EMBO reports

scientific report

The SOS response promotes *qnrB* quinolone-resistance determinant expression

Sandra Da Re[1,2], Fabien Garnier[1,3], Emilie Guérin[1,2], Susana Campoy[4], François Denis[1,2,3] & Marie-Cécile Ploy[1,2,3+]

[1]INSERM, Equipe Avenir, [2]Université de Limoges, Faculté de Médecine, EA3175, [3]CHU Limoges, Laboratoire de Bactériologie-Virologie-Hygiène, Limoges, France, and [4]Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Barcelona, Spain

The *qnr* genes are plasmid-borne fluoroquinolone-resistance determinants widespread in Enterobacteriaceae. Three families of *qnr* determinants (*qnrA, B* and *S*) have been described, but little is known about their expression and regulation. Two new determinants, *qnrC* and *qnrD*, have been found recently. Here, we describe the characterization of the *qnrB2* promoter and the identification of a LexA-binding site in the promoter region of all *qnrB* alleles. LexA is the central regulator of the SOS response to DNA damage. We show that *qnrB2* expression is regulated through the SOS response in a LexA/RecA-dependent manner, and that it can be induced by the quinolone ciprofloxacin, a known inducer of the SOS system. This is the first description of direct SOS-dependent regulation of an antibiotic-resistance mechanism in response to the antibiotic itself.

Keywords: antibiotic resistance; ciprofloxacin; *qnrB*; SOS response

EMBO reports (2009) **10**, 929–933. doi:10.1038/embor.2009.99

INTRODUCTION

Quinolones are synthetic broad-spectrum antibiotics used widely to treat a variety of infectious diseases. Bacterial resistance to quinolones emerged rapidly after their widespread use. In Gram-negative bacteria, quinolone resistance was for a long time considered to be entirely mediated by mutations in chromosomal genes encoding quinolone targets (that is, DNA gyrase and topoisomerase IV) and/or in regulatory genes of outer membrane proteins or efflux pumps (Jacoby, 2005). Recently, plasmid-encoded quinolone resistance has been described along with three mechanisms: (i) a quinolone-protective mechanism encoded by the *qnr* genes; (ii) a modifying enzyme, *aac(6′)-Ib-cr* (Robicsek *et al*, 2006b); and (iii) an efflux pump encoded by the *qepA* gene (Perichon *et al*, 2007; Yamane *et al*, 2007).

Since the identification of the first plasmid-mediated quinolone-resistance determinant, *qnrA*, a decade ago (Martinez-Martinez *et al*, 1998), many other plasmid-borne *qnr* determinants have been found, comprising three families of Qnr proteins (QnrA, QnrB and QnrS), for which, respectively, 6, 20 and 3 alleles have been described (Jacoby *et al*, 2008). Recently, two more *qnr* determinants have been identified: *qnrC* and *qnrD* (Cavaco *et al*, 2009; Wang *et al*, 2009a). The *qnr* determinants encode pentapeptide-repeat proteins, which bind to DNA gyrase, protecting it from quinolone inhibition. Several studies worldwide have shown that *qnr* genes are widespread in the Enterobacteriaceae often associated with extended-spectrum β-lactamases (Robicsek *et al*, 2006a). The *qnr* determinants are found on conjugative plasmids that often carry other antibiotic-resistance determinants, and all *qnrA* and most *qnrB* genes are located in complex *sul1*-type integrons downstream from the insertion sequence, IS*CR1* (insertion sequence common region 1; Nordmann & Poirel, 2005; Garnier *et al*, 2006). So far, the *qnr* promoter has been identified for only two determinants, *qnrA1* and *qnrB19* (Mammeri *et al*, 2005; Cattoir *et al*, 2008). Little is known about *qnr* gene expression and its regulation, except that *qnrA1* expression can be induced by ciprofloxacin, through an unknown mechanism (Rodriguez-Martinez *et al*, 2006).

We recently reported the presence of the *qnrB2* gene on a complex *sul1*-type integron in *Salmonella enterica* serovar Keurmassar (Garnier *et al*, 2006). Here, we characterized the *qnrB2* promoter and identified a LexA-binding site upstream from the start codon. LexA is the central regulator of the SOS response to DNA damage. We showed that *qnrB2* expression is repressed by LexA, and that ciprofloxacin, a known inducer of the SOS response, upregulates the expression of its own resistance determinant. This regulation seems to be a common trait of *qnrB* determinants, as the LexA site is conserved in all *qnrB* allele promoter regions described so far.

