Single and multiple point mutations were introduced to change the 12 glutamine residues within a 37-amino acid region of σ^d4. Multiple changes are shown to be required in order to interfere significantly with the function of this protein which is associated with enhancer-dependent bacterial transcription. Mutation of the central 4 glutamines leads to the production of less m-RNA, caused by an inability to fully open the promoter start site. DNA binding, however, is normal. Mutation of 4 other adjacent glutamines causes the promoter start site to open more readily than wild type, although this enhanced opening is not accompanied by more mRNA. The enhanced DNA melting is not caused by enhanced promoter binding, as indicated by normal protection of the polymerase-bound promoter against dimethyl sulfate attack. The results suggest that multiple glutamines play a role in transducing the melting signal from the enhancer protein to the polymerase.

σ^d4 is a minor bacterial transcription factor that is required for certain promoters whose activation elements can be moved far away and still retain function (Reitze and Magasanik, 1986; Birkman and Bock, 1989; Morett and Buck, 1988). The sequence of σ^d4 is not similar to other σ factors, and it also recognizes unique promoter elements located at positions -12 and -24. The glnAp2 promoter is transcribed by σ^d4 in association with RNA polymerase in response to nitrogen deprivation of Escherichia coli. The activator protein is phosphorylated NTRC which binds to sites approximately 100 base pairs upstream from the transcription start site (for reviews see Magasanik, 1988; Kustu et al., 1989, 1991). Other promoters use different activators and co-activators, but enhancer-dependent transcription appears to require the participation of σ^d4-associated RNA polymerase (see Collado-Vides et al., 1991).

The σ^d4 protein contains motifs reminiscent of those involved in enhancer-dependent eukaryotic transcription (Sasse-Dwight and Gralla, 1990). These include potential leucine zippers, a very acidic region, and a glutamine-rich region. We have been investigating the potential roles of these regions by assaying mutant forms of σ^d4. A repetitive element within the acidic region was shown recently to be required for fully opening the promoter start site and also for setting the rate at which this opening occurs (Wong and Gralla, 1992). Small deletions near the nonacidic N terminus of σ^d4, in which a majority of residues are either glutamine or leucine, lead to a loss of function (Sasse-Dwight and Gralla, 1990). The potential role of the glutamines in this region is of interest, since glutamine-rich regions were identified as critical parts of the mammalian transcription factors (Courey and Tjian, 1988; Courey et al., 1989; Dynlacht et al., 1989; and Maity et al., 1990; Gerster et al., 1990). Thus far, σ^d4 is the only bacterial transcription factor suggested to use a glutamine-rich domain, which could be related to its unique role in mediating eukaryotic-type mechanisms (see Gralla, 1991).

The main goal of this paper is to study the functional importance of glutamines in the N terminus of σ^d4. In this study a number of mutants were created and tested in vivo. The results showed that multiple glutamine substitutions lead to strong defects in σ^d4-dependent transcription. The major mechanistic alteration at the glnAp2 promoter was altered levels of start site melting. Some glutamine changes led to low levels of melting whereas others led to excessive levels of melting suggesting that the region plays a role in transducing the signal sent by the enhancer protein allowing appropriate DNA melting and mRNA initiation.

MATERIALS AND METHODS

The background strain, YMC109tk(thi, endA, har, Δlac U169, glzF.Tn10/F' pro lacI'ZU118, Tn5-102), was prepared by transducing YMC6 (Agln101) (Backman et al., 1981) (pro, thi, endA, har, pro ΔlacU169, kindly provided by Dr. Boris Magasanik, MIT) with P1 lysate from YMC18 (thi, endA, har, pro Δlac U169, glzF.Tn10). The resulting strain was then mated with RJ1399 (Δ pro lacI'ZU118, Tn5-102, kindly provided by Dr. Reid Johnson), to obtain YMC109tk carrying the lacI' gene on an episome and a Tn10 transposon inserted into the chromosomal rpoN (σ^d4) gene.

