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EXTREME ULTRAVIOLET RADIATION-SENSITIVITY OF RESPIRATORY ADAPTATION IN SACCHAROMYCES CEREVISIAE CELLS DURING TRANSITION FROM ANAEROBIC TO AEROBIC STATE

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SUMMARY

The respiratory adaptation process in both wild-type and UV-sensitive strains of Saccharomyces cerevisiae was sensitive to small doses of UV-radiation (10 and 0.7 J/m^2, respectively). These doses of irradiation were ineffective in arresting induced synthesis of acid phosphatase and catalase. Exposure of the irradiated cells to visible light (370 - 800 nm) could completely restitute the impaired respiratory adaptation process. UV irradiation at these doses affected DNA and RNA synthesis in maturing mitochondria in both the yeast strains. The UV-induced block could however be eliminated by exposure of the cells to visible light. These results suggest that the lesion in the UV-induced block in the respiratory adaptation may be in the DNA of promitochondria.

INTRODUCTION

Anaerobically grown cells of Saccharomyces cerevisiae are known to undergo respiratory adaptation during transition from the anaerobic to aerobic state and this has been shown to be due to transformation of promitochondria into functionally efficient mitochondria (1 - 3).

In this communication, we present evidence to show that the respiratory adaptation in S.cerevisiae is very sensitive to ultraviolet radiation (UV) and the impaired system can be restored to normalcy by exposure of the irradiated cells to visible light (370 - 800 nm). Other results suggest that the UV-induced impairment stems from lesions introduced in promitochondrial DNA which can be eliminated by photoreactivation.

RESULTS AND DISCUSSION

Two different strains of S.cerevisiae differing widely in their

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sensitivity to UV-radiation, viz., wild type strain (ATCC 3177) and sensitive strain UV 1-2/#3 were used.

The oxygen consumption by anaerobically-grown cells of both the wild and UV-sensitive strains of yeast was negligible (Fig. 1). It rose to the level of oxically grown cells during 2 hrs of aeration, which is indicative of the formation of functional mitochondria.

There was a marked difference in the susceptibility of anaerobically and aerobically grown cells to UV (Fig. 2). For wild strain, the D_{37} of aerobically grown cells and anaerobically grown cells was 125 and 75 J/m^2 and for UV-sensitive strain, the D_{37} was 1.1 and 0.9 J/m^2. The greater sensitivity of anaerobically grown cells to UV could be related to lower levels of ATP, the requirement of which has been implicated in some steps of DNA repair (6).

As seen in Table 1, relatively small doses of UV (10 - 20 J/m^2 and 0.7 to 1.1 J/m^2 respectively for wild and sensitive strain) could impair the respiratory adaptation in both the strains studied. On exposure of UV-treated cells to visible light (370 - 800 nm), however, the respiratory adaptation could be completely restored to normal signifying the elimination or bypassing of the UV-induced block. This would imply that the observed inhibitory effect could have been due to the formation of thymine dimers in DNA and these may have been monomerized in the presence of visible light by photoreactivation (7, 8). The damage could be conceived of as occurring either at the level of nuclear or mitochondrial DNA.

The extreme sensitivity of the respiratory adaptation process to UV is underlined from the result indicating that formation of catalase, which is known to be induced during aerobiosis (9), was not affected by exposure to the UV doses which were detrimental to respiratory adaptation (Fig. 3). Formation of acid phosphatase, known to be induced in phosphate-deficient medium (11), was also found to be unaltered when the cells were given similar degree of UV exposure as above (results not shown). Inductions of catalase and acid

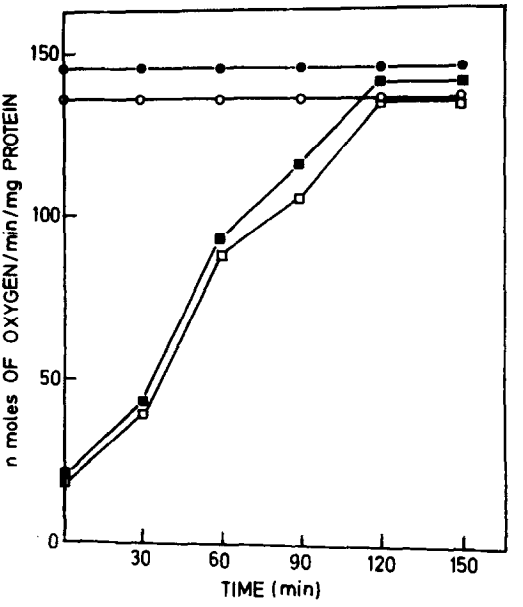

Fig. 1. Respiratory adaptation in S.cerevisiae cells during transition from anaerobic to aerobic growth.

