JOURNAL OF BACTERIOLOGY, Sept. 1996, p. 5263–5271 Vol. 178, No. 17
0021-9193/96/$04.00+0

Isolation, Purification, and In Vitro Characterization of Recessive-Lethal-Mutant RNA Polymerases from *Escherichia coli*

PATRICIA L. TAVORMINA,[1]† ROBERT LANDICK,[2]‡ AND CAROL A. GROSS[1]*

Department of Bacteriology, University of Wisconsin—Madison, Madison, Wisconsin 53706,[1] *and Departments of Biology, Biochemistry, and Molecular Biophysics, Washington University, St. Louis, Missouri 63130*[2]

Received 27 November 1995/Accepted 1 July 1996

The β subunit of prokaryotic RNA polymerase shares significant sequence similarity with its eukaryotic and archaeal counterparts across most of the protein. Nine segments of particularly high similarity have been identified and are termed segments A through I. We have isolated severely defective *Escherichia coli* RNA polymerase mutants, most of which are unable to support bacterial growth. The majority of the substitutions affect residues in one of the conserved segments of β, including invariant residues in segments D (amino acids 548 to 577), E (amino acids 660 to 678), and I (amino acids 1198 to 1296). In addition, recessive-lethal mutations that affect residues highly conserved only among prokaryotes were identified. They include a substitution in the extreme amino terminus of β, a region in which no substitutions have previously been identified, and one *rpoB* mutation that truncates the polypeptide without abolishing minimal polymerase function in vitro. To examine the recessive-lethal alleles in vitro, we devised a novel method to remove nonmutant enzyme from RNA polymerase preparations by affinity tagging the chromosomal *rpoB* gene. In vitro examination of a subset of purified recessive-lethal RNA polymerases revealed that several substitutions, including all of those altering conserved residues in segment I, severely decrease transcript elongation and increase termination. We discuss the insights these mutants lend to a structure-function analysis of RNA polymerase.

Cellular RNA polymerase is a key enzyme in gene expression and regulation. It not only carries out transcription but functions as a central receptor, integrating environmental signals to regulate gene expression. Identification and dissection of structurally and functionally important domains in the enzyme are necessary for an understanding of these processes.

The most thoroughly studied cellular RNA polymerase is that of *Escherichia coli*. Bacterial RNA polymerases are multisubunit enzymes that exist in two forms. The core RNA polymerase ($\alpha_2\beta\beta'$) carries out elongation and termination, whereas holoenzyme ($\alpha_2\beta\beta'\sigma$) carries out initiation (for a review, see reference 3). Both the β and β′ subunits of *E. coli* RNA polymerase exhibit a high degree of sequence conservation over their entire length when compared with their eukaryotic counterparts (1, 8, 36). In the case of β, nine highly conserved segments have been identified, with an average identity of roughly 35% and an average similarity of roughly 70% (36). Given this extensive homology, a structure-function analysis of the *E. coli* enzyme should provide insight into the organization of all cellular RNA polymerases, as well as into those features of RNA polymerase function that are specific to prokaryotes.

Because of the complexity of this essential enzyme, progress in determining its structural features has necessarily been slow. Low-resolution pictures of both *E. coli* and *Saccharomyces cerevisae* RNA polymerases have been provided by two-dimensional crystallographic studies (6, 7). These studies indicate that the enzyme contains a channel with dimensions that could accommodate double-stranded nucleic acid and branches with dimensions that could accommodate single-stranded nucleic acid (6).

A higher-resolution picture of specific features of polymerase structure comes from genetic studies and cross-linking analysis. Cross-linking studies have permitted identification of the regions of RNA polymerase capable of interacting with the transcript's 3′ terminus or a priming nucleotide analog. By using a procedure that selectively detects bound priming nucleotide analogs capable of reacting with an [α-^{32}P]nucleoside triphosphate (NTP) substrate (9, 10), Grachev and coworkers found that amino acid residues 1065 and 1237 of the β subunit are within 3 Å (0.3 nm) of the 5′ phosphate on the priming nucleotide during initiation (24). Interestingly, these two residues are located in regions highly conserved among all RNA polymerases. Site-directed changes of highly conserved residues around residue 1065 confirm that this region is important for RNA synthesis by the enzyme (32). Recently, cross-linking studies using chimeric rifampin-nucleotide compounds found that Lys-1065, positions −2 and −3 of the template DNA, a portion of σ, and bound rifampin, whose principal contacts localize to residues 500 to 575 of β, are all within 15 Å (1.5 nm) of each other in the open complex (25).

Genetic studies have localized the regions of RNA polymerase interacting with rifampin and streptolydigin, two small molecules with distinct effects on transcription (11, 14), and have identified regions of RNA polymerase that affect termination. Four distinct regions of the β polypeptide give rise to Rif^r

* Corresponding author. Present address: Departments of Microbiology and Stomatology, University of California—San Francisco, San Francisco, CA 94143. Phone: (415) 476-4161. Fax: (415) 476-4204. Electronic mail address: cgross@cgl.ucsf.edu.

† Present address: Department of Biological Chemistry, University of California—Irvine, Irvine, CA 92697.

‡Present address: Department of Bacteriology, University of Wisconsin–Madison, Madison, WI 53706.

mutants, suggesting that the binding site for rifampin is composed of distinct segments of the polypeptide (14, 20). Streptolydigin appears to contact both β and β′ (34). The contacts for streptolydigin in β appear to consist of a single set of contiguous amino acids (residues 543 to 546) located between two of the domains (residues 507 to 533 and residues 563 to 572) implicated in binding rifampin (11, 35). Mutations affecting termination are located in many regions of RNA polymerase. Many Rif^r mutants exhibit altered termination properties. In addition, a generalized approach involving the use of plasmids carrying discrete mutagenized intervals of *rpoB* (encoding the β subunit of RNA polymerase) or *rpoC* (encoding the β′ subunit of RNA polymerase) identified mutations in several intervals of β and β′ that alter termination at intrinsic terminators (19, 41). Features of the β subunit of RNA polymerase are diagrammed in Fig. 1.

In the present report, we describe the use of a terminator probe vector carrying a complex terminator to search for mutants exhibiting increased terminator read-through. Contrary to expectation, most of the mutants we obtained did not exhibit increased terminator read-through. Because these mutations conferred severe defects in RNA polymerase function, as judged by the inability of the mutant alleles to support cell growth, we characterized them further. Almost all of them occurred in highly conserved regions of RNA polymerase. After using a novel method to recover RNA polymerases with recessive-lethal mutations, we found that several of the mutations had profound effects on the elongation and termination functions of the enzyme when it was assayed in vitro. We discuss the implications of these mutant enzymes with regard to a structure-function analysis of RNA polymerase.

MATERIALS AND METHODS

Chemicals and enzymes. Hydroxylamine, isopropyl-β-D-thiogalactopyranoside (IPTG), heparin, human immunoglobulin G (IgG)-agarose, and heparin-agarose (type I) were purchased from Sigma. T4 DNA ligase, Klenow fragment, and restriction enzymes were purchased from New England Biolabs. Ultrapure nucleoside triphosphates and calf intestinal alkaline phosphatase were purchased from Boehringer Mannheim. DEAE-cellulose (DE52) was purchased from Whatman. [α-^{32}P]UTP was purchased from DuPont-NEN, and [α-^{32}P]GTP was purchased from Amersham. Low-melting-point agarose and gelase were purchased from Epicentre Technologies. Amplitaq was purchased from Perkin-Elmer Cetus. Sequenase version 2 was purchased from U.S. Biochemicals.

Strains and plasmids used. The strains and plasmids used in this study are listed in Table 1.

General bacterial techniques and media. Cells were grown in Luria-Bertani (LB) broth or SOC broth with shaking; growth of cultures was monitored by measuring the change in optical density (OD) at 600 nm.

LB broth, SOC broth, LB agar plates, and MacConkey galactose agar plates were made as described previously (33). Where indicated, ampicillin (50 μg/ml), tetracycline (10 μg/ml), and kanamycin (30 μg/ml) were added.

T7 D111 phage DNA was prepared following the protocol for λ DNA preparation, with MC1061 as a host (33).

Calcium shock transformations and electroporations were performed as described previously, and transformants were plated directly for ampicillin selection or after outgrowth for 45 min for tetracycline selection (21, 43).

