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Characterization of the Pleiotropic Phenotypes of Rifampin-Resistant *rpoB* Mutants of *Escherichia coli*

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We used our collection of 17 sequenced rifampin resistance alleles in *rpoB* to perform a systematic analysis of the phenotypes historically reported with this class of mutants, including growth phenotype, ability to support the growth of different bacteriophages, ability to maintain the F′ episome, interaction with mutant alleles at other loci, sensitivity to uracil, inhibition by 5-fluorouridine, and dominance. We found that mutational changes leading to the same phenotype were often located together and that certain phenotypes were associated with one another.

Historically, *Escherichia coli rpoB* mutations that confer rifampin resistance (Rif^r) have been used to probe the involvement of RNA polymerase in a variety of physiological processes. Rif^r mutations have been found to affect a wide variety of phenotypes, including altered growth properties (10, 16); the ability to support the growth of various bacteriophages (12, 18, 19); the ability to maintain the F′ episome (4); interaction with mutant alleles *dnaA*(Ts) (2), *rpoD*(Ts) (15), and *strA24* (3); and uracil sensitivity (13). We used our collection of 17 sequenced Rif^r mutations (8) to perform a systematic investigation of these phenotypes. Our goal was threefold. (i) We wished to determine whether the phenotypes historically associated with Rif^r mutants were also found in our collection of mutants. (ii) We wished to determine whether Rif^r mutations that confer the same phenotype were located together in the polypeptide, as this would suggest functional specialization within the region. (iii) We wished to determine whether certain groups of Rif^r alleles were associated with more than one phenotype, suggesting that the same structural or functional alteration is responsible for different phenotypes. The data from these studies (Table 1) indicate that our collection of Rif^r mutants exhibits a full spectrum of phenotypes reported for this class of mutants, that mutations that confer the same phenotype are often clustered, and that some phenotypes are associated with one another. These data complement our previous report on the effects of these Rif^r alleles on termination and antitermination (7, 9).

Growth phenotypes of Rif^r mutants. We examined the growth of Rif^r mutants in MC4100 on L broth plates at a variety of temperatures. Whereas the wild-type strain was able to form colonies at temperatures of 20 to 44°C, 12 of the 17 Rif^r mutants were unable to do so (Table 1, columns 3 and 4). Of the 17 Rif^r mutants, 6 were nonpermissive at low temperatures, 4 were nonpermissive at high temperatures, and 2 were nonpermissive at both low and high temperatures. Five of the six mutants nonpermissive at low temperatures were clustered and affected amino acids 531, 532, 533, 563, and 564. Interestingly, the group of Rif^r mutants that prevented growth at high temperature (those affecting amino acids 522 to 529) were located immediately 5′ to this group. Possibly, the changes in this region of the protein have large effects on the structure of the enzyme which are manifested as improper folding or functioning at the temperature extremes. Alternatively, clustering of mutations may indicate that this region is directly involved in growth at high temperature, perhaps by interaction with an alternate sigma factor or another factor(s). Of the 12 strains that restricted growth at some temperature, 10 grew significantly more slowly (>1.5-fold) than the wild-type strain (Table 1, columns 5 and 6), suggesting that RNA polymerase in these mutants did not function optimally, even at the permissive temperature.

Phage growth phenotypes of Rif^r mutants. We examined the abilities of Rif^r mutants to grow bacteriophages λ, T4, T7, and Mu. Only one allele, *rpoB111*, restricted the growth of either λ or T4. Nine of the Rif^r mutants gave λ plaques with a cocarde (clear center with turbid ring) appearance (Table 1, column 7). Since each of these mutants had a reduced growth rate, altered physiological conditions in the host cell may have been responsible for altered plaque morphology. The growth of bacteriophages T7 and Mu was not restricted by any of the Rif^r alleles. The Rif^r mutations previously reported to restrict T7 growth (18) may be absent from our collection either because they are very rare alleles or because their occurrence is strain specific.

