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The Effects of Propidium on Nuclear and Mitochondrial Mutation induced in Yeast by Manganese

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Manganese caused mitotic gene conversion and reverse mutation in nuclear genes of yeast *Saccharomyces cerevisiae* strain D7. Respiration-deficient (petite) mutation and antibiotic-resistant mutations were also induced by treatment with manganese. The presence of propidium had no effect on mitotic gene conversion and reverse mutation induced by manganese.

In the case of mitochondrial mutagenesis by manganese, additional petites were produced and induction of antibiotic-resistant mutants were reduced by half when propidium was present during culture. It seems that propidium affects only mitochondrial mutagenesis, not nuclear mutagenesis.

Keywords——manganese; *Saccharomyces cerevisiae*; propidium; recombination and mutation; respiration-deficient mutation; antibiotic-resistant mutation

Propidium [3,8-diamino-5-(3-diethylmethylammoniopropyl)-6-phenyl-phenanthridinium diiodide, PI], a phenanthridine derivative, has a chemical structure similar to that of ethidium [3,8-diamino-5-ethyl-6-phenyl-phenanthridinium bromide, EB], which can cause respiration-deficient (petite) mutation in the yeast *Saccharomyces cerevisiae* at high frequency. EB induces petite mutation in yeast during both resting and growing conditions.[1] In contrast, PI has mutagenic activity at a low level during growth but not in resting cells.[2] This suggests that inhibition of mitochondrial deoxyribonucleic acid (mit DNA) replication is involved in petite mutation by PI.

EB mutagenesis is highly stimulated during growth by the presence of PI, which acts as a co-mutagen, but PI competes with EB under resting conditions.[2] Mit DNA synthesis is inhibited by PI, and PI itself has no effect on the fragmentation of pre-existing DNA, but enhances mit DNA degradation provoked by EB.[2c] Moreover, the activity of mitochondrial nuclease is stimulated by EB but not by PI.[3] All of these results indicate that the enhancement of petite mutation by PI results from degradation of the mit DNA in petites induced by EB.

Manganese ($MnCl_2$) is known to induce petite mutation in yeast and also to induce point mutation such as antibiotic-resistant mutation and mit$^-$ mutation in yeast mitochondria.[4,5] The mechanism of such mutation induction by manganese is not clear. However, these mutations are induced through the interaction of manganese with the DNA polymerase.[5] Therefore, we examined the effects of PI on mutation induction by manganese to investigate the action of PI on mutagenesis in yeast mitochondria. We also examined the effect of PI on the mutagenic action of manganese on nuclear genes of yeast using strain D7, in which mitotic gene conversion, mitotic cross-over, and reverse mutation can be determined.[6]

Manganese caused reverse mutation and gene conversion at a high frequency but mitotic cross-over at a very low level. However, the presence of PI had no effect on any of these recombinations and mutations induced in nuclear genes by manganese. No enhancement of petite mutation induction by manganese was seen with the presence of PI, but induction of mitochondrial antibiotic-resistant mutants was reduced by half.

Experimental

Induction of Recombination and Mutation in Nuclear Genes by Manganese——A diploid yeast, *Saccharomyces cerevisiae* strain D7, which was kindly provided by Dr. F.K. Zimmermann, was used in these experiments. As described by Zimmermann,[6] strain D7 contains alleles *ade* 2-40/*ade* 2-119 to monitor mitotic cross-over, alleles *trp* 5-12/*trp* 5-27 to score mitotic gene conversion and *ilv* 1-92/*ilv* 1-92 for the detection of reverse mutation. Cells of D7 were spread onto YPD plates containing 1% yeast extracts (Difco), 2% bactopeptone (Difco), 2% dextrose and 2% agar (Difco) and incubated for 2 d at 30°C. Normal white colonies were selected and checked for amino acid requirement on a synthetic complete medium (Difco Yeast Nitrogen Base Medium) minus these amino acids. Thus a normal clone was selected and incubated on a YPD plate at 30°C. After incubation for 4 d, cells were harvested and washed twice with water. Cells were suspended at 5×10^6 cells/ml in 10 ml of YPD liquid medium containing manganese and/or PI at the desired concentration. Treatment with these drugs was continued for 24 h, then the cells were collected and washed twice in cold water. Recombinations and mutations were detected by spreading cells (10^6—10^7 cells/plate) onto plates of synthetic complete medium with 0.5% dextrose as recommended by Zimmermann.[6] Plates were incubated for 5 d at 30°C and scored for mitotic cross-over and mitotic gene conversion. For the detection of reverse mutation, incubation for 8 d was necessary.

