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The *GTS1* Gene Product Influences the Ultradian Oscillation of Glycolysis in Cell Suspension of the Yeast *Saccharomyces cerevisiae*

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We tested the effect of the *GTS1* gene of the yeast *Saccharomyces cerevisiae* on the cyanide-induced ultradian oscillation of the glycolytic metabolite NADH in cell suspension of strains with different copy numbers of the gene, that is, the wild-type, *GTS1*-disrupted and *GTS1*-overexpressing strains. The cells showed long-lasting oscillations when harvested between 1 and 2 hours after the diauxic shift from glucose to ethanol as a growth substrate. The frequencies of oscillation did not vary very much among the three strains tested. However, the amplitudes and durations of the oscillation were changed significantly as a function of the *GTS1* gene-dosage. The effect of GTS1 on the amplitude was not caused by changing rates of glucose incorporation into cells as the rates were the same among the three strains during the macroscopic oscillation.

We have reported that the gene *GTS1* isolated from the yeast *S. cerevisiae* (1, 2) shows pleiotropic effects on the yeast. *GTS1* lengthens the unbudding period which increases the cell volume (1), and affects the capacity of heat tolerance in the stationary phase and the speed leading to sporulation in a gene-dose dependent manner (2). In addition, the life-span of yeast was shortened by both inactivation and overexpression of the gene (2). We suggested that *GTS1* affects the biological clock of the yeast *S. cerevisiae* because the *GTS1*-related phenotypes are reportedly influenced by clock-affecting genes in other organisms, and because a gene-dose dependent control and pleiotropic phenotypes affecting various rhythmic biological processes (For reviews see 3, 4) are reportedly present in some mutant alleles of a typical clock gene. However, we could not synchronize cell division with a light-and-dark cycle, so it was premature to refer to *GTS1* as a yeast clock gene (1, 2).

In this communication, to investigate whether *GTS1* shows any effects on biochemical oscillations in yeast, we examined the effect of *GTS1* on the synchronization of ultradian oscillation of the concentration of the glycolytic metabolite NADH which is induced by addition of glucose followed by cyanide to a starved yeast (For review see 5). The macroscopic oscillation, i.e., synchronization of mutual cell oscillations, lasts longest when cells are harvested after the diauxic shift, from using glucose to ethanol as a growth substrate (6) and when incubated at a higher cell density (7, 8). We reported that the frequency and amplitude of the oscillation were modulated by *GTS1* in a gene-dose dependent manner.

MATERIALS AND METHODS

Yeast strains and culture of cells. Strains of the yeast *S. cerevisiae* W303 (*MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11, 15 can1-100*) were used for the transformation. Production of the gene disrupted transformant TMΔGts1(W303) and the high copy-number transformant TMpGTS1(W303) were produced from the strain W303 (*MAT*a) as described previously (2). Cells were cultured in a medium containing 10 g/l glucose, 6.7 g/l yeast nitrogen base without amino acids (Difco Lab., Detroit, USA) and 100 mM potassium phthalate at pH 5.0 (6) supplemented with 40 μg/ml adenine sulfate and essential amino acids.

Induction and monitoring of the oscillation. The cells harvested at various times before and after glucose exhaustion in the medium. They were washed and resuspended in 100 mM potassium phosphate, pH 6.8, to a concentration of 4 mg protein/ml before being starved for 3 hr at 30°C (6). Oscillations were induced by adding 20 mM glucose to the starved cells and then after 4 min 5 mM potassium cyanide was added. The oscillations were monitored by NADH fluorescence using a spectrofluorimeter (Hitachi F-4500) in a stirred and thermostatically regulated cuvette (9). The amplitude of pulse was estimated by subtracting the photometric value (PV) of valley from the PV of peak of the next pulse. The glucose concentration in the

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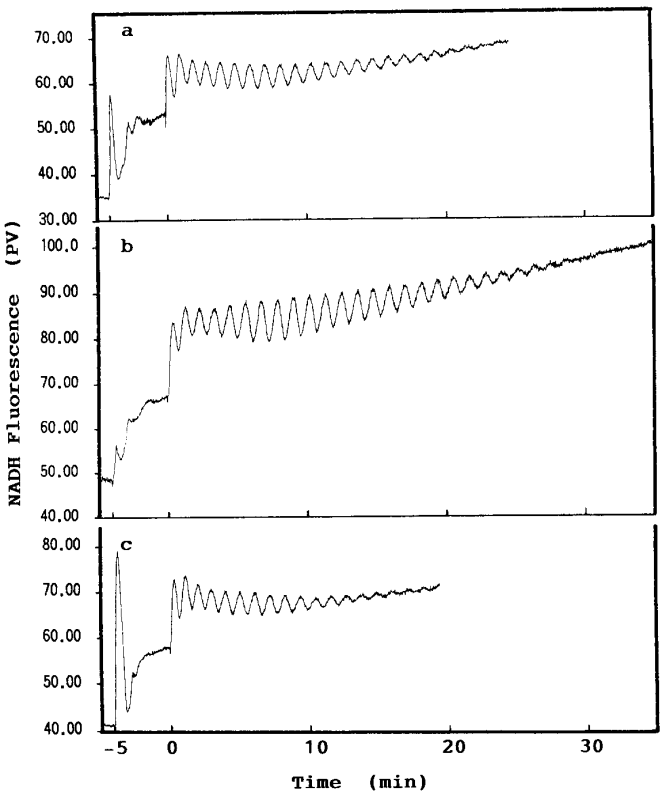