Construction of pPLT7. The terminator probe vector pES4 has been previously described (13). In brief, this pBR-based vector encodes the *rrnA* P1 promoter directing transcription of *galK*. The *rrnB* T1 and T_{IS2} terminators are interposed between the promoter and *galK*. These terminators do not allow sufficient expression of GalK to confer a *galK*$^+$ phenotype in *galK* mutant *E. coli.*

pPLT7 is identical to pES4 except that it contains the pACYC origin of replication and the *tet* gene in place of the pBR origin of replication and the *bla* gene. This construct was necessary to screen *rpoB* or *rpoBC*-encoding mutant plasmids with a pUC origin of replication. To construct pPLT7, a 2.5-kb *Eco*RI-*Nde*I fragment of pES4 carrying the *rrnA* P1 promoter, the *rrnB* T1 and T_{IS2} terminators, and the *galK* gene was ligated onto an *Eco*RI-*Xmn*I vector fragment of pACYC184. The overhangs from *Nde*I and *Xmn*I digestion were repaired with the Klenow fragment of DNA polymerase I prior to *Eco*RI digestion.

Mutagenesis and screening of *rpoB* and *rpoBC*. Plasmids pRL385 and pPLT2 encode *rpoB* and *rpoBC*, respectively. Aliquots (14 μg) of pPLT2 DNA were subjected to hydroxylamine mutagenesis for 30, 60, and 90 min as described previously (29). Bisulfite mutagenesis of gapped duplex derivatives of *rpoB*-encoding pRL385 was described previously (18).

To screen for RNA polymerase alleles capable of altering expression of *galK* from GalK reporter plasmid pES4 or pPLT7, 50 ng of mutagenized DNA was electroporated into strain N100 containing either pES4 or pPLT7. Transformants were plated on MacConkey galactose indicator plates and incubated for 16 to 40 h at 37°C.

Mapping strategy. pRL385 encodes *rpoB* under the control of the *lac* promoter. The initial selection of mutants was performed without repression; however, subsequent mapping and reconstruction were performed in the presence of *lacI*^{q} to prevent accumulation of suppressors on the mutant *rpoB* and *rpoBC* plasmids.

The following restriction fragment mapping strategies were used to locate the mutations. To determine whether the mutations isolated on pPLT2 were in *rpoB* or *rpoC*, a *Bgl*II fragment was deleted from each mutant plasmid. This removes most of *rpoC* while leaving *rpoB* intact. The resulting deletion derivatives were tested for the ability to confer the mutant phenotype. To map mutations within *rpoB*, the following restriction fragments, which span the gene, were exchanged between mutant and wild-type copies of *rpoB*:*Bam*HI-*Sty*I (263 bp), *Sty*I-*Bst*XI (791 bp), *Bst*XI-*Hpa*I (1,193 bp [within this fragment lies the 237-nucleotide *Bcl*I fragment which was also used for mapping]), *Hpa*I-*Bst*EII (1,466 bp; one mutation mapped within this large fragment and was then mapped to a 149-bp *Hpa*I-*Rsr*II fragment), and *Bst*EII-*Sac*I (420 bp). During the course of the mapping, alleles generated on pPLT2 were transferred to pRL385. The smallest restriction fragment capable of conferring the mutant phenotype was sequenced to identify all mutations present, and the otherwise wild-type *rpoB* plasmids carrying these fragments were retained as reconstructed versions of the mutant alleles of pRL385.

***rpoB* complementation analysis.** Strain RL585 encodes a nonpolar amber mutation in the chromosomal *rpoB* locus and a temperature-sensitive suppressor tRNA; therefore, this strain produces functional β at 36°C but not at 42°C (18, 28). Each unique reconstructed mutant allele was transformed into RL585 via electroporation. Transformants were purified on selective media, and the purified alleles were individually inoculated into 5 ml of LB broth. The cultures were grown to an OD of ~0.1, and 10-fold serial dilutions of each culture were plated at 36 and 42°C.

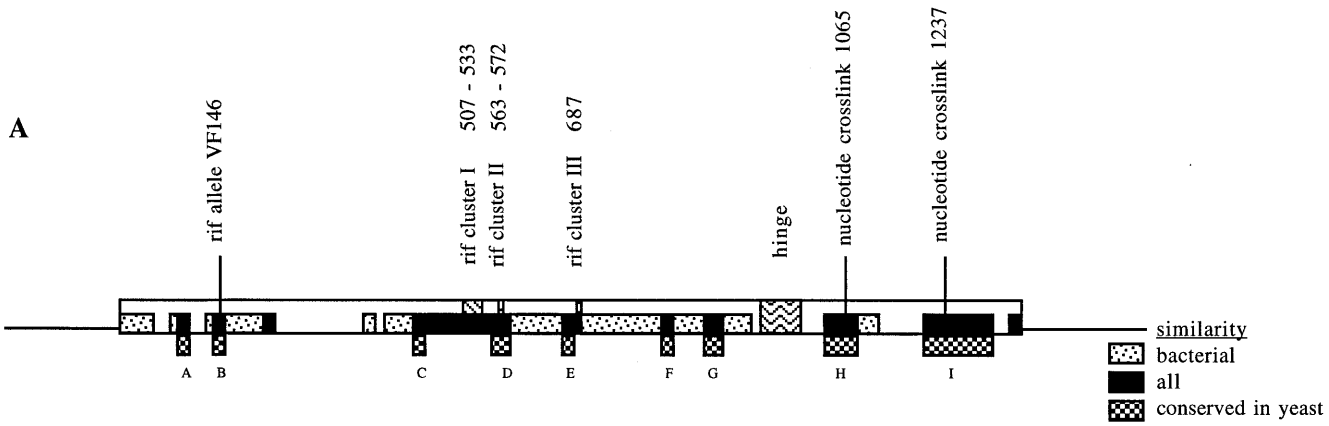
Construction of strain RL656. Strain RL656 was constructed to facilitate purification of RNA polymerase containing plasmid-encoded mutant β subunits. It contains a chromosomal *rpoB* gene tagged with a 234-amino-acid insertion consisting of four IgG-binding domains of *Staphylococcus aureus* protein A (SPA) inserted between Leu-998 and Glu-999.

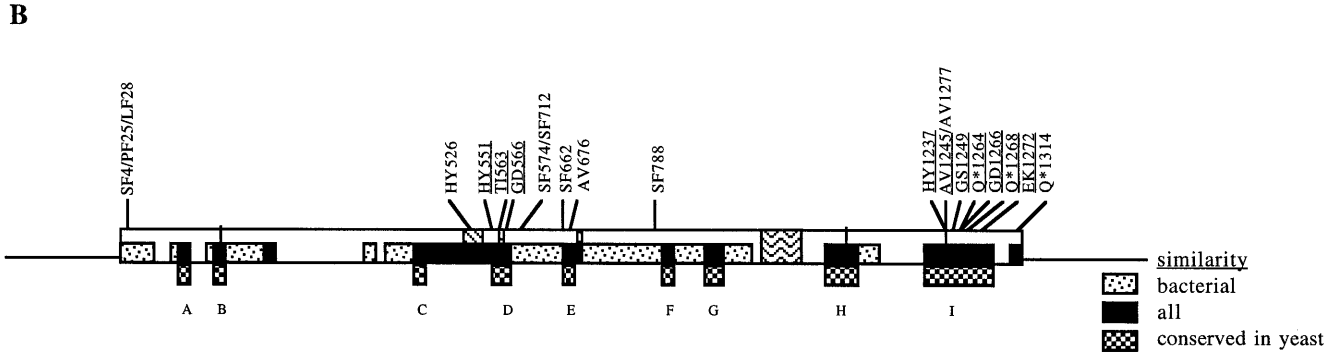
RL656 was derived from CY15001 (42) by selection for strong rifampin resistance after transformation with plasmid pRW237*rpoB*5142(Rif^r, SPA). After screening for loss of the plasmid by selecting for ampicillin sensitivity, the presence of the SPA insertion was confirmed by gel electrophoresis of whole-cell lysates to visualize the larger β subunit. This strain was transduced to Δ(*recA-srl*)306 *srl*::Tn*10* as described previously (17).

