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Identification of Rifampicin Resistance Mutations in *Escherichia coli*, Including an Unusual Deletion Mutation

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Antibiotic · Rifampicin · *Escherichia coli* · Mutation

Abstract
Rifampicin is an effective antibiotic against mycobacterial and other bacterial infections, but resistance readily emerges in laboratory and clinical settings. We screened *Escherichia coli* for rifampicin resistance and identified numerous mutations to the gene encoding the β-chain of RNA polymerase (*rpoB*), including an unusual 9-nucleotide deletion mutation. Structural modeling of the deletion mutant indicates locations of potential steric clashes with rifampicin. Sequence conservation in the region near the deletion mutation suggests a similar mutation may also confer resistance during the treatment of tuberculosis.

Introduction

Rifampicin (RIF) is a member of rifamycin antibiotics used in the treatment of tuberculosis and other bacterial infections [Sensi, 1983]. It targets bacterial RNA polymerases by binding near the polymerase active site and inhibiting the elongation of nascent mRNA [Campbell et al., 2001]. The catalytic core of bacterial RNA polymerases consists of α_2-, β-, β′-, and ω-chains, and RIF binds to a pocket formed by the β-chain (Fig. 1a) [Campbell et al., 2001; Molodtsov et al., 2013]. Although RIF binds in the RNA tract, many of the residues in the RIF binding pocket do not directly contact the RNA. Numerous mutations in the RNA polymerase β-chain confer RIF resistance (RIF^R) in *Escherichia coli* and *Mycobacterium tuberculosis* [Xu et al., 2005; Makiela-Dzbenska et al., 2011; Zhou et al., 2013; Jin and Gross, 1989; Sandgren et al., 2009; Barrick et al., 2010]. These mutations are clustered in 4 regions of RNA polymerase β, termed RIF^R-determining regions (RRDRs) N, I, II, and III [Molodtsov et al., 2016]. Crystal structures of RIF bound to RNA polymerases of *Thermus aquaticus* and *E. coli* show that all 4 RRDRs consist of residues located within approximately 10 Å of RIF [Campbell et al., 2001; Molodtsov et al., 2013]. RIF^R mutations and structural studies have provided detailed information on how *M. tuberculosis* and other bacteria evade the antibiotic action of RIF and other rifamycins [Artsimovitch et al., 2005; Molodtsov et al., 2017].

In this study, we selected *E. coli* for RIF^R in the absence of mutagens and sequenced a region of RNA polymerase β-chain encompassing RRDRs I, II, and III to identify new RIF^R mutations. Nonpathogenic *E. coli* serves as an excellent experimental system to probe RIF^R because of its ease of handling in the laboratory in contrast to *M. tuberculosis*, and there exists an abundance of structural

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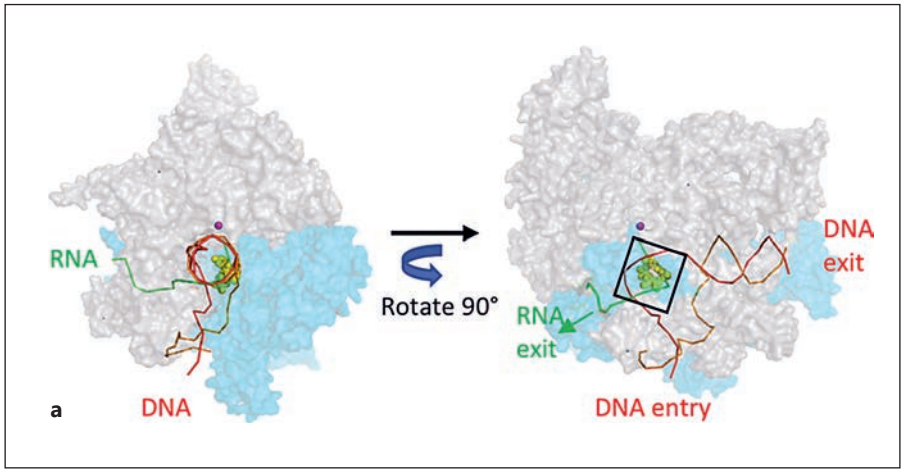

