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An Amber Mutation in the Gene Encoding the β' Subunit of *Escherichia coli* RNA Polymerase†

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An *Escherichia coli* strain carrying an amber mutation (UAG) in *rpoC*, the gene encoding the beta prime subunit of RNA polymerase, was isolated after mutagenesis with nitrosoguanidine. The mutation was moved into an unmutagenized strain carrying the *supD43,74* allele, which encodes a temperature-sensitive sul amber suppressor, and *sue* alleles, which enhance the efficiency of the suppressor. In this background, beta prime is not synthesized at high temperature. Suppression of the mutation by the non-temperature-sensitive amber suppressor sul^+ yields a protein which is functional at all temperatures examined (30, 37, and 42°C).

The RNA polymerase holoenzyme of *Escherichia coli* consists of one β, one β' one σ, and two α subunits (7, 8, 10). The operon containing the genes for β and β' also contains genes which encode ribosomal proteins L1, L7/L12, L10, and L11. The operon has the following structure: promoter P11, *rplK* (L11), *rplA* (L1), promoter P10, *rplJ* (L10), *rplL* (L7/L12), attenuator, *rpoB* (β), *rpoC* (β'), terminator (4, 6, 14, 29, 37, 43, 46). This operon is located at 89.5 min (3). The operon containing *rpoA*, the gene for α, is located at 72 min and also contains ribosomal protein genes (24, 28). The *rpoD* gene, encoding σ, maps at 66 min (17, 19, 35).

Mutants have proven highly useful for the study of the complex regulation of these genes. To date, the mutations reported include those in *rpoB* conferring drug resistance, temperature-sensitive (Ts) mutations in *rpoA*, *-B*, *-C*, and *-D*, and amber mutations in *rpoB* and *rpoD* (reviewed in 23 and 33; see also 1, 26, 30, 42).

The regulation of *rpoBC* is partially autogenous; consequently, subunits may have both catalytic and regulatory functions or may affect the catalytic or regulatory functioning of another subunit when in the intact enzyme (15, 25, 27; see also reviews [23, 33]). Because amber mutations coupled with compatible Ts amber suppressors make it possible to manipulate the concentration of a protein without simultaneously changing its functional capacity, they can be especially useful for studies involving autogenous regulation. We report here the isolation of an amber mutation in *rpoC* in a strain carrying a Ts amber suppressor and *sue* mutations, which increase the efficiency of the suppressor.

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MATERIALS AND METHODS

Organisms. The bacterial strains used in this work are listed in Table 1. Phage P1 *vir*, originally isolated by Tomizawa, was used for transductions and was the gift of John E. Cronan, Jr. P1 CM*clr*100 was the gift of Lee Rosner; phage BF23 was the gift of Robert Kadner.

Media. Haploid strains were routinely propagated on either of two complex media, G or R broth or agar (13, 38). Medium E (45) supplemented with glucose (2 mg/ml) and thiamine (13 μg/ml) was the base synthetic medium. For routine growth of merodiploid strains this medium was supplemented with tryptophan (0.5 mM) and 14 other amino acids (1.0 mM each; tyrosine, cysteine, phenylalanine, arginine, and methionine were omitted). For labeling with [^{14}C]tyrosine the merodiploid medium described above was supplemented with yeast extract digested with tyrosine decarboxylase (0.3%) (38). When required, the above media were supplemented with adenine to 125 μg/ml, thymine to 50 μg/ml, and amino acids to 1 mM with the exception of tryptophan (0.5 mM) and glutamic acid (30 mM).

The screening of suppressor activity was carried out on medium E agar supplemented with glucose (0.2%), thiamine (13 μg/ml), and Casamino Acids (1%) via the *trp* amber mutation carried by the strains. The ability to utilize sugars was monitored on MacConkey base agar supplemented with the test sugar to 0.5%.

GXG medium (M. P. Oeschger, unpublished data) was used to test haploid strains for the presence of amber mutations in essential genes. This medium consists of MacConkey base agar supplemented with maltose (0.9%), galactose (0.9%), isopropyl-β-D-thiogalactoside (1 mM), *N,N*-dimethyl formamide (3.5%),

