The Sensitivity of Suppressed and Unsuppressed *lon* Strains of *Escherichia coli* to Chemical Agents which Induce Filamentation

By R. JAMES AND N. E. GILLIES

*Department of Biology as Applied to Medicine,
The Middlesex Hospital Medical School, London, W1P 6DB*

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SUMMARY

The sensitivities of *lon* strains of *Escherichia coli* to the filament inducing agents nalidixic acid, gentian violet, crystal violet and penicillin were examined. The strains used included those in which the *lon* gene is suppressed by *sul*, *exr* and *rec* as well as strains in which the Lon phenotype is expressed. The sensitivity of the bacteria to agents which act on DNA was reduced when *lon* was suppressed, but the extent of the decrease in sensitivity was dependent on the mechanism by which suppression was effected. Sensitivity to penicillin, on the other hand, was reduced only in the *sul* strain and by a mechanism which appears to be independent of the direct suppression of *lon*. A model to account for these results indicates possible sites at which the *lon*, *sul*, *exrA* and *recA* genes are expressed.

INTRODUCTION

Many factors control the radiation sensitivity of strains of *Escherichia coli*. Apart from the well-established enzymatically controlled repair processes which reduce the expression of radiation damage (Boyce & Howard-Flanders, 1964; Setlow & Carrier, 1964; Rupp & Howard-Flanders, 1968), a major factor in determining radiation response is the sensitivity of processes directly involved in mechanisms controlling division of the bacteria. In some strains, septum formation, and therefore complete division of irradiated bacteria, is inhibited without significant concomitant effects on increase in mass, so that they grow into non-septate multinucleated filaments which eventually lyse (Witkin, 1947; Alper, 1957; Van de Putte, Westenbroek, & Rörsch, 1963; Adler & Hardigree, 1965; Brown & Gillies, 1972). This defect, the expression of a gene originally called *fil*⁺ in *E. coli* B and *lon* in strains of *E. coli* K12 but now generally referred to as *lon*, is mainly responsible for the high radio-sensitivity of such strains. In several strains of *E. coli* the Lon phenotype is suppressed by other genes such as *sul*, *exrA* and *recA* (Donch, Green & Greenberg, 1968; Donch, Chung & Greenberg, 1969; Green, Greenberg & Donch, 1969), so that after irradiation little or no filamentation is observed. In the *sul* strain *E. coli* B/r the suppression of *lon* confers radiation resistance (Donch *et al.* 1969), but the same effect is not observed in *exrA* and *recA* strains and this is presumably because of the marked radiation sensitivity arising directly from these mutations (Howard-Flanders & Boyce, 1966; Mattern, Zwenk & Rörsch, 1966). The mechanisms by which expression of the Lon phenotype is suppressed are unknown at present.

Several chemical agents also cause filamentation in unsuppressed *lon* strains, e.g. nalidixic acid (Kantor & Deering, 1968; Walker & Pardee, 1968), gentian violet (Normark & Westling, 1971), crystal violet (Walker, Shafiq & Allen, 1971), and penicillin (Leighton &

Table 1. *Relevant markers and source of Escherichia coli strains used*

Strain	Genotype	Source
B	<i>lon sul</i> ⁺	Laboratory stock
B ₈₋₂	<i>lon sul</i> ⁺ <i>exrA</i>	M.H.L. Green
B ₈₋₁₁	<i>lon sul</i> ⁺ <i>recA</i>	M.H.L. Green
B ₈₋₁₂	<i>lon sul</i> ⁺ <i>uvr</i>	M.H.L. Green
B/F' <i>lon</i> ⁺	<i>lon sul</i> ⁺ F' <i>lon</i> ⁺	Laboratory stock (Farnsworth & James, 1972)
B/r	<i>lon sul</i>	Laboratory stock
SB3100	<i>lon sul</i> /F' <i>ara</i> ⁺	E. Englesberg

Donachie, 1970). The sites of action of some of these agents are known. Nalidixic acid and gentian violet cause filamentation by inhibiting DNA synthesis, whereas the activity of penicillin is restricted to the bacterial envelope. The mechanism of the action of crystal violet is less well understood.