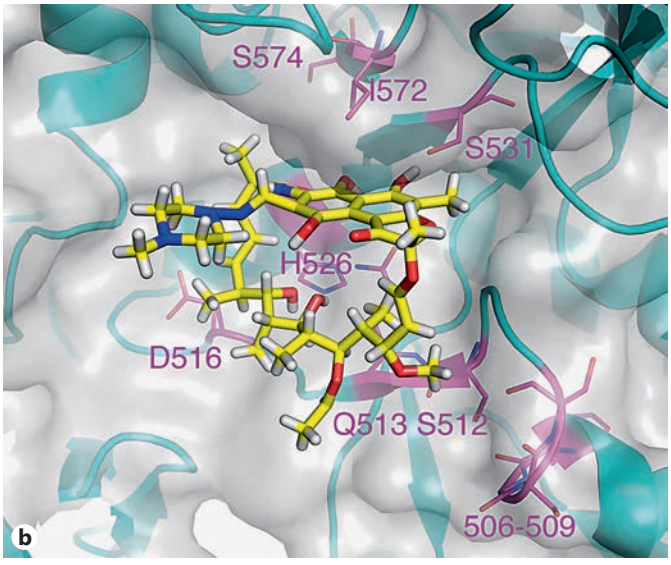

Fig. 1. Rifampicin binding to the bacterial transcriptional machinery. **a** Rifampicin (yellow spheres) binds inside the DNA-RNA tunnel of RNA polymerase elongation complex (gray surface representation; 5UPC.pdb) to the surface of the β-chain (cyan). The rifampicin binding site overlaps with the path of the nascent RNA chain (green). The template (red) and non-template (orange) DNA backbones are shown as lines, and the polymerase active site is shown as a magenta sphere. **b** The rifampicin (sticks representation; yellow carbons, blue nitrogens, red oxygens, white hydrogens) binding site (black box in **a**) and its solvent-exposed surface (gray) in *E. coli* RNA polymerase β-chain (cyan) from 4KMU.pdb. Locations of mutations identified in this study are depicted as magenta lines and labeled.

and biochemical information on its RNA polymerase [Campbell et al., 2001; Gill and Garcia, 2011; Kang et al., 2017; Molodtsov et al., 2013]. While most of the *E. coli* mutants contained previously characterized mutations, we identified a new mutation located in RRDR I, an unusual in-frame deletion that did not result in misfolding of RNA polymerase.

Results and Discussion

RIF^R in E. coli

Selection of *E. coli* K-12 MG1655 laboratory strain bacteria on Luria-Bertani broth (LB) plates containing RIF readily produced a large and variable number of RIF-resistant clones (results not shown). The number of RIF^R colonies and the total live bacteria in each of 64 cultures were counted and used to compute the mutation rate from Luria-Delbrück fluctuation tests [Gillet-Markowska et al., 2015; Luria and Delbrück, 1943; Ycart and Veziris, 2014]. We estimate the mutation rate of the MG1655 strain at 2.0×10^{-9}, which is in general agreement with other studies on *E. coli* lab strains and RIF^R [Bjedov et al., 2003; Galán et al., 2007] but lower than rates from clinical isolates [Baquero et al., 2004].

Identity of RIF^R Mutations

The chromosomal *rpoB* gene fragment containing the region that codes for residues 473–720 of the β-chain of RNA polymerase was directly amplified from RIF^R colonies using the polymerase chain reaction, resulting in a 744-base-pair band (results not shown). This section of