The rpoB5142 gene (Rif^r, SPA) was first constructed by using the plasmid pRL385*rpoB6*(HY526, SR643, Rif^r) (17). This rifampin resistance allele is a spontaneous pseudorevertant of the strong hypotermination rifampin resistance allele *rpoB2*(HY526) (14, 42). This plasmid was altered to contain an *Xho*I site between Leu-998 and Glu-999 by oligonucleotide-directed mutagenesis of CTG GAG to CTCGAG. This yielded plasmid pRL385*rpoB*5140. An *Xho*I-*Sal*I fragment encoding domains D, A, B, and C of SPA was created from pRIT2 (27) by PCR with the custom primer 5′CACCTCGAGGCTGATGCGCAACAAAATA ACTTC (the underlined triplet corresponds to Ala-57 of SPA) and a primer hybridizing downstream from the *Sal*I site in pRIT2, followed by digestion with *Xho*I and *Sal*I. This fragment was ligated into the *Xho*I site of pRL385*rpoB*5140 to produce pRL385*rpoB*5142. *rpoB*5142 was then excised from pRL385 by digestion with *Bam*HI, production of blunt ends with Klenow polymerase, and digestion with *Sac*I. This fragment was ligated between the *Nco*I site (ends blunted with Klenow fragment) and the *Sac*I site of pRW204, a derivative of pTrc99c (2) that contained a 450-bp fragment of M13 phage encoding its single-stranded origin of replication in the pTrc99c *Nsi*I site, to yield pRW237*rpoB*5142.

Purification of RNA polymerase. The chromosomal *rpoB* locus in strain RL656 contains an SPA insert and a rifampin resistance mutation. The insertion does not interfere with the function of the resulting RNA polymerase, and it allows selective removal of polymerase carrying chromosomally encoded β from polymerase carrying plasmid-encoded β. Mutant alleles carried on pRL385 were transformed into a *lacI*^{q} derivative of strain RL656. Transformants were purified on selective media; colonies were inoculated directly into 500 ml of LB broth and grown with shaking at 37°C. Upon reaching an OD of ~0.15 (~16 to 20 h after inoculation), IPTG was added to a final concentration of 0.7 mM, and growth was continued to an OD of ~0.6. Cells were harvested, quickly frozen in tubes in a dry ice-ethanol bath, and stored at −80°C. A sample of plasmid DNA was isolated from each pellet and sequenced to verify the mutant allele on the plasmid.

RNA polymerase was isolated from each cell pellet as described previously (18) with minor modifications. Prior to use, heparin-agarose and human IgG-agarose were washed four times with fourfold volumes of TEGD plus 0.3 M NaCl and TEGD plus 0.6 M NaCl, respectively. The step iv extract was dialyzed over a 2-h period against two 100-fold volumes of TEGD buffer plus 0.3 M NaCl and applied in batch to heparin agarose for 1 h with gentle rotation. The resulting slurry was poured into a 12-ml disposable column, the buffer was allowed to run





C

Segment D		
CPV POX VIRUS		RKYHPSQYLYFCSSDVPERGPQVGLVSQLSV
H. HALOBIUM		RDLHATQWGRICPSETPE-GPNCGLVKNFAQ
D. M. RP140		RQLHNTLWGMLCPAETPE-GAAVGLVKNLAL
RPB2 YEAST		RQLHNTHWGLVCPAETPE-GQACGLVKNLSL
TOBACCO CHL.		RDIHPSHYGRICPIDTSE-GINVGLIGSLAI
RPOB	548-578	RDV**H**PTHYGRVCPIE***T***PE-**G**PNIGLIN**S**LSV
Segment E		
CPV POX VIRUS		GYVASSLVGINHNSGPR
H. HALOBIUM		GIGAGMIPYPEHNASPR
D. M. RP140		GVCASIIPFPDHNQSPR
RPB2 YEAST		GVAASIIPFPDHNQSPR
TOBACCO CHL.		SIGASLIPFIEHNDANR
RPOB	662-678	**S**VGASLIPFLEHDD**A**NR
Segment I		
CPV POX VIRUS		RLRHLTQDKATVRCRGKKTKLIRQANEGRKRGGGIKFGEMERDCLIAH
H. HALOBIUM		KLYHMVSNKLHARSKGPVQVLTRQPTEGRAREGGLRVGEMERDTVIGH
D. M. RP140		RLKHMVDDKIHSRARGPVQILVRQPMEGRARDGGLRFGEMERDCQISH
RPB2 YEAST		RLRHMVDDKIHARARGPMQVLTRQPVEGRSRDGGLRFGEMERDCMIAH
TOBACCO CHL.		KLIHQVDDKIHGRSSGHYALVTQQPLRGRAKQGGQRVGEMEVWALEGF
RPOB	1234-1281	KLN**H**LVDDKMH**A**RST**G**SYSLVTQQPLGGKA**Q**F**G**G**Q**RFG**E**MEVW**A**LEAT

FIG. 1. Location of the amino acid changes within the β subunit of RNA polymerase. (A) Landmarks in the β subunit, including regions that display conservation between prokaryotic and eukaryotic homologs. Conserved segments A to I consist of the following residues in β: A, 83 to 106; B, 134 to 108, C, 438 to 453; D, 548 to 577; E, 660 to 678; F, 793 to 828; G, 859 to 889; H, 1047 to 1118; and I, 1198 to 1296. Of the residues within these segments, 43% are identical between *E. coli* and yeast homologs; conservative changes account for an additional 30%. (B) Locations of amino acid changes relative to these landmarks. Mutations that alter residues identical in *E. coli* and yeast homologs are underlined. (C) A partial sequence comparison for conserved segments D, E, and I. Of the 17 alleles in this study, 13 have at least one mutational change in one of these segments, indicated in boldface. Of those 13, 6 alter residues identical in all organisms listed; an additional 2 truncate the carboxy terminus, deleting residues identical in all organisms listed. These eight alleles are underlined. One other allele, indicated in italics, alters a residue that is identical in five of the six organisms listed. Three of the remaining five affected residues are part of multiple mutations. CPV, cowpox virus; H. halobium, *Halobacter halobium*; DM, *Drosophila melanogaster*; RPB2, *Saccharomyces cerevisiae* RNA polymerase 2 homolog; CHL, chloroplast; RPOB, *Escherichia coli* RNA polymerase β subunit.

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant markers	Source or reference
CAG 324	GalK$^-$ RecA$^-$	23
CAG 5200	CAG 324; pES4	38
CAG 5270	CAG 324; pPLT7	38
RL 585	MX 1494 *supD43,74*(Ts) *rpoBcl*(Am)	17
RL 656	W3110 Δ(*recA-srl*) *srl*::Tn*10 rpoB*5142 (Rif^r; SPA insert)	38
CAG 5434	RL 656; pPLT13 (*lacI*^{q})	38
pES4	Terminators *rrn*BT1 and t_{IS2} interposed between the *rrn*AP1 promoter and the *galK* reporter in a pBR plasmid; *bla*	13
pPLT7	Reporter construct from pES4 carried in pACYC; *tet*	38
pRL385	*rpoB* under *lacZp* control in pUC119; *bla*	17
pPLT2	9.7-kb *Nco*I-*Ssp*I fragment carrying *rpoB* and *rpoC* expressed from the upstream *rplJ* promoter inserted in pACYC184; *tet*	38
pPLT13	Mini-F supplying *lacI*^{q}; *kan*	38

out of the column, and the column bed was washed with 10 ml of TEGD plus 0.3 M NaCl. The RNA polymerase was eluted from the heparin-agarose with TEGD plus 0.6 M NaCl, and the eluate from the column was incubated three successive times for 1 h with 100 μl of human IgG-agarose with gentle rotation. DEAE-cellulose chromatography and storage of product were as described previously (18). Prior to storage, all manipulations were performed at 4°C.

To estimate the efficiency of removal of the SPA-containing RNA polymerase, we took advantage of the Rif^r marker on the allele to assay the percentage of rifampin-resistant transcription. Reconstruction experiments using wild-type RNA polymerase and varying amounts of SPA-containing polymerase from RL656 demonstrated that as little as 1% contaminating enzyme could be detected in these RNA polymerase preparations. RNA polymerase preparations from the strains with the mutant alleles routinely displayed no detectable rifampin-resistant transcription; however, the mutant RNA polymerase preparations generally displayed 50 to 100% of the activity displayed by the wild-type preparation. This indicates that SPA-containing RNA polymerase accounted for less than 2% of the total RNA polymerase in these preparations.

