Amino Acid Substitutions in the Two Largest Subunits of Escherichia coli RNA Polymerase That Suppress a Defective Rho Termination Factor Affect Different Parts of the Transcription Complex*

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Among the earliest rpoBC mutations identified are three suppressors of the conditional lethal rho allele, rho201. These three mutations are of particular interest because, unlike rpo88, they do not increase termination at all ρ-dependent and ρ-independent terminators. rpoB211 and rpoB212 both change Asn-1072 to His in conserved region H of rpoB (βN1072H), whereas rpoC214 changes Arg-352 to Cys in conserved region C of rpoC (β’R352C). Both substitutions significantly reduce the overall rate of transcript elongation in vitro relative to wild-type RNA polymerase; however, they probably slow elongation for different reasons. The nucleotide triphosphate concentrations required at the T7 A1 promoter for both abortive triphosphate synthesis and for promoter escape are much greater for βN1072H. In contrast, β’R352C and two adjacent substitutions (β’G351S and β’S350F), but not βN1072H, formed open complexes of greatly reduced stability. The sequence in this region of β’ modestly resembles a region of Escherichia coli DNA polymerase I that contacts the phosphate backbone of DNA in co-crystals. Core determinants affecting open complex formation do not reside exclusively in β’, however, since the Rif mutation rpoB2 in β also dramatically destabilized open complexes. We suggest that the principal defects of the two Rho-suppressing substitutions may differ, perhaps reflecting a greater role of β region H in nucleoside triphosphate-binding and nucleotide addition and of β’ region C in contacts to the DNA strands that could be important for translocation. Although both probably suppress rho201 by slowing RNA chain elongation, these differences may lead to terminator specificity that depends on the rate-limiting step at different sites.

The two largest subunits of Escherichia coli RNA polymerase, β’ (M, 155,063) and β (M, 150,538) are homologous to the largest and second largest subunits, respectively, of all multisubunit RNA polymerases (Allison et al., 1985; Briggs et al., 1985; Heisler et al., 1987; Hudson et al., 1988; Leffers et al., 1989; Patel and Pickup, 1989). Most catalytic and regulatory functions of RNA polymerase appear to involve these subunits. Thus β and β’ are likely to form an enzymatic platform for RNA synthesis common to all forms of life, making elucidation of structure/function relationships in these subunits in a well studied prokaryote an ideal approach to understanding the mechanism of transcription. Sequence comparisons and recent crystallographic studies of single subunit DNA and RNA polymerases reveal broadly similar catalytic regions for both classes of enzyme (Stitz et al., 1994; Pelletier et al., 1994; Beese et al., 1993; Sousa et al., 1993; Kohlstaedt et al., 1992). Although E. coli RNA polymerase has proven refractory to study by x-ray crystallography, owing principally to its large size and multisubunit complexity, low-resolution electron crystallography of E. coli RNA polymerase and yeast RNA polymerase II suggests that the multisubunit RNA polymerases also share this basic architecture, most notably a central nucleic acid binding channel large enough to encompass the DNA double helix (Darst et al., 1989, 1991; Poljakov et al., 1995). Most progress in identifying functionally important regions of the enzyme, however, has come through study of altered function mutants and sites of cross-linking between the subunits and nucleotide analogs.

Of the three core subunits, most is known about β, largely because it is the target of a widely studied antibiotic that inhibits transcription, rifampicin (Rif). Rif mutants in particular have been a focus of study for decades since they exhibit a number of pleiotropic phenotypes, most notably effects on pausing and termination (Fisher and Yanofsky, 1983; J in et al., 1988). Several more recent genetic studies have identified sites throughout rpoB, generally in evolutionarily conserved regions, that affect pausing and termination (Landick et al., 1990; T a vormina et al., 1996). In addition, substitutions in β have been isolated that suppress substitutions in Rho and NusA, suggesting that β is a site of functional, and possibly physical interaction with these transcriptional regulators (Guarente and Beckwith, 1978; Guarente, 1979; J in and Gross, 1989; Ito et al., 1991; Ito and Nakamura, 1993). Others have used reverse genetics and high resolution cross-linking to identify domains in β that affect a number of aspects of polymerase function.
Several lines of evidence suggest that the β′ subunit also is intimately involved in RNA chain synthesis. Early physical studies showed that purified β′ subunit is capable of binding DNA and heparin (Zillig et al., 1970), and several cross-linking studies have demonstrated that β′ is in the immediate vicinity of short and intermediate length transcripts (Hanna and Mears, 1983; Dissinger and Hanna, 1991; Borukhov et al., 1991a), as well as the DNA template (Okada et al., 1978; Chenckin et al., 1982). Substitutions in β′ affecting termination, suppression of nosA and rho mutants, DNA replication, and phage growth have been described, although, with a few exceptions, the particular amino acids affected have not been identified (Nomura et al., 1984; Rasmussen et al., 1983; Tanaka et al., 1983; Petersen and Hansen, 1991; Robledo et al., 1991). More recently we reported several clusters of substitutions in conserved regions of β′ that alter pausing and termination (Weiβbacher et al., 1994), streptolydigin-resistance was found to result from amino acid substitutions in one of these clusters that corresponds to the amanitin-resistance region of the largest subunit of RNA polymerase II (Severinov et al., 1995), and conserved aspartic acid residues that appear to chelate active-site Mg2+ ions were identified using the β′ homolog in yeast RNA polymerase III (Dieci et al., 1995).

To understand the roles of β′ and β′ in transcription better, we have studied mutations isolated as allele-specific suppressors of rho201 by Guarente (1979) and mapped to the rpoBC locus. These mutations were localized to either rpoB or rpoC in a previous study and renamed rpoB211, rpoB212, and rpoC214 (Jin and Gross, 1989). Unlike the rpoB8(Rif′) mutant that was also isolated as a suppressor of rho201 (Guarente and Beckwith, 1978) as well as independently by several other criteria (reviewed in Jin and Gross (1988)), these mutations do not cause general defects in termination, but rather alter termination only subtly at a few ρ-dependent and ρ-independent terminators (Jin and Gross, 1989).

To continue this investigation, we identified the precise amino acid substitutions in rpoB211, rpoB212, and rpoC214, finding that both rpoB211 and rpoB212 specify βN1072H and that rpoC214 specifies β′R352C, and purified both mutant RNA polymerases. A current model of Rho-dependent termination (Jin et al., 1992; Platt, 1994) postulates that the extent of termination is determined by “kinetic” coupling between extrusion of the nascent RNA by the elongating RNA polymerase molecule and movement of Rho along the RNA toward the complex. That this relationship is the basis of at least some modes of rho-suppression was demonstrated for rpoB8- (β′Q513P): β′Q513P RNA polymerase elongates at a significantly slower rate than wild type, and its hypertermination defect is correlated to its elongation rate (Jin and Gross, 1991). We wanted to determine whether a reduction in elongation rate was a plausible basis of isolation of these RNA polymerase mutants, or if they suppress rho201 by a different mechanism (such as disruption of interaction with Rho). If these mutants are defective in transcription itself, we wanted to ask whether they and β′Q513P exhibit similar or distinct transcriptional defects.

