contrary to our assumptions. This rate limitation could be either initiation of RNA chains by complexes or, less likely, elongation of these 67-base-length transcripts.

Direct Measurement of Initiation Rate. These possibilities are not distinguishable on the basis of these experiments, in part due to the possible effects of reinitiation by free RNA polymerase and the 10 μM UTP concentration. We minimized these effects by performing a prebinding experiment with transcription at higher nucleoside triphosphate concentration and in the presence of heparin, which inactivates free RNA polymerase. Figure 5A shows directly that preformed RNA polymerase–DNA complexes form product slowly upon addition of substrate ($t_{1/2}$ ca. 1 min).

This experiment shows unambiguously that the production of RNA from complexes is slower than formation of complexes (cf. Figure 3). Futhermore, the rate-limiting step is likely to be initiation since completion of the short 67-base RNA should be very rapid at the nucleoside triphosphate concentration used (see Discussion). This statement is supported by the experiment shown in Figure 5B which demonstrates that the formation of product is sensitive to rifamycin. Preinitiation but not elongation complexes are known to be inactivated by rifamycin (Sippel & Hartmann, 1968). Therefore, if all complexes had initiated but were elongating slowly, rifamycin would not inhibit the reaction. We suggest that rifamycin has inactivated those complexes which had not yet initiated at the time of drug addition.

Discussion

The particular advantage of the UV5 *lac* system used here is that the template contains a single promoter which directs a short, specific RNA species. Therefore, one may maximize for transcription specificity without complications due to the presence of multiple promoters or multiple transcripts. Most previous studies of the initiation mechanism have relied on whole phage templates [for a review, see Chamberlin (1976)]. Several recent studies using restriction fragment templates investigated complex formation rather than initiation (Majors, 1977; Seeburg et al., 1977; Reznikoff, 1976). The focus of these latter studies is understandable since the rate of initiation by complexes had been shown to be very fast for T7 (Mangel & Chamberlin, 1974a).

In contrast, we have shown that the production in vitro of UV5 *lac* mRNA is not limited by the rate of promoter-specific complex formation, but occurs subsequent to site selection. Below, we discuss the limited information we have available concerning the rate-limiting step and some implications of this discovery.

Initiation vs. Elongation. First we consider whether the slow production of RNA from complexes might be due to slow chain elongation. This explanation assumes that the transcription half-time of 1 min represents the time for complexes to elongate half the transcript length or 33 bases. The calculated elongation rate would then be approximately 0.5 nucleotides/s. In general, elongation rates are determined by the reaction of elongating complexes with substrate nucleoside triphosphates. Therefore, the rate depends on the nucleotide concentration. Rhodes (1974) has collected available literature data concerning elongation rates; these data vary widely and include measurements on many different templates. However, even a worst-case application of these data predicts an elongation rate more than 20 times faster than our rate calculated assuming elongation to be rate limiting (our nucleotide concentration was 200 μM, except CTP at 100 μM, Figure 4). Since relatively rapid elongation seems to be a general phenomenon, we conclude that our slow observed transcription is not likely to be rate limited by elongation. This conclusion is supported by the demonstrated sensitivity of the slow synthesis to rifamycin; elongation complexes should be resistant.

The alternative explanation is that the slow observed transcription represents the rate at which RNA polymerase–UV5 promoter complexes initiate when presented with nucleoside triphosphates. An initiation rate has been estimated by an indirect method using whole phage T7 DNA (Mangel & Chamberlin, 1974a) and found to be more than two orders of magnitude faster than the rate we observe using UV5 *lac* restriction fragment. This rapid rate has been derived from study of a single template, and other templates may well be different. The simplest interpretation of our data is that initiation of transcription by RNA polymerase–UV5 DNA complexes is slow and rate limiting for overall UV5 mRNA synthesis in vitro.

Nature of Rate Limitation

Strictly, the rate-determining step must occur subsequent to complex formation and likely occurs prior to formation of a continuously elongating complex. One possible explanation would be that abortive initiation complexes (Johnston & McClure, 1976) form at the promoter and only slowly escape into elongation complexes. Such a mechanism would predict the formation of small oligoribonucleotides in molar excess over transcript. Our preliminary gel analyses have not shown such products, but they might easily be obscured by the large excess of unreacted radioactive nucleoside triphosphate. Although we cannot exclude this possible mechanism, we consider it to be unlikely since all four nucleoside triphosphates are present in moderate concentration. The simplest explanation is that the dominant form of complexes formed in the absence of nucleoside triphosphates cannot rapidly initiate an RNA chain upon addition of a moderate concentration of substrate.

The rate-limiting mechanistic step in vitro may depend on

the choice of solution conditions. For example, we determined the complex formation rate at a single typical concentration of components and the "initiation" rate at a single typical nucleotide concentration. It is possible that each of these rates might increase along with the concentration (see Mangel & Chamberlin, 1974a). Therefore, we suggest that in vitro transcription experiments involve actual measurement of both rates rather than simply assuming their relative magnitude.

