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THE INDUCTION OF CYTOPLASMIC PETITE MUTANTS OF *SACCHAROMYCES CEREVISIAE* BY HYDROSTATIC PRESSURE

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SUMMARY

This study demonstrates that hydrostatic pressure is a potent inductive agent of the petite mutation in cultures of *Saccharomyces cerevisiae*. The inductive capacity of this mutagen is dependent on the magnitude and the duration of the pressure treatment. Furthermore, the extent of petite induction varies with the growth stage of the culture. Induction occurs in pressure-treated ($1{\cdot}4 \times 10^4$ lbf in.$^{-2}$ or $9{\cdot}66 \times 10^4$ kN m^{-2} for 4 h) log growth cultures but not in stationary or lag phase cultures. Petite induction and cell survival are also dependent on the particular strain of yeast which is pressure-treated. Tetrad analysis and complementation assays demonstrate that pressure-induced petite cells are cytoplasmic in nature. Moreover, induced petite cells show a wide range of suppressivity (2–99 %) with a large proportion of the petite cells being highly suppressive.

INTRODUCTION

Hydrostatic pressure has a profound effect on a variety of cellular structures and functions (cf. reviews: Zimmerman, 1970, 1971; Sleigh & Macdonald, 1972; Brauer, 1972; Johnson, Eyring & Stover, 1974; Macdonald, 1975; Zimmerman & Zimmerman, 1976, 1977). In general, the cytokinetic and mitotic activities of dividing cells are delayed or inhibited by pressure, depending on the magnitude and duration of the treatment (Zimmerman & Marsland, 1964; Marsland, 1970; Zimmerman, 1971). The form and function of the mitotic apparatus are altered by pressure (Zimmerman & Marsland, 1964; Forer & Zimmerman, 1974, 1976*a*, *b*; Forer, Kalnins & Zimmerman, 1977; Zimmerman, Zimmerman & Forer, 1977). In addition, hydrostatic pressure inhibits replication of DNA in bacteria (Landau, 1970), protozoa (Murakami & Zimmerman, 1973), marine eggs (Zimmerman, 1963; Zimmerman & Silberman, 1967) and mammalian cells (Landau, 1970). The possibility that treatment with hydrostatic pressure may have a direct effect on the expression of the genome has not been resolved.

The budding yeast *Saccharomyces cerevisiae* is a suitable test organism for studying the genetic effect of hydrostatic pressure. A genetic mutation, the cytoplasmic respiratory petite, has been well characterized biochemically and biophysically in this yeast (Mahler, 1973; Casey, Gordon & Rabinowitz, 1974; Gillham, 1974). Although this mutation occurs spontaneously, it may also be induced at high frequencies with

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a variety of mutagens (Nagai, Yanagishima & Nagai, 1961; Sager, 1972). In this paper, we discuss the induction of petite mutants by hydrostatic pressure and the characterization of these mutants.

MATERIALS AND METHODS

Strains

Strains of *Saccharomyces cerevisiae* employed in this study were NCYC 239 (diploid prototroph), C81-14A (α arg4), A364A (a adel ade2 ural gall tyrl his7 lys2), EK-6B (a Ma^+ leu ade ura lys Sup^-), 1412–4D (a $MA3^+$ $MG2^+$ ade2), AN33 (α thrl argl) and X3144–1D (a pet8 ade2 leu2 trpl arg9 his6 canl his2). Strains NCYC 239 and C81–14A were obtained from Dr B. F. Johnson (National Research Council, Ottawa, Ontario). Strain X3144–1D came from the Yeast Genetic Stock Center (Berkeley, California). All other strains were obtained from Dr J. D. Friesen (Dept of Biology, York University, Toronto, Ontario).

Culturing techniques and media

The complete basic medium used for culture growth was YEP (Difco yeast extract 1 %, Difco bacto-peptone 2 %, glucose 2 %). Minimal medium consisted of 2 % glucose and 0·67 % Difco yeast nitrogen base without amino acids. Agar (2 %) was added for plates.

Stock cultures were maintained on YEP agar slants at 4 °C. Axenic working stocks were prepared by inoculating from these slants into 50 ml of YEP medium in a 250-ml Erlenmeyer flask. Cultures were placed in a horizontal water bath (25 °C, 120 oscillations per min). Experimental cultures were prepared by inoculating 0·01 ml of a 48–52 h stock culture into fresh medium (50 ml YEP in a 250-ml Erlenmeyer flask). Experiments were performed on lag phase cultures (*ca.* 10^5 cells/ml) 14 min after inoculation and on log growth cultures (*ca.* 2×10^7 cells/ml) after 17 h of growth. Early stationary cultures (*ca.* 2×10^8 cells/ml) were obtained after 48 h of growth.