Here we show that βN1072H and β′R352C elongate more slowly in vitro and argue, based on their effects on abortive initiation and open complex stability, that they probably do so by principally affecting distinct interactions of RNA polymerase with different components of the transcription complex.

**TABLE I**

| Strain | Relevant genotype | Source/Reference |
|--------|------------------|-----------------|
| MG1655 | Wild-type K12    |                |
| MG1655poeB211 | bτB::Tn10 rpoB211 | J and Gross (1989) |
| MG1655poeB212 | bτB::Tn10 rpoB212 | J and Gross (1989) |
| MG1655poeC214 | bτB::Tn10 rpoC214 | J and Gross (1989) |
| CAG14179 | MG1655 + pPLT13  | This work      |
| CAG14180 | CAG14179poeB211  | This work      |
| CAG14181 | CAG14179poeC214  | This work      |
| RL676  | W3110 rpoC::S. aureus D 736 K14111, MG1655 | Weilbaecher et al. (1994) |
| 3215   | RL676 pRW308(β′Q530F) | This work      |
| 3216   | RL676 pRW308(β′G351D) | This work      |

**MATERIALS AND METHODS**

**Chemicals and Enzymes**

Adenosyl 5′-uridine (ApU) and isopropyl-β-D-thiogalactopyranoside were from Sigma, γ-ANS-UTP was a kind gift of M. Thomas Record. NTPs were purchased from Boehringer Mannheim or Pharmacia (high performance liquid chromatography-purified). Radioactive [α-32P]UTP (800 Ci/mmol) and [γ-32P]ATP (3000 Ci/mmol) were obtained from DuPont NEN, [α-32P]CTP (410 Ci/mmol) was from Amersham. Polynucleotide kinase and restriction enzymes were from New England Biolabs. Sequenase Version 2.0 was purchased from U.S. Biochemical Corp. AmpliTaq was obtained from Perkin-Elmer Instruments and Tfl polymerase from Epicentre.

**Bacterial Strains, Plasmids, and Bacteriophage**

The bacterial strains used in this study are E. coli K12 derivatives and are listed in Table I. Plasmids and bacteriophage used in this study are listed in Table I. T7ΔD11 phage stock was from Studier and phage DNA was prepared as described (Studier, 1975).

**Bacterial Techniques**

Cells were grown in L broth (Difco Laboratories). LB plates were prepared as described (Miller, 1992). Antibiotics were added at the following concentrations, unless otherwise indicated: ampicillin (Amp) 50 μg/ml; kanamycin (Kan) 30 μg/ml; tetracycline (Tet) 20 μg/ml. Competent cells were prepared by CaCl2 shock (Mandel and Higa, 1970), or by electroporation (Dower et al., 1988) and were stored at −80°C. Electroporation was performed with a Bio-Rad GenePulser using 0.2-cm Bio-Rad GenePulser cuvettes.

**Marker rescue of the cold-sensitive growth phenotype of rpoC214 was as described in Jin and Gross, 1989) using plasmids pRL611-pRL617 (Table I).**

**DNA Techniques**

Restriction enzyme digests and agarose gel electrophoresis were as described by Sambrook et al. (1989). Plasmid DNA purification was as described by Sambrook et al. (1989) or by purification on Qiagen or Magic Miniprep columns.

**DNA for Nitrocellulose Filter Binding—**End-labeled DNA was prepared by PCR essentially as described by Dombroski et al. (1992), using 30 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C and purifying the DNA by elution from nondenaturing 10% polyacrylamide gels as described by Sambrook et al. (1989). Quantitation of labeled DNA was as described by Dombroski et al. (1992).

**Nitrocellulose Filter Binding Assays—**DNA binding was determined by measuring retention of polymerase-DNA complexes on nitrocellulose filters. All data represent the average of at least 3 duplicate experiments, unless stated otherwise. Binding buffer (BB: 50 mM KCl, 40 mM Tris-HCl, pH 7.9, 0.1 mM EDTA, 1 mM β-mercaptoethanol) was made according to the specifications of Pfeffer et al. (1977) for the heparin competition experiments; for the excess DNA competition experiments, the KCl concentration was increased to 100 mM (see “Results”). The composition of the wash buffer (WB) was identical to that of BB, except that KCl was omitted. The experimental procedure was essentially as described (Pfeffer et al., 1977; Hinkle and Chamberlin, 1972). Briefly,
Plasmids used in this study

| Plasmid | Relevant markers | Source/reference |
|---------|-----------------|------------------|
| pCL185  | Contains T7A1 promoter with modified downstream sequence of the -17 from start site of transcription | Feng et al. (1994) |
| pH76    | Contains pRD under control of Pp on pBR322 | Hu (1987) |
| pRLT13  | Contains lacI on mini F | Tavormina (1994) |
| pRW308  | Contains rpc expression from Pp and controlled by lac repressor encoded on the same pBR322-based plasmid | Weilbaecher et al. (1994) |
| pRL611  | Derivative of pBR322 carrying only rpc codons | This work; see also Clerget et al. (1995) |
| pRL612  | As pRL611 with codons 1–292 | This work; see also Clerget et al. (1995) |
| pRL613  | As pRL611 with codons 293–544 | This work; see also Clerget et al. (1995) |
| pRL614  | As pRL611 with codons 878–1214 | This work; see also Clerget et al. (1995) |
| pRL615  | As pRL611 with codons 1214–1407 | This work; see also Clerget et al. (1995) |
| pRL616  | As pRL611 with codons 293–544 (opposite orientation of pRL612) | This work; see also Clerget et al. (1995) |
| pRL617  | As pRL611 with codons 546–877 (opposite orientation of pRL613) | This work; see also Clerget et al. (1995) |

0.25–1 nmol of end-labeled DNA (2 × 10^6 cpm/pmol) was preincubated with a 4-fold molar excess of active RNA polymerase in 1× BB at 37°C for 10 min. Duplicate 25-μl aliquots were filtered and washed with 250 μl of 1× WB, dried under an infrared heat source, and subjected to liquid scintillation counting. Background radioactivity was corrected for baseline dissociation when necessary (calculated from the control reactions described above) by subtracting the dissociation rate of the no-competitor control.

PCR Amplification and Sequencing of Chromosomal Mutations—Marker-rescue in a cold-sensitive growth phenotype of rpcC214 was done as described for rpcB211 and rpcB212 (J in and Gross, 1989). Mutations rpcB211, -212, and rpcC214 were sequenced by PCR amplification of the identified regions. Primers were made flanking the regions, and PCR reactions were essentially as described above. Double-stranded PCR product was gel purified from 2% agarose gels in Tris-acetate-EDTA buffer and eluted according to Sambrook et al. (1989). Purified fragment was sequenced according to the Sequenase procedure for sequencing double-stranded plasmid DNA.