Site-specific mutations were made at glutamines in Region 1 of the rpoN (σ^d4) gene by use of the Amersham Corp. in vitro mutagenesis kit. Briefly, the σ^d4-encoding plasmid pTH7, originally supplied by Dr. Boris Magasanik (MIT), was digested with EcoRI-EamHI, and the fragment containing the σ^d4 gene was cloned into the M13 mp18 vector to make single-stranded DNA. The mutant oligonucleotides were annealed to the single-stranded DNA template, and the mutagenesis procedures were then conducted according to the protocols. The EcoRI-BamHI fragment from the RF form of M13, containing the desired mutation, was then isolated and cloned back to the pTH7 plasmid.

Mutant σ^d4 protein expression levels were determined as described previously (Wong and Gralla, 1992). Examples are shown in Fig. 2. All mutants also inhibited growth on W-Gln-IPTG plates which is an indirect indication of expression (Sasse-Dwight and Gralla, 1990). To test growth, the YMC109tk cells were transformed with plasmids pTH7 carrying mutant σ^d4 gene. The transformants were plated on both W-Arg-X-gal plates (arginine, 0.01 mg/ml; X-gal, 40 μg/ml), which had arginine as the only nitrogen source and LB plates supplemented with kanamycin (40 μg/ml), tetracycline (15 μg/ml), and ampicillin (100 μg/ ml). In preparing W-Arg plates, W-salts media (a minimal medium without nitrogen) was prepared as described (Smith et al., 1971; Reitzer and Magasanik, 1985). After overnight incubation in 37 °C, the colonies on the LB plates were then picked up and streaked on the W-Arg/X-gal.
plates for further comparison. In both the transformation and the streak tests the W-Arg/X-gal plates were incubated at room temperature. We found that maximal differences in color development occurred at very low concentration of arginine and the incubation at the room temperature.

The dimethyl sulfate footprinting, potassium permanganate probing, and mRNA assays were all done in identical media (G-Gln) containing IPTG. For footprinting, the YMC109tk cells harboring the desired $\sigma^{4\mu}$ substitution plasmids were grown to OD$_{so90}$ 0.35–0.5 in the strong activating media G-Gln (Smith et al., 1971; Reitter and Magasanik, 1985) in the presence of the proper antibiotics. The expression of $\sigma^{4\mu}$ was then induced with IPTG for 1 h before probing with footprinting reagents. The procedures for permanganate and dimethyl sulfate footprinting and primer extension analysis were done as described (Sasse-Dwight and Gralla, 1991). The DNA concentration of all the samples was determined, and the same amount of the DNA was used for each primer extension. The samples were resolved on 6% DNA sequencing gels. To quantify the open complexes, the corresponding radioactive bands were excised and counted.

For mRNA analysis, YMC109tk cells bearing the desired substitution plasmids were grown in G-Gln media and induced with IPTG as described above. At 0–0.8 OD$_{so90}$, cells were resuspended in 350 ml of SA (0.15 m sucrose, 0.01 m sodium acetate) and then lysed in 10% SDS/SSA solution at 65 °C for 1 min (von Gabain et al., 1983). Sequentially, 60 ml of 2 m sodium acetate, pH 4, 0.5 ml of phenol (water saturated), and 100 ml of chloroform/isoamyl alcohol mixture (24:1) were added and shaken vigorously for 10 s and cooled on ice for 15 min. Samples were then centrifuged at 10,000 for 20 min at 4 °C. The aqueous phase was incubated with 20 units of RNase-free DNase I for 30 min at 37 °C. Phenol/chloroform/isoamyl alcohol extraction was repeated, and the RNA was precipitated with isopropyl alcohol twice. After washing with 75% ethanol, RNA pellet was dissolved in 40 ml of sterile water. The concentration of the total RNA was determined, and approximately 20 ug of total RNA was used for each primer extension. The primer extensions were done according to Baga et al. (1988). The same glnD primer used for footprinting was also used for RNA primer extension. The samples were resolved on 6% DNA sequencing gels.