Spontaneous petite colonies of the aforementioned strains were subcultured twice and used as petite stocks for pressure experiments. Pressure experiments were performed with petite cultures in log growth phase (17 h).

Cell counts were made on vortexed culture samples with a Model Z_2 Coulter Counter (Coulter Electronics, Hialeah, Fla.) fitted with a 70-μm aperture tube and a 500-μl manometer.

Pressure treatment

Pressure experiments were performed in a pressure–temperature apparatus similar in design to that of Marsland (1950). Cultures were placed in sterile glass containers (capacities of 4, 17, and 58 ml), capped with parafilm and put into a stainless steel pressure chamber. This pressure chamber was connected through stainless steel tubing to an Aminco hydraulic pump which operated on light paraffin oil (Zimmerman, 1971). Hydraulic pressures between $6{\cdot}0 \times 10^3$ lbf $in.^{-2}$ ($4{\cdot}13 \times 10^4$ kN m^{-2}) and $1{\cdot}8 \times 10^4$ lbf $in.^{-2}$ ($1{\cdot}24 \times 10^5$ kN m^{-2}) were applied at a rate of $5{\cdot}0 \times 10^3$ lbf $in.^{-2}$ ($3{\cdot}45 \times 10^4$ kN m^{-2}) per stroke per s. A needle valve was used to release the pressure virtually instantaneously. Temperature was maintained at 25 ± 0·1 °C. Control cultures were placed in parafilm-capped sterile glass containers and maintained at atmospheric pressure within the constant temperature housing for the duration of the experiment.

Culture samples were taken immediately before and after pressure treatment, diluted with 0·9 % NaCl and plated on YEP agar plates. Agar plates were incubated at 25 °C for 5–7 days prior to counting the colonies. The % cell survival of a pressure-treated culture was calculated as

$$\frac{\text{plate viable cells/ml after pressure}}{\text{theoretical plate viable cells/ml before pressure}^*} \times 100$$

where the theoretical plate viable cells/ml before pressure* was,

$$\text{the viable cells/ml before pressure} \times \frac{\text{Coulter count after pressure}}{\text{Coulter count before pressure}}.$$

The petite colonies were identified with the tetrazolium overlay technique (Ogur, St John & Nagai, 1957) with the overlay modified by addition of 1 % ethanol. The dye, 2,3,5-triphenyltetrazolium chloride, was purchased from Sigma Chemical Co., St Louis, Missouri.

Genetic analysis of induced petite mutants

A log growth culture of strain C81–14A was subjected to a pressure treatment of $1{\cdot}4 \times 10^4$ lbf in.$^{-2}$ ($9{\cdot}66 \times 10^4$ kN m^{-2}) for 4 h. Following decompression, the culture was diluted with 0·9 % NaCl and plated on YEP agar. Developing petite colonies were subcloned twice before using them to prepare log growth cultures for genetic analysis.

Zygotes for suppression studies were obtained with a modification of the mating procedure of Lee, Lusena & Johnson (1975). Log growth petite cells were mixed with an equal number of grande cells of strain EK-6B. This mixture was suspended in 50 ml mating medium (1 % Difco yeast extract, 2 % Difco bactopeptone, 10 % glucose) in a 250-ml Erlenmeyer flask and placed in a horizontal water bath (25 °C, 120 oscillations per min) for 4 h. The mixture was plated on minimal medium agar plates. After 5 days growth (25 °C) the clones were overlaid with tetrazolium agar and scored for petite or grande phenotype. The degree of suppressivity was calculated from the formula: $(x-y)/(100-y)$ (where x was the percentage of pure plus sectored petite diploid progeny and y was the percentage of spontaneous petite cells of the grande mater strain EK-6B).

The zygotes from suppression studies were subjected to tetrad analysis. Sporulation was induced by transferring diploid cells to presporulation medium (0·8 % Difco yeast extract, 0·3 % Difco bactopeptone, 10 % glucose, 2 % agar) for 3 days at 25 °C followed by 4–5 days growth on sporulation agar slants (0·1 % Difco yeast extract, 0·05 % glucose, 2 % agar, 1 % potassium acetate). Sporulated cells were digested with glusulase (Endo Laboratories Inc., Garden City, N.Y.) and dissected with the aid of a customized micromanipulator (Lawrence Precision Machine Co., Hayward, Calif.).