TABLE 1. *E. coli* K-12 strains

Designation	Relevant genotype[a]	Derivation or source
342-167	*argC32 ppc-1*	B. Bachmann (CGSC 3594) (16)
AJ53	F110 *rpoB70*[b]/*rpoBc1*[c]	J. Scaife (20, 21)
AJ131	F110 *rpoB70*[b]/*rpoB38*[d]	J. Scaife (20, 21)
KL16-99	*recA1* Hfr PO45 of KL16	K. B. Low (31)
NK5526	*hisG*::Tn*10*	N. Kleckner (CGSC 6170)
PA200	*argH1*	B. Bachmann (CGSC 1179) (2)
PA505-1-5	*argH1 metA90*	B. Bachmann (CGSC 4869) (5)
PCO132	*purH47*	B. Bachmann (CGSC 4496)
MX386	—[e] *supD43 metB1* (P1 lysogen)	41
MX397	—[e] *supD43,74 sueA metB1*	40
MX399	—[e] *supD43,74 sueB metB1*	40
MX414	*thyA* —[e] *supD43,74 sueA metB1*	From MX397, selected spontaneous trimethoprim-resistant derivative, screened Thy$^-$
MX448	*argH1* —[e] *supD43,74 sueA*	P1 · PA200 × MX397, selected Met$^+$, screened Arg$^-$
MX449	*argH1* —[e] *supD43,74 sueB*	P1 · PA200 × MX399, selected Met$^+$, screened Arg$^-$
MX515	*rpoBc1*[c] —[e] *supD43,74 sueB*	P1 · AJ53 × MX449, selected Arg$^+$, screened Ts
MX550	*rpoB38*[d] —[e] *supD43,74 sueA*	P1 · AJ131 × MX448, selected Arg$^+$, screened Ts
MX554	—[e] *supD43 metB1*	MX386 cured of P1 (see text)
MX589	Ts$^+$ —[e] *rpoB*[b] *rpoC*[f] *supD43, 74 sueB metB1*	From SR176B12, spontaneous Ts$^+$ derivative
MX595	—[e] *supD43,74 sueB*	P1 · MX515 × MX399, selected Met$^+$, screened Ts$^+$
MX600	*his* —[e] *supD43,74 sueA metB1*	From MX397 by nitrosoguanidine mutagenesis and penicillin enrichment, screened His$^-$ (40)
MX755	*hisG*::Tn*10* —[e] *supD43,74 sueB*	P1 · NK5526 × MX595, selected Tet^r, screened His$^-$
MX762	*supD43* —[e] *sueB*	P1 · MX554 × MX755, selected His$^+$, screened Tet^s Trp$^+$
MX778	*hisG*::Tn*10* —[e] *rpoC325 supD43,74 sueB btuB*[g]	P1 · NK5526 × MX782, selected Tet^r, screened His$^-$
MX782	*rpoC325 btuB*[g] —[e] *supD43,74 sueB*	P1 · SR249 × MX399, selected Met$^+$, screened Ts
MX784	*supD43* —[e] *rpoC325 sueB btuB*[g]	P1 · MX554 × MX778, selected His$^+$, screened Ts$^+$
SR176B12	Ts *rpoB*[b] *rpoC*[f] —[e] *supD43,74 sueB metB1*	From MX399, nitrosoguanidine-induced mutant, selected Rif^r, screened Ts and β′, polypeptide electrophoretic mobility
SR201	*ppc-1 argC32* —[e] *supD43*	P1 · 342-167 × MX554, selected Met$^+$, screened Glu$^-$ Arg$^-$
SR202	*metA90 argH1* —[e] *supD43*	P1 · PA505-1-5 × SR201, selected Glu$^+$, screened Met$^-$ Arg$^-$
SR208	*purH47* —[e] *supD43 argH1*	P1 · PCO132 × SR202, selected Met$^+$, screened Ade$^-$
SR214	*ppc-1* —[e] *supD43,74 sueA thyA*	P1 · 342-167 × MX414, selected Met$^+$, screened Glu$^-$
SR215	*argH1 metA90* —[e] *supD43,74 sueA thyA*	P1 · SR202 × SR214, selected Glu$^+$, screened Arg$^-$ Met$^-$
SR216	*rpoB38*,[d] r22[b] *btuB*[g] —[e] *supD43,74 sueA argH1 metA90 thyA*	P1 · SR227 × SR215, selected Rif^r, screened Ts and BF23^r
SR217	*rpoBc1*,[c] r24[b] *btuB*[g] —[e] *supD43,74 sueA metA90 thyA*	P1 · SR225 × SR215, selected Arg$^+$, screened Ts, BF23^r, and Rif^r
SR218	*recA1* —[e] *rpoBc1*,[c] r24[b] *supD43,74 sueA metA90 btuB*[g]	KL16-99 × SR217, selected Thy$^+$, screened UV^s
SR220	*recA1* —[e] *rpoB38*,[d] r22[b] *supD43,74 sueA argH1 metA90 btuB*[g]	KL16-99 × SR216, selected Thy$^+$, screened UV^s
SR224	*rpoBc1*,[c] r24[b] —[e] *supD43,74 sueB*	From MX515, spontaneous Rif^r derivative
SR225	*btuB*[g] —[e] *rpoBc1*[c] sr24[b] *supD43,74 sueB*	From SR224, spontaneous BF23^r derivative
SR226	*rpoB38*,[d] r22[b] —[e] *supD43,74 sueA*	From MX550, spontaneous Rif^r derivative
SR227	*btuB*[g] —[e] *rpoB38*,[d] r22[b] *supD43,74 sueA*	From SR226, spontaneous BF23^r derivative
SR228	*recA1* —[e] *supD43,74 sueA argH1 metA90*	KL16-99 × SR215, selected Thy$^+$, screened UV^s

TABLE 1—*Continued*

Designation	Relevant genotype[a]	Derivation or source
SR229	*amp* —[e] *rpoBc1,*[c] r24[b] *supD43,74 sueA metA90 recA1 btuB*[g]	From SR218, spontaneous Amp[r] derivative
SR230	*amp* —[e] *supD43,74 sueA argH1 metA90 recA1*	From SR228, spontaneous Amp[r] derivative
SR231	*amp* —[e] *rpoB38,*[d] r22[b] *supD43,74 sueA argH1 metA90 btuB*[c]	From SR220, spontaneous Amp[r] derivative
SR233	*argH1* —[e] *supD43,74 sueA his*	P1 · SR202 × MX600, selected Met$^+$, screened Arg$^-$
SR237	*btuB*[g] —[e] *supD43,74 sueA argH1 metA90 thyA*	From SR215, spontaneous BF23[r] derivative
SR242	*rpoB*[b] —[e] *supD43,74 sueA argH1 metA90 thyA btuB*[g]	From SR237, spontaneous Rif[r] derivative
SR243	*thyA* —[e] *rpoB*[b] *rpoC*[f] *supD43,74 sueB metB1*	From MX589, selected spontaneous trimethoprim-resistant derivative, screened Thy$^-$
SR246	*recA1* —[e] *rpoB*[b] *rpoC*[f] *supD43,74 sueB metB1*	KL16-99 × SR243, selected Thy$^+$, screened UV[s]
SR249	*rpoC325* —[e] *supD43 btuB*[g]	P1 · SR258 × SR208, selected Arg$^+$ Ade$^+$
SR258	*rpoC325* —[e] *supD43,74 sueA thyA btuB*[g]	P1 · Fsr1313/SR230 × SR242, selected Arg$^+$, screened Ts Rif[s]
F primes		
F110		K. B. Low (in strain CGSC 4261)
Fsr430	*rpoBr43*[b] *rpoC325*	From F110/SR220, nitrosoguanidine-induced mutant, selected Rif[r], screened Ts
Fsr1313	*rpoB*$^+$ *rpoC325 btuB*[g]	From Fsr430/SR237, a spontaneous recombinant between the F′ and the chromosome, selected BF23[r], screened in SR231 as Ts[p] and Rif[s]
Fsr1228	*rpoB*$^+$ *rpoC*$^+$ *btuB*[g]	From Fsr430/SR237, a spontaneous recombinant between the F′ and the chromosome, selected BF23[r], screened in SR231 as Ts$^+$ Rif[s]

