Isolation and Some Properties of Temperature-sensitive Mutants of *Micrococcus radiodurans* Defective in DNA Synthesis

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SUMMARY

Three temperature-sensitive mutants of *Micrococcus radiodurans* have been isolated which, unlike the wild-type, are unable to synthesize DNA at 39°. Synthesis of DNA stops immediately the bacteria are raised to the restrictive temperature. The mutants can be transformed normally for single genetic markers and can regain wild-type temperature resistance on incubation with DNA from wild-type at a frequency associated with single or very closely linked markers. Each mutant can also be transformed to wild-type temperature resistance with DNA from the other temperature-sensitive mutants. All three mutants are resistant to the lethal action of ionizing and ultraviolet radiations and at 39° are able to carry out all or most of the DNA repair functions associated with excision repair, but at a reduced rate compared with wild-type. The rate of excision at 39° of u.v.-induced thymine-containing pyrimidine dimers is four to five times slower in the mutants than in the wild-type.

INTRODUCTION

Micrococcus radiodurans is very resistant to the lethal action of ionizing and ultraviolet radiation (Anderson *et al.* 1956; Duggan, Anderson, Elliker & Cain, 1959), and to some other agents which cause single-strand damage to DNA, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Moseley, 1967), nitrous acid and hydroxylamine (unpublished observation). Its ability to recover from the action of these agents depends upon its DNA repair mechanisms. It has a very efficient excision repair mechanism with which it can excise from its DNA u.v.-induced pyrimidine dimers which could otherwise be lethal (Boling & Setlow, 1966) and presumably other altered bases (Hanawalt & Haynes, 1965). It also possesses a post-replication repair mechanism (Moseley & Mattingly, 1971) by which any unexcised dimers are effectively by-passed in the DNA by recombination, a mechanism discovered originally in *Escherichia coli* by Rupp & Howard-Flanders (1968). This mechanism appears to be defective in *E. coli* having a mutation in the *lex* or *exr* locus (Witkin, 1969) and in the mutant u.v. 17 of *M. radiodurans* (Moseley & Mattingly, 1971).

Temperature-sensitive mutants of *Escherichia coli* have been isolated which are unable to replicate their DNA at 42° (Bonhoeffer & Schaller, 1965), but which nevertheless are able to carry out unscheduled DNA synthesis, indicative of repair (Pettijohn & Hanawalt, 1964), at the restrictive temperature (Couch & Hanawalt, 1967). This paper describes the isolation of temperature-sensitive mutants of *Micrococcus radiodurans* which are unable to replicate their DNA at 39°, and some of their properties.

METHODS

Bacteria. The strain of *Micrococcus radiodurans* used was that originally isolated by Anderson *et al.* (1956).

Media. TGY broth for growth contained Bactotryptone (Difco), 5 g; glucose, 1 g; yeast extract (Difco), 3 g; distilled water, 1 l. TGY agar for colony counts and replica plating was made by solidifying this medium with 15 g Bactoagar/l.

An M/15 phosphate buffer, pH 7.0, was used for washing bacteria and for suspending them during irradiation. It contained Na_2HPO_4 , 4.73 g; KH_2PO_4 , 4.54 g; distilled water, 1 l.

Measurement of growth rates. Samples from 18 h cultures were diluted into 20 ml quantities of TGY broth to give a turbidity reading of about 0.08 in a nephelometer (Evans Electroselenium Ltd, Halstead, Essex) using an orange filter (this turbidity was equal to a concentration of 2.5×10^7 viable units/ml) and the cultures swirled in flasks at 30°. Turbidity readings were taken at various times until they reached about 0.80 (3.0×10^8 viable units/ml). The doubling times were calculated from the plotted data.

Preparation of bacteria for irradiation. Overnight cultures were diluted with TGY broth to a turbidity reading of 0.08 and swirled in flasks at 30° until the turbidities reached 0.50 to 0.55 (about 1.5×10^8 viable units/ml). The bacteria were centrifuged, washed, and resuspended in chilled M/15 phosphate buffer at a concentration of about 1.0×10^8 viable units/ml. For u.v. irradiation 5 ml samples of the washed bacterial suspension were agitated by means of a magnetic stirrer and irradiated in Petri dishes (9 cm diam) at a distance of 40 cm from a Hanovia germicidal lamp (model 12). The incident dose rate was 22.5 ergs/mm²/s. At intervals 0.1 ml samples were removed, suitably diluted into chilled TGY and 0.1 ml samples spread on TGY agar plates. Colonies derived from surviving cells were counted after incubation at 30° for two days.

