JOURNAL OF BACTERIOLOGY, June 1972, p. 1010-1016

Vol. 110, No. 3
Printed in U.S.A.

Repair of Ultraviolet Light-Induced Damage to the Deoxyribonucleic Acid of *Neurospora crassa*

THOMAS E. WORTHY AND J. L. EPLER

Institute of Radiation Biology, University of Tennessee, Knoxville, Tennessee 37916, and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

Received for publication 17 January 1972

A method is described for labeling a specific pyrimidine in the deoxyribonucleic acid (DNA) of *Neurospora crassa*. In cells grown in the presence of [5-^3H]-uridine, more than 97% of the radioactivity associated with the DNA had been incorporated into cytosine. The specific activity of the labeled DNA was approximately 3×10^3 counts per min per μg. The DNA was isolated by elution from hydroxyapatite columns with sodium phosphate buffer (0.40 M, *p*H 6.8). This procedure was used to demonstrate that in vegetative cells of *N. crassa* both photoreactivation and excision repair are operative, as measured by the removal of ultraviolet light-induced cytosine-containing dimers.

Although presumptive evidence is available, definitive biochemical evidence for the repair of radiation-induced damage to the deoxyribonucleic acid (DNA) of *Neurospora crassa* is lacking. The indirect evidence includes the following: (i) the initial zero slope of the survival curves of irradiated wild-type conidia (8); (ii) an increase in survival when wild-type conidia are incubated after irradiation either in buffer or in minimal medium (liquid holding recovery) (26); and (iii) a loss of photoreactivability upon holding in water after ultraviolet (UV) irradiation (35). Postirradiation treatment with known inhibitors of excision repair, such as acriflavines, changes in the shape of the initial portion of the survival curves (26). Additional evidence comes from the existence of UV-sensitive strains of *N. crassa* (7-9, 25, 31). These UV-sensitive strains generally are characterized by approximately exponential survival curves. Moreover, no increase in survival is seen under postirradiation conditions that lead to increased survival in wild-type conidia (26).

Unequivocal demonstration of excision repair in *N. crassa* has been hampered by two conditions. (i) Pyrimidine auxotrophs will not grow on minimal medium supplemented only with thymidine (12) but will grow on minimal medium supplemented with uracil, uridine, or cytidine (6, 20, 21). (ii) The radioactive label of thymidine is incorporated by *N. crassa* into nucleic acid but not specifically into the DNA (12-14). Thus, the usual experimental design—specifically labeling the DNA with [^{3}H]thymidine and monitoring, by radiochromatographic analysis, the removal of UV-induced thymine dimers—is not feasible.

Grivell and Jackson (15) accounted for the two observations discussed above by demonstrating that *N. crassa* and other fungi contain no detectable thymidine kinase activity (ATP: thymidine 5'-phosphotransferase; EC 2.1.21) and showing that incubation of *N. crassa* with either [2-^{14}C]uridine or [2-^{14}C]thymidine results in incorporation of radioactivity into both ribonucleic acid (RNA) and DNA. Analysis of the two classes of nucleic acid showed radioactivity associated with uracil and cytosine in RNA and with cytosine and thymine in DNA. They postulated that the exogenous radioactive pyrimidines were converted to a common uracil ribonucleotide intermediate, subsequent conversion of this intermediate to the various pyrimidines resulting in the observed distribution of radioactivity.

We have utilized the observation on the interconversion of pyrimidines to develop a procedure for specifically labeling one pyrimidine base, cytosine, in the DNA of *N. crassa*. Further, we have coupled our observations on the distribution of label in DNA to the report by Setlow and Carrier (29) that in DNA in which adenine plus thymine (A + T)/guanine plus cytosine (G + C) = 50% [in *N. crassa* DNA, (A + T)/(G + C) = 52 to 54% (33, 34)], approximately 50% of the pyrimidine dimers contain cytosine. Thus, we have been able to monitor the repair of UV-induced pyrimidine dimers in

N. crassa, and in this paper we report the direct demonstration of excision repair and photoreactivation in vegetative cells of *N. crassa*.

MATERIALS AND METHODS

Organism. The organism used in this study was a unicellular, osmotically sensitive strain of *N. crassa* (*fz;sg;os-1,agr-1,cr,aur:* Fungal Genetics Stock Center no. 326), commonly referred to as the slime mutant. The osmotic lability and unicellular morphology are a consequence of the absence of a cell wall. The strain was a gift from Frank Gaertner.

