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Host Cell Reactivation of *Bacillus subtilis* Bacteriophages

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Host cell reactivation of ultraviolet-irradiated phage can be used as a probe of the bacterial repair system and to determine phage and cellular contributions to the repair process. Using the *Bacillus subtilis* phages SPP1, SPO1, φe, and φ29, we found that the *uvr-1* and *polA* functions are involved in the host cell reactivation of the four phages. SPP1 was the only phage whose reactivation was also decreased in *recA*, *recD*, and *recF* mutant cells. We studied variations of host cell reactivation for SPP1 during spore outgrowth; at high ultraviolet doses the activity of a spore repair system requiring deoxyribonucleic acid polymerase I became evident. The spore repair system was completely replaced by the vegetative one by 120 min of outgrowth.

The repair process occurring in the dark after ultraviolet (UV) irradiation of deoxyribonucleic acid (DNA) involves the partial degradation of the regions containing photoproducts and the resynthesis of the removed portion (3, 7, 22, 28–30). The similarity and the overlapping of functions between the process of repair of UV-damaged DNA and that of genetic recombination in *Bacillus subtilis* are demonstrated by the isolation of *rec* mutants defective in both mechanisms (see reference 16 for a review and for the original references). *uvr-1* and *polA* functions are involved in the repair of UV lesions but not in recombination (10, 11, 13, 15, 18–20, 23, 24, 37). The same recovery process is assumed to operate in the repair of UV-induced lesions in phage DNA (host cell reactivation [HCR]) (12, 14, 27); this is strongly supported by the evidence that bacterial mutants isolated for their deficiency in HCR are themselves UV sensitive (8, 12, 14, 27).

HCR can thus be used as a probe to test the repair proficiency of a given strain and to analyze its variation at different physiological growth stages without the interference of the collateral effects of UV irradiation to the bacterial strain itself. In this paper we have studied the dependence of this process for different phages upon different host repair functions and the variation of HCR capacity during germination and outgrowth.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. The *B. subtilis* strains used in this work are listed in Table 1. For the nomenclature and properties of *rec* mutants see reference 16. *uvr-1* and *polA42* mutants are described in references 13 and 37, respectively. The bacteriophages used were SPP1 (26), SPO1 (21), φ29 (1), and φe (32). The phage lysates were produced by collecting confluent lysis obtained by the soft agar plating method.

Culture media. Difco nutrient broth (NB) supplemented with 10^{-5} M $MnCl_2$ was used to produce spores (31). For spore germination NB supplemented with 0.5% glucose (NBG) was used. Tryptone-yeast extract (TY; 2) medium was used for phage dilutions and plating.

Preparation of spores and germination. Spores were prepared as described by Siccardi et al. (31), suspended in distilled water, and stored at 4°C. The spores were heat activated at 70°C for 15 min. Germination was carried out at 37°C in NBG medium. Changes in culture turbidity (during germination and outgrowth) were followed by measuring the absorbance at 560 nm (A_{560}) with a Zeiss PMQ II spectrophotometer.

Irradiation and host cell reactivation. UV irradiation of phages was carried out with a Philips TUV 15-W germicidal lamp emitting mainly a 254-nm wavelength. Samples (5 ml) containing 10^8 plaque-forming units/ml in 0.01 M tris(hydroxymethyl)aminomethane (pH 7.0) and 0.01 M $MgCl_2$ were irradiated at an incident UV dose of 40 ergs/mm² per s, with gentle rocking, in 9-cm-diameter glass dishes. The thickness of the layer was approximately 1 mm.

