Effects of *Escherichia coli* Nus A Protein on Transcription Termination in Vitro Are Not Increased or Decreased by DNA Sequences Sufficient for Antitermination in Vivo

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ABSTRACT: The ability of *Escherichia coli* Nus A protein to recognize specific DNA or RNA sequences in vitro was tested by using transcription templates containing a variety of promoters, transcription terminators, and antitermination-conferring regions. We conclude that the effects of Nus A on termination are not qualitatively or quantitatively altered by sequences present in promoters, Rho-dependent terminators, or antitermination-conferring regions. Nus A was also shown to increase termination at the *rrnC* Rho-independent T_1 terminator by a mechanism that is independent of the promoter or sequences involved in antitermination. Altogether, these observations argue against a direct Nus A–nucleic acid interaction affecting termination in vitro. Together with the results described in the accompanying paper [Sigmund, C. D., & Morgan, E. A. (1988) *Biochemistry* (preceding paper in this issue)], these results suggest that the effects of Nus A on termination in vitro may not be related to the in vivo functions of Nus A.

Nus A is an essential protein of *Escherichia coli* (Nakamura & Uchida, 1983). Nus A has many in vivo and in vitro effects on transcription [reviewed in Platt (1986)]. Since detailed analysis of some or all of the in vivo effects of Nus A is complicated by the probable interplay of incompletely enumerated transcription factors, elucidation of the in vivo roles of Nus A has relied heavily on in vitro transcription experiments employing purified proteins. These in vitro experiments [reviewed in Platt (1986); this paper] show that Nus A increases transcriptional pausing, decreases termination at certain Rho-dependent termination sites, and increases termination at a Rho-independent termination site. Consequently, Nus A is commonly viewed as a protein that modulates termination events. Although Nus A by itself affects termination in vitro, it is not known if Nus A by itself affects termination events in vivo. However, it appears that Nus A affects at least some in vivo termination events by complex interactions with a variety of other transcription factors (Friedman et al., 1984; Stewart & Yanofsky, 1985; Greenblatt et al., 1981; Kuroki et al., 1982).

Some clues about how Nus A may affect termination have been obtained. In vitro binding studies suggest that Nus A binds specifically to RNA polymerase (Greenblatt & Li, 1981b), to Rho (Schmidt & Chamberlin, 1984b), to other proteins modifying transcription termination (Greenblatt & Li, 1981a), and to (or very near) box A (Tsugawa et al., 1985), a specific 14-base RNA sequence (Morgan, 1986) in regions that specify λ antitermination. Preliminary evidence has been presented which suggests that deletion of the box A preceding λ t_{R1} alters the effects of Nus A on termination at t_{R1} in vitro (Lau & Roberts, 1985). There is also genetic evidence for the participation of λ phage box A sequences in an in vivo interaction involving Nus A (Freidman & Olson, 1983). Thus, several types of experiments suggest that λ box A sequences are involved in the function of Nus A.

Box A sequences are also found in two regions of *rrn* operons that specify antitermination. The two box A sequences in *rrn* operons are identical at 13 of 14 positions and are located before the 16S and 23S rRNA genes in similar relationships to two RNA secondary structural features involved in RNase III processing (Morgan, 1986). There are no other sequences or structures besides the box A sequences and RNase III processing structures that appear to be strongly conserved in all *rrn* regions conferring antitermination. The conserved features of *rrn* box A sequence and placement strongly support the idea that box A is an important entity. In support of this conclusion, mutations in both box A sequences of *rrnC* affect *rrn* antitermination when assayed by using operon fusions and also reduce the synthesis of functional rRNA transcribed from an intact *rrnC* operon in vivo (Morgan et al., 1987; our unpublished data). Nus A may also be involved in *rrn* antitermination in vivo [reviewed in Morgan (1986)], further strengthening the correlation between box A, Nus A, and antitermination previously apparent from the λ antitermination system. Since the effects of *rrn* antitermination mechanisms assayed by using operon fusions in vivo are quantitatively and qualitatively very similar to antitermination caused by Nus A in vitro (Morgan, 1986; Sigmund & Morgan, 1988; see also below), it seemed possible that Nus A might be the only operon-specific transcription factor involved in *rrnC* antitermination and that *rrn* antitermination might result solely from direct box A–Nus A recognition events. We therefore wished to determine if *rrnC* sequences (containing box A) previously shown to be sufficient to cause *rrn* antitermination in vivo altered the effects of Nus A on termination in vitro. We also wished to qualitatively and quantitatively characterize the in vitro antitermination caused by Nus A as a necessary prelude to the characterization and identification of other *rrn* or λ antitermination factors.

Materials and Methods

Purification of Proteins. Sigma-saturated RNA polymerase was purified by chromatography (Gonzalez et al., 1977) starting with RNA polymerase purified by the method of Burgess and Jendrisak (1975) from *E. coli* strain CLB7 (Bassett & Rawson, 1983). Rho was purified through the affinity agarose chromatography step of Mott et al. (1985). Nus A was purified according to the method of Schmidt and Chamberlin (1984a) followed by purification on hydroxylapatite and Sephacryl S-300 according to the method of Olins

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et al. (1983). When the purified proteins were analyzed by SDS–polyacrylamide gel electrophoresis, their purity appeared at least equal to the purity of proteins shown in the papers presenting the purification schemes.

Preparation of DNA. Plasmid DNA to be used in transcription reactions was purified by lysozyme–sodium dodecyl sulfate lysis of cells grown to saturation, two CsCl density gradient centrifugations, phenol extraction, and ethanol precipitation. Aliquots of the predominantly supercoiled DNA were stored at −70 °C until use.