Growth of slime mutant. *N. crassa* slime mutant was routinely grown on Vogel's minimal medium (37) supplemented with vitamins, 0.05% arginine, 2.0% soluble starch, and 10.0% sucrose. The cells were inoculated into 25-ml flasks containing 10 ml of medium at an initial concentration of approximately 3×10^5 cells per ml and incubated at 30 C in a water bath shaker at approximately 100 rev/min. The cells were maintained either by daily transfer into fresh liquid medium or by periodic transfer on agar slants incubated at room temperature.

Radioactive label. Cultures grown as described were labeled with 7.5 μCi of [5-^3H]uridine/ml (20 Ci/mmole) for 24 hr. In certain experiments, cells were labeled with 5.0 μCi of [6-^3H]uridine/ml (17.0 Ci/mmole). Both radioactive isotopes were purchased from Schwarz BioResearch, Orangeburg, N.Y.

Distribution of radioactivity in RNA and DNA. Cells were grown for 24 hr in the presence of either [5-^3H]uridine or [6-^3H]uridine. The cultures were harvested separately by centrifugation and suspended in 2 ml of sodium phosphate buffer (0.02 M, *p*H 6.8). Nucleic acids were isolated by the hot salt method of Volkin and Astrachan (38), and the radioactive ribonucleotides were separated by ion-exchange chromatography (38). Radioactive DNA was precipitated with 0.2 N HCl. The radiochromatographic analysis of DNA is described below.

Irradiation and postirradiation conditions. Cultures grown for 24 hr and labeled with [5-^3H]uridine were harvested by centrifugation, washed once in sucrose (0.55 M)-sodium phosphate (0.02 M, *p*H 6.8), and resuspended in the same buffer at a concentration of approximately 10^7 cells per ml. A 5-ml sample was placed in a 10-cm diameter crystallizing dish at a thickness of 1 mm. A desk lamp containing two 15-watt germicidal lamps was positioned to give an incident intensity of 47 ergs per mm^2 per sec, as measured by a meter described by Jagger (17). The crystallizing dish was placed on ice, and the sample was irradiated to the appropriate dose with stirring. After the irradiation, the cells were treated as follows. In the dose-response experiments, samples were removed at each dose and centrifuged, and the DNA was isolated as described below. In excision experiments, the irradiated cells were centrifuged and resuspended in 10 ml of fresh growth medium, placed in a sterile 25-ml flask, and incubated in the dark in a water bath shaker at 30 C. At given times, 2.0-ml samples were removed and the DNA was isolated. In the in vivo photoreactivation experiments, the irradiated cells were exposed to illumination from two blacklights (G.E. 15w-BLB, which emits most of its radiation between 300 and 400 nm) with an intensity of 3.2×10^3 ergs per mm^2 per min. At various times, 1.0-ml samples were removed and treated as described below. All manipulations were carried out in dim yellow light to prevent undesired direct photoreactivation.

Assay for photoreactivating enzyme. Separate 18- and 72-hr cultures of unlabeled cells were harvested, washed once with sucrose (0.55 M)-sodium phosphate (0.02 M, *p*H 6.8), and resuspended and lysed in 3 ml of sodium phosphate (0.02 M, *p*H 6.8). The lysates were centrifuged to remove cellular debris, and the extracts were adjusted to equivalent protein concentrations (22). The assay for photoreactivating enzyme was that described by Cook and Worthy (11).

