NON-CHROMOSOMAL RESPIRATORY DEFICIENT MUTANTS INDUCED BY GUANIDINE HYDROCHLORIDE IN *Saccharomyces cerevisiae*

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Summary: Non-chromosomal petites can be produced in *Saccharomyces cerevisiae* by treatment with guanidine hydrochloride, a protein denaturing agent. Its efficiency in inducing petite mutants is comparable to the action of ethidium bromide. The high frequency of petite mutants observed is due to an induction effect rather than to a selection of preexisting mutants. Induction of petites by guanidine hydrochloride occurs even in non growing conditions, indicating that even parental cells are transformed in petites. Transformation depends upon the physiological properties of the cells, since repressed cells, cultivated in the presence of glucose, are more easily transformed than cells cultivated in ethanol.

Yeast respiratory deficient mutants can be induced by damage in the karyotic DNA (chromosomal petites) or in the mitochondrial DNA (cytoplasmic petites or non-chromosomal petites). The cytoplasmic mutants, also called "vegetative petites" (ρ^-), are completely deficient in cytochromes *a* and a_3, completely or almost completely deficient in cytochromes *b* and c_1, but contain normal or slightly higher amounts of cytochrome *c* (1, 2).

Among a wide variety of chemical agents capable of inducing non-chromosomal mutants in yeasts (3), the intercalating dyes acriflavin (4) and ethidium bromide (5) are the most efficient. Certain protein denaturing agents such as the detergents sodium dodecyl sulfate (6), sodium-N-palmitoyl sarcosinate and sodium-N-lauroyl sarcosinate (unpublished results from this laboratory) were shown to be inducers of respiratory deficient mutants in *S. cerevisiae*. Thus, it was of interest to study the induction of such mutations by protein denaturing agents since an association of DNA with the mitochondrial

inner membrane, which is a lipoprotein aggregate, seems highly probable to occur (7, 8).

In the present paper, preliminary studies on the effect of another protein denaturing agent, guanidine hydrochloride (GuHCl), are reported. Its efficiency in inducing petite mutants is much higher than the found for either sodium dodecyl sulfate or sodium-N-palmitoyl sarcosinate and is comparable to that of ethidium bromide.

Materials and Methods

The induction experiments with GuHCl were carried out with a respiratory sufficient strain of S. cerevisiae of ploidy higher than one (strain 1203, W. Slooff, Centraalbureau Voor Shimmelcultures, Delft, Netherlands), as well as with a respiratory sufficient haploid strain (D 58511 C, from F. Sherman). GuHCl (Eastman Organic Chemicals) was recrystallized according to the technique of Nozaki and Tanford (9).

The cells were usually grown in a medium containing 2.0 percent dextrose, 0.6 percent yeast extract and 0.8 percent peptone (YPD medium). The pH of this medium was not altered by the addition of GuHCl. Other media containing either 3.0 percent glycerol (YPG) or 0.2M ethanol (YPE), both non-fermentable substrates, instead of glucose, were also employed. The yield of petites was assayed in some cases as the absolute number of mutants in the population and in other cases as the percentage of mutants using the triphenyltetrazolium chloride (TTC) overlay technique of Ogur et al. (10) ; only those colonies which were entirely colorless were scored as petites. Complementation tests were carried out by overnight incubation in YPD of a mixture of the respiratory deficient strains induced by GuHCl with the chromosomal respiratory deficient strain D 6021 A (from F. Sherman) followed by a growth test on YPG.

Results and Discussion

There are two possibilities to explain the sigmoidal curve obtained when the frequency of petites was plotted against the concentration of GuHCl in YPD (Fig. 1) : - either several molecules of GuHCl are needed to produce a mutational event or several targets per cell are involved in such an effect. A similar "cooperative" effect was observed for the induction of petites by ethidium bromide (5).

The response to GuHCl (Fig. 2) was not immediate, and a lag time of about two hours was needed to observe the first effects. On the other hand, the total number of cells in the GuHCl treated population (normal plus petite cells) never equaled the number of cells in the control experiment (without GuHCl). This is an indication that besides inducing petites, GuHCl also interferes with cell growth. The proportion of petites increased from 5 to 90 percent in about 270 minutes, showing that GuHCl acts as a highly efficient inducer.

The appearance of petite cells in the presence of GuHCl is due to induction rather than to cell selection. This was demonstrated by carrying out the induction experiment in a medium containing glycerol as the sole carbon source. As it is shown in Fig. 3, the number of petite cells increased greatly in the presence of GuHCl. Since the growth medium is totally selective against mutant cells (petites are unable to grow in non-fermentable substrates such as glycerol), a possible selection for petites is excluded.