Dominance of Rif^r alleles. Rif^r alleles that confer resistance to rifampin although the cell also contains a wild-type *rpoB* (Rif^s) gene are termed Rif^d mutations (1, 14). To test this phenotype, we constructed *recA56 srl*::Tn*10* derivatives of each of the Rif^r alleles, mated in F′110 (carrying *rpoB*$^+$ linked to *btuB*::Tn*10kan*) and tested the merodiploid strains for growth on rifampin (in the presence of kanamycin and tetracycline to maintain selection for merodiploidy). Eleven of the Rif^r alleles were dominant, and two were partially dominant (Table 1, column 8). The prevalence of the Rif^d phenotype is consistent with the results reported by Ovchinnikov et al. (15) and contrary to an early report suggesting that the Rif^d phenotype is rare (11).

Stability of F′ maintenance. To examine the effects of the Rif^r mutations on F′ stability, we constructed Rif^r derivatives of CAG318 (Δ*pro-lac*/F′ *pro*$^+$ *lac*$^+$) and monitored the loss of the F′ *lac*$^+$ episome by plating serial dilutions on lactose indicator plates without selection for merodiploidy (Table 1, column 9). One mutation, *rpoB3445*, showed increased F′ maintenance, while five others resulted in at least a 10-fold decrease in F′ stability. Three of these mutations are located close to each other in β, affecting amino acids 517, 522, and 526. Interestingly, *rpoB111*, the Rif^r allele that promoted the greatest destabilization, is the

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TABLE 1. Characterization of pleiotropic phenotypes of rifampin-resistant mutants

Rif^r allele[a]	Amino acid(s) affected	Plating phenotype[b]		Doubling time (min)[c]		λ plaque morphology[d]	Rif^r dominance or recessiveness[e]	% which lost F'[f]	5 FU concn (μg/ml) required for inhibition[g]	Growth inhibition by uracil[h]	Enhanced Ts of *rpoD800*[i]	Suppressed Ts of *dnaA46*[j]
		20°C	44°C	M9 + glucose	L broth							
Wild type		+	+	40	19	T		0.8	20	−	−	−
Cluster I												
3445	507–511	−	−	80	35	CO	D	0.1	20	−	NT[k]	NT
101	513	+	+	46	20	T	D	3.0	20	−	−	−
8	513	−	+	80	30	CO	D	0.3	20	−	−	−
113	516	+	+	46	24	T	PD	0.6	20	−	−	−
148	516	+	+	42	20	T	D	0.8	20	−	−	−
3051	517	+	+	48	20	T	R	15.2	10[l]	+/−[l]	−	−
3595	522	+	−	48	27	TC[m]	D	10.3	1[n]	+[n]	NT	NT
2	526	+	−	46	21	T	D	20.3	3[n]	+/−[l]	NT	NT
3401	529	+	−	96	35	CO	D	0.4	20	−	NT	NT
3402	529	+	−	110	46	CO	R	0.3	20	−	NT	NT
114	531	−	+	56	30	CO	D	5.0	20	−	+	+
3449	532	−	+	52	34	CO	PD	1.1	20	−	+	+
3443	533	−	+	56	30	CO	D	0.6	20	−	+	+
Cluster II												
3370	563	−	+	100	48	CO	R	4.4	10[l]	+/−[l]	+	+
111	564	+/−	+	68	26	T[o]	D	75.3	10[p]	+/−[p]	−	−
7	572	+	+	48	25	T	D	1.2	20	−	−	−
3406	687	−	−	>200	48	CO	R	11.5	20	−	NT	NT

[a] *rpoB* mutations were described by Jin and Gross (8).
[b] Scored by viable counts on L broth plates after incubation overnight at 44°C or for 2 days at 20°C; efficiency of plating: +, 1; +/−, 0.3; −, <0.1.
[c] Assayed at 37°C.
[d] T, Turbid plaque; C, clear; CO, cocarde plaque.
[e] D, Dominant (efficiency of plating on rifampin plates, 1); PD, partially dominant (efficiency of plating on rifampin plates, 0.3 to 0.5); R, recessive (efficiency of plating on rifampin plates, <0.01).
[f] Approximately 1,000 colonies were scored.
[g] The number indicates the lowest concentration at which colony size was reduced by at least 75%.
[h] Scored on M9 glucose-uracil plates after overnight incubation. +, Colony size reduced at least 75%; +/−, colony size reduced 50%; −, colony size unaffected.
[i] Efficiency of plating at 37°C: −, 1; +, <0.1.
[j] +, Colonies grew at 40°C; −, colonies did not grow at 40°C.
[k] NT, Not tested because the Rif^r allele itself confers a Ts phenotype.
[l] Phenotype exhibited at 37 and 40°C.
[m] Turbid plaque at low temperature but clear plaque at high temperature.
[n] Phenotype exhibited at 30, 37, and 40°C.
[o] Supported phage growth at high temperature only.
[p] Phenotype exhibited only at 40°C.