[a] Gene symbols according to Bachmann and Low (3). For previously described strains only relevant genes are listed; for strains prepared as part of this work the complete genotype is given.
[b] Conveys resistance to rifampin.
[c] Nonpolar amber mutation.
[d] Polar amber mutation.
[e] *leu*(Am) *trp*(Am) *lacZ2210*(Am) *galK*(Am) *galE sueC rpsL* (conveys streptomycin resistance) *tsx*.
[f] Alters the electrophoretic mobility of the β′ subunit.
[g] Conveys resistance to phage BF23.

and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (3.6 μg/ml).

Antibiotics for genetic selections were used as follows: streptomycin at 50 and 100 μg/ml, rifampin at 40 μg/ml, and ampicillin at 10 or 20 μg/ml. *thyA* strains were selected by using medium E appropriately supplemented with trimethoprim and thymine, as described in Miller (34).

Chemicals. Yeast extract, Casamino Acids, tryptone, and MacConkey agar were obtained from Difco Laboratories. Sugars and amino acids were of the highest grade available from Sigma Chemical Co.; isopropyl-β-D-thiogalactoside was also from Sigma. Agar was obtained from BBL Microbiology Systems. Tyrosine decarboxylase-treated yeast extract was prepared as described in Oeschger (38). 5-Bromo-4-chloro-3-indolyl-β-D-galactoside was obtained from Sigma and Bachem.

Streptomycin sulfate and rifampin were obtained from Sigma. Rimactane (rifampin) was a gift of Hans Heymann of Ciba Geigy Pharmaceutical Co., and trimethoprim was a gift of George Hitchings of Burroughs-Wellcome and Co. Ampicillin (Polycillin-N) was obtained from Bristol Laboratories. *N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine (nitrosoguanidine) was obtained from Aldrich Chemical Co. BDH sodium lauryl sulfate was used for polyacrylamide gel electrophoresis.

L-[*U*-^{14}C]tyrosine with a specific activity of approximately 500 mCi/mmol and [^{35}S]methionine with a specific activity of 1,280 Ci/mmol were obtained from Amersham Corp. RNA polymerase purified by the method of Burgess and Jendrisak (9) was the gracious gift of James Sylvester.

All other chemicals were reagent grade.

Isolation of *rpoC* mutants. Mutagenesis by nitrosoguanidine and the collection of Rif[r] Ts derivatives of MX399 were conducted as described by Oeschger and Berlyn (39). For mutagenesis of F110/SR220, a final concentration of nitrosoguanidine of 0.01 mg/ml rather than 0.1 mg/ml was used. We find that this level of nitrosoguanidine reduces the killing and the number of

extraneous mutations but not the yield of clustered mutations.

The mutagenized cells were collected by centrifugation and suspended in medium E to their original volume, and 0.05-ml portions of 10^0 and 10^{-1} dilutions were spread onto merodiploid medium agar plates. After the plates had incubated for 18 h at 28°C, 1.0 ml of a 16% dimethyl sulfoxide solution containing rifampin (1.6 mg/ml) was injected under the agar. The plates were reincubated for several days. Colonies appeared at a frequency of 10 to 100 per plate.

Colonies were replica plated and the replicas were incubated at 42 and 27°C. After the 42°C plates had developed, they were replica plated and the second replicas were again incubated at 42°C. Colonies were scored for inability to maintain growth at 42°C.

F′ plasmids from the Ts derivatives were tested for the ability to complement *rpoBc1*, a nonpolar amber mutant allele, and *rpoB38*, a polar amber mutant allele, by being mated into SR229 (*rpoBc1*), SR230 (*rpoB*$^+$), and SR231 (*rpoB38*) (Table 2). Exconjugates carrying the F′ plasmids were scored for temperature sensitivity by replica plating.

Radiochemical assays. Mutants were screened for their ability to synthesize β′ subunit at 42°C with a radiochemical assay. Cells growing in liquid culture were pulsed with radiochemically labeled amino acids, and the cells were harvested. Extracts were prepared and fractionated on polyacrylamide gels as described by Maizel (32). The rate of polymerase subunit synthesis was judged by examining the intensity of labeling of the bands on autoradiograms of the dried gels.

Genetic and microbiological procedures. P1 phage transductions were carried out as described by Cronan et al. (12).

Sensitivity to BF23 phage was tested by patching the bacterial strain onto a G plate and then placing 1 drop (roughly 10^8 PFU) of a 1:1 mixture of the phage lysate and 30 mM $MgCl_2$–15 mM $CaCl_2$ onto the patch (J. L. Toffenetti, personal communication). Plates were incubated at between 30 and 34°C and scored for lysis. For testing merodiploid strains, appropriately supplemented medium E was used rather than G medium, and $CaCl_2$ was omitted from the phage mixture.

When BF23 was used as a selective agent, phage and bacteria were incubated in 10 mM $MgCl_2$ for 1 h at 34°C, soft agar was added, and the mixture was plated.

thyA mutants were selected with trimethoprim as described in Miller (34).

recA strains were prepared by mating a *thyA* derivative of the *recA*$^+$ strain with KL16-99 (*recA thyA*$^+$ Hfr) and screening the Thy$^+$ recombinants for *recA* by testing their UV sensitivity (31, 34).

P1 lysogens were cured of the phage by use of P1 CM*clr*100, according to the method of Rosner (44).

Cell densities were monitored at 600 nm with a Coleman Jr. II spectrophotometer, and cell numbers were calculated from a standard curve determined in this laboratory and relating absorbance to colony-forming units.