E. coli RNA polymerase encompasses the RIF-binding site and RIF^R-determining region, including clusters I (residues 507–533), II (residues 563–572), and III (residue 687), but not the N-terminal cluster (residue 146). Sequencing of polymerase chain reaction (PCR) products revealed 16 different mutations in clusters I and II (Table 1). All but 1 of the cluster I mutations have previously been reported in RIF-resistant *E. coli* mutants. The 9-bp deletion had not been previously reported, to our knowledge, but is similar to a previously characterized deletion that replaces 507-GSSQL-511 with V [Jin and Gross, 1989]. The deleted sequence (nucleotides 1517–1525 in the *rpoB* open reading frame, TCGGTTCCA) contained no obviously unusual features (e.g. A-T rich, long tracts of single nucleotides, self-annealing sequences). Mutations at I572 and S574 in cluster II have also previously been identified in RIF-resistant *E. coli* clones [Barrick et al., 2010; Jin and Gross, 1989; Makiela-Dzbenska et al., 2011; Sandgren et al., 2009; Zhou et al., 2013]. Several of these mutations occurred in positions that increased transcript deletions (Q513) or decreased transcriptional elongation slippage (D516 and I572) by *E. coli* RNA polymerase [Zhou et al., 2013].

Two of the clones (out of 81 total) contained no mutations to the coding region for residues 473–720. It is possible that these contained a mutation to the N-terminal cluster of rpoB or other regions of genes encoding RNA polymerase. Alternatively, mutations of other *E. coli* genes could lead to RIF secretion, or breakdown could also result in resistance. We did not subject these clones to genome sequencing to determine whether this was the case. Of the sequenced clones, the H526Y mutant was the most abundant (29/81). The mutations of nucleotides 1546 (GAC to AAC; D516N), 1547 (GAC to GGC; D516G), 1576 (CAC to TAC; H526Y), and 1592 (TCC to TTC; S531F) were the only transition mutations observed (35/81).

Table 1. Mutations to *rpoB* gene identified from rifampicin-resistant *E. coli*

Residue No.	Protein sequence change	Mutation	Observed clones, *n*
506–509	FGSS to C	9 bp (1517–1525) deletion	1
512	S to Y	TCT to TAT (1534)	1
513	Q to K	CAG to AAG (1537)	2
	Q to L	CAG to CTG (1538)	4
	Q to P	CAG to CCG (1538)	1
516	D to Y	GAC to TAC (1546)	3
	D to N	GAC to AAC (1546)	1
	D to V	GAC to GTC (1547)	11
	D to G	GAC to GGC (1547)	2
526	H to Y	CAC to TAC (1576)	29
	H to N	CAC to AAC (1576)	8
	H to D	CAC to GAC (1576)	1
	H to L	CAC to CTC (1577)	4
531	S to F	TCC to TTC (1592)	3
572	I to F	ATC to TTC (1714)	6
	I to L	ATC to CTC (1714)	1
574	S to Y	TCT to TAT (1721)	1

In mutation, nucleotide number in *E. coli rpoB* open reading frame in parentheses.

Relationship of Mutations to the RIF-Binding Site in RNA Polymerase

RIF-resistant mutants were mostly clustered to the RIF binding site in RNA polymerase β (Fig. 1b). Several of the residues where mutations occur are adjacent to where RIF binds (Q513, D516, H526, S531, I572). Mutations to these first shell residues are expected to alter the shape and/or chemical characteristics of the RIF binding site without substantially affecting the structure or function of RNA polymerase. According to the RNA polymerase-RIF complex structure [Molodtsov et al., 2013], the serine 512 and 574 side chains do not contact RIF, but point towards the interior of the protein. Mutation to a larger tyrosine at residue S512 or at S574 would require a change to the backbone structure, leading to possible changes in the RIF binding surface.

Unusual In-Frame Deletion Near RIF Binding Site

The deletion of 9 base pairs (1517–1525; Δ9) from the *rpoB* gene changes 506-FGSS-509 to C506. Because RNA polymerase is essential to the survival and growth of *E. coli*, we concluded that the Δ9 mutation did not create a nonfunctional protein and that the folding and structure of the enzyme were largely retained. In wild-type RNA polymerase, residues 506–509 reside at the end of an α-helix adjacent to the RIF binding site (Fig. 1b, 2a). However, none of the residues contact RIF in the crystal structure.

