Identification of Specific Contacts in T3 RNA Polymerase–Promoter Interactions: Kinetic Analysis Using Small Synthetic Promoters†

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Received September 2, 1992; Revised Manuscript Received February 11, 1993

ABSTRACT: The T7, T3, and SP6 RNA polymerases recognize very similar, yet distinct, promoter sequences. The high homology among the promoter sequences suggests that differential promoter recognition must derive from relatively small changes in the protein. Steady-state kinetic analyses of transcription from the T3 consensus promoter and from promoters modified in the region critical to specific recognition reveal details concerning which functional groups contribute to this recognition. Modifications include base pair substitutions, single base substitutions (mismatches), and simple functional group modifications at unique sites in the promoter. The results show that T3 RNA polymerase recognizes the amino group on the nontemplate cytidine in the major groove at position −10, while the identity of the base on the template strand is less critical to binding. In contrast, recognition at position −11 allows a greater range of modifications and seems to have a more complex recognition. The results do not seem to be consistent with a single recognition contact at this position; however, some groups may be ruled out as simple recognition contacts. While major groove modifications weaken binding at positions −10 and −11, the removal of an exocyclic amino group from the minor groove at either position does not disrupt binding, further supporting a model for promoter recognition in which the enzyme binds to one face of closed duplex DNA in this region. The effects of these changes in the DNA structure on the kinetics of initiation are compared to complementary results from the T7 system.

The RNA polymerases from the T3 and T7 family of bacteriophages present an ideal model system in which to probe the specificity of the molecular interactions involved in the initiation of transcription. The simplicity of structure of the enzymes, the ability to introduce subtle changes into both the DNA and the enzyme, and the easy isolation of both enzymes in high purity now permit a detailed kinetic study of the interaction of these enzymes with their promoters. These single-subunit DNA-dependent RNA polymerases are 884 amino acids in length, with a molecular mass of 100 kDa. Despite an 82% exact homology in protein sequence (Stahl & Zinn, 1981; Moffatt et al., 1984; McGraw et al., 1985) and a similar degree of conservation in their promoter sequences (Bailey et al., 1983; Basu et al., 1984), the two polymerases show a high degree of promoter specificity (Bailey et al., 1983; McAllister et al., 1983).

In the current study, interactions between T3 RNA polymerase and native and modified promoters are compared using an in vitro steady-state kinetic assay of initiation (Martin & Coleman, 1987). This assay allows direct determination of steady-state kinetic parameters associated with transcription from a short oligonucleotide promoter template (Martin & Coleman, 1987). Equation 1 shows the simplest steady-state equation that fully describes the observed kinetics.

$$\text{Enz} + \text{DNA} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Enz–DNA} \xrightarrow[\text{NTP's}]{k_{cat}} \text{Enz} + \text{DNA} + \text{RNA} \qquad K_m = \frac{k_{-1} + k_{cat}}{k_1} \quad (1)$$

Velocity of RNA synthesis is measured as a function of both total DNA and total enzyme concentration. A nonlinear least-squares algorithm then allows estimation of k_{cat} and K_m. To the extent that equilibration of the enzyme–DNA complex is fast relative to the rate-limiting step in catalysis ($k_{-1} \gg k_{cat}$), the Michaelis constant K_m approximates an apparent dissociation constant, K_d. In many of our experiments, K_m increases significantly, while k_{cat} remains unchanged, providing further support for the idea that K_m and k_{cat} may accurately reflect separate changes in binding and initiation kinetics, respectively. Changes in promoter function which result from changes in DNA, enzyme, or reaction conditions lead to measurable changes in K_m and/or k_{cat}. Comparison of K_m and k_{cat} values for a variety of individual, simple modifications of the enzyme–DNA system allows one to begin to establish the interactions involved in site-specific initiation.

The consensus sequences for the T3 and T7 promoters, compared in Figure 1, are highly conserved over a 17 base pair region from −17 to −1 relative to the transcription start site (Oakley & Coleman, 1977; Dunn & Studier, 1983). Comparison of the consensus sequences shows that the two promoters differ primarily at base pairs −10, −11, and −12, and it has been proposed that these three base pairs contribute most to the specific recognition of each polymerase for its respective promoter (Bailey et al., 1983; Klement et al., 1990). In the current work, we probe recognition of the −10 and −11 base pairs in T3 RNA polymerase in order to identify specific base functional groups which contribute to promoter recognition.

