Involvement of a Recombination Repair Function in Disciplined Cell Division of *Micrococcus radiodurans*

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SUMMARY

When a culture of the temperature-sensitive DNA mutant *Micrococcus radiodurans* ts1 is irradiated with a sublethal dose of ultraviolet or ionizing radiation and is plated immediately, all the bacteria give rise, after 36 h incubation, to colonies identical to those derived from unirradiated bacteria. However, when the irradiated population is held at its restrictive temperature (39 °C) (restrictive temperature holding) for 3 h before being plated, less than 0.1 % of the surviving bacteria give rise to normal colonies, the rest producing, after incubation for 96 h, small malformed colonies. Qualitatively, the same effect is observed when u.v.-irradiated wild-type *M. radiodurans* is incubated at 39 °C in the presence of nalidixic acid before plating. Compared with the loss of viability, the loss of normal colony development as a function of the radiation dose is sensitive, having 1/e values of 210 ergs/mm² for u.v. radiation and of 4 to 5 krad for ⁶⁰Co γ-radiation. These are identical to the radiation dose-response values of a recombination-deficient mutant of *M. radiodurans*. At first the abnormal colonies consist entirely of giant bacteria but eventually a few bacteria with normal morphology appear and because of their much faster generation time a highly sectorized colony results. These colonies can be 'rescued' by plating the irradiated bacteria held at 39 °C on agar containing pantoyl lactone, their growth being identical to that of unirradiated bacteria. Abnormal colony development is not a general phenomenon in temperature-sensitive mutants of *M. radiodurans* but occurs in those mutants which are sensitized to radiation when held at 39 °C. It is concluded that these abnormal colonies are produced as a result of a defect in a recombination function and that this function is also involved in the regulation of normal cell division.

INTRODUCTION

Wild-type *Micrococcus radiodurans* is extremely resistant to the lethal action of both u.v. and ionizing radiation (Anderson *et al.* 1956; Duggan, Anderson, Elliker & Cain, 1959) and to chemical damage to its DNA, e.g. by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) (Moseley, 1967). Thus the average doses of u.v. or ionizing radiation required to inactivate a single viable unit are 12 250 ergs/mm² or 720 krad respectively. The resistance of *M. radiodurans* is due, at least in part, to its ability to excise potentially lethal u.v.-induced pyrimidine dimers from its DNA (Boling & Setlow, 1966) and to a DNA repair mechanism involving a recombination function (Moseley, Mattingly & Copland, 1972a).

Survival in an irradiated or chemically-treated population of bacteria is usually measured by determining the proportion of bacteria able to give rise to colonies on a nutrient agar medium. In the case of *M. radiodurans* such colonies are usually identical to those derived from unirradiated bacteria, but it has been noted previously that some colonies which arise

following treatment with X-rays (Moseley, 1963) or NTG (Moseley, 1967) were smaller and 'showed abnormalities, e.g. sectoring caused by partial loss of pigment, roughness, and growth rate variations leading to the formation of very irregular edges to the colonies'. Such abnormal colonies seemed to arise sporadically and unpredictably following irradiation of cultures and the immediate conclusion was that the treatments, being potentially mutagenic, had caused extensive mutation in the populations in the abnormal colonies. Such an interpretation has been made by Driedger (1970). However, in spite of our initial inclination to this view we have been unable to demonstrate the presence of mutants, and have an alternative explanation.

A method is described which allows an irradiated population of *M. radiodurans* to produce either normal or abnormal colonies of the type described. The abnormal colonies appear to be produced as a result of a partial defect in the recombination-type repair which, although enabling an irradiated bacterium to remain viable, causes it to give rise to grossly enlarged and distorted progeny. It probably represents the expression of a normally repressed gene similar to *fil* or *lon* in *Escherichia coli* (Adler & Hardigree, 1964; Howard-Flanders, Simson & Theriot, 1964).

METHODS

Bacteria. *Micrococcus radiodurans* wild-type; *M. radiodurans* ts1, ts2 and ts3, temperature-sensitive mutants defective in DNA synthesis at 39 °C; *M. radiodurans* ts9 and ts11, temperature-sensitive mutants defective in cell wall synthesis at 39 °C. All these strains are very resistant to the lethal action of u.v. and ionizing radiation at 30 °C (Moseley, Mattingly & Shimmin, 1972*b*; Moseley *et al.* 1972*a*).