Cells grown anaerobically for 18 hrs in the medium containing peptone (2%), yeast extract (1%) and glucose (2%) were harvested, washed and suspended in equal volume of fresh growth medium with 0.8% glucose (10^7 cells/ml). The suspension was vigorously aerated and at the times of aeration indicated in the figure, aliquots of the cell suspensions (10 ml) were withdrawn and the cells harvested and washed. Aerobically grown cells subjected to aeration under the conditions described above served as the control. Rate of oxidation of succinate by these cells was measured using a GME oxygraph (4). Protein was estimated by Lowry's method (5).

■——■ Anaerobically grown ATCC 3177 cells.
●——● Aerobically grown ATCC 3177 cells.
□——□ Anaerobically grown UV 1-2/#3 cells.
○——○ Aerobically grown UV 1-2/#3 cells.

phosphatase are both the manifestations of nuclear transcription and hence it may be inferred that the inhibitory effect of UV stems from mitochondrial DNA damage rather than nuclear DNA damage.

This assumption was substantiated by experiments in which the effect of UV on mitochondrial DNA and RNA syntheses was investigated (Fig. 4). DNA and RNA syntheses in developing mitochondria were drastically arrested in both the S.cerevisiae strains at the doses of UV-radiation found to inhibit respiratory adaptation. The inhibition in mitochondrial nucleic acid synthesis

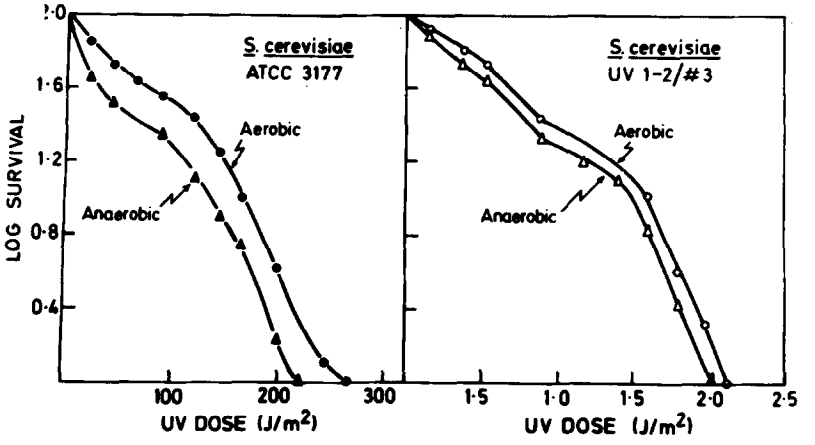

Fig. 2. Survival of anaerobically and aerobically grown cells of S.cerevisiae to UV radiation.

Cells of the two strains of S.cerevisiae were grown aerobically and anaerobically in the media containing 0.8% and 2% glucose respectively (see Fig.1). The cells were harvested, washed and suspended in phosphate buffer (pH 7.0). Five ml aliquots of the cell suspensions were spread out in petri dishes and exposed to different doses of UV at the dose rate of 0.18 $J/m^2/s$. Immediately after irradiation, 0.2 ml of suitable dilutions of samples were plated five-fold on yeast extract, peptone, glucose and agar in petri dishes. Macroscopic colonies were scored after 4 days of incubation at $30^{\circ}C$ to determine the surviving fractions.

could be completely reversed upon exposure to visible light. This can again be interpreted as the consequence of the monomerization of UV-induced thymine dimers by photoreactivation.

The doses of UV irradiation which block the mitochondrial biogenesis did not affect the respiratory rate of oxically grown cells indicating that the integrity of mitochondrial DNA may not be critical for mitochondrial function.