Four types of nucleotide modifications have been constructed: a dC → 5-methyl-dC base substitution, introducing a methyl group into the major groove of duplex DNA; a dG → dI base change, removing an amino group from the minor groove of duplex DNA; base pair mismatches to introduce (to

† Supported by Grant NP-722 from the American Cancer Society.
‡ Program in Molecular and Cellular Biology.
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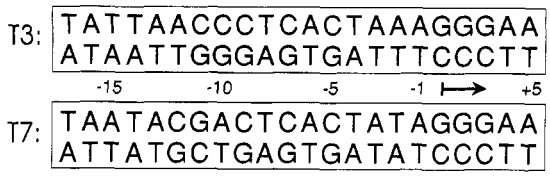

FIGURE 1: Comparison of T3 and T7 promoter sequences. All promoters contain the respective 17 base pair consensus promoter sequence from positions −17 to −1, followed by DNA to encode the five base message, GGGAA.

first order) localized changes in both the major and minor grooves; and full base pair substitutions introducing more substantial changes in the major and minor grooves of duplex DNA. The effects of these changes in DNA structure on the kinetics of initiation are compared to the kinetics of the native T3 promoter. Finally, these data are compared to complementary results for the T7 enzyme–promoter system.

EXPERIMENTAL PROCEDURES

RNA Polymerase. T3 RNA polymerase was prepared from *Escherichia coli* strain BL21 carrying the overproducing plasmid pCM56 (kindly supplied by W. T. McAllister), with the T3 gene 1 (RNA polymerase) cloned under inducible control of the *lac* UV5 promoter (Morris et al., 1986). The enzyme was purified as described for T7 RNA polymerase (King et al., 1986). The total number of aromatic residues in T3 and T7 RNA polymerase differs by 2–3%; therefore, a molar extinction of $\epsilon_{280} = 1.4 \times 10^5$ M^{-1}, previously determined for the T7 enzyme (King et al., 1986), was used to determine enzyme concentrations.

Oligonucleotides. Oligonucleotides were synthesized by the phosphoramidite method on a Milligen/Biosearch Cyclone Plus DNA synthesizer. All phosphoramidites were purchased from Cruachem. Single strands from a 1-μmol scale synthesis were purified trityl-on on an Amberchrome (TosoHaas) reverse-phase resin. Approximately 3 mL of resin was placed in a disposable syringe and equilibrated with 15 mL of acetonitrile, followed by a wash with 15 mL of 1.0 M triethylamine acetate, pH 7.0. After standard deprotection with 30% ammonium hydroxide, crude deprotected oligonucleotides were diluted with water (1:1, v/v), loaded onto the column and reloaded three times. Failure sequences lacking the trityl group were washed from the column in 15 mL of 3% ammonium hydroxide, and the ammonium hydroxide was removed from the column with 15 mL of water. The trityl group was then cleaved with 1 mL of 2% trifluoroacetic acid for 1 min, followed by a 10-mL wash with water. Finally, the retained oligonucleotides were eluted in six fractions with 5 mL of 20% acetonitrile. Peak fractions were dried down and then resuspended in a buffer containing 40 mM HEPES, pH 7.8, 1 mM EDTA,[1] and 10 mM NaCl. Concentrations of the single-stranded oligonucleotides were calculated using the weighted sums of the three different measured molar extinction coefficients for each base at 253, 259, and 267 nm. In general, the calculations at the different wavelengths were in good agreement (M. Maslak, unpublished results). DNA purity was verified by denaturing polyacrylamide gel electrophoresis.

