Multiplicity Reactivation of Bacteriophage T7 Inactivated by Methyl Methanesulfonate

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Experiments were conducted to determine whether phage T7 treated with methyl methanesulfonate used multiplicity reactivation to repair alkylation lesions. This type of repair was found to be operative at high multiplicities in actively growing wild-type Escherichia coli B cells.

Multiplicity reactivation (MR), a general DNA repair pathway characteristic of bacterial, animal, and human viruses (5, 6, 13), was first observed by Luria (13) as an increase, with increasing multiplicity of infection (MOI), in the titer of UV-irradiated phage preparations and was later shown to increase the survival of phages treated with monofunctional and bifunctional alkylating agents (8, 17). At the molecular level, it is now known that replication of undamaged segments of DNA in UV-irradiated phages leads to the formation of partial replicas (2, 18). Multiple infection could affect the production and fate of the pool of partial replicas in two ways. First, it would permit correction, by complementation, of functional damage to phage proteins essential for the replication and repair and, thus, for the production of partial replicas. Second, it would permit the establishment of a large and complete pool of partial replicas, assuming that the necessary proteins were present, which could recombine to form intact phage genomes (18).

It has been reported that phage T7, now one of the best-characterized model systems (4) in molecular virology, does not use MR to overcome lesions in its DNA (7, 13, 20). The purpose of this communication is to present data which demonstrate that MR does occur in phage T7.

The conditions for alkylation with methyl methanesulfonate (MMS) and for genetic crosses have been described previously (9, 10). For MR experiments, Escherichia coli B cells from an overnight culture were diluted 100-fold in fresh medium (T-broth supplemented with 1 mM MgSO₄ [9], 0.25% yeast extract, and 1% glucose) at 30°C and grown on a rotary shaker to a cell density of 5 × 10⁸ cells per ml (ca. 3 h). Samples (4 ml) of the culture were infected with alkylated or nonalkylated phage at an MOI of less than 1 (formation of monocomplexes) or much greater than 1 (formation of multicomplexes). The MOI was calculated from the input of viable nonalkylated phage and the CFU at the time of infection. At 7 min after infection, the cells were harvested on membrane filters, suspended, and diluted at room temperature for the measurement of infective centers. Bacterial survivors and nonadsorbed phage were also measured.

Because previous experiments (10) had not proven that two alkylated phage could actually infect the same cell, we looked for recombination between two alkylated T7 amber mutants (am37, gene 11, and am208, gene 4; alkylation dose, 0.006 M MMS). The results showed the frequency of wild-type recombinants to be 8% for the control cross and 20% for the cross in which both parents were alkylated.

Having shown that two alkylated phage could enter the same host cell, we next attempted to demonstrate MR in alkylated phage T7. The results of several experiments are presented in Table 1. They show that multiple infection enhanced phage survival, particularly at a dose of 0.02 M MMS. Superinfection did not appear to contribute in a quantitatively significant way to this enhanced survival (see Table 1 in reference 11). However, the increased ability to produce phage in multicomplexes did result, in part, from the increased probability of chance infection by surviving particles in the alkylated phage preparations. This contribution to multicomplex survival can be calculated as described in Table 1. The ratio of the observed multicomplex survival to the calculated multicomplex survival expected from chance infection by survivors is defined as the degree of MR; it is equal to 1 in the absence of MR.

The results in Table 1 show a degree of MR as high as 5.6. They thus demonstrate for the first time, to our knowledge, that MR can occur in phage T7. The degree of MR observed at a dose of 0.02 M MMS was similar to that observed by Johns et al. (8) for phage T4 treated with ethyl methanesulfonate. Variability at higher doses may be caused by the extensive loss of killing ability (40% for phage alkylated with 0.02 M MMS and greater for higher doses [data not shown]).

By comparison with the results of Johns et al. (8), we tentatively attribute the MR we have observed to a phenomenon of functional complementation. Indeed, it has been shown that the alkylation of phage T7 does interfere with phage-specific protein synthesis (11), and multiple infection might alleviate this situation.

Why has it taken so long to demonstrate MR in phage T7? We can propose several answers. First, we used favorable conditions, namely, enriched medium and a high MOI to favor adsorption. Studier (21) noted adsorption problems encountered in the past, and he and others (3, 19) used a high MOI (≥10) when working with phage T7. The use of a relatively low MOI for multicomplex formation may have prevented Schweiger et al. (20) from demonstrating MR in UV-irradiated phage T7. Another factor could be the decrease caused by alkylation in the ability of the phage to kill the bacterial host (9). It has long been known that X-rays and alkylating agents, but not UV light, decrease the killing ability of T-even phages; it was also observed that MR was much lower than in UV-treated phages (17, 22). Heavily UV-irradiated phage T7 particles lose their ability to kill host cells (7), in contrast to what is observed for T-even phages. This could explain why MR has not been demonstrated in UV-treated phage T7. Finally, host cell reactivation can interfere with the detection of MR (1). UV-irradiated phage T7 undergoes host cell reactivation (16), and this process has...
possibly masked MR in the past. However, despite that fact that host cell reactivation for alkylation lesions occurs (15), we have been able to demonstrate MR in alkylated T7 plaque. This suggests that, with host cells lacking essential enzymes in the base excision repair pathway (12), the degree of MR would even be enhanced.

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