sistently applied as new structures are included in the reference data set.

By independently checking the claim [Williams, 1983; retracted in Williams (1986)] that the present set of reference spectra allows distinction between parallel and antiparallel β-structure (it does not), we gain a clear statement of the accuracy one can expect in routine application of Raman spectroscopy to globular proteins.

Most likely the accuracy of the Raman-derived secondary structure estimate could be significantly improved by extending the set of reference spectra. It seems particularly important to include proteins of all topological classes, especially of proteins containing domains with both α-helices and β-sheets, such as parallel β-barrels and nucleotide binding folds. It is our hope that several of the groups involved in Raman spectroscopy of proteins will help to improve the method by contributing new reference spectra of proteins of known structure.

ACKNOWLEDGMENTS

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Registry No. DNase, 9003-98-9.

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Sequence-Dependent Termination of Bacteriophage T7 Transcription in Vitro by DNA-Binding Drugs†

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ABSTRACT: An in vitro T7 bacteriophage transcription system has been utilized in which the RNA was initiated to a specific length (defined by the absence of the appropriate nucleoside triphosphate). When the DNA–RNA–RNA polymerase ternary complex was exposed to nonsaturating levels of DNA-binding ligands (i.e., a small fractional occupancy at each site), and the RNA transcript then allowed to elongate in the presence of all four nucleoside triphosphates, there was a synchronous increase of RNA lengths up to sites occupied by ligands. A unique characteristic is that bacteriophage transcription was completely terminated at every ligand site, in contrast to bacterial RNA polymerases where "read-through" past drug sites occurs and results merely in a delay of transcription at each site due primarily to dissociation of drug from the DNA. Similar termination of transcription at each drug site was observed with T3 and SP6 RNA polymerases. The termination at drug sites in the bacteriophage system results in RNA of specific lengths which define the location of ligand sites, and the RNA concentration provides a measure of relative ligand occupancy at that site. Termination of transcription was observed with four drugs with relatively long DNA residence times (half-life ≥300 s at 20 °C for nogalamycin, actinomycin, mithramycin, and echinomycin) but to a lesser extent with drugs of intermediate residence times [a bis(thiadaunomycin) and an acridine–tripyrrole, with half-lives of 230 and 7 s, respectively, at 20 °C].

The technique of footprinting has been widely employed in recent years to define the sequence specificity of DNA-binding drugs and proteins. Several different DNA cleaving agents and procedures have been employed [e.g., DNase I, MPE-Fe(II), and EDTA-Fe(II)], and these have recently been reviewed (Dabrowiak, 1983; Dervan, 1986; Tullius, 1987). This technique has been especially successful in delineating the boundaries of large ligands with long residence times, such as specific DNA-binding proteins. For example, the size of

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the initiation and elongation complexes of *Escherichia coli* RNA polymerase has been defined as 70 and 30 base pairs (bp),[1] respectively, by DNase I footprinting (von Hippel et al., 1984).

Unfortunately, the technique of footprinting suffers several major limitations when applied to drug–DNA interactions (Fox & Waring, 1984; Lown et al., 1986). We have recently been developing an alternative procedure which offers advantages compared to classical footprinting. This method is based on an in vitro transcription assay employed to define the sequence specificity and dissociation kinetics of DNA-binding drugs (Phillips & Crothers, 1986; White & Phillips, 1988). These studies employed a bacterial RNA polymerase which yielded a stable ternary complex (DNA–RNA–RNA polymerase) but resulted in "read-through" of transcription from one drug site to the next. In the process of developing and examining the in vitro transcription assay, we utilized T7 RNA polymerase and the late promoter of bacteriophage T7 that had been cloned into a commercially available vector.

The molecular properties of RNA polymerase coded for by T7 and T7-like bacteriophages (T3 and SP6) have been reviewed by Chamberlin and Ryan (1982). However, a full understanding of the molecular events associated with complexes with promoter regions, and subsequent transcriptional events, is the subject of extensive current investigation (Basu & Maitra, 1986; Ikeda & Richardson, 1986; Smeekins & Romano, 1986; Gunderson et al., 1987; Shi et al., 1988). Less is known about phage RNA polymerase than bacterial RNA polymerases at the present time because the phage polymerases form less stable binary and ternary complexes and also because their RNA chain elongation rate and initiation rates are substantially faster (Chamberlin & Ryan, 1982).

We present here the results obtained using bacteriophage RNA polymerases in an in vitro transcription system. These phage transcription systems were unique in that no "read-though" was apparent at any drug-induced block site. These phage systems therefore serve as a simple, rapid means of defining the sequence specificity of DNA-binding ligands and also yield a quantitative ranking of relative affinity for those specific sites. The observed termination of transcription of these phage systems also suggests a new approach to the design of selective antiviral agents.