RESULTS

The Glutamine Point Mutants—Expression plasmids carrying either wild-type or mutant $\sigma^{4\mu}$ genes were introduced into cells that lack a chromosomal $\sigma^{4\mu}$ (YMC109tk). The cells contain two copies of the $\sigma^{4\mu}$-dependent glnAp2 promoter. One directs transcription of the glutamine synthetase gene, required for growth on minimal (W-Arg) plates where arginine is the sole nitrogen source; the other is fused to the $\beta$-galactosidase gene in order to indicate glnAp2 expression via blue color on X-gal plates.

The glutamine-rich region of $\sigma^{4\mu}$ contains 12 glutamines within the 37 N-terminal amino acids (Fig. 1). Initial results showed that single point mutations in glutamines 18, 20, 21, or 35 to serine did not affect the growth on W-Arg/X-gal plates and did not change the colony color from blue. This indicates that the single glutamine point mutations do not interfere strongly with the function of $\sigma^{4\mu}$.

Since multiple changes are needed to observe defects within the acidic region of $\sigma^{4\mu}$ (see Sasse-Dwight and Gralla, 1990; Wong and Gralla, 1992), we created multiple glutamine changes. The region was divided into three subregions, each containing 4 glutamines (see Fig. 1). The subregion 1 glutamines are the most N-terminal. Subregions 2 and 3 lie between proline 17 and proline 45 that form the boundaries of the putative coiled-coil motif associated with the leucine residues of $\sigma^{4\mu}$ (Sasse-Dwight and Gralla, 1990). All 4 glutamines in each of the three subregions were mutated simultaneously. In order to retain the hydrophilic character of the residues (to minimize disruptions of putative coiled-coil interactions), but to change possible hydrogen bonding patterns, we replaced the glutamines with either glutamate or serine. Thus this set consists of six mutants, each of the three subregions with changes to glutamate or changes to serine.

In addition, we combined these mutants so that 8 or 12 glutamines were mutated to either glutamate or to serine. These are named according to the type of change introduced and the subregions that are mutated; for example, simultaneously changing the 8 glutamines (Q) to glutamate (E) in subregions 1 and 2 generates mutant QE12. These mutants were expressed as judged by protein analysis and plate tests (see Fig. 2, for examples).

The mutants were tested for function by monitoring growth on W-Arg/X-gal plates. The results are summarized in Table I. All mutants can grow, but the color, indicative of the amount of functional $\sigma^{4\mu}$-holoenzyme, differed for different mutants. The color varied from the dark blue characteristic of wild type to light blue to white. The initial results indicate that these multiple-point mutants are potentially interesting.

The data suggest that the subregion 2 glutamines may be most critical for function. Within the less defective serine series, QS2 is the only mutation restricted to a single subregion that shows an obvious defect in this preliminary assay. Subregion 2 changes are also defective in the glutamate series (QE2). In the entire set only 2 mutants are so defective as to form white colonies and both of these contain subregion 2 changes (QE12 and QE123; see Table I). We also note two other inferences. One is that the glutamate changes are more damaging than the serine changes, even though the amino acids glutamine and glutamate have similar backbone structures. The other is the curiosity that QS2, which is light blue on its own, leads to dark blue colony formation when recombined with mutated flanking residues (see "Discussion").

