Vol. 84, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 29, 1978 Pages 501-507

PROPIDIUM: INDUCTION OF PETITES AND RECOVERY FROM ETHIDIUM MUTAGENESIS IN SACCHAROMYCES CEREVISIAE

Masahito Fukunaga and K. Lemone Yielding

Laboratory of Molecular Biology
University of Alabama in Birmingham
University Station
Birmingham, Alabama 35294 U.S.A.

Received August 21, 1978

SUMMARY: It was shown that petite induction in growing cells of Saccharomyces cerevisiae by ethidium was strongly stimulated by the presence of propidium, a phenanthridinium dye of similar structure to ethidium. Propidium itself also induced petites in growing but not in resting cells. Furthermore, propidium could prevent petite induction in resting cells and caused recovery from ethidium induction with prolonged incubation. A possible mode of action of propidium in the ethidium-induced petite mutagenesis is discussed.

INTRODUCTION:

Ethidium bromide (EBr)[1] is an effective mutagen for mitochondrial DNA (mtDNA)[2] in Saccharomyces cerevisiae in both growing and resting cells (1-4). The molecular events accompanying petite induction have been partially delineated, particularly by the studies of Grossman et al (5), Goldring et al (6,7), Bastos and Mahler (8-10), Hall et al (11) and Criddle et al (12).

These events include the inhibition of mtDNA synthesis and formation of a complex between the dye and mtDNA. In addition to a strong non-covalent complex between the dye and mtDNA there is also evidence that covalent attachment follows metabolic activation (8-10). Studies from this laboratory employing photosensitive derivative of ethidium which can be photolyzed to form covalent complexes without a requirement for metabolic activation have emphasized the importance of covalent binding in the petite induction process (13-15).

Propidium iodide (PI)[3] is similar to EBr in structure (Fig. 1) and

Abbreviations: [1]EBr - ethidium bromide; [2]mtDNA - mitochondrial DNA; [3]PI - propidium iodide.

0006-291X/78/0842-0501$01.00/0

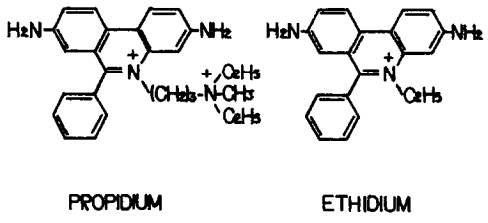

Fig. 1 Chemical structure of Propidium and Ethidium

also forms complexes with circular DNA like EBr (16). However, petite mutation was not induced by PI at levels normally employed in mutagenesis by EBr (17). It had not been determined whether this lack of petite induction was from lack of cell penetration, lack of metabolic activation, or subtle differences in binding. We have, therefore, performed additional studies of the effects of propidium and of propidium-ethidium combinations on petite induction.

As we reported here, we have now demonstrated that PI can induce petite formation with prolonged exposure of growing cells to high concentrations. EBr-induced formations of petites in growing cells was strongly stimulated by the additional presence of PI. In contrast, in the resting cells, formation of petite cells by EBr was inhibited by the presence of propidium.

MATERIALS AND METHODS:

A haploid strain of Saccharomyces cerevisiae DP1-1B/517 α his_1, trp_1 C^R $\omega+$ provided by Dr. N. Gunge was used. Cells were grown in a YPD medium containing 1% yeast extract, 2% peptone and 2% Dextrose as a carbon source. Cell numbers were counted in a haemocytometer and proportions of petites in a population was determined by tertazolium overlay method (18) and viability was measured by plating a suitable dilute aliquot of samples on 2% agar YPD plates.

Cultivation, incubations and treatment of ethidium and propidium were performed in the dark. More than 600 cells were plated for each sample and all experiments were duplicate. Other experimental details are described in the figure legends.

Ethidium and propidium were purchased from Sigma Chemical and Calbiochem. Yeast extract, peptone and agar were purchased from Difco Labs and all other chemicals were of reagent grade.

RESULTS:

Cells of Saccharomyces cerevisiae were incubated at 30^0 in liquid medium in the presence of 5, 10μM EBr and different concentrations of PI

alone and in combination with EBr. As can be seen in Fig. 2, cell growth was not inhibited by the presence of EBr and only a little by high concentrations of PI.