Samples (0.1 ml) of an appropriate dilution of the UV-irradiated phage were mixed with 0.2 ml of a suspension containing approximately 10^7 cells. After 5 min of absorption, 2.0 ml of TY soft agar (kept at 45°C) was added and plated on a solid TY agar layer. The cells used for phage plating were nonactivated spores or cells at different stages of germination and outgrowth for different experiments. No additional indicator strain was used. Plates were incubated at 37°C and counted the next morning. Every experimental value was obtained by duplicate plating. All

TABLE 1. *B. subtilis strains used*

Strain	Genotype[a]	Origin
SB25	*hisB2 trpC2*	J. Lederberg
SB202	*tyrA1 his B2 trpC2 aroB2*	J. Lederberg
PB1633	*tyrA1 his B2 trpC2 aroB2 recF33*	G. Mazza
PB1639	*tyrA1 his B2 trpC2 aroB2 recG39*	G. Mazza
PB1641	*tyrA1 his B2 trpC2 aroB2 recD41*	G. Mazza
PB1642	*tyrA1 his B2 trpC2 aroB2 polA42*	G. Mazza
PB1663	*hisB2 trpC2 recH342*	A. Prozorov
GSY228	*trpC2 metB4*	C. Anagnostopoulos
GSY1025	*trpC2 metB4 recA1*	C. Anagnostopoulos
GSY1028	*trpC2 metB4 recB2*	C. Anagnostopoulos
GSY1027	*trpC2 metB4 uvr-1*	C. Anagnostopoulos

[a] *his, tyr, trp, met, aro*: Requirement for hystidine, tyrosine, tryptophan, methionine, and shikimic acid, respectively. *rec*, Deficiency in recombination; *polA*, deficiency in DNA polymerase I; *uvr*, UV light sensitivity.

the strains used in this work showed the same plating efficiency (EOP) with nonirradiated phages.

Since survival curves are exponential, to compare the variations of the EOP with UV dose for a given phage, we have used the equation $N/N_0 = e^{-h}$, where N/N_0 is the survival fraction, N_0 is the initial phage titer, and h is the dose necessary to reduce the phage titer to e^{-1} of its original value.

Assay of DNA synthesis during germination. PB202 heat-activated spores were inoculated in NBG medium at an A_{560} of 0.4 to 0.5. During germination, DNA synthesis was monitored by the incorporation of [^{3}H]thymidine (5 μCi/ml) in 2-min pulses, using 0.5-ml samples of the culture. The pulses were performed in the presence or absence of either OHPhN$_2$Ura (30 μg/ml) or nalidixic acid (50 μg/ml) to distinguish between the replicative and nonreplicative fractions of DNA synthesis. After the pulse, the samples were diluted twofold with 2 N NaOH and incubated at room temperature overnight. The samples were neutralized and precipitated with 0.5 ml of cold 50% trichloroacetic acid, collected on membrane filters (Millipore Corp.; 0.45-μm pore size, 25-mm diameter), and washed twice with 10 ml of cold 5% trichloroacetic acid, twice with 5 ml of 2 N HCl, and once with 5 ml of ethanol. After drying, the radioactivity on the filters was measured with a Packard liquid scintillation counter.

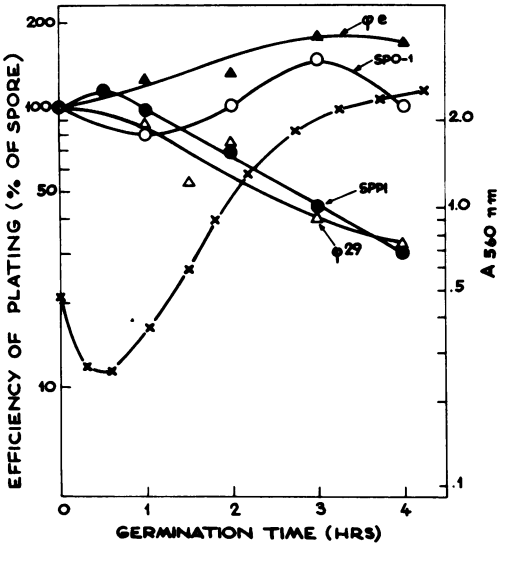

FIG. 1. *EOP of B. subtilis phages SPP1, SPO1, φe, and φ29 on cells at different stages of spore outgrowth. PB202 heat-activated spores were inoculated in NBG medium, and at the times indicated a sample of this culture was used for plating the phages. The curve (×) shows growth (A_{560}) of the germinating culture.*

RESULTS

As a prerequisite to our study, we looked for plating conditions as reproducible as possible for SPP1, SPO1, φe, and φ29 phages. We have evaluated the EOP of the four phages on PB202 indicator cells at different stages of growth. The results of these experiments are shown in Fig. 1. It is evident that the bacterial spore is a good indicator even at early stages of outgrowth.