Effects of PI on Mutation and Petite Induction by Manganese——Haploid strain DPI-1B/517 (α, his_1, trp_1, ρ^+) was used in these experiments. Cells were grown to the exponential phase in YPD liquid medium and harvested. Cells were washed twice and suspended at 10^5 cells/ml in fresh YPD medium containing manganese and/or PI. Treatment with drugs was performed in a shaking incubator at 30°C and aliquots were taken at intervals. Cell density was measured with a hemocytometer and cells were spread onto YPD 2% agar plates after suitable dilution. Petite mutation was scored by the tetrazolium overlay method[7] after incubation for 4 d on YPD plates. To detect erythromycin-resistant mutation, cells were spread onto YPG (3% glycerol was added instead of dextrose) 2% agar plates containing 3 mg/ml of erythromycin (Behringer). Erythromycin-resistant mutants (ERY^r) were scored after incubation for 10 d at 30°C and indicated as colonies of ERY^r mutants/10^7 ρ^+ colonies/division of cells.

Results

Effects of PI on Growth and Petite Mutation induced by Manganese

Yeast cells were grown in YPD liquid medium (Fig. 1). The presence of PI had no effect on growth and the cell population reached the stationary phase after incubation for 24 h. In contrast, manganese inhibited cell growth at concentrations of 3 to 5 mM but had almost no effect at 1 mM (data not shown). However, no significant cell killing effect was observed at the concentrations used in this experiment. Induction of petite mutation with PI and manganese is shown in Fig. 2. The frequency of spontaneous petite mutation was less than 2% throughout these experiments. PI (100 μM) induced more than 20% petites during incubation for 12 h. Manganese at 3 mM induced 40% petites and with 5 mM manganese, more than 60% petites were induced after incubation for 2 d. In the case of manganese mutagenesis, additional petites (about 20%) were produced in the presence of PI in culture. In these cases, when the cultures reached the late exponential phase, the proportion of petites decreased. Survivals in these experiments were more than 80%. Therefore, the decrease of petites at the stationary phase in every case of incubation was not caused by the selective killing of petites. The reason is so far unknown.

Effects of PI on the Induction of Antibiotic-Resistant Mutants by Manganese

Almost all antibiotic-resistant mutants of yeast induced by manganese are cytoplasmic[5] (mitochondrial, data not shown). Therefore, we examined the effects of PI on antibiotic-resistant mutation induced by manganese. The results are shown in Table I, in which the number of ERY^r mutants in 10^7 ρ^+ colonies was divided by the frequency of cell division from 10^5 cells of initial inoculum. Spontaneous ERY^r colonies numbered 1 to 3, and there were no differences on treatment with PI only. After the treatment with 3 mM manganese for 24 h, more than 1000 ERY^r colonies were produced, and more than 4000 mutants were induced by 5 mM manganese. However, on the addition of PI, the formation of ERY^r mutants was reduced to nearly half. This indicates that PI inhibits the induction of mutations by