Complementation assays were performed between each of pressure-induced petite mutants and the tester strains, X3144–1D (a nuclear petite) and EK-6B-S (an ethidium bromide-induced petite). The drug ethidium bromide has been shown to induce cytoplasmic petite mutants (Slonimski, Perrodin & Croft, 1968). Tester strains were streaked in parallel lines across a YEP agar plate. Pressure-induced petite mutants were streaked across another YEP agar plate. Plates were incubated for 3 days at 25 °C. At this time an impression of the tester strain plate was made on a velveteen pad. An agar plate with pressure-induced petite mutants on it was pressed on to the same velveteen pad, so that the streaks were perpendicular to the first set of streaks. The resulting checkerboard pattern was imprinted from the velveteen pad to another YEP agar plate. After 3 days incubation (25 °C), the pattern was replica-plated on to a minimal medium plate. Three days later, tetrazolium overlay of the latter plate was used to assay for complementation.

RESULTS

Evidence of induction of petite mutants by hydrostatic pressure

Pressure treatment ($1{\cdot}4 \times 10^4$ lbf in.$^{-2}$ ≡ $9{\cdot}66 \times 10^4$ kN m^{-2} for 4 h) of log growth cultures of strains C81–14A and NCYC 239 results in a significant increase in the number of petites, expressed as percent of survivors (Table 1). This increase in the number of petite cells is due to pressure induction of the mutation rather than selection of pre-existing spontaneous mutants. The evidence in support of this proposal is given in the following paragraph, where it is shown that spontaneous

petite cells and grande cells display similar sensitivity to the lethal effects of pressure. The number of petite cells present after pressure treatment is 9·4 and 5·5 times as great as the spontaneous petite frequency in log growth cultures of NCYC 239 and C81–14A respectively.

To ascertain the efficacy of hydrostatic pressure as a petite cell inducer it is necessary to determine the proportion of petite cells present at decompression which are due to: (1) induction of the mutation by the agent; or (2) the selective survival of spontaneous petite mutants to treatment by the agent. The latter value may be estimated by subjecting log growth cultures of spontaneous petite isolates of each strain to pressure treatment and determining cell survival after decompression. The average

Table 1. *Evidence of induction of petite mutants by hydrostatic pressure**

	Before pressure	After pressure
	NCYC 239	
Plate viable cells/ml	$22{\cdot}37 \times 10^{6}$	$31{\cdot}31 \times 10^{4}$
% petite	$0{\cdot}52 \times 10^{-1}$	$34{\cdot}82 \pm 2{\cdot}83$
Actual no. of petite cells/ml	$11{\cdot}63 \times 10^{3}$	$10{\cdot}90 \times 10^{4}$
	C81–14A	
Plate viable cells/ml	$33{\cdot}49 \pm 10^{6}$	$22{\cdot}44 \times 10^{5}$
% petite	0·50	$41{\cdot}23 \pm 2{\cdot}19$†
Actual no. of petite cells/ml	$16{\cdot}75 \times 10^{4}$	$92{\cdot}45 \times 10^{4}$

* Log growth cultures were subjected to $1{\cdot}4 \times 10^{4}$ lbf in.$^{-2}$ ($9{\cdot}66 \times 10^{4}$ kN m^{-2}) for 4 h at 25 °C.
† Petites, as % of survivors (n = 5).

Table 2. *Calculated contribution of spontaneous petite mutants to petite cell population present after pressure treatment**

Petite cells/ml before pressure	$11{\cdot}63 \times 10^{3}$
No. of these petite cells surviving treatment, i.e. 8·0 ± 0·9 %†	$9{\cdot}30 \times 10^{2}$
Total no. of petite cells/ml after treatment	$10{\cdot}90 \times 10^{4}$
Calculated % petites after pressure treatment due to survival of spontaneous petites is	

$$\frac{9{\cdot}30 \times 10^{2}}{10{\cdot}90 \times 10^{4}} \times 100 = 0{\cdot}85\,\%$$

* $1{\cdot}4 \times 10^{4}$ lbf in.$^{-2}$ ($9{\cdot}66 \times 10^{4}$ kN m^{-2}) for 4 h at 25 °C. Strain NCYC 239.
† n = 5.

% cell survival of petite cells (5 independent isolates) after a pressure treatment of $1{\cdot}4 \times 10^{4}$ lbf in.$^{-2}$ ($9{\cdot}66 \times 10^{4}$ kN m^{-2}) for 4 h was 8·0 ± 0·9 (n = 5) and 19·6 ± 2·1 (n = 5) for NCYC 239 and C81–14A petite mutants, respectively. This corresponds to a % cell survival for grande strains NCYC 239 and C81–14A of 1·4 ± 0·2 (n = 14) and 6·7 ± 0·6 (n = 5) respectively. With these values it is possible to estimate that only 0·85 % of the petite cells present after pressure treatment of strain NCYC 239 and 3·6 % of petite cells of treated strain C81–14A were due to survival of spontaneous petite mutants (see Table 2 for calculations).