For SPP1 and φ29 phages the EOP decreases during germination and vegetative growth; on the contrary, SPO1 and φe show better plating on vegetative cells. Still, for the latter phages, the optimum values are less than twice the value obtained on spores. All subsequent experiments were performed for all phages by plating them on spores (unless otherwise stated).

Phage and host contributions to phage recovery from UV irradiation. To check the dependence of HCR on different bacterial functions, the four phages were plated, after UV irradiation, on spores of *B. subtilis* mutant strains altered in recombination and/or repair functions. The HCR efficiency of a given bacterial strain has been expressed as a function of the HCR of its parental strain (assumed as 1.0)

by dividing the *h* (UV inactivation constant) value in the mutant by that obtained in the parent. The relative efficiency values are reported in Table 2 for different phages and strains.

The curves for SPP1 are reported in Fig. 2. On the basis of these curves, the UV-sensitive mutants of *B. subtilis* (*rec*, *pol*, *uvr*) can be classified into three groups, according to their HCR efficiency. Mutants *recB*, *recG*, and *recH* are indistinguishable from the parental strain, mutants *recA*, *recD*, *recF*, and *uvr-1* have significantly reduced efficiency, and the *polA* mutant is reduced even more (see also Table 2).

The products of *recA*, *recD*, and *recF* genes turn out to be important only in the HCR of SPP1. The ability of the strains carrying the above-mentioned mutations to perform efficient HCR of phages SPO1, $\phi 29$, and ϕe indicates that the basis of this different repair ability is not at the level of a repair pathway capable of distinguishing between cellular and phage damage; a likely explanation might be that some phages can induce phage-specific repair functions, which cooperate with the cellular enzymes.

Dependence of HCR efficiency for SPP1 on the growth stage of the infected cells. By performing HCR tests with spores and vegetative cells, we have observed that the efficiency of the repair process is strongly dependent upon the growth stage of the cells infected with irradiated phages (Fig. 3). In the first place, the nonirradiated phages show a higher EOP on

TABLE 2. *HCR of different strains of B. subtilis for different phages*

Strain	Relevant marker	Relative HCR values for different phages[a]			
		SPP1	SPO1	Ø29	Øe
GSY228	Parental	1.00	1.00	1.00	1.00
GSY1025	*recA1*	0.65	1.00	1.10	1.14
GSY1028	*recB2*	1.00	1.00	1.06	1.03
GSY1027	*uvr-1*	0.63	0.48	0.55	0.50
PB202	Parental	1.00	1.00	1.00	1.00
PB1641	*recD41*	0.65	0.95	1.07	1.00
PB1633	*recF33*	0.65	1.05	1.06	1.07
PB1639	*recG39*	0.92	1.04	1.00	0.96
PB1642	*polA42*	0.47	0.55	0.67	0.53
SB25	Parental	1.00	1.00	1.00	1.00
PB1663	*recH342*	1.06	1.00	1.00	1.12

[a] Relative HCR efficiency of each strain for phage is expressed as the ratio between the *h* value of the particular strain and that of the parental strain (see Materials and Methods).

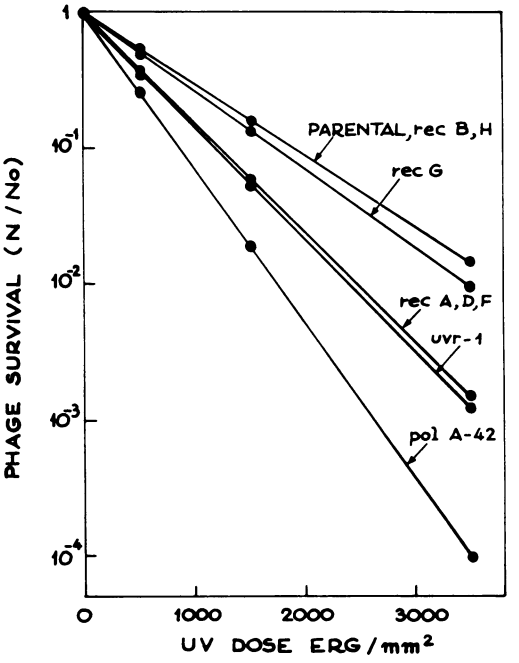

FIG. 2. *Survival of SPP1 phage as a function of UV doses when plated on spores of the bacterial mutants. The curve marked "parental" is common to the three different parental strains used (GSY228, PB202, SB25).*