Samples were taken at intervals indicated in Fig. 3 and plated onto YPD solid media for measurement of petite induction. The formation of petite mutants by EBr was stimulated by the presence of PI and PI alone produced petite mutants after longer times of incubation at 50 and 100μM. In these experiments, many sectored colonies were observed (Fig. 3).

The combined drug effects were also evaluated on resting cells. Less than 3% petites were produced after incubation for 12 hours with 100μM PI and also spontaneous mutations were less than 3% throughout the incubation of each experiment (data not shown). However, PI did influence petite induction by EBr illustrating its ability to penetrate cells. As shown in Fig. 4A, 10μM of EBr rapidly produced petite cells and 3 hr incubation resulted in nearly 100% petite formation in all cases. When PI concentrations in the range of 50-100μM were also used, continued exposure to drugs for 10 hours gave rise to a phase in which respiratory competence was recovered in 20-70% of the cells. A twofold excess of PI did not cause recovery. As can be seen in 4B, prolonged initial exposure to PI prior to addition of EBr prevented achievement of the maximum petite induction by EBr (seen with simultaneous addition in Fig. 4A) and accelerated somewhat the appearance of the recovery phase. Recovery in each instance was accompanied by production of sectored colonies. Loss of cell viability during exposure to either the EBr and/or PI was very low in each experiment (at least 90% viable counts). The decrease in percentage of cells which were respiratory incompetent during the recovery phase, therefore, could not be attributed to a selective killing of petite cells with prolonged incubation at high PI concentrations. Similar results have been obtained using other strains of starved or non starved cells.

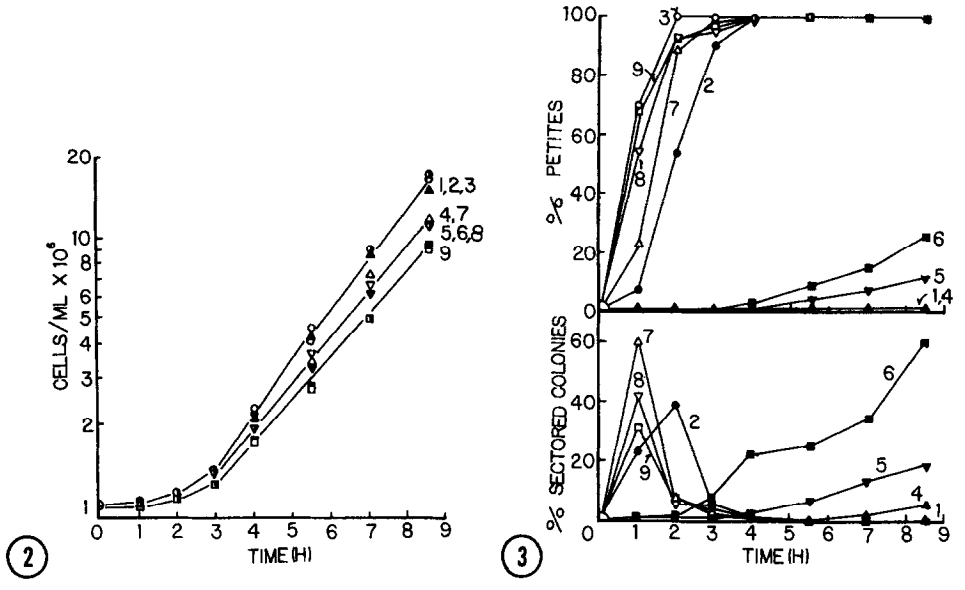

Fig. 2 Growth curve in the presence of ethidium and/or propidium.

Cells of Saccharomyces cerevisiae were grown at 30° in YPD medium to late exponential phase. Cells were harvested and resuspended in 10 ml of the same medium in a 50 ml foil covered flask containing ethidium and/or propidium at cell populations 10^6 cells/ml and cultured in the shaking water bath at 30° with: (1) no drug; 2,3) 5, 10μM ethidium; 4,5,6) 10, 50, 100μM propidium; 7) 10μM of propidium and 5μM ethidium; 8) 50μM of propidium and 5μM of ethidium; 9) 100μM of propidium and 5μM of ethidium. After each interval, samples of cells were taken and counted by haemocytometer after suitable dilutions.

