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Function of the SOS Process in Repair of DNA Damage Induced by Modern 4-Quinolones

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Abstract—The *recA13* mutant of *Escherichia coli* strain K-12, which lacks recombination and SOS error-prone DNA repair is hypersensitive to nalidixic acid and to the newer 4-quinolones ciprofloxacin, norfloxacin and ofloxacin. However, whereas recombination-proficient but SOS repair-deficient strains, such as those carrying the *lexA3* or *recA430* alleles are no more sensitive to nalidixic than the *lexA*$^+$ *recA*$^+$ parent, they are more sensitive to the newer quinolones, although not as sensitive as the *recA13* derivative. Nalidixic acid possesses only bactericidal mechanism A (which requires RNA and protein synthesis and is only effective on actively dividing cells), whereas the newer 4-quinolones exhibit additional mechanisms B (which does not require RNA and protein synthesis and is effective on bacteria unable to multiply) and C (which requires RNA and protein synthesis but does not depend on cell division). Results obtained with bacteria suspended in phosphate-buffered saline, which inhibits mechanism A, and with bacteria suspended in nutrient broth plus rifampicin, which inhibits mechanisms A and C, showed that the *lexA3* mutant was still more sensitive than the *lexA*$^+$ parent under these conditions. The results suggest that, unlike bactericidal mechanism A, DNA damage that results from bactericidal mechanisms B and C of the newer 4-quinolones is subject to SOS error-prone (mutagenic) repair.

Recombination of homologous DNA in *Escherichia coli* requires the products of several genes including *recA*, *recB*, *recC* and *recD* (reviewed by Smith 1988). The *recA* gene product promotes a variety of ATP-dependent DNA interactions, whereas the products of the *recB*, *recC* and *recD* genes together make up the ATP-dependent exonuclease V, which has DNA unwinding as well as nuclease and ATPase activities.

Some types of damage to bacterial DNA can induce a diverse set of effects, termed the SOS response (Walker 1984). The RecA protein also plays a role, in concert with the product of the *lexA* gene, in regulating this process. LexA and RecA proteins control transcription of a group of unlinked DNA damage-inducible (*din*) genes, which together constitute the SOS regulon. The LexA protein is the repressor of the SOS regulon, binding to similar sequences in all *din* genes and inhibiting their transcription (Walker 1984).

DNA damage causes bacteria to accumulate abnormal levels of deoxyribonucleotides and single-stranded DNA, which activate RecA protein (Craig & Roberts 1980) to stimulate autodigestion of the LexA protein (Slilaty et al 1986; Little 1991). The duration of the SOS response is therefore self-limiting, because once DNA is repaired the inducing stimulus is removed and levels of activated RecA consequently decline so that the LexA repressor levels rise to return the regulon back to its normal state of expression. Both nalidixic acid (Gudas & Pardee 1975), the prototype 4-quinolone, and the newer fluoroquinolones (Piddock & Wise 1987; Phillips et al 1987) induce the SOS response. Nalidixic acid induces high levels of RecA protein, and an early suggestion was that RecA could protect gapped DNA from attack by the RecBCD exonuclease (Ginsburg et al 1982). It is also possible that error-prone mutagenic DNA repair, which is induced as part of the SOS response (Walker 1984), could help protect cells from nalidixic acid-induced damage. On the other hand, SOS induction could even be responsible for quinolone-induced death. Walters et al (1989) pointed out that quinolones not only induce the SOS repair process, but also inhibit DNA gyrase, and the latter effect could prevent repair from going to completion. Incomplete DNA repair would lead to continuous expression of SOS genes such as the cell division inhibitor *sfiA*, causing cell filamentation and death. Therefore, it is not clear whether SOS induction helps protect cells from DNA damage or is, conversely, responsible for quinolone-induced death.

Lewin et al (1989) presented evidence suggesting that error-free recombination repair, but not the inducible mutagenic SOS system, is responsible for repairing nalidixic acid-induced damage. They showed that the *recA13* mutant, deficient in both recombination and SOS DNA repair, was hypersensitive to this quinolone, whereas a *lexA3* mutant, proficient in recombination repair but deficient in SOS repair, was no more sensitive to nalidixic acid than the *recA*$^+$ *lexA*$^+$ parent strain. We now report an investigation of the effects of SOS and recombination repair on the activities of the new 4-quinolones, ciprofloxacin, norfloxacin and ofloxacin.