Media. TGY medium for growth contained: Bacto-tryptone (Difco), 5 g; glucose, 1 g; yeast extract (Difco), 3 g; distilled water to 1 l. TGY agar was made by solidifying this medium with 15 g Bacto-agar/l. Pantoyl lactone agar was made by adding filter-sterilized pantoyl lactone solution to molten TGY agar to give a final concentration of 12 g/l ($\equiv 0.09$ M). The 0.067 M-phosphate buffer pH 7.0 contained 4.73 g Na₂HPO₄ and 4.54 g KH₂PO₄ per 1 l distilled water.

Growth of bacteria. Bacteria were grown in 20 ml quantities of TGY medium in 250 ml conical flasks which were swirled at 30 °C until the extinction of the cultures measured on a nephelometer (Evans Electroselenium Ltd, Halstead, Essex) with an orange filter, was between 0.25 and 0.30 (equivalent to 8×10^7 to 9×10^7 viable units/ml.).

Irradiation of bacteria. Ten ml samples of the bacteria were washed and resuspended in phosphate buffer at a concentration of about 10^8 viable units/ml. For u.v. irradiation a 5 ml sample of the washed suspension in a 9 cm Petri dish was agitated with a magnetic stirrer at a distance of 40 cm from a Hanovia model 12 germicidal lamp (incident dose rate 22.5 ergs/mm²/s). Viability was measured by removing 0.1 ml samples, suitably diluting, and spreading 0.1 ml quantities on TGY plates. Colonies were counted after 36 h at 30 °C.

Gamma irradiation was carried out with a ⁶⁰Co source at a dose rate of 5 to 6 krad/min. Three ml volumes of the washed bacterial suspension were irradiated, oxygen being bubbled during the irradiation. Viability was measured in the same way as for u.v. irradiation.

Post-irradiation treatment. Samples (0.1 ml) of the irradiated and unirradiated cultures were diluted into 10 ml amounts of TGY medium in 250 ml flasks and these were incubated on reciprocating shakers immersed in water baths at 30 or 39 °C as required. The large dilution factor was used to prevent the aggregation of bacteria which often follows the irradiation of relatively dense cultures, making quantitative analysis difficult. The viability of the bacteria in these cultures was measured by removing 0.1 ml samples at various times,

diluting appropriately in TGY medium and plating 0.1 ml samples on TGY plates. Colonies were counted after 36 h and again after 4 days' incubation at 30 °C. When necessary nalidixic acid or chloramphenicol solutions, sterilized by filtration, were added to the post-irradiation incubation TGY medium to give final concentrations of 10 and 15 µg/ml, respectively, before the bacteria were added.

RESULTS

Effect of increasing incubation times at 39 °C on cultures of Micrococcus radiodurans ts1 given a single radiation dose

The result of u.v.-irradiating an exponential-phase culture of *M. radiodurans* ts1 with a dose of 6600 ergs/mm², resuspending it in TGY medium at its restrictive temperature of 39 °C and plating on TGY agar after varying times at 39 °C is shown in Fig. 1. After incubation of the plates at 30 °C for 36 h the viability of the irradiated population appeared to have fallen from nearly 100 % at zero time to 0.03 % after 3 h at 39 °C. All the colonies at this stage were similar to those derived from unirradiated bacteria. However, when the plates were re-incubated for a further 48 h most of the 'missing' colonies had grown but were of an abnormal type, being much smaller and highly sectorized. After holding the irradiated population at 39 °C for 3 h the ratio of abnormal to normal colonies was 2000:1, although this value varied somewhat from one experiment to another, the minimum ratio being about 400:1. An unirradiated population held at 39 °C showed no loss of viability for the duration of the experiment, there being an initial increase in numbers presumably because those bacteria which had just completed a round of DNA replication before being put at 39 °C could proceed to division. However, about 50 % of these unirradiated bacteria held at 39 °C for 3 h gave abnormal colonies (see later section).

The phenomenon described was not considerably altered when the irradiated bacteria were incubated at 39 °C in TGY medium containing 15 µg chloramphenicol/ml (Table 1).

The bacteria from normal colonies had dimensions of about $2 \times 2 \times 4$ µm for the diplo-coccal stage while those from the abnormal colonies were mainly large distorted forms with typical dimensions of about $5 \times 5 \times 8$ µm, i.e. about twelve times larger in volume. During the early development of some of the abnormal colonies (up to the 128 cell stage) only very large forms were present but from that stage an increasing number of normal-sized bacteria became apparent, usually as a fringe of cells on the side of the colony. An indication of the relative sizes of the colonies and their development and of the bacterial morphology are shown in Figs. 2 to 5. When replated on TGY agar the small sectorized colonies gave rise to both normal and abnormal colonies in varying proportions.