When yeast cells were grown in a glucose containing medium, subsequently starved and then transferred to a solution of GuHCl in phosphate buffer (non-growing conditions) the number of normal cells decreased, while the number of petite cells increased as a function of the length of time of exposure to GuHCl (Fig. 4). Thus cell multiplication

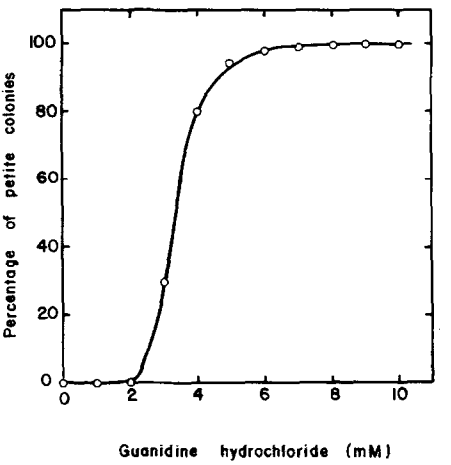


Fig. 1. The effect of GuHCl on the production of petites. S. cerevisiae, strain 1203, was grown in YPD medium for 24 hours at 28°C. Cells were then inoculated at a density of 2 x 10^4 cells per ml into a fresh medium of the same composition and containing GuHCl in concentrations varying from 1 to 10 mM. After 24 hours of growth at 28°C, cell suspensions from each tube were conveniently diluted and plated in Petri's dishes containing YPD medium. After two days growth of the colonies at 28°C, the differentiation between normal and petite colonies was carried out by tetrazolium overlay technique. The values plotted at the ordinate show percentage of petite colonies in relation to the total number of colonies (petites plus normal).

is not a prerequisite for the induction of petites by GuHCl. On the other hand, when cells cultivated in ethanol containing medium were exposed to GuHCl, a smaller difference was found between the total number of cells and the normal cells, which indicates a less effective induction of petites. Thus, at 36 hours of incubation with GuHCl (Fig. 4), the frequency of the petites averaged 53 percent in the case of cells cultivated in ethanol, compared with 98 percent obtained when cells were precultivated with glucose.

Yeast cells grown in the presence of high concentrations of glucose show a decreased number of mitochondria and impaired respiration. On the contrary, cells grown in the presence of ethanol contain the full complement of mitochondria and respiratory enzymes (11, 12, 13). Our

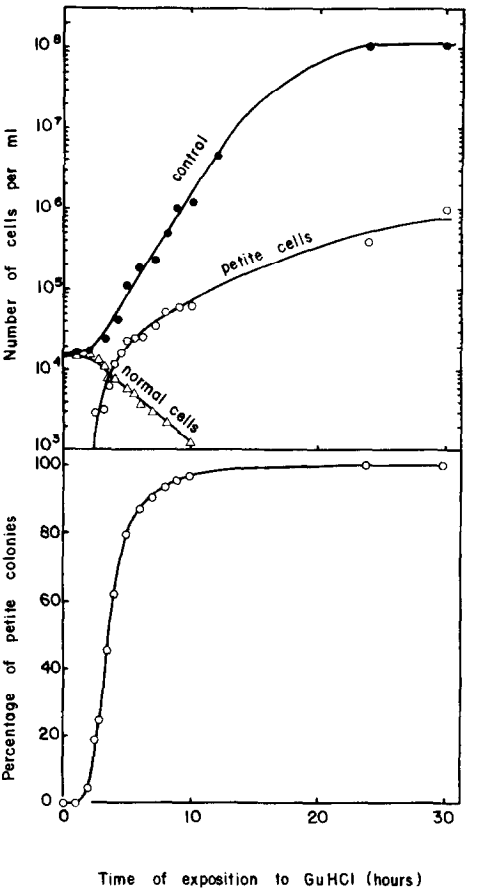
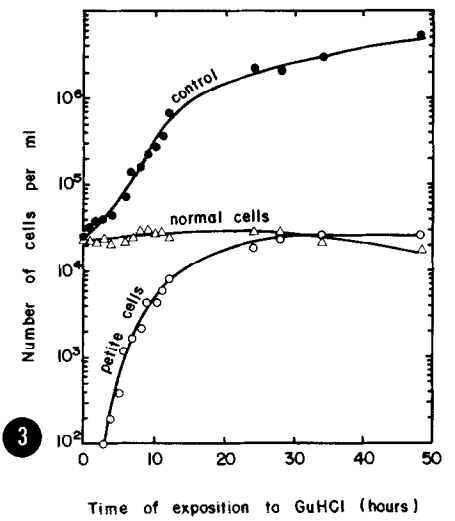


Fig. 2. The relationship between the length of exposition to GuHCl and the production of petites. S. cerevisiae, strain 1203, was grown in YPD medium for 24 hours at 28ºC. Cells were then inoculated at a density of 1 x 10^4 cells of GuHCl. The exposition of the cells to GuHCl was carried out by incubation of the suspension at 28ºC. At different intervals of time, aliquots were taken, diluted properly and plated in YPD medium. After 48 hours of incubation at 28ºC, differentiation between normal and petite colonies was carried out by the tetrazolium overlay technique. The lower figure shows percentage of petites in relation to the total number of cells (petite plus normal).