Materials and Methods

Antibacterial agents

Ciprofloxacin (Bayer, Newbury, UK) and norfloxacin (Merck, Sharp and Dohme, Hoddesden) were dissolved in sterile distilled water. Ofloxacin (Hoechst, Hounslow) was dissolved initially in 0·5 M NaOH solution (0·2 mL mg^{-1}) and then diluted in sterile distilled water. Stock solutions of rifampicin (Sigma, Poole) were prepared at 1 mg mL^{-1} in dimethylsulphoxide and then diluted in sterile distilled water.

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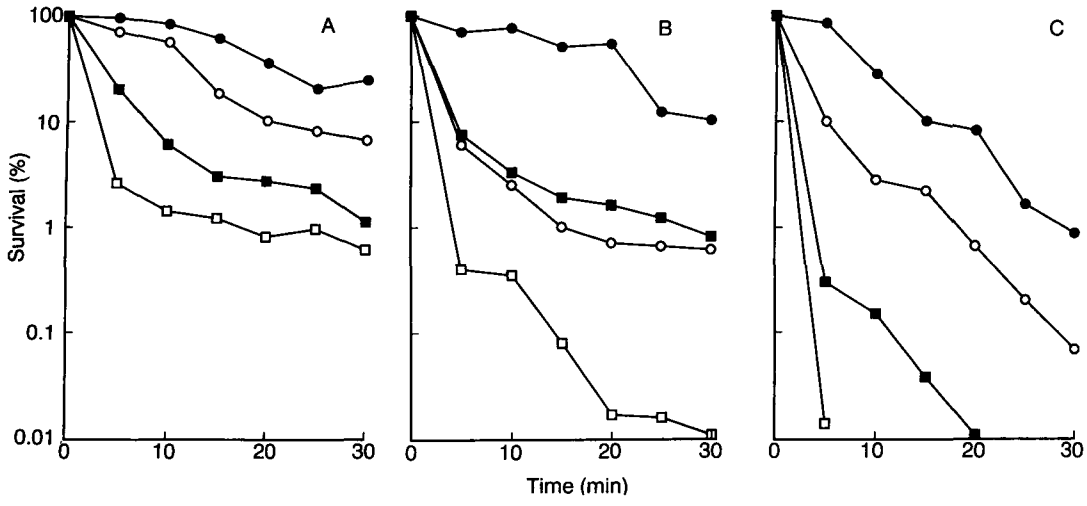

FIG. 1. Survival of *Escherichia coli* K-12 strains exposed to: A. 1·5 mg L^{-1} norfloxacin, B. 0·15 mg L^{-1} ciprofloxacin, or C. 0·9 mg L^{-1} ofloxacin in nutrient broth. Strains: AB1157 *recA*$^+$ *lexA*$^+$ (●), IC1657 *recA430 lexA*$^+$ (○), AB2463 *recA13 lexA*$^+$ (□), AB2494 *lexA3 recA*$^+$ (■).

Bacterial strains

All organisms used were derivatives of the *Escherichia coli* K-12 strain AB1157 *thr-1 leu-6 proA2 his4 thi-1 argE3 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx33 strA31 sup-37 recA*$^+$ *lexA*$^+$ (Bachmann 1972), which is fully proficient in all DNA processes. Strain AB2463 (Howard-Flanders & Theriot 1966) is a *recA13* derivative and strain AB2494 (Howard-Flanders 1968) carries the *lexA3* allele, whereas strain IC1657 (Blanco et al 1986) is *recA430*.

