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The influence of cell ploidy on the thermotolerance of *Saccharomyces cerevisiae*

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Summary. The resistance of *Saccharomyces cerevisiae* to inactivation by DNA damaging agents has long been known to be affected by cell ploidy. Resistance is greater for diploid than for haploid cells, but exhibits decreases for further increases in ploidy beyond diploid. In this study *S. cerevisiae* cells whose genomes differ only in their ploidy were employed to investigate how ploidy directly influences resistance to thermal killing. In virtually all species resistance to thermal killing is a cellular property that is elevated by heat shock and other agents that induce the heat shock response. We therefore investigated how ploidy affected the thermal killing of *S. cerevisiae* cells both before and after elevation of thermotolerance by means of a 40 min 25 °C to 38 °C heat shock. Without such induction of thermotolerance there was negligible effect of ploidy on thermal killing. In contrast in the heat shocked cultures there was an appreciable decrease in thermotolerance as ploidy increased. This difference indicates that the lethal thermal damage in the thermotolerance induced cultures is not totally equivalent to that in cells not given a prior heat shock, and that gene expression changes after heat shock result in a ploidy effect on heat tolerance which is absent from cells in which the heat shock response has not been induced.

Key words: Heat shock – Thermotolerance – Ploidy – Yeast

Introduction

The altered gene expression patterns that cells elicit in response to stressful conditions often appear to operate so as to increase tolerance to the stress (Walker 1984; Haynes and Kunz 1983; Neidhardt et al. 1984). When stressed through exposure to supra-optimal temperatures the cells of virtually all species display alterations in their physiology and protein synthesis (the "heat shock response"). Through these events they become better equipped to withstand high temperature. The study of the factors necessary for this enhancement or induction of thermotolerance has focussed mainly on the proteins synthesised in response to heat shock (heat shock proteins (HSPs)) and their roles (Walker 1984; Neidhardt et al. 1984; Pelham 1985; 1986). Recent studies have shown that inactivation of individual genes of the *S. cerevisiae* HSP70 gene family can have dramatic effects on temperature ranges over which *S. cerevisiae* cells will grow but does not affect the rate of killing at lethal temperatures (Craig and Jacobsen 1985).

Our primary objective in this study was to obtain evidence as to whether the thermal killing of *S. cerevisiae* is influenced by any of the properties of cells affected by their ploidy. The most serious effect of high temperature is thought to be protein denaturation damage (Pelham 1985, 1986). Another detrimental influence might be an inability of cells to maintain their ion or energy balance, and this may be exacerbated with the increased cells size associated with higher ploidy. Some of the glycolytic enzyme genes of *S. cerevisiae* are transcriptionally activated by heat shock (Piper et al. 1986) and this may be part of a protective mechanism serving to maintain ATP levels during heat stress. Finally, if single or double strand breaks in DNA are a major cause of the thermal killing of cells, then this killing might be markedly affected by ploidy in a manner comparable to the influences of ploidy on the inactivation of *S. cerevisiae* cells by γ-irradiation (Owen and Mortimer 1956; Mortimer 1958) and ultraviolet irradiation (Sarachek 1954). Whatever the primary events leading to killing at high

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Table 1. Properties of haploid (N), diploid (2N) and tetraploid (4N) cells of strain MD40-4c

	Mean DNA content/ cell (fg)	Mean cell vol (fl)	Cell doubling time (30 °C) on YEPD medium (min)
N	21 ± 2	97	82
2N	42 ± 2	87	87
4N	84 ± 11	103	103