RESULTS

Isolation of the mutant. No method for the positive selection of strains carrying mutations specifically and exclusively in the *rpoC* gene has yet been devised, but it is possible to increase efficiency of recovery of *rpoC* mutants from a mutagenesis. Two approaches designed to increase this efficiency were used. The first involved mutagenesis of a haploid strain, and the second involved mutagenesis of a merodiploid strain.

Mutagenesis by nitrosoguanidine followed by a selection for rifampin-resistant mutants was the initial step in both approaches. Because nitrosoguanidine produces clusters of mutations at the replication forks of the chromosome (11, 22), cells mutated in a particular gene are likely to carry mutations in genes located within a minute of the chromosome on either side of the affected gene (18, 39). Since *rpoB* is directly adjacent to *rpoC* and the majority of all mapped mutations conferring rifampin resistance (Rif^r) lie in the *rpoB* gene, selection of strains carrying Rif^r mutations induced by nitrosoguanidine yields a group of mutants with an increased probability of carrying *rpoC* mutations. With this in mind, we mutagenized a Rif^s strain with nitrosoguanidine and selected Rif^r derivatives.

Initially, we tried to isolate *rpoC* amber mutants from cultures of MX399 mutagenized with nitrosoguanidine. MX399 is a Rif^s haploid strain containing the *supD43,74* gene, which encodes a Ts amber suppressor sul^{Ts} (41), and the mutations *sueB* and *sueC*, which greatly enhance the efficiency of the suppressor (40). In MX399 an amber mutation in *rpoC* would be suppressed at low temperature but not at high temperature, thus rendering the strain temperature sensitive. Consequently, the Rif^r mutants of MX399 were screened for Ts growth by replica plating at 27 and 42°C. To identify those containing amber mutations in *rpoC*, we pulse-labeled the Ts isolates with radioactive tyrosine after they had been shifted from permissive temperature to 42°C; cell extracts were fractionated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Each isolate was checked for synthesis of the β′ subunit at high temperature by examination of autoradiograms of the gels. Those which were synthesizing β′ at the nonpermissive temperature would contain radioactive

TABLE 2. Strains used for screening of F′ plasmids for *rpoC* mutations[a]

Strain	Genes requiring complementation at 42°C and duplicated on F110	Growth at 42°C when F′ carries an *rpoC* mutation
SR230	*argH* and *metA*	+
SR229	*metA* and *rpoB*	+
SR231	*argH*, *metA*, *rpoB*, and *rpoC*	−

[a] Exconjugants containing the F′ to be tested were selected by their Arg$^+$ Met$^+$ Amp^r phenotypes.

β′, whereas any mutant unable to do so would not. Over 300 Rif^r Ts mutants (of which at least 200 were of independent origin) were isolated and screened in this manner. None exhibited the Ts synthesis of the β′ subunit indicative of an amber mutation in the *rpoC* gene, although comutagenesis of the *rpoB* and *rpoC* genes was demonstrated by the recovery of three mutants with altered mobilities of the β′ subunit on SDS-polyacrylamide gels as well as of mutants with altered β subunit mobilities.

A second isolation scheme which increased the specificity of the screening before the pulse-labeling was then developed. A set of strains designed to allow simple genetic detection of *rpoC* Ts and amber mutations was constructed. A merodiploid strain, F110/SR220, replaced MX399 as the parental strain. The chromosome of the strain carries the *supD*(Ts) mutation, the *sueA* and *sueC* suppressor-enhancing mutations, a spontaneous recessive Rif^r mutation in *rpoB*, and the polar amber mutation in *rpoB* reported by Hayward et al. (20). A polar amber mutation in *rpoB* was used to prevent expression of both the *rpoB* and *rpoC* chromosomal genes at 42°C, the nonpermissive temperature for the amber suppressor. Since the F′ carries wild-type copies of *rpoB* and *rpoC*, its presence enables the strain to grow well at the nonpermissive temperature. The chromosomal Rif^r allele is recessive to the Rif^s allele carried by the F′, and thus selection of Rif^r mutants after nitrosoguanidine mutagenesis predominately yields strains carrying mutations on the F′. At 42°C functional copies of all of the genes on the F′ are provided by the chromosome with the exceptions of *rpoB*, *rpoC*, *metA*, and *argH* (map of region shown in Fig. 1). As a result, mutations on the F′ causing Ts growth should either lie in one of these four genes or be dominant. These possibilities were distinguished by mating the mutant F′ plasmids into three related screening strains (see Table 2). The first strain, SR230, carries wild-type *rpoB* and *rpoC* genes; the second, SR229, carries a nonpolar amber mutation in *rpoB*; and the third, SR231, carries the polar amber mutation in *rpoB*. The presence of the *argH* and *metA* mutations made it possible to use a nutritional selection in the matings. The triplet sets of exconjugants were scored for temperature sensitivity of growth (see Table 2).

F′ plasmids with mutations in *rpoC* (rather than with dominant mutations or mutations in *rpoB*) were identified by their ability to complement the nonpolar but not the polar amber mutation in *rpoB*. The F′ plasmids from 17 of 170 Ts Rif^r derivatives of F110/SR220 showed such a complementation pattern; they were then mated into strain SR231, which contains the polar *rpoB* amber mutation. The resulting merodiploids

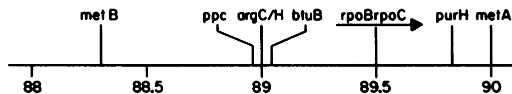

FIG. 1. Relative positions of the markers used in the region around the *rpoB,C* operon. The genetic map is redrawn from Bachmann and Low (3). F110 duplicates this entire region. The *rpoB* and *rpoC* genes are part of an operon which is transcribed in the order *rplK rplA rplJ rplL rpoB rpoC*.

were pulse-labeled with [^{14}C]tyrosine at 42°C, and the cell extracts were fractionated on SDS-polyacrylamide gels. One of these 17 merodiploids, Fsr430/SR231, showed a marked reduction in synthesis of the β′ subunit at 42°C (Fig. 2).