To understand the impact of the 3-residue deletion on the structure of RNA polymerase, the translated protein sequence from the Δ9 mutant of *rpoB* was threaded into known crystal structures of *E. coli* RNA polymerase using iterative threading assembly refinement (I-TASSER [Yang et al., 2015]). The Δ9 mutant model with the highest confidence score (C score of 1.65) produced by I-TASSER was highly similar to known *E. coli* RNA polymerase crystal structures with identical secondary struc-

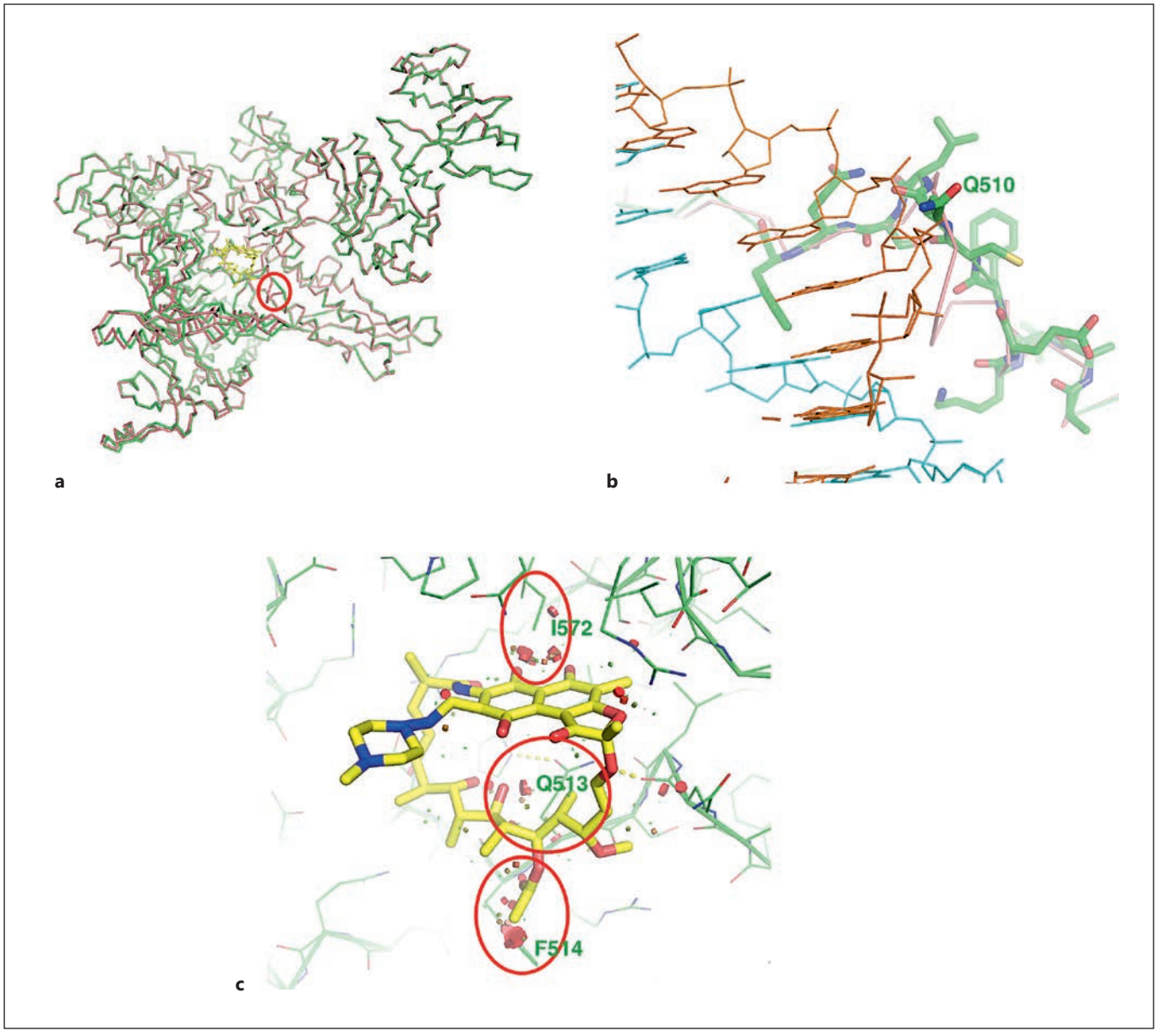

Fig. 2. The structure of the I-TASSER model of the Δ9 mutant. **a** The Δ9 mutant model protein backbone (green lines connecting α-carbons) is superimposed on the crystal structure of rifampicin (pink lines) bound to the wild-type *E. coli* RNA polymerase β-chain (4KMU.pdb; gray ribbon). Rifampicin is shown in yellow sticks. The red oval highlights the location of 506-FGSS-509 in the wild-type RNA polymerase, which is mutated to C506 in the Δ9 mutant. **b** The Δ9 mutant (green lines connecting α-carbons) is superimposed on the crystal structure of a DNA (blue)-RNA (brown) hybrid bound to the *E. coli* RNA polymerase transcriptional elongation complex (5UPC.pdb; pink lines). Residues 500–514 of the Δ9 mutant model are shown in sticks. **c** Superimposition of rifampicin (sticks with yellow carbons) onto the Δ9 mutant model indicates steric clashes (red disks) with Q513, F514, and I572 (highlighted in red ovals). The side chain of Q513 forms a hydrogen bond (yellow dotted line) with the backbone carbonyl of Q510 (wild-type numbering). **a–c** and Figure 1b are viewed in the same orientation. Figures and clashes were generated in PyMol [DeLano, 2014].