The incorporation of deoxyuridine modifications at specific positions in the promoter sequence was verified using the uracil specific enzyme uracil-*N*-glycosylase (Life Technologies). Single-stranded oligonucleotides containing uracil were individually incubated for 1 h with uracil-*N*-glycosylase at 37 °C to abstract the uracil base and then heated for 1 h at 95 °C. The reaction buffer (20 mM Tris-HCl, pH 8.0, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mg/mL BSA, and 1 unit of uracil-*N*-glycosylase) is slightly basic and at elevated temperatures promotes the cleavage of the DNA at sites where the uracil has been removed. Individual samples were then end-labeled with ^{32}P using polynucleotide kinase (New England Biolabs, Inc.) and visualized on a 20% denaturing (7 M urea) polyacrylamide gel (data not shown).

Double-stranded oligonucleotides were prepared by annealing a 1:1 mixture of complementary single strands in 40 mM HEPES, pH 7.8, and 1 mM EDTA. All synthetic templates used in the kinetic assays possessed 3′- and 5′-hydroxyl groups.

Kinetics. Kinetic assays of transcription (Martin & Coleman, 1987) on oligonucleotide templates were carried out in 20 μL of 30 mM HEPES, pH 7.8, 15 mM magnesium acetate, 100 mM potassium glutamate, 0.25 mM EDTA, 1 mM DTT, 0.1 mg/mL *N*,*N*-dimethylated casein, 0.05% TWEEN-20 (Calbiochem, 10% Protein grade), 0.8 mM GTP, and 0.4 mM ATP, as described in Maslak et al. (1993).

RESULTS

The synthetic promoters used in this study are derived from the sequence shown in Figure 1, consisting of the known T3 consensus sequence from positions −17 to −1 plus the DNA coding for the five-base message GGGAA. The bases at positions −12, −11, and −10 are believed to be primarily responsible for differential specificity between the T3 and T7 RNA polymerases (Klement et al., 1990). To analyze in detail which functional groups contribute to this recognition process, a variety of changes were made at positions −11 and −10. This region is likely to remain duplex during binding and catalysis, and the results are consistent with this assumption. The modifications presented below begin with very simple changes, in which functional groups are either added or removed from one region of the major or minor groove of duplex DNA, while leaving other regions unchanged to first order. With this additional perspective, more complex substitutions are then introduced in order to provide a smooth progression of functional group substitutions (Figure 2).

Steady-state kinetic parameters for the T3 RNA polymerase transcribing from its consensus promoter are $K_m = 1.0$ nM and $k_{cat} = 40$ min^{-1}. As might be expected, these kinetic constants are almost identical to the corresponding values for the T7 enzyme under the same conditions (Maslak et al., 1993). Changes in K_m and k_{cat} for various modified T3 promoters are compared in Table I.

Tests of Minor Groove Interactions. In order to probe for minor groove recognition elements in duplex DNA, one can effectively remove the 2-amino group from guanine via the single substitution dG → dI. The removal of this amino group changes the profile of a CG base pair in the minor groove to that of a TA base pair, while leaving the basic profile of the major groove unchanged. At positions −11 and −10 (template strand) neither of the dG → dI substitutions has a significant effect on K_m (0.8 and 0.2 nM, respectively) or k_{cat} (36 and 33 min^{-1}, respectively). This lack of any measurable perturbation to the kinetics strongly suggests that minor groove contacts do not contribute to binding. Of equal importance, this result indicates that substantial changes in the minor groove do not indirectly disrupt recognition contacts. Finally, the lack of interactions with the guanine 2-amino group makes the CI base pair an alternate platform from which to probe contacts in the major groove at positions −11 and −10.

[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol.