The result of raising the u.v. dose to 10600 ergs/mm², which reduced the initial viability to 50 %, is shown in Fig. 6. There was a similar loss of normal colony development following exposure to 39 °C. In this case 0.04 % of the initial population gave rise to normal colonies after 3 h at 39 °C compared with 17 % which gave abnormal colonies, i.e. a ratio of abnormal to normal colonies of about 400:1. The maximum rate of loss of normal colony development for both doses occurred between 40 and 140 min, i.e. the time of expression of this phenomenon was not dose-dependent.

There was a small effect on normal colony development when the wild-type strain of *M. radiodurans* was u.v.-irradiated with 10600 ergs/mm² and held for 3 h at 39 °C. In one such experiment the initial viability was reduced to 20 %, but after 3 h at 39 °C approximately half of the surviving population gave normal colonies.

Fig. 1. Normal and abnormal colony formation in u.v.-irradiated *M. radiodurans* ts1 as a result of post-irradiation incubation at 39 °C (restrictive temperature holding). The irradiation dose was 6600 ergs/mm². Normal colonies (●) were counted after incubation for 36 h and the total number (normal plus abnormal) (○) after incubation for 4 days. A control culture (■) was unirradiated but held at 39 °C and scored for normal colonies.

Ionizing radiation had the same effect as u.v. radiation. The result of irradiating *M. radiodurans* ts1 with 400 krad ⁶⁰Co γ-radiation and incubating at 39 °C for increasing times is shown in Fig. 7. The results are qualitatively similar to those for u.v. radiation, the loss of normal colony development being mainly completed by 3 h at 39 °C.

Effect of varying doses of radiation followed by incubation at 39 °C

Since the loss of normal colony development was mainly complete for different u.v. doses and for ionizing radiation after 3 h at 39 °C, this time was chosen as an end point for studying the effect of increasing doses of radiation on the sensitivity of normal colony formation. The results for u.v. and ionizing radiation are shown in Figs. 8 and 9. Compared with the

Table 1. *Effect of adding chloramphenicol (15 µg/ml) to the post-irradiation incubation medium on abnormal colony formation in Micrococcus radiodurans ts1*

Post-irradiation treatment	Colonies after 3 h at 39 °C (%)*	
	Normal	Abnormal
Held at 39 °C	0.2	80
Held at 39 °C in presence of chloramphenicol	0.8	63

* Taking the initial viable count (which was about 10^8 /ml) as 100 %.

loss of viability in the population as a whole, the initial loss of normal colony-forming ability was sensitive to u.v. radiation, the slope having a 1/e value of 210 ergs/mm², and very sensitive to ionizing radiation having a 1/e value of 4 to 5 krad. These values were equivalent to those for the inactivation of a recombination-deficient mutant (*rec30*) of *M. radiodurans*. The isolation and characterization of this mutant is to be the subject of another publication but the radiation dose-response curves have been superimposed on the data of Figs. 8 and 9. The loss of normal colony development, initially sensitive to both forms of radiation, became refractory and further loss was a function of the loss of viability in the population as a whole.

The effect of post-irradiation incubation of wild-type Micrococcus radiodurans at 39 °C in the presence of nalidixic acid

The effect of raising the temperature of *M. radiodurans* ts1 to 39 °C is to inhibit DNA synthesis while allowing RNA and protein synthesis to continue (Moseley *et al.* 1972*b*). We therefore investigated the effect of inhibiting DNA synthesis on colony formation in the wild-type strain of *M. radiodurans* using nalidixic acid under comparable conditions. Qualitatively the same effect was observed, but it was less marked than in the case of *M. radiodurans* ts1. Following a u.v.-irradiation dose of 10600 ergs/mm² the loss of normal colony development was less rapid, occurring between 60 and 210 min, normal colonies being produced by 0.2 % and abnormal colonies by 28 % of the original population. Unfortunately, nalidixic acid was not a good inhibitor of DNA synthesis in *M. radiodurans* because although DNA synthesis is 80 % inhibited in the presence of 2 µg nalidixic acid/ml, increasing the concentration to 200 µg/ml does not cause much further inhibition (Driedger & Grayston, 1971). Incubation of irradiated cells in nalidixic acid at 30 °C had almost no effect on colony development, at least 90 % giving rise to normal colonies.

A comparison of various treatments of irradiated and unirradiated strains of *M. radiodurans* on abnormal colony development is shown in Table 2.