γ -Irradiation was carried out in a ⁶⁰Co source at a dose rate of 7.5 to 8.0 krad/min. Three ml volumes of the washed bacterial suspension were irradiated, oxygen being bubbled during the irradiation. Samples were removed and viability measured in the same way as for u.v. irradiation.

Isolation of temperature-sensitive mutants. The procedure used was based on the method used to isolate radiation-sensitive mutants of *Micrococcus radiodurans* (Moseley 1967). Five ml of a log phase culture at a turbidity of 0.38 (1.0×10^8 viable units/ml) was washed and resuspended in 5 ml M/15 phosphate buffer and irradiated with 10000 ergs/mm² u.v. radiation. Four ml of the irradiated suspension was added to 5 ml TGY broth and swirled at 30° for 30 min. One ml of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) solution was then added to give a final concentration of 100 µg NG/ml. After incubation for a further 45 min, 0.2 ml of this culture was diluted 100-fold in TGY broth, to dilute out the mutagen, and incubated at 30° overnight. The culture was then suitably diluted and 0.1 ml samples spread on TGY plates to give about 80 to 100 colonies per plate after incubation at 30° for 2 days. Two replicate copies of each plate were made, one incubated at 39° and one at 30°. After incubation for 24 h, colonies which grew at 30° but not at 39° were isolated and inability to grow at 39° was confirmed.

Measurement of DNA, RNA and protein synthesis. Samples of the wild-type and the temperature-sensitive mutants were grown to a turbidity of about 0.30. To 0.12 ml quantities of the cultures were added equal quantities of TGY broth and an appropriate radioactive compound: 5 µCi tritiated thymidine (³H]dT specific activity 14 Ci/mM) to measure DNA synthesis, 5 µCi tritiated uridine (³H]U specific activity 17 Ci/mM) to measure RNA synthesis, or [¹⁴C]methionine to measure protein synthesis. To measure synthesis at

30° and 39° duplicate cultures were incubated at 30° for 80 min before one of each pair of tubes was raised to the restrictive temperature of 39°. The amount of radioactivity in the trichloroacetic acid-insoluble fraction of the bacteria was measured as a function of time by the paper-disc method of Bollum (1959).

Genetic transformation of the temperature-sensitive strains. The procedure was basically that described by Moseley & Mattingly (1971). Assay tubes were set up containing 0.05 ml autoclaved extract of *Micrococcus radiodurans*, 0.05 ml DNA and 0.20 ml recipient bacteria. The assay tubes were incubated at 30° for 3 h and diluted tenfold by the addition of 2.7 ml chilled TGY broth. Then 1.0 ml quantities were placed in Petri dishes and 9 ml amounts of molten TGY agar at 46° were added to each. The plates were swirled several times to ensure even distribution of the cells and the agar allowed to solidify. For transformation to wild-type temperature resistance a second layer of 10 ml molten agar was added, allowed to solidify and the plates were incubated at 39°. For transformation to streptomycin resistance a second layer of agar containing 200 µg streptomycin/ml was added and the plates incubated at 30°. Transformant colonies were counted after 4 to 5 days of incubation. Control plates from assay tubes containing no DNA were included in each experiment.