Materials and Methods

Materials. Actinomycin D was purchased from Calbiochem, and mithramycin was purchased from Sigma while echinomycin and nogalamycin was kindly supplied by Dr. L. P. G. Wakelin of the Cancer Institute, Melbourne, Australia. The bis(thiadaunomycin) and acridine–tripyrrole ($m = 2$, $n = 2$) were synthesized in our laboratories (Skorobogaty et al., 1988a; Eliades et al., 1988); adriamycin was a gift from Farmitalia Carlo Erba, Milan. T7 RNA polymerase, alkaline phosphatase (intestinal mucosa), ultrapure ribonucleotides, 3′-deoxynucleotides, ribonuclease inhibitor (human placenta), and BSA (RNase/DNase free) were obtained from Pharmacia. [α-^{32}P]ATP (3000 Ci/mmol and 10 mCi/mL) and X-ray film (Hyperfilm-β-Max) were obtained from Amersham. Urea, bis(acrylamide), acrylamide, ammonium persulfate, and TEMED were obtained from Bio-Rad as electrophoresis purity reagents. Restriction endonucleases were obtained from Boehringer Mannheim. T4 DNA ligase and SP6 RNA polymerase were obtained from Bresatec, Australia. The cloning vector pGEM-1 was obtained from Promega, T3 RNA polymerase was obtained from Bethesda Research Laboratories, and the cloning vector pIBI31 was from IBI. All other chemicals were analytical grade, and all solutions were prepared using distilled deionized and filtered water from a "Milli-Q" four-stage water purification system (Millipore).

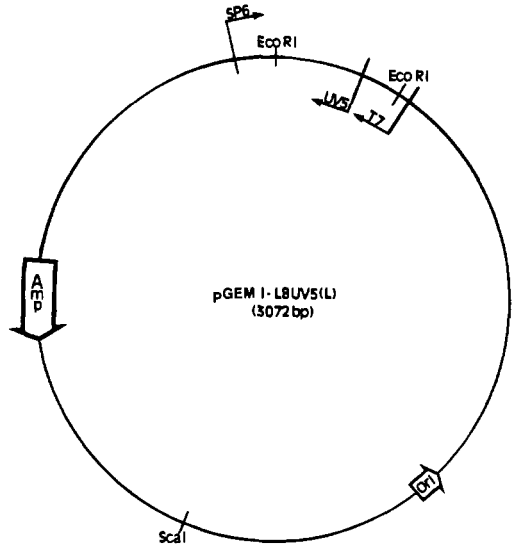

FIGURE 1: Plasmid pGEM-1-L8UV5(L) containing the bacteriophage promoters SP6 and T7.

DNA Source. A 203 bp *Eco*RI restriction fragment of *lac* DNA containing the L8-UV5 double mutant was supplied by Professor D. M. Crothers (Yale University). The 203 bp fragment was ligated into the unique *Eco*RI site of pGEM-1, following treatment of the vector with alkaline phosphatase. The desired orientation was selected for by restriction enzyme analysis and confirmed by RNA sequence analysis of the transcript derived from the T7 promoter, using the methods described below. The resulting plasmid, denoted pGEM-1-L8UV5(L) (Figure 1), was transformed into *E. coli* JM101 by using the calcium chloride/rubidium chloride procedure of Hanahan (1983). Harvesting of the plasmid involved amplification with 170 μg/mL chloramphenicol and isolation using a modification of the alkaline lysis procedure of Maniatis et al. (1982). The isolated plasmid was digested with *Sca*I to linearize the plasmid and to produce a full-length transcript of approximately 1850 bases, and used in studies with T7 RNA polymerase and SP6 polymerase. For studies with T3 RNA polymerase, the vector pIBI31 was also linearlized with *Sca*I to produce a full-length transcript of approximately 1200 bases.

Concentration and integrity of the DNA were determined by ethidium bromide titration by comparison to a plasmid standard determined spectrophotometrically with $E_{260} = 6600$ M^{-1} cm^{-1}.