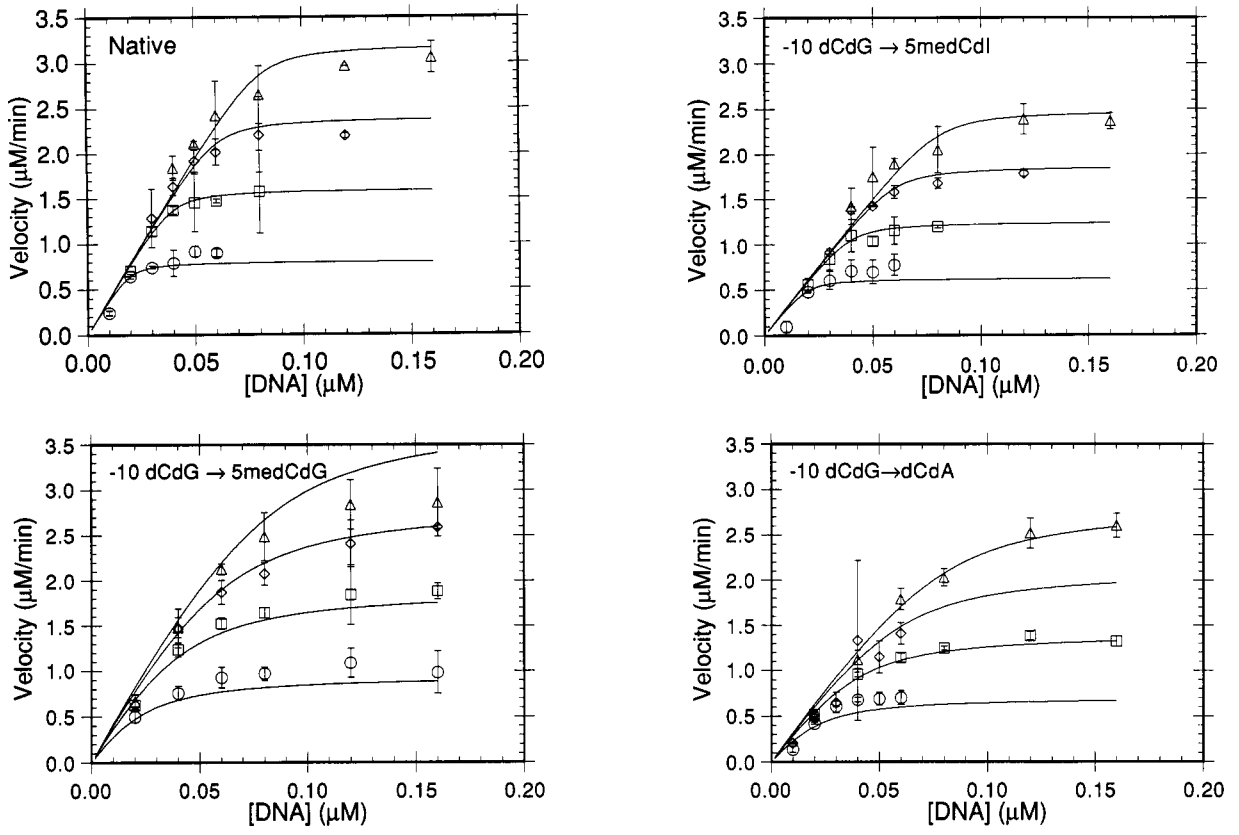

FIGURE 2: Representative fits to steady-state kinetic data from various templates. Each set of data includes the velocity curves predicted for the enzyme concentrations by the single set of best fit values for K_m and k_{cat}, as shown in Table I. The enzyme concentrations are (○) 0.02, (□) 0.04, (◇) 0.06, and (△) 0.08 μM.

Table I: Comparison of the Kinetic Parameters for Native and Modified Promoters[a]

DNA	K_m (nM)	k_{cat} (min^{-1})
native	1.0 (0.40–2.4)	40.2 (38.5–41.7)
−10dCdG → dCdI	0.2 (<0.01–1.5)	32.9 (31.1–34.5)
−10dCdG → 5medCdI	1.2 (0.47–2.7)	31.1 (29.7–32.2)
−10dCdG → 5medCdG	10 (7–16)	47.5 (44.3–50.8)
−10dCdG → dUdA	36.4 (25.2–53.2)	17.3 (15.9–18.7)
−10dCdG → dCdA	7.5 (4.4–12.7)	35.2 (32.6–37.8)
−10dCdG → dUdG	21.0 (13.0–34.0)	14.2 (13.1–15.3)
−11dCdG → dCdI	0.8 (0.01–3.1)	36.0 (33.8–37.6)
−11dCdG → 5medCdI	2.6 (1.4–4.9)	28.8 (27.3–30.2)
−11dCdG → 5medCdG	9.6 (5.1–17.8)	43.0 (38.8–47.0)
−11dCdG → dUdA	4.1 (1.9–8.3)	25.1 (23.2–26.8)
−11dCdG → dCdA	6.7 (4.2–10.6)	33.6 (31.5–35.7)
−11dCdG → dUdG	6.5 (4.2–10.0)	35.2 (33.4–37.0)
−11dCdG → TdA	11.2 (7.4–17.0)	40.0 (37.8–42.6)
−11dCdG → dGdC	26 (15–45)	30.9 (26.2–35.7)

[a] Indicated ranges represent a 65% joint confidence interval for the best fit parameters.