Characterization of the mutation. To facilitate further characterization of the mutation in Fsr430, we attempted to transfer it by phage P1 transduction from the F′ into the chromosome of a haploid Ts suppressor strain. Phage P1 propagated on Fsr430/SR230 was used to infect a Rif^s *argH supD43,74* strain, SR233. Arg^+ transductants were screened for rifampin resistance and temperature sensitivity. Because of the proximity of the *rpoB* and *rpoC* genes, rifampin resistance and temperature sensitivity were expected to cotransduce at a very high frequency. Nevertheless, no Rif^r Ts transductants were found and the *argH rpoB* cotransduction frequency was 10% (14 Rif^r of 142 Arg^+), much lower than the 73% (193 Rif^s of 263 Arg^+) cotransduction rate found with a pair of related strains (P1 · F110/SR230 × SR242). These results suggested that the mutation affecting *rpoC* or a closely linked secondary mutation might be lethal in the haploid state.

Possible secondary mutations lying near *rpoC* were then removed by two steps. First, a region of the F′ including at least the *btuB* (89.1 min) and *rpoB* (89.5 min) genes (and presumably the region between them) was replaced with unmutagenized DNA (see Fig. 1). The replacement was effected by recombination between the F′ and the chromosome in a merodiploid. Challenge by phage BF23 was used for the selection of recombinant F′ strains. The *btuB* gene encodes the receptor for BF23, and mutations which alter that receptor can confer resistance to the phage. When the mutation is recessive, *btuB/btuB* merodiploids can be selected from a mixed population of *btuB*$^+$/*btuB* and *btuB/btuB* strains by challenge with the phage. Fsr430 carries a wild-type *btuB* allele (*btuB*$^+$). It was mated into SR237, which is *btuB* (BF23 resistant) and *rpoB*$^+$ (Rif^s). After the exconjugants were grown for 18 h to allow for recombination, the F′ plasmids were mated into SR231, a Rif^r BF23-resistant *recA* strain with the Ts suppres-

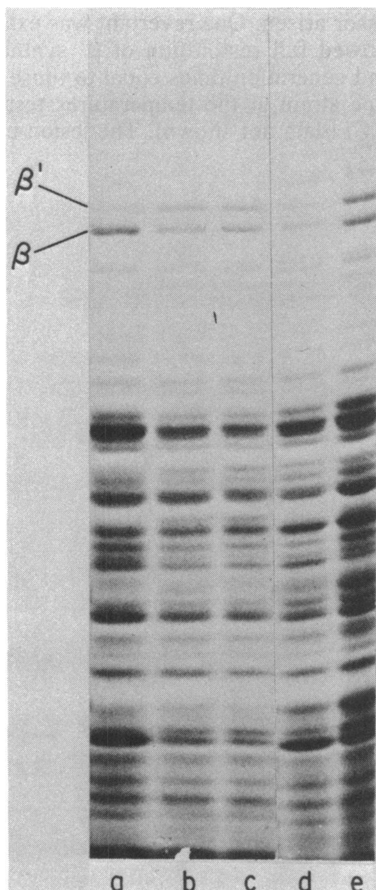

FIG. 2. Beta prime synthesis at 42°C in Fsr430/SR231. The autoradiogram shows pulse-labeled proteins from cell extracts fractionated on a 6% SDS-polyacrylamide gel. Cells were grown in medium E supplemented with glucose (0.4%) and adenine, cytosine, uracil, pantothenic acid, thiamine, and 17 amino acids (arginine, methionine, and tyrosine omitted) at the levels recommended by Neidhardt et al. (36) at 30°C. The cultures were shifted to 42°C when the cell densities reached 4×10^8 colony-forming units/ml. Upon shift to 42°C, strain Fsr430/SR231 was supplemented to 0.1% with tyrosine decarboxylase-digested yeast extract. Forty-five minutes later [^{14}C]tyrosine was added to 1.6 μCi/ml to label newly synthesized protein. After 4 min a 100-fold excess of cold tyrosine was added, and the incubation was continued for 4 additional min. The cells were then rapidly chilled to 4°C and harvested by centrifugation. Sample preparation and polyacrylamide gel electrophoresis were as described in the text. Two strains containing independent Ts F110 derivatives (Fsr414 and Fsr146) are shown for comparative purposes. F110/SR220 is the parental strain which was mutagenized. SR231 is a spontaneous ampicillin-resistant derivative of SR220. a, Fsr430/SR231 (mutant F'/*rpoB38*); b, Fsr414/SR231 (mutant F'/*rpoB38*); c, Fsr146/SR231 (mutant F'/*rpoB38*); d, SR231 (*rpoB38*); e, F110/SR220 (wild-type F'/*rpoB38*).

sor and the polar amber mutation in *rpoB*. The exconjugants were then challenged with BF23 phage. Isolates containing recombinant F' plasmids in which the $btuB^+$ allele had been replaced by the *btuB* allele were resistant to the phage.

After the phage challenge, the surviving SR231 merodiploids were screened for rifampin and temperature sensitivity. An SR231 merodiploid carrying an F' in which the episomal *rpoB* gene had been replaced should be Rif^s. If the episomal *rpoC* gene had not been replaced, the resulting SR231 merodiploid was expected to be temperature sensitive since neither the chromosomal nor the episomal *rpoC* gene was believed to direct sufficient synthesis of the β' subunit at high temperature for viability. The 634 merodiploids examined fell into three classes: Rif^r and Ts; Rif^s and not Ts (Ts^+); Rif^s and partially Ts (Ts^p). No Rif^s Ts strains were found. The Ts^p strains formed colonies at 42°C, but the colonies were noticeably smaller than those formed by the Ts^+ strains. The Ts^p strains represented 9% (28 of 329) of the Rif^s merodiploids examined. Representatives of each of the three classes were pulse-labeled after temperature shift to 42°C. Although the Rif^r Ts strains synthesized β' at the lowest rate, the Rif^s Ts^p strains synthesized β' more slowly than did the Rif^s Ts^+ strains (Fig. 3). This result confirmed that a lesion affecting *rpoC* was present in the Rif^s Ts^p merodiploids.