Biochemical Techniques

Purification of RNA Polymerase—Strains CAG 14179, 14180, and 14181 (Table I) were grown in LB + antibiotic in a 6-liter fermentor (University of Wisconsin Pilot Plant) and harvested in mid-log phase (OD_600 ~ 1). Protein purification of 25 g of cells (wet weight) was essentially as described by Lowe et al. (1979) and Burgess and J. Endrisak (1975), with several modifications: cell suspensions were sonicated in several 30-s intervals instead of being subjected to high speed shearing (described in Thompson et al. (1992)); the TGED + 2 M NaCl polymerin P eluate was precipitated with ammonium sulfate as described (Burgess and J. Endrisak, 1975) and resuspended in TGED to a conductivity equivalent to 0.25 M NaCl (in TGED), then loaded on a 10-ml single-stranded DNA-agarose column. Holoenzyme, eluted in TGED + 1 M NaCl, was precipitated, resuspended, and loaded on a 120-ml 5–30% HR sizing column. The holoenzyme fraction was pooled and eluted from an 8-ml Q-Sepharose FF column in TGED over a gradient from 0.25 to 0.75 M NaCl; the polymerase fraction typically eluted at 0.5 M NaCl. Highly pure fractions of holoenzyme (>95%) were pooled and dialyzed against polymerase storage buffer (TGED, containing 50% glycerol + 0.1 M NaCl) and stored in aliquots at −80°C.

RNA Polymerase Micropreparations—Crude preparations of β’530F and β’G351D RNA polymerases from strains RL676 were from strain RL676 transformed with appropriate plasmids (WeiBaecher et al., 1994) using a variation of the RNA polymerase microassays of Gross et al. (1976) described by Tavormina et al. (1996). Similar preparations of βG1074D and βH526Y (rpcB2) RNA polymerases were generously provided by P. Tavormina.

Polymerase Activity Assays—Activity of polymerase preparations was determined both by the Chamberlin T7ΔD111 assay and by the flow assay for abortive initiation (Bertrand-Berggraff et al. (1994), using γ-ANS-UTP (data not shown). The results of both assays indicated that the wild-type preparation was 45–50% active and the βN1072H and βR352C preparations were between 20 and 25% active. βG351D RNA polymerase was purified from strain C15023 (Horn and Yanofsky, 1981) by the method of Hager et al. (1990) and determined as described above to be ~25% active.

In Vitro Transcription Elongation Assays—T7ΔD111 assays (Chamberlin et al., 1979) were carried out at 30°C and at 0.4 mM NTPs. The λp<sub>α</sub>432s, original assays, originally described by Chen and Richardson (1987), were done according to the modifications described by Heisler et al. (1993). Reactions were carried out at 37°C, 1× NTPs equals 0.2 mM ATP, GTP, and 0.02 mM UTP.

Abortive Initiation Apparent NTP Km Assays—We used an abortive initiation assay similar to that originally developed by McClure et al. (1978), a more detailed description of which will be published separately. 0.25 pmol of the 142-bp fragment of pCL185 (T7A1 promoter; Feng et al. (1994); see Table I) was incubated with 1 pmol of active RNA polymerase in 1× T7A1 buffer (20 mM Tris acetate, pH 8.0, 20 mM NaCl, 14 mM MgCl<sub>2</sub>, 14 mM β-mercaptoethanol) and 25 μg of acetylated bovine serum albumin/ml at 37°C for 10 min. Reactions were initiated by the addition of ApU and [α-32P]CTP (final specific activity ~6 Ci/mmol) at the concentrations indicated. Aliquots were withdrawn at 2-min intervals (from 2 to 10 min) into a 4-fold excess of transcription stop buffer (final concentration, 1× TBE, 35 mM urea, 0.025% xylene cyanol, 0.025% bromphenol blue). 4.5-μl samples were electrophoresed on 15% denaturing PAGE, and wet gels were exposed to a Molecular Dynamics Phosphorimage scanner at ~80°C to prevent diffusion of the bands. The results were quantitated on a Molecular Dynamics Phosphorimage scanner and normalized to 10<sup>4</sup> C.T. Apparent substrate constants for nucleotides were determined assuming a random-order, rapid-equilibrium mechanism (Siegel, 1975). Since only the pathway in which NTP-binding preceded ApU-binding was significant, only the apparent equilibrium constants for binding NTP first (K<sub>NTP</sub>) and for binding ApU second (K<sub>ApU</sub>) are reported. The apparent equilibrium constant for binding CTP (K<sub>CTP</sub>) was determined by varying [GTP] at high [ApU].

Preselector Escape—Transcription assays and PAGE were essentially as described for abortive initiation assays, except that ApU was added at 0.5 mM in all reactions, and GTP, ATP, [α-32P]CTP, and 3-deoxy-UTP were added at the concentrations indicated. Aliquots were withdrawn into stop solution at the times indicated and electrophoresed on 15% urea-polyacrylamide gel, as described above. Control experiments done in the absence of 3'-dUTP (data not shown) demonstrated that the results were unchanged in the presence of the chain terminating NTP, except that there was no readthrough of the 17 nucleotide product (see also Sagot et al. (1993)). The identities of the products as indicated in Fig. 4 were confirmed by Feng et al. (1994) using 5'-AUC, 5'-AUGC, and 5'-AUCG<sub>A</sub> gene generated in transcription reactions with ApU and [α-32P]CTP only, ApU, CTP, and [α-32P]CTP only, or ApU, CTP, and [α-32P]CTP only, and showing (i) that they co-migrated with the abortive products as assigned in Fig. 6 and (ii) that addition of a 5'-PO<sub>4</sub> increased the mobility of these short transcripts to that of 5'-pAUC, 5'-pAUGC, and 5'-pAUCG<sub>A</sub>. That they were initiated with ApU. Note that the mobilities of short RNAs (less than 5 nucleotides in length) lacking a 5'-PO<sub>4</sub> are reversed on a 15% urea-polyacrylamide gel (Feng et al., 1994; Krummel, 1990; Levin et al., 1987; Cai and Luse, 1987).

RESULTS

rpcB211, -212, and rpcC214 Are Located in Conserved Regions Important in Transcription—In Gross (1989) de-
scribe the localization of rpoB211 and rpoB212 to a 650-bp region in the C-terminal portion of rpoB. We amplified this region by PCR and determined the sequence changes of these chromosomal mutations (see "Materials and Methods"). These two mutations contain identical single base pair substitutions and may have been isolated as siblings in the original selection, or as two independent isolates (Guarente, 1979). The amino acid change, N1072H, occurs in conserved region H, which has been subjected to extensive mutational analysis (Sagitov et al., 1993; Fig. 1). Mutations in this region affect many aspects of transcription.