FIG. 1. Representative patterns of ultradian oscillation of the NADH fluorescence at 20°C in cell suspensions of the wild type (a), *GTS1*-disrupted (TMΔgts1) (b), and *GTS1*-overexpressing (TMpGTS1) (c). Cells were harvested 1.5 hr after the diauxic shift. Glucose (20 mM at the final concentration) was added at −5 min and KCN (5 mM) was at time zero.

medium was determined with a commercial glucose kit (Sigma Chemicals, St. Louis, USA).

The level of *GTS1* gene product (Gts1p) was determined by Western blotting using anti-Gts1p antibodies as previously described (1).

RESULTS

The effect of GTS1 on the oscillation of NADH in the cells after the diauxic shift. The yeast strain used (W303) showed the longest macroscopic oscillation of NADH fluorescence when the cells were harvested at 1 to 2 hr after the diauxic shift and not before or after this period (data not shown), which is in good agreement with the previous results reported (6). To investigate whether or not *GTS1* has any effect on the oscillation, *GTS1*-disrupted and *GTS1*-overexpressing strains were harvested 1.5 hr after the diauxic shift and the patterns of the oscillation were compared with that of the wild-type strain (Fig. 1, Table 1). The Gts1p level in the *GTS1*-overexpressing strain used in this experiment was about 20 times that in the wild-type cell. This is in agreement with the previous report using cells in the exponential phase (1) (data not shown). The patterns of the oscillation apparently show that the duration of the oscillation was changed in both transformants as a function of the gene dosage, such that the oscillation of the *GTS1*-disrupted strain was lengthened by 26% while that of the *GTS1*-overexpressing strain was shortened by 20% compared with that of the wild-type based on the average number of pulses in one oscillation (Table 1). Further analysis of the patterns shows that, although the frequencies of the oscillation among the strains were little changed, the amplitudes were significantly changed as a function of the Gts1p levels because the amplitude of the *GTS1*-disrupted strain was 45% higher than that of the wild-type strain while that of the *GTS1*-overexpressing strain was 35% lower (Table 1). Temperature quotients of the oscillation measured by their frequencies between 15 and 25°C were about 2.8 in all strains suggesting that the oscillation was not temperature-compensated.

Incorporation rate of glucose into cells during the oscillation. As oscillatory reactions are influenced by the influx rate of the energy source into the system, the glucose incorporation rate during the oscillation of NADH was determined by measuring the glucose concentration in the medium (Fig. 2). The incorporation rates of glucose changed in a biphasic manner in all strains having the first slow (12 mg/L · min) and the second fast (50 mg/L · min) phases, however, the duration of the first phase changed in parallel with those of the oscillation (Fig. 2). These results suggested that the rates of glucose incorporation were the same among the strains until damping of the oscillation increased to some extent.

TABLE 1

Frequencies and Amplitudes of the Macroscopic Oscillations of NADH in the Wild-Type, *GTS1*-Disrupted (TMΔgts1) and *GTS1*-Overexpressing (TMpGTS1) Strains 1.5 hr after the Diauxic Shift

Strains	Nos of pulse[a] (pulses/run)	Frequency[a] (sec/pulse)	Amplitudes[b] (PV)
Wild type	19.0 ± 1.53	73.5 ± 2.69 (100)	6.61 ± 1.37 (100)
TMΔgts1	23.8 ± 1.46	69.9 ± 0.89 (95)	9.56 ± 1.58 (145)
TMpGTS1	15.0 ± 2.38	74.8 ± 3.50 (102)	5.23 ± 1.74 (79)

Note. Numbers in parentheses indicate % control.

[a] Average and standard deviation of the number of pulses in one oscillation and its frequency determined with 6 experimental values for each strain.