Effect of magnitude and duration of hydrostatic pressure on the extent of cell survival and petite induction

The extent of cell survival and petite induction by hydrostatic pressure is dependent on the magnitude and the duration of the pressure treatment. Cultures of NCYC 239 exposed to various magnitudes of pressure (6×10^3 lbf in.$^{-2}$, $4{\cdot}13 \times 10^4$ kN m^{-2}; 10^4 lbf in.$^{-2}$, $6{\cdot}89 \times 10^4$ kN m^{-2}; $1{\cdot}4 \times 10^4$ lbf in.$^{-2}$, $9{\cdot}66 \times 10^4$ kN m^{-2}; $1{\cdot}5 \times 10^4$ lbf in.$^{-2}$, $1{\cdot}035 \times 10^5$ kN m^{-2}; and $1{\cdot}8 \times 10^4$ lbf in.$^{-2}$, $1{\cdot}24 \times 10^5$ kN m^{-2}) for a constant duration (4 h) display a gradual decrease in cell viability accompanied by an increase in petite frequency with a maximum petite frequency obtained at $1{\cdot}4 \times 10^4$ lbf in.$^{-2}$ ($9{\cdot}66 \times 10^4$ kN m^{-2}). At higher magnitudes of pressure there is a decline in the petite frequency at decompression (Fig. 1). A similar pattern of increase followed by decrease in petite frequency is observed when cultures of NCYC 239 are exposed to increasing durations (0·5, 2, 3, 4 and 5 h) of a constant magnitude of pressure ($1{\cdot}4 \times 10^4$ lbf in.$^{-2}$, $9{\cdot}66 \times 10^4$ kN m^{-2}) (Fig. 2).

Pressure-treated petite cells

Log growth cultures of NCYC 239 petite C (a spontaneous isolate) show a greater survival capacity than grande cultures under all of the aforementioned pressure magnitudes and durations (see Fig. 3A, B). Furthermore, these survival values may be used to estimate the fraction of spontaneous petite mutants, in a grande culture, which survive a pressure treatment. To do this, it is necessary to assume that the survival of spontaneous petite C cultures to these pressure treatments is representative of the survival of all spontaneous petite mutants present in pressure-treated grande cultures. When this calculation is made it is apparent that induced rather than spontaneous petite mutants represent the majority of petite cells present after all of the durations and magnitudes of pressure studied.

Strain specificity of pressure action

The effect of pressure treatment ($1{\cdot}4 \times 10^4$ lbf in.$^{-2}$, $9{\cdot}66 \times 10^4$ kN m^{-2}, for 4 h) on cell survival and petite frequency varies with the strain of yeast investigated (Fig. 4). For example, the pressure-sensitive strain EK-6B has a cell survival value of $0{\cdot}07 \pm 0{\cdot}02\%$ ($n = 4$). In contrast, the strain AN33 has a cell survival value of $12{\cdot}3 \pm 1{\cdot}6\%$ ($n = 3$). Variability is also seen in the petite mutation frequency of pressure-treated yeast strains. Pressure-treated cultures of EK-6B display the smallest increase in petite frequency of the 6 strains tested.

The influence of culture age on mutagenic and lethal actions of pressure

The growth stage of the yeast culture is of prime importance in determining the mutagenic and lethal effects of hydrostatic pressure. Petite induction does not occur in pressure-treated ($1{\cdot}4 \times 10^4$ lbf in.$^{-2}$, $9{\cdot}66 \times 10^4$ kN m^{-2} for 4 h) lag phase and stationary growth cultures. Furthermore, mortality is absent in pressure-treated stationary cultures and negligible in lag phase cultures. The lethal and mutagenic