Prior to this study, rpoC214 had not been mapped within rpoC. Marker rescue of the cold-sensitive phenotype of this mutant by plasmids containing internal fragments of rpoC (see "Materials and Methods") allowed us to localize this mutation to an interval of rpoC between amino acids 305 and 695 (region 2; Weilbaecher et al., 1994). PCR amplification and sequencing identified an amino acid change from Arg to Cys at amino acid 352. This amino acid lies in a region of weak sequence similarity to a putative DNA-binding region in the Klenow fragment of DNA polymerase I (Ollis et al., 1985) and is adjacent to several rpoC mutations isolated for their effects on termination (Weilbaecher et al., 1994; Fig. 1).

βN1072H and β′R352C Decreased Elongation Rate in Vitro—
We purified the mutant enzymes according to variations in the procedures of Burgess and Endrissak (1975) and Lowe et al. (1979) (see "Materials and Methods") and examined their rates of elongation on T7ΔD111, a deletion of T7 phage DNA that contains only one E. coli E70 promoter, T7 A1 (Studier et al., 1975). In this assay, wild type RNA polymerase reached the T7 Terminator 6.5 min after addition of nucleotides and heparin or at 15 nucleotides/s, whereas βN1072H and β′R352C elongated RNA at 6 and 8 nucleotides/s, respectively (Fig. 2). We also isolated RNA polymerase from two rpoC mutants containing amino acid changes adjacent to R352C: S350F and G351S (Weilbaecher et al., 1994). These mutant enzymes elongated at rates comparable to that of β′R352C (β′S350F, 10.5 nucleotides/s and β′G351S, 8 nucleotides/s; data not shown).

To confirm the elongation defect of mutants βN1072H and β′R352C, we tested transcription on a λP, tr1 template. This template contains a number of well characterized pause sites in addition to the λtr1 terminator (Chen and Richardson, 1987). At 1× NTPs (0.2 mM), wild type reached the terminator by 2 min, while βN1072H required 4× NTPs and β′R352C, 2× NTPs, to match the transcription rate of wild type at 1× NTPs (Fig. 3). Neither mutant exhibited fundamental differences in the pattern of pausing at any concentration of nucleotides (the patterns are far more similar that different, especially since apparently new minor pauses in the mutant lanes may be difficult to detect in the 0.5-min wild-type sample; Fig. 3). Thus, the reduced elongation rates of these mutants result from slower elongation at pause sites that also are recognized by the wild-type enzyme, rather than recognition of new pause sites. Furthermore, these defects in elongation rate are magnified at lower NTP concentrations and do not appear to be template-specific since the magnitudes of their elongation de-
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ENZYMES

Table III

| Enzyme | \(K_{\text{ApU}}\) | \(K_{\text{CTP}}\) | \(K_{\text{GTP}}\) |
|--------|----------------|----------------|----------------|
| Wild type | 0.34 | 47 | 80 |
| \(\beta\)N1072H | 1.1 | 257 | 500 |
| \(\beta\)'R352C | 1.4 | 132 | 140 |

*See "Materials and Methods" for description of the analysis used to derive apparent equilibrium binding constants.

Double reciprocal plots of reaction velocity versus substrate concentration were linear over the entire range tested, indicating that there was no substrate activation or inhibition (data not shown). However, the kinetic parameters determined must be regarded as apparent substrate constants, rather than true dissociation constants for binding of ApU and NTPs since we cannot be certain the reactants bound in rapid equilibrium (see McClure et al., 1978; Smagowicz and Scheit, 1978). On both templates, the mutant RNA polymerases exhibited substantially high apparent substrate constants for both ApU primer and NTP substrates (Table III). \(K_{\text{ApU}}\) for wild-type was \(0.325 \text{ mM}\), in agreement with values determined for \(K_{\text{ATP}}\) in this promoter (Smagowicz and Scheit, 1978). Both mutants yielded slightly increased \(K_{\text{ApU}}\) values (\(\sim 1 \text{ mM}\); Table III), suggesting that these amino acid changes may somewhat perturb the primer sub-site of the catalytic center (site i, see Fig. 8; Erie et al. (1992)), either directly or indirectly.

In agreement with determinations on this and other promoters, the wild-type \(K_{\text{ATP}}\) values were approximately an order of magnitude smaller than \(K_{\text{CTP}}\) (\(K_{\text{CTP}}\) = 47 \(\mu\text{M}\), \(K_{\text{GTP}}\) = 80 \(\mu\text{M}\), Table II). Although both the \(\beta\) and \(\beta\)' substitutions increased these values, interestingly, they were approximately 6-fold greater for wild-type for \(\beta\)N1072H and about 2-3-fold greater for \(\beta\)'R352C, depending on the nucleotide in question (Table III).

Thus, the abortive initiation assay uncovered at least one component of the transcriptional defect of these mutant RNA polymerases, namely a decrease in apparent affinities for both priming nucleotide and substrate NTP that is consistent with their reduced elongation rate. We suspected, however, that the mutants might affect other aspects of RNA polymerase function because, although \(\beta\)N1072H has a somewhat greater defect in elongation rate than \(\beta\)' during abortive initiation, the growth rate of \(\beta\)'R352C is slower than that of \(\beta\)N1072H. If elongation were the only aspect of transcription affected, \(\beta\)N1072H would be the more deleterious allele. Furthermore, \(\beta\)'R352C demonstrated a more severe hypertermination phenotype in vivo (Jin and Gross, 1989), suggesting that its reduced elongation rate is not the sole determinant in its suppression of the rho-201 phenotype.

The Mutants Differed in the NTP Concentration Required for Efficient Promoter Escape—Promoter escape, or the transition from abortive to productive synthesis, is another component of the transcription cycle that is sometimes altered by amino acid changes in RNA polymerase. Reductions in promoter escape appear to be correlated with increased pausing and a reduced elongation rate (Kashlev et al., 1990; Sagitov et al., 1993). Interestingly, during initial assays of the \(\beta\)N1072H and \(\beta\)'R352C RNA polymerases, we found that both were defective in forming halted elongation complexes at position 16 of a T7 A1 promoter template at low nucleotide concentrations (pCL185 G16 complexes; data not shown; see Feng et al. (1994)). To characterize the NTP concentration dependence of promoter escape, we monitored the appearance of abortive and productive transcripts as a function of time in the presence of

Fig. 3. Transcript elongation from \(\lambda\)P, of WT, \(\beta\)N1072H, and \(\beta\)'R352C RNA polymerases. Transcription reactions were performed as described under "Materials and Methods" with 1, 2, or 4 NTPs (\(1 \times 0.2 \text{ mM ATP}, \text{CTP, GTP, and } 0.02 \text{ mM } \mu\text{P}\)) (Table III). The reactions were sampled at the times indicated above the lanes. The positions of the readthrough (RT) transcript and the \(\lambda\)P13 termination sites I (at nucleotide 288 relative to the start site of transcription), II, and III are indicated, as are the locations (\(\Delta\)) of previously characterized pause sites (Chen and Richardson, 1987).

