ABSCENCE OF K-REACTIVATION IN A RECOMBINATION-DEFICIENT MUTANT OF E. COLI

Hubert Kneser

Institut für Genetik der Universität zu Köln, Germany

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UV lesions in Escherichia coli are reactivated by two mechanisms working in the dark. (If a third mechanism of similar efficiency existed, it would probably have been detected during the extensive search for UV-sensitive mutants by several investigators. For references see Kneser, 1965.)
1) Darkreactivation (DR) or host cell reactivation acts on both phage and bacterial lesions and his inhibited specifically by caffeine (Metzger, 1964; Sauerbier, 1964). Mutants without DR are e.g. B_{s-1}, K 12 hcr$^-$ or uvr$^-$ or syn$^-$.
2) K-reactivation (KR) acts on bacterial lesions only and is found in strains K 12, K 12 hcr$^-$ or B/r. Under normal conditions it is inactive in strains B, BB or K 12 lon$^-$, but can be expressed with post irradiation treatments such as incubation at 45 °C instead of 37 °C or on media containing pantoyl lactone. After UV irradiation these strains, under normal growth conditions, form long filaments. They will be denonted as lon$^-$ type strains in this paper, though there may be minor differences between the lon gene of K 12 and the corresponding gene (fil) of B strains.

In a previous paper (Kneser, 1965) it has been concluded that lon$^-$ type strains lack a regulatory function that switches on KR and prevents filament formation; it can be substituted for by pantoyl lactone or heat treatment.

The experiments presented in this paper show that a recombination-deficient UV-sensitive mutant (similar to those described by Clark and Margulies, 1965) lacks an essential part of KR which cannot be replaced by pantoyl lactone or heat treatment. The regulatory KR function however seems to work and DR is unrestricted.

Materials and methods have been described earlier (Kneser, 1965). Strain 152 rec⁻ is a recombination-deficient mutant of strain W 3102 gal⁻ Str^r (Echols et al., 1963) isolated in the laboratory of Dr. M. Meselson. The parent strain shows the usual UV sensitivity of K 12 wild type with both DR and KR. Caffeine plates contained 0.1 % caffeine in tryptone (LT) agar. Phage λ was plated with a drop of overnight culture of strain 152 rec⁻ or 3102 in surface technique without preadsorption.

RESULTS

Figure 1 shows the survival curves of strain 152 rec⁻ under various conditions. The essential results are the following:

1) The UV survival curves for growing and resting cultures of strain 152 rec⁻ plated on tryptone agar are in the initial part similar to those for strain BB (DR-positive, KR-negative, lon⁻ type), especially in the pronounced difference in sensitivity between growing and resting cultures characteristic for lon⁻ type strains.

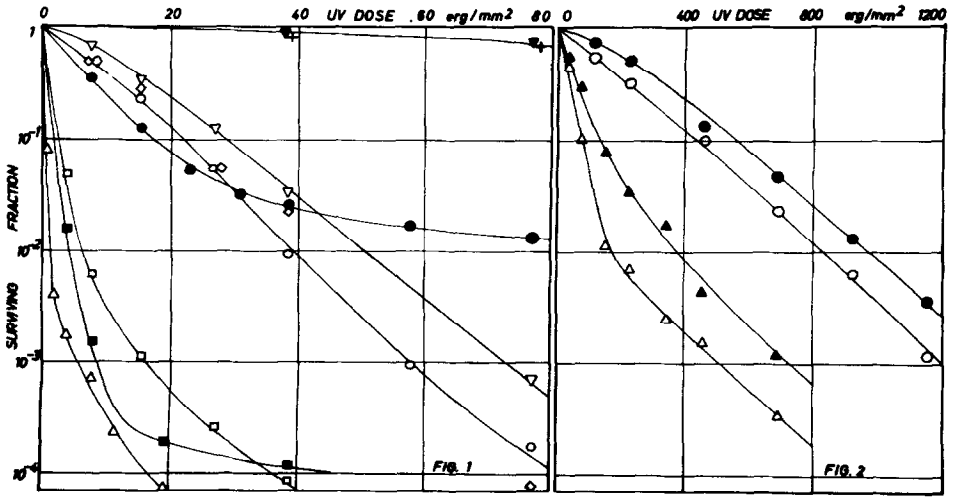

Fig. 1. Survival of UV irradiated bacteria under different conditions: Open symbols, strain 152 rec⁻; closed symbols, strain BB. □■, growing cells, plated on LT agar; ○●, resting, on LT; ▽▼, resting, on LT plus pantoyl lactone; ◇, resting, on LT at 45 °C (heat reactivation); △, resting, on LT plus caffeine. +, strain 3102, resting, on LT. Single representative experiments.

