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Putative Compensatory Mutations in the *rpoC* Gene of Rifampin-Resistant *Mycobacterium tuberculosis* Are Associated with Ongoing Transmission

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Rifampin resistance in clinical isolates of *Mycobacterium tuberculosis* arises primarily through the selection of bacterial variants harboring mutations in the 81-bp rifampin resistance-determining region of the *rpoB* gene. While these mutations were shown to infer a fitness cost in the absence of antibiotic pressure, compensatory mutations in *rpoA* and *rpoC* were identified which restore the fitness of rifampin-resistant bacteria carrying mutations in *rpoB*. To investigate the epidemiological relevance of these compensatory mutations, we analyzed 286 drug-resistant and 54 drug-susceptible clinical *M. tuberculosis* isolates from the Western Cape, South Africa, a high-incidence setting of multidrug-resistant tuberculosis. Sequencing of a portion of the RpoA-RpoC interaction region of the *rpoC* gene revealed that 23.5% of all rifampin-resistant isolates tested carried a nonsynonymous mutation in this region. These putative compensatory mutations in *rpoC* were associated with transmission, as 30.8% of all rifampin-resistant isolates with an IS*6110* restriction fragment length polymorphism (RFLP) pattern belonging to a recognized RFLP cluster harbored putative *rpoC* mutations. Such mutations were present in only 9.4% of rifampin-resistant isolates with unique RFLP patterns ($P < 0.01$). Moreover, these putative compensatory mutations were associated with specific strain genotypes and the *rpoB* S531L rifampin resistance mutation. Among isolates harboring this *rpoB* mutation, 44.1% also harbored *rpoC* mutations, while only 4.1% of the isolates with other *rpoB* mutations exhibited mutations in *rpoC* ($P < 0.001$). Our study supports a role for *rpoC* mutations in the transmission of multidrug-resistant tuberculosis and illustrates how epistatic interactions between drug resistance-conferring mutations, compensatory mutations, and different strain genetic backgrounds might influence compensatory evolution in drug-resistant *M. tuberculosis*.

Drug resistance in bacteria is often associated with reduced Darwinian fitness in the absence of drug pressure (1–4). However, this fitness cost is not universal. For example, some clinical isolates of drug-resistant *Mycobacterium tuberculosis* have been shown to have levels of fitness similar to those of wild-type bacteria (3, 5, 6). The heterogeneous fitness effects of drug resistance-conferring mutations can be attributed to several factors, including the preexisting differences in the genetic strain background, the presence of additional drug resistance-conferring mutations, and compensatory mutations (7, 8). Compensatory mutations alleviate the fitness cost associated with drug resistance mutations, resulting in overall fitness comparable to that of a wild-type strain in the absence of antibiotics (2, 5, 9). Compensatory mutations linked to isoniazid and aminoglycoside resistance have been described for *M. tuberculosis* (10, 11). However, these mutations are rarely seen in clinical isolates, thus their epidemiological importance appears to be minor.

M. tuberculosis can acquire resistance to rifampin through mutations in the gene encoding its target, *rpoB* (12). Mutations in an 81-bp core region of the *rpoB* gene, known as the rifampin resistance-determining region (RRDR), account for 95% of the rifampin resistance detected in clinical isolates (12). A number of studies have shown that the acquisition of rifampin resistance is associated with a fitness cost (3–5). However, in some cases clinical isolates harboring the *rpoB* S531L mutation had no fitness cost, like parent wild-type isolates (3, 5). For these clinical isolates, it was suggested that either the *rpoB* S531L mutation incurred no fitness cost in these particular strain genetic backgrounds or that secondary mutations were acquired that mitigate the initial low fitness cost of the *rpoB* S531L mutation (5). In support of the latter, a set of putative compensatory mutations in the genes of the RNA polymerase subunits RpoA and RpoC was described in rifampin-resistant *M. tuberculosis* isolates (13). These mutations were associated with increased *in vitro* fitness and were overrepresented among multidrug-resistant (MDR) strains from high-MDR-tuberculosis (TB)-burden countries. A subsequent study revealed that 14 out of 24 clinical isolates of *M. tuberculosis* harboring an *rpoB* mutation also carried nonsynonymous mutations in the genes *rpoA* or *rpoC* (14). These putative compensatory mutations were identified predominantly in the RpoA-RpoC interaction area of the *rpoC* gene (amino acid positions 356 to 756) (13). The gene sequences of the subunits of the RNA polymerase are