The attempt to move the lesion into a haploid background was then repeated. DNA from the recombinant episome Fsr1313 (which, when in an SR231 merodiploid, confers the Rif^s Ts^p phenotype) was transduced by P1 into SR242, a Rif^r strain carrying the Ts amber suppressor. The growth of SR242 is not temperature sensitive. Arg^+ transductants were selected and scored for temperature sensitivity. The transductants fell into only two classes: tightly Ts and Ts^+. Ten percent (31 of 307 of the Arg^+ transductants) were temperature sensitive. Figure 4 shows that three randomly selected Arg^+ Rif^s Ts transductants are unable to synthesize the β' subunit at 42°C. All three strains produce the β' subunit at 28°C (data not shown). Companion transductions with F' strains F110, Fsr1313, and Fsr430 as donors and bacterial strains SR237 and SR242 as recipients, with selection of either Met^+ or Arg^+ recombinants, were also carried out. The data from these transductions place the lesion affecting β' between *rpoB* and *metA* and thus probably in *rpoC* itself. The data also indicate that the combination of the *rpoB* and *rpoC* mutant alleles of Fsr430 (or perhaps a single mutation distal to the Rif^r mutation in *rpoB* and proximal to the mutation already described affecting *rpoC*) is lethal in a haploid

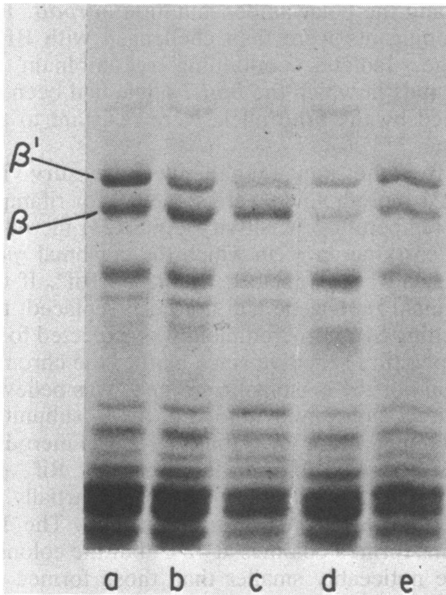

FIG. 3. Beta prime synthesis in merodiploid strains containing recombinant F' plasmids. Strains were grown at 30°C in the merodiploid medium (0.4% glucose) described in the text. The medium was supplemented with arginine for all strains; for SR231, which is a methionine auxotroph, the medium was also supplemented with methionine to 0.06 mM. Cultures were aerated by shaking at 400 rpm. After at least one generation at 30°C, the cultures were shifted to 42°C. After roughly a 20 to 50% increase in optical density, [^{35}S]methionine was added to 100 μCi per ml of culture. After 4 min a 1,000-fold excess of unlabeled methionine was added, and the incubation was continued for an additional 4 min. Cells were harvested and extracts were prepared and fractionated as described in the legend to Fig. 2. Strains described as Ts cannot form single colonies at 42°C, and those described as Ts$^+$ can form single colonies which are as large as those formed by the wild-type control F110/SR231. Strains described as Ts^p (partially Ts) can form single colonies at 42°C, but these single colonies are smaller than those formed by F110/SR231. a, Fsr1484/SR231 (Rif^s Ts$^+$); b, Fsr1313/SR231 (Rif^s Ts^p); c, Fsr430/SR231 (Rif^r Ts); d, SR231 (Rif^r Ts); e, F110/SR231 (Rif^s Ts$^+$).

strain and that there is a haploid lethal mutation in F' plasmids Fsr430 and Fsr1313 located between *rpoC* and *metA* (data not shown). Both of these problems are eliminated in the Rif^s Ts transductants derived from Fsr1313.

Spontaneous Ts$^+$ revertants of the Rif^s Ts transductant SR258 were isolated at 10^{-8} (10 Ts$^+$/3 × 10^8 cells), a frequency typical of single mutations. Since it carries a *trp* amber mutation, SR258 requires tryptophan at 42°C. That the revertants also still required tryptophan at 42°C suggests strongly that neither had the sul^{Ts} suppressor reverted to sul$^+$ nor had a new suppressor arisen. One revertant was examined and showed full restoration of β' synthesis at 42°C and generation times equal to those of the wild-type strain at the temperatures tested (22 and 42°C) (data not shown). The lesion causing

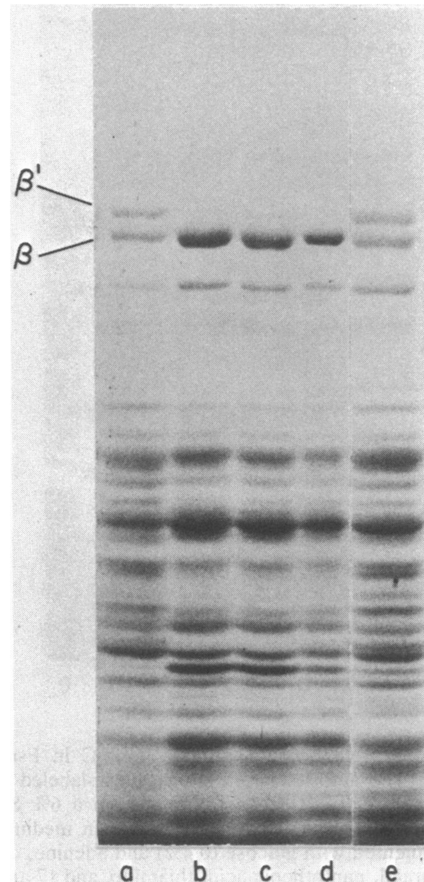

FIG. 4. Beta prime synthesis at 42°C in Ts transductants. The strains were grown at 30°C in the labeling medium described in the text supplemented with thymine and to 0.4% glucose. After two generations the cultures were shifted to 42°C, and 30 min later [^{14}C]tyrosine was added to 1.4 μCi/ml. After 4 min a 100-fold excess of unlabeled tyrosine was added, and the incubation was continued for an additional 2 min. Cells were harvested and extracts were prepared as in the legend to Fig. 2. An autoradiogram of the extracts fractionated on a 6% SDS-polyacrylamide gel is shown. SR242 (lane a) is the recipient strain used in the transductions. It is rifampin resistant but otherwise carries normal alleles of *rpoB* and *rpoC*. Fsr1313 served as the donor DNA. It is derived from Fsr430 and carries a wild-type *rpoB* gene (Rif^s) and *rpoC325*. The transductants shown in lanes b, c, and d are all Arg$^+$ Rif^s Ts. The companion transductant shown in lane f is also Arg$^+$ Rif^s but not Ts. a, SR242 (Arg$^-$ Rif^r Ts$^+$); b, c, and d, independent Arg$^+$ Rif^s Ts transductants from P1 · Fsr1313/SR230 × SR242; e, Arg$^+$ Rif^s Ts$^+$ transductant from P1 · Fsr1313/SR230 × SR242.

