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Mutations of Bacterial RNA Polymerase Leading to Resistance to Microcin J25*

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A mutation in the conserved segment of the *rpoC* gene, which codes for the largest RNA polymerase (RNAP) subunit, β′, was found to make *Escherichia coli* cells resistant to microcin J25 (MccJ25), a bactericidal 21-amino acid peptide active against Gram-negative bacteria (Delgado, M. A., Rintoul, M. R., Farias, R. N., and Salomon, R. A. (2001) *J. Bacteriol.* 183, 4543–4550). Here, we report that mutant RNAP prepared from MccJ25-resistant cells, but not the wild-type RNAP, is resistant to MccJ25 *in vitro*, thus establishing that RNAP is a true cellular target of MccJ25. We also report the isolation of additional *rpoC* mutations that lead to MccJ25 resistance *in vivo* and *in vitro*. The new mutations affect β′ amino acids in evolutionarily conserved segments G, G′, and F and are exposed into the RNAP secondary channel, a narrow opening that connects the enzyme surface with the catalytic center. We also report that previously known *rpoB* (RNAP β subunit) mutations that lead to streptolydigin resistance cause resistance to MccJ25. We hypothesize that MccJ25 inhibits transcription by binding in RNAP secondary channel and blocking substrate access to the catalytic center.

Bacterial RNA polymerase (RNAP)[1] is the central enzyme of gene expression and a target of genetic regulation. The catalytically proficient core enzyme is composed of five polypeptides: the largest subunit β′, the second largest subunit β, the dimer of identical α subunits, and a small subunit ω. Upon the binding of one of the several σ specificity subunits the core is converted to a holoenzyme that can specifically initiate transcription from promoters.

RNAP is a target of several inhibitors. Interest is attached to these low molecular weight compounds as they can be used as tools to reveal new information about RNAP mechanism and can also be used as antibacterial drugs. The best studied bacterial RNAP inhibitor, rifampicin, is widely used against mycobacterial infections. Rifampicin and its derivatives bind to the RNAP β subunit (1–4) and prevent synthesis of RNAs longer than two-three nucleotides in length by occluding the nascent RNA exit path (5, 6). An unrelated inhibitor, streptolydigin (Stl), either affects the binding of incoming NTP in the substrate binding site of RNAP or directly targets the catalysis of phosphodiester bond formation (7–9). Mutations causing RNAP resistance to Stl were mapped to both the *rpoB* and *rpoC* genes, coding for the β and β′ subunits, respectively (3, 10–12). The β′ site where Stl-resistant substitutions were localized overlaps the site in eukaryal RNAP II largest subunit where substitutions leading to resistance to α-amanitin, a peptide that specifically inhibits PNAP II transcription, map (7, 8, 12–15). Thus, Stl and α-amanitin may inhibit transcription in their respective systems through similar mechanisms, despite the lack of chemical similarity between the two drugs. The third antibiotic known to target bacterial RNAP, tagetitoxin, inhibits transcription by slowing the rate of RNAP elongation and promoting pausing (16). The site of RNAP that tagetitoxin interacts with is not known. Tagetitoxin also inhibits transcription by eukaryotic RNAP III (17), indicating that the tagetitoxin binding site may be evolutionarily conserved.

Recently, one of our groups (18) reported that *Escherichia coli* cells harboring a mutation in the *rpoC* gene, which codes for RNAP β′, became resistant to microcin J25 (18). Microcin J25 (MccJ25) is a bactericidal peptide made of 21 amino acids (19, 20). MccJ25-producing cells harbor a plasmid that is responsible for MccJ25 production and resistance of MccJ25-producing cells to the drug (21). MccJ25 production increases when cells reach stationary phase and nutrients become limiting, thus giving MccJ25-producing cells an advantage (19, 22).

Most of spontaneous MccJ25-resistant mutants affect genes encoding cytoplasmic membrane proteins and appear to be intake mutants (23). The fact that a rare microcin resistance mutation resulted in altered RNAP suggested that RNAP may be the cellular target of MccJ25. In agreement with this idea, it has been shown that *in vitro* activity of *E. coli* RNAP is reduced in the presence of micromolar concentrations of MccJ25 (18).

The *rpoC* mutation that resulted in MccJ25 resistance caused a substitution of an evolutionarily conserved β′ Thr^{931} for Ile. Thr^{931} is part of segment G, whose sequence is well conserved in largest (β′-like) RNAP subunits from bacteria to

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[1] The abbreviations used are: RNAP, RNA polymerase; Stl, streptolydigin; MccJ25, microcin J25; IPTG, isopropyl-1-thio-β-D-galactopyranoside.

man (15). In the structural model of RNAP core from thermophilic eubacterium *Thermus aquaticus* a residue equivalent to *E. coli* β′ Thr^{931} is exposed on the inner surface of RNAP secondary channel, a narrow opening that leads from RNAP surface to the catalytic center (24). Based on structural considerations, the secondary channel was hypothesized to direct substrates toward the enzyme active site and to accept the 3′-end-proximal portion of the nascent RNA in transcription elongation complexes that assumed the dead-end conformation (24–26). Thus, the location of the residue affected by *rpoC* MccJ25 resistance mutation suggests a novel mechanism of RNAP inhibition: occlusion of RNAP secondary channel. Here, we report the isolation of several MccJ25 resistance mutations in evolutionarily conserved segments G, G′, and F of cloned *E. coli rpoC*. The locations of the corresponding β′ residues on the *T. aquaticus* RNAP structure are exposed in the inside surface of RNAP secondary channel, strongly supporting the idea that MccJ25 inhibits transcription by binding to and occluding this channel.

EXPERIMENTAL PROCEDURES

Bacterial Techniques and DNA Manipulations—Plasmids pRW308 (27) and pRL663 (12), overproducing wild-type or C-terminally hexahistidine-tagged β′ subunit, respectively, were used to obtain MccJ25-resistant mutations. MccJ25 resistant *rpoC* mutants generated by error-prone PCR were selected from plasmid banks described by Weilbaecher *et al.* (27). To generate site-specific mutations in segment G, a derivative of pRW308 harboring a unique *Xho*I site at *rpoC* codon 943 was created by PCR mutagenesis. The β′ subunit encoded by the resultant plasmid, pRW308*Xho*_943, was wild-type because of the degeneracy of the genetic code. The *rpoC* positions 928, 929, 930, and 931 were next randomized using mutagenic oligonucleotides complementary to *rpoC* codons 922–946 and incorporating a *Xho*I site at codon 943. At the site of randomization, positions corresponding to the first and second bases of the codon were equimolar mixtures of A, G, C, and T, whereas positions corresponding to the third base of the codon was an equimolar mixture of G and C. Mutagenic oligonucleotides were used as primers in a PCR reaction with pRW308*Xho*_943 template. As a second primer, an oligonucleotide whose sequence corresponded to *rpoC* positions 2534–2555 was used. This primer anneals upstream of a unique pRW308 *Sal*I site located at *rpoC* position 2629. After amplification, PCR fragments were treated with *Sal*I and *Xho*I and ligated into appropriately treated pRW308*Xho*_943. Ligation mixtures were transformed in MccJ25-sensitive DH5α *E. coli* host cells, and transformants were plated on solid LB medium containing 200 μg/ml ampicillin. After overnight growth at 37 °C, recombinant colonies were replica-plated on LB plates containing 200 μg/ml ampicillin, 50 μg/ml MccJ25 (purified as described previously; see Ref. 20), and 1 mM IPTG to derepress the *lac* promoter that drives expression of plasmid-borne *rpoC*. MccJ25-resistant colonies were purified, and plasmid DNA was prepared and retransformed into DH5α *E. coli* cells. Transformants were plated on plates containing MccJ25 to confirm that resistance is plasmid-borne. An entire *Sal*I-*Xho*I *rpoC* fragment was next sequenced at the Rockefeller University DNA technology center to establish the nature of the mutational change leading to MccJ25 resistance.