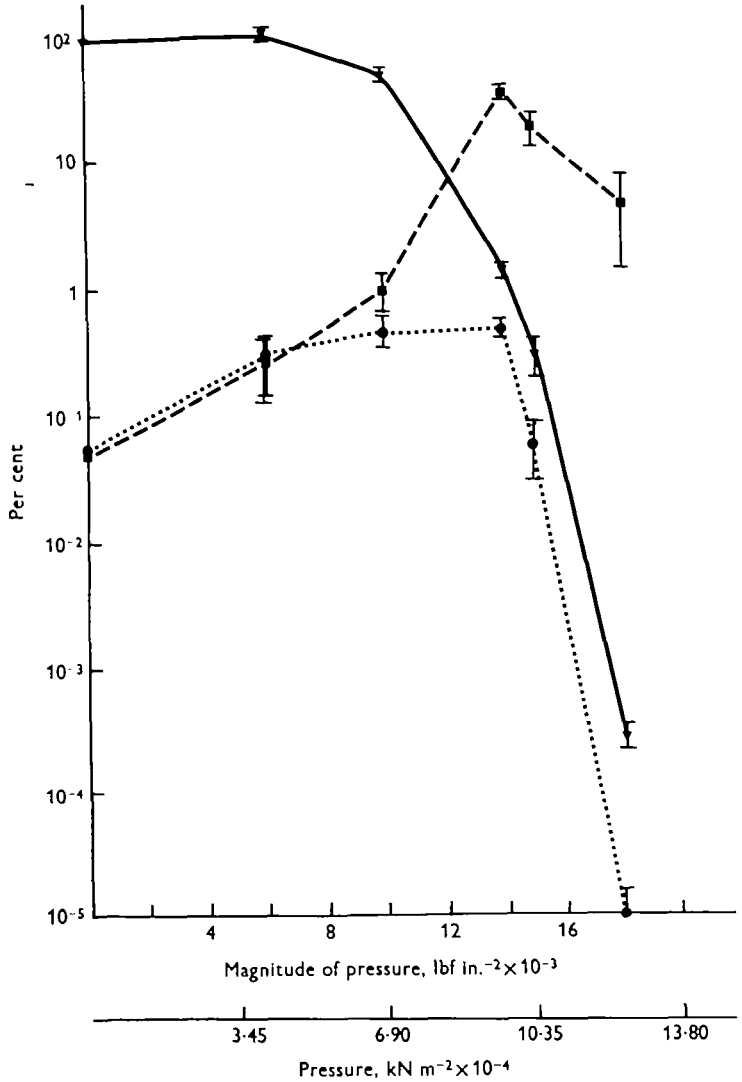

Fig. 1. Effect of the magnitude of applied pressure on cell survival and petite induction. Log growth cultures of NCYC 239 were subjected to various hydrostatic pressures for a constant duration of 4 h at 25 °C. The following indices were determined: % survival (▼——▼); and the number of petite cells expressed as % of survivors (■ – – – ■) and as % of pressure-treated population (●----●). Standard errors of all treatments are shown (n = 3 for all treatments except for $1{\cdot}4 \times 10^4$ lbf in.$^{-2}$ where n = 14).

actions of this pressure treatment appear to be confined to actively growing (log phase) cultures of yeast (Table 3).

Genetic studies of pressure-induced petite mutants

Tetrad analysis of zygotes obtained from crosses of pressure-induced petite mutants (C81–14A) and the grande tester strain (EK-6B) demonstrated the presence

of a cytoplasmic inheritance of the petite phenotype. A similar conclusion was obtained with complementation assays between the pressure-induced mutants and tester strains. Complementation occurred when petite mutants were crossed with cultures of the nuclear petite tester strain, X3144-1D; however, complementation was absent in crosses between the induced mutants and EK-6B-S, an ethidium bromide-induced petite.