Effects were similar on the two templates studied. In these regards, both mutants resemble \(\beta\)Q513P (\(\rho\)poB8) (\(\beta\)Q513P elongates at \(-0.25\) the rate of wild-type; Jin and Gross (1991)). \(\beta\)N1072H and \(\beta\)'R352C increased the Apparent \(K_m\) for both Priming and Substrate Nucleotides during Initiation—In principle, the defects in elongation we found could either reflect a fundamental catalytic defect, which should be evident at all nucleotide addition events, or might be specific to the elongation phase of transcription, such as would be the case if they affected movement of the DNA through the enzyme. In an attempt to distinguish these possibilities, we examined the mutant RNA polymerases using a steady-state abortive initiation assay first described by McClure et al. (1978). This assay takes advantage of the cycling of RNA polymerase in an open complex when the presence of a limited set of nucleotides forces the enzyme to synthesize and release a single short transcript (usually a dimer or trimer) repetitively. By restricting nucleotide addition to a single new phosphodiester bond in the open complex, we hoped to learn whether the defects affected the catalytic center and if they also were manifest during initiation. We measured the synthesis of the trinucleotides ApUPC or ApUpG on the T7A1 promoter (or a single bp variant, C + 3 \(\rightarrow\) G) by systematically varying the concentrations of the priming dinucleotide ApU and the substrate NTP (either CTP or GTP;
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ApU, 3'-dUTP, and several different concentrations of ATP, CTP, and GTP (see "Materials and Methods"). These conditions limited productive transcription to the formation of a dU17 complex. Representative time courses for the wild-type and two mutant polymerases at low (5 \( \mu \)M) and high (50 \( \mu \)M) NTP concentrations are shown in Fig. 4 and complete quantified data from these experiments is shown in Fig. 5. Productive initiation is reflected by appearance of the dU17 transcript, whereas abortive initiation yielded three short RNAs, ApUpC (the major product), ApUpCpG, and ApUpCpGpA (Fig. 4; see "Materials and Methods"). Other bands between the abortive and full-length products are most likely paused intermediates (Krummel and Chamberlin, 1989), since they chased through the time course of the experiment; these have not been included in the quantification. We first discuss the transition to productive initiation exhibited by wild-type polymerase and then consider how the mutants alter this pattern.

Quantification of the accumulation of productive and abortive products by wild type reveals that the transition from abortive to productive initiation is a time-dependent process (Fig. 5A). The lag observed in attaining the plateau level reflects the time required for escape from abortive cycling; with each round of initiation, a certain fraction of complexes clears the promoter. This switch between abortive and productive mode is demonstrated graphically by the biphasic accumulation of abortive transcripts. At early times (often <10 s), accumulation of abortive transcripts was rapid due to the relatively large number of complexes engaged in synthesizing abortive products. The inflection points in the accumulation of abortive products and full-length transcripts correspond to the point at which the limit of productive initiation was reached (less than 1 mol of transcript/mol of template, indicating that transcription has been limited to a single round). The small amount of abortive transcript that continued to be synthesized at a constant rate beyond the inflection point probably arose from a subpopulation of templates that somehow form another open complex at high ratio of active RNA polymerase to DNA (4:1 in our experiments) or from a subpopulation of polymerase molecules that are slow to clear the promoter. Kubori and Shimagami (1996) recently reported detection of the latter class of complexes using heparin to block initiation by multiple polymerases. However, rigorously establishing that initiating complexes can become trapped in abortive mode will require showing unambiguously that the template DNA does not harbor a second polymerase molecule that inhibits promoter escape by the first.

Nucleotide concentration influences the transition to productive synthesis in two ways, which are probably not independent. First, it takes longer to make the transition to productive transcription at lower [NTP] (Fig. 5, compare 5 and 50 \( \mu \)M). Second, many more abortive transcripts are made per productive transcript at lower [NTP]. These two points are demonstrated graphically in Fig. 6. The transition to productive mode is efficient only at higher nucleotide concentrations, requiring the production of >3 abortive transcripts/productive transcript at the lowest nucleotide concentration (5 \( \mu \)M; Fig. 6A). However, essentially every polymerase-promoter complex eventually made a productive transcript, even at the lowest nucleotide concentration (5 \( \mu \)M, Fig. 6B).

\( \beta \)N1072H is altered in its nucleotide dependence for the abortive to productive transition. This mutant dramatically over-synthesized abortive products (Fig. 5B) relative to the wild type (Fig. 5A). At the lowest nucleotide concentrations, \( \beta \)N1072H synthesized approximately 60 abortive products for every productive product, which is more than an order of magnitude greater than the abortive/productive ratio of wild-type polymerase (Fig. 6A). As a consequence, \( \beta \)N1072H was significantly slower than wild type in making the transition to pro-
productive initiation (compare Fig. 5, panels A and B). Indeed, at 5 μM NTP only one-fourth of the mutant complexes (as compared to wild type) succeeded in making the productive transcript (Fig. 6B). We note that the fraction of polymerases repetitively engaged in producing abortive transcripts at low NTP concentrations is not a dead-end population; addition of high nucleotide concentrations as much as 10 min after the reaction is started allows their transition to productive initiation (data not shown). These aberrant responses of βN1072H were ameliorated by higher nucleotide concentrations; higher NTP concentrations increased the fraction of polymerases that make productive transcripts (Fig. 6A) and decreased the ratio of abortive to productive transcripts (Fig. 6B) such that at the highest NTP concentration, the fraction of βN1072H complexes that made productive transcripts approached that made by the wild-type complexes.

βR352C is also defective in the abortive to productive transition (Fig. 5C); however, in this case the effect of increasing nucleotides is less straightforward. At low NTPs, a smaller fraction of mutant than wild-type polymerases made the transition to productive initiation (5 μM, Fig. 6A), and the abortive/productive ratio for the mutant was slightly greater than for wild type (Fig. 6B). Although these deficiencies were slightly ameliorated by increased nucleotide concentrations, much of the defect of βR352C for promoter escape did not appear to be responsive to increased nucleotides (Fig. 6A). We return to this point under “Discussion.”

Mutations in rpoC and in the Rif Region of rpoB Cause Defects in Promoter Binding—The rpoC mutations we investigated lie in a region of weak sequence similarity to a region in E. coli DNA polymerase I that may be in the vicinity of the DNA-binding channel. Because of the possible impact of DNA-binding defects on termination as well as on the promoter escape defect noted above, we examined the DNA-binding properties of these mutants. We used nitrocellulose filter binding to measure stability of RNA polymerase binding to an end-labeled DNA fragment containing the T7 A1 promoter. Chamberlin and co-workers (Pfeffer et al., 1977) have demonstrated that polymerase-promoter complexes at this promoter exhibit very different kinetics of dissociation depending on the choice of competitor; T7 A1 complexes are extremely sensitive to heparin, most likely owing to invasion of the open complex by the polyanion, while these same complexes are stable in the presence of passive competitors such as excess DNA (Hinkle and Chamberlin, 1972). In light of these observations, we examined the properties of wild-type and mutant complexes in the presence of each type of competitor. To test for possible correlations with an altered termination phenotype, we also tested the stability of complexes formed by Rif+ mutants with known termination defects: βQ513P(rpoB8), which hyperterminates on all terminators examined, and βH526V(rpoB2), which hypoterminates.