The effect of pantoyl lactone on rescuing abnormal colonies

The effect of plating irradiated wild-type *M. radiodurans*, held for increasing times at 39 °C in TGY broth containing nalidixic acid, on TGY agar containing pantoyl lactone (12 mg/ml) is shown in Fig. 10. The addition of pantoyl lactone to the medium slightly reduced the size of colonies from unirradiated bacteria but nevertheless 'rescued' the colonies which in its absence would have taken four days to develop and would have been highly sectorized. In the presence of pantoyl lactone all the colonies appeared after incubation at 30 °C for 36 h and were indistinguishable from colonies derived from unirradiated bacteria. All the bacteria in these colonies had normal morphology.

Fig. 2. Stages in the early development of a normal colony of *M. radiodurans* ts1. The sequence, (a) to (f), was photographed over a 4 h period.

Fig. 3. Stages in the early development of an abnormal colony of *M. radiodurans* ts1. The sequence, (a) to (f), was photographed over a 12 h period.

Fig. 4. A culture of *M. radiodurans* ts1, u.v.-irradiated (6600 ergs/mm²) and held at 39 °C for 3 h before plating. (a) This plate had been incubated at 30 °C for 48 h and had 15 normal colonies; the abnormal colonies are just visible. (b) The same plate after incubation at 30 °C for 4 days. The number of colonies, based on counts at higher dilutions, is about 5000.

Fig. 5. Abnormal colony development. (a) The arrow indicates a tetrad of bacteria with normal morphology on the colony edge. (b), (c) The normal-sized bacteria have given rise to sectors which are now growing faster than the portion of the colony occupied by 'giant' cells.

Fig. 6. Normal and abnormal colony formation in u.v.-irradiated *M. radiodurans* ts1 as a result of restrictive temperature holding. The irradiation dose was 10600 ergs/mm². The number of normal (●) and the total number (normal plus abnormal) (○) of colonies were counted after incubation for 36 h and 4 days respectively. A control culture (■) was unirradiated but subjected to restrictive temperature holding and scored for normal colonies.

The effect of pantoyl lactone on the recovery of irradiated *M. radiodurans* ts1 could not be investigated because the bacteria did not form colonies on agar containing pantoyl lactone at a concentration of 12 mg/ml. This strain did form colonies on agar containing 3 mg pantoyl lactone/ml but at this concentration pantoyl lactone had no rescuing effect on the abnormal colonies of either *M. radiodurans* ts1 or the wild-type. Spontaneous mutants of ts1 selected for resistance to pantoyl lactone (12 mg/ml) were also temperature-resistant (four out of four) and all spontaneous mutants selected for temperature-resistance were pantoyl lactone-resistant (eleven out of eleven). This surprised us. Since *M. radiodurans* ts1 can be transformed to temperature resistance with wild-type DNA at a frequency associated with a single marker, it is assumed that the same locus controls both properties in this

Fig. 7. Normal and abnormal colony formation in ^{60}Co γ -irradiated *M. radiodurans* ts1 as a result of restrictive temperature holding. The irradiation dose was 400 krad. Normal colonies (●) and the total number of colonies (normal plus abnormal) (○) were counted after incubation for 36 h and 4 days respectively.

mutant but that the permissive temperature for growth in pantoyl lactone is lower than for DNA synthesis. *Micrococcus radiodurans* ts2 mutant in another locus which affects DNA synthesis is able to grow on agar containing 12 mg pantoyl lactone/ml and gives the same results as the wild type, viz. pantoyl lactone rescues abnormal colonies formed as a result of holding the bacteria at 39 °C.

Effect of holding irradiated populations at 39 °C for 3 h on other temperature-sensitive mutants of M. radiodurans

Several temperature-sensitive mutants of *M. radiodurans* were tested for the loss of normal colony development by u.v.-irradiating exponential-phase populations with a dose of 6600 ergs/mm² and plating after 3 h at 39 °C. The proportion of surviving bacteria which gave rise to normal colonies is shown in Table 3. The effect described for ts1 is also true for the mutant ts2 but not for the mutants ts3, ts9 or ts11. Some other properties of these mutants relevant to the loss of normal colony development are included in the Table. Those mutants which gave predominantly normal colonies, i.e. ts3, ts9 and ts11, were not sensitized to radiation when held at their restrictive temperatures, while ts1 and ts2, which gave

Fig. 8. Ultraviolet radiation dose-response curve for survival of normal colony-forming ability in *M. radiodurans* ts1 subjected to restrictive temperature holding for 180 min after irradiation. Normal colonies (●) and the total number of colonies (○) were counted after 36 h and 4 days respectively. The dose-response curve for survival of a recombination-deficient mutant of *M. radiodurans* (*rec30*) is included for comparison (broken line).