[1]INSERM, Equipe Avenir, and
[2]Université de Limoges, Faculté de Médecine, EA3175, 2 Rue du Docteur Marcland, 87025 Limoges Cedex, France
[3]CHU Limoges, Laboratoire de Bactériologie-Virologie-Hygiène, 2 Avenue Martin Luther King, 87042 Limoges Cedex, France
[4]Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain
[+]Corresponding author. Tel: +33 5 55 05 67 27; Fax: +33 5 55 05 67 22;
E-mail: marie-cecile.ploy@unilim.fr

Received 23 October 2008; revised 20 March 2009; accepted 9 April 2009; published online 26 June 2009

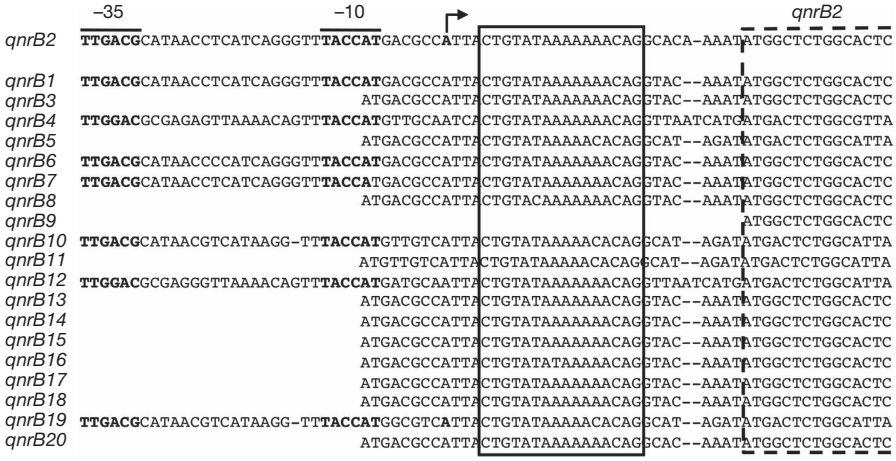

Fig 1 | Sequence alignment of the *qnrB2* promoter and *qnrB* alleles. The –35 and –10 promoter elements are indicated; the +1 start site is represented by an arrow; the start of the *qnrB* coding sequence is indicated by a dashed-open frame and the consensus sequence of the LexA-protein-binding site is boxed. *qnrB* allele sequences were aligned using the Geneious software. Sequence accession numbers EF682134, AM234698, EF683583, EF667294, EU052800, EU523120 and AB379831 for sequences with promoter regions for *qnrB1*, *qnrB2*, *qnrB4*, *qnrB6*, *qnrB10*, *qnrB19* and *qnrB20*, respectively; for the remaining determinants, see Jacoby *et al* (2008).

RESULTS

Characterization of the *qnrB2* promoter

We identified the *qnrB2* promoter by using the 5′ rapid amplification of cDNA ends technique (Frohman, 1993). The *qnrB2* transcription initiation site (+1) was mapped to position –28, upstream from the start codon (Fig 1). The –35 and –10 promoter elements (TTGACG and TACCAT, respectively), separated by 18 bp, were identified upstream from the +1 start site (Fig 1). This organization is in agreement with the recent description of the promoter of the *qnrB19* allele, albeit with a difference in the spacer between the –35 and –10 boxes (18 bp in *qnrB2*, 17 bp in *qnrB19*; Cattoir *et al*, 2008; Fig 1). Only six other potential *qnrB* promoter regions are available among all *qnrB* sequences deposited in databases. Their alignment with the *qnrB2* and *qnrB19* promoter sequences showed that the −10 box is strictly conserved among the various *qnr* alleles and is separated by 17–18 bp from the −35 box, of which two sequences can be found (Fig 1). Our results confirm that the second ATG initiation codon, common to all known *qnrB* gene variants, is the initiation codon for all *qnrB* alleles, as postulated by Cattoir *et al* (2008).