To randomize *rpoC* codons 1136 and 1137 (evolutionarily conserved segment G′) we made use of a unique *Sgr*AI recognition site at *rpoC* position 3402 (codon 1134). Mutagenic oligonucleotides spanned the *Sgr*AI site, as well as positions to be randomized. They were used as primers with pRL663 template and another primer, whose sequence was complementary to *rpoC* positions 3745–3777. This primer anneals downstream of a unique pRL663 *Bsp*EI site located at *rpoC* position 3639. PCR fragments were treated with *Sgr*AI and *Bsp*EI and ligated with appropriately treated pRL663, and MccJ25-resistant clones were selected and confirmed as above. In addition to the *Sgr*AI-*Bsp*EI fragment, a portion of *rpoC* coding for β′ segments F and G in mutant plasmids was also sequenced, and no changes from the published sequence were observed. Construction of the β′Δ(943–1130) mutation will be described elsewhere.[2]

Preparation of Mutant RNA Polymerases and in Vitro Transcription—Highly pure RNAP from MccJ25-resistant *E. coli* SBG231cells (18) and parental MccJ25-sensitive AB259 cells were purified as described (30). RNAP from *Xanthomonas oryzae* was purified as described in Ref. 31. RNAP from *Pseudomonas aeruginosa* 8882 strain (provided by Dr. A. Chakrabarty, University of Illinois College of Medicine) was purified by standard *E. coli* procedure without modifications. *Bacillus subtilis* RNAP was purified from *B. subtilis* PolHis cells harboring a genomic *rpoC* genetically fused to hexahistidine tag (generously provided by Drs. C. P. Moran and G. Schyns, Emory University School of Medicine). RNAP was purified from cell lysates by nickel-nitrilotriacetic acid affinity chromatography followed by ion-exchange on Resource Q (Amersham Biosciences) column. Recombinant RNAP from *T. aquaticus* was purified from overexpressing *E. coli* cells as described in Ref. 32. Yeast RNAP II and RNAP III were generous gifts of Dr. Sergei Borukhov (SUNY Brooklyn) and George Kassavetis (UCSD), respectively.

Mutant β′ Δ(943–1130) RNAP was purified by chitin-affinity chromatography and intein-mediated removal of the chitin binding domain tag, followed by heparin affinity column chromatography, as described elsewhere.[2] To partially purify RNAP containing β′ expressed from a plasmid, *E. coli* 397C cells (29) were transformed with pRW308, pRL663, or their derivatives, grown at 30 °C in 200 ml of LB medium containing 200 μg/ml ampicillin until A_{600} of 0.5, induced with 1 mM IPTG for 4 h, collected, disrupted by sonication, and polymin P fractionation was performed as described by Kashlev *et al.* (28). 1 M NaCl extract of polymin P pellet containing ~10% pure RNAP was precipitated with ammonium sulfate, and precipitate was stored at −80 °C. Before use, an aliquot of ammonium sulfate pellet was dissolved in transcription buffer (40 mM Tris-HCl, pH 7.9, 40 mM KCl, 10 mM $MgCl_2$, 5% glycerol) to give a final protein concentration of ~1 mg/ml, and this preparation was used in transcription assays.

Transcription from the T7 A1 promoter-containing DNA fragment was performed in 10-μl transcription buffer reactions containing 50 ng of DNA, 0.5 μg of wild-type or mutant RNAP, 0.5 mM CpA primer, 2.5 μM α-[^{32}P]UTP (300 Ci/mmol), and different concentrations of MccJ25. Reactions proceeded for 10 min at 37 °C and were terminated by the addition of urea-containing loading buffer. Products were analyzed by urea-PAGE electrophoresis (7 M urea, 20% polyacrylamide), followed by autoradiography and PhosphorImager analysis. Transcription from *B. subtilis vegA* promoter (32) was performed in a buffer containing 40 mM Tris-HCl, pH 7.9, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM dithiothreitol, 5% glycerol, 25 μg/ml bovine serum albumin using 0.5 mM UpA primer and 2.5 μM α-[^{32}P]GTP (300 Ci/mmol) substrate.

RESULTS

RNA Polymerase from Microcin-resistant E. coli Cells Is Resistant to MccJ25 in Vitro—Earlier, one of our groups (18) reported that *E. coli* cells harboring the *sjmA1* mutation, but not the wild-type *E. coli*, were able to grow on selective medium containing MccJ25. The *sjmA1* mutation was found to correspond to a substitution of Thr^{931} to Ile in the largest subunit of *E. coli* RNAP, the β′ subunit. The original report also established that MccJ25 partially inhibited a steady-state *in vitro* transcription by the wild-type *E. coli* RNAP, strongly implying that RNAP is a direct target of MccJ25. However, RNAP harboring the T931I substitution was not tested in these experiments. The experiment presented in Fig. 1 demonstrates that the mutant enzyme is indeed resistant to MccJ25 *in vitro*. As can be seen, MccJ25 inhibited T7 A1 promoter-directed synthesis of the CpApU abortive RNA product from the CpA dinucleotide primer and radioactively labeled UTP by the wild-type RNAP (compare *lanes 4* and *5*). In contrast, the CpApU synthesis by RNAP purified from cells harboring the *sjmA1* mutation was unaffected by the drug (compare *lanes 1* and *2*). Order-of-addition experiments established that MccJ25 inhibited abortive RNA synthesis when added either before or after the formation of open promoter complex on the T7 A1 promoter-containing DNA fragment used as a template in this experiment (compare *lanes 5* and *6*). We therefore conclude that (i) RNAP is a true cellular target of MccJ25, and (ii) MccJ25 does not act by preventing RNAP interaction with DNA.

Additional Substitutions in Conserved Segment G of the β′ Subunit Lead to MccJ25 Resistance—The genetic context of the

[2] I. Artsimovitch, V. Svetlov, K. Murakami, and R. Landick, manuscript in preparation.

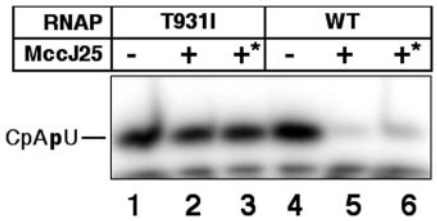

FIG. 1. **Transcription inhibition by MccJ25.** The indicated *E. coli* RNAP holoenzymes were combined with the T7 A1 promoter-containing DNA fragment, CpA primer, and [α-^{32}P]UTP in the presence and in the absence of 10 μM MccJ25. Reactions were incubated at 37 °C for 15 min, and the products were resolved by denaturing PAGE and revealed by autoradiography. In *lanes 2* and *5*, MccJ25 was added before promoter complex formation, and in *lanes 3* and *6*, it was added after promoter complex formation. *WT*, wild-type.

sjmA1 mutation is shown in Fig. 3. As can be seen, the corresponding substitution occurred in a highly conserved segment of the *E. coli* β' subunit, segment G. We hypothesize that the T931I substitution causes MccJ25 resistance by preventing MccJ25 binding to RNAP and that Thr^{931} is a part of MccJ25 binding site. Given the very high level of evolutionary conservation of segment G, the following two questions are of interest. First, can other MccJ25-resistant mutations in segment G be obtained? Second, will MccJ25 inhibit RNAPs from organisms other than *E. coli*?

To answer the first question, we obtained plasmids expressing mutant *rpoC* genes, transformed these plasmids into MccJ25-sensitive *E. coli* cells, and checked the ability of plasmid-bearing cells to grow on a medium containing MccJ25. In case when growth on selective medium was observed, we purified RNAPs containing mutant β' and confirmed that mutant RNAPs were indeed resistant to MccJ25. In cases when no *in vivo* resistance was observed, we considered the possibility that RNAP containing mutant β' could not support cell growth in the presence of MccJ25, when the wild-type, chromosomally encoded RNAP was inactivated. Therefore, RNAPs containing plasmid-borne β' were also purified, and their sensitivity to MccJ25 was tested *in vitro*. All mutants reported below were tested this way. Fig. 2 shows the results of *in vivo* and complementary *in vitro* testing with some of the mutants as an example.

A set of several point mutations in segment G of *E. coli rpoC* cloned on an expression plasmid was recovered in two unrelated screens, one aimed at obtaining termination-altering *rpoC* mutants (27) and another site-specifically mutating evolutionarily conserved β' positions 921 and 935,[2] is presented atop of the sequence alignment shown in Fig. 3. MccJ25-sensitive *E. coli* cells were transformed with plasmids expressing mutant *rpoC* genes, and the ability of plasmid-bearing cells to grow on MccJ25-containing medium was investigated. As controls, cells harboring plasmids expressing wild-type *rpoC* or MccJ25-resistant *rpoCT931I* allele were employed. As expected, cells expressing wild-type *rpoC* were sensitive to MccJ25, whereas cells expressing the *T931I* allele were resistant (Fig. 2 and data not shown). Cells harboring expression plasmids bearing the *F935S* allele were as resistant as control cells expressing *rpoC*^{T931I}, whereas cells expressing the *R933H,A946V* double mutant resulted in slow but detectable growth on MccJ25-containing medium (Fig. 2 and data not shown). In contrast, cells expressing *Q921P*, *T934M*, and *H936Y* alleles did not grow on selective medium (Fig. 2 and data not shown).