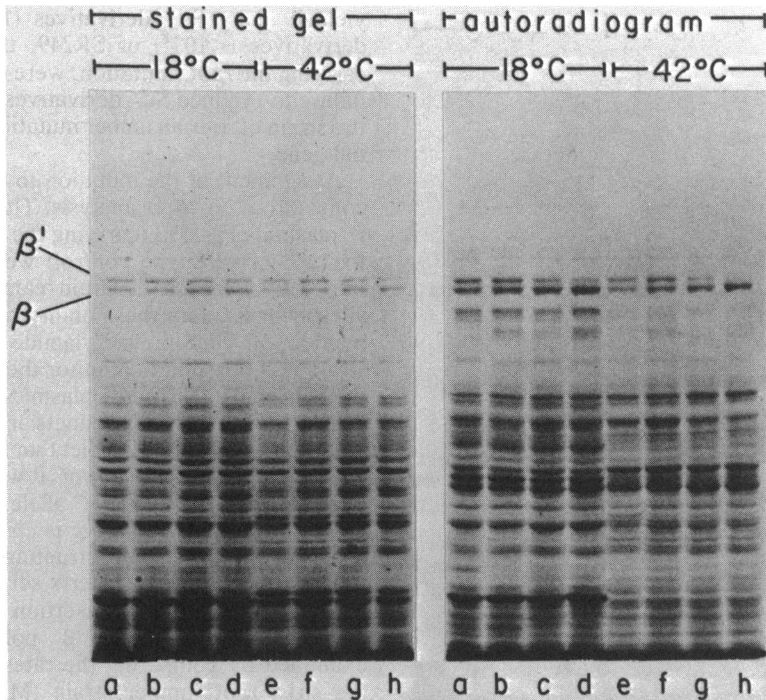

FIG. 5. Beta prime synthesis and steady-state level in Su$^+$ and Su(Ts) backgrounds. Cells were grown at 18°C in the labeling medium described in the text. Portions of the cultures were transferred to 42°C. Both sets of cultures were pulse-labeled with [^{14}C]tyrosine (1.6 μCi/ml) and chased with a 100-fold excess of cold tyrosine. The 18°C cultures were labeled for 14 min and chased for 8 min. The 42°C cultures were pulsed 35 min after transfer from 18°C for 2 min and chased for 2 min. Cells were harvested and extracts were prepared and fractionated as described in the legend to Fig. 2. The relative levels of β' subunit at the time of labeling can be estimated from the photograph of the stained gel (left-hand panel). The relative rate of β' subunit synthesis can be estimated from the autoradiogram of the same gel (right-hand panel). a and e, MX762 (sul$^+$ *rpo*$^+$); b and f, MX595 (sul^{Ts} *rpo*$^+$); c and g, MX784 (sul$^+$ *rpoC325*); d and h, MX782 (sul^{Ts} *rpoC325*).

the temperature sensitivity of SR258 is thus a single mutation.

The determination that the mutation is an amber mutation was a two-step process. The synthesis of a protein encoded by a gene carrying an amber mutation should not be Ts if the amber suppressor is not Ts. Consequently, the first step was to transduce the *rpoC* region from the sul^{Ts} strain into an sul$^+$ strain. SR208, an sul$^+$ strain, was infected with a P1 lysate of SR258, one of the Ts transductants described above. SR208 was chosen because it contains mutations in both the *argH* and *purH* genes, which bracket *rpoC*; thus, an Arg$^+$ Pur$^+$ selection could be used in the transduction. The resulting transductant, SR249, and its derivative strain, MX784, grow well and synthesize the β' subunit at 42°C, although the rate of β' synthesis relative to that of a congenic *rpoC*$^+$ strain, MX762, is reduced (Fig. 5).

The presence of the *rpoC* mutation in SR249 then had to be confirmed. This was done by transferring the mutation back into two Su^{Ts} strains, MX399 and SR237, by P1 phage transduction. Temperature sensitivity cotransduced with both *argH* (for SR237) and *metB* (for MX399). Figure 5 illustrates that the synthesis of the β' subunit is temperature sensitive in a representative transductant, MX782.

Further evidence that the mutation is an amber mutation came from a selection designed to yield Su$^-$ derivatives. SR249, which was used for this test, carries an amber mutation in the *galK* gene and a missense mutation in the *galE* gene. When the *galK* gene product is made, i.e., when the amber mutation is suppressed, the *galE* mutation renders the strains sensitive to galactose. Galactose is not lethal to the strains when the *galK* gene product is not made, i.e., when the amber mutation is not suppressed. Thus, when *galK*(Am) *galE* Su$^+$ strains are plated on medium containing galactose, the majority of the cells are killed, whereas spontaneous Su$^-$ mutants (along with other mutants blocked early in galactose metabolism or revertants of the *galE* mutation) survive and form