Interestingly, we also identified the CTGTATAAAAAAACAG sequence between the +1 start site and the initiation codon of *qnrB2*. This sequence is homologous to the gammaproteobacteria LexA-protein-binding site consensus, CTGTN$_8$ACAG (Erill *et al*, 2007), suggesting that *qnrB2* might be regulated by LexA. Furthermore, alignment of all available *qnrB* allele sequences upstream from the ATG (18 out of 20) showed that this potential LexA-binding site is fully conserved (Fig 1). The LexA protein is a member of the SOS regulatory network that represses a set of genes, the products of which are involved in several cellular processes (Little & Mount, 1982; Fernandez De Henestrosa *et al*, 2000). By binding specifically to a 16-bp palindromic motif typically located near or within the RNA-polymerase-binding site, the LexA dimer blocks gene transcription by interfering with RNA polymerase activity (Erill *et al*, 2007). The SOS response is induced in response to DNA damage, leading to RecA protein activation, which in turn promotes LexA autocatalytic cleavage and thereby derepresses LexA-controlled genes. To verify the functionality of the LexA site identified upstream from the *qnrB2* gene, we performed electrophoresis mobility shift assay (EMSA) experiments with purified *Escherichia coli* LexA protein and showed that LexA binds to the identified motif, but not a form mutated at crucial site positions (Fig 2). This strongly suggested a role for LexA in the regulation of *qnrB2* expression.

Negative regulation of *qnrB2* by LexA

To investigate whether *qnrB2* gene expression is regulated in a LexA-dependent manner, we constructed a *lacZ* reporter transcriptional fusion with the *qnrB2* promoter (P*qnrB2*). The recombinant plasmid, pP*qnrB2–lacZ*, was introduced into *E. coli* K12 MG1656 (a *lac*- derivative of MG1655), its *lexA*- and *recA*-deleted derivatives (MG1656Δ*lexA* and MG1656Δ*recA*, respectively), and *E. coli* K12 DM49, a strain carrying the *lexA3*(Ind-) allele encoding a non-cleavable LexA protein (Mount *et al*, 1972). Measurements of β-galactosidase activity showed that P*qnrB2* exhibited a basal level of expression in the MG1656 parent strain, its *recA*- derivative and in the DM49 *lexA3*(Ind-) strain, which was sixfold higher in the *lexA*- derivative (Fig 3). A fivefold increase was also observed (Fig 3) with strain MG1656(pP*qnrB2lexA*–lacZ*), which carries changes in the crucial positions of the LexA-binding site that impede LexA binding (Fig 2). Complementation experiments in which LexA was overexpressed by the arabinose-inducible plasmid pBad-*lexA* showed that, when induced in the MG1656Δ*lexA*(pP*qnrB2–lacZ*) strain, LexA repressed P*qnrB2*, nearly restoring its expression level to that of MG1656(pP*qnrB2–lacZ*; supplementary Fig S1 online).

We also performed real-time PCR assays with MG1656, MG1656Δ*lexA* and DM49 *lexA3*(Ind-) strains that carry a plasmid allowing QnrB2 expression from its own promoter (pP*qnrB2–qnrB2*; supplementary Table S1 online). We observed a similar number of transcripts for strains MG1656 and DM49 *lexA3*(Ind-), and a 5.3-fold increase in the MG1656Δ*lexA* strain.

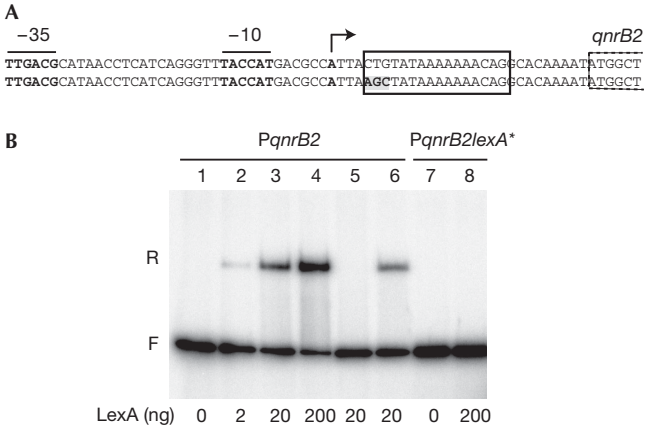

Fig 2 | LexA binds to the *qnrB2* promoter. (**A**) Sequence of the *qnrB2* promoter region with the LexA-binding site and the mutated version. The LexA-binding site sequence is boxed, the −35 and −10 promoter sequences are shown in bold, the mutated bases in the LexA site are grey-shaded and the *qnrB2* 5′ sequence indicated in a dashed open frame. (**B**) Electrophoresis mobility shift assay with the native (lanes 1–6) or mutated (lanes 7–8) *qnrB2* LexA-binding site (P*qnrB2* and P*qnrB2lexA**, respectively) in the presence or absence of LexA purified protein (amounts in nanograms are indicated). Competition experiments were performed with an excess of cold probe P*qnrB2* (lane 5) or P*qnrB2lexA** (lane 6). F, free DNA; R, retarded complex.