Determination of antibacterial effects

Quantities of 4-quinolone solution, sterile distilled water and rifampicin solution, if required, were added to 5 mL double-strength nutrient broth (Oxoid No. 2) to give the required concentration of quinolone in a final volume of 9·8 mL nutrient broth. All quinolones were tested at their most bactericidal concentrations (Smith 1984). Solutions were warmed to 37°C and 0·2 mL of an overnight nutrient broth culture of the strain to be tested was added to give an initial viable count of approximately 5×10^6 colony-forming units (cfu) mL^{-1}. Cultures were maintained at 37°C, diluted at intervals in nutrient broth and plated on nutrient agar (nutrient broth plus 1·5% Lab M agar) to determine viable counts. Plates were incubated for up to two days at 37°C and colonies counted. Phosphate-buffered saline (PBS) contained 9 g L^{-1} NaCl in 25 mM sodium phosphate buffer, pH 7·4. It was used double-strength in place of double-strength nutrient broth to produce the required solutions. Inocula for use in PBS experiments were prepared by washing overnight nutrient broth cultures twice, by centrifugation, in twice their original culture volume of PBS. Cells were then finally resuspended to their original culture volume in PBS before being used to inoculate reaction mixtures.

Results

Relative antibacterial activities in nutrient broth

Sufficient RecA protein is produced in uninduced *recA*$^+$ *lexA*$^+$ *Escherichia coli* strains, such as AB1157, for normal recombination functions. SOS error-prone, mutagenic DNA repair is also fully inducible in this wild-type strain (Walker 1984). However, the *recA13* mutant is completely defective in both DNA recombination and in inducible SOS repair functions, whereas strain IC1657, which carries the *recA430* allele, has almost normal recombination activity, but is deficient in SOS repair (Sassanfar & Roberts 1990). Similarly, the *lexA3* mutant strain has normal recombination activity, but because its LexA3 protein is resistant to SOS-induced cleavage, it is SOS-uninducible (Sassanfar & Roberts 1990).

As expected, the *recA13* mutant was more sensitive to norfloxacin than the parent *recA*$^+$ strain (Fig. 1). Surprisingly, however, both the *lexA3* and *recA430* mutants, though not as hypersensitive as the *recA13* mutant, were also more sensitive to norfloxacin than the wild-type *recA*$^+$ *lexA*$^+$ strain. This finding was not expected because previous results (Lewin et al 1989) had shown that the *lexA3* mutant was no more sensitive to nalidixic acid than its *lexA*$^+$ parent.

Ciprofloxacin was more active than norfloxacin (Fig. 1), with the *recA13* mutant being extremely hypersensitive compared with the *recA*$^+$ parent. However, once again the *lexA3* and *recA430* mutants, though not as hypersensitive as the *recA13* mutant, were also more sensitive to ciprofloxacin than the parent strain. Ofloxacin was even more active than ciprofloxacin (Fig. 1). Once again, as expected, the *recA13* mutant was much more sensitive than its *recA*$^+$ parent, but, yet again, the *recA430* and the *lexA3* mutants were also more sensitive than the *recA*$^+$ *lexA*$^+$ wild-type strain, but not as hypersensitive as the *recA13* mutant.

Antibacterial activities in conditions that inhibit cell division and RNA synthesis

The 4-quinolone antibacterials possess up to three mechanisms of kill (Smith 1984; Ratcliffe & Smith 1985; Lewin & Smith 1986). Mechanism A, which is common to all quinolones, requires protein and RNA synthesis and is only effective against actively-dividing bacteria. Mechanism B is active on bacteria unable to multiply, and therefore operates