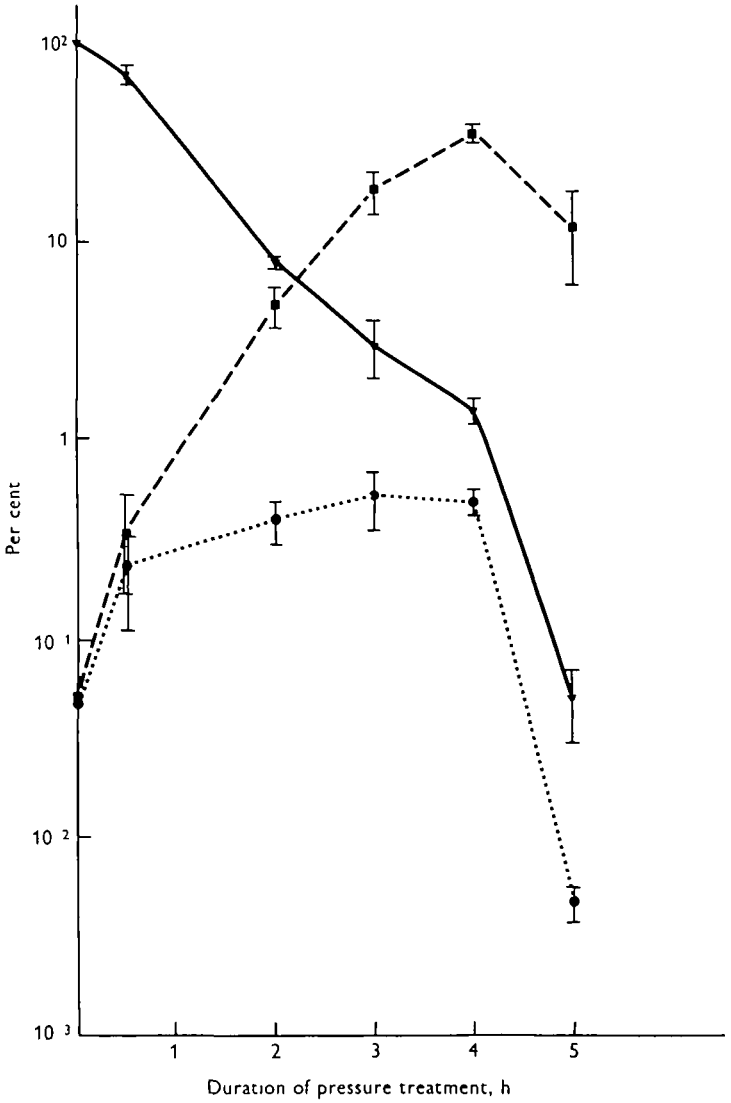

Fig. 2. Effect of the duration of applied pressure on cell survival and petite induction. Log growth cultures of NCYC 239 were subjected to various durations of a constant hydrostatic pressure ($1{\cdot}4 \times 10^4$ lbf in.$^{-2}$) at 25 °C. The following indices were determined: % survival (▼——▼); and the number of petite cells expressed as % of survivors (■ – – – ■) and as % of pressure-treated population (●----●). Standard errors for all treatments are shown (n = 3 for all treatments except the 4-h treatment, where n = 14).

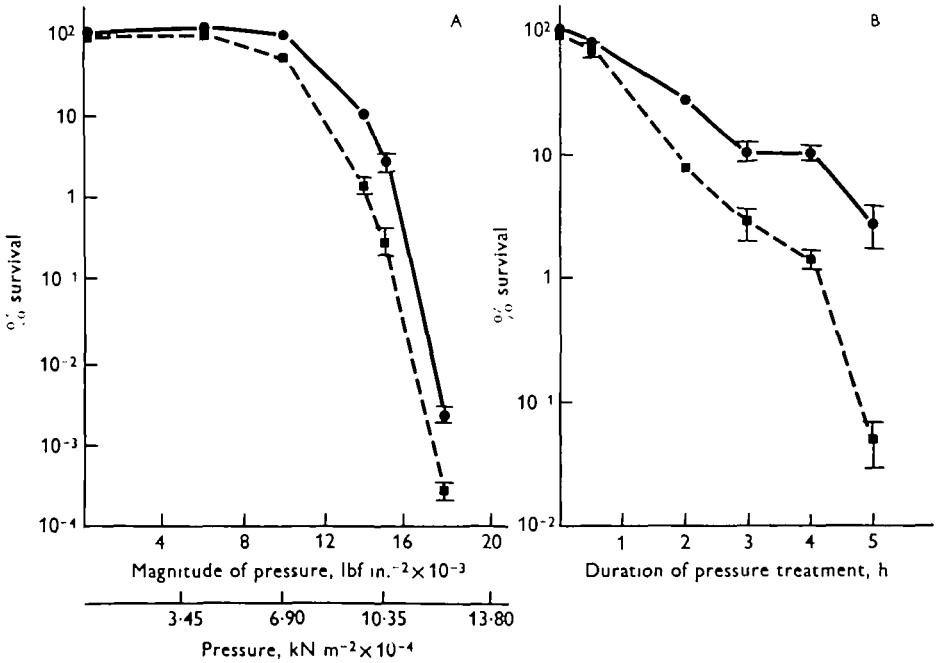

Fig. 3. Comparative cell survival of grande and petite cultures when subjected to various magnitudes and durations of pressure. Log growth cultures of NCYC 239 spontaneous petite C (●——●) and grande NCYC 239 (■ – – ■) were subjected to (A) various pressures for a constant duration of 4 h or (B) various durations of pressure at a constant magnitude of $1{\cdot}4 \times 10^4$ lbf in.$^{-2}$. After each treatment the % cell survival was calculated. Standard errors are shown for each treatment (n = 14 for treatment of grande culture with $1{\cdot}4 \times 10^4$ lbf in.$^{-2}$ for 4 h. In all other treatments n = 3).