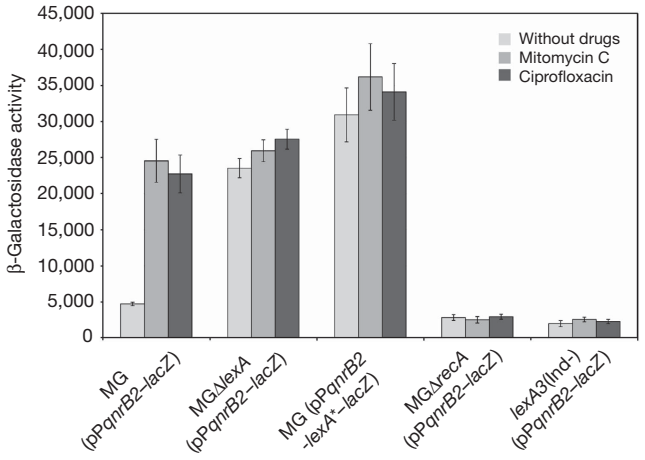

Fig 3 | Expression of *qnrB2* is regulated by LexA and the SOS response. β-Galactosidase activity was measured without drugs (light grey), in the presence of mitomycin C (medium grey) or ciprofloxacin (dark grey) in various genetic backgrounds: the parent *Escherichia coli* strain MG1656 (MG), its derivatives MG1656Δ*lexA* and MG1656Δ*recA*, as well as strain DM49 *lexA3*(Ind-). These strains contained a *lacZ*-fusion plasmid with either the native or the mutated *qnrB2* promoter, pP*qnrB2–lacZ* and pP*qnrB2lexA*–lacZ*, respectively. The error bars represent the standard deviation of at least four independent assays.

A 3.6-fold increase was consistently observed with the MG1656 (pP*qnrB2lexA*–qnrB2*) strain containing a mutation in the LexA-binding motif.

Together, these results confirmed that LexA is involved in *qnrB2* negative regulation.

Involvement of the SOS response in *qnrB2* regulation

To confirm the involvement of the SOS response in *qnrB2* regulation, we measured the β-galactosidase activity of MG1656(pP*qnrB2–lacZ*) after treatment with mitomycin C (a commonly used *in vitro* SOS activator) or ciprofloxacin (a quinolone known to induce the SOS response; Beaber *et al*, 2004; Bisognano *et al*, 2004). As shown in Fig 3, the addition of either mitomycin C or ciprofloxacin induced a fivefold increase in β-galactosidase activity in MG1656(pP*qnrB2–lacZ*) cultures, but not in MG1656Δ*recA*(pP*qnrB2–lacZ*) cultures, indicating that mitomycin C and ciprofloxacin regulation of P*qnrB2* is RecA dependent. As expected, neither drug had a significant effect on MG1656Δ*lexA*(pP*qnrB2–lacZ*) nor on MG1656(pP*qnrB2–lexA*–lacZ*) cultures, and there was no induction in DM49 *lexA3*(Ind-) (Fig 3).

These results confirmed that *qnrB2* expression is regulated by the SOS response in a LexA/RecA-dependent manner.

Effect of *qnrB2* regulation on quinolone resistance

To investigate the influence of the SOS-dependent regulation on the level of *qnrB2*-encoded quinolone resistance, we determined the minimal inhibitory concentration (MIC) of ciprofloxacin for each strain (Table 1). As ciprofloxacin itself is a well-known inducer of the SOS response, we expected to observe an induction of ciprofloxacin resistance with strain MG1656(pP*qnrB2–qnrB2*). Indeed, the MIC was 32-fold higher with MG1656(pP*qnrB2–qnrB2*) than with MG1656, and was comparable with the MIC of MG1656Δ*lexA*(pP*qnrB2–qnrB2*). Conversely, the MIC remained low for both the DM49 *lexA3*(Ind-)(pP*qnrB2–qnrB2*) and MG1656Δ*recA*(pP*qnrB2–qnrB2*) strains. Furthermore, both strains DM49 and MG1656Δ*recA* carrying plasmid pP*qnrB2lexA*–qnrB2*, in which the LexA-binding site is mutated, showed the same MIC (0.32 μg/ml) as MG1656Δ*lexA*(pP*qnrB2–qnrB2*).