During respiratory adaptation, the DNA of promitochondrial structures appears to replicate as evidenced by about 30% increase in mitochondrial DNA synthesis (15) and about 20-fold increase in the activity of DNA polymerase (16). This spurt in replicative DNA synthesis could be expected since anaerobically grown cells have much smaller content of DNA presumably due to smaller number of promitochondrial structures as compared to mitochondrial

Table 1

EFFECT OF UV-IRRADIATION ON RESPIRATORY ADAPTATION IN S.CEREVISIAE

Time of aeration of anaerobic cells	Oxygen uptake of Unirradiated cells aerated in dark	UV-irradiated cells aerated in dark	UV-irradiated cells aerated in light
(min)	(n moles of oxygen/min/mg protein)		
	S.CEREVISIAE ATCC 3177		
0	21	8	20
30	40	9	47
60	90	10	87
90	114	12	98
120	140	16	139
Aerobic cells	142	142	142
	S.CEREVISIAE UV 1-2/# 3		
0	20	8	19
30	40	10	42
60	90	10	78
90	104	13	98
120	134	14	133
Aerobic cells	134	134	134

S.cerevisiae cells grown anaerobically were washed and suspended in phosphate buffer (pH 7.0) to the cell density of 10^7 cells/ml. Portions of cell suspensions of the two yeast strains were exposed to UV (20 J/m^2 and 1 J/m^2 for ATCC 3177 and UV 1-2/# 3 strains respectively). The irradiated and unirradiated cell suspensions were transferred to fresh growth medium containing 0.8% glucose and aerated in dark. In another experiment, the irradiated cell suspensions were aerated in visible light emitted by fluorescent lamp (370 - 800 nm).

number of about 10-12 in oxically grown yeast and also perhaps due to fewer copies of DNA molecules per promitochondrial structure (17) as compared to reported 4-5 copies of DNA molecules per mitochondrion (18). Mitochondrial DNA with the molecular weight of 2.1 x 10^7 daltons has been shown to code for

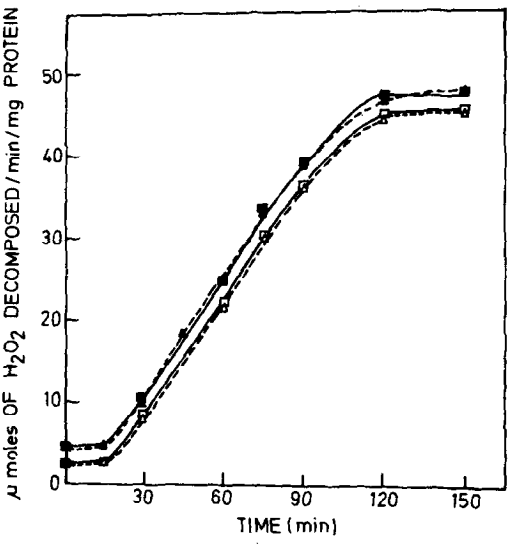

Fig. 3. Effect of UV on induction of catalase in S.cerevisiae. Cells grown anaerobically for 18 hrs were harvested, washed and suspended in 0.1 M sodium phosphate buffer (pH 7.0) to the cell density of 5 x 10^7 cells/ml. A batch of the cell suspension was exposed to UV (80 J/m^2 for ATCC 3177 strain and 10 J/m^2 for UV 1-2/# 3 strain) as described in the legend to Fig. 2. The unirradiated and irradiated cells were transferred to the nongrowth medium containing glucose 1%, KH_2PO_4 1%, $MgSO_4$ 0.01% and $CaCl_2$ 0.01% (pH 7.0). The suspensions were aerated vigorously in dark. Ten ml aliquots of cell suspensions were withdrawn at the times of aeration indicated in the figure, harvested and catalase activity by the cells was assayed in terms of the rate of hydrogen peroxide decomposition as determined at 240 nm using a double-beam spectrophotometer (10).

■——■ Unirradiated ATCC 3177 cells.
▲----▲ UV-irradiated ATCC 3177 cells.
□——□ Unirradiated UV 1-2/# 3 cells.
△----△ UV-irradiated UV 1-2/# 3 cells.

mitochondrial ribosomal RNA, several tRNAs specific for mitochondria, besides coding for several polypeptides (19, 20). Conceivably therefore both replication of promitochondrial DNA and its transcription may be essential for mitochondrial biogenesis. Once formed, mature mitochondria may not at all be dependent on mitochondrial DNA replication and also may not be so dependent on mitochondrial DNA transcription for their respiratory functions.