This paper describes the effect of these agents on the colony-forming ability of several *lon* mutants of *Escherichia coli* and derivatives which carry mutations at *sul*, *recA*, *exrA* and *uvr* loci. A model is put forward to account for the observations and to suggest sites in the bacterium at which these genes are expressed.

METHODS

Bacterial strains. The relevant genotypes and sources of the strains of *Escherichia coli* used are indicated in Table 1. Stock cultures of these strains were maintained on slopes of Oxoid Blood Agar Base (BAB) at 4 °C.

Growth conditions. Strains were grown overnight in Oxoid nutrient broth at 37 °C. They were then diluted 1:10 into fresh broth and incubation continued for a further 2 h. For treatment with gentian violet, crystal violet and benzyl penicillin, it was convenient to incubate the bacteria, at suitable dilution, for 24 h on plates of BAB containing different concentrations of the agents. Colony counts were made at this time. For treatment with nalidixic acid, a few experiments only were made in this way because growth on BAB containing as little as 5 µg of the drug/ml was too slow to allow colony counts to be made after 24 h of incubation. Instead, the bacteria were diluted 1:5 into Oxoid nutrient broth containing nalidixic acid (100 µg/ml) and incubation continued at 37 °C. Samples of the suspension were removed at intervals, diluted in 0.067 M-phosphate buffer and plated on BAB. The conclusions drawn concerning the relative sensitivity of the bacteria to nalidixic acid were unaffected by the particular method used.

RESULTS

Sensitivity to nalidixic acid. The effect on survival of incubating different strains of *Escherichia coli* in nutrient broth containing nalidixic acid (100 µg/ml) is shown in Fig. 1. The rate of killing of *E. coli* B was much more rapid than that of *E. coli* B/r because of the marked filamentation induced in the unsuppressed *lon* strain by nalidixic acid (Green, Donch, Chung & Greenberg, 1969). That this is the main cause of the difference in sensitivity of these strains was supported by the finding that F-duction of *lon*⁺ into *E. coli* B by the method of Farnsworth & James (1972) made this strain almost as resistant to nalidixic acid as *E. coli* B/r. *Escherichia coli* B₈₋₁₂, which lacks the repair enzyme system controlled by one of the *uvr* genes, was as sensitive to nalidixic acid as *E. coli* B, but sensitivity to this agent was markedly reduced in the *recA* strain (*E. coli* B₈₋₁₁) and in the *exrA* strain (*E. coli* B₈₋₂). Similar

Fig. 1

Fig. 2

Fig. 1. Survival of *Escherichia coli* strains incubated with nalidixic acid. Bacteria were incubated at 37 °C with shaking in nutrient broth containing 100 μg nalidixic acid/ml for the times indicated before plating on BAB. ○, *E. coli* B; ●, *E. coli* B/r; ◇, *E. coli* B/F' *lon*⁺; ◆, *E. coli* B₈₋₂; □, *E. coli* B₈₋₁₁; ■, *E. coli* B₈₋₁₂.

Fig. 2. Survival of *Escherichia coli* strains incubated with gentian violet. Bacteria were plated at suitable dilutions on BAB plates containing various concentrations of gentian violet and incubated overnight at 37 °C. Symbols as for Fig. 1.

observations on the *rec* and *exr* strains were made by Green, Donch, Chung & Greenberg (1969).

Sensitivity to gentian violet. Gentian violet produced a response similar to that caused by nalidixic acid in the different strains tested (see Fig. 2).

Sensitivity to crystal violet. The effect of this compound on viability was examined in three strains only (Fig. 3). Again *Escherichia coli* B/r was much more resistant to killing than was *E. coli* B, confirming the observations of Rörsch, Edelman, Van Der Kamp & Cohen (1962), but *E. coli* B₈₋₂ was almost as resistant as *E. coli* B/r to this agent.