These results showed that, *in vivo*, ciprofloxacin induced *qnrB2*-mediated quinolone resistance through LexA.

Table 1 | Minimal inhibitory concentrations of ciprofloxacin

***E. coli* strains**	**Plasmids**		
	pSU38Δ*tot*	**pP*qnrB2–qnrB2***	**pP*qnrB2lexA*–qnrB2***
MG1656	0.02†	0.64	0.64
MG1656Δ*lexA*	0.02	0.32	0.32
MG1656Δ*recA*	0.0025	0.005	0.32
DM49 *lexA3*(Ind-)	0.01	0.01	0.32

†Ciprofloxacin minimal inhibitory concentration (μg/ml).

DISCUSSION

Our results show that *qnrB2* has all the characteristics of an SOS-response LexA-regulated gene, as defined by Kelley (2006). These include: (i) induction in wild-type strains when exposed to DNA-damaging agents, and basal expression without drug exposure; (ii) no induction in *recA*-negative strain (no LexA derepression) or DM49 *lexA3*(Ind-) strain (uncleavable LexA); and (iii) constitutive

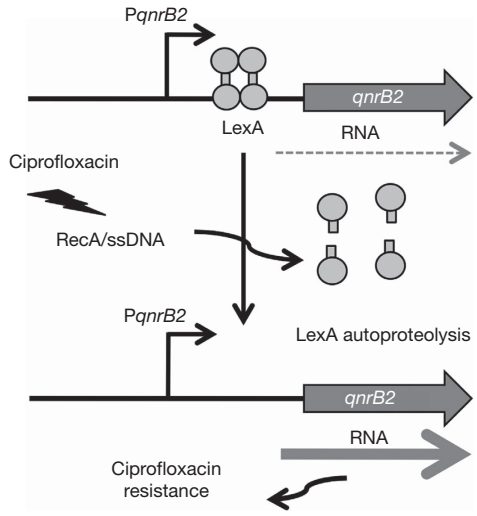

Fig 4 | Regulation of *qnrB2* gene expression. In the uninduced state, the LexA protein is bound to its site at the promoter region of *qnrB2*. The *qnrB2* gene is expressed at a basal level (dashed-line arrow). On induction of the SOS response, by ciprofloxacin for example, single-stranded DNA (ssDNA) is produced and the co-protease activity of the RecA protein is activated by binding to ssDNA. The interaction between LexA and the nucleoprotein filament RecA/ssDNA results in autoproteolytic cleavage of LexA and subsequently in *qnrB2* derepression (thick-line arrow). Induced expression of *qnrB2* leads to an increase in the ciprofloxacin minimal inhibitory concentration.

induction in Δ*lexA* strains. Furthermore, we performed EMSA assays definitely proving that the identified LexA-binding site is functional. This study shows that *qnrB2* expression is regulated directly by LexA and induced by SOS-response activators including ciprofloxacin (Fig 4).

When we analysed the available allele promoter sequences of the other *qnr* determinants (accession numbers AY070235 and EU495238 for *qnrA1* and *qnrA3*, DQ460733 and AB187515 for *qnrS1* and *qnrS2*, EU917444 for *qnrC* and FJ228229 for *qnrD*), we did not see any evidence for a LexA-binding site around the initiation codon of *qnrA*, *qnrS* and *qnrC* alleles, but we identified a potential LexA-binding site upstream from the *qnrD* gene. Interestingly, phylogenetic analysis showed that *qnrB* and *qnrD* are closer to one another than to the other *qnr* determinants (Baquirin & Barlow, 2008; Cavaco *et al*, 2009; Wang *et al*, 2009a). Furthermore, QnrB2 protein has been shown to protect DNA gyrase more efficiently than QnrA, and to inhibit DNA gyrase supercoiling activity at high concentrations (Jacoby *et al*, 2006). Other gyrase-protecting proteins, such as MfpA and GyrI, have also been shown to inhibit DNA gyrase supercoiling activity, and it has been suggested that this feature could have a cost in terms of bacterial fitness (Robicsek *et al*, 2006a). Furthermore, it has been speculated that Qnr proteins would have physiological functions other than quinolone resistance, such as resistance to naturally occurring toxins that inhibit DNA gyrase, such as CcdB and MccB17 (Ellington & Woodford, 2006). Indeed, it has been shown that McbG, a Qnr-like protein, protects DNA gyrase against MccB17, which also induces the SOS response (Herrero & Moreno, 1986). Thus, direct *qnrB* regulation through the SOS response might have two benefits, first by limiting the fitness cost of *qnrB* expression, and second by providing protection against natural stressors and toxins that are deleterious for DNA gyrase.