In Vitro Transcription Reactions. Transcription reactions (50 μL) contained 44.5 mM Tris-HCl (pH 8.0), 14 mM $MgCl_2$, 22.4 mM NaCl, 5% glycerol, 14 mM β-mercaptoethanol, 0.04 mM ethylenediaminetetraacetate, 40 μg/mL acetylated bovine serum albumin (Gonzalez et al., 1977), 200 μM ATP and GTP, 150 μM CTP, 10 μCi [α-^{32}P]UTP at a final concentration of 40 μM, 3.2 μg/mL Sigma-saturated RNA polymerase, and 0.5 pmol of DNA. Where indicated, reactions also contained 4 μg/mL Rho and 2 μg/mL Nus A. Reactions were prewarmed at 37 °C for 5 min, initiated by addition of DNA, and terminated after 10 min at 37 °C by the addition of 0.4 mL of 0.3 M ammonium acetate, 0.2% sodium dodecyl sulfate, 10 mM ethylenediaminetetraacetate, and 10 μg of yeast RNA. The RNA was then prepared for electrophoresis by phenol extraction, ethanol precipitation, and resuspension in 80% formamide, 10 mM Tris-HCl (pH 8.0), 10 mM boric acid, 0.3 mM ethylenediaminetetraacetate, 0.1% bromphenol blue, and 0.1% xylene cyanol. Transcripts were separated by electrophoresis on 5% polyacrylamide gels containing 8 M urea, 89 mM Tris, 89 mM boric acid, and 2.5 mM ethylenediaminetetraacetate. Polyacrylamide gels were 38 cm in length and 0.25 mm thick. Single-stranded DNA size markers were obtained by using [α-^{32}P]dCTP and Klenow fragment of DNA polymerase I to fill in the ends of *Hpa*II fragments of pBR322 and then denaturing the DNA by heating it to 90 °C in sample buffer.

Quantitation of Transcripts. Kodak BB1 X-ray film was exposed to acrylamide gels at −70 °C. The resulting exposures were scanned by using a densitometer. Termination efficiencies at each termination site were determined from peak area minus local background in the electrophoresis tracks with appropriate corrections for RNA length, base composition, and total RNA polymerase initiation events as estimated by summing the number of transcripts terminating at all termination sites.

S1 Nuclease Mapping. Labeled DNA used as a probe to map the 3′ end of transcripts was obtained by digesting 10 μg of pCDS70 or pCDS70Δ9 (depending on whether the t_{R1} region to be analyzed had the Δ9 deletion or not) with *Ava*I and end labeling the resulting fragments with 500 μCi of carrier-free [α-^{32}P]TTP using Klenow fragment of DNA polymerase I (Maniatis et al., 1982). An *Ava*I fragment extending from an *Ava*I site immediately before t_{R1} (see Figure 3) to an *Ava*I site in the polylinker downstream of the T_A terminator was end-labeled and then cut by *Pst*I in the polylinker between t_{R1} and T_A. The labeled *Ava*I–*Pst*I fragment containing t_{R1} then was purified by agarose gel electrophoresis. Sequencing ladders run next to S1 nuclease mapping lanes were obtained by chemical degradation of this fragment (Maxam & Gilbert, 1980). To obtain a single-stranded probe to use in S1 nuclease mapping experiments, the strands of the *Ava*I fragment were separated by electrophoresis at an ambient temperature of 9 °C on 5% acrylamide gels according to the method of Maniatis et al. (1982). The fastest migrating strand was complementary to the transcripts made in vitro. To S1 nuclease map transcripts made in vitro, the in vitro RNA from a single reaction was phenol-extracted and hybridized to 0.18 pmol (10^5 dpm) of end-labeled probe. After hybridization and S1 treatment according to the method of Postle and Good (1985), the samples were electrophoresed next to sequence ladders by using 30 cm × 115 cm × 0.25 mm 6% acrylamide gels.

To be certain that the transcripts separated on acrylamide gels could be assigned to the correct 3′ end points, transcripts separated on acrylamide gels were electroeluted from the gels and individual transcripts hybridized to 0.06 pmol (5×10^4 dpm) of probe, treated with S1 nuclease, and electrophoresed next to sequence ladders as described above.

Results

As discussed above, Nus A appears to be involved in both λ and *rrn* antitermination in vivo. Therefore, antitermination-conferring regions of these operons are more likely than any other available regions to contain specific sequences recognized by Nus A. The entire regions involved in antitermination may encompass promoter regions as well as regions far downstream of the promoter (Holben et al., 1985; Grayhack et al., 1985). In addition, terminators from transcription units affected by antitermination mechanisms might have special features that facilitate antitermination. Therefore, the effects of Nus A on transcription from templates containing promoters, antitermination regions, and terminators from operons that are unlikely to possess antitermination mechanisms must be compared to transcription from templates containing promoters and terminators from operons that do contain antitermination mechanisms. In the work described in this paper we tested the *rrnC* promoters and the *rrnC* leader region, both of which appear to independently confer some degree of antitermination in vivo (Holben & Morgan, 1984; Holben et al., 1985). We tested the λ t_{R1} terminator and adjacent antitermination-conferring region (Lau et al., 1982) and a derivative of the λ t_{R1} region containing an *Eco*RI linker in place of the most conserved 9 base pairs of box A (Lau & Roberts, 1985). We also tested the P_{tac} promoter (de Boer et al., 1983) and the *trp t′* terminator (Wu et al., 1981) because these DNA sequences are from operons unlikely to employ antitermination mechanisms.

Construction of Transcription Templates. To facilitate placement of the terminator, promoter, and antitermination regions in transcription templates, the individual regions were first cloned into polylinkers on plasmids. The polylinker sites flanking the resulting cassettes were then used to construct the plasmids used for *in vitro* transcription (Figure 1).

The experiments described in this paper employ superhelical templates because *rrn* promoters are superhelicity dependent (Glaser et al., 1983), and superhelical templates are probably closer to in vivo templates than are linear templates. In our experiments, we used tandem, strong Rho-independent *rrnC* T_1 terminators to efficiently terminate transcripts so that the efficiency of transcription termination at upstream Rho-dependent terminators could be measured. The first and second of these tandem T_1 terminators will subsequently be referred to as T_A and T_B. With the assumption that the T_A and T_B terminators function with equal efficiency, the efficiency of the Rho-independent T_A terminator can be calculated in a straightforward manner from measurements of the number of transcripts that terminate at T_A and T_B.