There was a wide variation in the suppressivity of pressure-induced petite mutants (2–99%). However, a large proportion of the mutants were highly suppressive (Fig. 5). The 2 spontaneous mutants assayed also displayed an extremely high suppressivity (99%).

DISCUSSION

In general, these studies demonstrate that hydrostatic pressure is a potent inductive agent of the cytoplasmic petite mutation in cultures of *Saccharomyces cerevisiae*. The inductive capacity of this mutagen is dependent on both the magnitude and the duration of the pressure treatment. Moreover, the extent of petite induction by pressure treatment varies with the strain of yeast and the growth stage of the culture.

There have been several reports of the inhibition by hydrostatic pressure of the incorporation of precursor molecules into the DNA of a variety of organisms: *Escherichia coli* (Pollard & Weller, 1966; Landau, 1970), fertilized eggs of *Arbacia punctulata* (Zimmerman, 1963; Zimmerman & Silberman, 1967) and the protozoa *Tetrahymena pyriformis* (Murakami & Zimmerman, 1973). In addition, Landau (1967, 1970) has shown that pressure treatment inhibits the processes of induction,

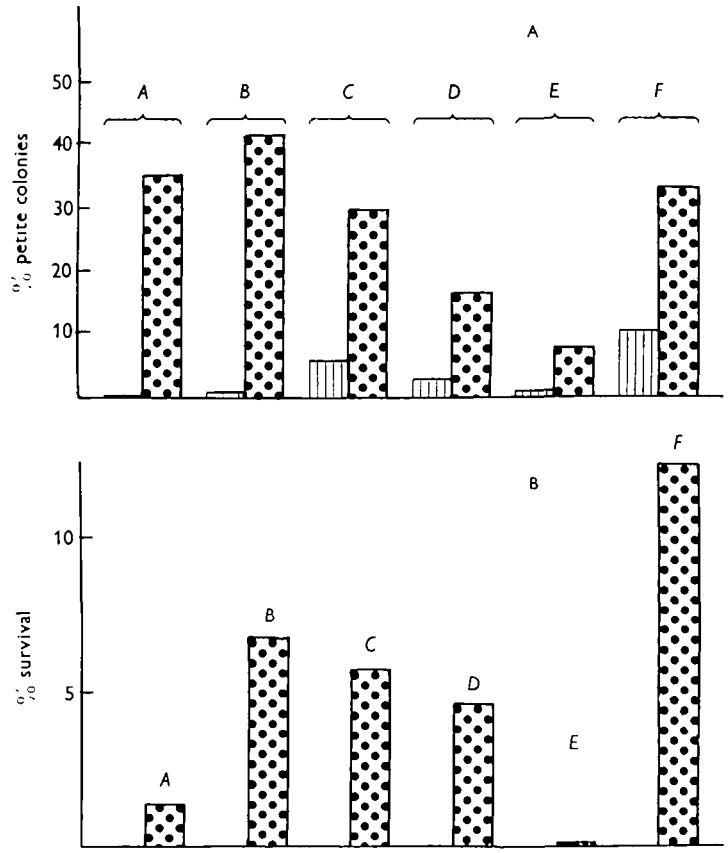

Fig. 4. The effect of hydrostatic pressure treatment on cell survival and petite frequency of several yeast strains: *A*, NCYC 239; *B*, C81–14A; *C*, A364A; *D*, 1412–4D; *E*, EK6B; and *F*, AN33. Log growth cultures of several yeast strains were subjected to $1{\cdot}4 \times 10^4$ lbf in.$^{-2}$ for 4 h at 25 °C. The following indices were measured for each yeast strain: A, % of colonies which were petite before (▥) and after (▩) pressure treatment; and B, % cell survival after pressure treatment.

Table 3. *Effect of hydrostatic pressure treatment* on various stages of culture growth*

Growth phase	% Budding	% Cell survival at decompression ± S.E.	% Petite/survivors at decompression ± S.E.
Lag	3·1	94·1 ± 6·9†	0
Log	89·3	1·4 ± 0·2‡	34·8 ± 2·8
Stationary	2·8	102·3 ± 6·6†	0

* $1{\cdot}4 \times 10^4$ lbf in.$^{-2}$ ($9{\cdot}66 \times 10^4$ kN m^{-2}) for 4 h.
† $n = 3$.
‡ $n = 14$.

translation and transcription of the enzyme β-galactosidase in *E. coli*. There have been indications that pressure affects not only the DNA replication and transcription but also the information content of the genome (McElroy & de la Haba, 1949; Rutberg & Hedén, 1960; Palmer, 1961; Hedén, 1964; Gross, 1965; Vacquier & Belser, 1965). The present study clearly shows that high-pressure treatment results in an inheritable genetic change.