Removal of pyrimidine-dimers from DNA at the restrictive temperature. Five 0.5 ml samples from a control culture at a turbidity of 0.08 were put in test tubes, 25 µCi [³H]dT added to each, and the cultures incubated at 30° until the turbidity in the control culture reached 0.60 to 0.70. The cultures were spun down, washed, and each resuspended in 0.5 ml M/15 phosphate buffer. One culture was not irradiated. The four remaining cultures were irradiated with u.v. light, centrifuged and resuspended in 0.5 ml TGY broth. Three were incubated for varying periods of time at 39° while the unirradiated and one irradiated sample were held in ice. The bacteria in each sample were centrifuged, washed in M/15 phosphate buffer and suspended in 0.2 ml buffer. Calf-thymus DNA (0.1 ml at 4 mg/ml) and 0.2 ml cold 10 % trichloroacetic acid (TCA) were added to each tube and the contents mixed. After 10 min the TCA precipitate was centrifuged and washed with 0.2 ml cold 5 % TCA. After centrifugation the TCA was removed and the precipitate washed with 95 % ethyl alcohol and dried. Each sample was hydrolysed with 0.2 ml 98 % formic acid in a sealed hydrolysis tube at 175° for 20 min. The hydrolysis procedure converts thymine-cytosine dimers to thymine-uracil dimers (Setlow, Carrier & Bollum, 1965), in which form they appear in subsequent chromatograms. The hydrolysed material was chromatographed by descending chromatography in two dimensions using butanol-acetic acid-water (80:12:30) for the first dimension and butanol-water (86:14) for the second. In the first dimension thymine had an R_f of 0.63, the thymine-thymine dimer 0.29 and the thymine-uracil dimer 0.22. The thymine region was assayed for radioactivity while the dimer region was run in the second dimension. The R_f of both dimers was 0.09, so that background counts were moved away from the dimer region. The dimer regions were cut into 1 cm strips and the radioactivity was assayed. The radioactivity in the dimers was calculated as a percent of the total radioactivity in the TCA-insoluble fraction.

RESULTS

The restrictive temperature. *Micrococcus radiodurans*, when originally isolated, showed good growth at 37° but none at 45° (Anderson *et al.* 1956). In practice we incubate liquid cultures at 30° because at 37° the bacteria tend to aggregate into large clumps and the culture is difficult to use for quantitative studies. However, good growth of single viable units into colonies was obtained on agar plates at temperatures up to 39°. At 41° there was no visible growth and so 39° was chosen as a possible restrictive temperature.

Fig. 1

Fig. 2

Fig. 3

Fig. 1. Uptake of [^3H]thymidine into the TCA-insoluble fraction of wild-type (O) and the temperature-sensitive mutants ts1 (x), ts2 (●) and ts3 (Δ) of *Micrococcus radiodurans* as a measure of DNA synthesis. The temperature was raised to 39° after 80 min at 30° as indicated by the arrow.

Fig. 2. Uptake of [^3H]uridine into the TCA-insoluble fraction of wild-type (O) and the temperature-sensitive mutants ts1 (x), ts2 (●) and ts3 (Δ) of *Micrococcus radiodurans* as a measure of RNA synthesis. The temperature was raised from 30° to 39° after 80 min.

Fig. 3. Uptake of [^3H]thymidine and [^{14}C]methionine in ts3 as a measure of DNA and protein synthesis respectively. One culture was incubated at 30° (O) and a second incubated at 30° for 80 min and then at 39° (●). The upper part of the graph gives the results for ^3H assay and the lower those for ^{14}C .

Isolation of mutants. Of about 6000 colonies derived from bacteria which survived the mutagenic treatment, 15 proved to have no growth at 39° but growth rates comparable to the wild type at 30° . These 15 mutants were screened to select for those which were unable to grow at 39° because of their inability to synthesize DNA at this temperature. On raising the temperature to 39° after approximately one generation time at 30° , the incorporation of [^3H]thymidine ceased in seven of the mutants. Of the seven, three have been examined in some detail. They have been designated *Micrococcus radiodurans* ts1, ts2 and ts3. The colonies of the wild-type and three mutants were similar in size and appearance and all contained the carotenoid pigment which has an absorption peak at 475 nm. Morphologically the bacteria were identical.

The uptake of [^3H]thymidine into the TCA-insoluble fraction of the bacteria, as a measure of DNA synthesis, is shown in Fig. 1. Synthesis of DNA ceased immediately the mutants were raised to 39° but continued in the wild-type. Fig. 2 shows the uptake of [^3H]uridine, as a measure of RNA synthesis, which continued at 39° in the wild-type and the mutants. Protein synthesis as measured by uptake of [^{14}C]methionine also continued in all three mutants at the restrictive temperature and results are shown in Fig. 3 for the mutant ts3.

Fig. 4

Fig. 5

Fig. 6

Fig. 4. Ultraviolet irradiation survival curves of wild-type (O) and the mutants ts1 (x), ts2 (●), and ts3 (Δ) of *Micrococcus radiodurans*.