Tests of Major Groove Determinants. Since minor groove contacts are not implicated in binding at positions −11 and −10, it is likely that major groove determinants are essential to recognition. The latter may be probed by placing a methyl group into the major groove, via a dC → 5-methyl-dC substitution. This simple change leaves the minor groove profile unchanged and does not directly disrupt simple Watson–Crick base pairing. The results show that adding a methyl group to the 5-substituent of cytosine at positions −10 or −11 has only a small effect on the reaction rate (k_{cat} = 48 and 43 min^{-1}, respectively) and a small but significant weakening effect on binding (K_m = 10 nM for both). Combined with the results of the dCdG → dCdI substitution above, these results indicate significant major groove interactions in this region of the promoter.

The simplest explanation for the effect of the addition of a methyl group at the 5-position of cytosine is steric hindrance. This may occur directly via a general crowding at the protein–DNA recognition interface or more indirectly via the displacement of a specific nearby favorable interaction. Since a CG base pair is more constrained than an AT base pair, there may be little possibility of local rearrangement to accommodate the new steric bulk. The substitution 5-methyl-dCdG → 5-methyl-dCdI might allow such a rearrangement by lowering the barrier to propeller motions of the bases. Incorporation of this modification at positions −11 and −10 has no effect on catalysis (k_{cat} = 29 and 31 min^{-1}, respectively) but does result in the restoration of tight binding (K_m = 2.6 and 1.2 nM, respectively). Since the modification dCdG → dCdI had no effect on recognition, this result suggests that energetic stress introduced into the major groove by the 5-methyl group can be relieved by subtle movements of the 5-methyl-dCdI base pair.

The implication of major groove contacts, combined with evidence suggesting a lack of minor groove contacts, focuses attention on the other functional groups in the major groove of a CG base pair, namely, the 4-amino group of cytosine and the 6-carbonyl of guanine. Starting from the base pair CI, which is recognized as well as CG, one can effectively swap only these two functional groups in the major groove by incorporating a dCdI → dUdA substitution (the minor groove profile of a UA base pair is identical to that of a CI base pair). At the −11 position, the dCdI → dUdA change has little effect on binding and catalysis (K_m = 4 nM and k_{cat} = 25 min^{-1}), suggesting that the positions of the carbonyl and the amino groups in the major groove at position −11 are not important in binding. In contrast, at the −10 position this major groove "swap" has a large effect on the kinetic