Isolation of DNA. Cells were harvested by centrifugation and resuspended in a lysing solution containing 0.1% duponol, 1.0 M $NaClO_4$, and sodium phosphate (0.02 M, *p*H 6.8). An equal volume of isoamyl alcohol–chloroform (1:24; Sevag solution) was added, and the solution was shaken overnight. The solutions were centrifuged, and the aqueous upper layer was saved. Ribonuclease (2 mg/ml) was added to the aqueous layer to a final concentration of 100 μg/ml, and the samples were incubated at 36 C for 60 min. After ribonuclease treatment, the samples were loaded onto individual 1-ml hydroxyapatite columns that had been prewashed with 2 ml of sodium chloride (0.5 M)-sodium phosphate (0.02 M, *p*H 6.8), followed by 20 ml of sodium phosphate (0.02 M, *p*H 6.8). The loaded columns were washed with 5 ml of the latter buffer, and the residual RNA was removed by elution with 60 ml (60 column volumes) of sodium perchlorate (1.0 M)-sodium phosphate (0.16 M, *p*H 6.8). The DNA was eluted with 6 ml of sodium phosphate (0.40 M, *p*H 6.8). The DNA solutions were heated to 100 C in a water bath for 10 min at neutral *p*H to deaminate the cytosine-containing dimers and then dialyzed against distilled water for 60 min at room temperature. Although the cytosine is converted to uracil, for clarity the dimers will be referred to as cytosine-containing. The DNA species were then precipitated with 10% trichloroacetic acid, with calf thymus DNA (1 mg/ml) as a carrier. The acid-precipitable material was hydrolyzed in 95 to 98% formic acid at 175 C (5) for 60 min, and the formic acid was evaporated at 65 C in an air stream. The hydrolysates were resuspended in 50 to 100 μliters of water, applied to individual strips of Amberlite IRC-50 H^+ paper (1 by 22 inches), and chromatographed for 2 hr in acetic acid (0.1 M, *p*H 4.8) (4). The chromatograms were removed, and the solvent fronts were marked and allowed to dry at room temperature. In this chromatographic system, cytosine has an R_F of 0.13; uracil, 0.60; and photoproducts have an R_F of 0.75.

Preparation of hydroxyapatite. Hydroxyapatite was prepared by the method of Miyazawa and Thomas (23).

Analysis of the chromatograms. The paper strips were marked and cut out from an R_F of 0.10 to

0.25 to include all of the cytosine region, cut into two equivalent pieces, and placed in scintillation vials. Similar cuts were made to include the uracil region (R_F 0.50 to 0.65) and the cytosine-dimer region (R_F 0.65 to 0.80). The samples were placed in scintillation vials; 1 ml of water and 10 ml of dioxane-BBOT (2,5-bis-[2-(5-*tert*-butylbenzoxazoyl)]-thiophene) were added; and the radioactivity was counted in a liquid scintillation counter. The total radioactivity in cytosine was determined by adding the radioactivity in the uracil region to that in the cytosine region.

RESULTS

Labeling of N. crassa DNA: (i) rationale of the labeling procedure. The procedure used for labeling *N. crassa* DNA was based on the observation of Grivell and Jackson (15) that the radioactive label 2-^{14}C on a pyrimidine ring is incorporated into all of the pyrimidines, and that the incorporation occurs via a uracil ribonucleotide intermediate. We hypothesized that, if *Neurospora* is grown in the presence of [5-^3H]uridine, upon conversion of the ribonucleotide intermediate to cytosine, the radioactive label should remain intact; only conversion to thymidine should result in the removal of the 5-^3H label. Conversely, radioactive label ^3H in any other position should be incorporated generally into all of the pyrimidine bases. Our labeling procedure should therefore be highly specific for the incorporation of [5-^3H]uridine into cytosine.

(ii) Distribution of radioactivity in the nucleic acids of N. crassa. Table 1 shows the results of an experiment designed to test our hypothesis. *N. crassa* was grown in the presence of either [5-^3H]uridine or [6-^3H]uridine. The labeled nucleic acids were isolated, and the amounts of radioactivity in the RNA and DNA and the fraction of radioactivity in each pyrimidine base of RNA and DNA were determined. The relative amount of radioactivity incorporated into DNA was the same regardless of the position of the radioactive label and comprised approximately 12% of the total acid-precipitable radioactivity. The distribution of radioactivity in the pyrimidine bases of RNA was the same for both labels: 50% for cytosine and 50% for uracil. These values agree well with those reported by Henney and Storck (16) and Storck (32) for RNA. The distribution of radioactivity between RNA and DNA is in agreement with the values given by Grivell and Jackson (15). When the cells were labeled with [5-^3H]uridine, more than 97% of the radioactivity was associated with cytosine, and only trace amounts were found to be associated with uracil and thymine. On the other hand, labeling with [6-^3H]uridine resulted in a general incorporation of radioactivity into all of the pyrimidines of DNA. The small amount of uracil found in the DNA labeled with either riboside is ascribed to the deamination of cytosine by the formic acid hydrolysis and may vary from 0.5 to 5% (24). The label associated with thymine in DNA labeled with [5-^3H]uridine was probably due to contamination of the isotope by trace amounts of [6-^3H]uridine during synthesis. It is evident from the data in Table 1 that the labeling procedure utilizing [5-^3H]uridine is very specific for the cytosine in DNA. The specific activity of the labeled DNA was approximately 3×10^3 counts per min per μg, based on the absorbance of DNA at 260 nm.