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TABLE 1 Population structure of clinical isolates selected in the Western Cape, South Africa[a]

Family	Subfamily	No. isolates by resistance status							
		DS	INH	Poly	RIF	MDR	PRE-XDR	XDR	All
Beijing	All	20	9	1	22	41	24	33	150
Beijing	Typical	20	8	1	15	27	9	2	82
Beijing	Atypical	0	1	0	1	11	14	31	58
LCC	All	23	5	7	3	22	4	7	71
LAM	All	10	8	1	13	21	8	3	64
T	All	0	7	0	11	5	5	1	29
Unknown	All	1	2	0	3	9	3	2	20
F28	All	0	0	0	0	2	0	0	2
Haarlem	All	0	0	0	0	0	0	2	2
CAS	All	0	0	0	0	0	0	0	0
EAI	All	0	0	0	1	0	0	0	1
F26	All	0	0	0	0	1	0	0	1
Total		54	31	9	53	101	44	48	340

[a] DS, drug susceptible; INH, isoniazid monoresistant; Poly, rifampin/isoniazid monoresistant plus other resistance to at least one other drug (not MDR); RIF, rifampin monoresistant; LCC, low-copy-clade lineage; LAM, Latin American Mediterranean lineage; CAS, Central Asian strains; EAI, East Africa India lineage; F26, S family.

generally highly conserved in *M. tuberculosis* (15). Hence, nonsynonymous variants in these genes likely represent recently acquired adaptive mutations rather than natural polymorphisms. Finally, using genetic reconstruction and competitive growth assays, a recent study of *Salmonella enterica* has shown that secondary mutations in *rpoA*, *rpoB*, and *rpoC* were necessary and sufficient to compensate for fitness costs incurred by rifampin resistance mutations (16). Here, we aimed to determine the epidemiological relevance of nonsynonymous mutations in *rpoA* and *rpoC* in a high-incidence setting of MDR-TB in South Africa.

MATERIALS AND METHODS

Study population and drug susceptibility testing. This study was approved by the Health Research Ethics Committee of Stellenbosch University. In total, 340 (54 drug-susceptible and 286 drug-resistant) well-characterized *M. tuberculosis* isolates were selected from an extensive longitudinal collection of drug-sensitive and suspected drug-resistant *M. tuberculosis* isolates collected in the Western Cape, South Africa, from 2000 to 2010 (17, 18). Patient isolates were submitted to the National Health Laboratory Services (NHLS) for routine culture and phenotypic drug susceptibility testing (DST) as described previously (18). Genotyping and sequencing of genes known to cause resistance was done at Stellenbosch University as described previously (18–20). Isolates were selected from the overall collection based on the availability of IS*6110* restriction fragment length polymorphism (RFLP) typing and/or spoligotyping fingerprints, as well as either phenotypic or genotypic drug susceptibility data. Only one isolate per patient was included. The sample of isolates included here represented a mixture of different types of drug resistance profiles, including isoniazid monoresistant, rifampin monoresistant, MDR *sensu stricto* (resistant to at least rifampin and isoniazid, excluding pre-XDR [extensively drug resistant] and XDR cases), polyresistant (non-MDR isolates resistant to more than one of the first-line drugs), XDR (MDR plus resistance to any fluoroquinolone and one of the second-line injectable drugs, i.e., amikacin, capreomycin, and kanamycin), and pre-XDR (MDR with resistance to either a fluoroquinolone or a second-line injectable drug but not both). The genetic population structure of our sample set is summarized in Table 1.