Elongation assays on T7 D111 template. Elongation rate measurements on the T7 D111 template were performed as described previously with minor modifications (5). In brief, the assay measures the time required to incorporate ribonucleotides into a long transcript, which initiates at the single σ^{70} promoter present on the T7 D111 template and terminates with 80% efficiency at the T7 terminator located 6 kb downstream of the promoter. Open-complex formation was synchronized by preincubating RNA polymerase (1 μg) with 40 μg of T7 D111 DNA and all reagents except nucleotides at 37°C. Transcription was initiated by the addition of prewarmed NTPs to 0.4 mM each, including [α-^{32}P]UTP (at a specific activity of 1,000 to 3,000 cpm/nmol), and heparin (to 50 μg/ml), which limits transcription to a single round. Aliquots (20 μl) were sampled onto DEAE filters over a time course such that at least six time points fell before and at least six fell after the population of polymerase molecules had traversed the T7 terminator. Estimates of elongation rate were made in triplicate and generally varied by less than 8%. This assay has been used extensively to measure elongation rate for two reasons: (i) the length of the transcript (6 kb) minimizes contributions from promoter clearance and allows multiple rate determinations of the transcription event, and (ii) the time required to reach the terminator provides a second measure of the elongation rate, which permits direct comparison of the activities of the RNA polymerase preparations. Two preparations with comparable specific activities will incorporate roughly the same amount of radioactivity upon traversing 6 kb of template.

PCR amplification. Templates for assaying termination efficiency were amplified by PCR as described previously (41), using plasmids generously provided by M. Chamberlin.

Termination assays. The derived T7 A1 promoter used in this study encodes the following initially transcribed region: +1 AUCGAGAGGGACACGGGG GAU +21. Providing substrates ApU, ATP, GTP, and CTP will cause a population of RNA polymerase molecules to stall at position +20. These stalled complexes are formed in order to synchronize transcription for subsequent manipulations—in this case, to limit transcription of a terminator-containing template to a single round. Termination reactions were performed as specified previously with the following modifications (41). Formation of A20 complexes was performed at the following nucleotide concentrations: 200 μM ApU; 25 μM (each) ATP, GTP, and CTP; and 36 Ci of [α-^{32}P]GTP per mmol. A20 complexes were extended with ATP, CTP, and GTP at 125 μM each, UTP at 100 μM, and heparin at 50 μg/ml. Radioactivity present in terminated and read-through bands was quantitated with a Molecular Dynamics phosphorimager. Because the A20 complexes have a 50% G content, and because the relative amount of labeled GTP was sixfold higher during A20 formation than during transcript elongation and termination, the specific activity of the first 20 bases of each transcript was estimated to be 12-fold higher than in the remainder of each transcript. Percent read-through was therefore calculated by correcting for length and specific activity in the terminated and read-through bands and then using the following formula: percent read-through = (moles of read-through transcript)/(moles of read-through transcript + moles of terminated transcript). Estimates of percent read-through were made in triplicate and generally varied by less than 2%.

RESULTS

Isolation of RNA polymerase mutants. To obtain RNA polymerase mutants altered in termination, we used the terminator probe plasmids pES4 and pPLT7. These GalK expression vectors differ only in their replication origin and antibiotic resistance genes (see Materials and Methods) and contain two strong terminators, one intrinsic and one rho dependent, interposed between the *rrnA* P1 promoter and the *galK* structural gene. *E. coli galK* mutants carrying these plasmids produce so little galactokinase that they give rise to white colonies on MacConkey galactose plates. RNA polymerase mutants with increased terminator read-through can be identified because they give rise to red colonies on these indicator plates. We chose this vector because its termination signals are strong and termination requires the auxiliary factors rho and NusA (13). Few of our existing mutants influenced these termination signals, suggesting that any mutants isolated would predominantly constitute a new class.

Increased terminator read-through is expected to be codominant with the wild type, permitting us to screen for this phenotype in merodiploid cells encoding wild-type RNA polymerase subunits on the chromosome and mutant subunits on a plasmid. Two different mutant-plasmid banks were used to obtain mutations in *rpoB* and *rpoC*. We used a bisulfite-mutagenized *rpoB* library, which contains contiguous mutagenized intervals of *rpoB* ranging in size from 200 to 500 bp, to assess whether defined intervals of *rpoB* were involved in the specified phenotype. Note, however, that we sometimes identified mutations outside of the interval targeted for mutagenesis (Table 2). We also screened hydroxylamine-mutagenized plasmid DNA carrying both *rpoB* and *rpoC* for functionally altered mutants. The latter approach extended the analysis by including *rpoC*. In addition, hydroxylamine increases the range of affected residues. Both hydroxylamine and bisulfite cause C-to-T transitions. However, because hydroxylamine mutagenesis is carried out on a double-stranded template, cytosines on both strands are subject to mutagenesis.

We screened 80,000 transformants carrying hydroxylamine-mutagenized *rpoBC* plasmid DNA and 15,000 transformants from each bisulfite-mutagenized *rpoB* interval for increased expression of galactokinase as judged by red color on MacConkey galactose plates. We found no candidates that were red on MacConkey galactose after short incubation times, as would be expected for a true termination-defective mutant. However, we identified 35 candidates from each mutagenesis protocol that exhibited pink to red phenotypes after extensive

TABLE 2. Mutational changes isolated

Original isolate				Reconstructed allele[c]	
Name[a]	No. of isolates	Identified sequence changes	GalK expression[b]	Sequence change	GalK expression[b]
Wild type	NA[d]	NA	−	NA	−
HA-6	2	GD566	+	GD566	+
HA-5	1	GS1249	++	GS1249	+
HA-4	1	GD1266	++	GD1266	+
HA-1	3	EK1272	++	EK1272	+
B10-1	1	SF4 PF25 LF28	++	SF4 PF25 LF28	+
B7-1	4	HY526	+	HY526	+
B7-6	1	HY526 AV532	+	HY526	+
B7-7	1	HY551	++	HY551	++
B7-4	3	TI563	++	TI563	++
B12-3	1	SF574 SF712	+	SF574 SF712	+
B12-2	1	SF662	++	SF662	++
B8-1	6	AV676 AV683 RC687	++	AV676	+
B12-4	2	AV676	+	AV676	+
B9-2	3	TI757 PS769 SF788	+	SF788	+
B13-2	1	HY1237 AV1284 AV1285	++	HY1237	+
B13-5	1	AV1245 AV1277	++	AV1245 AV1277	++
B13-1	1	Q*1264	++	Q*1264	++
B13-4	1	Q*1268	++	Q*1268	++
B13-3	1	Q*1314	++	Q*1314	++

[a] Isolates are designated as follows: HA, hydroxylamine mutagenesis, followed by isolate number; B, bisulfite mutagenesis, followed by the number of the interval targeted for mutagenesis and then the isolate number. The mutagenized intervals in the bisulfite-mutagenized plasmid bank correspond to β-subunit amino acid residues 1 to 74 (interval 1), 75 to 196 (interval 2), 197 to 262 (interval 3), 262 to 340 (interval 4), 341 to 433 (interval 5), 434 to 492 (interval 6), 493 to 571 (interval 7), 572 to 736 (interval 8), 737 to 846 (interval 9), 847 to 935 (interval 10), 936 to 1138 (interval 11), 1139 to 1223 (interval 12), and 1224 to 1342 (interval 13).

[b] GalK expression was assayed by colony color on MacConkey galactose plates after incubation at 37°. −, white after 30 h; +, pink after 16 to 30 h; ++, red after 16 to 30 h.

[c] Where possible, single mutational changes that demonstrate a mutant phenotype replaced multiple mutational changes isolated in the screen. The SF788 and HY1237 mutations were previously obtained as single mutational changes and were utilized here (18). The remainder of the reconstructed alleles were made for this study.

[d] NA, not applicable.

incubation of the plates (>36 h). Seven of the 35 candidates obtained from hydroxylamine mutagenesis and 28 of the 35 candidates obtained from bisulfite mutagenesis demonstrated linkage of this novel phenotype to the mutagenized plasmid and were studied further. All 35 mutants depend on both the GalK expression vector and the presence of galactose in the medium for manifestation of the phenotype. To characterize these unanticipated mutants and determine if any actually exhibited termination deficiency, we mapped and sequenced all 35 mutations and then purified representative mutant RNA polymerases.