Sensitivity to benzylpenicillin. *Escherichia coli* B/r was more resistant to inactivation by penicillin than was *E. coli* B, as was observed previously by Rörsch *et al.* (1962). However, *E. coli* B/F' *lon*⁺ and the suppressed *lon* strains *E. coli* B₈₋₂ and *E. coli* B₈₋₁₁ as well as the *uvr* strain *E. coli* B₈₋₁₂ were even more sensitive to the action of penicillin than was *E. coli* B (Fig. 4). *Escherichia coli* SB3100, an F' derivative of *E. coli* B/r was almost as resistant as the parent strain, indicating that the F' factor was not responsible for the high sensitivity of *E. coli* B/F' *lon*⁺ (Fig. 4).

Fig. 3

Fig. 4

Fig. 3. Survival of *Escherichia coli* strains incubated with crystal violet. Bacteria were plated at suitable dilution on BAB plates containing various concentrations of crystal violet and incubated overnight at 37 °C. ○, *E. coli* B; ●, *E. coli* B/r; ◆, *E. coli* B_{s-2}.

Fig. 4. Survival of *Escherichia coli* strains incubated with benzylpenicillin. Bacteria were plated at suitable dilutions on BAB plates containing various concentrations of benzylpenicillin and incubated overnight at 37 °C. ○, *E. coli* B; ●, *E. coli* B/r; ◇, *E. coli* B/F' *lon*⁺; ◆, *E. coli* B_{s-2}; □, *E. coli* B_{s-11}; ■, *E. coli* B_{s-12}; ▼, *E. coli* B/r/F' *ara*⁺.

DISCUSSION

The results show that there are marked differences in the relative sensitivities of *lon* strains of *Escherichia coli* to agents which induce filament formation.

Unsuppressed *lon* strains were more sensitive to agents which appear to induce filamentation by inhibition of DNA synthesis than were those in which the *Lon* phenotype is suppressed. Thus *Escherichia coli* B and *E. coli* B_{s-12} were much more sensitive to inactivation by nalidixic acid and gentian violet than was *E. coli* B/r (in which *lon* is suppressed by *sul*) or *E. coli* B/F' *lon*⁺ (in which *lon*⁺ is dominant). However, the degree of sensitivity to these agents appears to be dependent on the mechanism by which *lon* is suppressed. *Escherichia coli* B_{s-2} and *E. coli* B_{s-11} in which *lon* is suppressed by *exrA* and *recA* respectively were both more resistant to nalidixic acid and gentian violet than was *E. coli* B but they still were more sensitive to these compounds than was *E. coli* B/r.

The sensitivities of the strains to penicillin, however, reveal a rather different picture. Only suppression of *lon* by *sul* reduced sensitivity to penicillin. Suppression of *lon* by *exrA* or *recA* slightly increased rather than reduced sensitivity to penicillin. Therefore sensitivity to penicillin, which at low concentration induces filamentation by an effect on polymer murein biosynthesis (Schwarz, Asmus & Frank, 1969; Hartmann, Holtje & Schwarz, 1972),

was again highly dependent on the mechanism by which *lon* is suppressed. Moreover the introduction of F' *lon*⁺ into *Escherichia coli* B did not reduce sensitivity. Although the F' factor might have been responsible for this, this is unlikely, because the presence of F' in *E. coli* B/r F' had little effect on its sensitivity to penicillin. Therefore the resistance of *E. coli* B/r to penicillin is most likely the result of the expression of *sul* producing some effect in addition to direct suppression of *lon*. It is interesting that ten radiation-resistant mutants of the same u.v. sensitivity as *E. coli* B/r which we have isolated from *E. coli* B by the method of Witkin (1947) were all much more resistant to penicillin than was the parent strain (unpublished results).

Because of the differences shown between agents which induce filamentation in *lon* strains by a specific action on DNA and on the bacterial envelope, it may be possible to deduce the site of action of other filament inducing compounds. For example, the observed sensitivities of *Escherichia coli* B, *E. coli* B_{s-2} and *E. coli* B/r to crystal violet (Fig. 3) suggest that this agent induces filamentation in a manner analogous to nalidixic acid, i.e. by affecting DNA. This agrees with the conclusion of Witkin (1961) rather than that of Walker *et al.* (1971), who suggested that it acted on the bacterial envelope.