Figure 1 shows a typical radiochromatogram of hydrolyzed DNA labeled with [5-^3H]uridine. Cytosine migrates to an R_F of 0.13, in a sharply defined zone that is well separated from those of uracil and the cytosine-containing dimers. This chromatographic system utilizing ion-exchange paper is superior to systems more routinely used for measurement of cytosine-containing dimers, since the latter

TABLE 1. *Distribution of radioactivity in an acid hydrolysate of N. crassa DNA labeled with* [5-^3H] *uridine or with* [6-^3H]*uridine*

Label	Fraction of radioactivity in DNA		
	Cytosine	Uracil	Thymine
[5-^3H]Uridine	0.973	0.006	0.008
[6-^3H]Uridine	0.435	0.013	0.544

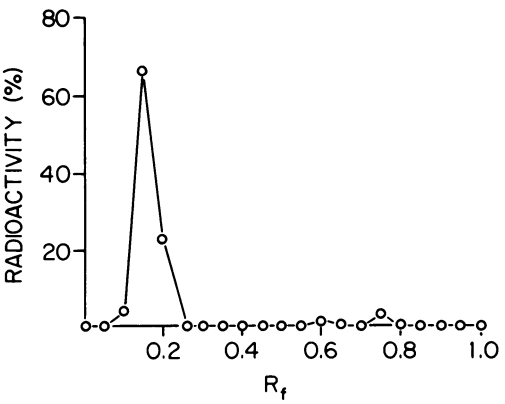

FIG. 1. *Radiochromatogram of Neurospora DNA isolated from cells labeled with* [5-^3H]*uridine. The DNA was hydrolyzed in formic acid and chromatographed on ion-exchange paper. Relevant R_F values: cytosine, 0.13; uracil, 0.60; cytosine-containing dimers, 0.75.*

migrate to a position in front of cytosine. This allows for improved quantitation of the observed photoproducts, since there is no interference from trailing counts from the cytosine zone, as there is in the butanol-acetic acid-water system (29). In addition, this system allows better separation of cytosine and cytosine-containing dimers than is possible with other chromatographic systems.

Separation of RNA and DNA. Because of the large amount of label incorporated into RNA, it was necessary to remove the RNA prior to the analysis of the DNA. Separation of RNA and DNA was based on the behavior of nucleic acids on hydroxyapatite columns, as described by Bernardi (1). We found that the resolution could be increased when the RNA was eluted with sodium phosphate (0.16 M, *p*H 6.8)-sodium perchlorate (1.0 M) (D. E. Graham, *personal communication*). Figure 2 shows a typical elution profile from a 1-ml hydroxyapatite column and indicates that elution with approximately 60 column volumes of buffer removes all the RNA from the column. To check the purity of the eluted DNA, a sample of the material eluted with sodium phosphate buffer (0.4 M) was precipitated with acid, hydrolyzed, and chromatographed. Analysis of such a chromatogram indicated that the DNA was free from contaminating RNA (Fig. 1). The absence of any appreciable radioactivity in the uracil region (R_F = 0.60) attests to the complete removal of the RNA. In addition, control experiments were performed that indicated the source of the uracil found in the chromatograms to be resistant to both ribonuclease and alkali; and the amount of label in the uracil region was about constant when cytosine was eluted, rehydrolyzed, and rechromatographed. These experiments all point to the deamination of cytosine as the source of the observed uracil, and not contamination with residual RNA.

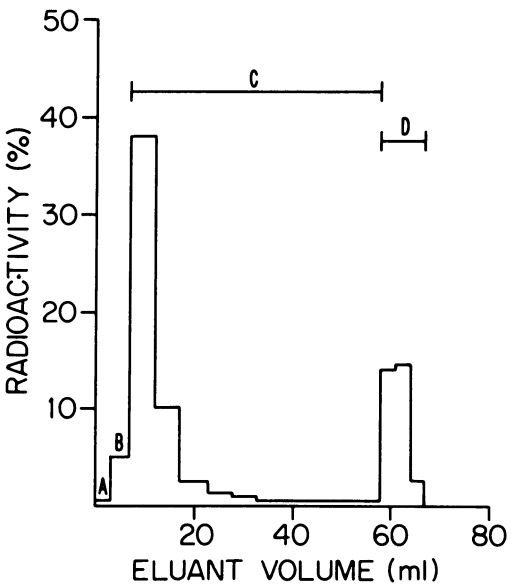

FIG. 2. *Histogram of the elution profile of separation of RNA and DNA with hydroxyapatite columns. Samples of known volumes were collected and counted, and the total counts per minute per fraction were calculated. Elution buffers (pH 6.8): (A) loading eluate; (B) 0.02 M sodium phosphate, 5 ml; (C) 0.16 sodium phosphate-1.0 M sodium perchlorate, 60 ml; (D) 0.40 M sodium phosphate, 6 ml.*