Transcription Conditions. The concentrations of RNA polymerase and Rho used in our in vitro transcription reactions are similar to those used in most previous studies of transcription termination in vitro. The concentration of Nus A

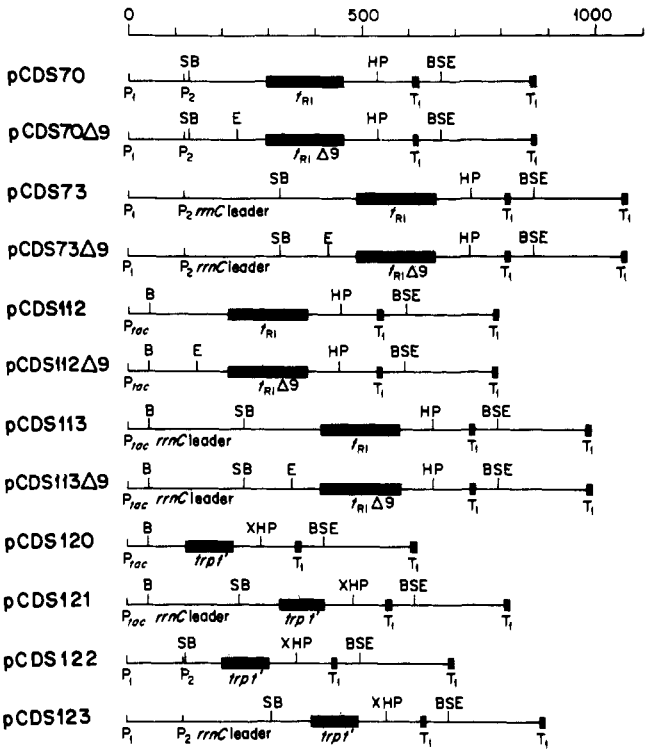

FIGURE 1: Structures of the in vitro transcription templates. Restriction enzyme recognition sites are as follows: B, *Bam*HI; S, *Sma*I; H, *Hin*cII; P, *Pst*I; E, *Eco*RI; X, *Xba*I. The transcription start points of the *rrnC* P_1 and P_2 promoters (Young & Steitz, 1979) and the P_{tac} promoter (de Boer et al., 1983) are known. Termination regions are indicated by a thickening of the line. The cassettes used for constructing these transcription templates were constructed as follows: the *rrnC* promoter cassette was constructed by replacing the *Sma*I–*Bam*HI *lac* fragment of pBH21-*lac* (Holben et al., 1985) with the *Sma*I–*Bam*HI polylinker segment of pUC9. The promoter cassette was then obtained from this plasmid as a *Hin*cII–*Bam*HI fragment. The *rrnC* promoter-leader cassette was constructed by replacing the small *Sma*I–*Bam*HI fragment of pBH16 (Holben et al., 1985) with the *Sma*I–*Bam*HI polylinker fragment of pUC9. The promoter-leader cassette was then obtained as a *Hin*cII–*Bam*HI fragment of this plasmid. The P_{tac} cassette was constructed by using Klenow fragment of DNA polymerase I to fill in the ends of an *Eco*RI fragment of pKM-*tac*1 (de Boer et al., 1983) and substituting this fragment for the *Hin*cII fragment containing the *rrnC* promoters of pBH12 (pBH12 is identical with pBH16 except that pBH12 does not contain the origin of F found in pBH16). Subsequent constructions using this plasmid involved only insertions of DNA into the polylinker between P_{tac} and the T_1 terminator on this plasmid. The t_{R1} terminator (Daniels et al., 1983) and $t_{R1}\Delta 9$ terminator (Lau & Roberts, 1985) cassettes were constructed by ligating a *Hae*III–*Hin*cII fragment of λ DNA into the *Hin*cII site of pUC9 (Viera & Messing, 1982) so that the *Bam*HI site of the pUC9 polylinker was located upstream of the terminators, allowing the terminators to be excised as *Bam*HI–*Hin*cII cassettes. The *trp t'* terminator module was constructed by filling in a *Bam*HI site downstream of *trp t'* in pSP65-*trp t'* (Wu et al., 1981) using Klenow fragment of DNA polymerase I. The *trp t'* region was then excised as an *Eco*RI–*Hin*cII fragment, the *Eco*RI end filled in by using Klenow fragment of DNA polymerase I, and the resulting fragment inserted in the *Hin*cII site of pUC8 so that the *Bam*HI site of pUC8 was located upstream. The *trp t'* terminator cassette was obtained as a *Bam*HI–*Hin*cII fragment of this plasmid. The *rrnC* T_1 terminator cassette (called T_A in the final plasmids) was constructed by inserting a *Hpa*II fragment containing T_1 (Holben et al., 1985) into the *Hin*cII site of pUC8 (Viera & Messing, 1982) so that the *Pst*I polylinker site of pUC8 was located upstream of the terminator. The T_1 cassette was then obtained as a *Pst*I–*Bam*HI fragment of this plasmid. The transcription template plasmids described in this figure were then constructed by a straightforward arrangement of cassettes in the polylinker of pBH12.

used is within the range where termination at t_{R1}, $t_{R1}\Delta 9$, *trp t'*, and T_1 exhibits approximately linear changes in response to changes in Nus A concentration and is a concentration at which Nus A causes half-maximal or lesser effects on termination (Sigmund & Morgan, 1988; data not shown). Therefore, DNA sequence elements that might merely increase the rate of association of Nus A with the transcription complex should be detectable at these Nus A concentrations.

Transcript Identification. As described under Materials and Methods, the 3′ ends of transcripts from pCDS70, pCDS70Δ9, pCDS73, and pCDS73Δ9 were determined by S1 nuclease mapping the mixture of transcripts from in vitro transcription reactions and by mapping individual transcripts electroeluted from gels (Figures 2 and 3). The autoradiograms of these end-mapping experiments have been presented elsewhere (Sigmund, 1987). These mapping experiments determined the end points of transcripts terminating at the T_1, t_{R1}, and $t_{R1}\Delta 9$ terminators (Figure 3). Since the P_1 and P_2 ribosomal promoters are separated by approximately 120 base pairs, identification of the 3′ end of transcripts also allowed the *rrnC* promoter used for each transcript to be identified.

The 3′ end mapping experiments revealed that there are large transcript structure-dependent changes in termination at t_{R1} (Figure 2). Early termination sites in t_{R1} and $t_{R1}\Delta 9$ are often inactive in certain templates (e.g., site I is inactive in pCDS70 and active in pCDS73). In addition, previously unreported termination sites in the t_{R1} region are active in some of the templates used in this study (e.g., sites IIIA and IVA in several of the templates). Previously unreported termination sites (labeled Δ9 I in Figure 3) are also activated by the deletion in $t_{R1}\Delta 9$. These differences in termination site usage are not consistently related to the size of transcripts or the presence of any specific DNA sequences in the transcription templates.

Although the termination sites within the *trp t'* terminators in our templates were not precisely mapped and differ in number from the termination sites previously identified by fingerprints of 3′ end-labeled transcripts (Wu et al., 1981), our size measurements of transcripts reveal that the same termination sites are used, albeit with differet relative efficiencies, in all of our transcription templates.