Fig. 5. γ-Irradiation survival curves of wild type (O) and the mutants ts1 (x), ts2 (●), and ts3 (Δ) of *Micrococcus radiodurans*.

Fig. 6. Uptake of [³H]thymidine as a measure of DNA synthesis in *Micrococcus radiodurans* wild-type in an unirradiated culture at 30° (x) and 39° (O) and in a culture irradiated with 5300 ergs/mm² u.v. radiation at 30° (Δ) and 39° (●). All the bacteria remain viable at this dose.

Table 1. Frequency of transformation to streptomycin resistance of wild-type and three temperature-sensitive mutants of *Micrococcus radiodurans* following incubation of the recipient bacteria with DNA at 30° and 39°

The bacteria were incubated with DNA for 2 to 3 h at 30° or 15 min at 39° since these times gave maximum transformation.

Strain	30°	39°
Wild-type	3.0×10^{-5}	1.5×10^{-6}
ts1	3.0×10^{-5}	1.0×10^{-5}
ts2	4.0×10^{-5}	4.0×10^{-6}
ts3	4.0×10^{-5}	2.0×10^{-6}

As a consequence of the continuing RNA and protein synthesis in the absence of DNA synthesis the mutant bacteria enlarged considerably but no cell division occurred.

Growth rates. The doubling times of the mutants were calculated from plots of increasing turbidity on a log scale against time. The mutants ts1 and ts2 had doubling times during exponential growth at 30° of 80 min, the same as wild-type, while ts3 was somewhat slower with a doubling time of 100 min.

Radiation survival. The survival curves of the three mutants compared with those of wild-type are shown in Fig. 4 and 5. All three are very resistant to both u.v. and ionizing radiations although ts1 and ts3 appear slightly more sensitive than ts2.

Transformation of the temperature-sensitive mutants to streptomycin resistance. All three mutants were transformable and results for the transformation of the streptomycin-sensitive ts mutants to streptomycin resistance are given in Table 1, where they are compared with those for wild-type. The uptake of DNA was carried out at both 30° and 39° but at 39°

Fig. 7. Uptake of [^3H]thymidine as a measure of DNA synthesis at 30° in $ts1$. (a) Unirradiated ($\circ$) and u.v.-irradiated (5300 ergs/mm^2) ($\bullet$) bacteria. (b) Unirradiated ($\circ$) and u.v.-irradiated bacteria which had been incubated for 50 ($\bullet$) and 75 ($\times$) min at 39° prior to uptake. (c) Unirradiated ($\circ$) and u.v.-irradiated bacteria incubated for 140 min at 39° prior to uptake ($\bullet$).

Table 2. Frequency of transformation to temperature resistance of $ts1$, $ts2$, and $ts3$ following incubation of the recipient bacteria with DNA from wild type, $ts1$, $ts2$, and $ts3$

Strain	DNA from				No DNA
	Wild-type	$ts1$	$ts2$	$ts3$	
$ts1$	6.0×10^{-5}	0	6.0×10^{-5}	2.5×10^{-5}	0
$ts2$	7.0×10^{-5}	1.2×10^{-4}	0	1.6×10^{-5}	0
$ts3$	2.0×10^{-5}	2.3×10^{-5}	3.0×10^{-6}	0	0

maximum transformation was obtained following 15 min incubation compared with 2 to 3 h at 30° . In all cases the transformation frequency was reduced at 39° , from 20-fold in the wild-type to twofold in $ts3$, although in the latter case the transformation frequency at 30° was much lower than in wild-type, $ts1$, or $ts2$.

Transformation of temperature-sensitive strains to temperature resistance. The three mutants were exposed to DNA extracted from the wild-type, $ts1$, $ts2$, and $ts3$, under normal transformation conditions, and the bacteria then incubated at 39° . Each of the three ts mutants could be transformed to wild-type temperature resistance with DNA from either the wild-type or either of the other ts mutants. The results are shown in Table 2.