Genotyping. Isolates were genotyped by spoligotyping according to standardized protocols as described previously (21–23). Isolates were also genotyped with IS*6110* RFLP fingerprinting as previously described (24). Beijing isolates were further differentiated into typical and atypical Beijing isolates by PCR (25). Isolates belonging to the atypical Beijing family of strains of the Western and Eastern Cape Provinces of South Africa were previously shown to belong to a single clonal group of bacteria, which was termed the R86 genotype of strains (26). Isolates belonging to a cluster exhibited IS*6110* RFLP patterns which were present at least twice in our overall database. Nonclustered patterns were unique and not detected in any other isolate of our full collection.

PCR amplification and DNA sequencing. Oligonucleotide primers (Table 2) were designed using Primer software 3, version 0.2 (Whitehead Scientific), for PCR amplification and sequencing of the entire *rpoA* locus (Rv3457c), the entire *rpoC* locus (Rv0668), and a portion of the region encoding the RpoA-RpoC interaction site in *rpoC* (Rv0668; amino acids 245 to 560) (26). PCRs were performed under the following thermocycling conditions: 15 min of denaturation at 95°C, followed by 40 amplification cycles (each cycle consisted of 94°C for 1 min, 62°C for 1 min, and a 1-min extension at 72°C) and an elongation step of 10 min at 72°C. PCR products were purified and sequenced with the ABI PRISM DNA Sequencer model 377 (PerkinElmer). Sequence polymorphisms were identified by comparing the consensus sequence of each isolate to the corresponding gene sequence of the H37Rv genome using BioEdit (v7.1.3) (27).

Statistical analyses. To test for an association between putative compensatory mutations and clustering, IS*6110* RFLP patterns of the 340 isolates included were compared to RFLP patterns obtained from our full

TABLE 2 Oligonucleotide primers used in this study for the detection of polymorphism in *rpoA* and *rpoC*

Gene	Gene range (amino acids)	Orientation	Oligonucleotide sequence	T_m (°C)
rpoA1	1–191 (6 bp upstream)	Forward	5′ GCATTCCAGTCGATTCCATC 3′	60.43
		Reverse	5′ CCAAGATCGCCTTCTGATGT 3′	60.22
rpoA2	167–348 (67 bp downstream)	Forward	5′ GGACGTCGAAAGGAAGAAGA 3′	59.41
		Reverse	5′ GTCTCCACGTCCAGGATCAG 3′	60.68
rpoC1	1–263 (72 bp upstream)	Forward	5′ AATCTGTCCCGCAACGAATC 3′	62.32
		Reverse	5′ CGTCGATGTCGAAGTTCTCG 3′	61.94
rpoC2	245–560	Forward	5′ CGAAAACCTCTACCGCGAAC 3′	62.02
		Reverse	5′ GCGACAGGATGTTGTTGGAG 3′	61.67
rpoC3	533–834	Forward	5′ TGGTGTGTGAGGCGTTCAAT 3′	62.55
		Reverse	5′ CACGGAAGGAGGACTTGACC 3′	62.01
rpoC4	825–1131	Forward	5′ GGTATGAAGGGCCTGGTGAC 3′	61.68
		Reverse	5′ ACCTCGCGAACCAGGTGTAT 3′	61.86
rpoC5	1070–1197 (131 bp downstream)	Forward	5′ TCACCATCGTTCCTGACGAC 3′	62.13
		Reverse	5′ GGGATTGCCACTCATGTTGA 3′	61.89