Transcript Quantitation. Densitometry of X-ray films (as shown in Figure 2) allowed quantitation of transcripts terminating at the individual termination sites within the termination regions used in this study. These data were then used to determine the termination efficiency at each termination site. The termination efficiencies of entire termination regions were also calculated (e.g., within *trp t'*, t_{R1}, $t_{R1}\Delta 9$, and *rrnC* T_1). Termination efficiency as used in this paper is defined as the percent termination of RNA polymerase molecules that enter the termination site (as distinct from the number of transcripts that terminate at each site). Calculation of the termination efficiencies of individual termination sites obtained by using data from many individual transcription reactions resulted in acceptably small standard errors of the mean for most termination events (see Table I and the legend to Figure 4). To show that these means are not influenced by systematic errors related to densitometry, termination within the t_{R1} or $t_{R1}\Delta 9$ termination regions of pCDS70, pCDS70Δ9, pCDS73, and pCDS73Δ9 was also determined by filter hybridization of in vitro transcripts to single-stranded DNA probes obtained from upstream and downstream of the t_{R1} and $t_{R1}\Delta 9$ terminators. Measurements of termination within the entire termination regions by filter hybridization gave means that fell within the standard errors of the means presented in Table I. The standard errors of the mean obtained by filter hybridization were also similar in magnitude to the standard

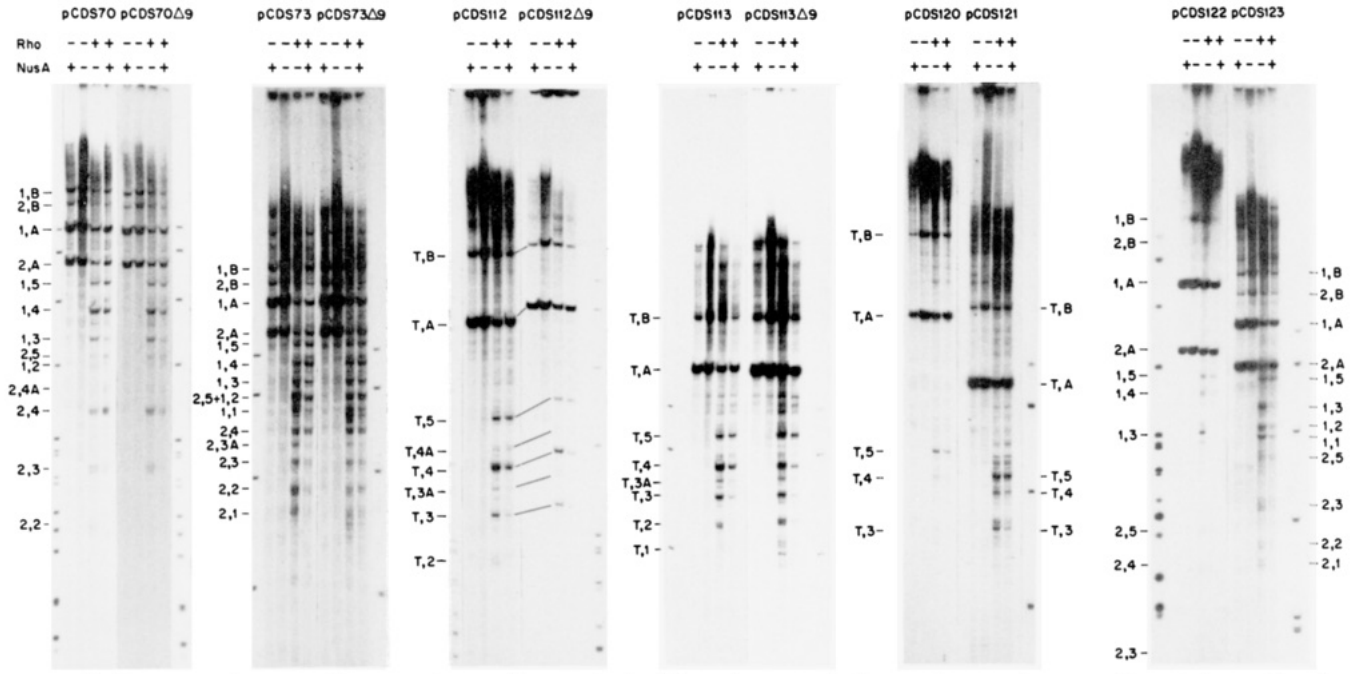

FIGURE 2: Representative autoradiograms of transcripts synthesized from the transcription templates and separated by gel electrophoresis. Each set of transcription reactions is flanked by a lane of single-stranded *Hpa*II fragments of pBR322. In the notations flanking lanes, the letter or number preceding the comma indicates the promoter responsible for the observed transcript (1 designates the *rrnC* P_1 promoter, 2 designates the *rrnC* P_2 promoter, and T designates P_{tac}), and the letter or number following the comma indicates the terminator responsible for the observed transcript [A designates the first T_1 terminator (T_A), B designates the second T_1 terminator (T_B), and 1–5 designate sites I–V of the t_{R1}, $t_{R1}\Delta 9$, or *trp t′* terminators].