In Vitro Transcription. The transcription buffer (Tc buffer) used was a modification of that used by Davanloo et al. (1984), containing 40 mM Tris-HCl (pH 7.9), 20 mM $MgCl_2$, 4 mM spermidine, 10 mM DTT, 500 μg/mL BSA, and 1.5 units/μL RNase inhibitor. A ternary complex was formed by incubating the linearized plasmid with T7 RNA polymerase in the presence of 200 μM GTP and 5 μM ATP and CTP, as well as [α-^{32}P]ATP at 37 °C for 10 min. The low level of NTPs was employed to maximize incorporation of the label and hence improve the sensitivity of the analysis (White & Phillips, 1988) and also to maximize the extent of a synchronized population of initiated complexes (i.e., to minimize misincorporation and/or effects of contaminating nucleotides). These conditions

[1] Abbreviations: BSA, bovine serum albumin; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; RNase, ribonuclease; TBE, Tris–borate–EDTA; bp, base pair(s).

resulted in a high yield of ternary complex with a nascent RNA of between 12 and 17 nucleotides due to exclusive initiation at the +1-position, but with apparent misincorporation to produce nascent RNA greater than the 12 bases expected on incubation with the 3 nucleotides. The initiated complex was then adjusted to 200 mM NaCl, to minimize nonspecific binding of the drugs as well as to minimize pausing and recycling by T7 RNA polymerase. The complex was then divided into two portions; to one was added the appropriate drug to a concentration between 0.5 and 15 μM (determined empirically as one which resulted in fractional occupancy at each drug-binding site) while to the other an equal volume of transcription buffer, followed by an incubation at 37 or 5 °C for 15 min. It should be noted that the drug:bp ratio cannot be directly compared to other studies since an unknown amount of the drug will be bound to the large amount of protein present in the transcription buffer. Following equilibration with drug, a nucleotide mix containing high levels of all nucleotides was added (to give a final concentration of all four nucleotides of 2.0 mM), the incubation was continued at 37 or 5 °C, and aliquots were removed at appropriate times. To terminate the reaction, and in preparation of electrophoresis, an equal volume of a solution containing 90% formamide, 50 mM EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue was added to the aliquots.

Transcription studies with SP6 and T3 RNA polymerases were carried out in a buffer similar to that used for T7 RNA polymerase, except that the levels of $MgCl_2$ and spermidine were reduced to 6 and 2 mM, respectively, while NaCl was included at 10 mM. When T3 RNA polymerase was used, an initiated complex was formed by incubating the polymerase and the linearized pIBI31 plasmid in the presence of 200 μM GTP and 5 μM ATP, UTP, and [α-^{32}P]ATP (to produce an 11-mer). For the SP6 RNA polymerase system, "run-off" transcripts were formed since a stable initiated complex could not be generated in the presence of GTP, ATP, and UTP (which produced a 5-mer) or by ApG, ATP, CTP, and UTP (which produced a 9-mer), when using the linearized pGEM-1-L8UV5(L) plasmid.

RNA Sequencing. Sequencing of the RNA was conducted to allow the location of drug-binding sites by direct comparison to sequencing lanes. The method used was a modification of Axelrod and Kramer (1985) using 3′-deoxyadenosine 5′-triphosphate and 3′-deoxycytidine 5′-triphosphate. To obtain sequence information over the first 200 nucleotides, a portion of the initiated complex was divided into two parts; to one was added an elongation nucleotide mix containing 500 μM 3′-dATP and 1 mM ATP, while to the other was added a mix containing 300 μM 3′-dCTP and 1 mM CTP. Each was elongated at 37 °C for 10 min and stopped by the addition of an equal volume of gel loading buffer.

Gel Electrophoresis of RNA. Following termination of the elongation and sequencing reactions, samples were placed at 90 °C for 5 min and then placed on ice. Samples were loaded onto a 0.35 mm × 40 cm 12% denaturing gel [19:1 acrylamide:bis(acrylamide) ratio] in TBE buffer (Maniatis et al., 1982). Gels were preelectrophoresed for 2 h at 2250 V and 100 W to bring the temperature of the gel to approximately 60 °C to ensure denaturing conditions. Electrophoresis was continued until the xylene cyanol had migrated approximately two-thirds the way down the gel.

Following electrophoresis, gels were fixed in 10% acetic acid/10% methanol for 30 min and dried for 30 min at 80 °C using a Bio-Rad 583 gel dryer. Autoradiography using Amersham Hyperfilm-β-Max or Kodak XAR-5 X-ray film without intensifying screens usually required an overnight exposure at room temperature.

Table I: Initiation Sequences of Bacteriophage Promoters[a]

promoter source	transcribed sequence
SP6[b,c]	+1 ↓ AGAATACAAGCTTGG
T3[c]	+1 ↓ AGGGAGAGAATTCGAGCT
T7[b,c]	+1 ↓ AGGGAGACCGGAATTCCGA

[a] Nucleotides present at high concentration in the initiation mixture have been underlined. The first nucleotide normally incorporated into RNA is denoted as +1. The first occurrence of a base not present in the initiation mixture is shown with an arrow and defines the major RNA sequence (up to that point) present in the initiated ternary complex. [b] Melton et al. (1984). [c] Chamberlin and Ryan (1982).