Table 2. Protein sequence alignment of several bacterial RNA polymerases in RRDR I and II

Bacterial species	*rpoB* residue	RDRR I and II protein sequence
Escherichia coli	505–533, 571–575	F**FGSS**QLSQFMDQNNPLSEITHKRRISAL LINSL
Pseudomonas aeruginosa	508–538, 574–578	F**FGSS**QLSQFMDQNNPLSEITHKRRVSAL LINSL
Neisseria meningitidis	527–557, 598–602	F**FGSS**QLSQFMDQTNPLSEVTHKRRVSAL LINSL
Clostridioides difficile	479–509, 548–552	F**FGSS**QLSQFMDQTNPLSELTHKRRLSAL LINSL
Streptococcus pneumoniae	475–505, 544–548	F**FGSS**QLSQFMDQHNPLSELSHKRRLSAL LINNL
Staphylococcus aureus	459–488, 525–529	F**FGSS**QLSQFMDQANPLAELTHKRRLSAL LINSL
Mycobacterium tuberculosis	422–452, 490–494	F**FGTS**QLSQFMDQNNPLSGLTHKRRLSAL LIGSL
Mycobacterium smegmatis	419–449, 487–491	F**FGTS**QLSQFMDQNNPLSGLTHKRRLSAL LIGSL
Chlamydia pneumoniae	448–478, 516–519	F**FGRS**QLSQFMDQTNPVAELTHKRRLSAL LITSL
Thermus thermophilus	383–413, 451–455	F**FSRS**QLSQFKDETNPLSSLRHKRRISAL LITSL
Thermus aquaticus	383–413, 451–455	F**FSRS**QLSQFKDETNPLSSLRHKRRISAL LITSL
Sequence conservation		**:****** * **: : ****:*** ** **
Mutations in this study		____ ^^ ^ ^ ^ ^ ^

Protein sequences are shown in 1-letter code. Any sequence corresponding with the Δ9 deletion mutation is shown in bold and denoted by dashes in the last row. Point mutations observed in this study are denoted by ^ in the last row. Sequence conservation: complete sequence identity amongst the RPOB sequences represented here denoted by an asterisk; conservation of amino acid type (positively or negatively charged, hydrophobic, hydrophilic, side chain size) denoted by a colon.

