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SHORT PAPERS

Effect of a *recA* gene on cell division and capsular polysaccharide production in a *lon* strain of *Escherichia coli*

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This report shows that the gene *recA* suppresses u.v.-induced filamentation in a *lon* strain without affecting capsular polysaccharide production.

Strains of *Escherichia coli* carrying the gene *lon* form long filaments after u.v. irradiation, and produce excess capsular polysaccharide during normal growth. Witkin (1967) has suggested that the process of filament induction by u.v. in a *lon* strain is an analogous process to prophage induction. The recombinationless u.v. sensitivity gene *recA* suppresses the induction of prophage by u.v. (Fuerst & Siminovitch, 1965; Brooks & Clark, 1967; Hertman & Luria, 1967). Recently we showed that the u.v. sensitivity gene *exrA* suppresses u.v.-induced filamentation but does not affect excess capsular polysaccharide production in a *lon* strain (Donch, Green & Greenberg, 1968) and that *exrA* suppresses the induction by u.v. of prophage (Donch, Greenberg & Green, 1969). These findings suggest that u.v. induction of prophage and filaments are more than analogous; they are related processes. A consequence of this hypothesis is that the *recA* mutation should suppress u.v.-induced filamentation in a *lon* strain. The present report confirms this prediction.

The present experiments were made possible by the gift of the *recA* Hfr strain JC 5088 *ilv str*$^+$ from Dr A. J. Clark. Exponentially growing broth cultures of this strain and strain AB1899 *lon lac his str* F$^-$ were mixed in the ratio 1:10 Hfr:F$^-$, and mated for 30 min at 37 °C. Mating was interrupted by violent agitation, and the mixture plated to select for *his*$^+$ *ilv*$^+$ *str* and *lac*$^+$ *ilv*$^+$ *str* recombinants. No *lac*$^+$ recombinants were obtained, indicating that mating had been interrupted before this region of the chromosome (which includes the *lon* gene) was transferred. Of several hundred *his*$^+$ recombinants, 100 were purified and tested for u.v. sensitivity by the rapid streak method (Greenberg, 1964). Two classes were found. About 60% of recombinants corresponded in u.v. sensitivity to AB1899 and about 40% to JC 5088. None were more sensitive than JC 5088.

Several recombinants from each class were repurified and tested for their ability to act as recipients in recombinations with strain HfrC *met*$^+$ *str*$^+$. In this cross *thr*$^+$ *leu*$^+$ *met*$^+$ *str* recombinants were selected. Mating was performed as in the original cross. Recombinants with u.v. sensitivity similar to JC 5088 showed over 1000-fold reduction in the capacity to act as recipients as compared with those resembling AB1899 in u.v. sensitivity. These observations confirmed that recombinants which resembled JC 5088 in u.v. sensitivity were *recA*.

All recombinants of the original cross (JC 5088 × AB1899), including the *recA*, showed excess production of capsular polysaccharide characteristic of the *lon* parent strain AB1899. From this and the already mentioned observation that none had inherited the *lac*$^+$ *lon*$^+$ region of donor, it was concluded that all recombinants were *lon*. Furthermore,

the *recA* gene did not appear to suppress the expression of mucoidy of the *lon* gene. This suggested that the *recA* gene did not affect the *lon* gene directly.

It can be seen in Fig. 1 that the *recA lon* recombinants resemble the *recA lon*$^+$ parent in u.v. sensitivity. This is in contrast to a *recA uvr* strain, which was much more sensitive to u.v. than *recA uvr*$^+$ or *recA*$^+$*uvr* strains (Howard-Flanders & Boyce, 1966). However, the fact that the *recA* and *lon* genes are not additive in lethality does not prove that the expression of the *lon* gene is suppressed by *recA*; both genes might cause lethality in the same fraction of the population.

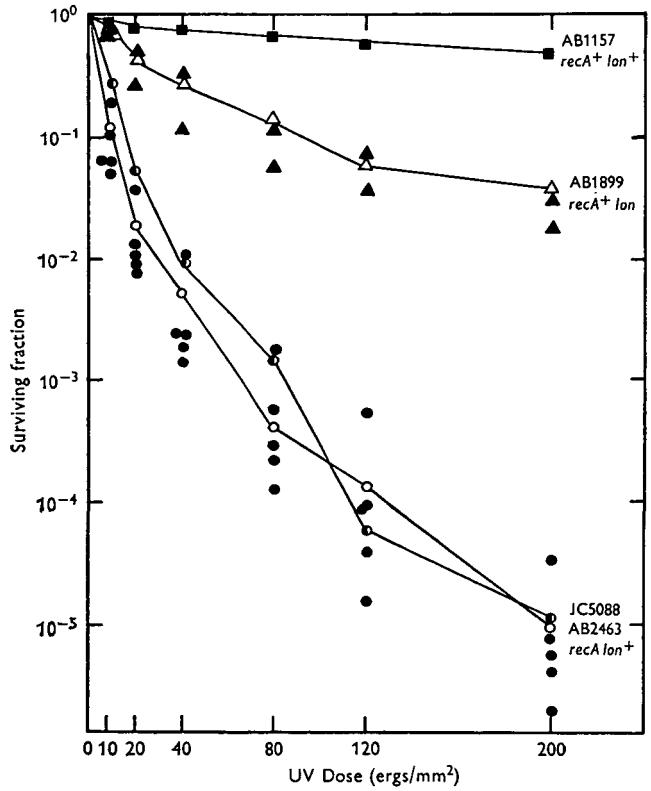

Fig. 1. Survival of *recA lon* recombinants after u.v. irradiation. ●, *recA lon* recombinants; ▲, *recA*$^+$*lon* recombinants; controls: ■, AB1157 *recA*$^+$*lon*$^+$; △, AB1899 *recA*$^+$*lon*; ○, AB2463 *recA lon*$^+$; ◐, JC 5088 *recA lon*$^+$. Viability was determined on complete medium with incubation at 37° C.

