〔Original〕

Respiration-Deficient Mutant Induction and Cell Killing Effects in Radiation-Sensitive Mutants of *Saccharomyces cerevisiae* by Ethidium Azide

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Abstract: Several UV-sensitive (*rad*) mutants were tested for their responses to photolytic binding of an azide analog of ethidium. All mutants were more sensitive to killing by the photolytic treatment with this drug than normal strain but this sensitivity was dependent on the growth phase of each mutant. In late exponential phase, two mutants were classified as supersensitive (*rad* 1, 3), while two (*rad* 6, 52) were more like the normal strain. Respiration-deficient (*petite*) mutation induction of late exponential cells was quite different from the cell killing effect. Almost all surviving cells of *rad* 1, 9 and normal strain were converted to *petite* mutants by photolytic treatment with 5–10 μ M of ethidium azide. In contrast, *rad* 3, *rad* 6 and *rad* 52 mutants were rather resistant. Early exponential cells of *rad* 6 and *rad* 52 were supersensitive to the killing effect while *petite* induction was the same as in normal strain.

Key words: yeast, UV-sensitive mutant, mitochondrial and nuclear mutation, ethidium azide.

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Introduction

Ethidium bromide (EB) has been used widely as a model compound for producing mitochondrial mutations (i.e. respiration-deficient, *petite* mutation) in yeast (Deutsch *et al.*, 1974; Fukunaga *et al.*, 1978; Perlman & Mahler, 1971; Slonimski *et al.*, 1968), but its mechanisms of action are still not clear. Ethidium monoazide (3-amino, 8-azido-5-ethyl-6-phenylphenanthridinium chloride, EA) was synthesized and used to study *petite* mutations in yeast by photoaffinity labeling (Graves *et al.*, 1977; Hixon *et al.*, 1975a, b; Fukunaga & Yielding, 1979a, b). Photolysis of this photosensitive analog with cells produced covalent adducts with mitochondrial DNA (Fukunaga & Yielding, 1979c) and enhanced mutagenesis over that of the parent EB (Fukunaga & Yielding, 1979b; Fukunaga *et al.*, 1980). Furthermore, EA can cause nuclear mutations in yeast (Hixon & Burnham, 1979). In the dark, the EA is expected to interact at the ethidium-binding sites in a manner similar to the parent compound. Upon photolysis, however, the azide substituent is activated, generating the reactive nitrene species which forms a covalent bond at the

binding site. Ideally, the qualitative aspects of the ethidium interaction should not be altered by the covalent attachment, but should be amplified due to the conversion of the reversible complex to an irreversible one. The drug receptor site association may be retrieved from any system and consequently may be analysed directly so that *in vivo* as well as the *in vitro* targets can be identified, characterized and correlated with biological effects.

This work has emphasized the importance of covalent binding of the drug to DNA in the mutagenic process and nuclear damage in yeast by the photolysis of EA is apparent from the increased killing effect in cells treated with EA as opposed to dark-treated cells. We have now compared cell killing and petite induction by this photosensitive ethidium analog in radiation-sensitive mutants because of their repair-deficiency in different steps of *Saccharomyces cerevisiae* to assist in identifying steps in the mutagenic sequence.

Materials and Methods

Yeast strain N 123 (a, his₁) provided from Dr. Moustacchi (via Dr. Hixon) was used as a normal strain, and *rad* mutants were obtained from the Yeast Genetic Stock Center, Berkeley. Cultures were grown in complete medium (YPD) consisting of 1% yeast extract, 2% peptone and 1% dextrose. All cultures were harvested at the intervals indicated in the figure legends and washed 3 times in phosphate buffer (0.067 M, pH 7.0). Treatments with EA were performed as described elsewhere (Fukunaga & Yielding, 1979a; b), and petite mutations were measured by the tetrazolium overlay method (Ogur *et al.*, 1957). Other experimental conditions are described in the figure legends. Yeast extract and agar were purchased from Difco Lab. and all other chemicals were of reagent grade.

Results and Discussion

The effects of photolytic treatment with EA on cell killing of repair-deficient nuclear mutants (Game & Cox, 1971; Ho, 1975; Resnick & Martin, 1976) from the late exponential cultures are shown in Fig. 1. *Rad* 1 and *rad* 3 (both of the mutants were excision-deficient) were most sensitive to the drug treatment, and *rad* 9 (error prone) showed intermediate sensitivity. The other two mutants (*rad* 6 and *rad* 52, error prone and ligase-deficient mutant, respectively) were sensitive to cell killing effects of this drug at a level similar to normal cells. Two other normal strains were tested for killing effects and petite mutation by EA, and all of these normal strains showed a similar sensitivity on cell killing and petite mutation (data not shown).