We present the results of the DNA-binding properties of wild type and mutants using heparin as a competitor in Table IV, second column. Results of a representative set of experiments are shown in Fig. 7. As demonstrated by Pfeffer et al. (1977), the wild type complexes were disrupted with a $t_{1/2}$ of approximately 30 min in the presence of 10 μg/ml heparin (Table IV). The mutants examined fell into 3 categories with respect to their stability in this assay. βN1072H and βG1074D, another elongation defective mutant in region H (Tavormina et al., 1996), exhibited $t_{1/2}$ values that are only slightly decreased relative to wild type (less than 2-fold faster), consistent with their primary defect being on the process of nucleotide addition. Two of the mutations in rpoC, βR352C, βS350F, as well as βH526Y, caused pronounced changes in $t_{1/2}$ of disruption, reducing the stability of the binary complex by approximately a factor of 5. We will discuss the implications of the magnitude of the defect for βH526Y below. βQ513P and βG351S exhibited intermediate defects ($t_{1/2}$ approximately twice as fast as wild
had a large effect in this assay, exhibited a total dU17 for WT (\(\beta\)N1072H, and \(\beta\)R352C RNA polymerases). Transcription reactions were performed and quantified as described in the legend to Fig. 5 and under "Materials and Methods." The amounts of productive and abortive RNAs present at 2 min were used to prepare these plots, which represent the averages of two experiments, that typically differed by less than 30%. Ten fmol is equivalent to 1 transcript per input template.

That both Rifr mutants affect DNA binding, and that it is the type value, and that there is no straightforward correlation between promoter binding and termination. The mutants that have a large effect binding and termination. The mutants that have a large effect in this assay, however, may profoundly affect the architecture of the open complex such that it is more susceptible to invasion by heparin.

Qualitatively similar results were seen when approximately 10 \(\mu\)g poly(dA-dT)/ml was used as a cold competitor (Table IV, third column; also Fig. 7). When these experiments were done under the same solution conditions as the heparin challenge experiments, we observed much slower dissociation rates for wild type than those measured when heparin was used as competitor, consistent with the hypothesis that heparin actively invades the open complex, while the DNA competitor acts as a trap for enzyme molecules that dissociate (data not shown). Because of the high degree of inaccuracy in these measurements, we increased the salt concentration (from 50 to 100 mm), thus increasing the dissociation rates.

Again, the region H mutant showing little deviation from the wild-type value, and \(\beta\)Q513P and \(\beta\)G351S falling between these two extremes (Table IV, third column).

**FIG. 7.** Dissociation of open complexes formed by WT, \(\beta\)N1072H, and \(\beta\)R352C RNA polymerases at the T7A1 promoter. RNA polymerase was incubated with the end-labeled 142-bp fragment of pCL185 containing the T7A1 promoter (see "Materials and Methods") at 37°C for 10 min prior to the addition of heparin to a final concentration of 10 \(\mu\)g/ml or poly(dA-dT) to 10 \(\mu\)g/ml. Aliquots were removed (in duplicate) and filtered through nitrocellulose filters at \(-10\) mm Hg vacuum pressure. Filters were washed once with 250 ml of 1 X wash buffer, dried, and counted. Background was subtracted from each measurement. Averages of at least 3 duplicate experiments (except where noted) are presented in Table III with standard deviations. M; WT; O, \(\beta\)N1072H; \(\Delta\), \(\beta\)R352C. A, dissociation of T7 A1 promoter open complexes in the presence of heparin. B, dissociation of T7 A1 promoter open complexes in the presence of poly(dA-dT).

**Table IV**

| Enzyme | Heparin t1/2 (min)\(^a\) | Poly(dA-dT)t1/2 (min)\(^b\) |
|--------|-----------------|-----------------|
| WT     | 29 (±4.5)       | 37 (±8)         |
| \(\beta\)R352C | 8 (±1)        | 9 (±1.5)        |
| \(\beta\)G351S | 17 (±2)\(^c\) | 14 (±2.5)       |
| \(\beta\)S350F | 6 (±0.5)\(^d\) | 8 (±2)          |
| \(\beta\)N1072H | 20 (±2)       | 40 (±4.5)       |
| \(\beta\)G1074D | 30\(^e\)       | ND\(^d\)        |
| \(\beta\)Q513P(B8) | 14 (±1)       | 14 (±1)         |
| \(\beta\)H526Y(B2) | 4 (±0.5)      | 8 (±0.1)        |

\(^a\) 50 mm KCl.
\(^b\) 100 mm KCl.
\(^c\) Done twice; duplicates typically varied <10%.
\(^d\) Done once.
\(^e\) Not done.

**DISCUSSION**

We identified the amino acid substitutions of three mutant alleles of rpoB and rpoC that were originally isolated by Guar-
**Fig. 8. Schematic model of the structure of a transcription complex.** The four regions indicated by the letters A-D correspond to possible parts of the transcription complex that might be affected by amino acid substitutions in the β or β′ subunits that alter chain elongation (see “Discussion”). The non-template strand is depicted as extruded from the transcription complex and reannealing with the template strand just after it emerges from the complex to reflect the fact that the position that exon II stops when digesting the template strand corresponds to the position of the non-template strand that becomes accessible to modification by the single-strand specific reagents KMnO₄ or diethylpyrocarbonate (see Lee and Landick, 1992; Lee et al., 1994). A, contacts to single-stranded RNA or DNA in exit channels. B, contacts to the RNA or template DNA strands that align them in the active site. Whether or not the RNA and DNA are extensively paired in this region (e.g., 8–12 bp) is controversial and not important to our analysis here. However, several results suggest their relationship to polymerase differs here from that in region A (see Lee and Landick, 1992; Borukhov et al., 1993; Feng et al., 1994; Lee et al., 1994; Nuñez et al., 1994; Chan and Landick, 1994; Wang et al., 1995; Chamberlin 1995; Zaychikov et al., 1995). C, catalytic center consisting of two subsites: i, for RNA terminus and i + 1, for NTP-binding site (see Eric et al., 1992). D, duplex DNA-entry channel and helix-unwinding site.