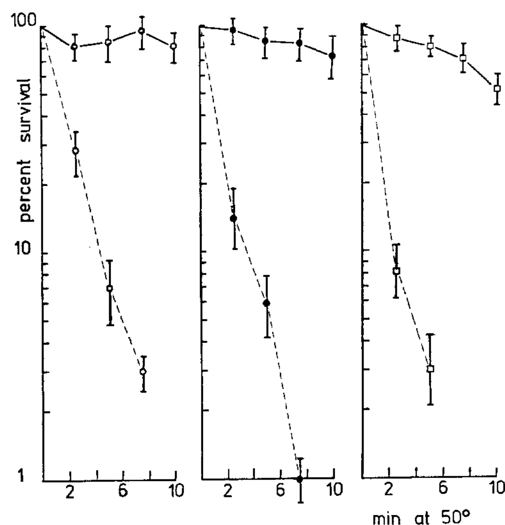

Fig. 1. The survival of haploid (○), diploid (●) and tetraploid (□) MD40-4c cells at 50 °C in YEPD medium. The broken lines represent the cultures rapidly shifted from 25 °C to 50 °C, and solid lines cultures whose thermotolerance had been enhanced by a heat shock (25 °C to 38 °C for 40 min) given prior to the 50 °C incubation

temperature, they are presumably the target of that part of the heat shock response that leads to enhanced thermotolerance.

Materials and methods

Strains. Commencing with the haploid (N) *S. cerevisiae* strain MD40-4c (MATα his3-11 his 3-15 trpl ura2 leu2-3 leu2-112), cells of higher ploidy (diploid (2N) and tetraploid (4N)) were constructed by the technique of transformation-associated cell fusion (Takagi et al. 1985) and characterised by size and by measurements of cellular content of DNA (Table 1). Although the volume of the cells increases with increased ploidy, it does not increase proportionally (Table 1). Higher ploidy also results in a small increase in cell doubling time (Table 1). The maximum growth temperature of cells on YEPD medium varies by less than 1 °C for MD40-4c cultures of N, 2N and 4N ploidy, and is between 38.5 °C and 39.5 °C.

All experiments were on cultures grown at 25 °C to mid-logarithmic phase of growth (3–7 x 10^6 cells/ml) on rich (2% bactopeptone, 1% yeast extract) medium (YEPD) containing 2% glucose as carbon source. The heat shock treatment used for induction of thermotolerance was a rapid shift of 2 ml of each culture (the "induced" cells) from 25 °C to 38 °C, maintaining these cells at 38 °C for 40 min prior to an immediate shift to 50 °C or 52 °C for a variable period. An identical 2 ml portion of each culture (the "uninduced" cells) was transferred immediately from 25 °C to either 48 °C or 50 °C for variable times. At intervals from 0 to 20 min whilst the cultures were at 48 °C, 50 °C or 52 °C 0.1 ml aliquots of both the induced and uninduced cultures were rapidly diluted into 2 ml YEPD at room temperature (21–23 °C), and, within 20 min of exposure to the high temperature, cells were plated on YEPD plates (300–700 cells per plate) so that killing could be measured as inhibition of 25 °C macrocolony formation. Unlike many experiments involving DNA damaging agents (Haynes and Kunz 1983) an extended post-injury holding of the cells prior to plating was not adopted since this was not employed in previous studies of ploidy and DNA damage (Sarachek 1954; Owen and Mortimer 1956; Mortimer 1958).

Results

S. cerevisiae cells grown at 25 °C prior to exposure to temperature of around 50 °C are killed rapidly at the higher temperature whereas a prior heat shock causes marked protection against 50 °C killing (McAlister and Finkelstein 1980; see also Fig. 1). This induction of thermotolerance by a heat shock to sublethal temperatures occurs in *S. cerevisiae* growing on both fermentative and gluconeogenic carbon sources (M. W. D. unpublished results) and is a property of the cells of many disparate species. Induction of thermotolerance also occurs in *S. cerevisiae* in the absence of heat shock during exposure to ethanol (Plesset at al. 1982), and following exposure to sub-lethal doses of ionising radiation (Mitchel and Morrison 1982). However, all the experiments on strains of different ploidy described in this communication utilized the same conditions of heat shock (25 °C to 38 °C for 40 min) to induce thermotolerance, and employed cells grown to similar density at 25 °C in medium containing 2% glucose. *S. cerevisiae* exhibits a higher maximal growth temperature in fermentative as compared to gluconeogenic media (Van Udem and Madiera-Lopes 1975), and is protected against killing at 44 °C by the presence of glucose (Madiera-Lopes 1982). It is therefore manifestly better equipped to withstand prolonged heat stress in media containing glucose. Also in investigations of how the genotype of different strains may affect their thermotolerance it is important to compare cultures of comparable physiological state. In this study we compared cultures which, prior to heat treatment, were in exponential growth. This was because the basal thermotolerance of cells, that is the thermotolerance without any elevation through heat shock to sublethal tempera-