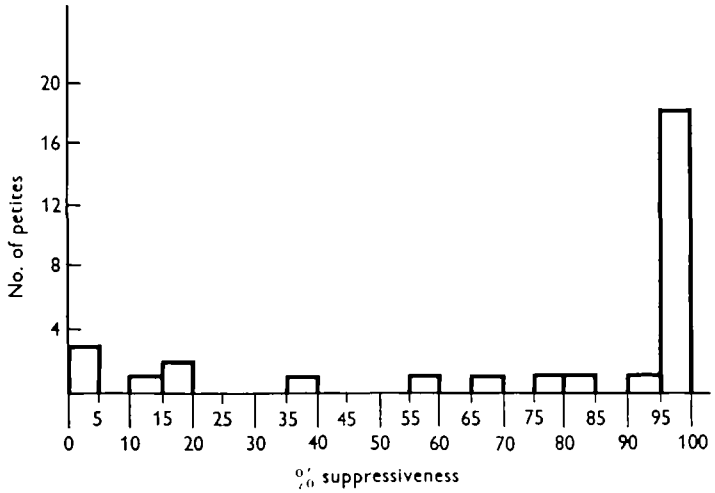

Fig. 5. The number of pressure-induced petite mutants which display particular degrees of suppressiveness. Petite mutants were induced with a hydrostatic pressure treatment ($1{\cdot}4 \times 10^4$ lbf in.$^{-2}$, $9{\cdot}66 \times 10^4$ kN m^{-2} for 4 h) of log growth cultures of strain C81–14A. The suppressivity of these mutants was determined by crossing them with the tester strain EK-6B and assaying the resulting zygotes for respiratory sufficiency.

Dose-response patterns of cell survival and petite induction are characteristic of the mutagen employed. With increasing doses (magnitude or duration) of hydrostatic pressure treatment, there is an increase in petite induction followed by a decrease in petite frequency at larger doses. A similar pattern of petite induction has been reported for yeast cells subjected to increasing durations of high-temperature treatment (Schenberg-Frascino & Moustacchi, 1972) or to high doses of H_2O_2 (Thacker, 1975).

There are several possible explanations for the decline in the petite mutation frequency following high doses of pressure. The petite mutation frequency may have declined with high mutagen doses due to a preferential inactivation of petite mutants under these conditions. However, preliminary results obtained with pressure-treated grande and petite (spontaneous and pressure-induced) cultures indicate a similar or greater survival capacity of petite mutants to a range of pressure treatments $4{\cdot}13 \times 10^4$ kN m^{-2} to $1{\cdot}24 \times 10^5$ kN m^{-2} for 0·5 to 5 h). Thacker (1975) has suggested the possibility that lesions produced in the DNA at high doses are reversing the initial potentially mutagenic lesions or alternatively, there may be a progressive inactivation of an error-prone repair system at high doses.

The lethal and mutagenic actions of pressure ($9{\cdot}66 \times 10^4$ kN m^{-2} for 4 h) appear to be limited to log growth cultures. Lag phase and stationary growth cultures are resistant to this pressure treatment. There may be several reasons for the resistance of stationary cells to pressure. Stationary phase cultures are characterized by the exhaustion of growth medium as well as modifications in the amino acid and nucleotide pools (Schenberg-Frascino & Moustacchi, 1972). Furthermore, stationary yeast cells are arrested just prior to nuclear DNA replication (Williamson & Scopes, 1960). Schenberg-Frascino & Moustacchi (1972) have reported that the single cell stage (interphase) of synchronized cultures is very resistant to both lethal and mutagenic actions of high temperature. There are indications that a similar variation in pressure sensitivity may be occurring during the yeast cell cycle (Rosin & Zimmerman, 1976).

All analysed pressure-induced petite mutants display extranuclear inheritance. Moreover, these petite mutants (strain C81–14A) have a wide range of suppressivity (2–99%) with the majority of the mutants displaying high suppression values (above 90%). The absence of neutral petites implies that all of the analysed mutants retained some mitochondrial DNA (Michaelis, Douglass, Tsai & Criddle, 1971). The wide range of suppression values may indicate that mitochondrial DNA is altered by pressure treatment to different extents in different cells.

The results of this study demonstrate that hydrostatic pressure may be a useful tool in the study of microbial control mechanisms. Hydrostatic pressure can be used as an agent to gain information on a specific class of cytoplasmic mutants, the respiratory petites.

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