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Transcription Modulation of *Salmonella enterica* Serovar Typhimurium Promoters by Sub-MIC Levels of Rifampin▽

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Promoter-*lux* fusions that showed rifampin-modulated transcription were identified from a *Salmonella enterica* serovar Typhimurium 14028 reporter library. The transformation of a subset of fusions into mutants that lacked one of six global regulatory proteins or were rifampin resistant showed that transcription modulation was independent of the global regulators, promoter specific, and dependent on the interaction of rifampin with RNA polymerase.

Antibiotics have been shown to alter global bacterial transcription patterns at concentrations below those that completely inhibit the growth of the bacterial cell (sub-MIC) (10, 29). Among the genes affected by antibiotics are those related to modes of action and bacterial stress responses. It has been suggested that characteristic gene expression patterns may be used to identify unknown small molecule inhibitors (2, 28). There have also been studies demonstrating antibiotic-induced transcription modulation of genes for accessory functions such as motility and virulence (reviewed in references 2 and 29).

Using a library of 6,528 promoter-reporter clones, we previously demonstrated dramatic up- and down-regulation of the

TABLE 1. Characteristics of rifampin-responsive promoters in *S. enterica* serovar Typhimurium 14028

Promoter	Luminescence (cps) with[a]:						Fold induction for LB with rif at[b]:		Putative function
	LB	SD	LB with rif 2.5 μg/ml	SD	LB with rif 5.0 μg/ml	SD	2.5 μg/ml	5.0 μg/ml	
STM2899/*invF*	61,605	15,125	1,139	244	295	72	↓ 54.1	↓ 209.2	Virulence, invasion
STM1091/*sopB*	44,671	10,443	853	309	274	105	↓ 52.3	↓ 163.0	Virulence, invasion
STM2066/*sopA*	16,133	5,246	224	141	140	61	↓ 72.1	↓ 115.2	Virulence, invasion
STM4255 to -4258	3,172	596	203	54	240	27	↓ 15.7	↓ 13.2	Virulence
STM1956/*fliA* (σ^F)	147,748	61,007	22,000	2,265	4,598	562	↓ 6.7	↓ 32.1	Flagellum synthesis
STM1914/*flhBA*	6,553	2,634	1,407	247	251	40	↓ 4.7	↓ 26.1	Flagellum synthesis
STM1183/*flgK*	93,550	8,524	25,533	3,462	5,222	2,221	↓ 3.7	↓ 17.9	Flagellum synthesis
STM2199/*cirA*	2,169	757	673	645	516	374	↓ 3.2	↓ 4.2	Iron metabolism
STM1328	2,480	150	113	46	100	38	↓ 21.9	↓ 24.9	Unknown
STM1248	2,806	386	150	72	148	54	↓ 18.7	↓ 19.0	Unknown
STM1444/*slyA*	1,029	316	2,558	787	5,223	473	2.5	5.1	Virulence, systemic
STM1154 to -1155/*yceE*, *htrB*	10,040	3,041	26,165	7,776	38,305	5,030	2.6	3.8	Virulence, systemic
pSLT041-39/*spvRAB*	3,688	515	7,017	5,170	13,230	3,942	1.9	3.6	Virulence, systemic
STM4118/*yijP*	2,633	619	3,941	626	8,688	1,821	1.5	3.3	Virulence
STM4454/*treB*	4,179	1,430	17,662	3,545	54,241	5,188	4.2	13.0	Carbon metabolism
STM2445/*ucpA*	12,867	3,627	26,283	6,120	46,325	11,770	2.0	3.6	Carbon metabolism
STM1597/*ydcW*	1,992	472	2,361	106	6,441	1,651	1.2	3.2	Carbon metabolism
STM2473/*talA*	3,149	660	4,543	1,054	9,194	1,340	1.4	2.9	Carbon metabolism
STM0425/*thiI*	1,927	324	5,245	2,685	15,918	2,758	2.7	8.3	RNA modification
STM3595, STM3084	824	29	2,157	165	19,686	775	3.0	24.0	Unknown
STM0389/*yaiA*	2,590	506	4,721	2,093	10,180	4,030	1.8	3.9	Unknown
STM2287	2,740	1,164	3,557	617	7,697	981	1.3	2.8	Unknown

[a] cps, counts per second; rif, rifampin; SD, standard deviation.
[b] Down arrows indicate RIDR.