GlnAp2 mRNA Formation by the Glutamine Mutants—Next, all multiple mutants were assayed for their ability to direct glnAp2 promoter in vivo. Total cellular RNA was isolated from

| Subregion | Mutant | Color |
|-----------|--------|-------|
| Subregion 1 | QS18 | S |
| Subregion 2 | QS20 | S |
| Subregion 3 | QS21 | S |
| Subregion 1,2 | QS5 | S |
| Subregion 1,3 | QS1 | SS |
| Subregion 2,3 | QS2 | SS |
| Subregion 1,2,3 | QS3 | SS |
| Subregion 1,2 | QS4 | SS |
| Subregion 1,3 | QS5 | SS |
| Subregion 2,3 | QS6 | SS |
| Subregion 1,2 | QE1 | EE EE |
| Subregion 1,3 | QE2 | EE EE |
| Subregion 2,3 | QE3 | EE EE |
| Subregion 1,2 | QE4 | EE EE |
| Subregion 1,3 | QE5 | EE EE |
| Subregion 2,3 | QE6 | EE EE |

Fig. 1. Amino acid sequences of the N-terminal glutamine substitution mutants. The upper sequence shows the 45 amino acids (in standard single letter code) of the N-terminal region of $\sigma^{4\mu}$ gene. The 12 glutamines are marked. Subregions 1, 2, and 3 are noted. The specific glutamine substitutions are indicated either from glutamine (Q) to serine (S) or from glutamine to glutamate (E). The first 4 mutants are the single point substitutions and named by the change from glutamine to serine and the amino acid position. The rest of the multiple change mutants are named by the amino acid change and the subregions.
the cells, and glnAp2-specific mRNA was assayed by extension of hybridized labeled primers using reverse transcriptase. In each experiment equal amounts of total RNA were loaded in each lane. The results show that all mutant forms of $\sigma^{54}$ polymerase direct transcription from the same initiation point (Fig. 3). This confirms that all mutant forms of polymerase are using the normal glnAp2 promoter (Reitzer and Magasanik, 1985; Hirschman et al., 1985) rather than recognizing some alternative region.

The amount of mRNA produced by the different $\sigma^{54}$ polymerases, however, differs. The assays were done in duplicate or triplicate, and the amount of mRNA for each mutant is shown in Table II. On the whole the results agree with the preliminary indications inferred from the plate tests. The $\sigma$E series of mutants shows a stronger defect in mRNA levels than does the QS series. The most defective of the group, as judged from the plate test, are QS12 and QE123 which produce less than 10% of the wild-type mRNA level. The functional defects of the QS mutants were not due to instability of the proteins, since the expression level of these mutants were comparable with that of wild type (Fig. 2). Other mutants showed intermediate effects which will be discussed in more detail below. We conclude that multiple glutamine substitutions can lead to significant changes in the ability of the $\sigma^{54}$ polymerase to produce mRNA from the glnAp2 promoter. In the next sections we will attempt to learn if these changes are caused by changes in promoter recognition or by changes in ability of the bound polymerase to melt the DNA.

**Changes in Open Complex Formation in Vivo Caused by Glutamine Changes**—The activating event at the glnAp2 promoter is the melting of the promoter start site by $\sigma^{54}$ polymerase (see Sasse-Dwight and Gralla, 1988 and Popham et al., 1989). The melting may be detected by pretreating cells with rifampicin to trap open complexes and then applying a permanganate assay in vivo. The assay has been done previously using both wild-type and deletion mutant forms of $\sigma^{54}$ at the glnAp2 promoter (Sasse-Dwight and Gralla, 1988, 1990; Wong and Gralla, 1992). We use the same experimental system to assay the glutamine mutant forms of $\sigma^{54}$ polymerase.

Examples of the in vivo permanganate assay are shown in Fig. 4. None of the $\sigma^{54}$ glutamine mutations changes the position of melting, consistent with the conservation of mRNA start sites in the series (Fig. 3). However, the degree of opening is different for the different mutant forms of $\sigma^{54}$ polymerase. These experiments were repeated two or three times, and the amount of open complex was calculated. These data are normalized to the amount of opening shown by wild-type $\sigma^{54}$ polymerase and are collected in Table III.