The results of *in vitro* transcription assays correlated with the *in vivo* results (Fig. 2) (data not shown). However, the *R933H,A946V* double mutant, which showed low levels of resistance *in vivo*, was highly resistant *in vitro*, suggesting that the mutant RNAP *in vivo* function is impaired. RNAP harboring the F935S substitution was found to be resistant to the drug, whereas other mutants were sensitive. Three RNAP harboring dominant lethal mutations in segment G, M932L, R933S, and T934A, were also tested for MccJ25 resistance. These mutants were obtained in the course of an independent mutagenesis effort[3] and were prepared by *in vitro* reconstitution.

Additional MccJ25-resistant mutants in segment G were also sought directly. Three *rpoC* codons immediately to the left of position 931 (928, 929, and 930) were randomized by site-directed PCR mutagenesis, libraries of recombinant plasmids were transformed in MccJ25-sensitive *E. coli* cells, and MccJ25-resistant clones were selected. As a control, position 931, the site of the original MccJ25-resistant mutation, was also randomized. MccJ25-resistant clones were only obtained in the control mutagenesis reaction. Sequencing of three resistant clones revealed the presence of the original mutation, T931I, as well as two new mutations, T931N and T931L. The corresponding enzymes were also resistant *in vitro* (data not shown). The result thus suggests that the identity of β' amino acids 928–930 is either not important for MccJ25 inhibition, or MccJ25-resistant substitutions at these positions lead to lethal phenotype.

MccJ25 Effect on RNAPs Other Than E. coli—MccJ25 is effective against Gram-negative bacteria but has no effect on Gram-positive bacteria (19). To determine the specificity of transcription inhibition by MccJ25, we assembled a panel of RNAPs prepared from several Gram-negative and Gram-positive bacteria and compared their ability to perform abortive RNA synthesis in the presence or in the absence of MccJ25 (Fig. 4). In the absence of MccJ25, RNAPs from Gram-negative bacteria demonstrated approximately equal specific activities on the T7 A1 promoter (0.9, 0.6, 1.2, and 0.8 pmol/min of CpApU synthesized by 1 pmol of wild-type *E. coli* RNAP, *E. coli* RNAP^{T931I}, *P. aeruginosa* RNAP, and *X. oryzae* RNAP, respectively). In agreement with the previously determined *in vivo* specificity, MccJ25 inhibited abortive synthesis of CpApU from the T7 A1 promoter-containing DNA fragment by RNAPs prepared from three Gram-negative bacteria, wild-type *E. coli*, *X. oryzae*, and *P. aeruginosa* (see Fig. 4; 10, 11, and 9% residual activity in the presence of 25 μM MccJ25, respectively). As expected, *E. coli* RNAP^{T931I} was active in the presence of 25 μM MccJ25 (85% activity). RNAP from *T. aquaticus* was assayed on the T7 A1 promoter at 60 °C and was considerably less active (0.2 pmol of CpApU synthesized per min per pmol of enzyme). MccJ25 had no effect on abortive synthesis by recombinant *T. aquaticus* RNAP at 60 °C (see Fig. 4; 100% activity in the presence of 25 μM MccJ25).

Because RNAP from *B. subtilis* displays only a very low level of activity on the T7 A1 promoter (data not shown), we assayed the effect of MccJ25 on this enzyme during the abortive synthesis of UpApG on *B. subtilis vegA* promoter (32). In the absence of MccJ25, *B. subtilis* RNAP, *E. coli* RNAP, and *E. coli* RNAP^{T931I} demonstrated comparable levels of activity on the *vegA* promoter (0.8, 0.3, and 0.1 pmol of UpApG synthesized per min per pmol of RNAP, respectively). MccJ25 had no effect on the *B. subtilis* enzyme (see Fig. 4; 110% activity in the presence of 25 μM MccJ25) and *E. coli* RNAP^{T931I} (see Fig. 4; 85% activity in the presence of 25 μM MccJ25) but was active against wild-type *E. coli* enzyme on this promoter (see Fig. 4; 11% activity in the presence of 25 μM MccJ25). Additional experiments demonstrated that MccJ25 had no effect on transcription by yeast RNAPs II and III (data not shown).

[3] V. Epshtein, A. Mustaev, and A. Goldfarb, submitted for publication.

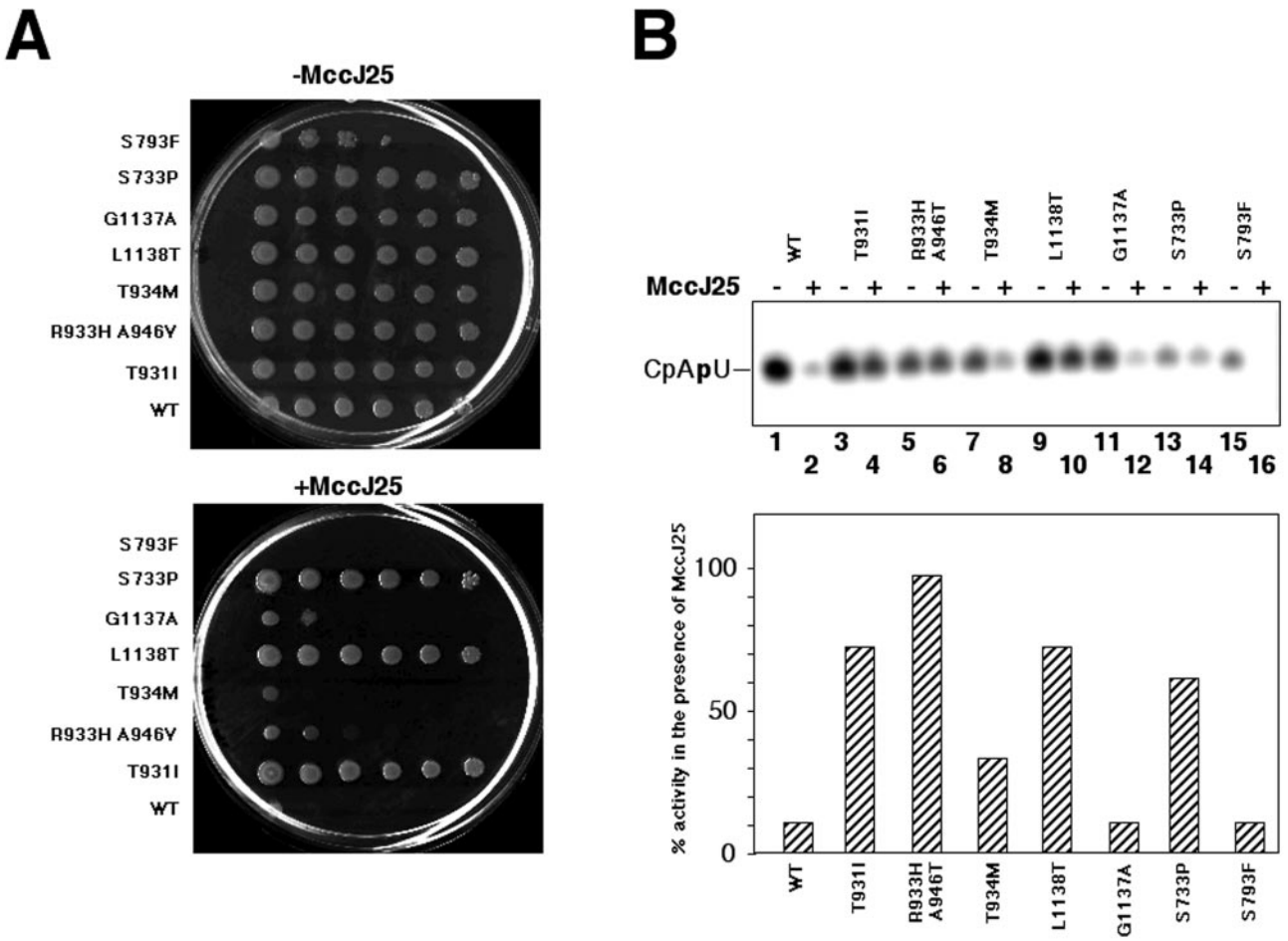

FIG. 2. ***In Vivo* and *in vitro* MccJ25 resistance of plasmid-borne *rpoC* mutants.** *A*, serial dilutions of the DH5α *E. coli* cells transformed with plasmids expressing the indicated *rpoC* mutants were spotted on plates in the presence or in the absence of MccJ25. Results of overnight growth are shown. *B*, RNAP harboring the indicated plasmid-borne β' mutations shown in *panel A* were purified and used in an *in vitro* transcription assay (see legend for Fig. 1) in the presence or in the absence of 10 μM MccJ25. Reaction products were resolved by denaturing PAGE and revealed by autoradiography (*top panel*). The residual activity in the presence of MccJ25 was quantified using a PhosphorImager (*bottom panel*). In the absence on MccJ25, the enzymes demonstrated the following levels of specific activity (calculated as pmol of abortive CpApU product synthesized by 1 pmol of RNAP per min of reaction): wild-type (*wt*) RNAP, 0.16; $RNAP^{T931I}$, 0.10; $RNAP^{R933H,A946T}$, 0.06; $RNAP^{T934M}$, 0.06; $RNAP^{L1138T}$, 0.10; $RNAP^{G1137A}$, 0.06; $RNAP^{S733P}$, 0.03; and $RNAP^{S793F}$, 0.03.