Excision repair of DNA at the restrictive temperature. The ability of ts mutants to repair their DNA at the restrictive temperature after u.v. or ionizing radiation damage was tested in the following way. Unirradiated bacteria incubated in broth in the presence of [^3H]thymidine incorporate radioactive label into their DNA immediately as a consequence of normal DNA replication. When the bacteria have been irradiated there is a delay in the uptake of label since there is a delay in DNA synthesis (Swenson & Setlow, 1966). Fig. 6 indicates that for wild-type *Micrococcus radiodurans* following a dose of 5300 ergs/mm^2 u.v. radiation there is a delay in DNA synthesis of 65 min at 39° and 80 min at 30° . The time of delay is linearly related to the radiation dose (Setlow & Boling, 1965). By analogy with *Escherichia coli* we may assume that the delay in DNA synthesis is due to damage to DNA, although damage to the replication system itself may conceivably be responsible. If irradiated bacteria which cannot incorporate label at 39° because they are unable to

Fig. 8. Uptake of [³H]thymidine as a measure of DNA synthesis at 30° in ts1. (a) Unirradiated (○) and γ-irradiated (175 krad) (●) bacteria. (b) Unirradiated (○) and γ-irradiated bacteria incubated for 120 min at 39° prior to uptake.

Fig. 9. Uptake of [³H]thymidine as a measure of DNA synthesis at 30° in ts2. (a) Unirradiated (○) and u.v.-irradiated (5300 ergs/mm²) (●) bacteria. (b) Unirradiated (○) and u.v.-irradiated bacteria incubated for 180 min at 39° prior to uptake.

replicate their DNA, are nevertheless able to complete excision repair of damaged DNA, then when they are returned to a temperature of 30° uptake of label will be immediate since normal DNA replication can resume immediately.

The three mutants were given sublethal doses of u.v. radiation that gave a reasonable delay in DNA synthesis. In all three cases the mutants were capable of carrying out some repair (presumably to their DNA) at 39° so that when the bacteria were returned to the permissive temperature uptake of label was seen either immediately or after a shorter time interval than would have been observed if no repair had taken place.

Fig. 7 gives the data for ts1 irradiated with 5300 ergs/mm² u.v. radiation. At 30° the delay in DNA synthesis was 80 min. (Fig. 7a), the same as for wild-type. There was no indication of repair until 75 min at 39° (Fig. 7b); after 140 min repair was complete, since there was

Fig. 10. Uptake of $[^3\text{H}]$ thymidine as a measure of DNA synthesis at 30° in *ts2*. (a) Unirradiated (○) and γ -irradiated (175 krad) (●). (b) Unirradiated (○) and γ -irradiated bacteria incubated for 180 min at 39° (●) prior to uptake.

Table 3. *Excision of thymine-containing pyrimidine dimers at 39° in wild-type and *ts* mutants*

The values represent the percentage of thymine as thymine-containing dimers in the TCA-insoluble fraction of the bacteria as a function of time of incubation of irradiated cells at 39° . The bacteria were given a u.v. dose of 5300 ergs/mm 2 .

Incubation at 39° (min)	Strain			
	Wild-type	<i>ts1</i>	<i>ts2</i>	<i>ts3</i>
0	0.52	0.57	0.57	0.40
4	0.16	—	—	—
7.5	0.04	—	0.37	—
15	—	0.14	0.14	0.11
30	—	0.07	—	0.05

uptake of radioactive label immediately the temperature was reduced (Fig. 7c). To test the repair of γ -radiation induced damage the bacteria were given a dose of 175 krad which in the wild-type and *ts1* gave a delay in DNA synthesis of 85 min at 30° . By holding irradiated cells of *ts1* for 120 min at 39° uptake of label was immediate when the temperature was reduced to 30° (Fig. 8).

When *ts2* was irradiated with a dose of 5300 ergs/mm 2 the delay in DNA replication at 30° was 85 min (Fig. 9a). However, it was not possible to show complete repair for u.v.-induced damage at the restrictive temperature; even after 180 min the delay was 30 min and uptake of label appeared to be at a slower rate than in the wild-type when it did resume (Fig. 9b). For γ -radiation induced damage repair was complete by 120 min incubation at 39° , since uptake of label at 30° was normal (Fig. 10).