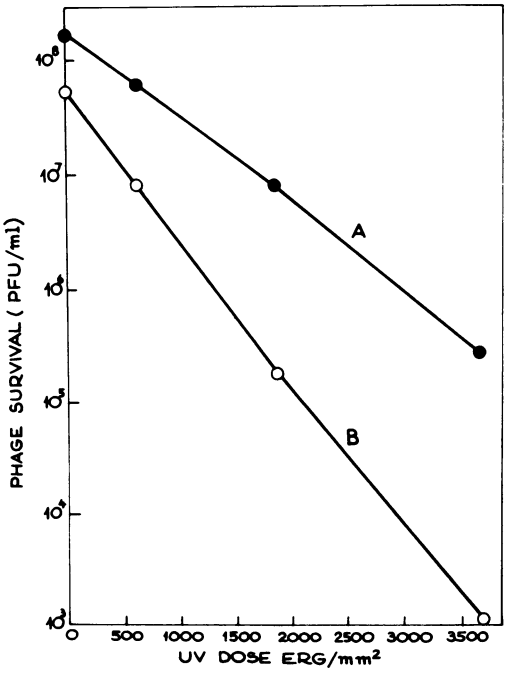

FIG. 3. *Survival of phage SPP1 as a function of UV dose when plated on nonactivated spores (curve A) and exponentially growing cells.*

spores than on vegetative cells, as can also be seen in Fig. 1. Second, the UV inactivation curve obtained on vegetative cells is much steeper than that obtained on spores. Similar results were obtained with bacterial strains PB25, PB202, and GSY228, i.e., with all the parental strains used (Table 2). The HCR efficiency of vegetative cells can be expressed as a function of that of the spore (by dividing its inactivation constant by that of the spores). This turns out to be approximately 0.6.

Variation of the HCR efficiency for SPP1 during outgrowth. These observations prompted us to study more closely the variations in HCR efficiency during spore germination and outgrowth (Fig. 4). At low doses of irradiation (900 ergs/mm^2; a survival of 10%), the curve obtained maintains the same pattern as the control (nonirradiated phage) curve, and the relative HCR proficiency is thus constant throughout the spore outgrowth process. The conclusion is that the host cellular repair systems acts upon this level of UV damage with the same efficiency throughout spore germination and outgrowth. A different pattern is obtained at higher doses of UV (2,200 to 3,600 ergs/mm^2). Both curves represent two conditions, before and after 120 min of germination and outgrowth; after 120 min from spore activation, both curves run parallel to the control and 900-erg curves, but until 120 min they both show a higher HCR efficiency, with peaks at 0 and 60 min. The observed behavior in HCR variation during outgrowth for the higher UV doses of irradiation could be interpreted as the result of a spore repair system that is more efficient in the repair of the kind of damage that becomes prevalent at the higher UV doses.

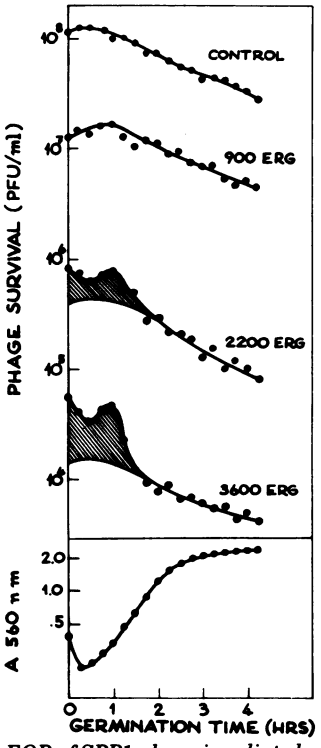

FIG. 4. *EOP of SPP1 phage irradiated at different UV doses (control; 900, 2,200, and 3,600 ergs/mm^2). PB202 heat-activated spores, germinating and outgrowing in NBG medium, were used at the time marked on the figure as indicators for plating samples. The lower panel shows the variation of absorbance (A_{560}) of the culture. The shaded areas are attributed to increased HCR efficiency by comparison of the curve shape with that for unirradiated phage.*

