JOURNAL OF BACTERIOLOGY, June 1988, p. 2858–2859
0021-9193/88/062858-02$02.00/0

Vol. 170, No. 6

Absence of Transient Elevated UV Resistance during Germination of *Bacillus subtilis* Spores Lacking Small, Acid-Soluble Spore Proteins α and β

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Received 14 December 1987/Accepted 8 March 1988

Germinating spores of *Bacillus subtilis* mutants which lack small, acid-soluble spore proteins α and β did not exhibit the transient elevated UV resistance seen during germination of wild-type spores.

Dormant spores of various *Bacillus* species are much more resistant to UV irradiation than are the corresponding vegetative cells (2). This elevated spore UV resistance appears to have two causes. First, UV irradiation of spores does not produce the pyrimidine dimers formed in vegetative-cell DNA, but rather produces several other photoproducts, the most predominant of which is termed the spore photoproduct, a 5-thyminyl-5,6-dihydrothymine adduct (1, 10). Second, spores have at least two mechanisms which efficiently repair this spore photoproduct during spore germination, including one which monomerizes the adduct back to two thymines (7, 12).

Surprisingly, when spores germinate they go through a transient stage of extremely high UV resistance, much higher than that of the dormant spore (3, 9, 13). This stage lasts only 1 to 6 min for individual spores (4). In spores of *B. megaterium* and *B. cereus*, this period of high UV resistance is correlated with a low efficiency of formation of spore photoproducts as well as of pyrimidine dimers in DNA (9, 13). It was suggested that this was due to a transient change in DNA structure or environment which rendered the DNA photochemically unreactive (9). In contrast to these results, the transient UV resistance of germinating *B. subtilis* spores was suggested to be due to a highly active germination repair system which is specific for pyrimidine dimers (11). However, detailed kinetic analysis of changes in UV resistance during *B. subtilis* spore germination suggested that the UV-resistant stage in this organism was also accompanied by a period of diminished photoreactivity of spore DNA (3, 4).

Recently, it has been shown that spores of a *B. subtilis* mutant which lacks two closely related major small, acid-soluble spore proteins (SASP), termed SASP-α and -β, are actually more UV sensitive than are vegetative cells of this mutant. However, the vegetative cells of the mutant (termed $\alpha^-\beta^-$) have UV resistance identical to that of wild-type cells. Spores of a mutant lacking only SASP-α (termed α^-) exhibit intermediate UV resistance (5). Strikingly, UV irradiation of $\alpha^-\beta^-$ spores generates significant levels of pyrimidine dimers in DNA, suggesting that SASP-α and -β are somehow involved in the change in spore DNA which alters its UV photochemistry in vivo (8). Since formation of pyrimidine dimers in dormant spore DNA was accompanied by increased UV sensitivity, this suggested that the dimers were not efficiently repaired by a germination-specific repair mechanism which caused the transient UV resistance during spore germination. If efficient repair of dimers formed in dormant spores did take place, then the $\alpha^-\beta^-$ spores would have been UV resistant, not UV sensitive. This further led to the prediction that the $\alpha^-\beta^-$ spores would not show a transient increase in UV resistance upon spore germination.

To test this prediction, spores of *B. subtilis* 168 and its isogenic α^- and $\alpha^-\beta^-$ derivatives were prepared as described previously (5). Spores were heat shocked in water for 15 min at 70°C. After cooling on ice, spores were germinated at an A_{600} of ca. 0.75 in 28 mM glucose–5.6 mM L-alanine–20 mM $NaPO_4$ (pH 7.2) at 37°C. At various times, samples were diluted 75-fold in cold 0.15 M NaCl–20 mM $NaPO_4$ (pH 7.2), irradiated as previously described (5) for 30 s with a dose of 390 J/m^2 (wild-type and α^- spores) or 76 J/m^2 ($\alpha^-\beta^-$ spores), and plated as previously described (5) (Fig. 1). The initiation of spore germination was also measured by following the fall in A_{600} of the spore suspension (Fig. 1, inset). While most spores had initiated germination by 20 min, some spores were still initiating germination between 20 and 60 min, as was seen by a decrease in the percentage of phase-bright spores in the phase-contrast microscope (data not shown). However, by 60 min ca. 99% of the spores had initiated germination.

As found by others (4, 9, 13), germinating wild-type spores showed a transient elevated UV resistance (Fig. 1). The slow decline in elevated resistance was probably due to the slow germination of a small percentage of the spores. Strikingly, $\alpha^-\beta^-$ spores exhibited no transient elevated UV resistance but only showed the small increase in resistance seen in going from the $\alpha^-\beta^-$ dormant spore to the vegetative cell (5) (Fig. 1; note different doses used). α^- spores showed an extremely small transient increase in UV resistance. However, this minute increase may be caused by the small percentage of α^- dormant spores which have wild-type UV resistance (data not shown), possibly due to high levels of SASP-β, since there is a threshold level of SASP-α plus -β which is needed for significant spore UV resistance (6).