Quantitation of RNA. Autoradiograms were scanned with a Biomed SL-504-XL laser densitometer linked to a Spectra Physics SP4270 integrator. Each drug-induced inhibition site was expressed as a fraction of the total radioactivity in that lane, and these values therefore represent the mole fraction of RNA of differing lengths (A_i).

Since the value of A_i will be underestimated after the first drug-induced blockage site (i.e., less RNA polymerase is able to reach other downstream drug sites), a correction must be applied to the value of A_i for all subsequent sites. The corrected relative concentration of RNA lengths (A'_i) is defined by

$$A'_i = \frac{A_i}{1 - \sum_{1}^{i-1} A_i}$$

and allows for the probability that the i'th site lies downstream of at least one of the $i - 1$ upstream sites which will block the RNA polymerase. The corrected relative RNA concentrations were summed and then normalized as a percentage and plotted in Figure 4 at each drug-induced blockage site.

Results

Selective initiation of the T7 promoter from the +1-position would ideally result in all RNA in the initiated complex being 12 bases long (Table I). Considerable misincorporation of nucleotides appears to occur, and the RNA exists as 12–17-mers (data not shown), with no evidence of RNA of any greater length (Figure 2). Addition of all four nucleoside triphosphates resulted in rapid elongation to mainly full-length transcripts, with only minimal pausing evident for transcripts less than 200 bases in length (Figure 2). Elongation was complete within 3 min, and additional elongation times did not reveal any significant decrease of pausing (Figure 2). A comparison of the initiation to elongation lanes also revealed that a high proportion of initiated complexes did not elongate (data not shown).

Sequence Specificity. When different DNA-binding drugs were added to the initiated T7 ternary complex at 37 °C, a range of blocked transcripts was observed for each drug system after 3 min of elongation (Figure 2). There was no evidence of any "read-through" of the RNA polymerase past any drug site, and the 10-min elongation lanes were identical with the 3-min lanes for all drugs studied. Further elongation times up to 30 min were also performed that confirmed this observation (data not shown). The RNA lengths where drug-induced blockage occurred were defined by appropriate sequencing lanes and the known sequence of the transcribed DNA.[2] This sequence-specific termination of transcription

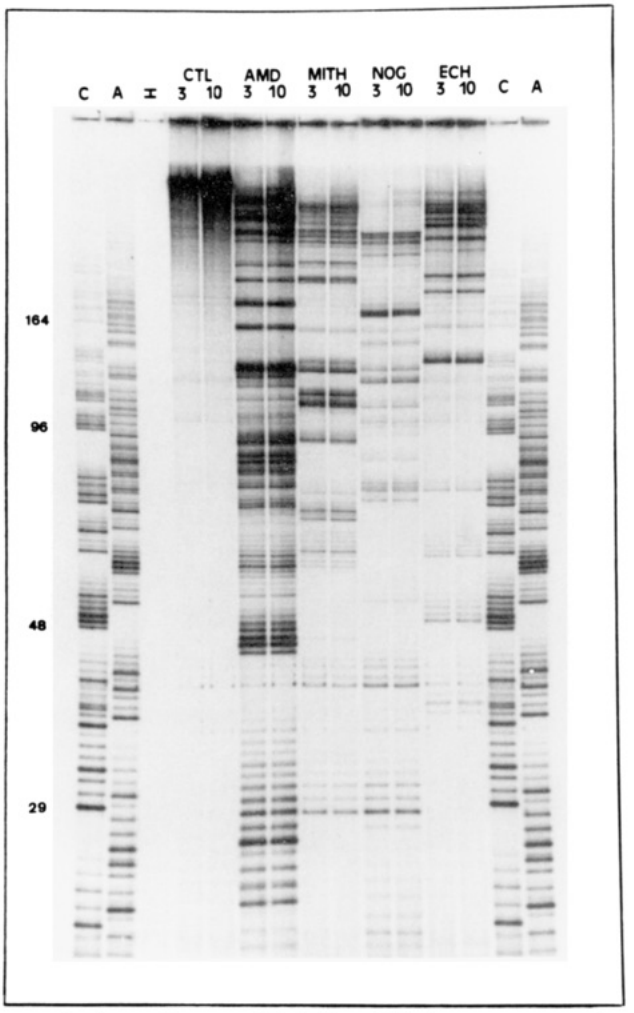

FIGURE 2: Autoradiogram of the initiated T7 phage ternary complex (I), C and A sequencing lanes, control lanes in the absence of drug (CTL) (3 and 10 min after elongation at 37 °C), and elongation in the presence of actinomycin D (AMD, 6 μM), mithramycin (MITH, 10 μM), nogalamycin (NOG, 15 μM), and echinomycin (ECH, 15 μM), also 3 and 10 min after elongation at 37 °C.

is summarized for all four drugs in Figure 3.