In vivo dose-response curve. Figure 3 shows the production of cytosine-containing dimers as a function of dose. The rate of formation of dimers is linear with increasing dose of 254-nm radiation. Setlow and Carrier (29) have shown that, in *Escherichia coli* DNA [(A + T)/(G + C) = 50%] irradiated at 280 nm, the rate of formation of cytosine-containing dimers is linear to 10^4 ergs/mm^2, beyond which the curve becomes nonlinear. The nonlinearity above 10^4 ergs/mm^2 is due to the saturation of the overall reaction as a result of the relatively high absorption coefficient of cytosine dimers (28).

Photoreactivation. Terry and Setlow (36) have demonstrated the presence of photoreactivating enzyme in conidial extracts of *N. crassa*, but the presence of the enzyme has not been reported in vegetative cells. Two separate criteria have been used to measure photoreactivating enzyme in *Neurospora*. Extracts have been assayed for enzyme activity, as measured by the ability of the extracts to monomerize acetophenone-photosensitized *E. coli* DNA; and the monomerization of cytosine-con-

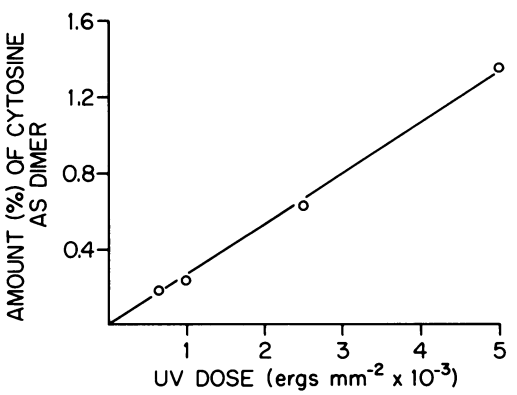

FIG. 3. *Production of cytosine-containing dimers in vivo as a function of irradiation at 254 nm.*

taining dimers by intact cells has also been monitored.

In vivo photoreactivation. Figure 4 shows the monomerization of cytosine-containing dimers as a function of time of exposure to visible light of effective photoreactivating wavelengths. Such exposure results in an approximately first-order decrease for the first 20 min, after which the number of dimers monomerized by the enzyme does not increase appreciably.

In vitro photoreactivation. Extracts of vegetative cells from *Neurospora* were assayed for their ability to photoreactivate thymine-containing dimers in acetophenone-photosensitized *E. coli* DNA under appropriate conditions. Figure 5A shows the kinetics of photoreactivating enzyme from *Neurospora* in this assay. The reaction is characterized initially by a steep slope, which breaks at about 20% (based on extrapolation to the ordinate); the rate subsequently slows by at least a factor of two. This curve is characteristic of the assay system and appears to be due to the inaccessibility of some of the dimers (11).

Boling and Setlow (2) observed that in *Saccharomyces cerevisiae*, a unicellular ascomycete, the activity of photoreactivating enzyme varied with the phase of growth. No activity could be demonstrated in log-phase cells; enzyme activity was not observed until the end of the log phase and reached a maximum in stationary phase. To see whether this observation was true for another ascomycete, *N. crassa* cultures were prepared in log and stationary phases, and extracts were made, adjusted to equivalent protein concentrations and assayed (Fig. 5*B*). There was no demonstrable difference in activity between log-phase and stationary-phase cells. It appears that the enzyme is active throughout the cell growth cycle of *N. crassa*, and that the observation in yeast (2) may be a unique function of its unicellular life cycle.

Excision. The disappearance of cytosine-containing dimers in acid-precipitable DNA from cells incubated in the dark in growth medium is shown in Fig. 6. There is a rapid decrease for the first 60 min, after which the excision process slows down. After 240 min, almost 96% of the cytosine-containing dimers have been excised.