Early exponential cells of *rad* 6 and *rad* 52 showed enhanced cell killing effects from EA treatment (Fig. 2), while normal cells were equally sensitive to EA whether from early or late exponential cultures. Thus the effects of EA also appeared to depend on the growth phase of the culture, especially for *rad* 6 and *rad* 52 because of their different

biological conditions.

EA treatment in the dark did not cause cell killing in either the mutants or normal yeast cells. These results indicated that covalent binding of the drug with target by the photolysis is essential and also excision repair is correlated mostly on that mutagenic process.

Fig. 1. Cell survival during late exponential growth of *rad* mutants following photolytic treatment with ethidium azide. Late exponential phase cells were washed and resuspended in a phosphate buffer (0.067 M, pH 7.0) at 10^8 cells/ml containing various concentrations of EA. Cell suspensions were held in the dark for 60 min at 30°C and then divided into two portions. One half was exposed to visible light and the other half was held in the dark for another 60 min. After photolytic treatment, cells were washed and plated on YPD solid media (2% agar) and incubated for 3–5 days at 30°C. More than 500 surviving cells were counted and cell killing effects were calculated from the original cell number held in the dark. All experiments were duplicated.

Fig. 2. Cell survival during early exponential growth of *rad* mutants following photolytic treatment with EA. Stationary phase cells in YPD liquid medium were harvested and resuspended in fresh medium, and cultivated for 2.5 hours at 30°C. Cells were washed and treated with EA as described in the legend for Fig. 1.

Petite mutation induction in late exponential cultures by EA was also different for *rad* mutants (Fig. 3). *Rad* 1 and *rad* 9 were similar to normal strain, while the others, especially *rad* 6, were more resistant to petite formation. It was observed that petite formation from EA was very low in *rad* 3, but this strain was very sensitive to cell killing. Two mutants (*rad* 6 and *rad* 52) showed a resistant killing response similar to normal cells. In contrast, early exponential cells of these mutants were mutagenized much like normal cells with a change in their killing response (Fig. 4).

These experiments have shown that the *rad* mutants tested are all sensitive to photolytic treatment with EA, but the killing and mutagenic responses can vary independently and similar results were obtained by others (Game & Cox, 1971; Ho, 1975; Lawrence *et al.*, 1974) using chemical mutagens and UV treatment. Furthermore, these responses to covalent drug adducts formation appear to be strongly dependent on the growth phase, probably DNA synthesizing steps, of the cells tested.

Frameshift mutations in Salmonella tester strain have been obtained with EA in the light but not in the dark (Yielding *et al.*, 1976). Moreover, EA appears to intercalate DNA and compete with EB for DNA binding sites (Fukunaga & Yielding, 1979a). In yeast, while EB binds to both nuclear and mitochondrial DNA it is only mutagenic for mitochondrial DNA presumably because of its activation inside mitochondria which results in the formation of covalent DNA adducts.

Fig. 3. Petite mutagenesis induced during late exponential growth in *rad* mutants following photolytic treatment with EA. Experiments were done as in the legend of Fig. 1 and petite mutants were scored in survivors by the tetrazolium overlay method (Ogur *et al.* 1957).

Fig. 4. Petite mutagenesis during early exponential growth of *rad* mutants following photolytic treatment with EA. Early exponential phase cells were prepared as indicated in the legend of Fig. 2 and treated with EA. Petite mutations were measured as described in Fig. 3.

As shown here, EA was cytotoxic to nuclear only if light activated. These experiments suggest that drug intercalation alone is insufficient for causing mutations; apparently an additional covalent attachment is required.

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エチジウムアジドによる紫外線感受性酵母の呼吸欠損変異の誘発と殺菌効果

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要 旨： 紫外線感受性酵母 (*rad* mutant) を用いて、エチジウムアジドの変異誘発能および生存率の変化を検討した。生存率においては、用いた全ての変異株で正常酵母より低く、特に *rad* 1 と *rad* 3 が最も低く、また *rad* 6 と *rad* 52 は正常酵母に近い値を示した。呼吸欠損変異の誘発においては、生存率の場合と異なり、*rad* 1, *rad* 9 および正常酵母では生存した全ての細胞が呼吸欠損変異を起こした。これに対し、*rad* 3, *rad* 6, *rad* 52 では呼吸欠損変異の誘発に対して抵抗性を示した。しかしながらこれらの変異株も、対数増殖期におけるエチジウムアジド処理により、生存率が低下し、呼吸欠損変異の誘発も正常酵母のそれと同様の値を示した。

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