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SOS Regulation of *qnrB* Expression[▽]

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In the sequence upstream from *qnrB* (but not *qnrA* or *qnrS*) is a LexA binding site. *qnrB* was shown to be under SOS control by demonstrating that quinolone susceptibility decreased with increasing temperature in a strain with a *recA441*(Ts) allele, whereas *qnrB* expression increased in response to ciprofloxacin or mitomycin C in strains with an intact *lexA* gene.

Plasmid-mediated Qnr proteins provide low-level quinolone resistance and protect bacterial DNA gyrase and topoisomerase IV from quinolone inhibition (21, 22). QnrA, QnrB, and QnrS are currently known (7, 10, 14). All are pentapeptide repeat proteins differing from each other by 40% or more in amino acid sequence, while within each type minor variations in sequence define alleles such as QnrB1 and QnrB2 (18). In addition to protecting DNA gyrase, QnrB1 (but not QnrA1) at high concentrations has been shown to inhibit the enzyme in vitro, which may explain the bacterial growth inhibition observed when the gene is maximally expressed (10). We have discovered that *qnrB* is regulated by the SOS system so that quinolone exposure augments its expression.

Table 1 shows the DNA sequences at the starts of the *qnr* genes. Two in-phase ATG start codons are present in *qnrB1*, *qnrB3*, and *qnrB5.* In *qnrB2* and *qnrB4* the first ATG is out of phase with the remainder of the reading frame, suggesting that for all five alleles translation may be initiated at the second ATG codon. Between the two start codons is a LexA binding site or box, the canonical sequence of which is TA**CTGT**ATA TATAT**ACAG**TA with the 5′-CTGT essential and the central $(AT)_4$ known to vary in different LexA boxes (4, 23). No LexA binding site was found upstream from *qnrA1* or *qnrS1.* A similar LexA box, however, is found upstream from all those *qnrB* alleles in GenBank for which this region of the sequence has been reported, including *qnrB6*, *qnrB10*, *qnrB12*, *qnrB13*, *qnrB14*, *qnrB15*, *qnrB16*, *qnrB17*, and *qnrB18* (8).

To determine whether expression of *qnrB* alleles is under SOS control, plasmids were introduced into *Escherichia coli* GW1000 (11) with *recA441*, which encodes a RecA protease that is more easily activated, so that the strain would be SOS inducible at 30°C but constitutive at 42°C, and into *E. coli* J53 azide^r (9), which has wild-type *lexA* and *recA* alleles. *qnrB1* plasmid pMG298 (10), *qnrB2* plasmid pMG301 (10), *qnrB3* plasmid pMG317 (19), *qnrB4* plasmid pMG319 (19), *qnrB5* plasmid pMG305 (6), and *qnrA1* plasmid pMG252 (14) (all natural plasmids) were introduced by conjugation. Tra$^-$ *qnrS1* plasmid pMG306 (6) was introduced into GW1000 by transformation.

As shown in Table 2 GW1000 derivatives containing plasmids with *qnrB* alleles demonstrated two- to eightfold decreases in ciprofloxacin susceptibility as the growth temperature increased. R$^-$ GW1000 also showed a decrease in susceptibility with rising temperature, but the decrease was less than that observed in *qnrB* derivatives. A two- to threefold decrease in susceptibility was also seen in strains with

TABLE 1. Nucleotide sequences at the starts of plasmid-mediated *qnrB*, *qnrA*, and *qnrS* alleles

Allele	Sequence[a]	GenBank accession no.
	1↓ 2↓	
qnrB1	ATGACGCCATTA**CTGT**ATAAAAAA**ACAG**GTACAA--AT-ATGGCTCTGGC	DQ351241
qnrB2	ATGACGCCATTA**CTGT**ATAAAAAA**ACAG**GTACAA-AAT-ATGGCTCTGGC	DQ351242
qnrB3	ATGACGCCATTA**CTGT**ATAAAAAA**ACAG**GTACAA--AT-ATGGCTCTGGC	DQ303920
qntB4	ATGTTGCAATCA**CTGT**ATAAAAAA**ACAG**GT-TAATCATGATGACTCTGGC	DQ303921
qnrB5	ATGACGCCATTA**CTGT**ATAAAAAC**ACAG**GCATAG--AT-ATGACTCTGGC	DQ303919
	↓	
qnrA1	CCGAAAGAGTTAGCACCCTCCCTGATTAAAGGAAGCCGTATGGATATTAT	AY070235
qnrS1	ATAATGGTAGTCTAGCCCTCCTTTCAACAAGGAGTACTCATGGAAACCTA	AB187515