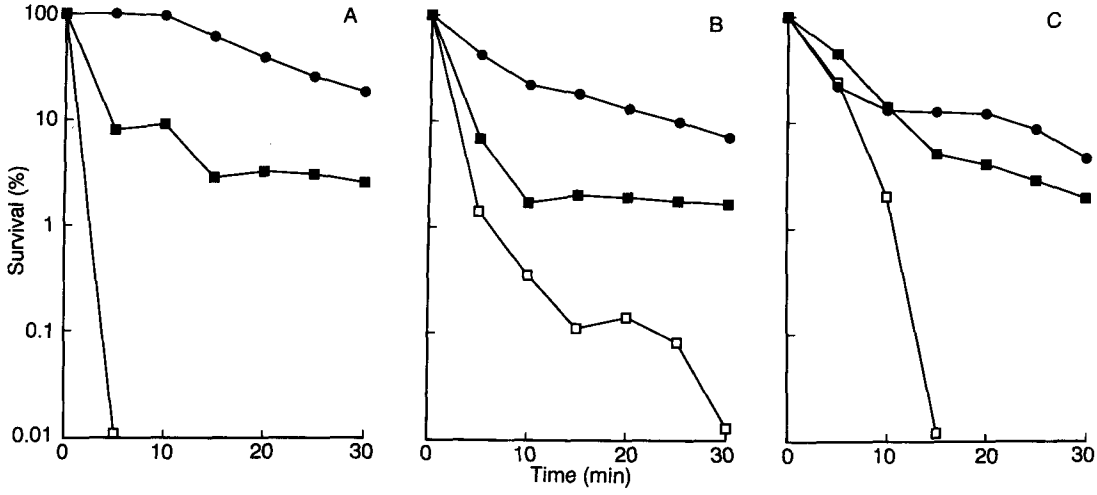

FIG. 2. Survival of *Escherichia coli* K-12 strains exposed to: A. 1·5 mg L^{-1} norfloxacin, B. 0·15 mg L^{-1} ciprofloxacin, or C. 0·9 mg L^{-1} ofloxacin in phosphate-buffered saline. Strains: AB1157 *recA*$^+$ *lexA*$^+$ (●), AB2463 *recA13 lexA*$^+$ (□), AB2494 *lexA3 recA*$^+$ (■).

against bacteria suspended in PBS. It is also effective against bacteria in which protein or RNA synthesis has been inhibited, and will therefore operate on bacteria suspended in nutrient broth in the presence of rifampicin. Mechanism C requires protein and RNA synthesis for activity, but does not require cell division. It is therefore effective on bacteria suspended in PBS, but is not effective on bacteria suspended in nutrient broth in the presence of rifampicin.

Nalidixic acid possesses only mechanism A, whereas all three of the newer quinolones studied possess mechanism C (Ratcliffe & Smith 1985; Lewin & Smith 1986), while ciprofloxacin and ofloxacin also exhibit mechanism B (Smith 1984). We therefore tested the possibility that the unexpected hypersensitivity of the *lexA3* and *recA430* mutants to the fluorinated quinolones could be due to them possessing mechanisms B or C. Norfloxacin does not possess mechanism B, and therefore any lethal effect in PBS could only be due to mechanism C. Fig. 2 shows that the *recA13* mutant, deficient in recombination and SOS repair was extremely hypersensitive to norfloxacin in PBS. The *lexA3* mutant, which is recombination repair-proficient but SOS repair-deficient was also more sensitive than the wild-type *recA*$^+$ strain, although not as hypersensitive as the *recA13* mutant. The extra hypersensitivity conferred by *recA13* over *lexA3*, reflects the contribution of recombination repair to recovery from norfloxacin-induced DNA damage caused by bactericidal mechanism C. However, the greater sensitivity of the *lexA3* mutant, compared with its *recA*$^+$ *lexA*$^+$ parent, suggests that the SOS response is also acting to repair damage caused by norfloxacin's mechanism C.

Experiments with ciprofloxacin and ofloxacin (Fig. 2) in PBS gave similar results to those obtained with norfloxacin, which may suggest that damage due to mechanism C was also being repaired by recombination repair and by the SOS response. However, since ciprofloxacin and ofloxacin also possess mechanism B in PBS, the experiments were repeated in nutrient broth with the addition of a bacteriostatic concentration of rifampicin. Since rifampicin inhibits mechanisms A and C by its action on RNA synthesis, any activity in its presence must be due solely to mechanism B. Fig. 3 shows that the *recA13* mutant retained its hypersensitivity to ciprofloxacin and to ofloxacin in the presence of rifampicin, and that the *lexA3* mutant was also more sensitive to both drugs than the *recA*$^+$ *lexA*$^+$ parent strain, but not as hypersensitive as the *recA13* mutant. These results therefore indicate that damage caused by mechanism B is also repairable by SOS error-prone DNA repair as well as by the recombination pathway.