The data in Table III show that the two mutants that are most defective in mRNA production (above) are also the most defective in open complex formation; QE12 and QE123 produce less than 10% of the wild-type level. The effect of mutations within subregion 2 in lowering mRNA expression (Table II) is also reflected in the lesser melting directed by mutants QS2.
and Q52. This is a property of changes to either serine or to glutamate, suggesting that the subregion 2 glutamines play an important role in open complex formation. In principle these losses could be caused by a loss of either promoter binding or a loss in ability of the bound enzyme to catalyze open complex formation; we will distinguish between these possibilities.

The behavior of changes within subregion 3 is quite different in this assay. Surprisingly, changes to either serine or to glutamate within this subregion lead to elevated levels of promoter start site melting. Both Q53 and Q53 melt the DNA to the 150% level compared with wild type (Table III). Thus, mutation of the glutamines in subregion 3 leads to an enhanced ability of the Q54 polymerase to open the promoter start site. We have not seen this property previously in our studies of Q54 mutations (Sasse-Dwight and Gralla, 1990; Wong and Gralla, 1992). In principle it could be caused either by more polymerase collecting at the promoter or by an enhanced melting capability associated with the same amount of bound polymerase. We will also distinguish between these possibilities in the next section.

**Assay for DNA Binding in Vivo**—The altered melting and mRNA production directed by some of these mutant forms of Q54 could be due to altered DNA binding by the Q54 polymerase at the glnAP2 promoter. This can be tested by *in vivo* footprinting with dimethyl sulfate. We showed previously that such experiments can reveal promoter occupancy by assessing protection of bands near the -24 region of the glnAP2 promoter (Sasse-Dwight and Gralla, 1988, 1990). This assay was repeated using the mutant forms of Q54 with glutamine substitutions.

The results of probing promoter occupancy are shown in Fig. 5, A and B. The extent of promoter occupancy is indicated by the lessening intensity of the bands near -24 compared with the -19 reference band. 100% occupancy is considered to be the difference in signal between lanes 1 and 2, corresponding to no Q54 and wild-type Q54, respectively. The very significant protection of -24 compared with -19 in all lanes confirms that the mutant forms of Q54 are stable enough to provide a very substantial level of occupancy of the promoter.

The results using three serine mutants, Q51, Q52, and Q53, are shown in lanes 3-5 of Fig. 5A. In all cases the low -24 to -19 ratio suggests that the mutants protect the DNA as well as does wild type. This set includes the Q52 mutant (lane 4) that is defective in both promoter start site melting and mRNA production. We conclude that the 40-60% reduction in mRNA production and promoter opening using Q52 (Tables II and III) is not caused by a reduction in polymerase binding by the Q52 polymerase. In the case of the QE series of mutants, DNA binding is also largely intact, as evidenced by the protection at -24 compared with -19 (Fig. 5, B and A, lanes 6-8). For mutant QE2 there appears to be a small loss of DNA binding (compare lane 7 with lanes 1 and 2). This mutant has 70% loss of mRNA (Table II) and a 60% loss of melting (Table III). The data for QE2 and Q52 taken together indicate that the subregion 2 glutamines have a role in setting the ability of bound Q54 polymerase to catalyze open complex formation. The very strong down mutants QE12 and QE123 also have nearly full DNA binding (lanes 3 and 6 of Fig. 5B), recall that these yield less than 10% of wild-type mRNA and melting levels (Table II and

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**Table III**

| Mutant | Level of melting |
|--------|------------------|
| WT     | 1.0              |
| Q51    | 1.2              |
| Q52    | 0.6              |
| Q53    | 1.5              |
| Q512   | 1.0              |
| Q513   | 1.2              |
| Q523   | 0.8              |
| Q5123  | 1.2              |

**Fig. 4. Potassium permanganate probing in vivo.** The following forms of Q54 were probed: wild type (lane 1), Q51 (lane 2), Q52 (lane 3), Q53 (lane 4), Q512 (lane 5), Q513 (lane 6), Q523 (lane 7), Q5123 (lane 8), Q51 (lane 9), Q52 (lane 10), Q53 (lane 11), Q51 (lane 12), Q513 (lane 13), Q523 (lane 14), and Q5123 (lane 15).