The presence of *qnr* determinants only leads to slightly increased resistance to fluoroquinolones, but these determinants considerably facilitate the emergence of higher-level resistance (Jacoby, 2005). In *E. coli*, this latter effect depends on the increased mutation ability conferred by the non-essential polymerases Pol II, Pol IV and Pol V on LexA-cleavage-mediated derepression of their respective genes (*polB, dinB* and *umuDC*; Cirz *et al*, 2005). Thus, *qnrB*-mediated quinolone resistance and increased mutation ability are two events triggered by the same signal, namely the SOS response. Upregulation of the quinolone-resistance gene *qnrB* by ciprofloxacin in a RecA/LexA-dependent manner is thus one more example of the involvement of the SOS system in the evolution of bacterial antibiotic resistance. Indeed, the SOS system is known to be induced by a variety of antibiotics (ciprofloxacin, rifampicin, β-lactams and trimethoprim) and has been implicated in the spread of antibiotic resistance by promoting horizontal dissemination of antibiotic-resistance genes (Beaber *et al*, 2004) or mutations (Cirz *et al*, 2005). All these observations identify LexA as a potential target for the development of inhibitor molecules to delay the emergence of multidrug-resistant bacteria.

This description of the direct SOS-dependent regulation of an antibiotic-resistance mechanism has clinical implications. Indeed, we found that *qnrB2*-mediated quinolone resistance is induced in response to the antibiotic itself even at sub-inhibitory concentrations. This is probably the case for all *qnrB* genes given the presence of a conserved LexA-binding site. Indeed, during the reviewing process of this study, Wang *et al* also identified the LexA-binding site upstream from *qnrB* alleles and showed by PCR experiments after reverse transcription that expression of several *qnrB* determinants (*qnrB1–4*) was increased in response to SOS inducers including ciprofloxacin (Wang *et al*, 2009b). Thus, a *qnrB*-containing strain does not express quinolone resistance in non-inducing conditions (silent resistance gene), but this resistance will be activated under selective antibiotic pressure. This is an important observation with respect to preventing the dissemination of resistance genes, and should be taken into account in the management of infectious disease treatments and in future antibiotic policies.

METHODS

Bacterial strains and culture conditions. All bacteria were grown in Brain Heart Infusion at 37 °C. Antibiotics, when required, were used at the following concentrations: kanamycin 25 μg/ml and ampicillin 100 μg/ml. Mitomycin C was added at a final concentration of 0.8 μg/ml, ciprofloxacin at 0.025 μg/ml, and glucose and arabinose at 1%. MG1656Δ*sfiA*Δ*lexA* (referred to as MG1656Δ*lexA*) and MG1656Δ*recA* were constructed by three-step PCR as described in Chaveroche *et al* (2000) and at http://www.pasteur.fr/recherche/unites/Ggb/3SPCRprotocol.html. Bacterial strains and plasmids used in this study are summarized in supplementary Table S2 online.

Plasmid construction. Plasmids were constructed as detailed in the supplementary information online.

5′ rapid amplification of cDNA ends. 5′ rapid amplification of cDNA ends was performed as recommended by the manufacturer (Invitrogen, Cergy Pontoise, France). Total RNAs were extracted from cultures of *S. enterica* serovar Keurmassar (Garnier *et al*,

2006). Gene-specific primers GSP1-*qnrB2*, GSP2-*qnrB2* and GSP3-*qnrB2* were used (supplementary Table S3 online).