The delay in DNA replication at 30° when *ts3* was irradiated with 5300 ergs/mm 2 u.v. radiation was too long to be experimentally useful so the dose was halved to 2650 ergs/mm 2 giving a delay of 70 min. The results of raising the bacteria to a temperature of 39° were similar to those of *ts1* after the higher dose. For γ -ray induced damage the time required appeared about the same as for *ts1* and *ts2*. A dose of 75 krad gave a delay in DNA replication of about 40 min at 30° , and after 120 min incubation at 39° repair was almost complete.

Irradiated cells did not show normal growth after repair at 39° because RNA and protein synthesis continued at this temperature and the cells became swollen. As a result of this imbalance of synthesis, when the bacteria were returned to a temperature at which DNA replication was possible, the time required for the culture to reach the same status as one kept at 30° in terms of viable units per ml and eventual optical density was equal to the time held at 39°. Thus it appeared that a period of DNA synthesis equal to the period the bacteria were held at the restrictive temperature was necessary before cell division could occur.

Excision of pyrimidine dimers from DNA at 39°. All three mutants were able to excise u.v.-induced pyrimidine dimers at the restrictive temperature. Table 3 shows the specific loss of thymine-containing dimers from the TCA-insoluble fraction of the irradiated bacteria compared with wild-type. The dimers are excised exponentially and when plotted the results indicate that wild-type can excise 90 % of thymine containing dimers in 7 min, while the three mutants take 32 min, i.e. the process is 4 to 5 times faster in the wild-type at 39°.

DISCUSSION

The three temperature-sensitive mutants isolated were unable to replicate their DNA at 39° as wild-type does but behaved typically in other respects although ts3 did have a somewhat slower growth rate and lower frequency of transformation. The mutations leading to the isolation of these strains were either single mutations or very closely linked mutations since they could be transformed to wild-type temperature resistance with wild-type DNA with the frequency of transformation associated with the transfer of a single marker. The mutations were different since each of the ts strains could be transformed to temperature resistance with DNA from either of the other two. They also behaved differently at 39° in terms of repair capability. The temperature-sensitive steps were not connected with the initiation of new rounds of DNA replication since DNA synthesis ceased immediately the bacteria were raised to the restrictive temperature. If the initiation step was involved then one would expect continuing DNA synthesis until all rounds of replication were complete (Maaløe & Hanawalt, 1961).

The mutants, ts1 and ts2, which have essentially the same radiation resistance as the wild-type, could be transformed for single genetic markers, e.g. streptomycin resistance, with the same frequency as the wild-type at 30° so that the recombination mechanism operated normally at this temperature. This is in contrast to the radiation-sensitive mutant u.v. 17 isolated by the same technique which showed a reduced frequency of recombination (measured by transformation) commensurate with the reduction in radiation resistance and which was defective in recombination repair (Moseley & Mattingly, 1971). The lower transformation frequency in ts3, which was not radiation-sensitive, may be connected with its slower growth rate rather than with a defect in recombination. This has been observed for other slow-growing strains of *Micrococcus radiodurans*.

Excision is possible in the mutants at the restrictive temperature. Hence it follows that the enzymes which have become temperature-sensitive, preventing the bacteria from replicating their DNA at 39°, are not involved in the excision step.

The repair at 39° is not as efficient as in the wild-type. In the latter the delay in DNA synthesis following ionizing or u.v. radiation is shorter at 39° than at 30°, indicating a faster rate of repair at 39°. In the mutants ts1 and ts2 the rate of repair at 30°, as indicated by the resumption of DNA synthesis, is the same as in the wild-type, but at 39° even in those cases where excision repair appeared to be capable of completion, the time required was considerably longer than at 30°. One specific step in the excision repair of u.v.-induced damage

is the removal of pyrimidine dimers. This step occurs in all three mutants at the same rate but is 4 to 5 times slower at 39° than the corresponding excision in wild-type. The reduced rate of dimer excision is the same in all three mutants of *Micrococcus radiodurans*, even though in ts3 the delay in DNA synthesis is about twice as long at 30° as in ts1 and ts2 for the same u.v. dose and very much longer at 39°. Thus the delay is not dependent upon dimer excision but on some other factor. It could conceivably be due to a defect in the repolymerization step resulting from the ts3 mutation. The only comparable work with ts DNA mutants has been that of Couch & Hanawalt (1967) with *Escherichia coli*, who showed that repair replication after u.v. did occur at the restrictive temperature.