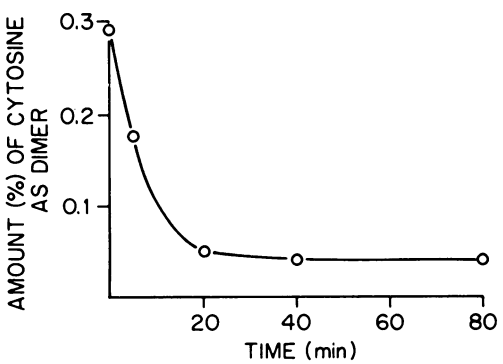

FIG. 4. *In vivo photoreactivation of vegetative cells of Neurospora. Cells were grown and labeled as usual, and irradiated as a thin suspension with 10^3 ergs/mm^2 at 254 nm. The irradiated cells were exposed to blacklights at an intensity of 3.2×10^3 ergs per mm^2 per min at room temperature for various periods of time.*

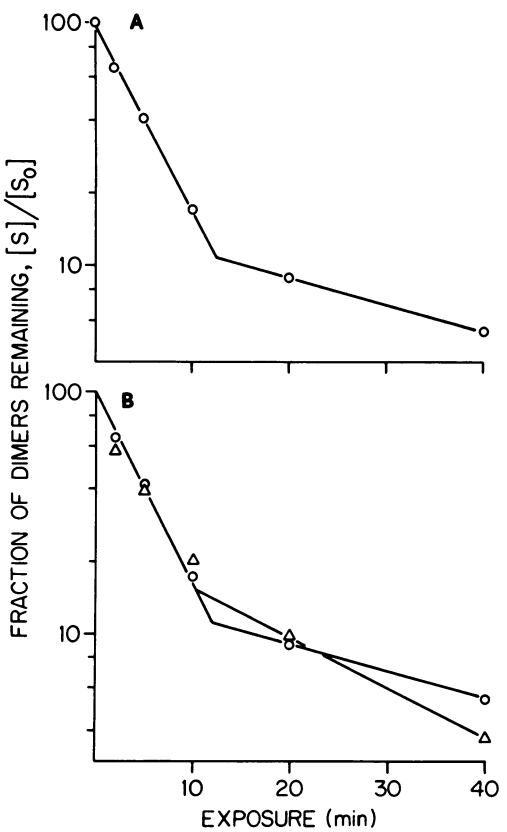

FIG. 5. *Assay for photoreactivating enzyme activity in extracts of Neurospora. Vegetative cells were grown for either 18 to 20 or 72 hr, and extracts were prepared and adjusted to equivalent protein concentrations. Samples of the extracts were assayed for their ability to monomerize thymine dimers in E. coli DNA that had been photosensitized with acetophenone. Samples were removed at various times, and the DNA was hydrolyzed, chromatographed, and analyzed for the fraction of thymine dimers remaining. Symbols: 18- to 20-hr culture, O; 72-hr culture, Δ.*

Preliminary experiments indicated that the irradiated cells must be incubated in growth medium in order for maximum excision to take place. Incubation in 0.55 M sucrose–0.02 M sodium phosphate after UV irradiation resulted in a decreased amount of excision. Excision was characterized by a lag of 30 min prior to any observable decrease in dimers, and after 120 min only 33% of the dimers had been removed. The decreased excision in sucrose buffer is unexplained at this stage.

Neither the fate of the excised dimers nor the size of the excised region has been investigated. Experiments designed to follow the appearance of the dimers in either the acid-soluble material or the medium were not attempted at this time because of the large background of cytosine from cellular RNA. However, experiments investigating the size of the excised region are in progress.

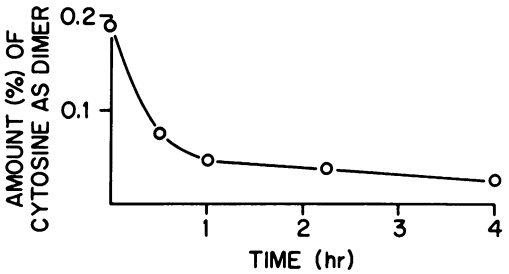

FIG. 6. *In vivo excision of cytosine-containing dimers by vegetative cells of Neurospora. Vegetative cells were grown and labeled as usual and irradiated with 650 ergs/mm² at 254 nm. The irradiated cells were suspended in fresh growth medium and incubated at 30 C in the dark, with shaking, and samples were removed at various times.*

DISCUSSION

The excision repair system first described by Setlow and Carrier (27) and Boyce and Howard-Flanders (3) in bacteria has been implicated previously in the repair of chemically induced damage to DNA (18, 19). Evidence is available indicating that xeroderma pigmentosum, a human genetic disease characterized by extreme sensitivity to UV light and a resultant high incidence of skin cancer, may have its etiology in a nonfunctional UV-specific endonuclease (10, 30) that normally carries out the putative initial step in the excision repair system.