The detailed processes involved in bacterial division are poorly understood but many authors (Clark, 1968; Helmstetter & Pierucci, 1968; Donachie, 1969; Inouye, 1969) are agreed that there is probably a close relationship between completion of DNA synthesis and septum formation. Zusman, Inouye, & Pardee (1972) have suggested that a specific protein produced at the end of a round of DNA replication may be involved in the co-ordination of these processes, possibly by binding to the membrane site at which septation is initiated. Although the mechanism by which *lon* interferes with the formation of a normal septum is unknown, Farnsworth & James (1972) proposed that *lon* caused a defect in the bacterial envelope – a suggestion supported by the recent finding by Leighton (1972) that there is a deficiency in a protein component of the envelope of a *lon* strain of *Escherichia coli* K12.

We propose that the *lon*-controlled envelope defect reduces the affinity of the septum-initiation site to bind the septum-promoting protein. This results effectively in an increase in the amount of the protein required to trigger septum formation, an increase which is normally met. However, because of the sensitivity of the binding site to reductions in the production of the protein, prevention of septum initiation readily occurs following inhibition of DNA synthesis.

Because of the marked effect of *sul* in reducing sensitivity to penicillin, which acts directly on the bacterial envelope, we propose that *sul* is also expressed as an alteration in the envelope. *Sul* may act, therefore, by countering the higher concentration of septum-promoting protein required in *lon* mutants, and thereby suppressing the Lon phenotype.

The mechanism of suppression of *lon* by the DNA repair mutations *recA* and *exrA* is unknown. Farnsworth & James (1972) found that these mutations affect the permeability properties of *Escherichia coli* B, thus suggesting some interaction with the bacterial envelope. However, our results show that although these mutations reduce the lethal effect of filament inducing agents which act directly on the DNA (Fig. 1, 2) they do not modify the penicillin sensitivity of *E. coli* B. This suggests that their site of action may also involve the coupling between DNA replication and septum formation, but is of a completely different nature from that involving *sul*.

The sensitivities of *Escherichia coli* B and *E. coli* B_{s-12} to each of the agents used were very similar, indicating that the *uvr* gene had no effect on modifying survival under conditions of filament induction. This is as expected from our knowledge of the reactions controlled by

Fig. 5. A model of the control of bacterial division in *lon* strains of *Escherichia coli*.
 →, Site of action of filament-inducing agents; —×, site of suppression by mutant genes.

the *uvr* genes and is consistent with the observation by Green *et al.* (1969) that *uvr* does not suppress filament formation.

These conclusions are summarized in the model presented in Fig. 5, in which we indicate proposed sites of action of the filament inducing agents, the location of the *lon* lesion and the points at which suppression of *lon* by *sul*, *exrA* and *recA* may occur. This model contains features of similarity to that proposed by Zusman *et al.* (1972). Whereas these authors postulated that in filamentous strains the *lon* mutation was expressed as a reduction in the amount of the septum-promoting protein produced following treatments which inhibit DNA synthesis, we suggest that the reduction in the amount of septum-initiation protein made is a consequence of the inhibition of DNA synthesis in both the *Lon*⁻ and *Lon*⁺ strains. However, this leads to filamentation only in *Lon*⁻ strains because of the reduced affinity of the septum site for the binding protein.

Nothing is known concerning the nature of the expression of the *sul* gene, but if, as we suggest, it interacts with *lon* by producing further alterations in the bacterial envelope, then it may be possible to detect these. This possibility is being tested by an examination of the

envelope proteins of *Escherichia coli* B and one of the radiation-resistant mutants derived by us from *E. coli* B.

The mechanisms by which *exr* and *rec* suppress filamentation are also unknown, but our model predicts that these are not the same as those involving *sul*. Certainly, Holland & Tuckett (1972) found that *recA* did not alter the envelope protein profile of *Escherichia coli*. *RecA* and *exrA* suppress production of bacteriophage in *E. coli* (Donch *et al.* 1968; Green, Greenberg & Donch, 1969) whereas *sul* does not, but the reverse is true for suppression of mucoidy in *E. coli* K12 1899 (Donch *et al.* 1968; Donch *et al.* 1971), adding support to the view that *rec* and *exr* have no direct effect on the envelope but are involved in modifying effects on the bacterial genome.

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