tural elements and a similar RIF binding site (Fig. 2a). The 506-FGSS-509 sequence lies at the C-terminal end of an α-helix pointing to where RIF binds, with glycine 507 marking the end of the helix and the start of a loop. The threaded model generated by I-TASSER indicates that shortening of the β-chain sequence by 3 residues leads to the truncation of the α-helix by one turn, but no substantial changes to the rest of the protein backbone. A comparison of the RNA polymerase-RIF complex crystal structure (4KMU [Molodtsov et al., 2013]) with the *E. coli* RNA polymerase elongation complex cryoelectron microscopy structure (5UPC [Kang et al., 2017]) indicates that RIF inhibits RNA synthesis by blocking the path of the nascent RNA chain [Campbell et al., 2001; Lin et al., 2017; McClure, and Cech, 1978] and that residues 506–509 of the β-chain lie near where the backbone of the transcript and template strand would lie in the transcriptional elongation complex (Fig. 2b). The side chain of Q510 (wild-type numbering) in the Δ9 mutant makes contact with the RNA transcript backbone (Fig. 2b), but the clash could be alleviated by a rotamer change. The observation that the Δ9 *rpoB* mutant formed a colony suggests that RNA polymerase activity is not substantially decreased by the unusual mutation.

Although the Δ9 mutant does not result in large structural rearrangements near the mutation site, the sequence change does lead to RIF^R. The backbone of the next 5 residues after the Δ9 mutation (QLSQF, numbered residues 510–514 in the wild-type sequence) follows closely the structure of full-length, wild-type *rpoB* (Fig. 2c), but subtle structural changes in the Δ9 mutant model lead to steric clashes with RIF, particularly at Q513, F514, and I572 (wild-type numbering). The strong overlap between RIF atoms and the Δ9 mutant model denoted by red disks in Figure 2c suggests that RIF may not bind well to the mutant without alterations to the protein surface. One such change could be the rotations of side chains away from RIF if there is space. The clashes between RIF and F514 and I572 can be resolved by rotamer changes in the 2 side chains. However, the model indicates that the side chain of Q513 forms hydrogen bonds with the Q510 backbone and H526 side chain. We note that the hydrogen bond between Q510 and Q513 in the Δ9 mutant model is not present in wild-type crystal structures (4KMU.pdb and 5UPC.pdb). This interaction could prevent the Q513 side chain from swinging out of the way of RIF. Without an experimental atomic structure of this Δ9 mutant of RNA polymerase, we cannot be sure how this mutation confers RIF^R, but we speculate that the subtle changes in structure immediately after residue 506, specifically the formation of a new backbone-side chain hydrogen bond, lock the β-chain into a conformation that has a lower binding affinity to RIF, thereby conferring resistance.

Implications for RIF^R in M. tuberculosis *and Other Bacteria*

RIF^R is a major problem for the treatment of human infections by *Staphyloccocus aureus* and *M. tuberculosis*. Many RIF-resistant clinical isolates of *Staphyloccocus* contain nonsynonymous single-nucleotide substitutions that result in amino acid substitutions in the RIF binding site (e.g. S455A, L466S, H481N) [Hellmark et al., 2009; Murugan et al., 2015; Zhou et al., 2012], but a 3-nucleotide insertion mutant of *rpoB* has also been isolated [Hellmark et al., 2009]. A 9-nucleotide deletion similar to the Δ9 mutant identified in our study may also generate a RIF^R mutant, as the RNA polymerase β amino acid sequence corresponding to 506-FGSS-509 in *E. coli* is the same in *S. aureus* (Table 2). A comparison of crystal structures of RIF bound to RNA polymerases of *M. tuberculosis* (*Mt*rpoB) and *E. coli* (*Ec*rpoB) indicates that RIF binds to highly conserved sites with nearly identical structures in both enzymes [Lin et al., 2017]. The most clinically important mutation in the treatment of tuberculosis, serine 450 (or 531 in *E. coli*) to leucine [Telenti et al., 1993; Williams et al., 1998], was not observed in our screen due to the codon-encoding serine (TCT) requiring 2 mutations to mutate the residue to leucine. We did observe a similar S531F mutant, which required only a single mutation (TCC to TTC). The Δ9 RIF-resistant mutant in *E. coli* converts 506-FGSS-509 to C506. Homologous residues in RNA polymerase β-chains of 10 other bacteria are almost identical to *E. coli* RNA polymerase (Table 2). A similar 9-nucleotide deletion in *Mt*rpoB would change the corresponding sequence to 425-SQLSQF-428 instead of CQLSQF in *Ec*rpoB. Serine and cysteine differ only by 1 atom (oxygen replaced by sulfur) and are chemically similar. Thus, we hypothesize that a similar 9-nucleotide deletion mutation may also confer resistance in *M. tuberculosis* and other bacteria.