Thus, because of the apparent general ability of the excision repair system to recognize damage to DNA and its central role in the maintenance of cellular integrity, it is of interest to characterize the system as fully as possible in eukaryotes. Any characterization must of necessity include a study of the genetics of the repair system. Unfortunately, no suitable eukaryotic system has been available for such a study. Eukaryotic systems in which studies of repair phenomena are possible lack the required genetic facilities. Conversely, studies of systems with the prerequisite genetic facilities, such as fungi, have been restricted to indirect measurement of repair because of the inability to measure repair directly and to assign relative importance to the various repair systems.

The finding that the DNA of organisms deficient in thymidine kinase can be labeled by growth on [5-^3H]uridine now makes *N. crassa* a system of choice for studies of the genetics of repair in eukaryotes. More generally, this system may be useful in other studies concerned with various aspects of DNA function and metabolism in fungi.

ACKNOWLEDGMENTS

This investigation was sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

We wish to thank C. G. Mead, M. A. Resnick, and M. C. Paterson for helpful advice and stimulating discussions, and R. B. Setlow and F. J. de Serres for their critical review of the manuscript. Further, we thank J. S. Cook for the use of the equipment and materials required for the photoreactivating enzyme assay.

One of us (T.E.W.) is a Laboratory Graduate Participant from the University of Tennessee under appointment from the Oak Ridge Associated Universities.

LITERATURE CITED

1. Bernardi, G. 1971. Chromatography of nucleic acid on hydroxyapatite columns, p. 95–139. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 21: part D. Academic Press Inc., New York.
2. Boling, M. E., and J. K. Setlow. 1967. Photoreactivating enzyme in logarithmic-phase and stationary phase yeast cells. Biochim. Biophys. Acta. **145**:502–505.
3. Boyce, R. P., and P. Howard-Flanders. 1964. Release of ultraviolet light-induced thymine dimers from DNA in *E. coli* K-12. Proc. Nat. Acad. Sci. U.S.A. **51**:293–300.
4. Brown, R. D., and C. E. Holt. 1967. Rapid separation of thymine dimer and pyrimidines on ion-exchange paper. Anal. Biochem. **20**:358–360.
5. Carrier, W. L., and R. B. Setlow. 1971. The excision of pyrimidine dimers (the detection of dimers in small amounts), p. 230–237. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol 21: part D. Academic Press Inc., New York.
6. Chakraborty, K. P., and H. S. Loring. 1960. Incorporation of uracil-2-^{14}C into the nucleic acids of *Neurospora crassa*. J. Biol. Chem. **235**:2122–2126.
7. Chang, L. T., J. E. Lennox, and R. W. Tuveson. 1968. Induced mutation in UV-sensitive mutants of *Aspergillus nidulans* and *Neurospora crassa*. Mutat. Res. **5**: 217–224.
8. Chang, L. T., and R. W. Tuveson. 1967. Ultraviolet-sensitive mutants in *Neurospora crassa*. Genetics **56**:801–