results suggest that cells containing fewer mitochondria are more readily induced by GuHCl. In addition, this compound acts even upon parental cells in the absence of cell division (Fig. 4). The transformation of ρ^+ into ρ^- cells by ethidium bromide (5) has been observed to occur not only in the absence of cell multiplication but also in cells containing the full complement



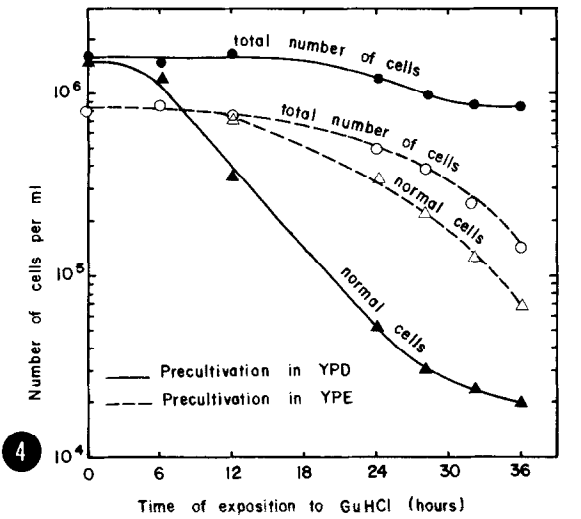


Fig. 3. Induction of petite cells in a glycerol containing medium as the sole carbon source. S. cerevisiae , strain 1203, was grown for 24 hours in YPD at 28ºC. The cells were then washed with 0.1 M phosphate buffer pH 5.7, and finally suspended in YPG medium containing 10 mM GuHCl at a density of about 2.0×10^4 cells per ml. The cell suspension was incubated at 28ºC and aliquots were taken at different intervals of time, diluted, plated in YPD, and incubated 48 hours at 28ºC. The differentiation between normal and petite cells was carried out by means of the TTC overlay technique.

Fig. 4. The effect of precultivation in glucose or in ethanol on the induction of respiratory deficient mutants from S. cerevisiae, strain 1203. Yeast cells were grown in either YPD or YPE media up to the exponential phase. After being harvested and thoroughly washed with 0.1 M phosphate buffer pH 5.7, they were incubated in the same buffer containing 0.1 M glucose. After 20 hours of incubation, cells were harvested, washed and suspended in phosphate buffer, pH 5.7 with no carbon or nitrogen source but containing 10 mM GuHCl. Both suspensions were then incubated at 28º C and aliquots were taken at different intervals of time, diluted and plated on YPD. After an incubation of 48 hours at 28ºC, normal cells and petites were counted by the use of the tetrazolium overlay technique. The total number of cells corresponds to normal cells plus the petites.

of mitochondria, e.g., cells precultivated in ethanol medium. On the other hand, in the induction by acriflavin the parental cells are not transformed into petites (14).

The crossings of 12 haploid strains of petite mutants induced

by GuHCl obtained from the yeast D 58511C (a, ρ^+, lys_1) with chromosomal petite mutants of opposite mating type D 6021 A (α, ρ^+, pet, lys_2, his_1, trp_2) , constantly yielded hybrid cells which could respire and grow on glycerol medium. This fact and the high frequency of petite induction, as described before, indicate that mutants induced by GuHCl are of the ρ^- type.

The cells obtained by the action of GuHCl did not oxidize non-fermentable substrates (acetate, lactate and glycerol) and did not show any respiratory activity as was determined with an oxygen electrode, with ethanol as the substrate. The difference spectrum between a suspension of GuHCl induced petite cells and a suspension of mutant cells saturated with atmospheric oxygen, confirmed the absence of cytochromes a and a_3 and probably b and c_1, which are characteristic properties of the petite phenotype.

It is known that sex (F) and drug resistance (R) factors in Escherichia coli and Shigella flexneri can be eliminated by treatment with acriflavin and ethidium bromide (15-18). Elimination of the penicillinase plasmid, in Staphylococcus aureus, by acridine dyes and ethidium bromide was also reported (18, 19). Sodium dodecyl sulfate and urea are also effective in eliminating the F factor in Escherichia coli (20, 21) , and sodium dodecyl sulfate is effective in eliminating the penicillinase plasmid of Staphylococcus aureus (22). Thus, many protein denaturing agents fall in the category of drugs able to induce cytoplasmic petites in yeast as well as to eliminate extrachromosomal elements in bacteria, suggesting that the ρ determinant in yeast and the bacterial plasmids may have common features (23, 24).

Sonstein and Baldwin (22) suggested that sodium dodecyl sulfate eliminates the penicillinase plasmid by disrupting the membrane

sites of the plasmid attachment. Structures similar to the bacterial mesosome have been shown in yeast mitochondria (25) and the mitochondrial DNA has been considered to be bound to the inner mitochondrial membrane (7, 8). It therefore seems reasonable to suggest that the integrity of the association between mitochondrial DNA and the membrane are involved in the induction of ρ^- mutants by protein denaturing agents, such as GuHCl.

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