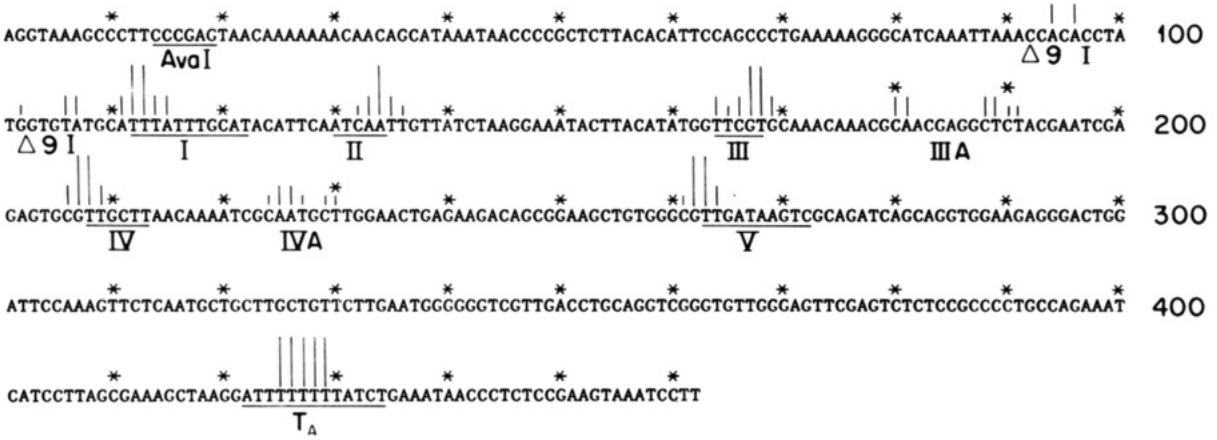

FIGURE 3: Termination sites within t_{R1}, $t_{R1}\Delta 9$, and T_1. The underlined regions are the t_{R1} and T_1 termination sites previously reported (Morgan et al., 1983; Young, 1979). The tick marks above the sequence indicate the termination points and approximate relative magnitudes of termination at each point as determined by our analysis. The termination sites unique to $t_{R1}\Delta 9$ (indicated by $\Delta 9$) are superimposed on the t_{R1} sequence and are included with site I transcripts in the quantitative analysis in this paper. All other termination sites in this region are common to t_{R1} and $t_{R1}\Delta 9$. The $\Delta 9$ deletion of box A replaces nucleotides 50–64 of this sequence with a 14-base-pair *Eco*R1 linker.

errors obtained by densitometry. These filter hybridization data have been presented elsewhere (Sigmund, 1987). The similar means and small standard errors of the mean obtained by using both filter hybridization and densitometry indicate that the measured differences in termination site utilization are real, reproducible differences.

Effect of Nus A on Termination. It has been previously established that Nus A reduces termination at early termination sites in the t_{R1} and *trp t′* termination regions (Lau & Roberts, 1985; Wu et al., 1981). Our experiments extend these data by qualitatively and quantitatively comparing the effect of Nus A on these two terminators and by examining whether different promoters and antitermination-conferring regions can enhance the ability of Nus A to affect termination. As seen in Figure 4, Nus A preferentially reduces termination at the upstream termination sites in the linear array of termination sites in the t_{R1}, $t_{R1}\Delta 9$, and *trp t′* termination regions regardless of which sites were active in the particular transcription template and regardless of the overall efficiency of the terminator. The overall similarity of the Nus A caused reduction of termination at *trp t′*, t_{R1}, and $t_{R1}\Delta 9$ in all templates indicates that Nus A affects all terminators by a similar mechanism regardless of the type of promoter or Rho-dependent terminator or the presence of antitermination-conferring regions.

The effects of Nus A on total termination within the entire t_{R1} and *trp t′* termination regions were calculated from the data presented above (Table I). To enable these calculations, the effects of Nus A on terminators were analyzed in a manner similar to the way enzyme–substrate interactions are analyzed. According to this method of analysis, Nus A is analogous to an enzyme acting upon a substrate, the substrate being defined as RNA polymerase that enters the termination region and is destined for termination in the absence of Nus A. This definition of substrate does not include RNA polymerase

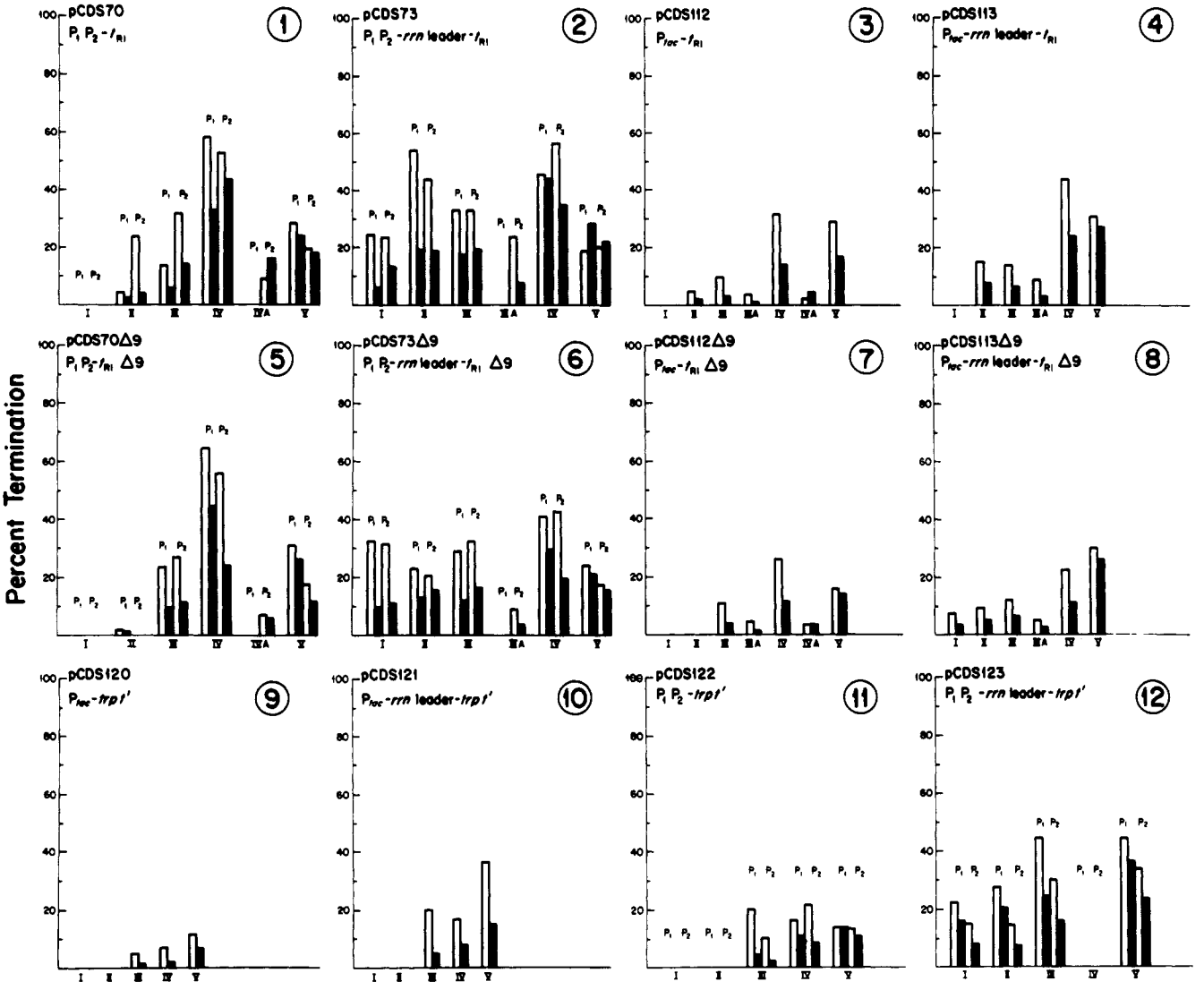

FIGURE 4: Percent termination at individual termination sites. For termination at sites I–V in t_{R1}, $t_{R1}\Delta 9$, and *trp t'*, white bars indicate termination in the presence of Rho and absence of Nus A and black bars indicate termination in the presence of both Rho and Nus A. SEMs are in all cases less than 1% termination for termination efficiencies less than 5%, less than 4% termination for termination efficiencies less than 50%, and less than 8% termination for greater termination efficiencies.