Effect of Substitutions in and around Conserved Region G′ on MccJ25 Resistance—Because segment G positions affected by MccJ25-resistant substitutions are identical in β' homologues from Gram-positive and Gram-negative organisms, the result implies that other regions of RNAP may also contribute to MccJ25 binding. In RNAP from Gram-negative bacteria, segment G is followed by a long stretch of amino acid sequence that is hypervariable in evolution (33). The hypervariable region is missing in RNAPs from Gram-positive bacteria and eukaryal RNAPs. To test whether the presence of the evolutionarily hypervariable region of β' contributes to the MccJ25 sensitivity of RNAP from Gram-negative bacteria, we tested the ability of *rpoC*Δ(*943–1130*) allele that lacks the entire hypervariable region and thus resembles homologues from Gram-positive microorganisms to confer MccJ25 resistance *in vivo*. The mutant β' poorly assembles into RNAP, presumably because of its inability to compete with chromosomally encoded wild-type β'.[4] We therefore tested the ability of MccJ25-sensitive cells harboring plasmid pIA331, which, in the presence of IPTG, co-overexpresses wild-type *rpoA* (α), *rpoB* (β), and *rpoC*Δ(*943–1130*) and thus increases the efficiency of the mutant enzyme assembly to grow on MccJ25-containing medium. As controls, plasmid pIA423, which co-overexpresses wild-type *rpoA*, *rpoB*, and *rpoC*, and pRL663*rpoC*$^{+}$ and pRL663*rpoCT931I* plasmids, were used. As can be seen from Fig. 5*A*, cells harboring pIA331 and pRL663*T931I*, but not cells harboring pIA423 and pRL663, formed colonies in the presence of MccJ25 and IPTG. Colonies formed by cells harboring pIA331 were minute as compared with colonies formed by cells harboring pRL663*rpoCT931I* cells, but the efficiency of plating was comparable. Plasmid pIA331, but not other plasmids, significantly inhibited cell growth in the presence of IPTG only, suggesting that $RNAP^{\Delta(943-1130)}$ was defective in some cellular function(s) unrelated to MccJ25 resistance. Be that as it may, the results demonstrate that hypervariable region indeed contributes to MccJ25 sensitivity and may be partially dispensable for cell viability at our conditions, because RNAP lacking β' residues 943–1130 is presumably the only transcriptionally active enzyme in the presence of MccJ25.

E. coli $RNAP^{\Delta(943-1130)}$ was prepared from cells harboring pIA331 and tested for the ability to transcribe from the T7 A1 promoter in the presence or in the absence of MccJ25 (Fig. 5*B*). The results showed that the mutant was more resistant to the drug than wild-type *E. coli* RNAP (26 and 3% residual activity in the presence of 50 μM MccJ25). Because RNAPs from Gram-positive bacteria are highly resistant to MccJ25 *in vitro*, other

[4] I. Artsimovitch, personal observation.

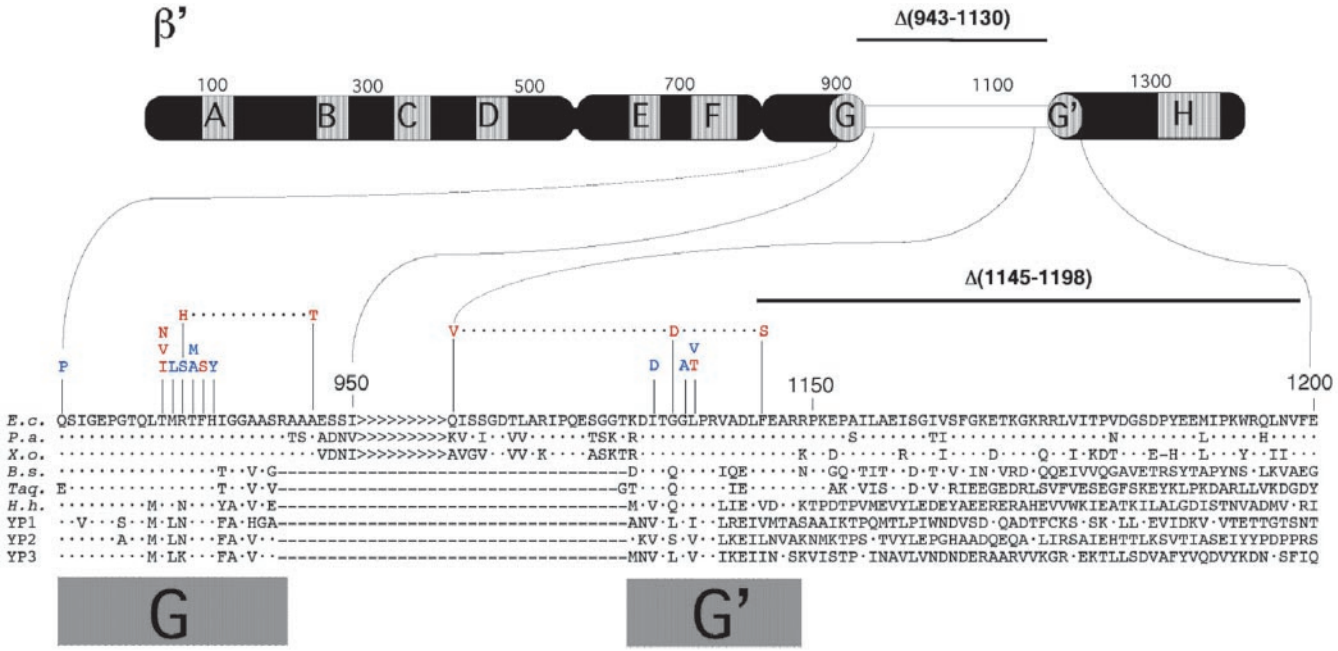

FIG. 3. **Genetic context of *rpoCT931I*.** The *heavy bar* represents the 1407 amino acid β′ subunit of *E. coli* RNAP. *Constrictions* indicate the sites of natural splits in homologues from chloroplasts and archaea. *Hatched boxes* labeled from *A* to *H* represent segments of β′ highly conserved in evolution. The amino acid sequences of *E. coli* β′ subunit conserved segments G and G′ are expanded *underneath*. Mutations that conferred MccJ25 resistance (either *in vivo* or *in vitro*) are shown *above* the *E. coli* sequence in *red*. Mutations that did not confer MccJ25 resistance either *in vivo* or *in vitro* are shown in *blue*. Homologous amino acid sequences from *P. aeruginosa* (*P.a.*), *X. oryzae* (*X.o.*), *B. subtilis* (*B.s.*), *T. aquaticus* (*Taq*.), *Halobacterium halobium* (*H.h.*), and yeast RNAPs I, II, and III (*YP1*, *YP2*, and *YP3*, respectively) are aligned with the *E. coli* sequence. The *dots* symbolize identity to the *E. coli* sequence, and the *hyphens* represent gaps. The evolutionarily variable sequence that separates segments G and G′ in β′ subunits from Gram-negative bacteria is shown as a *white box*. Deletion Δ(943–1130) is shown as a *black line above* the β′ subunit and is drawn to scale. Deletion Δ(1045–1098) is shown as a *black line above* the sequence alignment.

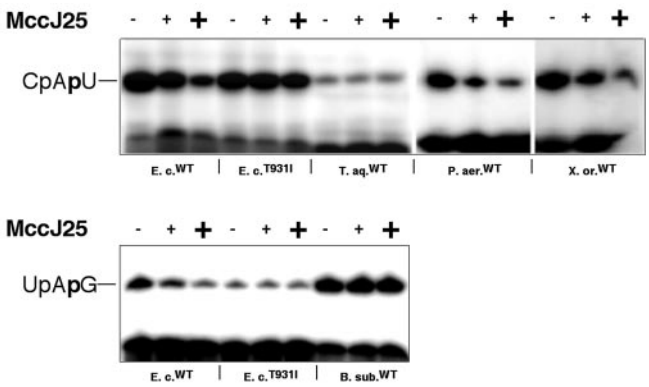

FIG. 4. **Effect of MccJ25 on transcription by RNAPs from different bacteria.** Results of *in vitro* transcription by the indicated RNAPs in the absence of MccJ25 or in the presence of 8 and 25 μM MccJ25 are shown. In the *top panel*, abortive transcription was performed from the T7 A1 promoter-containing DNA template using CpA primer and [α-^{32}P]UTP to generate the CpApU product. In the *bottom panel*, transcription was performed from the *B. subtilis vegA* promoter-containing template using UpA primer and [α-^{32}P]GTP to generate UpApG product. *WT*, wild-type; *E.c.*, *E. coli*; *T. aq.*, *T. aquaticus*; *P. aer.*, *P. aeruginosa*, *X. or.*, *X. oryzae*; *B. sub.*, *B. subtilis*.

sites in these RNAPs must contribute to MccJ25 resistance.