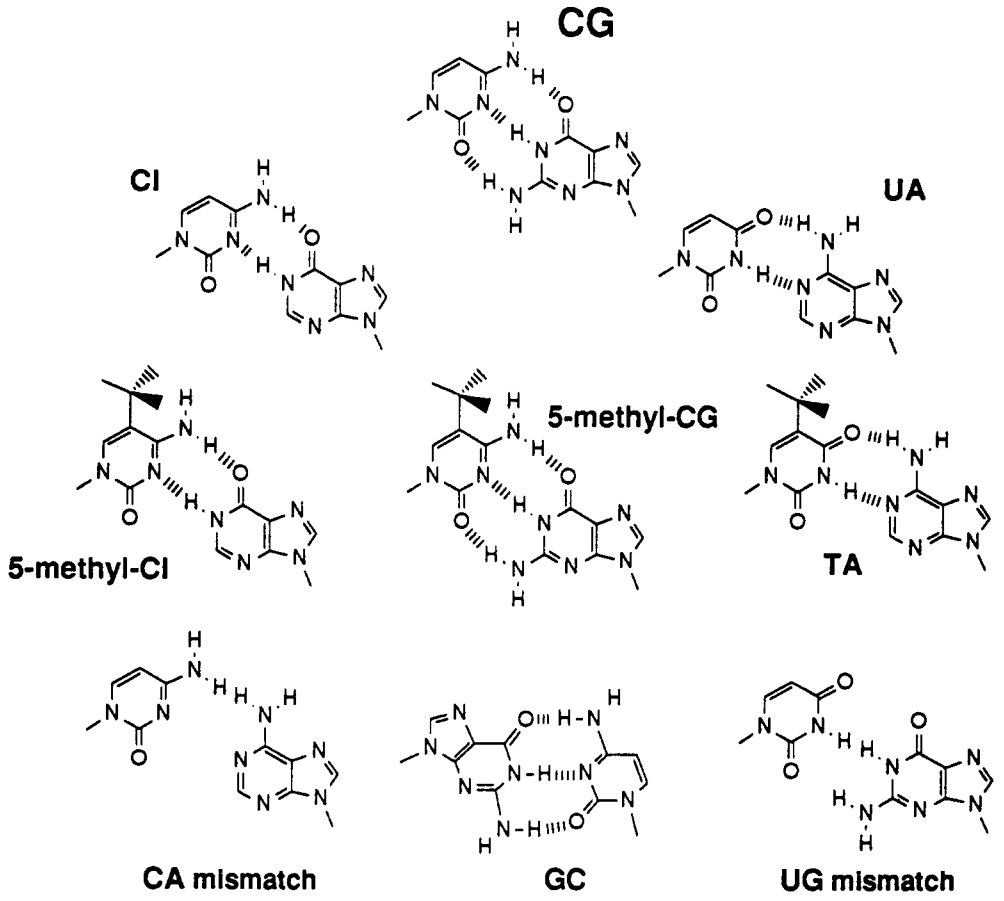

FIGURE 3: Modified base pairs used in this study. Note the profile of the major and minor grooves when comparing base pairs.

parameters (K_m = 36 nM and k_{cat} = 17 min^{-1}), suggesting that the positions of the carbonyl and/or amino groups in the major groove at position −10 are important for recognition.

To determine more specifically which of the major groove functional groups are involved in recognition at positions −10 and −11, we continued with mismatched base pair studies. Although potentially more disruptive of local structure, mismatches allow one to easily place a modified base in one strand of the duplex while leaving the native base on the other strand. In the major groove, the dCdG → dCdA mismatch substitution replaces the guanine 6-carbonyl with an amino group, while the dCdG → dUdG mismatch replaces the cytosine 4-amino group in the major groove with a carbonyl. At position −11, both CA and UG have similar small effects on binding (K_m = 7 nM) and no significant effect on catalysis (k_{cat} = 34 and 35 min^{-1}, respectively). This small change in binding most likely reflects secondary effects due to the mismatch created. At position −10 the CA and UG substitutions have different effects. The CA mismatch results in only a small weakening in binding (K_m = 8 nM; k_{cat} = 35 min^{-1}), while the UG mismatch retains the larger disruption characteristic of the UA base pair (K_m = 21 nM; k_{cat} = 14 min^{-1}). These data strongly suggest that it is the cytidine of the nontemplate strand at position −10 that is important for binding, specifically the 4-amino group of cytosine.

The remaining functional group candidates at position −11 are the 5- and 6-hydrogen of cytosine and the 7-imino nitrogen of guanine, both in the major groove, and the 2-carbonyl of cytosine in the minor groove. The involvement of these groups can be tested with complete base pair changes which either preserve or swap purines and pyrimidines. We have already seen that the substitution dCdG → dUdA, which preserves the placement of purine and pyrimidine (and therefore the positioning of these functional groups) has little effect at position −11 but a large effect at position −10. At position −11, the substitution dCdG → TdA has a small effect on binding (K_m = 11 nM), comparable to the effect of adding a pyrimidine 5-methyl in the dCdG → 5-methyl-dCdG substitution at this position (K_m = 10 nM). Finally, the substitution dCdG → dGdC swaps purine and pyrimidine and therefore substantially rearranges the major and minor grooves. This substitution at position −11 has a large effect on binding (K_m = 26 nM).