Mapping and sequencing revealed that the mutations are located in conserved regions of *rpoB*. Plasmid pPLT2 contains both *rpoB* and *rpoC*. To determine whether any of the mutations generated in this plasmid were in *rpoC*, we deleted a 2.6-kb internal fragment of *rpoC*. This deletion did not destroy the mutant phenotype of any isolate (see Materials and Methods), indicating that all seven of the hydroxylamine-generated mutations were located in *rpoB*.

Many of the mutants proved to be inviable (see below), making classic genetic approaches to mapping difficult. We therefore located the mutations within *rpoB* by molecular methods. Mutations were mapped by exchanging restriction fragments between mutant and wild-type plasmids to obtain reconstructed genes with a single mutagenized interval that conferred a mutant phenotype (see Materials and Methods for a description of the restriction fragments exchanged). The entire mutagenized interval was then sequenced to identify the mutational changes conferring the phenotype.

The changes identified in the 35 *rpoB* mutants characterized are indicated in Table 2. In some cases, the reconstructed mutational changes did not account for the entire phenotype. For example, the reconstructed clone encoding the GS1249 change identified in mutant HA5 did not exhibit as severe a plate phenotype as HA5 itself. We have not yet located and sequenced the additional mutations that contribute to the complete phenotype in these cases. In some cases, we obtained a family of mutants, all of which contained the same mutation and some of which also contained additional mutations (for example, see clones B8-1 and B12-4 in Table 2). In these cases, we have characterized mutants encoding the single mutation in common among the isolates. Three cases (B10-1, B12-3, and B13-5) contained multiple mutations within a single interval. We have not reconstructed them as single alleles. Instead, the characterization described in this report were performed on the multiple mutants. Characterization of the 35 mutants resulted in the identification of 17 different reconstructed alleles in the β subunit (Table 2; Fig. 1B). Six of these alleles, HY526, HY551, TI563, SF788, HY1237, and AV1277, have been previously isolated (14, 18).

Alignment of prokaryotic and eukaryotic RNA polymerase sequences indicates that the β subunit of prokaryotic RNA polymerase shares significant sequence similarity with its eukaryotic counterparts across most of the protein. Nine segments with a particularly high degree of conservation, exhibiting about 70% similarity, have been designated segments A through I (Fig. 1A). Of the 17 mutants described in this report, 13 exhibit changes in one of the highly conserved segments, D, E, or I (Fig. 1B and C). Six of the 13 mutational changes, TI563, GD566, HY1237, GS1249, GD1266, and EK1272, alter a residue that is identical in all 22 prokaryotic and eukaryotic homologs examined, and many of the others alter residues exhibiting conservative substitutions among homologs. The changes in the other four mutants are located in regions that are conserved among prokaryotes (Fig. 1B). The fact that these mutations are located in such highly conserved regions of the

TABLE 3. Growth phenotypes of mutants

rpoB allele[a]	Plating efficiency in RL585 at 42°C/36°C[b]	Growth inhibition by mutant alleles[c]
Wild type	0.5–1.0	−
SF4/PF25/LF28	<0.005	−
HY526	0.5–1.0	−
HY551	<0.005	−
TI563	<0.005	−
GD566	<0.005	+
SF574/SF712	<0.005	−
SF662	<0.005	++
AV676	<0.005	+
SF788	0.5–1.0	−
HY1237	<0.005	+++
AV1245/AV1277	<0.005	++
GS1249	<0.005	++
Q*1264	<0.005	++
GD1266	<0.005	+
Q*1268	<0.005	++
EK1272	<0.005	−
Q*1314	<0.005	++

[a] The *rpoB* allele is carried on plasmid pRL385.

[b] Because RL585 does not produce wild-type RNA polymerase at 42°C, the efficiency of plating at 42°/36° reflects the ability of the mutant alleles to support growth in the absence of wild-type RNA polymerase.

[c] Inhibition of growth of a wild-type strain (CAG324) by plasmid-encoded mutant RNA polymerase alleles (dominant-negative phenotype), assayed by time of appearance of visible colonies on MacConkey galactose plates at 30°C. −, 22 h; +, 26 h; ++, 30 h; +++, 36 h. Similar trends were observed on LB agar for all alleles except HY551, which grew significantly more slowly than the wild type on that medium.

β subunit suggests that they alter residues with important roles in RNA polymerase function.

Many of these mutants are inviable. The *rpoB* mutants described in this report were isolated in *trans* from a wild-type copy of *rpoB*. To determine whether the mutant RNA polymerases are capable of supporting growth in the absence of the wild-type RNA polymerase, we used RL585, a strain of *E. coli* that encodes a nonpolar amber mutation in the chromosomal copy of *rpoB* and a temperature-sensitive suppressor tRNA. In this strain, σ, α, and β′ are made at all temperatures but functional β is made only at low temperature, permitting us to determine whether the plasmid-encoded mutant β subunit could provide all functions necessary for growth at 42°C. Of the 17 alleles tested, 15 were unable to restore growth at high temperature (Table 3). Furthermore, many of these alleles interfere with growth in the presence of wild-type RNA polymerase (Table 3). The fact that the vast majority of the mutants we isolated had recessive-lethal mutations and that some had a partial dominant-negative phenotype provided further evidence that the mutations alter residues that are functionally or structurally important for RNA polymerase. We therefore turned to further characterization of the mutant polymerases in vitro.

Purification of the mutant RNA polymerases. To obtain an RNA polymerase preparation essentially free of the wild-type RNA polymerase, we purified the mutant RNA polymerases from a strain in which the chromosomal copy of *rpoB* contains an SPA insertion in the nonessential hinge region of the protein as well as a mutation that confers resistance to rifampin (RL656; see Materials and Methods). The SPA insertion permits elimination of wild-type RNA polymerase by affinity chromatography on IgG-agarose, and the rifampin resistance allele allows one to assay for extent of contamination by determining the fraction of transcription that is rifampin resistant. We routinely find that following purification, >98% of the RNA polymerase is mutant (see Materials and Methods).

We purified RNA polymerase from the 11 mutants isolated in this study that had not been described previously. Three of these preparations were subsequently eliminated. SF662 was eliminated because it could not be propagated without acquiring revertants, even under conditions in which expression of the mutant subunit should be significantly repressed. Two additional alleles, which truncate the β peptide at positions 1264 and 1268, were eliminated because the mutant proteins were difficult to purify. These two mutant proteins consistently yielded 5% or less mutant RNA polymerase.

Elongation rate of mutant RNA polymerases. The T7 D111 template has been used extensively to characterize the elongation rate of RNA polymerase (5, 11, 15). $E\sigma^{70}$ transcription on this template initiates from a single promoter and terminates with about 80% efficiency approximately 6 kb downstream from the initiation site. Determining the time it takes the polymerase to reach this terminator in a synchronized transcription assay permits a rough estimation of the elongation rate of RNA polymerase. An example of data generated in this assay, shown in Fig. 2A, indicates that under standard experimental conditions (37°C and 0.4 mM each nucleotide), wild-type RNA polymerase required about 4 min to reach the terminator whereas GD1266 required over 30 min to traverse the 6 kb. In addition to GD1266, four other mutant RNA poly-

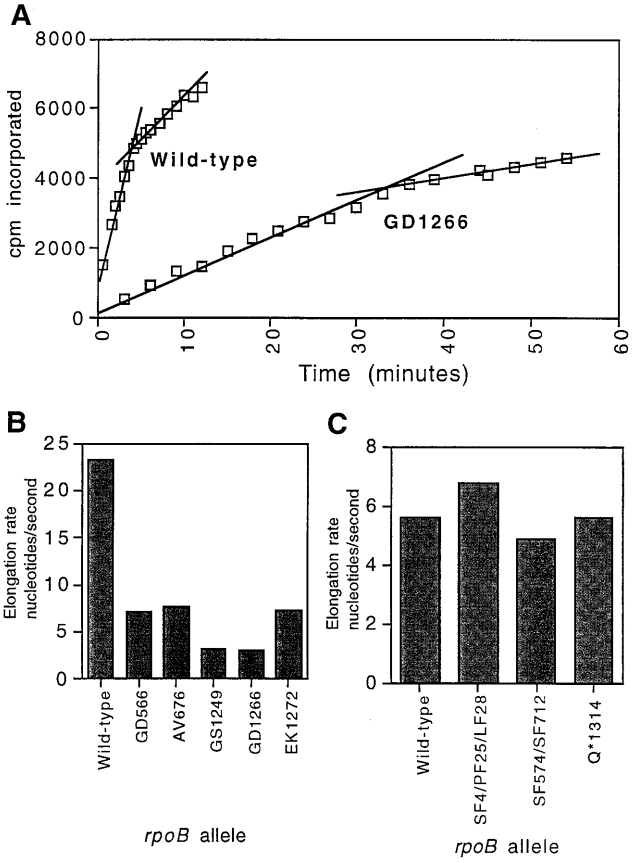


FIG. 2. The elongation rates of mutant RNA polymerases. (A) Example of a typical T7 elongation assay comparing transcription by wild-type polymerase to that of the GD1266 mutant. (B) Elongation rates of the wild type and five mutant RNA polymerases assayed under standard conditions (37°C, 0.4 mM each NTP). (C) Elongation rates of the wild type and three mutant RNA polymerases assayed at 30°C and 0.1 mM each NTP.