**Fig. 5. Dimethyl sulfate footprint of wild-type and six glutamine mutants at the glnAp2 promoter.** Critical guanines at the -12 and -24 regions are indicated by the "<" sign, and the -19 position is indicated by the arrowhead as the reference band. A, protection patterns are shown with no plasmids (lane 1), wild type (lane 2), Q51 (lane 3), Q52 (lane 4), Q53 (lane 5), QE1 (lane 6), QE2 (lane 7), and Q53 (lane 8), respectively. B, protection patterns are shown with no plasmids (lane 1), wild type (lane 2), QE12 (lane 3), QE13 (lane 4), QE23 (lane 5), and QE123 (lane 6), respectively.
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III. We conclude that these losses are not due to a lack of binding.

For mutants QS3 and QE3 (lanes 5 and 8 in Fig. 5A), which showed elevated levels of open complex formation, the $-24$ protection is not greater than that of wild-type polymerase (lane 2). There is no evidence of any extra protection that could account for the elevated amounts of DNA melting. We infer that the elevated levels of melting caused by either serine or glutamate conversions in the subregion 3 glutamines are caused by an enhanced ability of the bound mutant polymerases to melt the DNA start site. Thus subregions 2 and 3 have in common that they alter the ability of the polymerase to open the DNA, but they differ in that mutations in one subregion boost this ability, whereas mutations in the adjacent subregion dampen it.

Previous experiments showed that deletion of subregion 2 and part of subregion 3 led to a severe loss of $-12$ promoter protection (Sasse-Dwight and Gralla, 1990). This is assessed by the ratio of the $-12$ region band intensity to that of the $-19$ reference band. The protection by wild-type $\sigma^{54}$ is substantial ($-12$ to $-19$ ratio in lanes 2 compared with lanes 1 in Fig. 5, A and B), although it is not as strong as reported previously using plasmids to carry the promoter. The QS series shows no loss of $-15$ protection (lane 5 of Fig. 5A), whereas significant losses are seen with some of the QE mutants (lanes 6 and 7 of Fig. 5A and lanes 3-6 of Fig. 5B).

**DISCUSSION**

These data have shown that the central four glutamines within the glutamine-rich region of $\sigma^{54}$ are important for function. The primary evidence for this is the 60–95% reduction of mRNA production by a number of mutant forms containing point mutations within these glutamines. The question arises concerning whether the cause of these losses is different from the losses caused by other mutant forms of $\sigma^{54}$; such differences would indicate a unique role for the glutamine-rich region.

We have covered the $\sigma^{54}$ gene with small deletions, and deletions in the N-terminal region bear some resemblance to the glutamine point mutants. Deletion of the acid trimer repeat region adjacent to the glutamines leads to less mRNA production without a significant loss of DNA binding (Wong and Gralla, 1992). This deletion also leads to a reduction in start site melting. These properties resemble the effects of mutation in glutamine subregion 2. They are quite different, however, from the enhanced melting shown by changing glutamines within subregion 3. Such enhanced melting is also not seen when the acidic subregion is tandemly duplicated (Wong and Gralla, 1992). Deletion of the glutamine-rich region shows only some of the properties associated with the introduction of multiple glutamine point mutations. Deletion and some substitutions have in common a loss of ability to melt the DNA coupled with a preservation of promoter occupancy. However, as just discussed, the enhanced melting associated with subregion 3 glutamine changes is not a property of this or any other deletion. A further difference is that the deletion shows a severe loss of protection of the $-12$ promoter element; this protection is largely preserved in the QS mutants and is only partly lost in the QE series. The protection is lost, however, when the leucines within this region are mutated selectively. We infer that the glutamines have some properties associated with other motifs and some properties that are unique.