destined to read through the termination region in the absence of Nus A (actual events in vitro may be more complicated than indicated by this method of analysis, but any reasonable alternative analysis we have considered results in similar overall conclusions). According to this method of analysis, the percent of termination relieved by Nus A (the right-most column in Table I) is defined as the fraction of substrate prevented from terminating by Nus A. As can be seen from Table I, Nus A prevents from 0.15 to 0.53 of the RNA polymerase molecules from terminating at the *trp t'*, t_{R1}, and $t_{R1}\Delta 9$ termination regions. Although the magnitude of the reduction of termination caused by Nus A varies with the transcription template, detailed examination of the data in Table I indicates no clear quantitative enhancement of the effects of Nus A by the transcription of any single DNA sequence present in the transcription templates. In particular, Nus A strongly reduces termination during transcription of a template (pCDS120) composed entirely of sequences from operons unlikely to have antitermination mechanisms and therefore unlikely to have specific sequences involved in Nus A recognition. However, there is much template-dependent variation in the efficiency of termination in the absence of Nus A and in the reduction of termination caused by Nus A, which makes interpretation of these data difficult at first glance. Fortunately, much of the variation in the magnitude of the reduction of termination caused by Nus A has a systematic and understandable cause that, when recognized, allows interpretation of the data. As shown in Figure 5, the Nus A caused reduction of termination at *trp t'*, t_{R1}, and $t_{R1}\Delta 9$ is greater when the terminator is in a sequence context where it functions less well. This behavior of terminators is a straightforward consequence of the fact that, for all Rho-dependent termination regions tested, Nus A preferentially reduces termination at about the same number of active upstream termination sites independently of the number or identity of the sites (see above). Since, even in the absence of Nus A, a terminator that functions weakly in a particular sequence context is a weak terminator because upstream termination sites are not active (see above and Figure 4), it follows that the termination at a weaker termination region will be on the average reduced more by Nus A than at the same terminator in a context where it functions strongly simply because Nus A reduces termination at a greater percentage of the active termination sites of the weaker termination regions. A theoretical straight line drawn through the data obtained by using all the constructions tested describes this relationship between terminator strength in the absence of Nus A and the reduction of termination by Nus A (Figure 5). The fact that no promoter, antitermination region, or

Table I: Effects of Nus A on the t_{R1} and *trp t'* Rho-Dependent Terminators[a]

DNA	promoter	*rrn* leader	terminator	read-through with Rho	read-through with Rho + Nus A	percent of termination relieved by Nus A
pCDS70	P_1	−	t_{R1}	0.24 ± 0.01	0.47 ± 0.03	0.27 ± 0.04
	P_2	−	t_{R1}	0.18 ± 0.02	0.32 ± 0.03	0.20 ± 0.03
	$P_1 + P_2$	−	t_{R1}	0.21 ± 0.01	0.40 ± 0.03	0.25 ± 0.03
pCDS70Δ9	P_1	−	$t_{R1}\Delta 9$	0.19 ± 0.02	0.37 ± 0.04	0.19 ± 0.03
	P_2	−	$t_{R1}\Delta 9$	0.25 ± 0.03	0.57 ± 0.05	0.44 ± 0.05
	$P_1 + P_2$	−	$t_{R1}\Delta 9$	0.22 ± 0.02	0.46 ± 0.04	0.31 ± 0.03
pCDS73	P_1	+	t_{R1}	0.10 ± 0.01	0.24 ± 0.02	0.15 ± 0.02
	P_2	+	t_{R1}	0.09 ± 0.01	0.27 ± 0.03	0.22 ± 0.02
	$P_1 + P_2$	+	t_{R1}	0.10 ± 0.01	0.25 ± 0.02	0.18 ± 0.02
pCDS73Δ9	P_1	+	$t_{R1}\Delta 9$	0.17 ± 0.02	0.39 ± 0.03	0.25 ± 0.03
	P_2	+	$t_{R1}\Delta 9$	0.17 ± 0.02	0.40 ± 0.03	0.30 ± 0.04
	$P_1 + P_2$	+	$t_{R1}\Delta 9$	0.17 ± 0.02	0.39 ± 0.03	0.27 ± 0.03
pCDS112	P_{tac}	−	t_{R1}	0.40 ± 0.03	0.67 ± 0.01	0.44 ± 0.03
pCDS112Δ9	P_{tac}	−	$t_{R}1\Delta 9$	0.52 ± 0.03	0.70 ± 0.03	0.38 ± 0.04
pCDS113	P_{tac}	+	t_{R1}	0.29 ± 0.02	0.49 ± 0.01	0.28 ± 0.03
pCDS113Δ9	P_{tac}	+	$t_{R1}\Delta 9$	0.38 ± 0.03	0.55 ± 0.02	0.27 ± 0.04
pCDS120	P_{tac}	−	*trp t'*	0.80 ± 0.01	0.90 ± 0.01	0.51 ± 0.05
pCDS121	P_{tac}	+	*trp t'*	0.42 ± 0.02	0.73 ± 0.02	0.53 ± 0.04
pCDS122	P_1	−	*trp t'*	0.59 ± 0.07	0.73 ± 0.04	0.30 ± 0.08
	P_2	−	*trp t'*	0.61 ± 0.06	0.81 ± 0.03	0.38 ± 0.18
	$P_1 + P_2$	−	*trp t'*	0.60 ± 0.06	0.77 ± 0.05	0.35 ± 0.11
pCDS123	P_1	+	*trp t'*	0.17 ± 0.01	0.32 ± 0.02	0.18 ± 0.03
	P_2	+	*trp t'*	0.34 ± 0.01	0.55 ± 0.02	0.35 ± 0.03
	$P_1 + P_2$	+	*trp t'*	0.24 ± 0.03	0.41 ± 0.02	0.23 ± 0.03

[a] The data are presented as the mean ± SEM. These values are derived from the data in Figure 4. See the text for the definition of percent of termination relieved by Nus A.