In the β′ subunits from Gram-negative bacteria, the hypervariable segment is followed by evolutionarily conserved segment G′ (33). Because there is no hypervariable region in homologues from Gram-positive bacteria, segments G and G′ form a continuous stretch of evolutionary conserved sequence in the β′ subunits from these organisms (Fig. 3). Segment G residues that cause MccJ25 resistance are part of the so-called G-loop in RNAP structures from thermophilic bacteria of the *Thermus* genus (24, 34, 35). Residues of segment G′ are also part of the G-loop. In particular, *Thermus* RNAP residues corresponding to *E. coli* β′ amino acids 1137 and 1138 are in direct contact with the residue corresponding to *E. coli* Thr^{931} and are located at the base of the G-loop. We therefore considered a possibility that substitutions in positions 1137 and 1138 will make *E. coli* RNAP MccJ25-resistant. Accordingly, codons 1137 and 1138 of plasmid-borne *rpoC* were randomized, mutant plasmid libraries were transformed in MccJ25-sensitive *E. coli*, and transformants were plated on selective medium containing MccJ25. No MccJ25-resistant mutants were obtained when codon 1137 was randomized. One clone was picked up at random and found to encode a G1137A substitution; the corresponding RNAP was MccJ25-sensitive *in vitro*. One resistant clone, coding for L1138T, was recovered from codon 1138 mutagenesis. The corresponding RNAP was purified and found to be MccJ25-resistant *in vitro* (Fig. 2). Because no changes from the published *rpoC* sequence in segment G was observed in this mutant (data not shown), we conclude that a substitution in segment G′ is indeed responsible for MccJ25 resistance. One MccJ25-sensitive clone from the 1138 mutagenesis reaction was picked up at random and sequenced and found to contain a mutation coding for L1138V substitution. The corresponding RNAP was purified and found to be MccJ25-sensitive *in vitro* (data not shown).

A functional deletion of β′ amino acids 1145–1198 immediately to the right of segment G′ was described by us previously (33). This deletion did not result in MccJ25 resistance *in vivo* and *in vitro* (data not shown).

A double mutation coding for E1030K and I1134D substitutions was isolated in an independent PCR-based screen for termination-altering *rpoC* mutations (27). The first substitution, of Glu^{1030}, occurred in the hypervariable region; the second substitution, of Ile^{1134}, occurred in segment G′. We tested the ability of plasmid-borne *E1030K,I1134D* allele to confer MccJ25 resistance *in vivo* and *in vitro* and observed no resist-

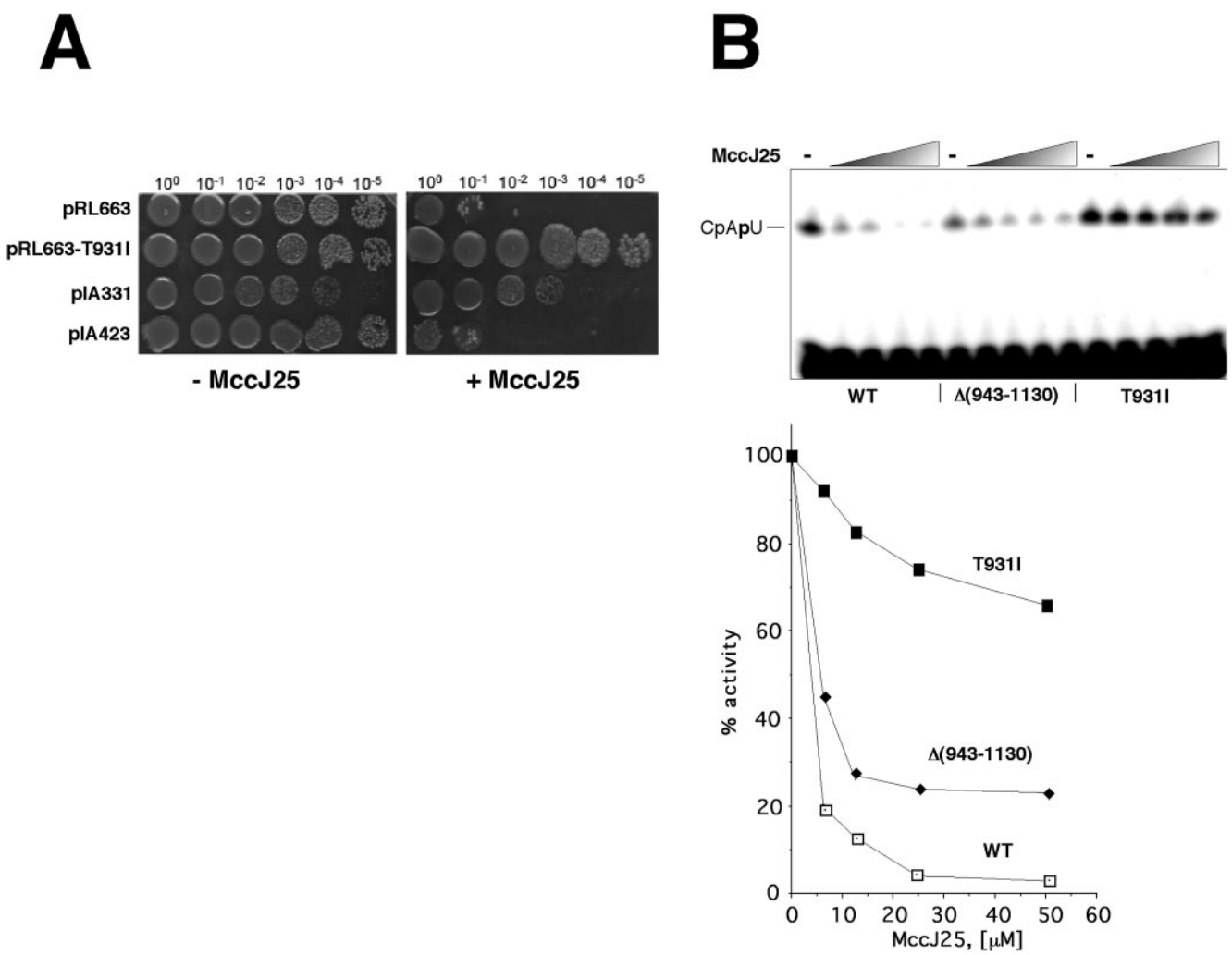

FIG. 5. **Removal of β′ hypervariable region leads to low level MccJ25 resistance.** *A*, expression of *rpoC*Δ(*943–1130*) allele allows growth in the presence of MccJ25. Indicated serial dilutions of *E. coli* DH5α cultures transformed with plasmids expressing wild-type *rpoC* (*pRL663*) and *rpoCT931I* (*pRL663-T931I*), co-overexpressing wild-type *rpoA*, *rpoB*, and *rpoC* (*pIA423*), or co-overexpressing wild-type *rpoA*, *rpoB*, and *rpoC*Δ(*943–1130*) (*pIA331*) were spotted on IPTG-containing LB plates in the presence or in the absence of MccJ25. Plates were incubated at 37 °C and photographed after 24 h (in the absence of MccJ25) or after 48 h (in the presence of MccJ25). *B*, effect of MccJ25 on transcription by *E. coli* RNAP lacking the β′ hypervariable region. $RNAP^{\Delta(943-1030)}$ was purified, and transcription from the T7 A1 promoter-containing DNA template using CpA primer and [α-^{32}P]UTP substrate was performed at increasing concentrations of MccJ25 (from 6.25 to 50 μM). $RNAP^{WT}$ and $RNAP^{T931I}$ were used as controls. Reaction products were resolved by denaturing PAGE and revealed by autoradiography (*top*). Reaction products were quantified using phosphorimagery, and transcription activity in the presence of MccJ25 was plotted as a percent of the activity in the absence of MccJ25 (*bottom*).

ance (data not shown). We also looked for additional MccJ25-resistant mutations within the bank of *rpoC* expression plasmids subjected to error-prone PCR at and around segments G and G′ (*rpoC* codons 876–1213; see Ref. 27). A triple mutation coding for I1115V, G1136D, and F1145S substitutions was recovered in this way. Of the three residues affected, one (β′ Phe^{1145}) is removed by MccJ25-sensitive Δ(1145–1198) deletion. Substitutions I1115V and/or G1136D are thus likely responsible for MccJ25 resistance. Because I1115V is a conservative substitution, substitution of evolutionarily conserved Gly^{1136} in segment G′ is the probable cause of MccJ25 resistance.