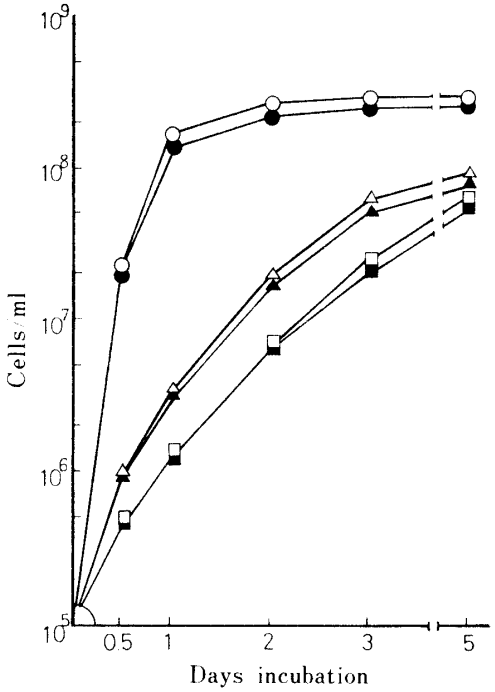

Fig. 1. Growth of Yeast in the Presence of Manganese and/or Propidium

Overnight culture of yeast cells (DPI-1B/517) in YPD liquid medium was harvested and washed in water. The cells were inoculated in 10 ml of fresh YPD medium with or without manganese and/or propidium at a cell density of 10^5 cells/ml and incubated at 30°C on a shaker. At various intervals of incubation, a small aliquot of culture was taken and the cell density was measured with a hemocytometer.

Symbols: control (○); 100 μM propidium (●); 3 mM manganese (△); 3 mM manganese + 100 μM propidium (▲); 5 mM manganese (□); 5 mM manganese+100 μM propidium. (■),

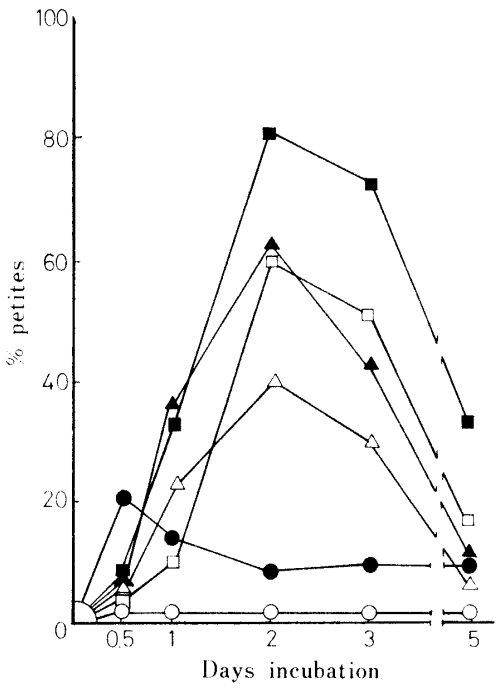

Fig. 2. Effects of Propidium on Petitegenesis by Manganese

Cells of yeast were treated with propidium and/or manganese as described in Fig. 1. Aliquots of cell cultures were taken at intervals and plated onto YPD plates after suitable dilution. Petite colonies were scored by the tetrazolium salt overlay method[7] and the percentage of survivals is indicated. Sectored colonies were counted as normal colonies. The results given here were averages of three independent experiments. Symbols are the same as in Fig. 1.

Table I. Effects of Propidium on the Induction of Erythromycin-Resistant Mutants by Manganese in Yeast

Treatment		Erythromycin-resistant mutants (ERY^r)[a] Incubation (h)			
Manganese (mM)	Propidium (μM)	24	48	72	120
—	—	3	1	1	1
—	100	3	1	2	2
3	—	1112	660	98	32
3	100	695	395	52	21
5	—	4048	4135	410	22
5	100	2850	2349	179	9

a) ERY^r mutants/10^7 ρ^+ colonies/cell division. All cultures were grown in 10 ml of YPD liquid medium with or without drugs at the concentrations indicated. After cultivation, cells were washed and plated on YPG 2 % agar plates containing 3 mg/ml of erythromycin after suitable dilution. Plates were incubated for 10 d at 30°C and ERY^r colonies were counted. Viable counts and the number of petites were estimated by plating the same samples on YPD agar plates as described in the legend to Fig. 2. Numbers of ERY^r colonies are the averages of three independent experiments.

manganese. At both concentrations used, the mid-exponential phase was most effective for the production of ERY^r mutants, and fewer mutants were obtained on further incubation. These results are similar to those for petite induction shown in Fig. 2.

