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Stimulation of φX174 Production in Mitomycin C-treated *Escherichia coli* Cells by Caffeine

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After pretreatment of an *Escherichia coli* C culture with mitomycin C, the deoxyribonucleic acid (DNA) synthesis of the bacteria, as well as that of infecting phage φX174, is very largely inhibited. Although the bacterial DNA synthesis is similarly inhibited by mitomycin C in *uvr*⁻ mutants (P. Howard-Flanders, R. P. Boyce, and L. Theriot, Genetics **53**:1119, 1966), phage DNA synthesis and progeny formation take place in a normal manner (B. H. Lindqvist and R. L. Sinsheimer, J. Mol. Biol. **30**:69, 1967). The reason for this behavior of the *uvr*⁻ cells is not understood. Since it is known that *uvr*⁺ cells do not reactivate double-stranded ultraviolet-damaged phage DNA in the presence of adequate concentrations of caffeine (W. Sauerbier, Biochem. Biophys. Res. Commun. **14**:340, 1964), it was of interest to test the ability of mitomycin C-treated *uvr*⁺ cells to produce phage under these conditions.

A thymine-requiring mutant CT⁻ (isolated by M. Azegami) was used in the experiments. The CT⁻ bacteria were grown with aeration at 37 C in a modified TPG medium (R. L. Sinsheimer et al., J. Mol. Biol. **4**:142, 1962; 1 g/liter of KH_2PO_4, 0.147 g/liter of $CaCl_2 \cdot 2H_2O \equiv 10^{-3}$ M), supplemented with 2.7 g/liter of a special mixture of the 20 natural L-amino acids (Nutritional Biochemicals Corp., Cleveland, Ohio) and 0.01 g/liter of thymine. At a titer of approximately 6×10^7 colony formers per ml, the bacteria were concentrated fivefold in the same medium but with only one-fifth of the thymine concentration. The cells were treated with mitomycin C in a dim-yellow light without aeration for 15 min at 50 μg/ml and at 37 C. The caffeine concentrations employed were 1, 3, and 10 mg/ml; normally the caffeine treatment began with the addition of the mitomycin. Mitomycin was then removed by centrifugation, and phage was added ($m \approx 5$) to the resuspended cells.

Phage adsorption took place (in the presence of caffeine) during 7.5 or 10 min, whereupon the bacteria were diluted 1:100 in the same medium and incubated until lysis (1 hr after addition of wild-type phage) or artificial opening (at 40 or 60 min after addition of the lysis-defective mutant, *am*3; C. Hutchison and R. L. Sinsheimer, J. Mol. Biol. **18**:429, 1966).

TABLE 1. *Relative phage yield*

Caffeine	Plaque titer	
	Without mitomycin	With mitomycin
mg/ml		
0	100	1
1		6–10
3	60	6–10
10		0.1

With wild-type φX, a 6- to 10-fold increase of plaque titer was found with 1 and 3 mg/ml of caffeine (Table 1), but the titer decreased to less than one-tenth of the zero value in 10 mg/ml. The φX mutant *am*3 gave a sevenfold increase with 1 mg/ml of caffeine. The caffeine treatment of the cells could either start 15 min before or together with the addition of mitomycin, or immediately after its removal, with equal stimulation of phage production.

To decide between an increase of the burst size, or of the number of bursts, or both, unlysed complexes (of wild type) were plated on caffeine-agar and, in a parallel culture, liberated phage were determined after lysis of the complexes in caffeine medium. Both with (1 and 3 mg/ml) and without caffeine, only about 20% of the mitomycin-pretreated cells were found to produce a plaque. Thus, it can be concluded that caffeine mainly enlarges the phage yield of mitomycin-pretreated *uvr*⁺ cells.

The parallel to the effectiveness of *uvr*⁻ mutation is clearly only partial. This is most likely a consequence of a toxicity of caffeine in the concentrations necessary to block the reactivation process. At 3 mg/ml, caffeine reduced the ability of untreated cells to produce φX by about 40%.

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