The question of whether recombination as well as excision repair occurs in the ts mutants at 39° is difficult to answer since at 39° the bacteria cannot grow and the temperature has to be reduced to 30° for the genetic marker to be expressed. Integration of DNA could then occur at this lower temperature. In both wild-type and the mutants ts1 and ts2 the marker for streptomycin resistance can be transformed at 30° with the normal frequency although in ts3 it is somewhat lower. At 39°, however, the frequency is reduced considerably in all four strains. One possible method for showing recombination (by transformation) at 39° in the mutants would be to transform them for temperature resistance so that the bacteria could be exposed to DNA from wild-type at 39°, overlaid in agar at 39° and incubated at 39°. Such experiments have so far proved negative.

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REFERENCES

- ANDERSON, A. W., NORDAN, H. C., CANIN, R. F., PARRISH, G. & DUGGAN, D. (1956). Studies on a radio-resistant micrococcus. Isolation, morphology, cultural characteristics, and resistance to gamma radiation. *Food Technology* **10**, 575-578.
- BOLING, M. E. & SETLOW, J. K. (1966). The resistance of *Micrococcus radiodurans* to ultraviolet radiation. III. A repair mechanism. *Biochimica et biophysica acta* **123**, 26-33.
- BOLLUM, F. J. (1959). Thermal conversion of non-pairing deoxyribonucleic acid to primer. *Journal of Biological Chemistry* **234**, 2733-2744.
- BONHOEFFER, F. & SCHALLER, H. (1965). A method for the selective enrichment of mutants based on the high UV sensitivity of DNA containing 5-bromouracil. *Biochemical and Biophysical Research Communications* **20**, 93-97.
- COUCH, J. & HANAWALT, P. C. (1967). DNA repair replication in temperature-sensitive DNA synthesis deficient bacteria. *Biochemical and Biophysical Research Communications* **29**, 779-784.
- DUGGAN, D. E., ANDERSON, A. W., ELLIKER, P. R. & CAIN, R. F. (1959). Ultraviolet exposure studies on a gamma radiation resistant micrococcus isolated from food. *Food Research* **24**, 376-382.
- HANAWALT, P. C. & HAYNES, R. H. (1965). Repair replication of DNA in bacteria: irrelevance of chemical nature of base defect. *Biochemical and Biophysical Research Communications* **19**, 462-467.
- MAALØE, O. & HANAWALT, P. C. (1961). Thymine deficiency and the normal DNA replication cycle. *Journal of Molecular Biology* **3**, 144-155.
- MOSELEY, B. E. B. (1967). The isolation and some properties of radiation-sensitive mutants of *Micrococcus radiodurans*. *Journal of General Microbiology* **49**, 293-300.
- MOSELEY, B. E. B. & MATTINGLY, A. (1971). Repair of irradiated transforming deoxyribonucleic acid in wild type and a radiation-sensitive mutant of *Micrococcus radiodurans*. *Journal of Bacteriology* **105**, 976-983.
- PETTIJOHN, D. & HANAWALT, P. (1964). Evidence for repair-replication of ultraviolet damaged DNA in bacteria. *Journal of Molecular Biology* **9**, 395-410.
- RUPP, W. D. & HOWARD-FLANDERS, P. (1968). Discontinuities in the DNA synthesised in an excision defective strain of *Escherichia coli* following ultraviolet irradiation. *Journal of Molecular Biology* **31**, 291-304.

- SETLOW, J. K. & BOLING, M. E. (1965). The resistance of *Micrococcus radiodurans* to ultraviolet radiation. II. Action spectra for killing, delay in DNA synthesis, and thymine dimerisation. *Biochimica et biophysica acta* **108**, 259–265.
- SETLOW, R. B., CARRIER, W. L. & BOLLUM, F. J. (1965). Pyrimidine dimers in UV-irradiated poly dI:dC. *Proceedings of the National Academy of Sciences of the United States of America* **53**, 1111–1118.
- SWENSON, P. A. & SETLOW, R. B. (1966). Effect of ultraviolet radiation on macromolecular synthesis in *Escherichia coli*. *Journal of Molecular Biology* **15**, 201–219.
- WITKIN, E. M. (1969). Ultraviolet mutation and DNA repair. *Annual Review of Microbiology* **23**, 487–514.