810.
9. Chang, L. T., R. W. Tuveson, and M. H. Munroe. 1968. Non-nuclear inheritance of UV-sensitivity in *Neurospora crassa*. Can. J. Genet. Cytol. **10**:920-927.
10. Cleaver, J. E. 1969. Xeroderma pigmentosum: a human disease in which an initial stage of DNA repair is defective. Proc. Nat. Acad. Sci. U.S.A. **63**:428-435.
11. Cook, J. S., and T. E. Worthy. 1972. Photoreactivating enzyme: a new quantitative assay and estimation of molecular weight of the yeast enzyme. Biochemistry. **11**:388-393.
12. Fink, R. M., and K. Fink. 1961. Biosynthesis of radioactive RNA and DNA pyrimidines from thymidine-2-^{14}C. Biochem. Biophys. Res. Commun. **6**:7-10.
13. Fink, R. M., and K. Fink. 1962. Utilization of radiocarbon from thymidine and other precursors of ribonucleic acid in *Neurospora crassa*. J. Biol. Chem. **237**: 2289-2290.
14. Fink, R. M., and K. Fink. 1962. Relative retention of ^{3}H and ^{14}C labels of nucleosides incorporated into nucleic acids of *Neurospora crassa*. J. Biol. Chem. **237**:2889-2891.
15. Grivell, A. R., and J. F. Jackson. 1968. Thymidine kinase: evidence for its absence from *Neurospora crassa* and some other micro-organisms, and the relevance of this to the specific labelling of deoxyribonucleic acid. J. Gen. Microbiol. **54**:307-317.
16. Henney, H., and R. Storck. 1963. Nucleotide composition of ribonucleic acid from *Neurospora crassa*. J. Bacteriol. **85**:822-826.
17. Jagger, J. 1961. A small and inexpensive ultraviolet dose-rate meter useful in biological experiments. Radiat. Res. **14**:394-403.
18. Lawley, P. D., and P. Brookes. 1965. Molecular mechanism of the cytotoxic action of di-functional alkylating agents and of resistance to this action. Nature (London) **206**:480-483.
19. Lawley, P. D., and P. Brookes. 1968. Cytotoxicity of alkylating agents toward sensitive and resistant strains of *Escherichia coli* in relation to extent and mode of alkylation of cellular macromolecules and repair of alkylation lesions in deoxyribonucleic acids. Biochem. J. **109**:433-447.
20. Loring, H. S., M. L. Hammell, W. Levy, and H. W. Bortner. 1952. The chemistry of the isomeric cytidylic acids: enzymatic hydrolysis to cytidine. J. Biol. Chem. **196**:821-828.
21. Loring, H. S., and J. G. Pierce. 1944. Pyrimidine nucleosides and nucleotides as growth factors for mutant strains of *Neurospora*. J. Biol. Chem. **153**:61-69.
22. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
23. Miyazawa, Y., and C. A. Thomas. 1965. Nucleotide composition of short segments of DNA molecules. J. Mol. Biol. **11**:223-237.
24. Schein, A. H. 1966. Uracil in formic acid hydrolysates of deoxyribonucleic acid. Biochem. J. **98**:311-316.
25. Schroeder, A. L. 1970. Ultraviolet-sensitive mutants of *Neurospora*. I. Genetic basis and effect on recombination. Mol. Gen. Genet. **107**:291-304.
26. Schroeder, A. L. 1970. Ultraviolet-sensitive mutants of *Neurospora*. II. Radiation studies. Mol. Gen. Genet. **107**:305-320.
27. Setlow, R. B., and W. L. Carrier. 1964. The disappearance of thymine dimers from DNA: an error-correcting mechanism. Proc. Nat. Acad. Sci. U.S.A. **51**: 226-231.
28. Setlow, R. B., W. L. Carrier, and F. J. Bollum. 1965. Pyrimidine dimers in UV-irradiated poly dI:dC. Proc. Nat. Acad. Sci. U.S.A. **53**:1111-1118.
29. Setlow, R. B., and W. L. Carrier. 1966. Pyrimidine dimers in ultraviolet-irradiated DNA's. J. Mol. Biol. **17**: 237-254.
30. Setlow, R. B., J. D. Regan, J. German, and W. L. Carrier. 1970. Evidence that xeroderma pigmentosum cells do not perform the first step in the repair of ultraviolet damage to their DNA. Proc. Nat. Acad. Sci. U.S.A. **64**:1035-1041.
31. Stadler, D. R., and D. A. Smith. 1968. A new mutation in *Neurospora* for sensitivity to ultraviolet. Can. J. Genet. Cytol. **10**:916-919.
32. Storck, R. 1965. Nucleotide composition of nucleic acids of fungi. I. Ribonucleic acids. J. Bacteriol. **90**:1260-1264.
33. Stock, R. 1966. Nucleotide composition of nucleic acids or fungi. II. Deoxyribonucleic acids. J. Bacteriol. **91**: 227-230.
34. Storck, R., and C. J. Alexopoulos. 1970. Deoxyribonucleic acid of fungi. Bacteriol. Rev. **34**:126-154.
35. Terry, C. E., B. J. Kilbey, and H. B. Howe. 1967. The nature of photoreactivation in *Neurospora crassa*. Radiat. Res. **30**:739-747.
36. Terry, C. E., and J. K. Setlow. 1967. Photoreactivating enzyme from *Neurospora crassa*. Photochem. Photobiol. **6**:799-803.
37. Vogel, H. J. 1956. A convenient growth medium for *Neurospora* (Medium N). Microbiol. Genet. Bull. **13**: 42.
38. Volkin, E., and L. Astrachan. 1956. Phosphorous incorporation in *Escherichia coli* ribonucleic acid after infection with bacteriophage T2. Virology **2**:149-161.