The fact that $\alpha^-\beta^-$ spores did not show a transient increase in UV resistance upon germination is consistent with this phenomenon in wild-type spores being due to a transient change in spore DNA structure or environment, from "spore type" to "cell type," which renders the DNA photochemically unreactive. This further suggests that this change does not take place in $\alpha^-\beta^-$ spores, since much of the DNA in these spores is already in a cell-type environment or structure as evidenced by the production of pyrimidine dimers upon UV irradiation. This finding is also inconsistent with the transient UV resistance in germination being

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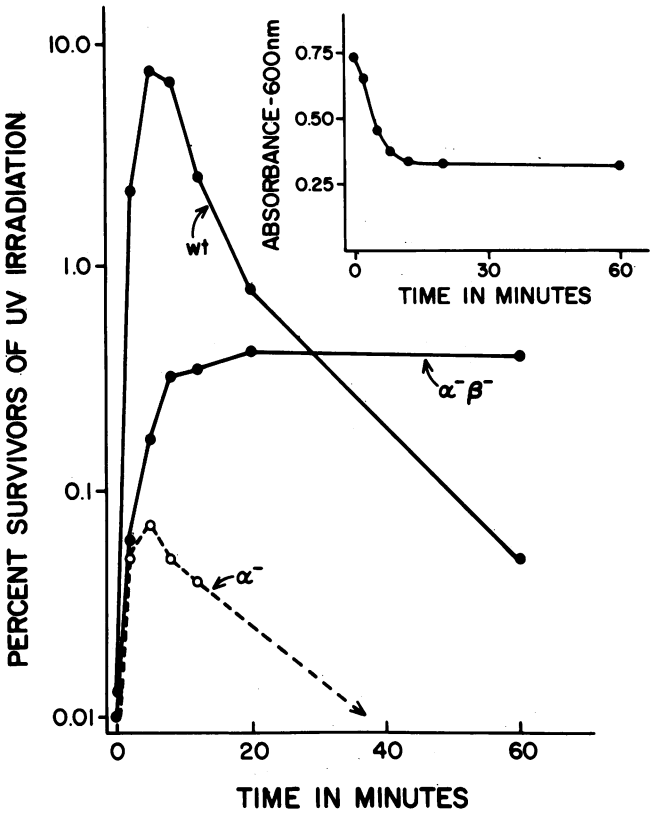

FIG. 1. UV resistance during germination of spores of various *B. subtilis* strains. Details are given in the text. wt, Wild type. The inset gives the change in absorbance of the germinating spores of the $\alpha^-\beta^-$ strain; wild-type and α^- spores gave essentially the same curve (data not shown).

due to a highly efficient germination-specific repair system. This work of course does not indicate that such a germination-specific repair system does not exist, but only that it is not the reason for the transient UV resistance during spore germination, unless it is argued that only DNA to which SASP-α and -β are bound is a substrate for this repair process. Even then, the lack of significant UV resistance of germinating SASP-α^- spores argues against this possibility, since these spores have at least half the wild-type level of SASP-α plus -β (6). These results strongly suggest that SASP-α and -β play a key role in the structure and/or environment of spore DNA in both the dormant and germinating spore. The details of this role are currently under investigation.

This work was supported by a grant from the Army Research Office.

We are grateful for advice and suggestions from Jane K. Setlow and Richard B. Setlow.

LITERATURE CITED

1. **Donnellan, J. E., Jr., and R. B. Setlow.** 1965. Thymine photoproducts but not thymine dimers found in ultraviolet irradiated bacterial spores. Science **149**:308–310.
2. **Gould, G. W.** 1983. Mechanisms of resistance and dormancy, p. 173–209. *In* A. Hurst and G. W. Gould (ed.), The bacterial spore, vol. 2. Academic Press, Inc., New York.
3. **Irie, R., T. Okamoto, and Y. Fujita.** 1982. Estimation of photoproduct formation in germinating spores of *Bacillus subtilis*. Photochem. Photobiol. **35**:783–787.
4. **Irie, R., N. Yano, and H. Kembo.** 1986. Kinetic analysis of the UV-resistant transient stage in spore germination of *Bacillus subtilis*. J. Gen. Appl. Microbiol. **14**:279–293.
5. **Mason, J. M., and P. Setlow.** 1986. Essential role of small, acid-soluble spore proteins in resistance of *Bacillus subtilis* spores to UV light. J. Bacteriol. **167**:174–178.
6. **Mason, J. M., and P. Setlow.** 1987. Different small, acid-soluble proteins of the α/β type have interchangeable roles in the heat and UV radiation resistance of *Bacillus subtilis* spores. J. Bacteriol. **169**:3633–3637.
7. **Munakata, N., and C. S. Rupert.** 1972. Genetically controlled removal of "spore photoproduct" from deoxyribonucleic acid of ultraviolet-irradiated *Bacillus subtilis* spores. J. Bacteriol. **111**:192–198.
8. **Setlow, B., and P. Setlow.** 1987. Thymine-containing dimers as well as spore photoproducts are found in ultraviolet-irradiated *Bacillus subtilis* spores that lack small, acid-soluble proteins. Proc. Natl. Acad. Sci. USA **84**:421–423.
9. **Stafford, R. S., and J. E. Donnellan, Jr.** 1968. Photochemical evidence for conformation changes in DNA during germination of bacterial spores. Proc. Natl. Acad. Sci. USA **59**:822–829.
10. **Varghese, A.** 1970. 5-Thyminyl-5,6-dihydrothymine from DNA irradiated with ultraviolet light. Biochem. Biophys. Res. Commun. **38**:484–490.
11. **Wang, T.-Z. V., and C. S. Rupert.** 1977. Transitory germinative excision repair in *Bacillus subtilis*. J. Bacteriol. **129**:1313–1319.
12. **Wang, T.-Z. V., and C. S. Rupert.** 1977. Evidence for the monomerization of spore photoproduct to two thymines by the light independent "spore repair" process in *Bacillus subtilis*. Photochem. Photobiol. **25**:123–127.
13. **Weinberger, S., Z. Evenchik, and I. Hertman.** 1984. Transitory UV resistance during germination of UV-sensitive spores produced by a mutant of *Bacillus cereus* 569. Photochem. Photobiol. **39**:775–780.