Minimal inhibitory concentration determination. MICs were determined as recommended by CLSI (Clinical and Laboratory Standards Institute; http://www.clsi.org), and were evaluated three times.

β-Galactosidase assay. Overnight (o/n) cultures of cells containing pP*qnrB2–lacZ* were diluted 1:100 in Brain Heart Infusion supplemented with kanamycin and grown for 2 h before adding mitomycin C or ciprofloxacin. Cells were then grown for a further hour before the assay. β-Galactosidase-specific activity was measured as described in Miller (1992), except that the temperature was set at 37 °C.

Electrophoresis mobility shift assay. Overexpression and purification of the *E. coli* LexA protein encoded by pUA1107 were performed as described earlier (Abella *et al*, 2004). EMSA probes were obtained by PCR amplification using oligonucleotides qnrB-EMSA-5 and qnrB-EMSA-3 (supplementary Table S3 online), and were end-labelled with [γ^{32}P]ATP (Amersham, Saclay, France) using T4 polynucleotide kinase (Promega, Charbonnières, France). EMSA experiments were performed as described elsewhere (Abella *et al*, 2004), using various amounts of purified LexA, 20 ng of one of the radiolabelled DNA probes in the binding mixture and 1.4 μg of unlabelled probe for competition experiments. Samples were separated in 5% non-denaturing Tris–glycine–EDTA polyacrylamide gel, then dried and exposed to storage Phosphor Screen (Perkin-Elmer, Courtaboeuf, France). Images were digitized with a Cyclone scanner.

Supplementary information is available at *EMBO reports* online (http://www.emboreports.org).

ACKNOWLEDGEMENTS

We thank A. Tabesse and A. Chanut for technical assistance, T. Jové and E. Pinaud for helpful discussions, and D. Mazel and S. Raherisson for critical reading of the paper. This work was supported by grants from the French Ministère de la Recherche, from the Conseil Régional du Limousin, from the Fondation pour la Recherche Médicale and from the Institut National de la Santé et de la Recherche Médicale (Inserm). S.D.R. is supported by Inserm, E.G. by the Conseil Régional du Limousin and S.C. by grants BFU2008-01078/BMC from the Ministerio de Ciencia e Innovación (MICINN) and by Generalitat de Catalunya.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abella M, Erill I, Jara M, Mazon G, Campoy S, Barbe J (2004) Widespread distribution of a *lexA*-regulated DNA damage-inducible multiple gene cassette in the Proteobacteria phylum. *Mol Microbiol* **54:** 212–222

Baquirin MH, Barlow M (2008) Evolution and recombination of the plasmidic *qnr* alleles. *J Mol Evol* **67:** 103–110

Beaber JW, Hochhut B, Waldor MK (2004) SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* **427:** 72–74

Bisognano C, Kelley WL, Estoppey T, Francois P, Schrenzel J, Li D, Lew DP, Hooper DC, Cheung AL, Vaudaux P (2004) A recA-LexA-dependent pathway mediates ciprofloxacin-induced fibronectin binding in *Staphylococcus aureus*. *J Biol Chem* **279:** 9064–9071

Cattoir V, Nordmann P, Silva-Sanchez J, Espinal P, Poirel L (2008) ISEcp1-mediated transposition of *qnrB*-like gene in *Escherichia coli*. *Antimicrob Agents Chemother* **52:** 2929–2932

Cavaco LM, Hasman H, Xia S, Aarestrup FM (2009) *qnrD*, a novel gene conferring transferable quinolone resistance in *Salmonella enterica* serovar Kentucky and Bovismorbificans strains of human origin. *Antimicrob Agents Chemother* **53:** 603–608

Chaveroche MK, Ghigo JM, d'Enfert C (2000) A rapid method for efficient gene replacement in the filamentous fungus *Aspergillus nidulans*. *Nucleic Acids Res* **28:** E97

Cirz RT, Chin JK, Andes DR, de Crecy-Lagard V, Craig WA, Romesberg FE (2005) Inhibition of mutation and combating the evolution of antibiotic resistance. *PLoS Biol* **3:** e176

Ellington MJ, Woodford N (2006) Fluoroquinolone resistance and plasmid addiction systems: self-imposed selection pressure? *J Antimicrob Chemother* **57:** 1026–1029

Erill I, Campoy S, Barbe J (2007) Aeons of distress: an evolutionary perspective on the bacterial SOS response. *FEMS Microbiol Rev* **31:** 637–656