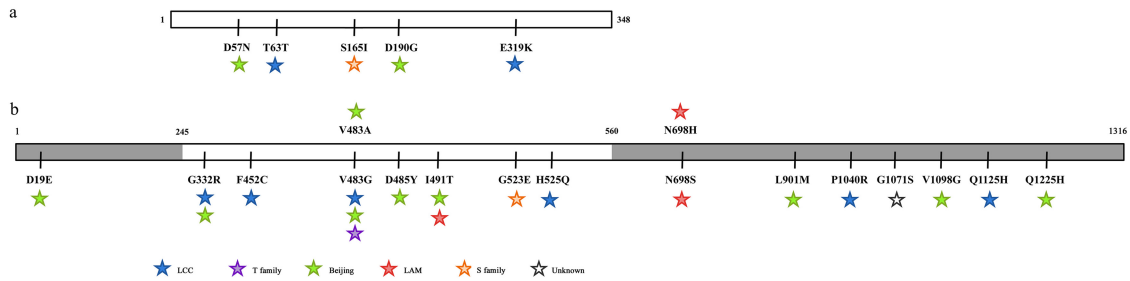

FIG 1 Synonymous and nonsynonymous mutations identified in *rpoA* (a) and *rpoC* (b) of isolates collected from the Western Cape, South Africa. Each star indicates the presence of a mutation in a specific family of strains.

sample bank. Statistical analyses were performed in IC Stata 10.0 (StataCorp LP, College Station, TX). The Fisher's exact test and univariate and multivariate logistic regression analyses were done to identify associations of putative compensatory mutations in *rpoC* with clustering, level of drug resistance, strain genotype, and different rifampin resistance mutations in *rpoB*. $P < 0.05$ was considered statistically significant.

RESULTS

A well-characterized convenience sample of 340 clinical *M. tuberculosis* isolates was used in this study. These included 54 drug-susceptible isolates, 247 rifampin-resistant isolates (including 53 rifampin-monoresistant, 1 polyresistant, 101 MDR *sensu stricto*, 44 pre-XDR, and 48 XDR isolates) and 39 isoniazid-monoresistant or polyresistant isolates. We tested for the presence of nonsynonymous, potential compensatory mutations in *rpoA* and a region of *rpoC* (amino acid positions 245 to 560) previously shown to be prone to acquire compensatory mutations and including the RpoA-RpoC interaction area of *rpoC* (13). Only 8 (3.5%) out of 227 rifampin-resistant isolates tested showed nonsynonymous mutations in *rpoA*. Altogether, 4 distinct nonsynonymous mutations (D57N, S165I, D190G, and E319K) were identified (Fig. 1A); in addition, 1 isolate harbored a synonymous mutation in *rpoA* (T63T). Given their infrequent occurrence, mutations identified in *rpoA* were excluded from further analyses.

Overall, 23.5% (58 isolates) of 247 rifampin-resistant clinical isolates tested harbored nonsynonymous mutations in the sequenced portion of *rpoC*. All isolates exhibiting nonsynonymous mutations in *rpoC* also harbored mutations in the RRDR of *rpoB* (Fig. 1B). Eight different nonsynonymous mutations in *rpoC* (G332R, F452C, D485Y, V483A, V483G, I491T, Q523E, and H525Q) accounted for all nonsynonymous mutations detected. In addition, one synonymous substitution (A542A) was identified. This mutation was previously reported to be a phylogenetic marker for the Latin American Mediterranean family of *M. tuberculosis* strains and therefore was excluded from further analyses (13). All of the mutations described above were absent from all 93 rifampin-sensitive isolates tested.

Nonsynonymous mutations in *rpoC* at identical amino acid positions were previously detected in MDR *M. tuberculosis* strains from various countries, including Russia, Ghana, Abkhazia/Georgia, Kazakhstan, and Uzbeksitan, but not in rifampin-susceptible isolates from these countries (13, 14). This demonstrates the repeated and independent emergence of these mutations in geographically distant settings and convergent evolution across phylogenetically distinct lineages of *M. tuberculosis*, indicating that these *rpoC* mutations confer a selective advantage to rifampin-resistant strains and probably represent compensatory mutations, as suggested previously (13, 14). A high propensity for homoplastic mutations at these amino acid positions also is demonstrated directly by our data. For example, three (G332R, V483G, and I491T) of the eight nonsynonymous mutations detected within our sample set evolved independently in phylogenetically distantly related strain families as defined by spoligotyping (Fig. 1).