Materials and Methods

Selection of RIF^R Strains of E. coli

E. coli K-12 MG1655 was streaked on LB agar plates to yield single colonies. Colonies were picked and dissolved in 200 μL sterile saline. 10 μL of bacteria were diluted to individual cultures of 25 mL of LB and shaken overnight at 37°C. 200 μL of each bacterial culture was plated on LB agar plates containing 100 μg/mL RIF and cultured at 37°C overnight. Plates generally contained between 5 and 250 colonies. In addition, each individual 25-mL culture was serially diluted to 10^{-5} or 10^{-6} and plated on LB agar plates without antibiotics to determine the concentration of live bacteria in the culture.

Sequencing of E. coli *rpoB Gene Fragments Form RIF^R Strains*

Colonies were restreaked onto LB + RIF plates to obtain freshly grown bacteria. Single colonies were picked and dissolved in 1 × GoTaq Green Master Mix (Promega Corporation, Madison, WI, USA) with 1 μM rpoBforward (5′-CGT GCG GTG AAA GAG CGT CTG TCT-3′) and rpoBreverse (5′-ACG TTT AGC TAC CGC AGT TAC ACC-3′) primers. The primers are designed to amplify nucleotides 1417–2160 of the *E. coli* K-12 MG1655 rpoB open reading frame. The rpoB fragment was amplified directly from colonies with 30 cycles of 95°C for 30 s, 57°C for 45 s, and 72°C for 1 min after initial denaturation at 95°C for 2 min. PCR amplification was confirmed by agarose gel electrophoresis. PCR products were purified using the MinElute PCR Purification Kit (Qiagen Sciences, Germantown, MD, USA) and sequenced by Sanger DNA sequencing using the rpoBforward primer (Genewiz Inc., South Plainfield, NJ, USA).

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Disclosure Statement

The authors have no conflicts of interest to declare.

References

Artsimovitch I, Vassylyeva MN, Svetlov D, Svetlov V, Perederina A, Igarashi N, et al: Allosteric modulation of the RNA polymerase catalytic reaction is an essential component of transcription control by rifamycins. Cell 2005;122:351–363.

Baquero MR, Nilsson AI, Turrientes MDC, Sandvang D, Galán JC, Martínez JL, et al: Polymorphic mutation frequencies in *Escherichia coli*: emergence of weak mutators in clinical isolates. J Bacteriol 2004;186:5538–5542.

Barrick JE, Kauth MR, Strelioff CC, Lenski RE: *Escherichia coli rpoB* mutants have increased evolvability in proportion to their fitness defects. Mol Biol Evol 2010;27:1338–1347.

Bjedov I, Tenaillon O, Souza V, Denamur E, Radman M, Gerard B, et al: Stress-induced mutagenesis in bacteria. Science 2003;300:1404–1409.

Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, et al: Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell 2001;104:901–912.

DeLano WL: The PyMOL Molecular Graphics System, Version 1.8. Schrödinger LLC. 2014. http://www.pymol.org.

Galán JC, Turrientes MC, Baquero MR, Rodríguez-Alcayna M, Martínez-Amado J, Martínez JL, et al: Mutation rate is reduced by increased dosage of *mutL* gene in *Escherichia coli* K-12. FEMS Microbiol Lett 2007;275:263–269.

Gill SK, Garcia GA: Rifamycin inhibition of WT and Rif-resistant *Mycobacterium tuberculosis* and *Escherichia coli* RNA polymerases in vitro. Tuberculosis 2011;91:361–369.

Gillet-Markowska A, Louvel G, Fischer G: bz-rates: a web tool to estimate mutation rates from fluctuation analysis. G3 (Bethesda) 2015;5:2323–2327.

