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The Pleiotropic Effect of the *GTS1* Gene Product on Heat Tolerance, Sporulation and the Life Span of *Saccharomyces cerevisiae*

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We investigated whether or not the potential clock gene, *GTS1,* of the yeast *Saccharomyces cerevisiae,* shows pleiotropic effects on the yeast cellular processes. We tested the effect of the Gts1 protein on heat tolerance, sporulation and life-span, by characterizing the phenotypes of transformants with different copy numbers of the gene. We found that the Gts1 protein affects the capacity of heat tolerance in the stationary phase and the speed leading to sporulation in a gene-dose dependent manner, and that both inactivation and overexpression of the gene shortened the life-span of yeast. These results supported the notion that *GTS1* affects the biological clock of the yeast *S. cerevisiae,* although this cannot be definitively concluded because the strain cannot be synchronized with circadian or ultradian rhythms.

Biological clocks are considered to be ubiquitous in eukaryotic and prokaryotic organisms and are involved in various cellular and organismal processes. Understanding of the molecular mechanism underlying the clock has improved rapidly by studies in molecular biology and genetics (1,2). Studies on the period (per) mutants of Drosophila melanogaster have indicated that a gene-dose dependent control (3–5) and pleiotropic phenotypes affecting various rhythmic biological processes (6–10) are present in some mutant alleles of a typical clock gene.

We isolated a potential clock gene, named *GTS1,* from the yeast *S. cerevisiae* (11) using synthetic oligonucleotides encoding three GT repeats which are shared by the clock-affecting genes, *per* (12,13), and the *frequency* gene (*frq*) of *Neurospora crassa* (14). The function of the putative clock gene was examined by characterizing the phenotypes of transformants with different copy numbers of the gene produced either by inactivating the gene by gene-disruption or by transformation with a multicopy plasmid harboring the gene (11). Analyzing the phenotypes of the transformants of the *GTS1* gene revealed that the unbudding period and the cell volume changed as a function of gene-dosage. However, as we could not synchronize cell division with a light-and-dark cycle, it was premature to refer to the *GTS1* gene as a yeast clock gene (11). In this study, to investigate whether the Gts1 protein shows pleiotropic effects on other cellular processes than the timing of budding and cell size (11), we tested the effects of the Gts1 protein using transformants of the *GTS1* gene on heat tolerance, sporulation and life-span as they are reportedly affected by clock-affecting genes in other organisms (10,14–17).

MATERIALS AND METHODS

Yeast strains and Media. Strains of the yeast S. cerevisiae, IFO10151 (*MAT*a, *ade2, his3-532, trp1-289, ura3-1.2, Can*^{r} Inos$^-$) and IFO10102 (*MAT*α*, ade1, arg4, his6, ilv3, leu2-1, lys7, ura3, trp5, met*) were obtained from the Institute for Fermentation (Osaka, Japan) and used for transformation. Cells were either cultured in YPAD (rich) medium or in a synthetic medium as described (11). The media for sporulation, PSP2 (acetate growth medium) and SPM (sporulation medium) were formulated according to Simchen et al. (18). PSP2 was supplemented with the required amino acids, adenine and uracil (20 μg/ml).

Production of transformants of the gene GTS1. The gene disrupted transformant TMΔ gts1 and the high copy-number transformant TMpGTS1 were produced from the strains IFO10151 (*MATa*) and IFO10102 (*MAT*α) as described (11).

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Western blots showed that transformant TMpGTS1 contained about 20 times more Gts1 proteins than did the wild type cell, whereas transformant TMΔgts1 contained none (11). For sporulation experiments, the wild-type cells and the transformants produced from the strains were mated with each other obtaining a/α diploid cells with the nutritional marker, *ura3*.

Heat exposure. Cells were cultured in the synthetic medium at 30°C and those in either exponential growth or the stationary phase were heated at 55°C and 0.2 ml-samples were removed at 2-min intervals. To determine the frequency of viable cells, a known number of cells was plated and cultured on YAPD agar plates at 30°C and colonies were counted after 2 days.

Determination of the parameters related to sporulation. Sporulation proceeded essentially according to Simchen et al. (18). Cells cultured in PSP2 at 30°C were harvested, washed in water and resuspended in SPM at a density of 7×10^6 cells/ml. Two 2 ml-samples of cells were taken at hourly intervals. One sample was resuspended in 0.5 ml of distilled water to test readiness and the other was resuspended in 0.5 ml of PSP2, without uracil, to test whether the cells were committed to form asci. Cells were incubated at 30°C and the frequency (%) of asci was determined using a hemocytometer under a light microscope.