Involvement of DNA polymerase I in spore repair. The survival values observed at early germination times for high UV doses are relatively higher (as shown in Fig. 4) than at later germination times; this is in agreement with the different slopes of the curves of Fig. 3, since the difference in survival between spores and vegetative cells increases with the UV dose. This situation offers a simple test to identify host functions involved in the spore repair system, since a mutant defective at some stage of the process should give parallel survival curves with spores and vegetative cells. This result is obtained with the *polA* mutant, whereas all other tested mutants (*recA*, *recD*, *recF*, and *uvr-1*) show divergent curves similar to those shown in Fig. 3 for the parental strain. Figure 5 shows a kinetic analysis of HCR (3,000 ergs) during spore outgrowth of the *polA* and *recF33* mutants and their parental strain. As expected from the above-mentioned results, the *recF* strain shows the contribution of spore repair to phage recovery.

The specific activity of DNA polymerase I in the spores is similar to that of vegetative cells (9); it is thus improbable that higher levels of HCR efficiency at early stages of outgrowth are due to higher levels of this activity.

DISCUSSION

We have evaluated phage and host contributions to the recovery from UV irradiation for four phages of *B. subtilis*. *uvr-1* and *polA* products are involved in the repair of all the tested phages, and thus they are probably key functions always required and not replaceable by phage information. SPO1 is known to code for a new DNA polymerase (39, 40). Our data suggest that the specific phage polymerase induced after infection cannot replace the host DNA

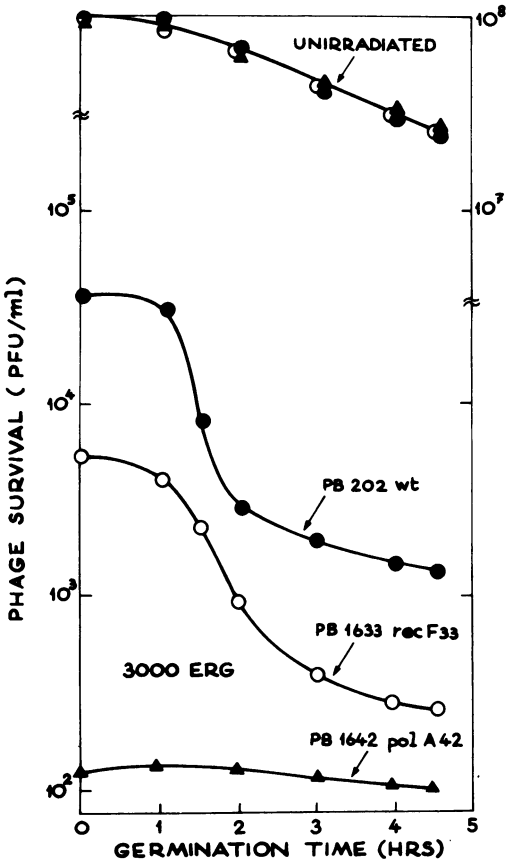

FIG. 5. *EOP of SPP1 phage irradiated at 3,000 ergs/mm² and tested on outgrowing spores of mutants strains.*

polymerase I in the repair process. Among the phages tested, SPP1 turns out to be the one that depends upon the greatest number of host functions (*recA*, *recD*, *recF*, *polA*, and *uvr-1*). This phage has been used to test the variation of HCR efficiency during spore outgrowth.