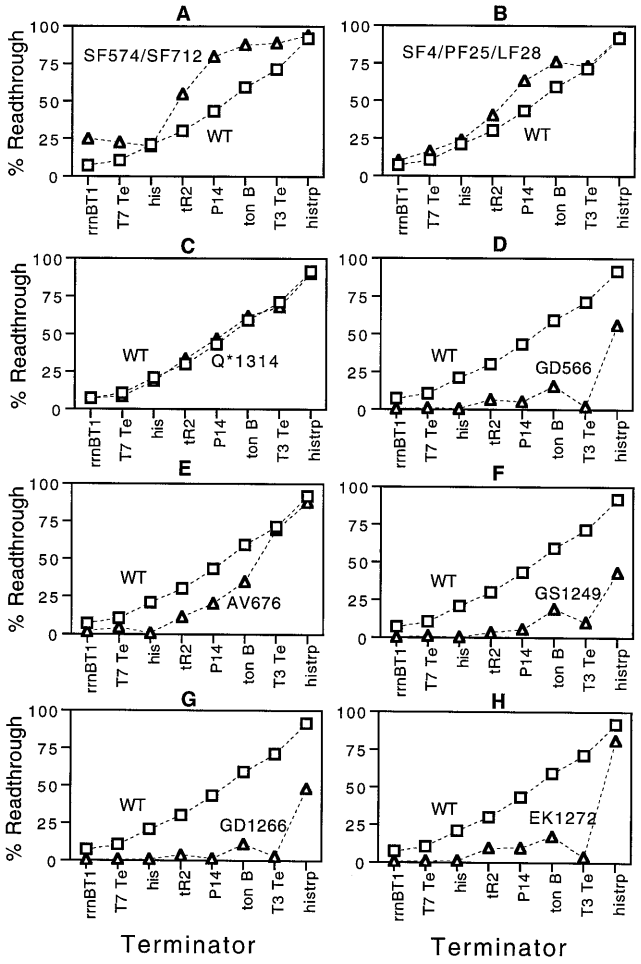


FIG. 3. Termination of eight mutant RNA polymerases relative to the wild type (WT). Each mutant polymerase was assayed on a panel of eight different terminators in a single-round assay. The termination capacity of each RNA polymerase was determined by quantifying the amount of radioactivity in the read-through and terminated RNA species. In each panel, termination of wild-type RNA polymerase is represented by open squares and that of the mutant RNA polymerase is indicated by open triangles. (A) SF574/SF712; (B) SF4/PF25/LF28; (C) Q*1314; (D) GD566; (E) AV676; (F) GS1249; (G) GD1266; (H) EK1272.

merases elongated significantly more slowly than did the wild type (Fig. 2B). One of these mutants, GD566, was also examined at nucleotide concentrations twice those believed to exist in vivo (5.4 mM ATP, 2.2 mM GTP, 2.8 mM UTP, and 1.4 mM CTP). Although GD566 elongated more slowly (50% of the wild-type rate), the magnitude of the defect was reduced about twofold compared with that exhibited at 0.4 mM each NTP (data not shown). The other three RNA polymerases elongated at rates that were indistinguishable from that of the wild type at 0.4 mM each NTP (data not shown). In addition, their elongation rates were indistinguishable from that of the wild type when the sensitivity of the assay was increased by using lower nucleotide concentrations (Fig. 2C).

Termination phenotypes of mutant RNA polymerases. It has previously been found that mutant polymerases respond differently to different terminators (16, 18, 41). We therefore used eight different terminators, including *rrnB*T1, the intrinsic terminator present in pES4, to characterize the termination properties of the purified *rpoB* mutants.

Two of the mutant RNA polymerases displayed increased read-through. SF574/SF712 exhibited a significant increase in read-through at six out of eight terminators (Fig. 3A). Most importantly, it exhibited a fivefold increase in read-through at *rrnB*T1, which may be sufficient to explain the isolation of SF574/SF712 in our screen. A second allele, SF4/PF25/LF28, the first RNA polymerase mutation located in the extreme N terminus of β, exhibited a slight increase in read-through at several terminators; however, it is questionable whether this is sufficient to confer the in vivo phenotype observed (Fig. 3B).

Of the remaining six mutant polymerases, one had termination properties identical to those of the wild type (Fig. 3C). The other five exhibited large increases in termination (Fig. 3D to H). They were the same five polymerases that displayed severe elongation defects (Fig. 2). In many cases, termination was increased 30- to 50-fold and essentially no read-through transcript could be observed.

To test whether the defects of the mutant RNA polymerases were related to interaction with NusA, a protein that often enhances termination, we examined polymerase interaction with NusA in vitro. Extracts containing the mutant polymerases were bound to NusA immobilized on Affigel and eluted with a salt gradient. In general, we saw no reproducible differences in elution between wild-type and mutant RNA polymerases from these columns (37). One possible exception was Q*1314. This polymerase bound well to the column but appeared to elute at a lower salt concentration than did the wild type, suggesting that defective interaction with NusA may contribute to the phenotype of Q*1314 (37).

Q*1314 and SF4/PF25/LF28 are located in regions conserved among prokaryotes but not eukaryotes. To further characterize the phenotypes of Q*1314 and SF4/PF25/LF28, we asked whether they were altered in interaction with NusG and rho. In assays performed by the Platt laboratory, it was shown that SF4/PF25/LF28 and Q*1314 are indistinguishable from the wild type in NusG- and rho-mediated termination at the *trpt'* terminator (26a).

DISCUSSION

We have described 17 new alleles of *rpoB*. These alleles are predominantly located in highly conserved regions of β and, with two exceptions, are not capable of supporting cellular growth. These characteristics suggested that the mutant polymerases were worthy of detailed in vitro analysis. We devised a novel method for purifying mutant RNA polymerases even though coexpression of a fully functional RNA polymerase is necessary for viability. This method allowed purification of most of the mutant RNA polymerases. It failed in only two cases (Q*1264 and Q*1268) in which very little mutant protein was recovered. Analysis of the mutant polymerases in vitro indicated that most exhibit severe defects in RNA polymerase function. We discuss below possible reasons for the isolation of these mutants and the insights into RNA polymerase function that these mutants provide.

Mutant isolation. Our screen was set up to identify RNA polymerase mutants with reduced termination. The terminator probe plasmid pES4 contains two strong terminators interposed between the *rrnA* P1 promoter and the *galK* gene. Alleles of RNA polymerase with reduced termination should exhibit increased expression of galactokinase and consequently red colony color on MacConkey galactose plates. Our initial characterization of the mutants had suggested to us that they were not proficient at reading through the terminators on the pES4 plasmid. Whereas NusA mutant cells known to read through these terminators exhibited red colonies on MacConkey galactose after only 10 h, our mutant cells required 36 h to give a pink-red phenotype. The fact that none of the *rpoB*

mutant strains exhibited a significant increase in galactokinase expression (data not shown) is consistent with their slow production of red color. However, we note that it is difficult to directly measure activity of the mutant enzyme in vivo because measurements must be conducted in merodiploid cells and depend upon relative levels of mutant and wild-type enzyme. The color change on MacConkey galactose plates requires the presence of the pES4 plasmid and galactose as a carbon source, indicating that cellular production of galactokinase contributes to the phenotype. However, enhanced color change in the mutant cells could still be an indirect effect of severely deleterious RNA polymerase mutations that altered stationary-phase physiology. Among the possible alterations that could lead to red color on MacConkey galactose plates are (i) increased permeability due to partial cell lysis or improper assembly of membrane components that created a more acidic environment around the colonies, (ii) a membrane-independent change in cellular pH during late stationary phase that mimicked fermentation of galactose, and (iii) an event resulting in increased plasmid replication, increased *rrnA* P1-promoted initiation, or increased *galK* transcription or translation late in stationary phase in mutant cells.