Transcription complexes containing forms of $\sigma^{54}$ in which a variety of glutamines are mutated have in common a changed ability to melt the DNA start site. This appears to be the primary broadly defined function of this motif, since most of the mutant $\sigma^{54}$ polymerases do not show strong changes in promoter DNA binding. Because DNA binding does not depend critically on the activator NTRC, but the subsequent melting does (Sasse-Dwight and Gralla, 1988; Popham et al., 1989; Buck and Cannon, 1992), the glutamines appear to be involved in the steps that are activator-dependent. In addition several of the mutants that are deficient in producing mRNA do not show a corresponding reduction in melting. Thus the region appears to be necessary not only to respond to NTRC and melt the DNA but also for optimal initiation. Initiation involves ribonucleotide condensation as well as promoter clearance and polymerase escape (Carposis and Gralla, 1985; Knaus and Bujard, 1988) and could conceivably involve NTRC as a co-factor in these processes.

Melting is the key activating event at the $\sigma^{54}$-dependent glnAp2 promoter, because a stable closed complex exists prior to activation (Sasse-Dwight and Gralla, 1988). The $\sigma^{54}$-associated bound closed complex was speculated to be a key to allowing enhancer-dependent activation, since it provides an easy target for enhancer activation (see Gralla, 1991); this is known to occur by a looping mechanism after the NTRC protein is phosphorylated (Su et al., 1990). The regulation of melting associated with the glutamine-rich region seems to reflect a role for this region in mediating the enhancer-dependent activation, which is uniquely associated with $\sigma^{54}$ in E. coli. Glutamine-rich domains have not been reported in other bacterial transcription factors, but are associated with many factors involved in enhancer-dependent eukaryotic transcription (see below).

The glutamine-rich region of $\sigma^{54}$ is included in a proposed coiled-coil leucine zipper motif (Sasse-Dwight and Gralla, 1990). It is interesting to note that an $\alpha$-helical wheel representation shows that all 8 glutamines in subregions 2 and 3 lie on the hydrophilic side of the proposed $\alpha$-helix. In addition the region as a whole is bounded by a pair of proline residues (residues 15 and 45), further suggesting that it may act as a complex but single unit. Our data do not provide any further tests of the existence of this structure but do imply that the unit plays an important role in mediating the NTRC-dependent melting of the DNA.

Subregion 1 lies N-terminal to the motif containing subregion 2 and 3. The data do not firmly establish an important role for subregion 1, but do hint its involvement. Mutant QS1 is normal in all our tests. However, when QS1 is combined with QS2, it rescues the defect of QS2 in melting (but not in transcription). Mutant QE1 has a normal level of melting but is defective in producing mRNA, like QS12. These complexities might be explained if subregion 1 glutamines would support peripherally both the masking of melting done by subregion 3 and the assistance in melting done by subregion 2.

Domains rich in glutamines are important for the function of various mammalian factors, most notably SP1 (Courey and Tjian, 1988; Courey et al., 1989; Gerster et al., 1990). The glutamine-rich regions are required for transcription and likely for forming active multimers in the case of SP1 (Pascal and Tjian, 1991). The lack of SP1 binding sites leads to an inability of the mammalian machinery to open the mammalian DNA start site (Jiang et al., 1993), although there is no direct evidence that this is caused by the lack of glutamine-rich regions.

In the case of $\sigma^{54}$ the glutamines have a role in the signal transduction pathway between the binding by the activator NTRC and the melting and initiation by the polymerase. Presumably this involves mediating changes in interactions between protein domains, which could include domains on $\sigma$, core polymerase and NTRC. Glutamine is an appropriate amino acid to mediate such conformational switches because it has a

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2 C. Wong, unpublished data.

3 M. Hsieh and J. D. Gralla, unpublished data.
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relatively flexible side chain and can be paired as either a hydrogen bond donor or acceptor with other amino acids. It will be interesting to determine if glutamine-rich domains have a common role in mediating selective interactions between different proteins or protein domains that are required to lead to DNA melting.

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