[a] Potential initiation codons are indicated by arrows, and the essential components of a LexA binding site are shown in bold.

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TABLE 2. Quinolone susceptibility as a function of temperature in strains with varying SOS response regulation

E. coli strain and plasmid	Ciprofloxacin MIC (μg/ml) at[a]:			
	21°C	30°C	37°C	43°C
GW1000 (*lexA*$^+$ *recA441*)	0.0005	0.001	0.002	0.004
pMG298 (*qnrB1*)	0.004	0.012	0.023	0.023
pMG301 (*qnrB2*)	0.003	0.012	0.012	0.023
pMG317 (*qnrB3*)	0.094	0.125	0.125	0.19
pMG320 (*qnrB5*)	0.023	0.032	0.047	0.094
pMG252 (*qnrA1*)	0.023	0.032	0.047	0.064
pMG306 (*qnrS1*)	0.064	0.094	0.125	0.125
J53 (*lexA*$^+$ *recA*$^+$)	0.004	0.008	0.012	0.023
pMG298 (*qnrB1*)	0.19	0.38	0.38	0.38
pMG252 (*qnrA1*)	0.19	0.38	0.38	0.38

[a] Measured by Etest on Mueller-Hinton agar plates at the indicated temperatures except for R$^-$ GW1000 and J53, for which MICs were performed by agar dilution. Determinations were repeated at least twice.

plasmids carrying *qnrA1* or *qnrS1* alleles. In *E. coli* J53 with unmodified SOS regulation, temperature had only a twofold effect on the level of *qnrB1*-mediated ciprofloxacin resistance.

While the trend observed suggested that *qnrB* alleles are specifically regulated by the SOS system, the MIC results were not clear-cut because of a background effect of temperature on quinolone susceptibility. To document SOS regulation directly, the expression of *qnr* genes was measured by real-time quantitative PCR after a 15- to 30-min exposure to agents known to trigger the SOS response. Strains were grown in LB broth at 37°C to exponential phase in triplicate. When the optical density at 600 nm reached 0.08 to 0.1, 0.1 μg/ml ciprofloxacin or 0.2 μg/ml mitomycin C was added, leaving one culture as a control. Aliquots (200 to 300 μl) were treated with RNAprotect bacteria reagent (Qiagen, Valencia, CA) and centrifuged, the pellet was briefly frozen and treated with lysozyme, and the RNA was extracted with an RNeasy Mini kit (Qiagen) and treated with Turbo DNA-free (Ambion, Austin, TX). cDNA synthesis was performed with a Verso reverse transcription-PCR kit (Abgene, Epsom, United Kingdom) using gene-specific reverse primers (Table 3). Quantitative PCR amplification

TABLE 3. Primers used for RT-PCR

Primer	Direction	Sequence (5′→3′)
qnrB1	Forward	GGTACAAATATGGCTCTGGCACTCG
	Reverse	GACAGCCGATAAATTCAGTGCCG
qnrB2	Forward	CTCTGGCACTCGTTGGCGAA
	Reverse	AATTCAGTGCCGCTCAGGTCG
qnrB3	Forward	CTCTGGCACTCGTTGGCGAA
	Reverse	AATTCAGTGCCGCTCAGGTCG
qnrB4	Forward	TCAAAAGTTGTGATCTCTCCATGGC
	Reverse	AACTTGCGCCGCGAAAATCT
qnrA1	Forward	TTTTCAGCAAGAGGATTTCTCACGC
	Reverse	GCTTTCAATGAAACTGCAATCCTCG
mdh	Forward	CTGCGTAACATCCAGGACACTAACG
	Reverse	CGACGGTTGGGGTATAAATAACAGG