Termination behavior of mutant RNA polymerases. Only two alleles (SF574/SF712 and HY526) exhibit a significant increase in terminator read-through. HY526 has previously been isolated several times (14, 42) and exhibits the highest read-through of any rifampin resistance mutation on a wide spectrum of terminators (16). Three additional alleles, TI563, SF788, and SF4/PF25/LF28, exhibit slight increases in read-through at some terminators (18) (Fig. 3B). The increased read-through displayed by SF4/PF25/LF28 is particularly noteworthy, for this represents the first demonstration that the extreme amino terminus of β may play a role in termination. We find it interesting that we did not identify more mutants exhibiting increased terminator read-through, either in this screen involving *rrnB*T1 or in those involving other terminators (18). Although it is dangerous to extrapolate from negative results, one possible explanation is that mutations dramatically increasing read-through are deleterious to the cell.

Five mutants, GD566, AV676, GS1249, GD1266, and EK1272, exhibited moderate to severe increases in termination at all eight intrinsic terminators examined. Given the complex nature of the termination process, different steps could be rate limiting at different terminators. Thus, the pattern of response of the mutant polymerases to the panel of terminators is one means of assessing whether the mutants affect similar steps in termination. By this criterion, the defect in AV676 is mechanistically distinct from that in the other four mutant polymerases.

Relationship between elongation and termination. The process of termination is complex and involves interactions between polymerase and (i) the nascent transcript, (ii) DNA regions both upstream and downstream of the terminator region, and (iii) elongation and termination factors (13, 26, 30, 31, 39, 40). The observation that the altered termination properties of the slowly elongating RpoB8 polymerase and the quickly elongating RpoB3595 polymerase at rho-dependent terminators can be eliminated by restoring their elongation rates to normal suggests that there is a kinetic component to termination efficiency at rho-dependent terminators (12). The fact that the nucleotide concentration can influence the pattern and efficiency of termination at intrinsic terminators also suggests that there is a kinetic component to these termination events (22).

The termination phenotypes of the mutants described in this report provide further evidence of a link between elongation rate and termination at intrinsic terminators. Five of the mutants identified in this screen had very slow elongation rates. Four of these polymerases also terminated significantly better than the wild type at all eight terminators examined; the fifth terminated significantly better than the wild type at six of these terminators. Despite the fact that we neither selected nor screened for increased termination, some of the mutants exhibited as much as a 20- to 50-fold reduction in read-through at many terminators, far greater than that exhibited by any existing termination-proficient mutants. Furthermore, we have shown that intragenic second-site suppressors of AV676 simultaneously restore both elongation and termination to the wild-type rates (38). Taken together, our results suggest that the strong correlation we observe between reduced elongation rate and enhanced termination is significant.

How general is the correlation between elongation rate and termination proficiency? One of our groups (R.L.) has examined intrinsic termination for a number of *rpoB* and *rpoC* mutants, most of which have smaller defects in elongation rate than the mutants described above (41). In general, there is a correlation between slower elongation and increased termination, although it is not absolute and is not manifest for all terminators examined. However, some of these mutants are exceptions to this correlation (41). These mutants will be particularly interesting to study further, as their particular defects may override the coupling between elongation and termination.

Why might elongation rate influence termination proficiency at intrinsic terminators? A conformational change in the polymerase may be required for termination. Since termination is limited to relatively few sites, the template positions permitting this conformational change may be limited. If the polymerase elongates slowly, there may be a high probability that it completes the conformational change before leaving the locale where it is possible. Alternatively, there may simply be a kinetic competition between the elongation and termination processes at potential termination sites.

Contributions of the mutants to a structure-function analysis of β. Sequence comparisons indicate that nine small regions of β (called segments A to I) (Fig. 1) are highly conserved among prokaryotic and eukaryotic homologs. Arg-1065 (segment H) and His-1237 (segment I) have been identified as residues capable of cross-linking to the 5′ phosphate on a priming nucleotide, leading to the proposal that these two segments are likely to be involved in some way in the nucleotide addition reaction. Genetic support for the involvement of segment H in the catalytic process comes from the work of Goldfarb and collaborators (32), which shows that mutants with an alanine substitution at or around amino acid 1065 usually have a decreased rate of elongation, exhibit altered patterns of abortive initiation, and may be impeded in the transition to productive elongation. A mutant with an alanine substitution at amino acid 1237 in region I also exhibits an altered pattern of abortive initiation and may be impeded in the transition to elongation, although the effects appear to be less severe (24). Our work provides additional genetic support for the involvement of segment I in this same process. The HY1237, GS1249, GD1266, and EK1272 mutations isolated in this study affect residues in the vicinity of the amino acid 1237 cross-link that are completely conserved among all *rpoB* homologs. We have characterized the transcription phenotypes of the last three and find that they all elongate much more slowly than wild-type polymerase. In fact, their elongation defects are among the most severe that have yet been documented. Our results do not permit a mechanistic interpretation of this defect. However, they are certainly consistent with the idea that some or all of these residues participate in the intrinsic catalytic function of the enzyme.

The N and C termini of β are conserved among prokaryotes but not among their eukaryotic counterparts. We have identified recessive lethal mutations in both the N and C termini, indicating that these regions are essential for polymerase function. We do not yet understand the nature of the defects of these mutant polymerases. RNA polymerase from both the N-terminal mutant SF4/PF25/LF28 and the mutant Q*1314, which has a C-terminal truncation that removes 28 amino acids, showed normal yields in RNA polymerase preparations, implying that the mutated subunits are stable and assemble well. Their content of σ was in the same range as other polymerase preparations, indicating that affinity for σ is not dramatically altered. In addition, they exhibited essentially normal interaction with rho and NusG and normal rates of elongation. Further studies are planned to characterize the contribution of these regions of the polypeptide to RNA polymerase function.

ACKNOWLEDGMENTS

We thank Jack Greenblatt for instructing us in microaffinity chromatography techniques. We also thank Keith Nehrke and Terry Platt for examining the effects of several mutants on NusG- and rho-mediated termination.