Discussion

Functional *recABCD* genes are required for induction of the SOS response by nalidixic acid (Walker 1984). Mutations inactivating any of these genes increase sensitivity to the drug (McDaniel et al 1978; Lewin et al 1989). Drlica (1984) proposed that SOS gene products are involved in repair of nalidixic acid-induced damage. However, Ratcliffe & Smith (1986) found that a *lexA3* mutant, which is recombination-proficient but SOS-deficient, was no more sensitive to nalidixic acid than its *lecA*$^+$ parent. Therefore the results of Ratcliffe & Smith (1985) suggest that SOS gene function is involved neither in repair of nalidixic acid-induced damage nor in the bactericidal activity of nalidixic acid. The *recA430* mutant, like strains carrying the *lexA3* allele, is recombination-proficient but SOS-deficient, and like *lexA3*, is no more sensitive than its parent to nalidixic acid (Howard 1991). This tends to confirm the suggestion of Ratcliffe & Smith (1985) that the SOS response is not involved in repair of nalidixic acid-induced damage, nor does it contribute to the bactericidal activity of nalidixic acid. The *recA430* allele confers, in fact, a double block to SOS-induction; not only cannot RecA430 protein facilitate the digestion of LexA repressor, but it is also deficient in the proteolytic function required for the processing of UmuD protein to UmuD′, which is necessary for the induction of SOS DNA repair (Burkhardt et al 1988; Nohmi et al 1988).

SOS DNA repair has been proposed, on the one hand, to contribute to quinolone-induced death (McDaniel et al 1978;

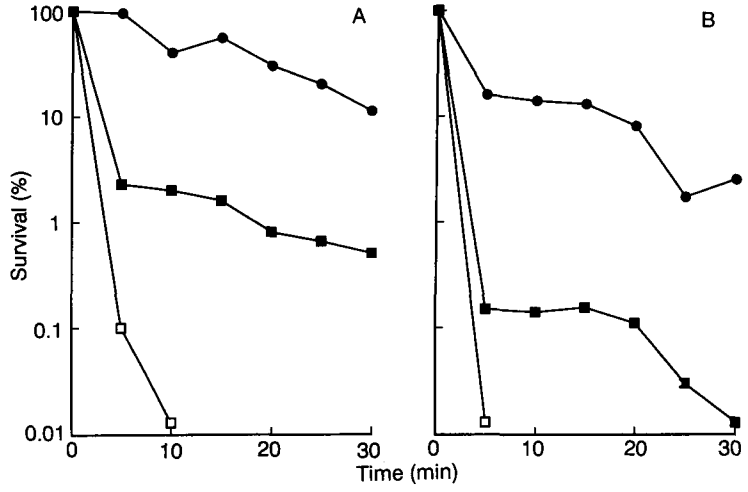

FIG. 3. Survival of *Escherichia coli* K-12 strains exposed to: A. 0·15 mg L^{-1} ciprofloxacin, or B. 0·9 mg L^{-1} ofloxacin in nutrient broth containing 150 mg L^{-1} rifampicin. Strains: AB1157 *recA*$^+$ *lexA*$^+$ (●), AB2463 *recA13* *lexA*$^+$ (□), AB2494 *lexA3* *recA*$^+$ (■).

Piddock & Wise 1987; Phillips et al 1987; Piddock et al 1990) and, on the other hand, to repair DNA damage initiated by quinolone activity (Ginsburg et al 1982; Drlica 1984; Walters et al 1989). The work of Smith and co-workers (Smith 1984; Ratcliffe & Smith 1985; Lewin & Smith 1986) showed that, unlike nalidixic acid, the newer 4-quinolones have more than one mechanism of action. Damage induced by mechanism A, which is common to all quinolones and which requires RNA and protein synthesis for activity (Smith 1984), appears to be repaired by recombination repair, but not by the SOS response (Lewin et al 1989). This explains why ciprofloxacin, norfloxacin and ofloxacin are more bactericidal to the recombination-deficient *recA13* strain than to the *recA*$^+$ parent (Figs 1, 2, 3). *lexA3* and *recA430* mutants are proficient in recombination repair, and therefore are less hypersensitive to these 4-quinolones than is *recA13* (Fig. 1). However, the *recA430* and *lexA3* derivatives are still significantly more sensitive than their *recA*$^+$ *lexA*$^+$ wild-type parent strain (Fig. 1), suggesting that SOS repair, which is lacking in these mutants, has a role in the repair of damage induced by ciprofloxacin, norfloxacin and ofloxacin.