Table II: Effects of Nus A on the *rrnC* T_1 Rho-Independent Terminator[a]

DNA	promoter	termination, no additions	termination + Nus A	Nus A caused increase in termination	termination + Rho	termination + Rho + Nus A	Nus A caused increase in termination
pCDS53	P_1	0.67 ± 0.00	0.87 ± 0.01	0.61 ± 0.00	0.65 ± 0.00	0.89 ± 0.03	0.69 ± 0.07
	P_2	0.74 ± 0.00	0.92 ± 0.00	0.72 ± 0.00	0.68 ± 0.01	0.92 ± 0.01	0.74 ± 0.02
pCDS70	P_1	0.85 ± 0.04	0.87 ± 0.02	NS	0.88 ± 0.03	0.92 ± 0.01	0.27 ± 0.18
	P_2	0.90 ± 0.03	0.94 ± 0.01	0.30 ± 0.13	0.84 ± 0.03	0.93 ± 0.02	0.35 ± 0.32
pCDS70Δ9	P_1	0.86 ± 0.04	0.92 ± 0.01	0.20 ± 0.18	0.89 ± 0.03	0.92 ± 0.02	NS
	P_2	0.89 ± 0.03	0.96 ± 0.01	0.46 ± 0.13	0.91 ± 0.02	0.93 ± 0.02	NS
pCDS73	P_1	0.87 ± 0.04	0.91 ± 0.03	NS	0.65 ± 0.06	0.86 ± 0.02	NS
	P_2	0.82 ± 0.07	0.92 ± 0.03	NS	0.81 ± 0.03	0.92 ± 0.02	0.56 ± 0.11
pCDS73Δ9	P_1	0.83 ± 0.06	0.89 ± 0.04	0.23 ± 0.15	0.80 ± 0.06	0.86 ± 0.03	NS
	P_2	0.81 ± 0.07	0.90 ± 0.03	NS	0.90 ± 0.03	0.94 ± 0.02	NS
pCDS112	P_{tac}	0.58 ± 0.03	0.81 ± 0.03	0.56 ± 0.05	0.72 ± 0.02	0.83 ± 0.01	0.41 ± 0.05
pCDS112Δ9	P_{tac}	0.68 ± 0.02	0.94 ± 0.02	0.82 ± 0.06	0.73 ± 0.03	0.91 ± 0.02	0.73 ± 0.06
pCDS113	P_{tac}	0.51 ± 0.03	0.76 ± 0.04	0.52 ± 0.05	0.67 ± 0.03	0.87 ± 0.02	0.59 ± 0.06
pCDS113Δ9	P_{tac}	0.66 ± 0.01	0.71 ± 0.02	0.17 ± 0.04	0.78 ± 0.01	0.78 ± 0.01	NS
pCDS120	P_{tac}	0.67 ± 0.01	0.88 ± 0.00	0.68 ± 0.04	0.69 ± 0.02	0.80 ± 0.01	0.36 ± 0.05
pCDS121	P_{tac}	0.77 ± 0.03	0.94 ± 0.01	0.73 ± 0.04	0.82 ± 0.02	0.83 ± 0.02	NS
pCDS122	P_1	0.88 ± 0.01	0.97 ± 0.01	0.77 ± 0.06	0.80 ± 0.02	0.90 ± 0.02	0.52 ± 0.07
	P_2	0.97 ± 0.01	0.99 ± 0.00	0.68 ± 0.05	0.95 ± 0.01	0.97 ± 0.01	NS
pCDS123	P_1	0.80 ± 0.01	0.91 ± 0.01	0.54 ± 0.03	0.74 ± 0.03	0.84 ± 0.01	0.33 ± 0.10
	P_2	0.76 ± 0.01	0.89 ± 0.01	0.54 ± 0.04	0.81 ± 0.02	0.86 ± 0.01	0.21 ± 0.10

[a] The data are presented as the mean ± SEM. pCDS53 is not described elsewhere in the paper, but its structure is like pCDS70 except that it lacks the t_{R1} region and thus contains no terminator except for T_1. NS means that statistically significant values could not be provided due to technical difficulties that result from attempting to measure differences in read-through of T_1 when the read-through is very small. See the text for the definition of Nus A caused increase in termination.

terminator consistently results in significant deviation to one side of the line shows that the Nus A caused reduction of termination at Rho-dependent terminators is sequence independent.

We also discovered that Nus A affects termination at the *rrnC* T_1 Rho-independent terminator. However, unlike the Nus A caused reduction of termination at Rho-dependent terminators, Nus A causes an increase in termination at T_1. The data in Table II clearly illustrate this effect and show that the source of promoters or inclusion of antitermination-conferring sequences does not significantly alter the ability of Nus A to increase termination at T_1. In the analysis presented in Table II, RNA polymerase destined to read through T_1 in the absence of Nus A is considered to be the substrate for Nus A and therefore the Nus A caused increase in termination (headings of columns in Table II) is the effect of Nus A on this fraction of RNA polymerase. The data in Table II also show that Rho has no effect on termination at T_1 in either the presence or absence of Nus A and that the efficiency of T_1, like the efficiency of Rho-dependent terminators, fluctuates due to unknown reasons related to structural differences of the transcription templates.