Substitutions in Evolutionarily Conserved Segment F Lead to MccJ25 Resistance—Residues of β′ segments G and G′ that are important for MccJ25 inhibition are exposed on the surface of narrow RNAP secondary channel that opens on the downstream face of the enzyme and leads to the catalytic site (24). In addition to β′ segments G and G′, conserved segment F also participates in the formation of the secondary channel. We were therefore interested in whether MccJ25-resistant mutations in segment F can be obtained. Toward this end, we tested two segment F mutants, F773I and S793F, that were shown previously to cause resistance to the elongation inhibitor, streptolydigin (12). These mutants did not result in appreciable MccJ25 resistance *in vivo* or *in vitro* (Fig. 2) (data not shown). We therefore looked for MccJ25-resistant region F mutants directly, by incorporating an error-prone PCR-amplified *rpoC* fragment coding for region F (*rpoC* codons 544–875) into an *rpoC* expression plasmid, transforming mutant plasmids in MccJ25-sensitive host, and selecting MccJ25-resistant colonies. Several independent MccJ25-resistant colonies were obtained, and the plasmid-borne nature of MccJ25 resistance was confirmed by retransforming of *rpoC* expression plasmids from MccJ25-resistant clones into sensitive host and replating on selective medium. Four independent clones were obtained, and their sequence at and around segments F, G, and G′ was determined. No changes in segment G/G′ sequences was detected. In contrast, changes from the published sequence leading to substitutions of segment F residues Ser^{733} for Pro, Leu^{783} for Gln, and a double substitution of Leu^{746} for Pro and

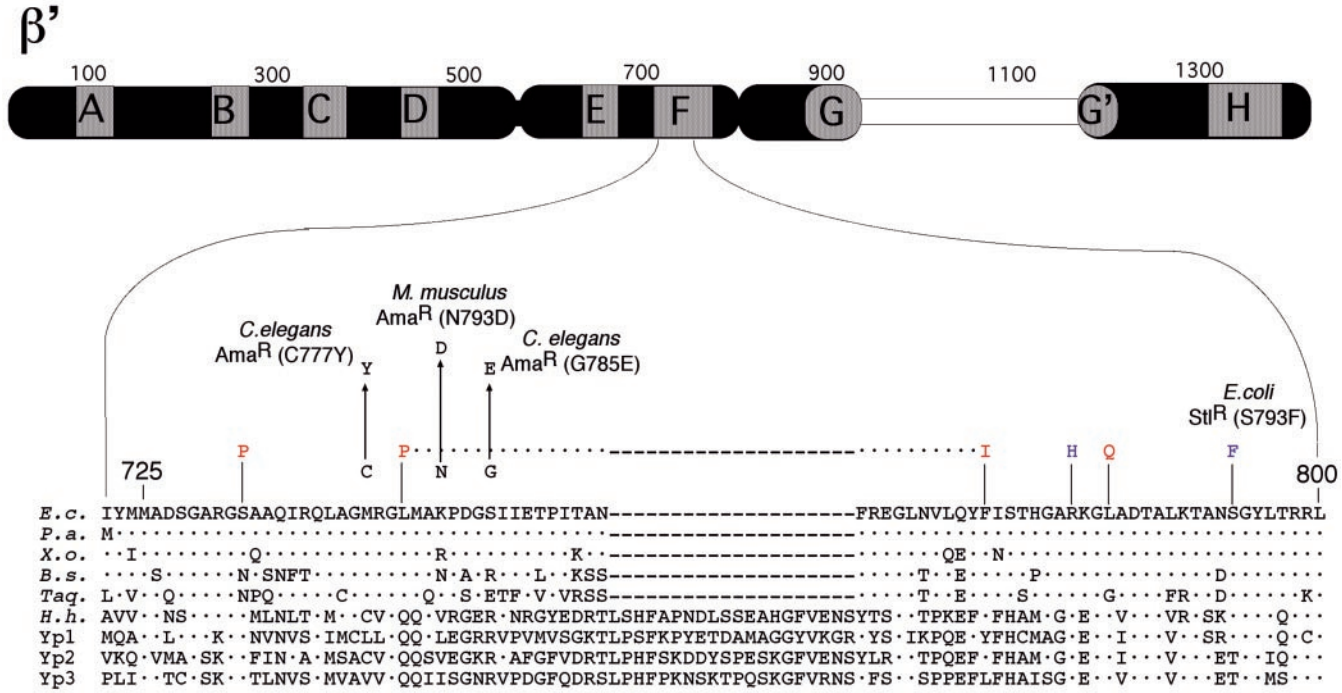

FIG. 6. **Mutations in β′ conserved segment F result in MccJ25 resistance.** Genetic context of β′ segment F. See legend for Fig. 3 for details. Mutations that conferred MccJ25 resistance are shown *above* the *E. coli* sequence in *red*. Mutations that did not confer MccJ25 resistance are shown in *blue*. *E. coli* mutation that cause streptolydigin resistance and mutations in eukaryal RNAPs that cause α-amanitin resistance are shown *above* the *E. coli* sequence.

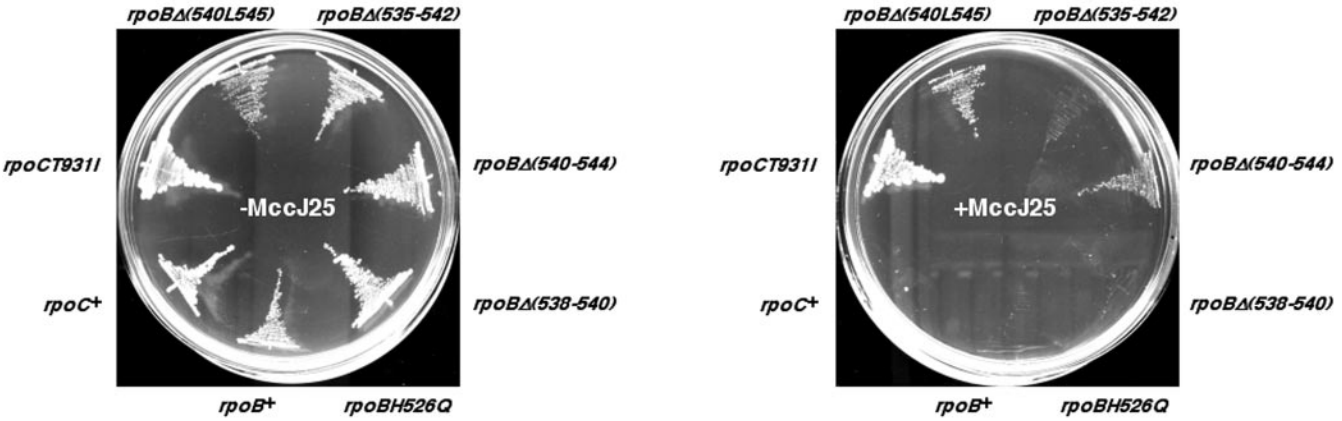

FIG. 7. **Stl-resistant *rpoB* mutations cause MccJ25 resistance.** *E. coli* DH5α cells transformed with plasmids expressing the indicated *rpoC* and *rpoB* alleles were streaked on plates in the presence or in the absence of MccJ25. Results of 30-h growth at 37 °C are shown.

Phe[773] for Ile were observed (Fig. 6). *In vitro* analysis confirmed that RNAPs carrying mutations in segment F are resistant to MccJ25 (Fig. 2) (data not shown). We conclude that substitutions in RNAP β′ segment F lead to MccJ25 resistance.

Stl-resistant Mutations in the β Subunit Lead to MccJ25 Resistance—Substitutions in β′ segment F that lead to MccJ25 resistance occurred close to segment F sites that, when mutated, cause Stl resistance (12). The main cluster of Stl-resistant mutations is located in the β subunit, between Rif clusters I and II (3). To further investigate the relationship between MccJ25 and Stl resistance we tested the ability of *E. coli* cells expressing several MccJ25-resistant alleles to grow in the presence of Stl, and we tested the ability of cells expressing Stl-resistant *rpoB*(β) alleles to grow in the presence of MccJ25. As expected, cells overproducing Stl-resistant β′ harboring S793F substitution, as well as cells overproducing Stl-resistant β subunit microdeletions Δ(*540–544*) and Δ(*540L545*) (3), but not cells overproducing wild-type β′ or β, grew on plates containing Stl. Also as expected, cells overproducing partially Stl-resistant $\beta^{\Delta(535-542)}$ formed minute colonies in the presence of Stl, whereas cells overproducing Stl-sensitive $\beta^{\Delta(538-540)}$ did not grow (3) (data not shown). None of the segment F, segment G, or segment G′ MccJ25-resistant *rpoC* mutations tested allowed growth on Stl-containing plates (data not shown).