Spores and vegetative cells exhibit a significant difference in HCR efficiency, which parallels the finding of Tyrrell et al. (35) that the physiological state of the cell can influence repair proficiency in *Escherichia coli* cells. At low UV doses, the same relative HCR efficiency for SPP1 is maintained throughout spore outgrowth and vegetative growth. At high UV doses (2,200 to 3,600 ergs/mm²), the process is much more efficient during the first 2 h of outgrowth than with cells at later stages of vegetative growth. Apparently, a spore repair system, which becomes more frequently utilized at higher UV doses, is active during outgrowth, with two peaks of maximal activity (at 0 and 60 min). Such a system depends upon DNA polymerase I and is independent of the other repair functions tested (*recA*, *recD*, *recF*, and *uvr-1*). Van Wang and Rupert (36) have arrived at similar conclusions regarding a repair system acting during spore outgrowth ("germination excision repair").

The kind of UV damage most studied is the pyrimidine dimer, but besides this a variety of photochemical effects on DNA has been observed, including local denaturation, thymine derivatives of the dihydro-dyhydroxy type, chain breakage, and intermolecular and intramolecular cross-linkage (for a review see 6). Several types of damage are observed only at relatively high UV doses. Evidence has been presented to support the hypothesis that, whereas the dimers play a significant role in the effect of UV irradiation on bacterial cells, they may be of less importance for bacteriophages (25) and spores (38); in both cases, the DNA is in a relatively dry state. The concentration of thymine dimers formed after UV irradiation of spores (which are more UV resistant than vegetative cells) is one-tenth that formed in DNA irradiated in aqueous solution (4, 5), and kinetic studies on the repair of UV-induced damage during spore outgrowth have shown that it occurs through two independent repair systems, namely, "spore repair" and germinative excision repair (17, 36).

Single-stranded breaks produced in DNA of *B. subtilis* spores by ionizing radiation can be repaired during postirradiation germination in the absence of replicative DNA synthesis and also in the absence of protein synthesis, suggesting that this repair is performed by spore enzymes (33, 34).

The increase in HCR efficiency during the early stages of germination, observed by us at high doses of UV irradiation of the bacteriophage, may thus be the result of a spore repair system that is more active in the repair of damage produced by high doses.

The spore repair system might involve spore-specific enzymes or a different regulation of DNA metabolism during outgrowth, or both. One can thus expect to observe different profiles of nonreplicative synthesis during the early outgrowth phase compared with those at later stages, after the beginning of replication. In the experimental conditions used for testing HCR in outgrowing spores, replicative DNA synthesis starts at 30 min and is sensitive to DNA replication inhibitors (31). We have studied DNA synthesis in outgrowing spores by pulse-label incorporation of radioactive precursors in both the presence and absence (during the pulse) of the DNA synthesis inhibitors

$OHPhN_2Ura$ and nalidixic acid, with the aim of evaluating the replicative and nonreplicative fractions of DNA synthesis (data not shown). In vegetative cells, the residual incorporation in the presence of the inhibitors is of the order of 5% of the untreated control. During the first 120 min of spore outgrowth, the pattern of incorporation of the pulse label is somewhat variable in repeated experiments, and the fraction of nonreplicative synthesis is even more variable. Although we have not been able to establish a reproducible pattern of variation of the rate of synthesis during outgrowth, preliminary data from several experiments suggest a continuous decrease of the proportion of nonreplicative DNA synthesis from values higher than 50% down to a value (5%, at 120 min) similar to that found in vegetative cells.