Fig. 9. Ionizing radiation dose-response curve for survival of normal colony-forming ability in *M. radiodurans* ts1 subjected to restrictive temperature holding for 180 min after irradiation. Normal colonies (●) and the total number of colonies (○) were scored after incubation for 36 h and 4 days respectively. The dose-response curve for survival of a recombination-deficient mutant of *M. radiodurans* (*rec30*) is included for comparison (broken line).

Table 2. *Effect of various incubation conditions on normal colony development in M. radiodurans*

Strain of <i>M. radiodurans</i>	Irradiation dose (ergs/mm ²)	Incubation temperature* (°C)	Nalidixic acid present	Percentage of survivors giving normal colonies
ts1	0	30	+	94
ts1	0	39	—	40
ts1	6600	30	—	100
ts1	6600	30	+	82
ts1	6600	39	—	0.43
Wild-type	6600	39	—	100
Wild-type	6600	39	+	2

* All incubations were for 3 h.

Fig. 10. Normal and abnormal colony formation in u.v.-irradiated wild-type *M. radiodurans* as a result of restrictive temperature holding in the presence of nalidixic acid, and the effect of plating on pantoyl lactone agar. The irradiation dose was 10600 ergs/mm². Normal (●) and total (○) colonies in the absence of pantoyl lactone were counted after incubation for 36 h and 4 days respectively. Normal colonies (△) in the presence of pantoyl lactone were counted after 36 h incubation. An unirradiated culture (■) was held at 39 °C in the presence of nalidixic acid and scored only for normal colonies.

Fig. 11. Effect of restrictive temperature holding before u.v.-irradiation on normal colony-forming ability in *Micrococcus radiodurans* ts1. One culture (○) was irradiated after growth at 30 °C, then held at 39 °C. A second culture (●) was held at 39 °C for 75 min, irradiated, and held at 39 °C. The irradiation dose was 2650 ergs/mm². Only the results for normal colonies are shown. The total number of colonies did not fall below 60 %.

Table 3. Effect on colony type of holding u.v.-irradiated populations (6600 ergs/mm²) of temperature-sensitive mutants of *M. radiodurans* at 39 °C for 180 min before being plated

Mutant	ts defect	Survival (%)	Percentage of survivors giving normal colonies	Population sensitized to radiation by holding at 39 °C
ts1	DNA synthesis	68	0.12	+
ts2	DNA synthesis	72	0.66	+
ts3	DNA synthesis	74	83	—
ts9	Cell wall	58	76	—
ts11	Cell wall	98	100	—

predominantly abnormal colonies, were sensitized to radiation when held at 39 °C (Moseley *et al.* 1972).

Effect of holding M. radiodurans ts1 at 39 °C before irradiation

Since the loss of normal colony-forming ability is associated with those strains of *M. radiodurans* which are sensitized to radiation when subject to restrictive temperature holding, it was assumed that the loss of normal cell division was the result of the decay of a recombination function which also leads to sensitization. If so, the time of expression of loss of normal colony-forming ability should depend on the length of time the bacteria have been at 39 °C and not on the timing of the irradiation. A log-phase culture of *M. radiodurans* ts1 grown at 30 °C was held at 39 °C for 75 min, irradiated, diluted into TGY medium and

returned to 39 °C. A control culture was irradiated after growth at 30 °C and then held at 39 °C. Samples were taken from both cultures at various times, plated on TGY agar and incubated at 30 °C. Normal and abnormal colonies were counted after 36 h and 4 days respectively (Fig. 11). Since the initial period at 39 °C sensitizes the bacteria to radiation (Moseley *et al.* 1972*a*) the dose of u.v. radiation was kept relatively low, at 2650 ergs/mm². In the culture held at 39 °C for 75 min before irradiation, the loss of the ability to give rise to normal colonies began immediately after irradiation and occurred about 75 min earlier (measured from the time of irradiation) than in the control culture.

DISCUSSION

Micrococcus radiodurans can be induced to form giant cells which give rise to abnormal colonies. The maximum loss of normal colony formation occurs in irradiated populations of *M. radiodurans* in which DNA synthesis is inhibited at 39 °C. Inhibition of DNA synthesis at 30 °C or holding strains with temperature-resistant DNA synthesis at 39 °C does not lead to any significant loss of normal colony development.