Fernandez De Henestrosa AR, Ogi T, Aoyagi S, Chafin D, Hayes JJ, Ohmori H, Woodgate R (2000) Identification of additional genes belonging to the LexA regulon in *Escherichia coli*. *Mol Microbiol* **35:** 1560–1572

Frohman MA (1993) Rapid amplification of complementary DNA ends for generation of full-length complementary DNAs: thermal RACE. *Methods Enzymol* **218:** 340–356

Garnier F, Raked N, Gassama A, Denis F, Ploy MC (2006) Genetic environment of quinolone resistance gene *qnrB2* in a complex sul1-type integron in the newly described *Salmonella enterica* serovar Keurmassar. *Antimicrob Agents Chemother* **50:** 3200–3202

Herrero M, Moreno F (1986) Microcin B17 blocks DNA replication and induces the SOS system in *Escherichia coli*. *J Gen Microbiol* **132:** 393–402

Jacoby G, Cattoir V, Hooper D, Martinez-Martinez L, Nordmann P, Pascual A, Poirel L, Wang M (2008) *qnr* Gene nomenclature. *Antimicrob Agents Chemother* **52:** 2297–2299

Jacoby GA (2005) Mechanisms of resistance to quinolones. *Clin Infect Dis* **41** (**Suppl 2**)**:** S120–S126

Jacoby GA, Walsh KE, Mills DM, Walker VJ, Oh H, Robicsek A, Hooper DC (2006) *qnrB*, another plasmid-mediated gene for quinolone resistance. *Antimicrob Agents Chemother* **50:** 1178–1182

Kelley WL (2006) Lex marks the spot: the virulent side of SOS and a closer look at the LexA regulon. *Mol Microbiol* **62:** 1228–1238

Little JW, Mount DW (1982) The SOS regulatory system of *Escherichia coli*. *Cell* **29:** 11–22

Mammeri H, Van De Loo M, Poirel L, Martinez-Martinez L, Nordmann P (2005) Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. *Antimicrob Agents Chemother* **49:** 71–76

Martinez-Martinez L, Pascual A, Jacoby GA (1998) Quinolone resistance from a transferable plasmid. *Lancet* **351:** 797–799

Miller JH (1992) *A Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for Escherichia coli and Related Bacteria*. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press

Mount DW, Low KB, Edmiston SJ (1972) Dominant mutations (lex) in *Escherichia coli* K-12 which affect radiation sensitivity and frequency of ultraviolet lght-induced mutations. *J Bacteriol* **112:** 886–893

Nordmann P, Poirel L (2005) Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. *J Antimicrob Chemother* **56:** 463–469

Perichon B, Courvalin P, Galimand M (2007) Transferable resistance to aminoglycosides by methylation of G1405 in 16S rRNA and to hydrophilic fluoroquinolones by QepA-mediated efflux in *Escherichia coli*. *Antimicrob Agents Chemother* **51:** 2464–2469

Robicsek A, Jacoby GA, Hooper DC (2006a) The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infect Dis* **6:** 629–640

Robicsek A, Strahilevitz J, Jacoby GA, Macielag M, Abbanat D, Park CH, Bush K, Hooper DC (2006b) Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nat Med* **12:** 83–88

Rodriguez-Martinez JM, Velasco C, Pascual A, Garcia I, Martinez-Martinez L (2006) Correlation of quinolone resistance levels and differences in basal and quinolone-induced expression from three *qnrA*-containing plasmids. *Clin Microbiol Infect* **12:** 440–445

Wang M, Guo Q, Xu X, Wang X, Ye X, Wu S, Hooper DC (2009a) New plasmid-mediated quinolone resistance Gene, *qnrC*, found in a clinical isolate of *Proteus mirabilis*. *Antimicrob Agents Chemother* **53:** 821–823

Wang M, Jacoby GA, Mills DM, Hooper DC (2009b) SOS regulation of qnrB expression. *Antimicrob Agents Chemother* **53:** 821–823

Yamane K, Wachino J, Suzuki S, Kimura K, Shibata N, Kato H, Shibayama K, Konda T, Arakawa Y (2007) New plasmid-mediated fluoroquinolone efflux pump, QepA, found in an *Escherichia coli* clinical isolate. *Antimicrob Agents Chemother* **51:** 3354–3360