DISCUSSION

The T3 and T7 RNA polymerases share a high degree of protein primary sequence conservation (Stahl & Zinn, 1981; Moffatt et al., 1984; McGraw et al., 1985), and their consensus promoter sequences differ primarily only at positions −10, −11, and −12 (Bailey et al., 1983; Basu et al., 1984). Nevertheless, the two enzymes are highly specific for their respective promoters (Bailey et al., 1983; McAllister et al., 1983). Studies of artificially constructed hybrid polymerases have located a region in the protein, from amino acid residues 674 to 754, that determines the specificity for each promoter (Joho et al., 1990). Only 11 amino acids in this region are not conserved between the two species. Recently, Raskin et al. (1992) have identified a specific amino acid involved in differential recognition of the two polymerases. By changing one amino acid in each of the T3 and the T7 RNA polymerases (in T3 Asp749 → Asn; in T7 Asn748 → Asp), they have been able to switch the specificities of the polymerases at position −11 to the heterologous sequence. From these studies, they have developed a model of differential recognition which involves major groove contacts with the base pairs at positions −11 and −10.

Footprinting studies in the T7 system have suggested that the polymerase binds primarily to one face of a fully or partially duplex form of the promoter (Basu & Maitra, 1986; Ikeda & Richardson, 1986; Gunderson et al., 1987; Muller et al., 1989). The hydroxyl radical protection pattern suggests that the bound protein spans the major groove of primarily duplex DNA in the region of differential specificity (Muller et al., 1989). The strong difference between predicted major and minor groove effects observed in the current studies is consistent with the idea that this region is duplex DNA in a recognition complex. The dCdG → dCdI substitution results, in particular, suggest that no minor groove contacts are occurring in this region. Furthermore, ethylation and methylation interference data on T7 and T3 promoters (Jorgensen et al., 1989, 1991), along with kinetic data from T7 promoters (Martin & Coleman, 1987; Maslak et al., 1993) suggest that the polymerase may be binding this major groove asymmetrically, interacting preferentially with the base on the nontemplate strand. The dCdG → dUdA → dCdA changes at the −10 position, presented here, provide strong evidence to support this asymmetric binding. All of the data presented to date are consistent with a simple read out of functional groups in the major groove. The fact that substantial changes in the minor groove are tolerated supports this view.

Position −10 Contacts. The substitutions at −10 strongly implicate contacts in the major groove nontemplate strand and, in particular, most strongly support interactions with the cytosine 4-amino group. In the mismatch studies, replacement of the 4-amino group of cytosine by the carbonyl of uracil (dCdG,dCdI → dUdG) disrupts binding substantially, while the corresponding substitution of inosine by adenosine in the opposite strand (dCdI → dCdA) produces only a small effect. This same interaction with the cytosine 4-amino group may explain the sensitivity of recognition to the dC → 5-methyl-dC substitution. Addition of the 5-methyl group to cytosine may sterically restrict access to the 4-amino group. However, subsequent removal of one of the Watson–Crick hydrogen bonds in the 5-methyl-dCdG → 5-methyl-dCdI substitution might alleviate this secondary restriction by allowing a propeller motion of the bases. In any case, recognition of the nontemplate strand base is demonstrated by these results.

The current data show that changes in the template strand at position −10 have little or no effect in the T3 system. Removal of the methyl group of the template strand thymine in the T7 system, through the placement of a uridine at position −10, also has no effect on binding (Maslak et al., 1993), and Martin and Coleman (1987) demonstrated in the T7 system that the nature of the base in the nontemplate strand at position −10 is more critical to binding than is that of the template strand. Together these results agree with the recent proposal (Raskin et al., 1992) that Asp749 of the T3 enzyme makes a contact with the 4-amino group on the nontemplate strand at position −10 of the T3 promoter.

