RecA-MEDIATED ASYMMETRIC REPAIR OF LETHAL DNA LESIONS IN 

E. coli

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ABSTRACT

Mechanisms of action of DNA lesions produced by decay of genetically incorporated $^{125}$I or by 313-nm photolysis of incorporated 5-bromouracil (5BU) in exponential phase cultures of Escherichia coli were studied by inducing lesions after subsequent periods of DNA replication. The analogues were incorporated during brief labeling periods to provide prelesional substrates for later induction of the lesions, and label concentrations were chosen to give cell survivals of about 10% when lesions were induced immediately after labeling. When the same number of lesions were induced at intervals during the first generation after labeling survival levels decreased. After replication of the labeled regions, however, survival levels increased sharply in the wildtype strains. Three very different patterns of recovery were observed.

1. 5BU photolysis, wildtype strains. Recovery was essentially complete.

2. $^{125}$I decay, wildtype strain. Approximately half of the cells recovered at the end of the first round of DNA replication.

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3. $^{125}$I decay, recA strain. No significant increase in recovery occurred at the first generation, but recovery increased gradually during the second generation, as expected for production of irreparable damage in labeled regions.

The results are consistent with recA-mediated asymmetric repair of $^{125}$I decay-induced lesions.

INTRODUCTION

Lethal effects of DNA lesions produced by incorporation of base analogues usually have been studied either in cells labeled continuously for many generations or in cells tested immediately after pulse-labeling. We have employed a new approach in which pulse labeled cells were grown for various periods before inducing the DNA lesions$^{1,2}$. In this way, modifying effects of cell growth, DNA replication, nuclear separation, and cell division can, in principle, be examined. Our results do indeed show that cell survival depends upon the intervening growth period between labeling and lesion induction.

METHODS

Cultures of Escherichia coli, strains B/r K (wildtype), B/r TT (thy trp), or B/r WP10 (thy trp recA), were grown overnight to exponential phase in minimal medium (M9-salts plus 2 mg/ml glucose). For both auxotrophs the growth medium was supplemented with thymine (20 µg/ml) and tryptophan (50 µg/ml, and arginine, methionine, and proline were also added to 100 µg/ml for cultures of strain WP10. Doubling times of these cultures ranged from 40 to 87 min. Cultures were labeled for 2-4 min with 5-bromouracil (5BU) or with 5-ido-$^{125}$I-2'-deoxyuridine, washed, and resuspended in growth medium. There was no detectable change in growth rate of the labeled cells.

Periodically during culture growth, samples were removed and DNA lesions were induced in 5BU-labeled cultures by exposure to 313-nm radiation at a fluence of 5 x 10 J/m$^2$. Survival was determined from colony counts after plating labeled cells and those from an unlabeled control culture on nutrient agar. With $^{125}$I, samples of the labeled culture and an unlabeled control were frozen in liquid nitrogen to permit accumulation of decay-induced DNA lesions. Samples were thawed and plated after a period sufficient to give approximately 10% survival for cells frozen immediately after labeling. Further details were described earlier$^{1,2}$. 