Plating of cells expressing Stl-resistant *rpoB* alleles on MccJ25 gave an unexpected result. As expected cells expressing *rpoCT931I*, but not cells expressing wild-type *rpoC* or Stl-resistant *rpoCS793F*, grew in the presence of MccJ25 (Fig. 7). Likewise, cells expressing wild-type *rpoB* and Stl-sensitive *rpoB*Δ(*538–540*) did not grow in the presence of MccJ25. In contrast, cells expressing highly Stl-resistant Δ(*540–544*) and Δ(*540L545*) grew in the presence of MccJ25, whereas cells expressing low-level Stl resistance allele *rpoB*Δ(*535–542*) formed minute colonies. The results of the plating assay were supported by the results of *in vitro* transcription experiments (data not shown). We also tested several plasmid-borne Rifam-

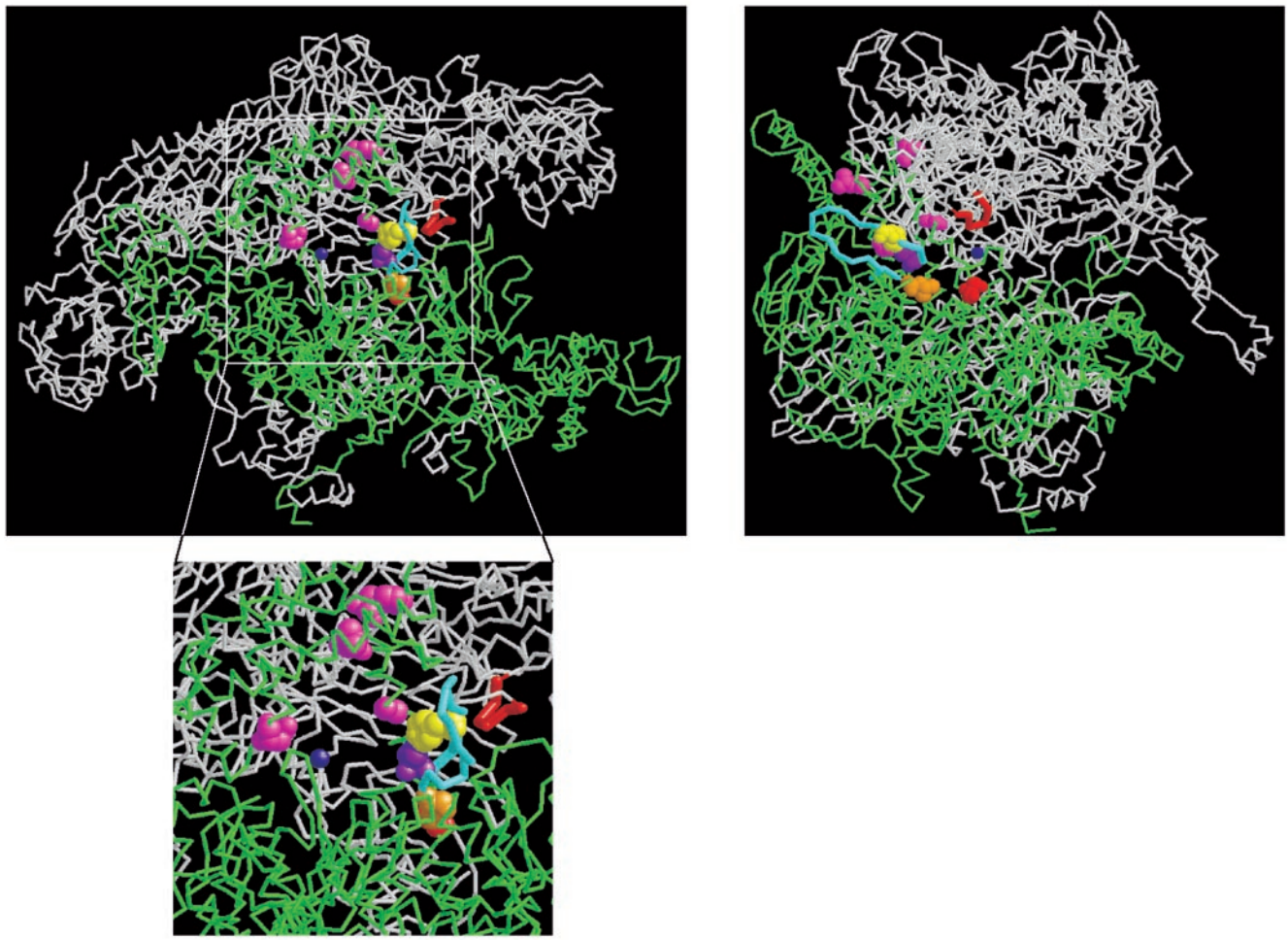

FIG. 8. **Structural context of MccJ25 resistance mutations.** At the *top left* a backbone representation of *T. aquaticus* RNAP core is shown. The β′ subunit is *green*, and other subunits are *white*. The active-center Mg^{2+} is shown in SPACEFILL representation and is colored *blue*. The *boxed area* is expanded at the *bottom*. Amino acid corresponding to *E. coli* β′ Thr^{931} the site of the original MccJ25 mutation (18) is shown in *purple* and SPACEFILL. Amino acid homologous to *E. coli* segment G β′ Phe^{935} that can be mutated to result in MccJ25 resistance is shown in *yellow* and SPACEFILL. Amino acid homologous to *E. coli* segment G′ Leu^{1138} that can be altered to result in MccJ25 resistance is shown in *orange* and SPACEFILL. The G-loop is shown in *cyan*. The corresponding loop in RNAPs from Gram-negative bacteria contains an insertion of more than two hundred amino acids (see Fig. 3). Segment F amino acids whose homologues in *E. coli* β′ can be altered to result inMccJ25 resistance are shown in *magenta*. Amino acid homologous to *E. coli* segment F Ser^{793} that can be altered to result in Stl resistance is shown in *red* and SPACEFILL. A stretch of β subunit amino acids whose homologues in *E. coli* can be mutated toward Stl resistance mutation is colored *red*. The view on the *right* is perpendicular to the main DNA binding channel of the enzyme and was obtained from the *left* view by ~90° clockwise rotation around the vertical axis.

picin-resistant *rpoB* mutants and found that none of them were able to support growth in the presence of MccJ25 (data not shown). We conclude that Stl-resistant mutations in the *rpoB* gene lead to MccJ25 resistance.

CONCLUSIONS

The principal result of this work is the demonstration that transcription by mutant RNAP purified from MccJ25-resistant *E. coli* cells is resistant to MccJ25, whereas transcription by RNAP purified from wild-type cells is MccJ25-sensitive. This result proves that *E. coli* RNAP is the cellular target of MccJ25. In the best understood case of Rifampicin, mutations toward resistance affect RNAP residues that are removed from each other in primary sequence but that cluster in the enzyme quaternary structure (1–3, 24). Structural analysis demonstrates that Rif-resistance mutation define the Rifampicin binding site (4). We hypothesized that the original MccJ25-resistance mutation, that changed evolutionarily conserved amino acid inside RNAP secondary channel, likewise defines the MccJ25 binding site on RNAP. According to this view, MccJ25 inhibits transcription by binding in the secondary channel and preventing the traffic of NTP substrates to the catalytic center of the enzyme. Indeed, molecular modeling using *T. aquaticus* RNAP structure and a reported MccJ25 structure (20) shows that 21-amino acid MccJ25 can fit into RNAP secondary channel with little or no steric clashes (data not shown).

If MccJ25 were indeed binding in the secondary channel, it should be possible to isolate additional MccJ25-resistant mutations located in the channel. Further, at least some of these residues must be evolutionarily variable, to explain the observed restriction of MccJ25 action to RNAP from Gram-negative bacteria. Both of these predictions are fulfilled. Here, we report the isolation of additional MccJ25-resistant mutants in segment G, as well as mutations in conserved segments G′ and F. Structural analysis indicates that in *T. aquaticus* RNAP core enzyme structure, residues homologous to those affected in *E. coli* are exposed in the secondary channel (Fig. 8). As expected, residues in segments G and G′ are located close to the original Thr^{931} and to each other, at the base of the G loop. However, residues in segment F (*magenta*) are spatially isolated from

each other and are located on two opposing sides of the secondary channel, as well as on the roof of the channel. Residues in segments G and G′ are identical in RNAPs from Gram-positive and Gram-negative bacteria and therefore could not be responsible for differential action of MccJ25 on these enzymes. On the other hand, residues in segment F are different between RNAPs from Gram-negative and Gram-positive bacteria, and these differences could account for observed specificity of MccJ25 inhibition. In addition, our data indicate that the presence of hypervariable region in β' contributes to MccJ25 sensitivity of RNAP from Gram-negative bacteria. The hypervariable region is inserted in RNAP G loop, which appears to be flexible. In *T. aquaticus* core enzyme structure, G loop is in a "closed" conformation and takes part in the formation of the secondary channel wall (Fig. 8, *cyan*). In the holoenzyme structure, G loop is in an "open" conformation, turned almost 90 degrees from its position in the core. The opening of the G loop shortens the secondary channel and may affect MccJ25 binding. It is conceivable that the hypervariable region restricts the mobility of the G-loop and thus allows better binding of MccJ25 to RNAP from Gram-negative bacteria.