one that prevented the growth of both λ and T4. Other work (7) has suggested that this mutation may alter interactions between NusA and RNA polymerase, raising the possibility that this functional defect underlies these diverse phenotypes.

Growth inhibition of Rif^r mutants by 5FU and uracil. Because RNA polymerase mutants resistant to 5-fluorouridine (5FU) have altered K_ms for UTP and ATP (5, 6), we tested our Rif^r mutants for this phenotype by plating fresh overnight cultures on L broth plates containing 1 to 100 μg of 5FU per ml and comparing colony sizes with or without 5FU after overnight incubation. Although none of the Rif^r mutants were more resistant to this analog, five were hypersensitive to 5FU (Table 1, column 10). These same five mutants were also sensitive to inhibition by uracil (50 μg/ml in M9 glucose minimal plates) (Table 1, column 11); this is believed to result from underexpression of the *pyrA* gene (13). The uracil sensitivity of all five of these mutants was relieved by arginine but not ornithine, as expected if the phenotype results from altered expression of *pyrA* (13). The mutations that produce these phenotypes are clustered at two positions; three Rif^r alleles affect amino acids 517 to 526, and the remaining two affect amino acids 563 to 564. The Rif^r alleles that affect amino acids 517 to 526 also result in F' destabilization.

Interaction of Rif^r mutations with the *strA24* allele. The Rif^r *rpoB180* mutation was reported by Chakrabarti and Gorini (3) to confer a Ts phenotype on *strA24* but not on wild-type strains at 42°C, possibly because of altered transcriptional-translational coupling. In our collection, only the *rpoB2* allele produced this phenotype. Preliminary sequence data suggest that *rpoB2* has the same mutational change as *rpoB180*.

Interaction of Rif^r mutations with the *rpoD800* and *dnaA46* mutations. Ovchinnikov et al. (15) reported that the two Rif^r mutations enhanced the Ts phenotype of strains containing the σ^{70} mutation *rpoD800* and suppressed that of strains containing *dnaA46*. Among our collection, the four Rif^r mutations that enhanced the Ts phenotype of *rpoD800* also suppressed that of *dnaA46* (Table 1, columns 12 and 13). Three of the Rif^r mutations affect the adjacent amino acids 531, 532, and 533, while the fourth affects the next observed amino acid change, 563, leading to Rif^r. In other work, we have found that the three Rif^r mutations that alter amino acids 531 to 533 were the only ones to completely suppress the termination defects of *nusA* mutants and to alter the cellular antitermination system involved in rRNA synthesis (7). These functional alterations may contribute to the altered Ts phenotypes exhibited by these three mutant strains in combination with other mutant alleles.

One Rif^r allele, *rpoB2*, is incompatible with the *dnaA46* mutation, as previously reported by Schaus et al. (17). It is noteworthy that the same *rpoB2* allele is also incompatible with *rho-15*, *nusA10*(Cs), and *nusA11*(Ts) (7, 9). Other in vivo studies indicate that strains containing *rpoB2* are very defective in termination (9). This defect may underlie the incompatibility of the *rpoB2* allele with many of the mutant strains.

In conclusion, these studies demonstrate that there is functional specialization within the region of the β polypeptide defined by Rif^r mutations. In many cases, Rif^r mutations that lead to the same phenotype (for example, the Ts or Cs growth phenotype, inhibition by 5FU or uracil, an altered interaction with *rpoD800* or *dnaA46*) are clustered. In addition, certain phenotypes (such as inhibition by 5FU and uracil or altered interaction with *rpoD800* and *dnaA46*) are associated with each other. However, the actual defects in RNA polymerase function responsible for these phenotypes remain to be determined.

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