Fig. 3 Petite inductions by ethidium and propidium

Yeast cells were grown as indicated in Fig. 2 in the presence of ethidium and propidium. Samples of cells were taken at the times indicated, diluted in saline, and plated on 2% agar YPD medium. After 3 days incubations at 30° petite colonies and sectored colonies were determined by tetrazolium overlay method (18). Symbols and conditions were the same as in Fig. 2

DISCUSSION:

Propidium produced petite mutants in complete glucose medium without inhibition of the growth but not in resting cells. Therefore, petite induction by PI may be caused by disruption of normal mtDNA synthesis and not by fragmentation or binding covalently with preformed mtDNA.

PI has similar chemical structure to EBr (Fig. 1) and can form similar non-covalent complexes with DNA (16). Metabolic activation and covalent

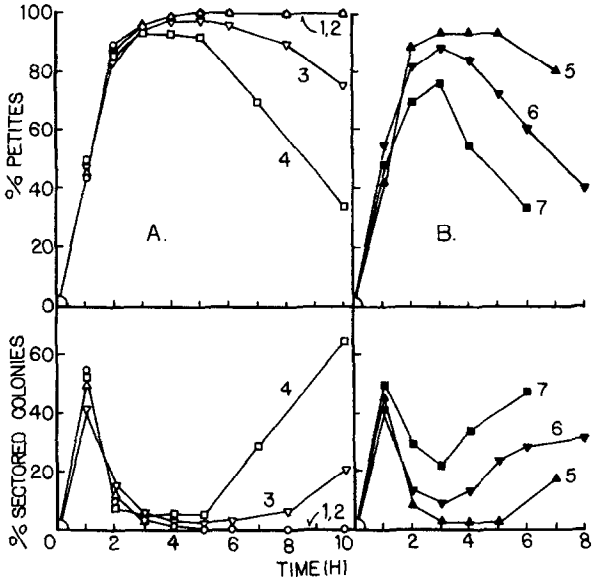

Fig. 4 Recovery of ethidium induced mutagenic events by propidium.

Yeast cells were grown to late exponential phase in YPD medium, washed twice in 0.067M phosphate buffer pH 7.0, and starved overnight in the same buffer, and then suspensions were made at cell population 10^6 cells/ml in the same buffer.

Fig. 4A 10 ml samples were incubated in 50 ml foil covered flasks for 1 hr with: (1) no drug; (2) 20μM; (3) 50μM; (4) 100μM propidium; and then 10μM of ethidium was added and incubations were continued at 30^0 for the time intervals indicated followed by plating on for determination of petites as in Fig. 3.

Fig. 4B 10μM of ethidium was added: (5) at the same time; (6) after 3 hrs; (7) after 5 hrs incubation with 100μM of propidium. Petite colonies were determined at each time indicated as in Fig. 3. Petite inductions by PI were less than 3% even at 12 hr incubation with 100μM of PI.

binding of PI to mtDNA or other cellular targets may not occur because of its complex side chain. Mahler (17) has reported that formation of petite mutants by phenanthridinium dyes required short alkyl chain on ring nitrogen (N^5).

The action of PI in producing petites in growing cells only was similar to that reported for acridine dyes. Its ability to act as a co-mutagen for

mutagenesis by EBr on growing yeast cells was also of interest since EBr, in contrast to PI and the acridines, is a mutagen for resting cells.

A great deal is known about the events which take place following EBr treatment of cells. The importance of covalent drug attachment which was postulated following metabolic activation of ethidium has been emphasized through photoaffinity labeling studies with ethidium analogs (13-15). MtDNA is fragmented following exposure to EBr, and subsequently fragments are lost either by further fragmentation (2) or by segregation (3). Lack of a complete mtDNA complement results with production of respiratory incompetence. The molecular events have been divided into phases due to observations that high drug concentrations could promote late recovery from respiratory incompetence (11, 12, 19). Recovery was also reported following incubation of cells at elevated temperature in phosphate buffer (20). It was proposed previously that the early or premutational state for EBr consisted of intracellular complex with DNA in resting cells which was followed by an additional ATP requiring irreversible step (9, 21, 22). It has not been possible previously to determine whether high concentrations of ethidium served to reverse the initial phase or to inhibit the irreversible step. Our observations that PI can cause recovery but is not mutagenic for resting cells may aid in distinguishing between alternate models in subsequent experiments. The complex side chain of propidium could lead to delayed cell penetration and account for the delayed recovery observed. However, the observation of its co-mutagenic effect with EBr at early time intervals in growing cells suggests that cells take up the drug as early as 1 hr.