To test whether the putative compensatory mutations in *rpoC* identified here were associated with increased transmissibility of drug-resistant *M. tuberculosis*, we compared the presence of these *rpoC* mutations between isolates belonging to a recognized IS*6110* RFLP cluster to those of isolates showing unique RFLP patterns. Among rifampin-resistant isolates with clustered RFLP patterns, 30.8% (48/156 isolates; 95% confidence interval [CI], 23.6 to 38.6%) harbored mutations in *rpoC*, significantly more than among rifampin-resistant isolates with nonclustered RFLP patterns (5/53 isolates; 9.4%; 95% CI, 3.1 to 20.7%; $P = 0.002$ by Fisher's exact test) (Fig. 2). Mutations in *rpoC* were detected in one-third of the RFLP clusters present among rifampin-resistant isolates (in 12/36 RFLP clusters) (Fig. 2). Two of these RFLP clusters showed two distinct *rpoC* mutations, and a third cluster harbored four different *rpoC* mutations. Thus, mutations in *rpoC* were acquired at least 17 times among the 36 RFLP clusters, while only 5 of 53 nonclustered RFLP types harbored *rpoC* mutations.

The entire *rpoC* gene from 54 isolates showing nonclustered IS*6110* RFLP patterns was sequenced in order to assess the presence of mutations independent of transmission and outside the genetic region covered by our initial survey. Altogether, 9 iso-

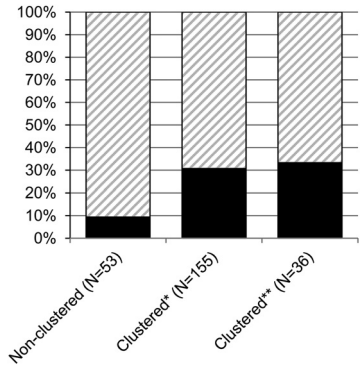

FIG 2 Proportion of rifampin-resistant isolates or IS*6110* RFLP types/clusters harboring *rpoC* mutations. *, The number of isolates classified as clustered, based on IS*6110* RFLP type, harboring the *rpoC* mutation ($P < 0.05$ by Fisher's exact test); **, the number of IS*6110* RFLP types classified as clustered harboring an *rpoC* mutation ($P < 0.05$ by Fisher's exact test).

TABLE 3 Logistic regression analysis for factors influencing the presence of compensatory mutations in *rpoC*[d]

Characteristics and variants	Regression analysis findings					
	Unadjusted			Adjusted[a]		
	Freq	OR	*P* value	Freq	OR	*P* value
Drug resistance group[b]						
R mono	53	NA	NA	20	NA	NA
MDR s.s.	101	5.5	0.026	71	1.0	0.958
Pre-XDR	44	19.4	<0.001	37	4.7	0.085
XDR	48	16.7	<0.001	46	0.9	0.926
Strain family[c]						
Non-atypical (non-R86)	177	NA	NA	118	NA	NA
Atypical (R86)	57	8.8	<0.001	56	28.0	<0.001
Resistance mutation[c]						
Other *rpoB* mutations	49	NA	NA	49	NA	NA
rpoB S531L mutation	127	18.5	0.001	125	125.2	<0.001

[a] Analysis adjusted for drug resistance group, strain family, and rifampin resistance mutation.
[b] Mutations in *rpoC* were only present among rifampin-resistant isolates. Other drug resistance groups were excluded from the analysis.
[c] Only rifampin-resistant isolates were included.
[d] Freq, frequency of isolates showing a given characteristic included in the logistic regression analysis; OR, odds ratio; NA, not applicable; R mono, rifampin monoresistant; MDR s.s., multidrug-resistant *sensu stricto* (excluding identified pre-XDR and XDR isolates).