Exposure of bacteria in PBS inhibits bactericidal mechanism A, but permits bactericidal mechanism B or C of the quinolones to function. The *recA13* mutant, lacking both recombination and SOS DNA repair was extremely hypersensitive to ciprofloxacin, norfloxacin and ofloxacin in PBS (Fig. 2). However, unlike nalidixic acid (Lewin et al 1989) these three drugs were more bactericidal against the SOS repair-defective *lexA3* mutant in PBS than against its *lexA*$^+$ wild-type parent strain (Fig. 2). Ciprofloxacin and ofloxacin possess mechanisms A, B and C, while norfloxacin possesses mechanisms A and C. Therefore, the results in Fig. 2 suggest that DNA damage due to norfloxacin's mechanism C is repairable not only by the recombination pathway but also by error-prone SOS DNA repair. The same conclusions can be applied to the repair of DNA damage caused by bactericidal mechanisms B or C of ciprofloxacin and ofloxacin (Fig. 2). However, similar results were obtained with ciprofloxacin and ofloxacin in nutrient broth plus rifampicin (Fig. 3), conditions which abolish both mechanisms A and C. The *lexA3* mutant was, once again, more sensitive than its parent to ciprofloxacin and ofloxacin in the presence of rifampicin (Fig. 3). This suggests that the SOS process plays a role in the repair of DNA damage due to mechanism B in addition to repair mediated by the recombination pathway.

The results presented in this paper provide further evidence that, unlike nalidixic acid, modern 4-quinolones have more than one mechanism of action. Also, our results suggest that whereas DNA damage resulting from nalidixic acid exposure is repaired only by error-free recombination repair, DNA damage caused by ciprofloxacin, norfloxacin or ofloxacin is further subject to SOS error-prone (mutagenic) DNA repair. Urios et al (1991) have also recently reported evidence that an SOS-dependent process contributes to the repair of ciprofloxacin-induced damage.

We suggest that mutagenic SOS repair is induced in response to damage caused by bactericidal mechanisms B and C of the newer fluoroquinolones. In agreement with these findings and contrary to previous reports, the fluoroquinolones have recently been shown to be positive mutagens in Ames strains of *Salmonella typhimurium*, providing that excision repair is functioning (Ysern et al 1990; Gocke 1991).

References

Bachmann, B. J. (1972) Pedigrees of some mutant strains of *Escherichia coli* K-12. J. Bacteriol. 36: 525–557

Blanco, M., Herrera, G., Aleixandre, V. (1986) Different efficiency of UmuDC and MucAB proteins in UV light induced mutagenesis in *Escherichia coli*. Mol. Gen. Genet. 205: 234–239

Burkhardt, S. E., Woodgate, R., Scheuermann, R. H., Echols, H. (1988) UmuD mutagenesis protein of *Escherichia coli*: overproduction, purification and cleavage by RecA. Proc. Natl. Acad. Sci. USA 85: 1811–1815

Craig, N. L., Roberts, J. W. (1980) *E. coli recA* protein-directed cleavage of phage λ repressor requires polynucleotide. Nature (Lond.) 283: 26–30

Drlica, K. (1984) Biology of bacterial deoxyribonucleic acid topoisomerases. Microbiol. Rev. 48: 273–289

Ginsburg, H., Edmiston, S. H., Harper, J., Mount, D. W. (1982) Isolation and characterisation of an operator-constitutive mutation in the *recA* gene of *E. coli* K12. Mol. Gen. Genet. 187: 4–11

Gocke, E. (1991) Mechanism of quinolone mutagenicity in bacteria. Mut. Res. 248: 135–143