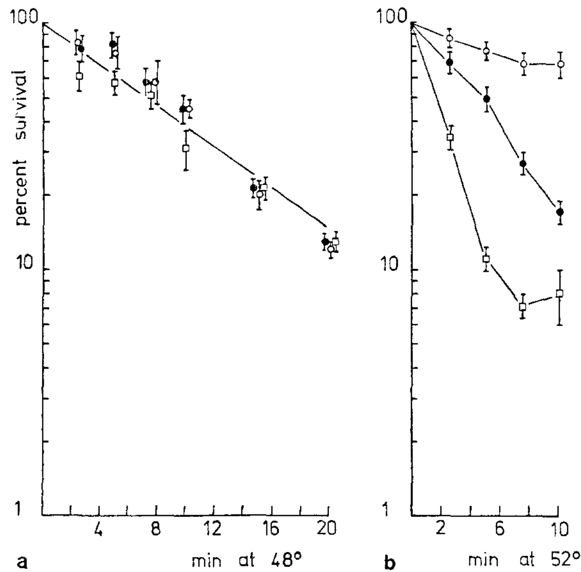

Fig. 2a, b. Survival of haploid (○), diploid (●), and tetraploid (□) MD40-4c cells at 48 °C (*a*) and 52 °C (*b*). The 48 °C viability measurements (*a*) were on cultures uninduced for thermotolerance and shifted to this temperature from 25 °C. The 52 °C viability measurements (*b*) were on cultures whose thermotolerance had been increased by a prior heat shock from 25 °C to 38 °C for 40 min

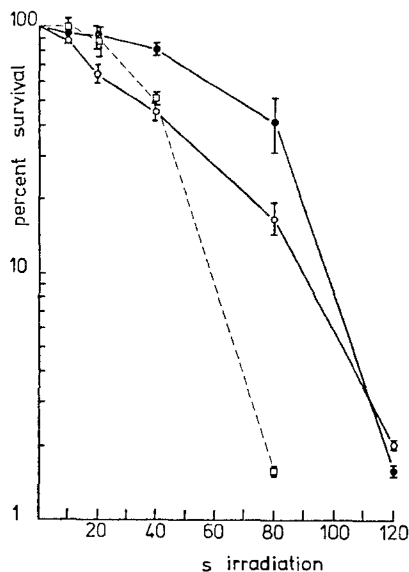

Fig. 3. Survival of haploid (○), diploid (●) and tetraploid (□) MD40-4c cells following ultraviolet irradiation for 0–120 s

tures, increases considerably as cells enter stationary phase (Iida and Yahara 1985). Also as cells approach stationary phase their ability to further elevate their thermotolerance in response to heat shock diminishes (Iida and Yahara 1985).

We measured initially the kinetics of cell killing for MD40-4c cultures of differing ploidy at 50 °C as shown in Fig. 1 Without a prior heat shock to induce their thermotolerance cells were killed rapidly at 50 °C, while after a heat shock they were considerably more resistant to 50 °C inactivation. Since the killing of the non heat shock adapted cells was rather too rapid at 50 °C to be measured accurately by our protocol we subsequently measured loss of viability of these cells at the slightly lower temperature of 48 °C (Fig. 2a). This revealed that, within the accuracy of our measurements, their survival was unaffected by increased ploidy.