Earlier (12), we proposed that the presence of mutations that cause resistance to Stl and α-amanitin in conserved segment F of bacterial RNAP β' subunits and eukaryal RNAP II largest subunits indicated that the two drugs may function similarly in their respective systems despite the lack of common chemical structure. Here, we show that mutations in β' segment F also cause resistance to MccJ25. Amino acids that, when mutated, cause resistance to all three drugs are exposed on the surface of the secondary channel. Analysis of a structural model of bacterial RNAP elongation complex reveals that RNAP secondary channel provides the only unobstructed way from the solvent to RNAP catalytic center, because access from the main DNA binding channel is blocked by nucleic acids (25). Therefore, it is possible that substitutions in the secondary channel can cause resistance to transcription elongation inhibitors whose actual mechanisms of action are different, but all of whom have to pass through the secondary channel to get access to the catalytic center.

Analysis of Stl-resistant *rpoB* revealed, unexpectedly, that they cause MccJ25 resistance. The result appears to strengthen the idea that MccJ25 and Stl may have a common inhibition mechanism, despite the lack of structural similarity. On the other hand, the presence of MccJ25-resistant mutations in β is difficult to reconcile with the notion of MccJ25 binding in the secondary channel, because in RNAP structure, the site of Stl-resistant mutations in the β subunit (Fig. 8, *red*) is located slightly upstream of the catalytic center and should become inaccessible from the secondary channel in the elongation complex (25). Therefore, it is possible that lesions in this site cause MccJ25 resistance indirectly. For example, the G-loop, when opened, may interact with the site of Stl resistance mutations in β, and Stl resistance mutations could therefore affect the position of the G-loop and thus cause resistance to MccJ25. Alternatively, the β Stl site can undergo a conformational change upon transcription complex formation that brings it closer to the secondary channel.

Obviously, further studies will be necessary to determine the site of MccJ25 interaction with RNAP, the mechanism of transcription inhibition by MccJ25, and its relationship, if any, to transcription inhibition by Stl. If MccJ25 were indeed binding in the secondary channel, several very specific predictions concerning the biochemical effects of its interactions with transcription complex could be made. The secondary channel is thought to conduct NTP substrates to the RNAP catalytic center, to accept the 3′-end proximal portion of the nascent RNA in the back-tracked, dead-end conformation of the elongation complex, and to accept transcript cleavage factors GreA and GreB (24). MccJ25 binding should interfere with all of these activities. Experiments aimed at testing these predictions are currently underway.

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REFERENCES

1. Ovchinnikov, Y. A., Monastyrskaya, G. S., Guriev, S. O., Kalinina, N. F., Sverdlov, E. D., Gragerov, A. I., Bass, I. A., Kiver, I. F., Moiseyeva, E. P., Igumnov, V. N., Mindlin. S. Z., Nikiforov, V. G., and Khesin, R. B. (1983) *Mol. Gen. Genet.* **190,** 344–348
2. Jin, D. J., and Gross, C. A. (1988) *J. Mol. Biol.* **202,** 45–58
3. Severinov, K., Soushko, M., Goldfarb, A., and Nikiforov, V. (1993) *J. Biol. Chem.* **268,** 14280–14825
4. Campbell, E. A., Korzheva, N., Mustaev, A., Murakami, K., Nair, S., Goldfarb, A., and Darst, S. A. (2001) *Cell* **104,** 901–912
5. McClure, W. R., and Cech, C. L. (1978) *J. Biol. Chem.* **253,** 8949–8956
6. Schulz, W., and Zillig, W. (1981) *Nucleic Acids Res.* **9,** 6889–6906
7. Cassani, G., Burgess, R. R., Goodman, H. M., and Gold, L. (1971) *Nat. New Biol.* **230,** 197–200
8. Helm, K. v. d., and Krakow, J. S. (1972) *Nat. New Biol.* **235,** 82–83
9. McClure, W. R. (1980) *J. Biol. Chem.* **255,** 1610–1616
10. Lisitsyn, N. A., Sverdlov, E. D., Moiseyeva, E. P., and Nikiforov, V. G. (1985) *Bioorg. Khim.* **11,** 132–134
11. Heisler, L. M., Suzuki, H., Landick, R., and Gross, C. A. (1993) *J. Biol. Chem.* **268,** 25369–25375
12. Severinov, K., Markov, D., Nikiforov, V., Severinova, E., Landick, R., Darst, S. A., and Goldfarb, A. (1995) *J. Biol. Chem.* **270,** 23926–23929
13. Bartolomei, M., and Corden, J. (1987) *Mol. Cell. Biol.* **7,** 586–594
14. Bartolomei, M. S., and Corden, J. L. (1995) *Mol. Gen. Genet.* **246,** 778–782
15. Allison, L. A., Moyle, M., Shales, M., and Ingles, C. J. (1985) *Cell* **42,** 599–610
16. Mathews, D. E., and Durbin, R. D. (1990) *J. Biol. Chem.* **265,** 493–498
17. Steinberg, T. H., Mathews, D. E., Durbin, R. D., and Burgess, R. R. (1990) *J. Biol. Chem.* **265,** 499–505
18. Delgado, M. A., Rintoul, M. R., Farias, R. N., and Salomon, R. A. (2001) *J. Bacteriol.* **183,** 4543–4550
19. Salomon, R. A., and Farias, R. N. (1992) *J. Bacteriol.* **174,** 7428–7435
20. Blond, A., Cheminant, M., Segalas-Milazzo, I., Peduzzi, J., Barthelemy, M., Goulard, C., Salomon, R., Moreno, F., Farias, R., and Rebuffat, S. (2001) *Eur. J. Biochem.* **268,** 2124–2133
21. Solbiati, J. O., Ciaccio, M., Farias, R. N., and Salomon, R. A. (1996) *J. Bacteriol.* **178,** 3661–3663
22. Chiuchiolo, M. J., Delgado, M. A., Farias, R. N., and Salomon, R. A. (2001) *J. Bacteriol.* **183,** 1755–1764
23. Salomon, R. A., and Farias, R. N. (1995) *J. Bacteriol.* **177,** 3323–3325
24. Zhang, G., Campbell, E. A., Minakhin, L., Richter, C., Severinov, K., and Darst, S. A. (1999) *Cell* **98,** 811–824
25. Korzheva, N., Mustaev, A., Kozlov, M., Malhotra, A., Nikiforov, V., Goldfarb, A., and Darst, S. A. (2000) *Science* **289,** 619–625
26. Severinov, K. (2000) *Curr. Opin. Microbiol.* **3,** 118–125
27. Weilbaecher, R., Hebron, C., Feng, G., and Landick, R. (1994) *Genes Dev.* **8,** 2913–2927
28. Kashlev, M., Nudler, E., Severinov, K., Borukhov, S., Komissarova, N., and Goldfarb, A. (1996) *Methods Enzymol.* **274,** 326–334
29. Nedea, E. C., Markov, D., Naryshkina, T., and Severinov, K. (1999) *J. Bacteriol.* **181,** 2663–2665
30. Polyakov, A., Severinova, E., and Darst, S. A. (1995) *Cell* **8,** 365–373
31. Nechaev, S., Yuzhenkova, Y., Niedziela-Majka, A., Heyduk, T., and Severinov, K. (2002) *J. Mol. Biol.* **320,** 11–22
32. Le Grice, S. F., Shih, C. C., Whipple, F., and Sonenshein, A. L. (1986) *Mol. Gen. Genet.* **204,** 229–236
33. Zakharova, N., Bass, A., Arsenieva, E., Nikiforov, V., and Severinov, K. (1998) *J. Biol. Chem.* **273,** 24912–24920
34. Vassylyev, D. G., Sekine, S., Laptenko, O., Lee, J., Vassylyeva, M. N., Borukhov, S., and Yokoyama, S. (2002) *Nature* **417,** 712–719
35. Murakami, K. S., Masuda, S., and Darst, S. A. (2002) *Science* **296,** 1280–1284

Mutations of Bacterial RNA Polymerase Leading to Resistance to Microcin J25

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