Position −11 Contacts. The implications of the modifications at position −11 are less clear. Introduction of a methyl group into the major groove at position −11 (dCdG → 5-methyl-dCdG) results in a small disruption in binding (as evidenced by changes in K_m), suggesting contacts on the nontemplate strand at this position. Substitution by 5-methyl-dCdI restores the interaction, similar to what is observed at position −10. A slight repositioning of the base pair, allowed by the removal of one Watson–Crick hydrogen bond, may relieve the steric stress imposed by the added methyl group. However, unlike the result at position −10, replacement of the 4-amino group at position −11, with the 4-carbonyl of uracil results in very little disruption of binding. In base pair mismatch studies, the CA mismatch has an effect on binding comparable to that of the UG mismatch. This slight effect may not be due to the loss of a specific contact, but rather to a more general disruption of the site resulting from the mismatch (as seen in the CA mismatch at position −10). Moreover, a complete swap of exocyclic amino and carbonyl groups in the major groove at position −11 (dCdG → dUdA) causes little or no disruption in binding. Taken at face value, this would appear to argue against a recent proposal that recognition at position −11 occurs via the 4-amino group of cytosine (Raskin et al., 1992).

It has been previously shown that methylation of the 7-imino group of the template strand guanosine at position −11 does not interfere with binding (Jorgensen et al., 1991), thereby ruling out the third of the common candidates for direct read out through hydrogen bonding. Results from the incorporation of 5-methyl-dC at this position, presented above, however, show that introduction of a pyrimidine 5-methyl does weaken binding (increases K_m). Indeed, in the progression dCdG → dUdA → TdA, the change dUdA → TdA results in a significant increase in K_m, similar to that observed for the dCdG → 5-methyl-dCdG substitution. These results lead to the proposal that the 5-hydrogen position of cytosine may be recognized on the nontemplate strand at position −11, via either steric considerations or van der Waals contacts. As at position −10, the effects of the dCdI → 5-methyl-dCdI substitution suggest a role for base pair rigidity in this mode of recognition. It is intriguing to propose that the 4-amino group of cytosine at position −11 may be a normal recognition contact but that, in a UA pair (replacing the cytosine amino group by a carbonyl), the 5-hydrogen of uracil might serve as an alternate contact. Although weak hydrogen bond donation of the 5-hydrogen is theoretically possible, model studies of nucleic acid base analogs have been unable to observe such interactions (Bruskov et al., 1989).

Implications for Protein–DNA Interactions. At positions −11 and −10, the lack of sensitivity to some of the mismatches and whole base changes argue against any recognition based on subtle structural differences in the DNA. The data summarized above strongly suggest a local and specific interaction with the cytosine 4-amino group at position −10. Significant alterations of other functional groups have little or no effect. At position −11, recognition may not be as straightforward but certainly involves contacts along the same nontemplate strand wall of the major groove (the 4- and 5-positions of cytosine). An interesting proposal is that recognition occurs via an interaction with the 4-amino group of cytosine but that, in an appropriate promoter variant, the 5-hydrogen of uracil can serve as an alternate contact.

On the basis of recent studies of mutant enzymes and promoters containing single base pair substitutions, Raskin et al. (1992) have proposed that Asp749 in the T3 RNA polymerase makes a bidentate contact with the cytosine 4-amino group at position −10 and the cytosine 4-amino group at position −11. The results presented here strongly support involvement of the 4-amino group at position −10 and provide some support for the involvement of the 4-amino group at position −11. Analysis of model B-form DNA suggests that simple movements would allow the same aspartate carboxylate to form a bidentate interaction with the 4-amino group of cytosine at position −10 and either the 4-amino of cytosine or the 5-hydrogen of uracil at position −11.

By similar dissection of recognition elements in the T7 promoter, other protein–DNA contacts will be identified, and

ambiguities in our understanding of recognition at the −11 position may be resolved. The addition to this study of an array of T7 and T3 enzymes containing directed mutations in the proposed recognition region of the protein (Raskin & McAllister, 1992; Raskin et al., 1992) will further refine the developing model of this central region of the promoter.

ACKNOWLEDGMENT

We are grateful to Maribeth Maslak for valuable discussions and to Dr. William McAllister for communicating results prior to publication. We thank Dr. Amos Heckendorf of The Nest Group (Southboro, MA) for suggesting the use of Amberchrome resin in the purification of synthetic oligonucleotides.

SUPPLEMENTARY MATERIAL AVAILABLE

Steady-state kinetic fits for constructs listed in Table I but not included in Figure 2 (3 pages). Ordering information is given on any current masthead page.

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