Discussion

Comparison of in vitro termination at the Rho-dependent t_{R1}, $t_{R1}\Delta 9$, and *trp t'* terminators indicates that the qualitative

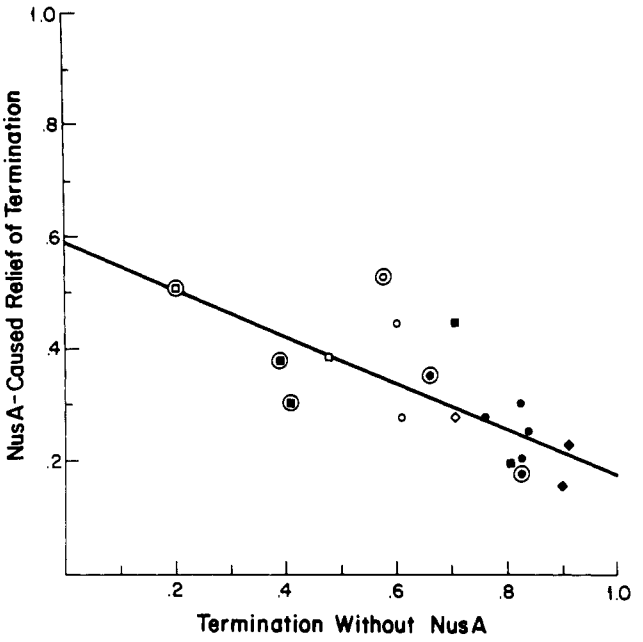

FIGURE 5: Relationship of the reduction of Rho-dependent termination by Nus A to termination region efficiency in the absence of Nus A. The symbol shape, coloring, and circling are arranged so that the promoter, terminator, and number of box A sequences in the transcription template responsible for each data point may be discerned. The symbols indicate there are (■) no, (●) one, and (◆) two box A sequences in the template. The symbols are filled in if the *rrnC* promoters direct transcription and are not filled in if the P_{tac} promoter directs transcription. Termination is at the *trp t'* terminator if each symbol is circled and at the t_{R1} terminator if it is not circled. The linear regression line through these points has a correlation coefficient of −0.75.

and quantitative effects of Nus A on termination are similar for all of these terminators. In addition, analysis of termination using different template DNA reveals that the effects of Nus A on termination are not qualitatively or quantitatively altered by the source of the promoters (either *rrnC* promoters or the *tac* promoter), the presence or absence of box A sequences from the λ t_{R1} region, or the presence or absence of a region of the *rrnC* leader capable of conferring antitermination in vivo. We have also shown that Nus A causes sequence-independent increases in termination at the *rrnC* Rho-independent T_1 terminator, thereby establishing that Nus A affects termination at a Rho-independent terminator in a manner opposite to the previously well-documented Nus A caused decrease in termination at Rho-dependent terminators. Altogether, our data show that, at least under the conditions of in vitro transcription we employed, specific DNA or RNA sequences are unlikely to increase the association of Nus A with the transcription complex or trigger an activity of Nus A important to termination.

It is at present unclear if any functions of Nus A require specific RNA or DNA sequences. Although the preferential action of Nus A at specific pause and termination sites in vitro implies some sequence specificity in pausing and termination, the fact that these sites are usually also recognized as pause and termination sites in the absence of Nus A suggests that interactions of RNA polymerase with RNA or DNA may entirely determine the sequence specificity of the effects of Nus A on pausing and termination. Therefore, Nus A might simply modify the properties of RNA polymerase transcribing all operons, a role consistent with the widespread defects in *nusA* mutants [reviewed in Platt (1986) and Freidman et al. (1984)]. This proposal is also supported by data showing sequence independence of Nus A induced pausing (Schmidt & Chamberlin, 1984a) and Nus A binding to RNA polymerase (Greenblatt & Li, 1981b).

It remains entirely possible that Nus A does facilitate antitermination in vivo by direct recognition of specific RNA or DNA sequences and the subsequent participation of other transcription factors. Alternatively, the effects of Nus A on pausing and termination in vitro may be physiologically meaningful but not require sequences required in vivo simply due to a failure to establish physiological conditions in vitro. However, there are at present no compelling data which indicate that any of the observed in vitro effects of Nus A on transcription are physiologically relevant. In the accompanying paper (Sigmund & Morgan, 1988) we provide data which support the proposal that the effects of Nus A on termination in vitro may not involve events important in vivo and discuss the possibility that modified in vitro transcription conditions might help reveal roles of Nus A (and perhaps also box A) that are important in vivo.

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Structural Changes of Nucleosomal Particles and Isolated Core-Histone Octamers Induced by Chemical Modification of Lysine Residues†

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ABSTRACT: Treatment of nucleosomal particles and isolated core-histone octamers with dimethylmaleic anhydride, but not with acetic anhydride, is accompanied by a biphasic release of the two H2A·H2B dimers, the first dimer being more easily released than the second. With both kinds of particles, 50% of histones H2A and H2B are released for modification of approximately 35% of the histone amino groups. The similar behavior of nucleosomal particles and isolated core-histone octamers is consistent with the same structure of the histone octamer in the nucleosomal particle and in the free octamer in 2 M NaCl. The described release of H2A·H2B dimers allows the preparation of nucleosomal particles deficient in one H2A·H2B dimer and of the histone hexamers H2A·H2B·$(H3·H4)_2$. For more extensive modifications, both reagents, acetic and dimethylmaleic anhydrides, cause the dissociation of nucleosomal particles with liberation of double-stranded DNA, which suggests that lysine amino groups are involved in the binding of histones to DNA. The modified nucleosomal particles are more sensitive to ionic strength than those untreated, and the presence of salt (NaCl) increases the extent of DNA release. The histones corresponding to the liberated DNA, except H2A and H2B released with dimethylmaleic anhydride, are apparently bound to the DNA-containing particles as extra histones.

The forces that bind double-stranded DNA to the core-histone octamer to form nucleosomal particles appear to be essentially electrostatic. Changes in these electrostatic interactions, as those produced by the physiological acetylation of lysine residues, might cause structural alterations of functional significance (Weisbrod, 1982; Reeves, 1984). The electrostatic forces within the nucleosomal particle are weakened by increasing the ionic strength and by modification of the interacting charged groups. Acylation of lysine residues has been used to change the interaction of histones with DNA. The chemical acetylation of chromatin decreases the salt concentration needed to release core histones (Wong & Marushige, 1976). Treatment of nucleosomal particles with dimethylmaleic anhydride causes rearrangement of the nucleosomal components, with release of histones H2A and H2B and of single-stranded DNA, and formation of residual particles deficient in histones H2A and H2B but containing an excess of H3 and H4 (Jordano et al., 1984a,c). Structural studies of the residual particles are consistent with the stabilization by histones H2A and H2B of a DNA length of 50–70 base pairs per nucleosome (Jordano et al., 1984b). Regeneration of the modified amino groups of the residual particles plus the complementary fraction containing histones H2A and H2B is accompanied by reconstitution of nucleosomal particles with the structural properties of the original nucleosomes (Jordano et al., 1984a,b). Recently, we have

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