[b] Average and standard deviation of amplitudes of all pulses (90, 72 and 120 for wild type, TMΔgts1 and TMpGTS1 strains, respectively) observed in the 6 experiments eliminating the last several pulses to avoid the damping effect as much as possible.

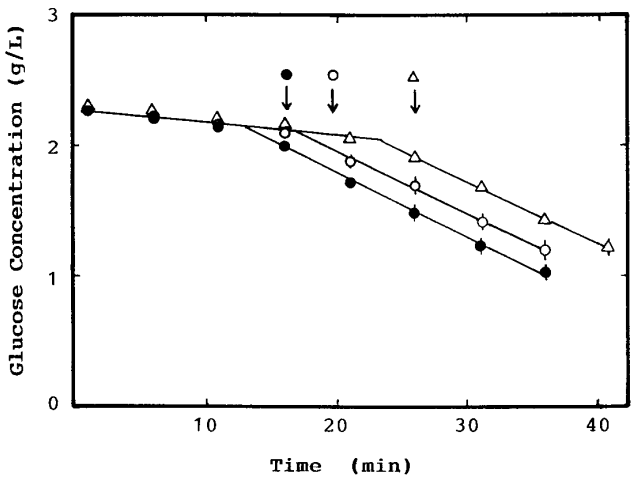

FIG. 2. Time courses of the glucose concentration in the medium after the addition of KCN in cell suspensions of the wild type (○), *GTS1*-disrupted (TMΔgts1) (△) and *GTS1*-overexpressing (TMpGTS1) (●) strains. Vertical lines attached to the symbols indicate standard deviations determined with 3 experimental values. The arrows indicate approximate time points when the oscillation of each strain ceased.

DISCUSSION

The ultradian oscillation of the glycolytic pathway in populations of yeast cells is known to occur autonomously under the primary control of phosphofructokinase (5, 10, 11) but little is known about whether and how the oscillation is regulated at molecular level *in vivo* (5). This is the first report on the gene which affects the glycolytic oscillation although the exact mechanism remains to be clarified. We showed that with an increasing gene-dosage *GTS1* affects the oscillation by decreasing its amplitude and shortening the duration. It is likely that Gts1p suppresses the amplitude of the oscillation leading to a shortening of the duration time. The changes caused by *GTS1* were not mediated through affects on the rate of glucose influx although the duration of the first slow incorporation phase was changed among the strains in parallel with that of the oscillation. The shift of the glucose incorporation rate may reflect the shift from the macroscopic oscillation at the cell population level to the microscopic oscillation at the individual cell level (8) as damping of the macroscopic oscillation increased prior to the shift of the incorporation rate.

Gts1p is conventionally classified as a transcription factor in the yeast genome database (12) probably because it contains a zinc finger similar to that of GATA-transcription factors (13) and a glutamine-rich sequence. However, it is unlikely that Gts1p modulates the glycolytic oscillation by affecting the expression of glycolytic enzyme genes because the zinc finger motif of Gts1p lacks basic amino acid residues conserved in GATA-transcription factors for DNA binding (14) and because Gts1p bound to neither oligomers containing the consensus binding motif of GATA-transcription factor nor the *Sau*3AI fragments derived from yeast genome when we analyzed by gel mobility shift assays (data not shown). Furthermore, mRNA levels of glycolytic enzymes like pyruvate kinase and phosphofructokinase were hardly detectable by Northern blot analysis in the wild-type and transformant cells 1.5 h after the glucose exhaustion (data not shown) while Gts1p was expressed continuously after the diauxic shift. In agreement with this result, Boy-Marcotte *et al.* reported using 2-dimensional gel electrophoresis that the glycolytic enzymes identified on the gel were not newly synthesized at the diauxic shift (15). In addition, the fact that the glucose incorporation rate was almost the same among the strains suggested that the activity of glycolytic pathway was not so changed among them.

As the change in frequency of the oscillation was not temperature-compensated, the cyanide-induced ultradian oscillation seems not to be clock-controlled. This, however, does not mean that the glycolytic oscillator is not linked to the machinery of the biological clock. There have been some proposals based on the ubiquitous distribution of the glycolytic pathway that the oscillator could mediate various oscillatory phenomena, including insulin release from the β islet cells of the pancreas (16), slow waves of contraction in stomach and intestine (17), and oscillations of membrane potential in cardiac cells (18). In yeast, we previously reported that the timing of budding, cell size, heat tolerance and the timing of sporulation were affected by *GTS1* in a dose dependent manner (1, 2) similar to the glycolytic oscillation reported here. In addition the lifespan of both *GTS1*-transformants were shortened (2). As yeast is an organism which is easy to analyze genetically and cytologically, it may be a good system for studying the relationship between the glycolytic oscillation and other biochemical processes or oscillations.

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