We observed, however, that the *recA lon* recombinants did not form filaments after u.v. irradiation. The formation of filaments requires that cellular growth continues in the absence of cell division. The *recA* gene might prevent filamentation in a *lon* strain either by inhibiting growth or by permitting cell division. It was likely that the *recA* gene would affect growth since it causes almost complete inhibition of DNA synthesis after u.v. irradiation (Howard-Flanders & Boyce, 1966) which will indirectly inhibit protein and RNA synthesis (Luzzati, 1966). However we were able to show that the *recA* gene also affects cell division as follows.

A characteristic of *lon* strains is that even those cells that survive u.v. irradiation re-initiate cell division only after a lag of several hours. If the *recA* gene suppressed the

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filamenting effect of the *lon* gene by permitting cell division to occur, survivors in a *recA lon* strain should re-initiate cell division after irradiation much more rapidly than in a *recA*$^+$*lon* strain. To test this, exponentially growing broth cultures of each type were embedded in soft nutrient agar on Perma-Slides (Laboratory Specialties Corp., Woodbury, NY 11797). The cells were given a nominal dose of 10 ergs/mm^2 u.v. irradiation (the actual dose was less, due to shielding by the agar). Coverslips were added and the slides incubated at 37 °C over wet tissue in Petri dishes. At intervals slides were removed and 100 cells examined for cell division. Figure 2 shows the fraction of cells dividing in a *recA lon* and a *recA*$^+$*lon* strain. It can be seen that the inhibition by the *lon* gene of cell

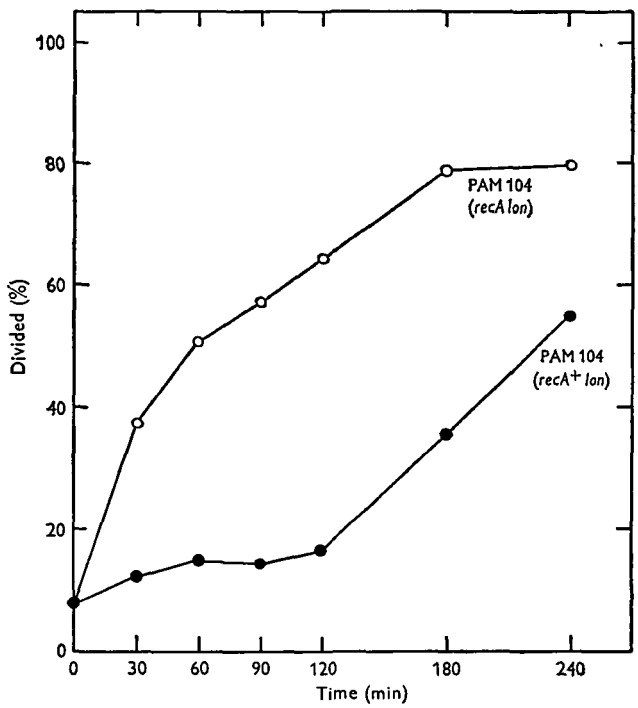

Fig. 2. Percentage of a cell population which has undergone cell division at different times after 10 ergs/mm^2 u.v. irradiation. ●, PAM 101 *recA*$^+$*lon*; ○, PAM 104 *recA lon*.

division after u.v. irradiation is indeed suppressed by *recA*. This is in spite of the *recA lon* strain being much more sensitive to u.v. irradiation. Similar results were obtained following inhibition of DNA synthesis with nalidixic acid and were also obtained with *exrA lon* strains.

Thus the *recA* and *exrA* genes resemble each other in suppressing u.v. induction of prophages and u.v. induction of filaments. In addition E. M. Witkin has found that both *exrA* (1968) and *recA* (personal communication) suppress u.v.-induced mutation. Thus these three effects—u.v. induction of filaments, prophage and mutations—would appear to be related. Since neither *recA* nor *exrA* suppresses capsular polysaccharide production in a *lon* strain, they do not appear to affect the *lon* gene directly. Nor do they appear to suppress filamentation merely by inhibiting growth after u.v. irradiation. Rather, they would seem to prevent the stimulus that causes a *lon* strain to filament. The nature of this stimulus is not known, nor is its connection with the DNA repair performed by

recA$^+$ and *exrA*$^+$ strains understood. One suggestion, by Kirby, Jacob & Goldthwait (1967) is that the stimulus may be related to variation in levels of DNA precursors. At present, all that is clear is that both *exrA*$^+$ and *recA*$^+$ functions are required for u.v. induction of filaments, mutations and prophages.

SUMMARY

In *Escherichia coli* the u.v. sensitivity gene *recA* suppressed u.v.-induced filamentation in a *lon* u.v. sensitive strain without affecting capsular polysaccharide production. *recA* appears to prevent the stimulus that leads to filamentation in a *lon* strain.

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