It is interesting to note that exposure of UV-irradiated cells to visible light completely restores impaired biogenesis of mitochondria. This would imply that UV-induced thymine dimers are formed in mitochondrial DNA

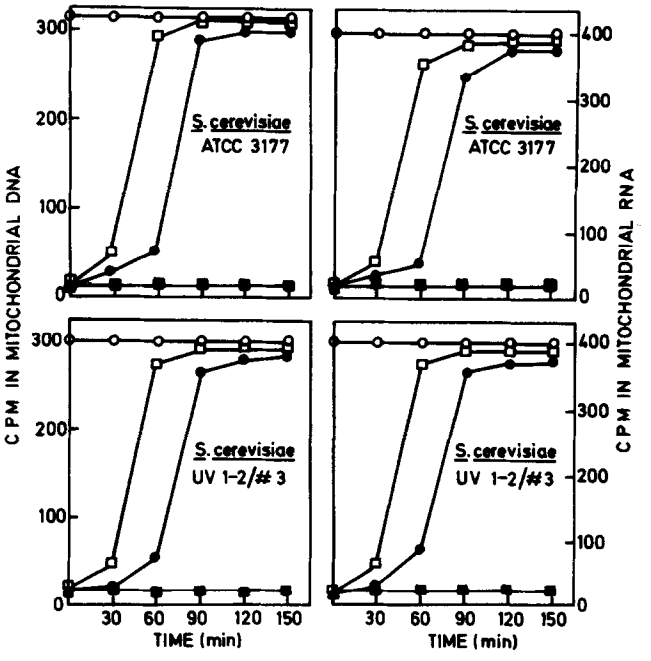

Fig. 4. Effect of UV on mitochondrial DNA and RNA synthesis during respiratory adaptation in S.cerevisiae. Details essentially as in Table 1. Anaerobically grown cells of the two strains were suspended in phosphate buffer and exposed to UV. The unirradiated and irradiated cells suspensions were then transferred to fresh growth medium containing 0.8% glucose (5×10^8 cells/ml and vigorously aerated. Thirty ml aliquots of cell suspensions were withdrawn at various times during aeration and to these 60 µCi of (^{3}H) uridine (sp. activity 6500 mCi/mmole) were added. After 15 min labelling, the cells were processed for the isolation of mitochondria (1, 12). After DNase and RNase treatments of mitochondria, DNA and RNA were isolated (13, 14) and radioactivity incorporated into them was determined in a liquid scintillation spectrometer. The ordinates represent radioactivity (cpm) incorporated into DNA (left-hand panels) and RNA (right-hand panels) of mitochondria derived from cells corresponding to 30 ml of cell suspensions.

o——o Unirradiated aerobic cells.
□——□ Unirradiated anaerobic cells.
■——■ UV-irradiated anaerobic cells (aerated in dark).
●——● UV-irradiated anaerobic cells (aerated in light).

and are then monomerised in the presence of visible light by a photoreactivating enzyme. In this context it is pertinent to mention that DNA of yeast mitochondria contains as high as 83% AT. A recent report suggests that UV-induced pyrimidine dimers formed in mitochondrial DNA in yeast are photoreactivable although yeast mitochondria do not contain photoreactivating enzyme (21). Photoreactivation of UV-damaged promitochondrial DNA may seem

to have been brought about by a photoreactivating enzyme of either cytoplasmic or nuclear origin.

Since UV-treated cells of both wild-type and UV-sensitive strains of S.cerevisiae fail to undergo mitochondrial biogenesis during aeration in dark, it may be supposed that dimers in promitochondrial DNA are not excised. This conclusion is also supported by a recent report indicating the absence of DNA repair enzymes in mitochondria (22). In the wild strain, the enzymes responsible for DNA repair are either confined to the nucleus or do not have access even to promitochondrial DNA to bring about repair of the resident DNA. This seems to be in contrast to the behaviour exhibited by the enzyme responsible for photoreactivation which appears to have access to promitochondrial DNA.

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