ente (1979) as suppressors of rho201, rpoB211 and -212 encode a change from Asn to His at amino acid 1072 in conserved region H of rpoB, and rpoC214 encodes a change from Arg to Cys at amino acid 352 in conserved region C of rpoC. Unlike another suppressor of rho201, rpoB88(βQ513P), neither βN1072H nor β′R352C greatly increase termination at most -dependent and -independent terminators, but both appear to be somewhat specific for the -dependent terminator, trpt, used for their isolation. Furthermore, all three of the rho201-suppressing mutants, βQ513P, βN1072H, and β′R352C fail to suppress another conditional lethal rho mutation, rho-15 (Das et al., 1978), whereas another class of polymerase mutants, is specific for rho-15 (Jin and Gross, 1989). We have purified the βN1072H and β′R352C mutant enzymes and investigated their properties in vitro. Our most important findings are that (i) although their phenotypes might have been consistent with specific interactions at trpt, βN1072H and β′R352C exhibit general defects in elongation and transcripational activity that are consistent with their compensating for a reduced activity of Rho by slower elongation, (ii) the precise nature of the defects caused by these substitutions (and thus the likely role in transcription of the corresponding conserved regions of the β and β′ subunits) differ; (iii) the specificity of βN1072H and β′R352C for trpt and rpo201 most probably reflect differences in the rate-limiting steps for termination in these cases that are affected differently by the particular structural perturbations caused by these two substitutions. To discuss these points, we refer to a simple diagram of the structure of a transcription elongation complex (Fig. 8).

βN1072H and Conserved Region H in β May Be Located Near the Catalytic Center—Our studies of βN1072H are consistent with the idea that conserved region H may be in the proximity of the catalytic center (C, Fig. 8). Our failure to detect significant effects of this mutation on the stability of the open complex (Table IV; Fig. 7) and of the ternary complex (data not shown) suggests that DNA binding is not perturbed in this mutant. Although this region could interact with the nascent transcript, at least some of the mutant defect is manifest even at the initial nucleotide binding step. The initiating βN1072H mutant encounters the same decision points as the wild-type enzyme but releases abortive transcripts much more often, resulting in a higher ratio of abortive to productive transcripts and inefficient conversion to an elongation complex. The decreased affinity of the mutant for NTPs clearly could contribute to lowering release over elongation since the defect is greatly reduced at higher concentrations of NTPs. Finally, βN1072H increases apparent Km for NTP by 3-fold and Km for -6-fold during abortive initiation. Perturbation of the catalytic center appears able to explain all of these effects of this mutant. However, apparent, but only moderate, perturbation of binding sites both for the priming nucleotide and the substrate NTP argues against direct contacts between βN1072H and either reactant. Instead, a general deformation of the active site caused by βN1072H could be an indirect effect of the mutation. For example, βN1072H could alter one amino acid contact that positions one of the secondary structure elements that actually form the active site.

This interpretation is consistent with other studies of conserved region H, one of nine collinear regions of sequence conservation found in β homologs from bacteria to humans (Sagitov et al., 1993 and references therein). Cross-linking studies indicate that conserved region H is likely to be positioned close to the 5′ face of the priming nucleotide; an evolutionarily invariant lysine residue within this segment (Lys-1065 in β and probably Lys-979 in Saccharomyces cerevisae polII) is within 2 Å of the α-phosphate of the initiating NTP (Grachev et al., 1987, 1989; Mustaev et al., 1991; Riva et al., 1990). The observation that the nascent chain can be elongated by only two residues after cross-linking to this site is further evidence for its proximity to the nucleotide binding site (Mustaev et al., 1993).

Studies of site-directed mutants in this region by the Goldfarb group also support the location of this region of β near the catalytic center. The K1065R mutation exhibits a severe lethal phenotype. Although it forms open complexes, it catalyzes the formation of only one or two phosphodiester bonds, an effect similar to inhibition by Rif. However, replacement of a number of conserved amino acids between 1063 and 1073, including Lys-1065, with alanine caused a less severe phenotype (Sagitov et al., 1993). Single alanine substitutions all retained significant transcriptional activity on natural templates (≈15% of the wild-type polymerase in multiround assays), although they deviated from wild type in a number of steps in transcription, including promoter escape and the extent and pattern of pausing. The mild phenotypes of most Ala substitutions in region H are consistent with the view that it does not form the nucleotide addition site per se, but is probably in its immediate vicinity and is likely to affect RNA polymerase by perturbing the catalytic center indirectly. For instance, changes in charged residues (e.g., Lys-1065 to Arg and Asn-1072 to His) could disrupt catalytic function by forming new H-bonds or charge-charge interactions that reposition side chains near the active center.

Sagitov et al. (1993) have suggested that mutations in this region distort the active center, possibly by affecting the coupling of nucleotide addition and translocation. Although our experiments do not define the function of the region, they are most consistent with this idea. Jin and Turnbough have recently described a Rif β polymerase (R529C in β) that exhibits an increased apparent K for pyrimidines in promoter escape (Jin and Turnbough, 1994). However, the fact that the βN1072H mutant is more defective than β′R352C in the process of promoter escape, but exhibits a comparable increase in K for (cf. ~6-fold for βN1072H and ~2–3-fold for β′R352C), suggests that additional factors, such as context-dependent conformational changes, may contribute to the phenotype of
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\(\beta\)N1072H at such decision points. A more thorough investigation of the block to elongation at these points is necessary to better articulate the nature of the \(\beta\)N1072H defect.

\(\beta\)R352C and Conserved Region C in \(\beta\) May Contact DNA Like a Weakly Similar Sequence in a DNA-binding Region of DNA Polymerase I—Our selection of experiments on \(\beta\)R352C were motivated in part by finding it located adjacent to two termination-altering mutations at amino acids 350 and 351 (Weilbaecher et al., 1994) in a region of weak sequence similarity to a putative DNA-binding region in DNA polymerase I. Our results support the view that this weak sequence similarity reflects functional similarity of these two sequences: substitutions in this region profoundly destabilize polymerase-promoter complexes in the presence of competitor molecules.

In DNA polymerase I, this region comprises \(\beta\)-sheets 7 and 8 of the thumb domain of the DNA-binding cleft and is immediately upstream of helices J and K. The region homologous to amino acids 350–352 in \(\beta\) forms part of a loop between these two \(\beta\) sheets. Polesky et al. (1990) have generated an alanine substitution of the arginine (R668A) corresponding to Arg-352 in rpoC. This mutation has profound effects on \(K_{\text{cat}}\) (400-fold decrease), \(K_{\text{m}}\) for DNA (20-fold decrease), and slight effects on \(K_{\text{m}}\) for dNTPs, suggesting that this arginine side chain forms part of the catalytic site of polymerase I and may also be in close proximity to the phosphate backbone of the DNA (Polesky et al., 1990).

Our results suggest that this region of \(\beta\) may, like the R668A mutation in DNA polymerase I, be involved in more than one important function of polymerase. The convergence of effects on \(K_{\text{cat}}\), with those on DNA binding might be predicted in a region involved in nucleotide addition; at some point in the process, addition of NTPs must be tightly coupled to DNA contacts. Similarly, the failure of \(\beta\)R352C to convert efficiently from the abortive to productive mode (Figs. 4–6), while not due to an obvious increase in the release of abortive products, could reflect aberrant contacts between the polymerase and either the transcript or the template. These defects could, however, also be the indirect result of alterations in the conformational changes that accompany the switch to productive initiation.