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LITERATURE CITED

1. **Anderson, D. L., D. D. Hickman, and B. E. Reilly.** 1966. Structure of *Bacillus subtilis* bacteriophage $\phi 29$ and the length of $\phi 29$ deoxyribonucleic acid. J. Bacteriol. 91:2081-2089.
2. **Biswal, N., A. K. Kleinschmidt, H. C. Spatz, and T. A. Trautner.** 1967. Physical properties of the DNA of bacteriophage SP 50. Mol. Gen. Genet. 100:39-55.
3. **Boyce, R. P., and P. Howard-Flanders.** 1964. Release of ultraviolet light-induced thymine dimers from DNA in *E. coli* K12. Proc. Natl. Acad. Sci. U.S.A. 51:293-300.
4. **Donnellan, J., Jr., and R. Setlow.** 1965. Thymine photoproducts but not thymine dimers found in ultraviolet-irradiated bacterial spores. Science 149:308-310.
5. **Donnellan, J., and R. Stafford.** 1968. The ultraviolet photochemistry and photobiology of vegetative cells and spores of *Bacillus megaterium*. Biophys. J. 8:17-28.
6. **Doudney, C. O.** 1968. Ultraviolet light effects on the bacterial cell. Cur. Top. Microbiol. Immunol. 46:116-175.
7. **Elder, R. L., and R. E. Beers, Jr.** 1965. Nonphotoreactivating repair of ultraviolet light-damaged transforming deoxyribonucleic acid by *Micrococcus lysodeikticus* extracts. J. Bacteriol. 90:681-686.
8. **Ellison, S. A., R. R. Feiner, and R. F. Hill.** 1960. A host effect on bacteriophage survival after ultraviolet irradiation. Virology 11:294-296.
9. **Falaschi, A., and A. Kornberg.** 1966. Biochemical studies of bacterial sporulation. II. Deoxyribonucleic acid polymerase in spores of *Bacillus subtilis*. J. Biol. Chem. 241:1478-1482.
10. **Ganesan, A. T., C. O. Yehle, and C. C. Yu.** 1973. DNA replication in a polymerase I deficient mutant and the identification of DNA polymerase II and III in *Bacillus subtilis*. Biochem. Biophys. Res. Commun. 50:155-163.
11. **Gass, K. B., T. C. Hill, M. Goulian, B. S. Strauss, and N. R. Cozzarelli.** 1971. Altered deoxyribonucleic acid polymerase activity in a methyl-methan sulfonate-sensitive mutant of *Bacillus subtilis*. J. Bacteriol. 108:364-374.
12. **Harm, W.** 1963. On the relationship between host-cell reactivation and UV reactivation in UV-inactivated phages. Z. Vererbungsl. 94:67-79.
13. **Hoch, J. A., and C. Anagnostopoulos.** 1970. Chromosomal location and properties of radiation sensitivity mutations in *Bacillus subtilis*. J. Bacteriol. 103:295-301.
14. **Howard-Flanders, P., R. P. Boyce, E. Simson, and L. Theriot.** 1962. A genetic locus in *E. coli* K12 that controls the reactivation of UV-photoproducts associated with thymine in DNA. Proc. Natl. Acad. Sci. U.S.A. 48:2109-2115.
15. **Laipis, P., and A. T. Ganesan.** 1972. A deoxyribonucleic acid polymerase I-deficient mutant of *Bacillus subtilis*. J. Biol. Chem. 247:5867-5871.
16. **Mazza, G., A. Fortunato, E. Ferrari, U. Canosi, A. Falaschi, and M. Polsinelli.** 1975. Genetic and enzymic studies on the recombination process in *Bacillus subtilis*. Mol. Gen. Genet. 136:9-30.
17. **Munakata, N., and C. S. Rupert.** 1974. Dark repair of DNA containing "spore photoproduct" in *Bacillus subtilis*. Mol. Gen. Genet. 130:239-250.
18. **Munakata, N., and C. S. Rupert.** 1975. Effects of DNA-polymerase-defective and recombination-deficient mutations on the ultraviolet sensitivity of *Bacillus subtilis* spores. Mutat. Res. 27:157-169.
19. **Okubo, S., and W. R. Romig.** 1965. Comparison of ultraviolet sensitivity of *Bacillus subtilis* bacteriophage SPO2 and its infectious DNA. J. Mol. Biol. 14:130-142.
20. **Okubo, S., and W. R. Romig.** 1966. Impaired transformability of *Bacillus subtilis* mutant sensitive to mitomycin C and ultraviolet radiation. J. Mol. Biol. 15:440-454.
21. **Okubo, S., B. Strauss, and M. Stodolsky.** 1964. The possible role of recombination in the infection of competent *Bacillus subtilis* by bacteriophage deoxyribonucleic acid. Virology 24:552-562.
22. **Pettijohn, D., and P. Hanawalt.** 1964. Evidence for repair-replication of ultraviolet damaged DNA in bacteria. J. Mol. Biol. 9:395-410.
23. **Polsinelli, M., G. Mazza, U. Canosi, and A. Falaschi.** 1973. Genetical and biochemical characterization of *Bacillus subtilis* mutants altered in transformation, p. 27-44. *In* L. Archer (ed.), Bacterial transformation. Academic Press Inc., London.
24. **Reiter, H., and B. Strauss.** 1965. Repair of damage induced by a monofunctional alkylating agent in a transformable ultraviolet-sensitive strain of *Bacillus subtilis*. J. Mol. Biol. 14:179-194.
25. **Riklis, E.** 1965. Studies on mechanism of repair of ultraviolet-irradiated viral and bacterial DNA *in vivo* and *in vitro*. Can. J. Biochem. 43:1207-1217.
26. **Riva, S., M. Polsinelli, and A. Falaschi.** 1968. A new phage of *Bacillus subtilis* with infectious DNA having separable strands. J. Mol. Biol. 35:347-356.
27. **Rörsch, A., A. Edelman, and J. A. Cohen.** 1963. The gene-controlled radiation sensitivity in *Escherichia coli*. Biochim. Biophys. Acta 68:263-270.
28. **Rörsch, A., C. V. D. Kamp, and J. Adema.** 1964. Dark reactivation of ultraviolet irradiated bacteriophage deoxyribonucleic acid *in vitro*. Biochim. Biophys. Acta 80:346-348.
29. **Sauerbier, W.** 1962. Evidence for a nonrecombinational mechanism of host cell reactivation of phage. Virology 16:398-404.
30. **Setlow, R. B., and W. L. Carrier.** 1964. The disappearance of thymine dimers from DNA: an error-correcting mechanism. Proc. Natl. Acad. Sci. U.S.A. 51:226-231.
31. **Siccardi, A. G., A. Galizzi, G. Mazza, A. Clivio, and A. M. Albertini.** 1975. Synchronous germination and outgrowth of fractionated *Bacillus subtilis* spores:

tool for the analysis of differentiation and division of bacterial cells. J. Bacteriol. 121:13–19.
32. Sonenshein, A. L., and D. H. Roscoe. 1969. The course of phage φe infection in sporulating cells of *Bacillus subtilis* strain 3610, Virology 39:265–276.
33. Terano, H., H. Tanooka, and H. Kadota. 1969. Germination-induced repair of single-strand breaks of DNA in irradiated *Bacillus subtilis* spores. Biochem. Biophys. Res. Commun. 37:66–71.
34. Terano, H., H. Tanooka, and H. Kadota. 1971. Repair of radiation damage to deoxyribonucleic acid in germinating spores of *Bacillus subtilis*. J. Bacteriol. 106:925–930.
35. Tyrrell, R. M., S. H. Moss, and D. J. G. Davies. 1972. The variation in UV sensitivity of four K12 strains of *Escherichia coli* as a function of their stage of growth. Mutat. Res. 16:1–12.
36. Van Wang, T., and C. S. Rupert. 1977. Transitory germinative excision repair in *Bacillus subtilis*. J. Bacteriol. 129:1313–1319.
37. Villani, G., U. Canosi, A. Fortunato, G. Mazza, M. Polsinelli, and A. Falaschi. 1974. Properties of a *Bacillus subtilis* strain lacking DNA polymerase I. Nucleic Acids Res. 1:461–477.
38. Zamenhof, S., and T. K. R. Reddy. 1967. Induction of mutations by ultraviolet irradiation of spores of *Bacillus subtilis*. Radiat. Res. 31:112–120.
39. Yehle, C. O., and A. T. Ganesan. 1972. Deoxyribonucleic acid synthesis in bacteriophage SPO1-infected *Bacillus subtilis*. I. Bacteriophage deoxyribonucleic acid synthesis and fate of host deoxyribonucleic acid in normal and polymerase-deficient strains. J. Virol. 9:263–272.
40. Yehle, C. O., and A. T. Ganesan. 1973. Deoxyribonucleic acid synthesis in bacteriophage SPO1-infected *Bacillus subtilis*. II. Purification and catalytic properties of a deoxyribonucleic acid polymerase induced after infection. J. Biol. Chem. 248:7456–7463.