Determination of the life-span. Virgin cells were generated and their life-span was determined according to Egilmez et al. (19) on YPAD agar plates using a micromanipulator (Narishige, MN-151).

RESULTS

The effect of Gts1 on the heat tolerance of the yeast. To test whether the Gts1 protein affects the heat tolerance of the yeast *S. cerevisiae*, the wild-type and transformant cells harvested at either exponentially-growing or stationary phase were heated at 55°C and the surviving cells were counted at 2-min intervals (Fig. 1). The cells in the stationary phase were more resistant to the heat than the exponentially-growing cells (compare Fig. 1a with 1b), in agreement with the results published by Schenberg-Frascino and Moustacchi (20). The sensitivity of the transformants was affected in the stationary phase. For example, TMpGTS1 and TMΔgts1 cells in the stationary phase were more and less resistant, respectively, than those of the wild-type. This suggested that the Gts1 protein plays a role in increasing the heat tolerance in the yeast cells at the stationary phase in a gene-dose dependent manner.

The effect of Gts1 on yeast sporulation. The changes leading to sporulation in *S. cerevisiae* which are expressed either as reversible readiness, or as the irreversible commitment of the cells to the process, occur in a synchronized fashion (18), indicating that their timing is controlled by a biological clock. As the sporulation ability of the strains used was at most about 50% after an overnight incubation, their synchrony was much poorer than that of the strain used by Simchen et al. (18) (Fig. 2). Apparently however, the time courses of the readiness and commitment of TMpGTS1 were reproducibly delayed for 1 and 2 hours, respectively, compared with those of the

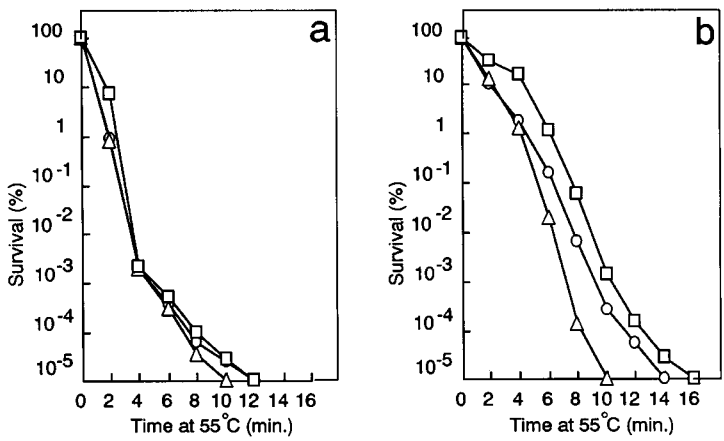

FIG. 1. Heat tolerance of the wild type (○), TMΔgts1 (△) and TMpGTS1 (□) of cells at exponential growth (a) and in the stationary-phase (b). Cells cultured in the synthetic medium were heated at 55°C. At 2-min intervals, samples were removed and a known number of cells were plated and cultured on YAPD agar plates at 30°C. The frequency of viable cells was determined by counting colonies after 2 days.

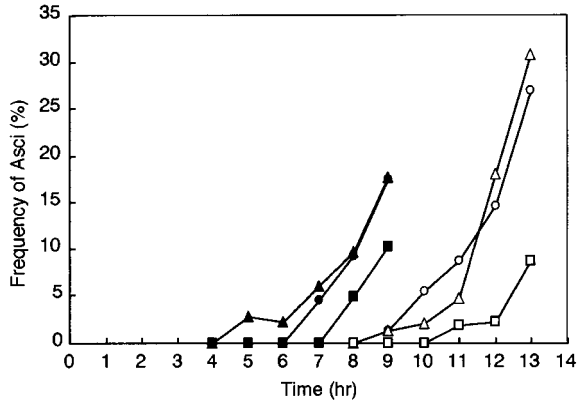

FIG. 2. Sporulation of the wild type (○, ●), TMΔgts1 (△, ▲) and TMpGTS1 (□, ■). Determination of the time of the readiness for (○, △, □) and commitment to sporulation (●, ▲, ■) proceeded essentially according to Simchen et al. (18).

wild type cells. On the other hand, although the time-courses of TMΔ gts1 tended to precede those of the wild-type, the difference between them was not reproducible. Thus, it was suggested that the Gts1 protein affects the timing of sporulation at least on overexpression.