TABLE 4. Expression of *qnr* alleles

Strain and plasmid	Inducing agent	Relative RNA transcript level at[a]:	
		15 min	30 min
J53 (*lexA*$^+$ *recA*$^+$)			
pMG298 (*qnrB1*)	Ciprofloxacin	3.7	3.2
	Mitomycin C	2.6	2.8
pMG301 (*qnrB2*)	Ciprofloxacin		5.9
pMG317 (*qnrB3*)	Ciprofloxacin		2.1
pMG319 (*qnrB4*)	Ciprofloxacin	9.9	9.0
	Mitomycin C	8.2	7.9
pMG252 (*qnrA1*)	Ciprofloxacin		0.9–1.0
	Mitomycin C		0.9–1.5
AB1157 (*lexA*$^+$ *recA*$^+$)			
pMG319 (*qnrB4*)	Ciprofloxacin		3.9
	Mitomycin C		2.0
AB1157 LexA300::spec (*lexA*[Def] *recA*$^+$)			
pMG319 (*qnrB4*)	Ciprofloxacin		1.1
	Mitomycin C		1.0
DM49 (*lexA3*[Ind] *recA*$^+$)			
pMG319 (*qnrB4*)	Ciprofloxacin[b]		1.2
	Mitomycin C		1.0

[a] All measurements were repeated at least twice.
[b] The ciprofloxacin MIC of DM49 pMG319 was 0.064 μg/ml, compared to the MIC of of 0.25 μg/ml for AB1157 pMG319 and AB1157 LexA300::spec pMG319, and so a ciprofloxacin concentration of 0.05 μg/ml was used for induction.

was conducted in an MJ Research PTC-200 thermal cycler with a Chromo4 detector (Bio-Rad, Hercules, CA) using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) and the listed primers (Table 3). The housekeeping *mdh* (malate dehydrogenase) gene was used as an internal control and did not change in response to the inducing agents. Experiments were repeated at least twice. RNA transcript levels were calculated using the $2^{-\Delta\Delta CT}$ method (12), where C_T is the cycle number of the detection threshold, and are expressed relative to levels in the unsupplemented control.

Table 4 shows that in *E. coli* J53 with intact *lexA* and *recA* genes expression of *qnrB* alleles increased between 2.1- and 9.9-fold in response to the inducing agents while expression of *qnrA1* was unchanged. Proof that this increase in *qnrB* expression required an intact SOS system was obtained with a set of related strains. Expression of *qnrB4* increased in response to ciprofloxacin or mitomycin C in *E. coli* AB1157 with wild-type *lexA* and *recA* genes but not in two strains derived from it: strain AB1157 LexA300::spec, which has a defective LexA protein so that LexA-regulated genes are constitutively expressed, or strain DM49 (15), which has a protease-resistant LexA product and consequently is defective in SOS induction.

The SOS response is triggered by DNA damage, such as that generated by quinolones (13, 24). The RecA protein is activated by single-stranded DNA and acts as a coprotease to cleave the LexA protein, which otherwise binds as a dimer to LexA boxes, repressing expression of adjacent genes. More than 40 genes or operators on the chromosome of *E. coli* are so regulated (4, 5). Most are involved in DNA repair or regulation of cell division. The native function of *qnr* genes is not known. They have been found on the chromosome of both

gram-negative and gram-positive bacteria (1, 2, 16, 17, 20). SOS regulation of QnrB could be a carryover reflecting a role for this topoisomerase-interacting protein in response to DNA damage. Alternatively, SOS regulation serves to protect the host cell from the potentially toxic effects of QnrB while allowing augmented production upon exposure to quinolone antimicrobial agents. Since the SOS response also results in derepression of specialized DNA polymerases that promote quinolone resistance by mutations (3), it thus coordinates both *qnrB* plasmid-mediated and chromosomal target-derived resistance.

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