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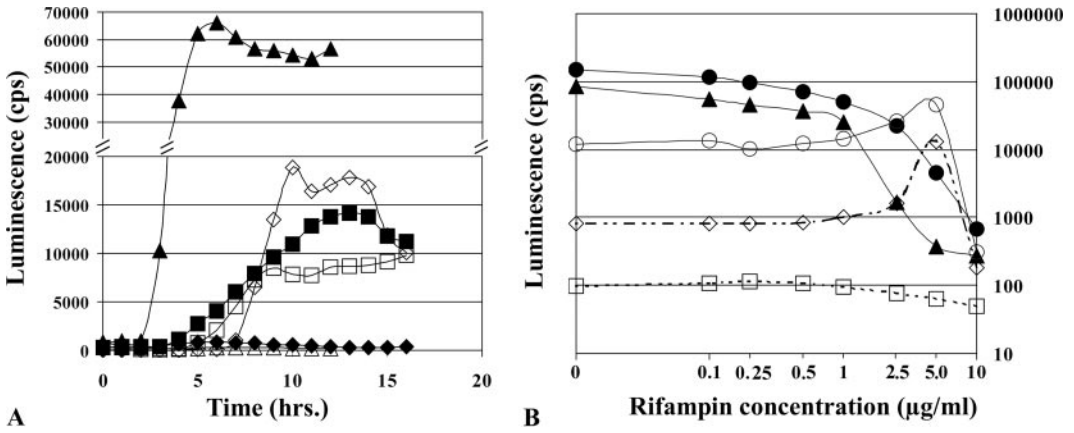

FIG. 1. (A) Luminescence profiles of promoter-*lux* reporters. Luminescence patterns for serovar Typhimurium 14028 reporters STM3595::*luxCDABE* (◇), *invF*::*luxCDABE* (△), and STM2091::*luxCDABE* (□) in LB supplemented with kanamycin at 25 μg/ml (for plasmid maintenance) and rifampin at 5.0 μg/ml (open symbols) or LB supplemented with kanamycin at 25 μg/ml in the absence of rifampin (filled symbols) are shown. Promoters drive expression from the *luxCDABE* operon, producing luminescence without any exogenous substrate (19). (B) Concentration dependence of rifampin-induced transcription modulation. Serovar Typhimurium 14028 with *fliA* (○), *invF* (▲), STM3595 (◇), and *ucpA* (○) *luxCDABE* reporters and pCS26 without an insert (□) grown in LB supplemented with kanamycin at 25 μg/ml and the indicated concentrations of rifampin were grown for at least 14 h. Average peak luminescence values from a minimum of three time courses are plotted.

transcription induced by the antibiotic rifampin (a transcription inhibitor [3, 14]) and the macrolide class of antibiotics on a global scale in *Salmonella enterica* serovar Typhimurium 14028 (10, 28). In this communication, we further examine rifampin-responsive promoter-*luxCDABE* fusion clones from our initial screen (~5% of library clones [10]) to explore the effects of known global transcription regulators on rifampin-induced transcription modulation (RITM).

Fusions whose activity was modulated by rifampin in our initial screens (10) were screened continuously by inoculating cultures grown at 37°C in a white, opaque microtiter plate (Costar; Fisher Scientific, Ottawa, Ontario, Canada) sealed with a breathable sealing membrane (Nalge Nunc, Naperville, IL). Luminescence was followed during 12 to 16 h of growth in the presence or absence of rifampin by using a Victor II multilabel counter (PerkinElmer, Boston, MA). Clones were discarded if luminescence readings were below 1,000 cps in medium with and without rifampin. This resulted in the identification of a subset of 22 moderately to strongly affected promoters that displayed between 200-fold down-regulation and 24-fold up-regulation (Table 1). Figure 1A shows the patterns of luminescence produced from two promoters that were sensitive to rifampin and from an unaffected promoter. The strain carrying the vector alone produced very low levels of luminescence (~100 cps [data not shown]). Note that both stimulation and inhibition of the transcription by the inhibitor were found and that the timings of the maximum response differed between promoters. As a result, promoters were screened for a minimum of 12 h to identify the maximum response. The effect of rifampin was observed to be concentration dependent and generally maximal at 5 μg/ml rifampin (Fig. 1B).