Gudas, L. J., Pardee, A. B. (1975) Model for regulation of *Escherichia coli* DNA repair functions. Proc. Natl. Acad. Sci. USA 72: 2330–2334

Howard, B. M. H. (1991) Studies on the Bactericidal Activities of the 4-Quinolones. PhD Thesis, University of London

Howard-Flanders, P. (1968) Genes that control DNA repair and genetic recombinaton in *Escherichia coli*. Adv. Biol. Med. Phys. 12: 299–317

Howard-Flanders, P., Theriot, L. (1966) Mutants of *Escherichia coli* K-12 defective in DNA repair and in genetic recombination. Genetics 53: 1137–1150

Lewin, C. S., Smith, J. T. (1986) Detection of a third bactericidal mechanism in ciprofloxacin and ofloxacin. J. Pharm. Pharmacol. 38 (Suppl.): 44P

Lewin, C. S., Howard, B. M. A., Ratcliffe, N. T., Smith, J. T. (1989) 4-Quinolones and the SOS response. J. Med. Microbiol. 29: 139–144

Little, J. W. (1991) Mechanisms of specific LexA cleavage: autodigestion and the role of RecA coprotease. Biochimie 73: 411–422

McDaniel, L. S., Rogers, L. H., Hill, W. (1978) Survival of recombination deficient mutants of *Escherichia coli* during incubation with nalidixic acid. J. Bacteriol. 134: 1195–1198

Nohmi, T., Battista, J. R., Dodson, L. A., Walker, G. C. (1988) RecA-mediated cleavage activates UmuD for mutagenesis: mechanistic relationship between transcriptional drepression and post-translational activation. Proc. Natl. Acad. Sci. USA 85: 1816–1820

Phillips, I., Culebras, E., Moreno, F., Baguero, F. (1987) Induction of the SOS response by new 4-quinolones. J. Antimicrob. Chemother. 20: 631–638

Piddock, L. J., Wise, C. (1987) Induction of the SOS response in *Escherichia coli* by 4-quinolone antimicrobial agents. FEMS Microbiol. Lett. 41: 289–294

Piddock, L. J. V., Walters, R. N., Diver, J. M. (1990) Correlation of quinolone MIC and inhibition of DNA, RNA, and protein synthesis and induction of the SOS response in *Escherichia coli*. Antimicrob. Agents Chemother. 34: 2331–2336

Ratcliffe, N. T., Smith, J. T. (1985) Norfloxacin has a novel bactericidal mechanism unrelated to that of other 4-quinolones. J. Pharm. Pharmacol. 37 (Suppl.): 92P

Ratcliffe, N. T., Smith, J. T. (1986) Effect of nalidixic acid on bacteria possessing mutations in the *recA* and *lexA* genes. J. Pharm. Pharmacol. 38 (Suppl.): 45P

Sassanfar, M., Roberts, J. W. (1990) Nature of the SOS-inducing signal in the involvement of DNA replication. J. Mol. Biol. 212: 79–96

Slilaty, S. N., Rupley, J. A., Little, J. W. (1986) Intramolecular cleavage of LexA and phage lambda repressors: dependence of kinetics on repressor concentration, pH, temperature and solvent. Biochemistry 25: 6866–6875

Smith, G. R. (1988) Homologous recombination in procaryotes. Microbiol. Rev. 52: 1–28

Smith, J. T. (1984) Awakening the slumbering potential of the 4-quinolone antibacterials. Pharm. J. 233: 299–305

Urios, A., Herrera, G., Aleixandre, V., Blanco, M. (1991) Influence of *recA* mutations on *gyrA* dependent quinolone resistance. Biochimie 73: 519–521

Walker, G. C. (1984) Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. 48: 60–93

Walters, R. N., Piddock, L. J. V., Wise, R. (1989) The effect of mutations in the SOS response on the kinetics of quinolone killing. J. Antimicrob. Chemother. 24: 863–873

Ysern, P., Clerch, B., Castano, M., Gilbert, I., Barbe, J., Llagostera, M. (1990) Induction of SOS genes in *Escherichia coli* and mutagenesis in *Salmonella typhimurium*. Mutagenesis 5: 63–66