REFERENCES

1. **Allison, L., M. Moyle, M. Shales, and C. Ingles.** 1985. Extensive homology among the largest subunits of eukaryotic and prokaryotic RNA polymerases. Cell **42:**599–610.
2. **Amann, E., B. Ochs, and K.-J. Abel.** 1988. Tightly regulated tac promoter vectors useful for the expression of unfused proteins in *Escherichia coli*. Gene **69:**301–315.
3. **Burgess, R., B. Erickson, D. Gentry, M. Gribskov, D. Hager, S. Lesley, M. Strickland, and N. Thompson.** 1987. Bacterial RNA polymerase subunits and genes. Elsevier, New York.
4. **Casadaban, M. J., and S. N. Cohen.** 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. J. Mol. Biol. **138:**179–207.
5. **Chamberlin, M. J., W. E. Nierman, J. Wiggs, and N. Neff.** 1979. A quantitative assay for bacterial RNA polymerases. J. Biol. Chem. **254:**10061–10069.
6. **Darst, S. A., A. M. Edwards, E. W. Kubalek, and R. D. Kornberg.** 1991. Three-dimensional structure of yeast RNA polymerase II at 16Å resolution. Cell **66:**121–128.
7. **Darst, S. A., E. W. Kubalek, and R. D. Kornberg.** 1989. Three-dimensional structure of *Escherichia coli* RNA polymerase holoenzyme determined by electron crystallography. Nature (London) **340:**730–732.
8. **Falkenberg, D., B. Dworniczak, D. M. Faust, and E. K. F. Bautz.** 1987. RNA polymerase II of *Drosophila*. Relationship of its 140,000 Mr subunit to the β subunit of *Escherichia coli* RNA polymerase. J. Mol. Biol. **195:**929–937.
9. **Grachev, M. A., T. I. Kolocheva, E. A. Lukhtanov, and A. A. Mustaev.** 1987. Studies on the functional topography of *Escherichia coli* RNA polymerase. Highly selective affinity labelling by analogues of initiating substrates. Eur. J. Biochem. **163:**113–121.
10. **Grachev, M. A., E. A. Lukhtanov, A. A. Mustaev, E. F. Zaychikov, M. N. Abdukayumov, I. V. Rabinov, V. I. Richter, Y. S. Skoblov, and P. G. Christyakov.** 1989. Studies on the functional topography of *Escherichia coli* RNA polymerase. A method for localization of the sites of affinity labelling. Eur. J. Biochem. **180:**577–585.
11. **Heisler, L. M., H. Suzuki, R. Landick, and C. A. Gross.** 1993. Four contiguous amino acids define the target for streptolydigin resistance in the β subunit of *Escherichia coli* RNA polymerase. J. Biol. Chem. **268:**25369–25375.
12. **Jin, D. J., R. R. Burgess, J. P. Richardson, and C. A. Gross.** 1992. Termination efficiency at rho-dependent terminators depends on kinetic coupling between RNA polymerase and rho. Proc. Natl. Acad. Sci. USA **89:**1453–1457.
13. **Jin, D. J., M. Cashel, D. Friedman, Y. Nakamura, W. Walter, and C. A. Gross.** 1988. The effects of rifampicin resistant *rpoB* mutations on antitermination and interaction with NusA in *Escherichia coli*. J. Mol. Biol. **204:**247–261.
14. **Jin, D. J., and C. A. Gross.** 1988. Mapping and sequencing of mutations in the *Escherichia coli rpoB* gene that lead to rifampicin resistance. J. Mol. Biol. **202:**45–58.
15. **Jin, D. J., and C. A. Gross.** 1991. RpoB8, a rifampicin-resistant termination proficient RNA polymerase, has an increased Km for purine nucleotides during transcription elongation. J. Biol. Chem. **266:**14478–14485.
16. **Jin, D. J., W. Walter, and C. A. Gross.** 1988. Characterization of the termination phenotypes of rifampicin resistant *rpoB* mutants in *Escherichia coli*. J. Mol. Biol. **202:**245–263.
17. **Landick, R., A. Colwell, and J. Stewart.** 1990. Insertional mutagenesis of a plasmid-borne *Escherichia coli rpoB* gene reveals alterations that inhibit β-subunit assembly into RNA polymerase. J. Bacteriol. **172:**2844–2854.
18. **Landick, R., J. Stewart, and D. N. Lee.** 1990. Amino acid changes in conserved regions of the β subunit of *Escherichia coli*. Genes Dev. **4:**1623–1636.
19. **Landick, R., C. Yanofsky, K. Choo, and L. Phung.** 1990. Replacement of the *Escherichia coli trp* operon attenuation control codons alters operon expression. J. Mol. Biol. **216:**25–37.
20. **Lisitsyn, N. A., E. D. Sverdlov, E. P. Moiseyeva, O. N. Danilevskaya, and V. G. Nikiforov.** 1984. Mutation to rifampicin resistance at the beginning of the RNA polymerase β subunit gene in *Escherichia coli*. Mol. Gen. Genet. **196:**173–174.
21. **Mandel, M., and A. Higa.** 1970. Calcium-dependent bacteriophage DNA infection. J. Mol. Biol. **53:**159–162.
22. **McDowell, J., J. Roberts, D. J. Jin, and C. A. Gross.** 1994. Determination of intrinsic transcription termination efficiency by RNA polymerase elongation rate. Science **266:**822–825.
23. **McKenney, K., H. Shimatake, D. Court, U. Schmeissner, C. Brady, and M. Rosenberg.** 1981. A system to study promoter and terminator signals recognized by Escherichia coli RNA polymerase, p. 383–415. *In* J. G. Chirikjian and T. S. Papas (ed.), Gene amplification and analysis II: analysis of nucleic acids by enzymatic methods. Elsevier/North-Holland, New York.
24. **Mustaev, A., M. Kashlev, J. Y. Lee, A. Polyakov, A. Lebedev, K. Zalenskaya, M. Grachev, A. Goldfarb, and V. Nikiforov.** 1991. Mapping of the priming substrate contacts in the active center of *Escherichia coli* RNA polymerase. J. Biol. Chem. **266:**23927–23931.
25. **Mustaev, A., E. Zaychikov, K. Severinov, M. Kashlev, A. Polyakov, V. Nikiforov, and A. Goldfarb.** 1994. Topology of RNA polymerase active center probed by chimeric rifampicin-nucleotide compounds. Proc. Natl. Acad. Sci. USA **91:**12036–12040.
26. **Nehrke, K. W., and T. Platt.** 1993. NusG alters rho-dependent termination of transcription in vitro independent of kinetic coupling. Gene Expr. **3:**119–133.

26a. **Nehrke, K. W., and T. Platt.** Personal communication.

27. **Nilsson, B., L. Abrahmsen, and M. Uhlen.** 1985. Immobilization and purification of enzymes with staphylococcal protein A gene fusion vectors. EMBO J. **4:**1075–1080.
28. **Oeschger, M. P., and G. T. Wiprud.** 1980. High efficiency temperature-sensitive amber suppressor strains of *Escherichia coli* K12: construction and characterization of recombinant strains with suppressor-enhancing mutations. Mol. Gen. Genet. **178:**293–299.
29. **Oosawa, K., J. F. Hess, and M. I. Simon.** 1988. Mutants defective in bacterial chemotaxis show modified protein phosphorylation. Cell **53:**89–96.
30. **Reynolds, R., R. M. Bermuda-Cruz, and M. Chamberlin.** 1992. Parameters affecting transcription termination by *Escherichia coli* RNA polymerase. I. Analysis of thirteen rho independent terminators. J. Mol. Biol. **224:**31–51.
31. **Reynolds, R., and M. Chamberlin.** 1992. Parameters affecting transcription termination by *Escherichia coli* RNA polymerase. II. Construction and analysis of hybrid terminators. J. Mol. Biol. **224:**53–63.
32. **Sagitov, V., V. Nikiforov, and A. Goldfarb.** 1993. Dominant lethal mutations near the 5′ substrate binding site affect RNA polymerase propagation. J. Biol. Chem. **268:**2195–2202.
33. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **Severinov, K., D. Markov, E. Severinova, V. Nikiforov, R. Landick, S. A. Darst, and A. Goldfarb.** 1995. Streptolydigin-resistant mutants in an evolutionarily conserved region of the β′ subunit. J. Biol. Chem. **270:**23926–23929.
35. **Severinov, K., M. Suoshko, A. Goldfarb, and V. Nikiforov.** 1993. Rifampicin region revisited. New rifampicin-resistant and streptolydigin-resistant mutants in the β subunit of *Escherichia coli* RNA polymerase. J. Biol. Chem. **268:**14820–14825.
36. **Sweetser, D., M. Nonet, and R. A. Young.** 1987. Prokaryotic and eukaryotic RNA polymerases have homologous core subunits. Proc. Natl. Acad. Sci. USA **84:**1192–1196.
37. **Tavormina, P.** 1994. Ph.D. thesis. University of Wisconsin, Madison.
38. **Tavormina, P. L., W. S. Reznikoff, and C. A. Gross.** 1995. Identifying interacting regions in the β subunit of *Escherichia coli* RNA polymerase. J. Mol. Biol. **258:**213–223.
39. **Telesnitsky, A. P. W., and M. J. Chamberlin.** 1989. Sequences linked to prokaryotic promoters can affect the efficiency of downstream terminators. J. Mol. Biol. **205:**315–330.
40. **Telesnitsky, A. P. W., and M. J. Chamberlin.** 1989. Terminator-distal sequences determine the in vitro efficiency of the early terminators of bacteriophages T3 and T7. Biochemistry **28:**5210–5218.
41. **Weilbaecher, R., C. Hebron, G. Feng, and R. Landick.** 1994. Termination-altering amino acid substitutions in the β′ subunit of *Escherichia coli* RNA polymerase identify regions involved in RNA chain elongation. Genes Dev. **8:**2913–2927.
42. **Yanofsky, C., and V. Horn.** 1981. Rifampin resistance mutations that alter the efficiency of transcript termination at the tryptophan operon attenuator. J. Bacteriol. **145:**1334–1341.
43. **Zabarovsky, E. R., and G. Winberg.** 1990. Highly efficient electroporation of ligated DNA into bacteria. Nucleic Acids Res. **18:**5912.