Hellmark B, Söderquist B, Unemo M: Simultaneous species identification and detection of rifampicin resistance in staphylococci by sequencing of the *rpoB* gene. Eur J Clin Microbiol Infect Dis 2009;28:183–190.

Jin DJ, Gross CA: Characterization of the pleiotropic phenotypes of rifampin-resistant *rpoB* mutants of *Escherichia coli*. J Bacteriol 1989;171:5229–5231.

Kang JY, Olinares PDB, Chen J, Campbell EA, Mustaev A, Chait BT, et al: Structural basis of transcription arrest by coliphage HK022 Nun in an *Escherichia coli* RNA polymerase elongation complex. Elife 2017;6:e25478.

Lin W, Mandal S, Degen D, Liu Y, Ebright YW, Li S, et al: Structural basis of *Mycobacterium tuberculosis* transcription and transcription inhibition. Mol Cell 2017;66:169–179.e8.

Luria S, Delbrück M: Mutations of bacteria from virus sensitivity to virus resistance. Genetics 1943;28:491–511.

Makiela-Dzbenska K, Jonczyk P, Schaaper RM, Fijalkowska IJ: Proofreading deficiency of Pol I increases the levels of spontaneous *rpoB* mutations in *E. coli*. Mutat Res Fundam Mol Mech Mutagen 2011;712:28–32.

McClure WR, Cech CL: On the mechanism of rifampicin inhibition of RNA synthesis. J Biol Chem 1978;253:8949–8956.

Molodtsov V, Nawarathne IN, Scharf NT, Kirchhoff PD, Showalter HDH, Garcia GA, et al: X-ray crystal structures of the *Escherichia coli* RNA polymerase in complex with benzoxazinorifamycins. J Med Chem 2013;56:4758–4763.

Molodtsov V, Scharf NT, Stefan MA, Garcia GA, Murakami KS: Structural basis for rifamycin resistance of bacterial RNA polymerase by the three most clinically important *RpoB* mutations found in *Mycobacterium tuberculosis*. Mol Microbiol 2017;103:1034–1045.

Murugan K, Kavitha K, Al-Sohaibani S: Rifampicin resistance among multi-resistant MRSA clinical isolates from Chennai, India, and their molecular characterization. Genet Mol Res 2015;14:2716–2725.

Sandgren A, Strong M, Muthukrishnan P, Weiner BK, Church GM, Murray MB: Tuberculosis drug resistance mutation database. PLoS Med 2009;6:0132–0136.

Sensi P: History of the development of rifampin. Rev Infect Dis 1983;5:S402–S406.

Telenti A, Imboden P, Marchesi F, Matter L, Schopfer K, Bodmer T, et al: Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. Lancet 1993;341:647–651.

Williams DL, Spring L, Collins L, Miller LP, Heifets LB, Gangadharam PRJ, et al: Contribution of rpoB mutations to development of rifamycin cross-resistance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 1998;42:1853–1857.

Xu M, Zhou YN, Goldstein BP, Jin DJ: Cross-resistance of *Escherichia coli* RNA polymerases conferring rifampin resistance to different antibiotics. J Bacteriol 2005;187:2783–2792.

Yang J, Yan R, Roy A, Xu D, Poisson J, Zhang Y: The I-TASSER suite: protein structure and function prediction. Nat Meth 2015;12:7–8.

Ycart B, Veziris N: Unbiased estimation of mutation rates under fluctuating final counts. PLoS One 2014;9.e101434.

Zhou W, Shan W, Ma X, Chang W, Zhou X, Lu H, et al: Molecular characterization of rifampicin-resistant *Staphylococcus aureus* isolates in a Chinese teaching hospital from Anhui, China. BMC Microbiol 2012;12:240.

Zhou YN, Lubkowska L, Hui M, Court C, Chen S, Court DL, et al: Isolation and characterization of RNA polymerase *rpoB* mutations that alter transcription slippage during elongation in *Escherichia coli*. J Biol Chem 2013;288:2700–2710.