The dependence of the termination phenomena on drug loading is shown in Figure 4. With decreasing drug level, there is a decrease of the number of observed drug-induced block sites due to a loss of occupancy at low-affinity sites. The higher affinity sites are therefore most clearly revealed at low drug loadings (GpC positions 131, 163, and 190) and correspond to high occupancy sites observed for actinomycin at 8 μM (Figures 2 and 3).

Drug-independent pausing was observed at two A-T-rich regions in the presence of actinomycin (45–49, 80–89, and 99–105; Figures 2 and 4). Both sites exhibited broad pausing not characteristic of drug-induced blockage, and is thought to be due to DNA structural perturbations since actinomycin is known to exhibit significant transmitted effects along the DNA (Pardi et al., 1983). The enhanced amount of label evident in those lanes with relatively high levels of actinomycin (Figures 2 and 4) suggests that the initiated ternary complex has been stabilized by actinomycin but not by mithramycin, nogalamycin, or echinomycin.

Limited termination of transcription was also observed at 37 °C with a bis(daunomycin) and a bifunctional acridine–tripyrrole (data not shown). Full-length transcripts were reduced to approximately half that of the control elongation lanes, as compared to complete loss of the full-length transcript in the case of actinomycin, mithramycin, nogalamycin, and echinomycin (Figure 2). When the elongation was carried out at 5 °C, many more block sites were observed (compared to 37 °C) with the bis(daunomycin) and the acridine–tripyrrole (Figure 5). All of these sites therefore appear to exhibit similar termination characteristics as seen with the other four drugs at 37 °C (i.e., identical degree of site-specific blockage after 3 and 10 min of elongation). No termination was observed with adriamycin at 5 °C (Figure 5).

The L8UV5 fragment was inserted into the pGEM-1 vector in the same orientation as the T7 promoter so that sequence-specific interactions between the T7 system and the drugs could be directly compared to that observed with *E. coli* RNA polymerase (Phillips & Crothers, 1986; White & Phillips, 1988). *E. coli* RNA polymerase was initiated from the UV5 promoter and the sequence probed by both polymerase starts at position 167. In the region probed by both polymerases, the binding site detected is identical for actinomycin (Phillips & Crothers, 1986; White & Phillips, 1988) and nogalamycin (unpublished results). The detection of drug sequence specificity is therefore, as expected, independent of the complexity of the RNA polymerase employed in this assay.

SP6 and T3 RNA Polymerases. The drug-induced termination phenomena observed with T7 RNA polymerase were also seen with SP6 RNA polymerase. When this system was initiated with ApG together with ATP, UTP, and CTP, the resulting RNA expected (see Table I) was a 9-mer (Axelrod & Kramer, 1985). This initiated complex was presumably unstable, and little labeled transcript was observed when all four nucleotides were subsequently added. To use this system, actinomycin D was equilibrated with the DNA for 30 min at 37 °C, and then all four nucleotides and labeled nucleotide were added simultaneously. As the label was diluted considerably by this procedure, the method lacked sensitivity. Quantitation was also more difficult because label was continually being added to the growing chain. T3 RNA polymerase also exhibited similar deficiencies even though an initiated complex with an RNA 11-mer suggested that it would be more stable (Table I). The T7 RNA polymerase system did not suffer from these two problems, and was therefore the system of choice.

Relative Occupancy. Each of the 3-min drug lanes was scanned by densitometry and the area of each band integrated and expressed as a percentage of the total integrated area in that lane. The percentage area of each band therefore represents the mole fraction (as a percent) of each RNA length present in each transcription mixture. It should be noted that this follows because incorporation of label was restricted only to RNA in the initiated complex (i.e., 12-17-mers), by diluting out the label some 400-fold in the elongation phase with a high level of unlabeled ATP.

The mole fraction of RNA at each drug block site cannot be used as a direct measure of drug occupancy since downstream sites are not exposed to as many RNA polymerases as is the first drug site. This is a direct result of termination of transcription at each drug site. The apparent occupancy at all downstream sites (i.e, past the first drug site) is therefore underestimated and has been corrected to allow for the probability that downstream sites are behind occupied upstream sites.

Discussion

Sequence Specificity. The observed sequence specificity of each drug is consistent with their known preferred DNA-binding sites and with transcription proceeding mainly up to

[2] All sequences shown employ the 5′–3′ notation.

