RecA\textsuperscript{+}-Dependent Repair of Gamma-Ray Damage to Escherichia coli Does Not Require Recombination Between Existing Homologous Chromosomes

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Received for publication 16 August 1971

Chromosomal regions.

Enchanced survival of ultraviolet-irradiated Escherichia coli dependent upon the recA\textsuperscript{+} gene appears to result from the ability to seal gaps in newly synthesized deoxyribonucleic acid (DNA) produced when DNA containing pyrimidine dimers is replicated (8). Strains which are recombination-deficient (recA) do not seal these gaps and degrade their DNA to a considerable extent. Rupp and Howard-Flanders (8) postulated that the gap-sealing process involves genetic recombination between the two daughter chromosomes produced at replication.

Recombination-deficient bacteria are also sensitive to ionizing radiation, although here the repair process dependent upon the recA\textsuperscript{+} gene appears to involve the sealing of single-strand DNA breaks formed directly by the radiation (5).

We may therefore ask whether recA\textsuperscript{+}-dependent repair of ionizing radiation damage requires recombination between existing homologous chromosomes, i.e., before replication, analogous to that postulated to occur after replication of ultraviolet photoproducts. If such a hypothesis is correct, recA\textsuperscript{+} bacteria with a single unreplicated chromosome should be as sensitive as recA bacteria, as there would be no possibility of recombination between homologous chromosomal regions.

In testing this hypothesis, we found that, contrary to a widely held belief, stationary-phase bacteria rarely contained a single unreplicated chromosome. We grew E. coli B/r WP2 trp recA\textsuperscript{+} and a recA derivative in a chemostat at 37 C at a generation time of about 4.5 hr. The apparatus was that described by Baker (1) but with a covering of black paper to exclude light. The medium was M9 salts plus 100 \( \mu \)g of glucose/ml and 10 \( \mu \)g of tryptophan/ml. Plate count viability exceeded 90% for the recA\textsuperscript{+} strain. After cultures were run for at least 48 hr, bacteria were removed and suspended in phosphate buffer (0.067 M, pH 6.8) for irradiation with cobalt-60 gamma rays.

According to Kubitschek, Bendigkeit, and Loken (6), bacteria grown in this way possess a single chromosome which replicates immediately before division. Measurements of DNA by the method of Burton (3) based on seven cultures indicated an average DNA content of 5.66 \( \pm \) 0.26 \( \times \) 10\textsuperscript{-15} g per bacterium. This corresponds to 1.23 times the amount of DNA in an unreplicated E. coli genome, assuming that one genome-equivalent is 2.8 \( \times \) 10\textsuperscript{9} daltons (4), or 4.6 \( \times \) 10\textsuperscript{15} g of DNA. Therefore, at least 77% of the genes in the population are present as only one copy per cell. If DNA replication occurs just before cell division, in an asynchronous population, approximately 50 to 70% (depending on the proportion of bacteria which have completed replication) of the bacteria would have no regions duplicated as a result of replication.

Measurements of bacteria growing freely at a generation time of about 45 min in the same medium with nonlimiting glucose (0.4%) contained 1.11 \( \pm \) 0.01 \( \times \) 10\textsuperscript{-15} g of DNA per bacterium (based on four cultures). This is equivalent to about 2.4 genome-equivalents of DNA. If each nucleus contains 1/1n2 (1.44) genome-
equivalents of DNA, this would indicate an average of 1.6 to 1.7 nuclei per bacterium, which is within the range normally found for fast-growing bacteria in minimal medium (2).

Figure 1 shows the survival of fast-growing and slow-growing bacteria after exposure to Co-60 gamma irradiation under aerobic conditions. The small shoulder on the curve for fast-growing recA+ bacteria has been shown to be attributable to their genome multiplicity (7), and, as expected, was not detectable on the curve for slow-growing bacteria. The final slopes of the curves for recA+ bacteria did not, however, differ significantly, indicating that the sensitivity of genomes was similar under fast- and slow-growing conditions. Slow-growing chemostat cultures of strain WP2 recA were considerably more sensitive than recA+ cultures. Fast-growing recA bacteria (not shown) were about 20% more sensitive than slow-growing recA bacteria.

As the slow-growing population presumably is a mixture of organisms with chromosomes in different stages of replication, the radiation response of a recA+ strain, on the basis of the hypothesis under test, would be expected to be that of a mixed population. The dotted line (Fig. 1) shows approximately what survival curve might be expected if those slow-growing recA+ bacteria with no replicated DNA region had the sensitivity of the recA strain and those with partially replicated chromosomes had sensitivities intermediate between those of the recA strain and the fast-growing recA+, depending on the proportion of the chromosome that was replicated. The precise form of the expected curve may vary slightly, depending on the precise timing of replication within the cell cycle, but it was not at all similar to the observed survival curve of the slow-growing recA+ bacteria. More convincing results might be obtained if conditions could be found in which a larger proportion of the population were single genome cells. We have not been able to achieve this. Nevertheless, the present results suggest that recombination between existing homologous chromosomes before replication is not necessary for manifestation of the recA+-dependent resistance in E. coli.

I acknowledge the technical assistance of R. P. Mottershead and advice on chemostat technology from J. Drozd. Anne Rothwell constructed the WP2 recA strain by conjugation between K-12 Hfr JC5088 recA and B/r WP2 thy F−.

FIG. 1. Survival of Escherichia coli after exposure to gamma radiation. Fast-growing multigenome WP2 (O), slow-growing WP2 population containing single genome bacteria (.), slow-growing population of WP2 recA (O). Dotted line indicates predicted survival of slow-growing WP2 population if presence of duplicated chromosomal regions is necessary for resistance conferred by recA+ allele.

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