Upon sequencing the promoters sensitive to RITM (Table 1), we noticed that a number of serovar Typhimurium virulence genes were included. The affected promoters were also grouped by the involvement of the associated genes in two distinct regulons. Promoters from virulence genes associated with intracellular growth and survival in macrophages, *slyA* (5, 13, 16), *spvAB* (11, 13, 17), *somA* (7), *htrB* (15), and SPI-2 genes (4, 12, 13), showed rifampin-induced up-regulation (RIUR). Promoters from genes involved in intestinal invasion, those associated with the type III secretion system encoded on

TABLE 2. Summary of rifampin-induced transcription modulation[a]

Strain description	Modulation in indicated reporter							
	fliA	*flhB*	*cirA*	*STM1328*	*STM3595*	*spvAB*	*ucpA*	*talA*
14028	↓↓	↓↓	↓↓	↓				
14028 *crp*::Tn*10*	↓	*[b]	↓↓	↓		**[c]		
14028 *fis*::*tet*	↓	*	↓	↓	↓**	↓↓**		**
14028 *hns*::Tn*10*	↓↓↓	↓↓	↓	↓				
14028 *ihfB*::*cat*	↓↓↓	↓↓	↓	↓				*
14028 *fnr*::Tn*10*	↓↓	↓↓	↓↓	↓↓↓	↑			
14028 *rpoS*::*amp*	↓↓	↓	↓↓	↓↓↓		*		

[a] The modulation is depicted by up arrows (RIUR) and down arrows (RIDR) as follows: a 2.5- to 5-fold effect is depicted as one arrow, a 6- to 15-fold effect by two arrows, and a 16-fold or greater effect as three arrows. No arrow indicates there was a less-than-2.5-fold difference.

[b] In four cases, indicated by single asterisks, the introduction of the mutations changed RITM from strong RIDR to no effect.

[c] In four cases, indicated by double asterisks, the mutation of a global regulator either blocked RIUR or switched the response from RIUR to RIDR, indicating that the regulator was involved in RIUR for that promoter.