Fig. 2. Influence of caffeine on survival of UV irradiated phage λ using different hosts: Open symbols, indicator strain 152 rec⁻; closed symbols, indicator strain 3102. ○●, plated on LT plates; △▲, on LT plus caffeine. Single representative experiments.

2) Caffeine, a specific DR inhibitor, sharply reduces survival of UV irradiated strain 152 rec⁻.

3) Pantoyl lactone and heat treatment scarcely influence survival of strain 152 rec⁻ (while they do so powerfully with strain BB).

Figure 2 shows the survival of UV irradiated phage λ in strain 152 rec⁻ and the influence of caffeine on it. It is seen that phage λ survives almost to the same extent in the mutant as in the parental strain. Caffeine cuts down λ survival in both strains.

Discussion

These results show clearly that DR is intact in the recombination-deficient mutant 152 rec⁻. Three arguments lead to this conclusion:

1) UV irradiated phage λ survives to almost the same extent in the mutant as in the parent strain, where DR is known to be active (host cell reactivation).

2) Survival of UV irradiated phage λ is reduced by the specific DR inhibitor caffeine (Metzger, 1964) to similar degrees in the mutant and in the parent strain.

3) Survival of the mutant bacteria themselves is strongly reduced by caffeine.

Since the recombination-deficient mutant 152 rec⁻ performs full DR, its high UV sensitivity must be due to the lack of KR, assuming that no third reactivation mechanism of similar effeciency exists. This view is confirmed by the similarity of the survival curves of the mutant and of strain BB, which normally does not perform KR. Especially the great difference in sensitivity between growing and resting cultures is known only with KR-negative DR-positive strains. Therefore KR probably has one step in common with the process leading to genetic recombination.

There is however an important difference between the recombination-deficient mutant 152 rec⁻ on the one hand and the KR-negative strains of the lon⁻-type hitherto known (B, BB, K 12 lon⁻) on the other hand: While these strains

can perform KR under certain conditions (pantoyl lactone or heat), the present mutant does not do so. It is concluded, that the step of KR lost in this mutant (which is involved also in recombination) is an essential one for KR. In the lon⁻ type strains on the other hand all essential stops of KR can be performed, but they are not switched on under normal conditions, because some regulatory compnent does not work (Kneser, 1965). This regulatory function can be replaced by pantoyl lactone or heat treatment in lon⁻ type strains. In strain 152 rec⁻ however these treatments have no effect on UV survival. The reason is a twofold one: 1. An essential part of KR is deficient (see above). 2. The regulatory function itself seems to be irtact (so that additionally imitating it by these treatments changes nothing). This can be seen from another effect of this regulatory function, viz. the inhibition of filament formation: In normal medium after UV irradiation long filaments are formed by lon⁻ type strains which lack the regulatory KR component. Filament formation can be inhibeted by pantoyl lactone (Van de Putte et al., 1963). No filaments are found with KR performers after UV. This is not due to KR itself, since the mutant 152 rec⁻ does not form filaments after UV either, although it cannot perform KR and has a similar UV sensitivity as the filament forming lon⁻ type strains. Here the regulatory KR function seems to be intact and to inhibit filament formation without accomplishing KR.

The mechanism of KR remains unknown, but hypotheses can be ruled out that involve filament formation as an essential step in UV killing of KR-negative strains. KR apparently shares a common step with genetic recombination; KR must be switched on by a special regulatory function or by environmental conditions; and it seems to work only on lethal lesions in bacterial DNA (for references see Kneser 1965).

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