lates (16.7%) harbored 10 additional nonsynonymous mutations (D19E, G594E, N698H, N698S, L901M, P1040R, G1071S, V1098G, Q1125H, and Q1225H) (see Table S1 in the supplemental material). Interestingly, two isolates showed two distinct mutations at amino acid position 698 (N698H and N698S), and mutations at this position were observed previously among strains from other settings (13). The remaining mutations were not detected in earlier studies (13, 14). In addition, the V1098G mutation was found in a rifampin-monoresistant isolate not harboring any mutation in the RRDR of *rpoB*. Thus, except for the mutations at amino acid position 698, there is little evidence that these mutations play a role in compensatory evolution. Possibly, only mutations in the RpoA-RpoC interaction region of the *rpoC* gene contribute to ameliorating the fitness cost of *rpoB* resistance mutations.

Analyzing the proportion of *rpoC* mutations across isolates with various degrees of drug resistance showed that this proportion increased significantly toward higher levels of drug resistance, with 39.6% of XDR-TB isolates harboring nonsynonymous *rpoC* mutations ($P < 0.001$) (Table 3). Similarly, *rpoC* mutations were nonrandomly distributed among different families of strains. In particular, a genotype of atypical Beijing strains known as the R86 cluster showed a more than four times higher proportion of isolates harboring *rpoC* mutations than isolates belonging to other genotypes (57.9 versus 13.6% among rifampin-resistant isolates; $P < 0.001$) (Table 3).

Interestingly, the presence of nonsynonymous mutations in *rpoC* was significantly associated with the *rpoB* S531L mutation ($P < 0.001$; Table 3). Among isolates harboring this mutation, 44.1% also harbored *rpoC* mutations, while only 4.1% of the isolates with other *rpoB* mutations exhibited mutations in *rpoC*. A broad range of *rpoC* mutations was detected among isolates with an *rpoB* S531L mutation, with the *rpoC* V483G mutation being detected most frequently. Only two isolates with *rpoC* mutations did not show an *rpoB* S531L mutation. Instead, these isolates harbored the L511P and D516V mutations in *rpoB*, and both showed a V483G mutation in *rpoC*. A multivariate logistic regression model adjusted for different types of *rpoB* mutations, strain genotype, and degree of drug resistance confirmed strong independent associations between nonsynonymous mutations in *rpoC* and the R86 genotype on the one hand and the *rpoB* S531L mutation on the other (Table 3). In contrast, the association between XDR-TB and *rpoC* mutations was not supported in this multivariate model, probably because it was confounded by an underlying association between the R86 genotype and XDR-TB.

To further investigate the link between the *rpoB* S531L mutation and nonsynonymous mutations in *rpoC*, we analyzed two previously published data sets, one from high-MDR-TB-burden countries in central Asia and one from a global collection of MDR *M. tuberculosis* isolates (13). Among isolates with an *rpoB* S531L mutation from these collections, 33.3 and 21.7%, respectively, harbored *rpoC* mutations. Conversely, in only 4.3 and 2.3%, respectively, of the isolates with other *rpoB* resistance mutations were *rpoC* mutations detected. This association remained significant when adjusted for the different *M. tuberculosis* lineages in a multiple logistic regression analysis ($P = 0.001$ and $P = 0.015$ for the high-burden and global data set, respectively). The fact that the association of *rpoB* S531L with *rpoC* mutations was observed in three independent data sets supports a biological basis for this association.

DISCUSSION

A recent study has proposed a role of putative compensatory mutations in the genes *rpoA* and *rpoC* to alleviate the fitness cost incurred by rifampin resistance-conferring mutations in *rpoB* (16). However, as yet, little is known about the epidemiological relevance of compensatory evolution in drug-resistant *M. tuberculosis*. This study shows that nonsynonymous mutations in the region of *rpoC* that we analyzed are prevalent among rifampin-resistant isolates in a high-burden setting in South Africa and strongly associated with transmission of rifampin-resistant strains. Moreover, the presented data confirm the convergent evo-

lution of specific compensatory *rpoC* mutations, indicating the positive selection of these mutations, as shown previously (13). Taken together, our findings support a role for nonsynonymous mutations in *rpoC* in the compensatory evolution of rifampin-resistant *M. tuberculosis*, thereby contributing to the spread of drug resistance.