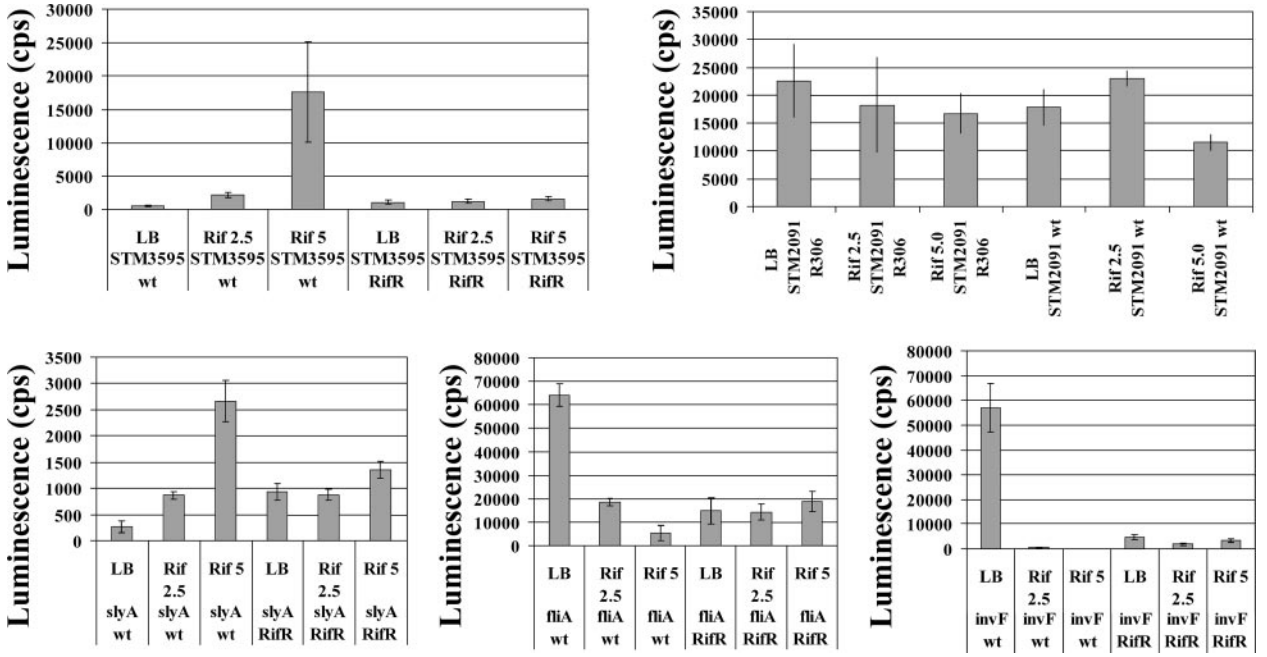

FIG. 2. Luminescence profiles of promoter-*lux* fusions in serovar Typhimurium 14028 and a rifampin-resistant isogenic mutant, R306, in response to rifampin (Rif). Two rifampin-up-regulated promoters (STM3595 and *slyA*), two rifampin-down-regulated promoters (*fliA* and *invF*), and one promoter unaffected by rifampin (STM2091) were assayed. Luminescence was monitored during a minimum of 14 h of growth in microtiter plates containing LB with kanamycin (25 μg/ml) and the indicated amount (μg/ml) of rifampin; the plates were sealed with a Mylar plate sealer (Thermo Labsystems, Franklin, MA) and incubated at 37°C in a Victor II multilabel counter. Average peak luminescence values from eight replicate time courses are plotted. Error bars indicate standard deviations. wt, wild type; RifR, rifampin-resistant strain.

SPI-1 and its secreted effectors, showed rifampin-induced down-regulation (RIDR). These genes included *invF* (6, 9, 18), *sopA*, and *sopB* (8, 24). Promoters from genes in SPI-4 (STM4255 to 4258), which have been shown to be coordinately regulated with SPI-1 by HilA (1), also displayed RIDR. Furthermore, rifampin also down-regulated the promoters from three operons involved in flagellum synthesis.

It seemed possible that RITM might be due to antibiotic effects on one of the known global transcription regulators or to the activation of one or more stress responses. To examine these possibilities, eight different representative rifampin-responsive promoter (RRP) fusions were transformed into serovar Typhimurium 14028 strains, each carrying a mutation in the gene for one of six major global regulators: CRP (25), FNR (27), FIS (22), H-NS (21), IHF (23), or σ^S (25). Mutant alleles were introduced into the 14028 background by using P22HT*int*-mediated generalized transduction (26) and confirmed by PCR or inverse PCR (20), followed by nucleotide sequencing and phenotypic analysis. The promoter activity of four rifampin-up-regulated and four rifampin-down-regulated *lux* fusions was examined in the presence or absence of rifampin (Table 2). The change in expression (*n*-fold) in response to rifampin was calculated by dividing the amount of luminescence observed with rifampin by the amount of luminescence observed without rifampin. In the majority of promoter-mutant combinations, although the magnitude of RITM may have changed, RITM levels were similar for both the wild-type and mutant host backgrounds. In four cases, the introduction of the mutations changed RITM from strong RIDR to no effect (Table 2). However, in these cases, the introduction of regulatory mutations may have reduced the basal expression of the *lux* reporter to the lower limit of detection, preventing a clear conclusion regarding its involvement in RIDR. Overall, in 44/48 of the combinations tested, rifampin modulation of transcription was not altered by the loss of one of the regulatory proteins. In four cases, the mutation of a global regulator either blocked RIUR or switched the response from RIUR to RIDR, indicating that the regulator was involved in RIUR for that promoter (Table 2). We note that Fis was involved in three of the four cases in which RITM was altered by the loss of a regulator. This involvement may indicate that Fis has a role in RIUR, but as it did not affect all RRPs, no model involving Fis regulation of RITM was readily apparent. We also tested the effects of rifampin in a rifampin-resistant host (resistance was conferred by a mutation in the β subunit of RNA polymerase). The mutation conferring rifampin resistance eliminated RITM in all of the RRP fusions tested (Fig. 2). Thus, RITM is a transcription-specific effect and is not due to separate effects on cell physiology. Further analysis of this novel regulation will require the identification of the specific nucleotide sequences at the RRP responsible for rifampin sensitivity.

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