The effect on the life-span of the yeast. The life-spans of the wild-type and the transformants, understood as the budding capacity of the cells in budding yeast, were determined on agar plates containing synthetic medium (Fig. 3). The life-spans of the wild-type, TMpGTS1 and TMΔ gts1, expressed as 50% mortality, were 26, 16 and 24, respectively, indicating that the life-spans of both transformants were shortened although the difference between those of the wild-type and TMΔ gts1 was not so significant.

DISCUSSION

We described that heat tolerance of the yeast *S. cerevisiae* was affected by the *GTS1* gene transformants in the stationary phase in a gene-dose dependent manner. Using *Schizosaccharomyces pombe*, Kippert reported that the capacity of stationary-phase cells for heat tolerance changes with a circadian rhythm (16), suggesting that genes which affect the biological clock also control heat tolerance in yeast. Thus, we suggested that Gts1 protein is a potential clock protein in the yeast cells, although whether it is controlled by a circadian rhythm could not be tested.

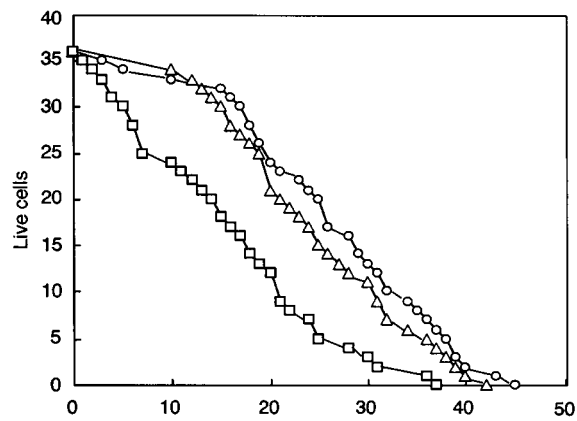

FIG. 3. Life-span of the wild type (○), TMΔgts1 (△) and TMpGTS1 (□). Virgin cells were generated as described (11) and the life-span of 36 cells from each strain was determined according to Egilmez et al. (19).

We also showed that the Gts1 protein affects the timing of sporulation at least in terms of overexpression. The rhythm of asexual spore formation in *Neurospora crassa*, referred to as conidiation, is controlled by a circadian rhythm and is affected by *frq* and some other clock affecting genes (14,17). Unlike conidiation in *Neurospora*, sporulation of the yeast *S. cerevisiae* is an inducible process under defined conditions in an a/α diploid. However, the changes are expressed either as reversible readiness or the commitment of the cells to the process, occur in a synchronized fashion (18). Thus, the process may be controlled by a biological clock in which the Gts1 protein is involved.

We also showed that the life time of TMpGTS1 was severely shortened whereas that of TMΔ gts1 was slightly decreased, suggesting that aberrant expression of the Gts1 protein affects the life-span of the yeast. Ewer et al. have reported that both *per*^{s} and *per*^{l} mutants of *Drosophila* had a shorter life-span than the wild-type fly (10) and claimed that *per* does not have any effect on life-span because *per*^{l}, which lengthen various biological processes did not lengthen the life-span. These findings together with our results indicate that mutants in clock-affecting genes reduce the life-span due to various stresses caused by an imbalance between biological and environmental rhythms. However, mutants of the *clk-1* gene of *Caenorhabditis elegans* which reportedly lengthened the developmental timing and organismal periods of defecation, swimming and pumping cycles, also lengthened life-span (21). Furthermore, deuterium oxide, which lengthens the free-running circadian period of locomotor activity in *Drosophila* shortens its life-span at low concentrations (below 30%) and lengthens it at high concentrations (above 40%) (22). Thus, the effects of clock-affecting genes on life-span are probably indirect and complex.

In summary, the results described here supported the notion that Gts1 protein affects the clock in yeast and that its mutation exerts a pleiotropic effect upon its phenotype. However, as we have not synchronized the yeast cell cycle to a circadian rhythm, it remains premature to conclude that the Gts1 protein is a clock-affecting protein.

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