This study has several limitations. The sample analyzed consists of a convenience sample which does not accurately represent the overall population structure of *M. tuberculosis* in this setting. Thus, the true proportions of *M. tuberculosis* isolates with *rpoC* mutations may deviate from the proportions herein reported. However, this does not affect the main conclusions of this study. Furthermore, defining transmission chains on the basis of identical IS*6110* RFLP patterns is not ideal. For example, two of the five isolates showing nonclustered RFLP types belonged to the R86 genotype, as suggested by other phylogenetic markers (see Table S1 in the supplemental material). Thus, rather than being rarely transmitted, these strains are representative of new RFLP variants, which emerged from a fast-spreading clone of *M. tuberculosis*, herein identified to be associated with *rpoC* mutations. Similarly, the remaining isolates with nonclustered RFLP patterns harboring *rpoC* mutations also belonged to well-recognized strain families. Thus, this study probably overestimates the proportion of putative compensatory mutations among relatively infrequently transmitted strains. Only a portion of the *rpoC* gene was analyzed for most isolates in this study. Notwithstanding these details, the conclusions presented here remain valid at least for nonsynonymous mutations detected between amino acid positions 245 and 560 of *rpoC*. However, sequencing of the entire *rpoC* gene of a subset of nonclustered isolates showed no evidence of convergent evolution among nonsynonymous mutations detected outside the RpoA-RpoC interaction region.

Multivariate logistic regression analysis revealed a strong association between putative compensatory mutations in *rpoC* and strain genotype (odds ratio [OR], 28.0; $P < 0.001$) as well as the specific rifampin resistance mutation acquired (OR, 125.2; $P < 0.001$) (Table 3). The observation that the variability in terms of the presence of these *rpoC* mutations is critically influenced by purely genetic properties may illustrate how the direction of compensatory evolution is shaped by epistatic interactions with the strain genetic background. Indeed, studies of several bacterial species have shown how genetic background and primary drug resistance mutations predetermine subsequently acquired mutations that are favored within a particular strain background (28–31).

The association between the *rpoB* S531L mutation and these *rpoC* mutations could be explained by at least three models. First, compensatory mutations in *rpoC* could act by restoring structural interactions between the β′, β, and α subunits of the RNA polymerase, which is distorted after the acquisition of a resistance mutation in *rpoB*. Under these conditions, resistance mutations other than the *rpoB* S531L mutation could, in theory, affect the structural properties of the RNA polymerase complex in ways such that mutations in *rpoC* are not able to restore the interaction between the α, β′, and/or β subunit. This could explain the relative underrepresentation of *rpoC* mutations in strains harboring other *rpoB* mutations. This concept is supported by the observation that distinct mutations in *rpoC* have been shown to compensate for the *rpoB* R529C mutation in *Salmonella enterica* (16). Second, the *rpoB* S531L mutation may allow for a wider range of compensatory mutations, including mutations in *rpoC*, while other rifampin resistance mutations could require a more complex or a more limited number of possible compensatory mechanisms outside the genetic regions investigated here. This is supported by the variety of compensatory mutations detected among strains with an *rpoB* S531L mutation. Finally, *rpoB* S531L has been shown to cause a small fitness defect compared to other *rpoB* mutations (5). This low fitness cost might be easier to compensate for than relatively higher costs incurred by other rifampin resistance mutations. More work is required to differentiate between these hypotheses.

In conclusion, nonsynonymous *rpoC* mutations may facilitate restoration of fitness in some clinical strains of drug-resistant *M. tuberculosis*, thereby enhancing their spread. The fact that not all successful MDR/XDR strains carry such mutations suggests that alternative mechanisms of fitness compensation exist. The observation of an association between strain genotype as well as the *rpoB* S531L mutation and *rpoC* mutations supports a role for mutation-specific epistatic effects in driving the compensatory events described in this study.

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