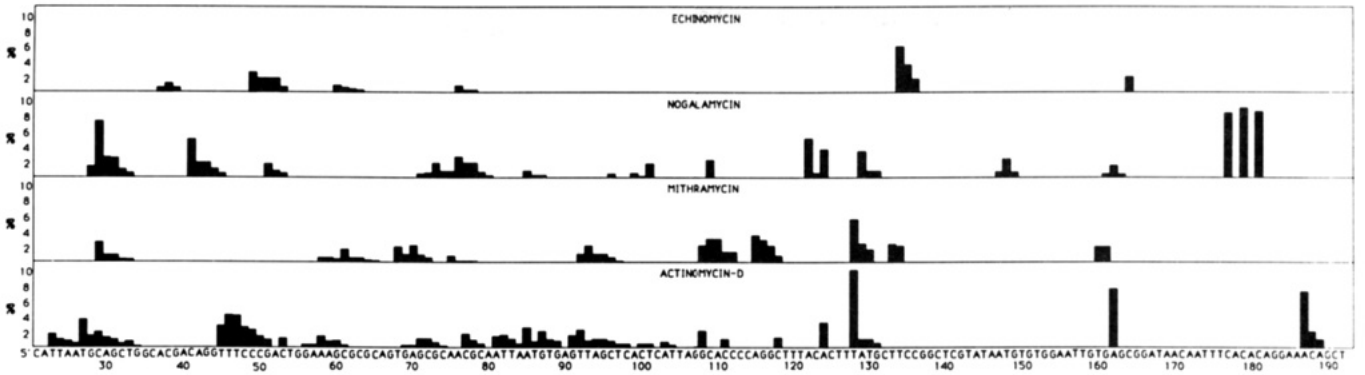

FIGURE 3: Relative occupancy of drug-induced termination sites observed with T7 RNA polymerase at 37 °C.

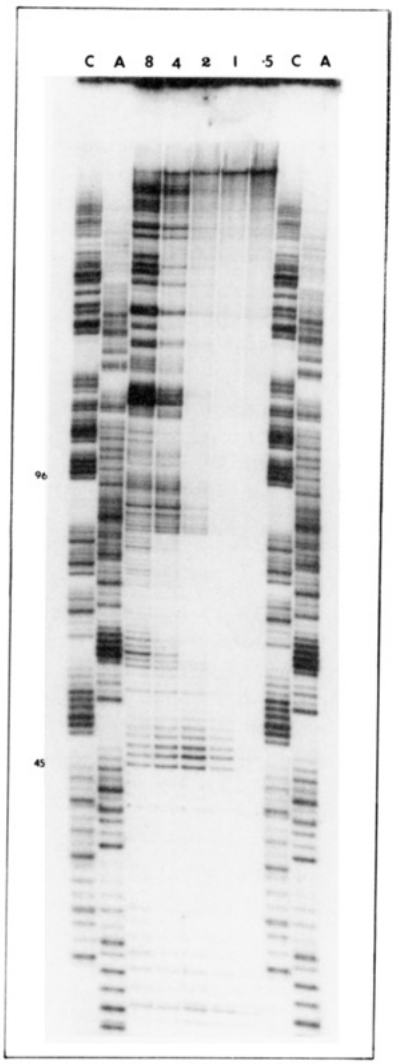

FIGURE 4: Autoradiogram of the initiated ternary complex 3 min after elongation at 37 °C in the presence of 8, 4, 2, 1, and 0.5 μM actinomycin. C and A are sequencing lanes.