This result has been obtained with holoenzyme rather than with core which has been inferred to initiate slowly. Our enzyme has a high σ content and forms complexes with DNA which are resistant to heparin; core–DNA complexes are reported to be heparin sensitive (Zillig et al., 1971). Furthermore, our transcription is highly promoter specific.

Models for Promoter Utilization. The conclusion that complex formation is not rate limiting for this system forces us to reevaluate models for promoter utilization (see references in the introduction). The assumption that the rate of site selection determines the efficiency of promoter utilization in vitro is inconsistent with our results for the UV5 *lac* promoter. However, we have shown that the rate of site selection is of similar magnitude to other promoters and we have no reason to doubt that this rate may vary among promoters and influence mRNA levels. Furthermore, our results do not address the question of the nature of steps leading to site selection. Since completion of all of these steps is faster than our observed initiation rate, they are of secondary importance in UV5 *lac* mRNA production in our in vitro system.

Can promoters specify the rate at which bound RNA polymerase initiates transcription and thereby influence the level of maximal operon expression? Such a conclusion is clearly premature, though the measured UV5 *lac* rate clearly differs from that of T7 DNA. However, the two rates were measured by very different experiments. Furthermore, our experiments are qualitative and do not definitively characterize the slow observed rate. This will require extensive studies involving variation in concentration of each nucleotide, sensitivity to inhibitors, and observation of RNA species produced during the reaction.

Other Implications. Our observation of a slow initiation rate at the UV5 *lac* promoter has serious implications concerning measurements of transcription rates using less well-defined templates. For example, experiments of the type reported here would not be interpretable using whole phage DNA templates. Slow initiation from one promoter would be masked by rapid initiation at others. Direct measurement of the initiation half-time (as in Figure 5) would be obscured by the time required to elongate lengthy, possibly heterogeneous transcripts. Rifamycin-challenge experiments on various templates must also be very carefully interpreted. In these experiments one forms complexes and then challenges with a mixture of nucleoside triphosphates and rifamycin (Mangel & Chamberlin, 1974a). If initiation is rapid, as with T7 DNA, transcription proceeds, while if it is slow, as in *lac* UV5, transcription is halted. Thus, in experiments which measure simple incorporation, synthesis is dominated by the fastest initiating component in a multipromoter system. This assay has been adapted [for example, see Hirose et al. (1976)] to measure the number of initiation sites for *E. coli* RNA polymerase in chromatin. We urge caution in the application of this adapted assay since any sites with slow initiation rates will be seriously underestimated.

References

Chamberlin, M. J. (1976) in *RNA Polymerase*, Vol. 159, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Clewel, D. B. (1972) *J. Bacteriol. 110*, 66.

Dickson, R. C., Abelson, J., Barnes, W. M., & Reznikoff, W. S. (1975) *Science 187*, 27.

Gilbert, W., & Maxam, A. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 3581.

Gilbert, W., Gralla, J., Majors, J., & Maxam, A. (1975) in *Protein-Ligand Interactions* (H. Sund & G. Blauer, Eds.) pp 193–210, Walter de Gruyter & Co., Berlin.

Gray, D. M., & Tinoco, I. (1970) *Biopolymers 9*, 223.

Guerry, P., LeBlanc, D. J., & Falkow, S. (1973) *J. Bacteriol. 116*, 1061.

Hinkle, D. C., & Chamberlin, M. J. (1972) *J. Mol. Biol. 70*, 187.

Hirose, M., Tsai, M., & O'Malley, B. (1976) *J. Biol. Chem. 251*, 1137.

Hirsch, J., & Schleif, R. (1976) *J. Mol. Biol. 108*, 471.

Johnston, E. E., & McClure, W. R. (1976) in *RNA Polymerase*, Vol. 413, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Maizels, N. M. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 3585.

Majors, J. (1977) Doctoral Dissertation, Harvard University, Cambridge, MA.

Mangel, W. F., & Chamberlin, M. J. (1974a) *J. Biol. Chem. 249*, 2995.

Mangel, W. F., & Chamberlin, M. J. (1974b) *J. Biol. Chem. 249*, 3002.

Mangel, W. F., & Chamberlin, M. J. (1974c) *J. Biol. Chem. 249*, 3007.

Maniatis, T., Jeffrey, A., & Van de Sande, H. (1975) *Biochemistry 14*, 3787.

Pribnow, D. (1975) *J. Mol. Biol. 99*, 419.

Reznikoff, W. S. (1976) in *RNA Polymerase*, Vol. 441, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Rhodes, G. (1974) Doctoral Dissertation, University of California at Berkeley.

Schaller, H., Gray, C., & Herrmann, K. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 737.

Seeburg, P. H., Nüsslein, C., & Schaller, H. (1977) *Eur. J. Biochem. 74*, 107.

Sippel, A., & Hartmann, G. (1968) *Biochem. Biophys. Acta 157*, 218.

Zillig, W., Zechel, K., Rabussay, D., Schachner, M., Sethi, V. S., Palm, P., Heil, A., & Seifert, W. (1971) *Cold Spring Harbor Symp. Quant. Biol. 35*, 47.