Although the data in Fig. 1 show that a prior heat shock from 25 °C to 38 °C for 40 min confers substantial protection against 50 °C killing, they would only reveal a large ploidy effect on survival of these thermotolerance induced cultures. That the thermotolerance of these cells displays a decline with increased ploidy was shown in subsequent experiments in which their killing at the slightly higher temperature of 52 °C was determined (Fig. 2b). At 52 °C the induced haploid cells were the most thermotolerant, and survival decreased with increased ploidy. In these cultures induced for thermotolerance diploid cells were killed at 52 °C almost 3.5 times as rapidly as haploid cells, and tetraploid cells were still less resistant to thermal stress (Fig. 2b).

To confirm that the cells employed for thermotolerance measurements display a similar sensitivity to DNA damaging agents as other *S. cerevisiae* ploidy series that have been investigated we measured their sensitivity to ultraviolet radiation as is shown in Fig. 3. Their ultraviolet sensitivity was affected by ploidy in the manner that would be expected for cells not synchronised in their cell cycle on the basis of the results obtained by other investigators (Sarachek 1954; Owen and Mortimer 1956; Mortimer 1958). As is discussed below neither the basal nor the heat shock induced tolerance of *S. cerevisiae* to heat (Figs. 1 and 2) is affected by ploidy in a manner comparable to this effect of ploidy on tolerance to DNA damaging agents.

Discussion

Following a 25 °C to 38 °C shock *S. cerevisiae* displays a transient synthesis of heat shock proteins (McAlister et al. 1979; Miller et al. 1979). It also acquires an increased ability to withstand heat stress and this depends on protein synthesis since it is blocked by cycloheximide (McAlister and Finkelstein 1980; Mitchel and Morrison 1982; Craig and Jacobsen 1985). This HSP synthesis will occur in haploid MD40-4c during heat shocks to 42 °C–43 °C, but no protein synthesis at all occurs at higher temperatures irrespective of whether the cells have been previously heat-shocked (Piper et al. 1986). At 48 °C to 52 °C there is therefore no adaptation to heat stress through HSP synthesis, and the killing of cells

uninduced or induced for thermotolerance approximates to exponential kinetics (Figs. 1 and 2). The thermotolerance of *S. cerevisiae* cultures in which the heat shock response has been induced decreases with increased ploidy (Fig. 2b). This is in marked contrast to the negligible effect of ploidy on thermal death in the uninduced cultures (Fig. 2a). It provides an indication that the lethal thermal damage in cultures adapted through heat shock differs in some undefined manner from the damage that kills cultures not adapted for increased thermotolerance. The alterations in cellular properties caused by the heat shock response lead to a ploidy effect on thermotolerance which is not displayed by cells uninduced for this stress response.

Cells of higher ploidy have a larger volume (Table 1) and through their larger surface area may be less able to maintain energy or ionic balance under stressful conditions. Since ploidy does not affect thermal killing of non heat shocked *S. cerevisiae*, cells size can only be considered as a factor that may affect thermotolerance in cultures adapted through heat shock. Whether increased cell size is associated with more rapid thermal death in heat shock adapted cultures could be investigated further using the *whi-1* mutant, since diploid cells homozygous for the *whi-1* mutation are approximately the same size as $WHI1^+$ haploid cells (Sudbery et al. 1980).