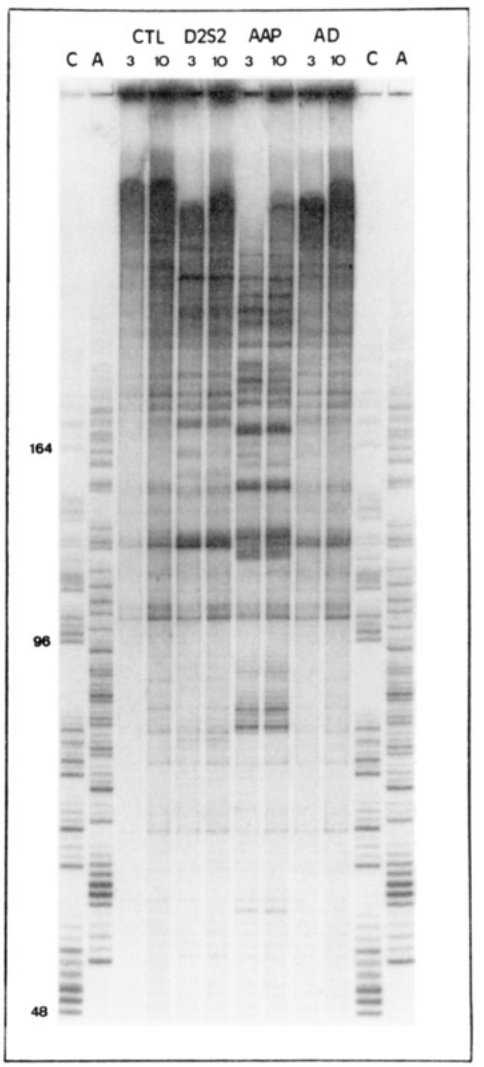

FIGURE 5: Autoradiogram of the initiated T7 phage ternary complex, elongated for 3 or 10 min at 5 °C in the absence of drug (CTL), or in the presence of bis(thiadaunomycin) (D2S2), acridine–tripyrrole (AAP), and adriamycin (AD). Lanes C and A denote C and A sequencing lanes, respectively.

the 5′ side of drug-binding sites (Phillips & Crothers, 1986; Skorobagaty et al., 1988c; White & Phillips, 1988). This was confirmed by the observation that T7 RNA polymerase was inhibited by actinomycin D at exactly the same position as observed with *E. coli* RNA polymerase (i.e., the AGCT site at position 190 on the template; see Figure 3). The terminated transcripts were observed adjacent to and upstream of preferred CpG sites for echinomycin (Low et al., 1984; Van Dyke & Dervan, 1984) (6 out of 6 sites), CpA for nogalamycin (Fox & Waring, 1986) (12 out of 14 sites), probably GpC for mithramycin (Van Dyke & Dervan, 1983; Fox & Howarth, 1985) (7 out of 10 sites), and GpC for actinomycin (Aivasashvilli & Beabealashvilli, 1983; Neidle & Abraham, 1984; Chen, 1988) (9 out of 14 sites). Furthermore, the highest occupancies for isolated sites correspond to the known sequence specificity for all four drugs (e.g., 48, 134, and 154 for echinomycin, with CpG sites at 50, 136, and 155, respectively; 128, 162, and 187 for actinomycin D, with GpC sites at 131, 163, and 190, respectively).

There are several instances in Figure 3 where a small degree of transcription appears to have proceeded one or two nu-

Table II: Half-Lives for Dissociation of Drugs from DNA[a]

drug	dissociation half-life (s)	drug	dissociation half-life (s)
nogalamycin	2990[b]	bis(thiadaunomycin)	230[e]
actinomycin D	374[c]	acridine–tripyrrole	6.9[f]
mithramycin	~375[d]	adriamycin	0.8[g,h,i]
echinomycin	297[d]		

[a] All were at 20 °C except for nogalamycin, which was at 40 °C. [b] Fox et al. (1985). [c] Bresloff and Crothers (1975). [d] Van Dyke and Dervan (1983). [e] Skorobogaty et al. (1988a). [f] Eliades, Phillips, and Reiss (unpublished results). [g] Fox et al. (1985). [h] Chaires et al. (1987). [i] Phillips et al. (1988).

cleotides past a known binding site (e.g., A-80 of the nogalamycin CpA site; G-50 and A-51 of the CpG site of echinomycin). This has not been observed with *E. coli* RNA polymerase (Phillips & Crothers, 1986; White & Phillips, 1988) but has been reported recently with T7 RNA polymerase (Shi et al., 1988) and has been attributed to a random incorporation of bases at the 3′ end of the paused transcript. It is also interesting to note that T7 RNA polymerase can transcribe to the bases adjacent to the preferred intercalation site, suggesting as has been found for *E. coli* RNA polymerase that the catalytic site and the melted region of the RNA–DNA hybrid in the ternary complex virtually coincide (Shi et al., 1987; White & Phillips, 1988).