Other chemicals which have shown protective effects for petite mutation by EBr include nalidixate (23-25) and caffeine (26, 27).

The mechanisms for recovery of respiration competence are also unknown for these drugs. Their understanding could also assist in elucidating the pathways for induction in yeast.

REFERENCES:

1. Slonimski, P. P., Perrodin, G. and Croft, J. H., Biochem. Biophys. Res. Commun. 30: 232-239 (1968).
2. Perlman, P. S. and Mahler, H. R., Nature New Biol. 231: 12-16 (1971).
3. Deutsch, J., Dujon, B., Netter, P., Petrochilo, E., Slonimski, P. P., Bolotin-Fukuhara, M. and Coen, D., Genetics 76: 195-219 (1974).
4. Fukunaga, M., Morita, T., and Mifuchi, I., J. gen. Appl. Microbiol. 24: 135-142 (1978).
5. Grossman, L. I., Goldring, E. S. and Marmur, J., J. Mol. Biol. 46: 367-376 (1968).
6. Goldring, E. S., Grossman, L. I., Krupnick, D., Cryer, D. R. and Marmur, J., J. Mol. Biol. 52: 323-335 (1970).
7. Goldring, E. S., Grossman, L. I. and Marmur, J., J. Bacteriol. 107: 377-382 (1971).
8. Mahler, H. R. and Bastos, R. N., FEBS Lett. 39: 27-34 (1973).
9. Bastos, R. N. and Mahler, H. R., J. Biol. Chem. 249: 6617-6627 (1974).
10. Mahler, H. R. and Bastos, R. N., Proc. Nat. Acad. Sci. 71: 2241-2245 (1974).
11. Hall, R. M., Trembath, M. K., Linnane, A. W., Wheelis, L. and Criddle, R. S., Molec. gen. Genet. 144: 253-262 (1976).
12. Criddle, R. S., Wheelis, L., Trembath, M. K. and Linnane, A. W., Molec. gen. Genet. 144: 263-272 (1976).
13. Hixon, S. C., White, W. E., Jr. and Yielding, K. L., J. Mol. Biol. 92: 319-329 (1975).
14. Hixon, S. C., White, W. E., Jr. and Yielding, K. L., Biochem. Biophys. Res. Commun. 66: 31-35 (1975).
15. Morita, T. and Yielding, K. L., Mutation Res. (in press) (1978).
16. Waring, M., J. Mol. Biol. 54: 247-279 (1970).
17. Mahler, H. R., J. Supramol. Struct. 1: 449-460 (1972).
18. Ogur, M., John, S. and Nagai, S., Science 125: 928-929 (1957).
19. Wheelis, L., Trembath, M. K. and Criddle, R. S., Biochem. Biophys. Res. Commun. 65: 838-845 (1975).
20. Perlman, P. S. and Mahler, H. R., Biochem. Biophys. Res. Commun. 44: 261-267 (1971).
21. Mahler, H. R. and Perlman, P. S., J. Supramol. Struct. 1: 105-124 (1972).
22. Bastos, R. N. and Mahler, H. R., Biochem. Biophys. Res. Commun. 69: 528-537 (1976).
23. Hollenberg, C. P. and Borst, P., Biochem. Biophys. Res. Commun. 45: 1250-1254 (1971).
24. Whittaker, P. A., Hammond, R. C. and Luha, A. A., Nature New Biol. 238: 266-268 (1972).
25. Vidova, M. and Kovac, L., FEBS Lett. 22: 347-351 (1972).
26. Hixon, S. C. and Yielding, K. L., Mutation Res. 34: 195-200 (1976).
27. Wolf, K. and Kandewitz, F., Molec. gen. Genet. 146: 89-93 (1976).