Effects of PI on Nuclear Mutation induced by Manganese

Cells of strain D7 without drugs or with PI were grown to the stationary phase for 24 h. Five to 10 mM manganese inhibited cell growth and survivals decreased to 80%. As a result of the treatment, giant cells appeared and cell survivals decreased after prolonged incubation or with higher concentrations (data not shown).

As shown in Table II, treatment with manganese and/or PI was not effective on mitotic cross-over. Mitotic gene conversion was increased 5 to 6 times after the treatment with manganese, and the number of revertants was also increased, but the increase of reverse mutation was greatest. However, the addition of PI had no effect on nuclear mutation and gene conversion induced by manganese.

Table II. Effects of Propidium on Nuclear Mutation induced by Manganese

Treatment		Growth[a]	Survivals		Convertants		Revertants	
$MnCl_2$ (mM)	Propidium (μM)		Survivals (%)	Crossover (%)	Per 10^5 surviving cells	Crossover (%)	Per 10^6 surviving cells	Croosover (%)
—	—	66.2	98.2	0.2	1.0	5.7	0.2	0.3
—	100	62.2	101.9	0.4	1.1	7.1	0.3	0.2
5	—	1.8	100.2	0.2	5.3	17.3	183.3	0.1
5	100	1.9	102.4	0.3	5.4	16.4	174.0	0.2
10	—	1.2	82.6	0.4	6.6	13.6	159.0	0.5
10	100	1.3	83.2	0.3	6.0	14.3	163.2	0.6

a) Growth denotes the number of cells after treatment divided by the initial cell density. Cells were treated with drugs at the concentrations indicated in 10 ml of YPD liquid medium for 24 h at 30 °C on a shaker. Cells were harvested and suspended in water. The number of cells was counted with a hemocytometer and the growth rate for 24 h (initial cell population, 5×10^6 cells/ml) was calculated. The surviving cells were measured by plating on synthetic complete medium. For the determination of conversion or reversion, each sample was spread onto synthetic complete medium minus tryptophan or isoleucine. After incubation of 4 to 10 d, convertant or revertant colonies were counted. Adenine requiring colonies are indicated as crossovers in percent. These results were the averags of 4 to 6 independent experiments.

Discussion

Our results showed that manganese induced mitochondrial antibiotic-resistant mutation and nuclear mutation at the *ilv* region and recombination at the *trp* region. These results indicated that manganese acts on both nuclear and mitochondorial genes to cause reverse mutation on nuclear genes and antibiotic resistant mutation on mitochondria. Furthermore, manganese caused both nuclear and mitochondorial mutations only in growing cells, not in resting cells, as in the case of mitochondrial mutation by PI.

The effects of PI on mitochondrial mutation of yeast, such as inhibition of mit DNA synthesis, stimulation of petitegenesis by EB during growth and competition with EB under resting conditions, have been reported.[2] In the present study, PI did not inhibit nuclear mutation and recombination and did prevent the induction of mitochondrial mutation. Petites were induced by manganese in exponential phase cell culture and additional petites were produced in the presence of PI.

Studies on mitochondrial mutation with manganese have been done by Putrament *et al.*[5] They reported that manganese caused petite mutation and induced mitochondrial antibiotic-resistant mutation in yeast. They also reported that the mutation by manganese resulted from a decrease in the fidelity of DNA synthesis due to disturbance and inhibition of DNA polymerase.

The relationship between these results and the mechanisms of action of these two compounds on mitochondria are far from clear. Nevertheless, it seems possible that PI inhibits only mit DNA polymerase but not nuclear DNA polymerases, and that the action of PI on mitochondria is similar to that of manganese. Further studies on the mutagenic activities of these compounds should prove useful in deciphering the details of mutation mechanisms in yeast.

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