Drug Dissociation Kinetics. The most striking aspect of the drug-induced blockage of transcription with phage RNA polymerase is that six different drugs resulted in termination of transcription at every observed drug site. This is in direct contrast to the effect observed with bacterial RNA polymerases, where the presence of a ligand generally results in a pausing of transcription, with "read-through" of polymerase past the drug site being limited by the rate of dissociation of drug from the DNA (Phillips & Crothers, 1986). This indicates that the elongating DNA–RNA–T7 RNA polymerase ternary complex is inherently less stable than the corresponding bacterial complex. This conclusion has also been drawn from the limited stability of phage RNA polymerase–DNA complexes, relative to long-lived bacterial systems (von Hippel et al., 1984; Basu & Maitra, 1986), and is further supported by the present observation that only a fraction of the initiated complex is able to be elongated after an initial 15-min incubation period. This differing stability has also been documented by recent observations that *E. coli* transcription complexes survive nondenaturing polyacrylamide gel electrophoresis (Straney & Crothers, 1985), whereas T7 complexes dissociated under similar treatment (Shi et al., 1988). Furthermore, nitrocellulose filter assays have shown a 4–8 times greater retention of the *E. coli* complex than the T7 transcription complex (Smeekens & Romano, 1986). The general implication then is that the presence of a drug on DNA is sufficient to destabilize the phage ternary complex, presumably by a local conformational perturbation, or as a direct consequence of arresting of the elongation complex by the drug, conditions that do not disrupt the more stable bacterial complex. Those ligands which have a rapid rate of dissociation from DNA (e.g., adriamycin, $t_{1/2}$ ~0.8 s) have been observed to delay bacterial transcription at each drug site (Skorobogaty et al., 1988b,c) and exhibit normal first-order dissociation kinetics (unpublished results) but do not exhibit any delay or termination of the T7 phage transcription system. This suggests that there is a minimal residence time required of the drug on DNA in order to induce termination of the phage transcription. The two ligands with intermediate residence times (dissociation half-lives of 7 and 230 s) exhibited intermediate termination characteristics. Limited termination was evident at 37 °C, but when the residence time was increased by a decrease of temperature to 5 °C, site-specific termination increased. All four ligands with longer residence times (i.e., half-life ≥300 s; Table II) gave rise to complete termination of transcription at every drug site at physiological temperatures.

On the basis of the present data, we propose a tentative model for drug-induced termination of transcription involving T7-like phage RNA polymerases and possibly any relatively unstable transcriptional system. Those ligands with relatively slow dissociation rates ($t_{1/2}$ ≥300 s) delay the normally rapidly transcribing polymerase for a sufficient length of time for local deformations of DNA secondary structure, or simple blockage, to result in decreased polymerase–DNA contacts, and hence a less stable ternary complex. The complex dissociates and releases RNA which cannot be elongated further. This model therefore assumes a minimal ligand residence time, and possibly some minimal degree of local perturbation to DNA secondary structure. The notion that induction of termination by a ligand requires a critical residence time at the block site (of the order of 300 s) also comes from the work of Shi et al. (1988). From an analysis of the stability of T7 RNA polymerase transcripts blocked by covalent psoralen adducts, they have estimated that the lifetime for arrested elongation complexes is approximately 5 min, and this is similar to the half-life of 5 min estimated from the present work, based on reversibly binding ligands. This particular phenomenon of drug-directed termination is particularly interesting in light of the fact that termination signals for these polymerases are rare and when they do occur are often inefficient (Studier & Moffatt, 1986).

Biological Implications. The fact that all slowly dissociating drugs result in complete termination of transcription by phage RNA polymerase at every occupied site, whereas those same drugs merely delay transcription of bacterial DNA, suggests a potential mechanism for the design of selective agents to act on ternary complexes of relatively low stability, prokaryotic or eukaryotic transcription systems. Selective destabilization of the ternary transcription complex is possible in principle, and this may be achieved by DNA-binding agents which offer the appropriate combination of kinetic parameters and perturbation of DNA secondary structure.

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Laser Temperature-Jump, Spectroscopic, and Thermodynamic Study of Salt Effects on Duplex Formation by dGCATGC†

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ABSTRACT: Salt effects on duplex formation by dGCATGC have been studied with spectroscopic, thermodynamic, and kinetic methods. Circular dichroism spectra indicate different salt conditions have little effect on the structures of the duplex and single strand. NMR chemical shifts indicate the structure of the duplex in 1 M NaCl is similar to that of the B-form determined previously in 0.5 M KCl [Nilges, M., Clore, G. M., Gronenborn, A. M., Brunger, A. T., Karplus, M., & Nilsson, L. (1987) *Biochemistry 26*, 3718–3733]. Optical melting experiments indicate the effect of Na^+ concentration on melting temperature is similar to that expected for a polynucleotide with the same GC content. Laser temperature-jump experiments indicate the effect of Na^+ concentration on the rate of duplex formation is much less than is observed for polynucleotides. The observations are consistent with expectations based on a counterion condensation model. This is surprising for a duplex with only 10 phosphates.

The salt dependence of duplex formation by short nucleic acids is important for the prediction of nucleic acid structure and dynamics under different buffer conditions. An understanding of salt effects is also important for the interpretation and prediction of the influence of salt on processes that may involve formation of short duplex regions. Such processes include RNA processing and control of gene expression by "antisense" nucleic acids. For example, reactions of both RNase P (Altman et al., 1986) and the self-splicing RNA from *Tetrahymena thermophila* (Cech, 1987) have been shown to depend on salt in unusual ways (Reich et al., 1988; Sugimoto et al., 1988). Effects of site-directed mutagenesis that disrupts base pairing can also be dependent on salt. For example, Burke et al. (1986) found that disrupting conserved pairs in

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