The inducible response systems for DNA repair in *S. cerevisiae* may share certain gene functions with the heat shock response since there is induction of ionising radiation resistance by heat shock, and the acquisition of thermotolerance during a short low temperature incubation after exposure to ionising radiation (Mitchel and Morrison 1982). In *Escherichia coli* many HSPs are induced, not only by heat and ethanol, but also by agents (e.g. ultraviolet light and DNA gyrase inhibitors) that induce the proteins of the SOS or error-prone DNA repair system (Neidhardt et al. 1984). One *E. coli* HSP, *Lon*, has a role in recovery from the SOS state by degrading the *SulA* inhibitor of cell division (Mizusawa and Gottesman 1983). In eukaryotic organisms there have been few reports of proteins that are induced by both heat and DNA damaging agents, and the most abundant HSP (HSP70) is thought to act primarily against protein denaturation damage (Pelham 1985; 1986). Two genes of *S. cerevisiae* are induced by both DNA damage and heat shock, yet most yeast HSP genes are not induced by DNA damage (McClanahan and McEntee 1986). Indeed, heat shock appears to suppress an inducible error-prone DNA repair system in yeast (Mitchell and Morrison 1986). Pioneering studies on the sensitivity of *S. cerevisiae* to ionising radiation revealed that resistance to cell inactivation is markedly influenced by ploidy (Sarachek 1954; Owen and Mortimer 1956; Mortimer 1958). As for the ploidy series investigated in this study (Fig. 3), diploid cells were found to exhibit a sigmoidal survival curve and to be considerably more resistant to irradiation than haploids. This is due primarily to the ability to repair double-strand DNA breaks in diploids but not haploids through recombination. With further increases in ploidy from diploid to hexaploid radiation resistance decreases progessively (Mortimer 1958). In cells capable of the DNA recombination that requires genes of the RAD52 epistasis group (Mitchel and Morrision 1986) thermal sensitivity does not display the same changes with ploidy as radiation sensitivity (Figs. 1–3). Most notably there is no increase in the thermotolerance of a diploid as compared to a haploid strain. This indicates that survival following exposure to lethal temperatures is not determined primarily by repair of chromosome breakages through homologous recombination.

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References

Craig EA, Jacobsen K (1985) Mol Cell Biol 5:3517–3524
Haynes RH, Kunz BA, Strathern JN, Jones EW, Broach JR (eds) (1983) In: The molecular biology of the yeast Saccharomyces. Cold Spring Harbor Laboratory, New York, pp 371–414
Iida H, Yahara I (1985) Nature 315:688–690
Madiera-Lopes A (1982) Cienc Biol (Portugal) 7:59–64
McAlister L, Finkelstein DB (1980) Biochem Biophys Res Commun 93:819–824
McAlister L, Strausberg S, Kulaga A, Finkelstein D (1979) Curr Genet 1:63–74
McClanahan T, McEntee K (1986) Mol Cell Biol 6:90–96
Miller MJ, Xuong NH, Geiduschek EP (1979) Proc Natl Acad Sci USA 76:5222–5225
Mitchel REJ, Morrison DP (1982) Radiat Res 92:192–199
Mitchel REJ, Morrison DP (1986) Mutat Res 159:31–39
Mizusawa S, Gottesman S (1983) Proc Natl Acad Sci USA 80:358–362
Mortimer RK (1958) Radiat Res 9:312–326
Neidhardt FC, VanBogelan RA, Vaughn V (1984) Ann Rev Genet 18:295–329
Owen ME, Mortimer RK (1956) Nature 177:625–626
Pelham H (1985) Trends Genet 1:31–35
Pelham H (1986) Cell 46:959–961
Piper PW, Curran B, Davies W, Lockheart A, Reid G (1986) Eur J Biochem 161:525–531
Plesset J, Palm C, McLaughlin CS (1982) Biochem Biophys Res Commun 108:1340–1345
Sarachek A (1954) Cytologia 19:77–87
Sudbery PE, Goodey AR, Carter BLA (1980) Nature 288: 401–404
Takagi A, Harashima S, Oshima Y (1985) Appl Environ Microbiol 49:244–246
Van Uden N, Madiera-Lopes A (1975) Arch Microbiol 104: 23–28
Walker GC (1984) Microbiol Rev 48:60–93

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