It is premature to conclude that this region of \(\beta\) is involved in nucleotide addition or stabilization of contacts to the template during this process: the mutants that are described in this study do not have strong enough phenotypes to unequivocally determine what aspects of transcription are primarily altered. However, further study of this region of \(\beta\) is warranted, particularly with a view to uncovering the relationship between defects in DNA binding and nucleotide addition. The relatively weak phenotype of \(\beta\)R352C could stem from its having been isolated as a haploid, altered-function mutation, necessitating that it retain significant function. We are currently generating the R352A mutation in rpoC as well as several other site-directed changes to evaluate more stringently the function of this region. Interestingly, no significant differences were detected between \(\beta\)R352C and wild type in an elongation assay designed to measure dissociation of the ternary complex during elongation (Arndt and Chamberlin, 1988; data not shown). It is conceivable that in the ternary complex additional contacts between the polymerase and the RNA or DNA mask the putative destabilizing effect of the mutation at \(\beta\)R352C but that the structural perturbation still affects transcript elongation.

DNA-binding Defects of Substitutions in \(\beta\) and in the Rif Region of \(\beta\) Suggest the Existence of DNA-binding Domains in Each Subunit—We have presented results implying that regions in both rpoB and rpoC are important in DNA binding, although we do not yet have sufficient evidence to suggest that these regions contact DNA directly. Prior to this study, only a few workers had addressed the DNA binding properties of the core subunits of RNA polymerase. Martin et al. (1992) describe the DNA binding defect of a deletion mutant of rpoB (in conserved region C, Fig. 1) that fails to form stable complexes at the promoter. Several groups have described the isolation and partial in vitro characterization of cold-sensitive mutations in \(\beta\) and \(\beta\) that appear to be defective in open complex formation (Larionov et al., 1979; Gragerov et al., 1980; Panny et al., 1974). Interestingly, Nomura et al. (1984) describe mutations in rpoC, as well as a double mutation in \(\beta\) which includes the H526Y change, discussed below, that appear to be important in promoter selectivity. Unfortunately, none of the rpoC mutations examined in these earlier studies have been mapped or sequenced.

The three mutations in rpoC and two mutations in the Rif region of rpoB that we analyzed significantly affected the stability of the open complex to challenge by both heparin and poly(dA-dT) (Table IV). In both cases, \(\beta\)R352C and \(\beta\)S350F, as well as \(\beta\)H526Y, formed open complexes that were significantly less stable than wild type in the presence of competitors, consistent with, but not proving that these residues stabilize contacts to the DNA.

The result obtained with \(\beta\)H526Y is particularly interesting. The T7 A1 open complex containing \(\beta\)H526Y is as susceptible to both types of competition as the rpoC mutants. Recent experiments by Goldfarb and co-workers (Mustaev et al., 1995) in which Rif, substituted with a cross-linkable side chain, is bound to RNA polymerase at the T7 A1 promoter indicate that the bound Rif molecule cross-links to the template strand of the DNA between positions 2 and 3 relative to the start site of transcription. These observations, taken with our findings for \(\beta\)H526Y and \(\beta\)Q513P, suggest that the Rif region of rpoB is in the immediate vicinity of the DNA when polymerase is bound at the promoter and may be involved in stabilizing contacts to the DNA in the open complex. In another study, we found evidence suggesting that the Rif region, or at least the residue affected by the \(\beta\)Q513P mutation, is important in correctly positioning the 3′ end of the nascent transcript in the ternary complex (i.e. makes contacts in the promoter distal segment of region B, Fig. 8). This same region could be involved in contacting the DNA in the absence of transcription (i.e. in the binary promoter complex). An interaction between the Rif region and DNA is also consistent with observations by Chamberlin and co-workers (Hinkle et al., 1972) that the affinity of RNA polymerase for Rif is reduced by 2 orders of magnitude when polymerase is bound to DNA. Whether or not these regions contact DNA directly, their effects on the stability of binary complexes suggest that they must at least communicate with regions that stabilize polymerase-DNA interactions.

Suppression of rho Mutations May Occur by Distinct Mechanisms—Jin et al. (1992) previously described a model to explain the kinetic interplay between RNA polymerase and Rho. In this view, the movements of polymerase and Rho on DNA and RNA, respectively, are kinetically coupled such that alterations in the translocation rate of either polymerase or Rho affect termination efficiency. This model has been invoked to explain the hypotermperature phenotype of rpoB (\(\beta\)Q513P), which elongates more slowly in vivo and in vitro, and exhibits increased termination on all Rho-dependent (and intrinsic) terminators tested. Indeed, rpoB8 was also isolated (as rpoB203) as a suppressor of rho201 (Guarente and Beckwith, 1978).

Both Guarente and Beckwith (1978) and our previous study (Jin and Gross, 1989) concluded that the rpoB211 and rpoC214 mutations do not exhibit a general termination defect such as that observed for rpoB8. Although reconstruction experiments...
verified that both mutants increased termination at the trpt' terminators used in their selection, only rpoC214 (β′R352C) increased termination on other terminators, and this defect was not particularly pronounced. Furthermore, in contrast to βN1072H, these mutants show only a marginal increase in termination at intrinsic terminators in vitro (data not shown). These differences in phenotype suggested that a study of the defects of these two mutants might reveal additional characteristics of the termination decision.

We were therefore not surprised to discover that while both βN1072H and β′R352C elongate more slowly in vitro, neither demonstrates an elongation defect as severe as that measured for βN1072P. Furthermore, although βN1072H demonstrates the greater elongation defect of the two, β′R352C exhibits the more significant termination-altering properties. We suggest that the specific effects of the mutants on the trpt' terminator can best be understood in the context of the mutant defects elaborated above for the transition between abortive synthesis and productive elongation. Termination versus elongation is a decision similar to abortive versus productive initiation. In both, release competes with RNA chain elongation. The same properties of the mutants invoked to explain their defects in making the transition to productive elongation may account for their altered termination properties. Thus, the defect of βN1072H in nucleotide addition, and the altered template binding properties of β′R352C may affect their ability to terminate. Moreover, such defects are likely to be manifest only at those terminators whose particular rate-limiting steps are affected by this defect, giving rise to the terminator specific properties of the mutants. For similar reasons, region H mutants exhibit severe defects in forming productive elongation complexes only at certain promoters (Sagot et al., 1993). Our findings complement other recent studies examining the elongation properties of termination-terminating mutants that suggest that elongation rate is not the sole determinant of the probability of termination (Landick et al., 1990; Weilbaecher et al., 1994). Furthermore, it is becoming increasingly clear that contacts to additional protein factors, particularly NusG, play an important role in determining the efficiency of Rho-dependent termination, at least at some terminators. Further studies are underway, both in our groups as well as several others, to unravel the intricacies of the elongation to termination decision at both Rho-dependent and intrinsic termination sites.

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