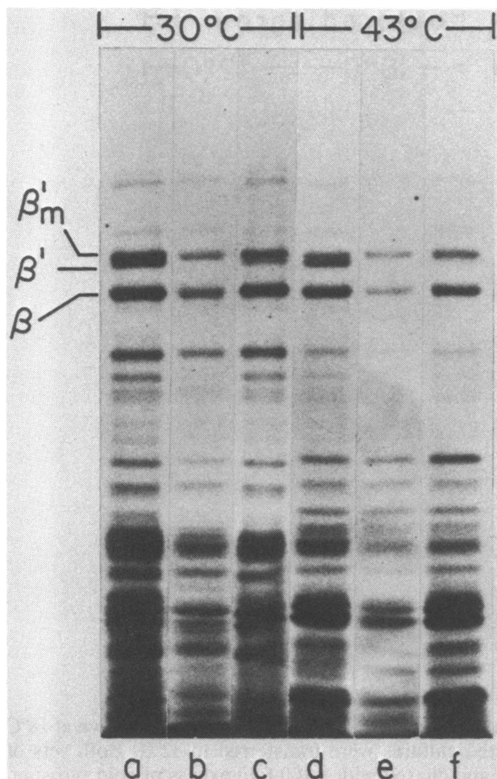

FIG. 6. Beta prime synthesis in merodiploid strains. Strains were grown at 30°C in the merodiploid medium (0.4% glucose) described in the text. For SR246, which is a methionine auxotroph, the medium was supplemented with 0.06 mM methionine. After at least two generations at 30°C, portions of each culture were transferred to 43°C. Thirty minutes after the cultures were split, [^{35}S]methionine was added to 280 μCi/ml to each merodiploid culture and five times that amount was added to SR246. After 4 min a 1,000-fold excess of unlabeled methionine was added, and the incubation was continued for an additional 4 min. Cells were harvested and extracts were prepared and fractionated as described in the legend to Fig. 2. The normal positions of the β and β′ subunits are so marked; the position of the mobility mutant β′ is marked by the symbol β′$_m$. The Ts amber suppressor in SR246 is approximately 30% efficient at 30°C and <1% efficient at 43°C (40). These values are consistent with the extent of synthesis of β′ from the amber mutant containing F′ Fsr1313 (lanes c and f). Lane f also shows that at 42°C both copies of the *rpoB* gene are being expressed since the intensity of the β band is approximately twice that of the β′ band. a, Fsr1228/SR246 30°C (wild-type F′); b, SR246 30°C; c, Fsr1313/SR246 30°C (amber mutant F′); d, Fsr1228/SR246 43°C (wild-type F′); e, SR246 43°C; f, Fsr1313/SR246 43°C (amber mutant F′).

colonies. On the medium devised for this test (GXG medium; Oeschger, unpublished data) Su$^-$ revertants can be distinguished from other galactose-insensitive mutants by their color. Although the parental Su$^+$ *rpo*$^+$ strain SR208 yielded many Su$^-$ derivatives (10^{-6}), no Su$^-$ derivatives ($<10^{-8}$) of SR249, the Su$^+$ strain carrying the *rpoC* mutation, were observed. The failure to produce Su$^-$ derivatives indicates that the strain carries an amber mutation in an essential gene.

Assignment of the mutation to the *rpoC* gene comes from *cis-trans* analysis. The recombinant F′ plasmids Fsr1313 (carrying the mutation) and Fsr1228 (a wild-type control) were transferred into SR246, an sul^{Ts} strain carrying an *rpoC* allele which alters the mobility of the β′ polypeptide on SDS-polyacrylamide gels. In this strain it is possible to monitor the expression of the chromosomal and plasmid-carried *rpoC* genes since their gene products are separable on SDS-gels. One would predict that if the mutation was in a gene other than *rpoC* it would affect the expression of the two *rpoC* alleles equally. Figure 6 shows that the amber is *cis* acting and so must lie within the *rpoC* structural gene.

The sul suppressor inserts serine. The compatibility of the serine insertion with the biochemical activity of the β′ polypeptide was examined by comparing the rates of growth of the sul$^+$ *rpoC* amber strain (MX784) and its sul$^+$ *rpoC*$^+$ cognate (MX762). Cultures of the two strains were grown at 30, 37, and 42°C, and their growth rates were determined. The only detectable difference was that the growth of the mutant was restricted at high cell density at 42°C. This effect was reversed on dilution of the culture and may be due to the *btuB* allele carried by MX784 (data not shown). These results indicate that the serine insertion is compatible with biochemical function at all temperatures, although it may not restore total wild-type activity to the β′ subunit.

DISCUSSION

A temperature-sensitive sul^{Ts} strain unable to synthesize full-length β′ at high temperatures was isolated and extraneous mutations were removed. Restoration of the ability to both grow and synthesize full-length β′ at high temperature occurred simultaneously at a spontaneous rate typical of single mutations (10^{-8}). The lack of suppression at 42°C of a known amber mutation in these revertants indicated that the suppressor had remained temperature sensitive. P1 transductions showed that the lesion affecting β′ synthesis lay within a sequence beginning in the *rpoB* gene and ending before the *purH* gene, a region encompassing *rpoC*. *cis-trans* analysis, using merodiploids, showed that the mutation only affects the synthesis of β′ from the DNA carrying the mutation, ruling out that the lesion lies in a gene outside *rpoC* which produces a protein required for *rpoC* gene expression. When this lesion was coupled with an amber

suppressor which is not Ts (sul$^+$), growth was no longer temperature sensitive and β′ was made at all temperatures, although not at wild-type levels. All evidence thus indicates that the lesion is a single amber mutation lying within the *rpoC* gene.

The puzzling ability of the sul$^+$ merodiploid Fsr1313/SR231 [*rpoB*$^+$ *rpoC*(Am)/*rpoB*(polar Am) *rpoC*$^+$] to form small colonies at 42°C may be due to a partial loss of the polarity of the amber mutation in the *rpoB* gene, the proposed loss having been caused by the spontaneous Rif^r mutation in the same gene. When the *rpoC* amber was moved into a haploid Su^{Ts} strain, the strain was unable to form colonies at 42°C.

The amber mutation in *rpoC* was sought for use in regulation studies. Work with this mutant has shown that a declining rate of synthesis of the β′ subunit results in an increasing rate of synthesis of the β subunit (Oeschger and Ridley, manuscript in preparation).

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