Since the formation of abnormal colonies is enhanced by irradiation with either u.v. or ionizing radiation, what mechanism related to the repair of both forms of damage occurs in ts1 held at 39 °C? The most likely repair mechanism which operates both for u.v. and ionizing radiation damage is that involving recombination (Rupp & Howard-Flanders, 1968), the excision process making very little contribution to the repair of ionizing radiation damage (Howard-Flanders, Theirot & Stedeford, 1969). In an unirradiated population of ts1 at 39 °C, after an initial lag of about 20 min there is an exponential loss of a recombination function and the culture becomes progressively more sensitive to u.v. or ionizing radiation, leading eventually to the loss of the shoulders from the dose-response curves (Moseley *et al.* 1972*a*) which is not prevented by the presence of chloramphenicol. This suggested that the large shoulder of the survival curves is due to a repair system involving recombination.

That the loss of this recombination function plays a role in the formation of the giant bacteria and hence abnormal colonies is based on three observations. Firstly, the formation of abnormal colonies by the majority of surviving bacteria is found in the temperature-sensitive DNA mutants ts1 and ts2, which both become sensitized to irradiation at 39 °C as a result of the loss of a recombination function, but is not found in the temperature-sensitive DNA mutant ts3 or in the temperature-sensitive wall mutants ts9 and ts11, none of which are sensitized to radiation by being held at 39 °C (Moseley *et al.* 1972*a*). An anomaly arises in the case of *M. radiodurans* ts3, which is unable to synthesize DNA at 39 °C but does not form abnormal colonies when subjected to restrictive temperature holding. However, it is also not sensitized to radiation by restrictive temperature holding (Moseley *et al.* 1972*a*) suggesting that either the recombination function does not decay at 39 °C in this mutant or, more likely, that it is not defective in the step of DNA synthesis which is involved in the restrictive temperature holding effect and which presumably is sensitive to nalidixic acid. Secondly, the expression of abnormal colony formation depends on the time the culture of ts1 has been held at 39 °C and is independent of the timing of the irradiation. Thirdly, the loss of normal colony development as a function of irradiation dose is similar to the dose-response curves of a recombination-deficient mutant (*rec30*) but not to any of the other ten radiation-sensitive mutants we possess. Thus the region of the survival curves (Figs. 8 and 9) where colonies are abnormal is that part which is absent in the recombination deficient mutant. We conclude that the recombination events which lead to survival are not

absent but temporarily defective. Since the loss of the recombination function is dependent only on the length of time the bacteria are held at 39 °C, it is not surprising that the rate of loss of normal colony development is independent of the irradiation dose. It also follows that since inhibition of protein synthesis by chloramphenicol does not prevent the loss of the recombination function at 39 °C, it does not prevent the loss of normal colony-forming ability.

Recombination functions have been implicated in the process of cell division in *Escherichia coli*. Thus mutation in the *lon* or *fil* gene causes irradiated cells to form long filaments in recombination-proficient bacteria (Adler & Hardigree, 1964). A second mutation in the *recA* gene however suppresses this effect so that after irradiation little or no filamentation is observed (Green, Greenberg & Donch, 1969). Inouye (1971), using a *recA* *thy* strain of *E. coli*, showed that under conditions where DNA synthesis was inhibited, either by thymine deprivation or by the presence of nalidixic acid, the RecA phenotype uncoupled cell division from DNA replication so that cell division continued, eventually producing cells containing no DNA. The situation is different in *Micrococcus radiodurans*. In the normal bacterium the recombination function, and not its absence, suppresses the tendency to produce giant bacteria and a reduction in its level does not uncouple DNA replication and cell division since the bacteria enlarge enormously but do not divide in the absence of DNA synthesis. It is, of course, possible that the recombination lesion in ts1 after 39 °C incubation is more akin to the *recB* or *recC* lesion of *E. coli*.

The presence of pantoyl lactone in the plating medium causes surviving bacteria which would otherwise have given rise to abnormal colonies to give normal colonies. In *E. coli*, strains which have an unsuppressed *lon* or *fil* gene and form long filaments after u.v. irradiation, leading to loss of viability, can be rescued by plating on pantoyl lactone agar when they are induced to form septa. The mechanism by which pantoyl lactone exerts this effect is unknown. We conclude that *M. radiodurans* is inherently capable of forming giant cells following DNA damage, equivalent to the expression of *lon* or *fil* in *E. coli*, but that it is normally repressed by a recombination function.

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