The Trehalose Pathway Regulates Mitochondrial Respiratory Chain Content through Hexokinase 2 and cAMP in Saccharomyces cerevisiae*

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Abdelmajid Noubhani†§§1, Odile Bunoust†§§1, Beatriz Monge Bonini†¶, Johan M. Thevelein†¶¶, Anne Devin†§§, and Michel Rigoulet†§§2

From †CNRS, UMR5095 Institut de Biochimie et Génétique Cellulaire, 1 rue Camille Saint-Saëns, 33077 Bordeaux Cedex, France, the §§Université Victor Segalen Bordeaux2, 146 rue Léo Saignat, 33076 Bordeaux Cedex, France, the ¶¶Laboratory of Molecular Cell Biology, Institute of Botany and Microbiology, Katholieke Universiteit Leuven, Arenberg 31, B-3001 Leuven-Heverlee, Flanders, Belgium, and the 1Department of Molecular Microbiology, Flanders Institute of Biotechnology, Kasteelpark Arenberg 31, B-3001 Leuven-Heverlee, Flanders, Belgium

In yeast, trehalose is synthesized by a multimeric enzymatic complex: TPS1 encodes trehalose 6-phosphate synthase, which belongs to a complex that is composed of at least three other subunits, including trehalose 6-phosphate phosphatase Tps2 and the redundant regulatory subunits Tps3 and Tsl1. The product of the TPS1 gene plays an essential role in the control of the glycolytic pathway by restricting the influx of glucose into glycolysis. In this paper, we investigated whether the trehalose synthesis pathway could be involved in the control of the other energy-generating pathway: oxidative phosphorylation. We show that the different mutants of the trehalose synthesis pathway (tps1Δ, tps2Δ, and tps1,2Δ) exhibit modulation in the amount of respiratory chains, in terms of cytochrome content and maximal respiratory activity. Furthermore, these variations in mitochondrial enzymatic content are positively linked to the intracellular concentration in cAMP that is modulated by Tps1p through hexokinase2. This is the first time that a pathway involved in sugar storage, i.e. trehalose, is shown to regulate the mitochondrial enzymatic content.

The control of glycolysis in the yeast Saccharomyces cerevisiae has been extensively studied. First, allosteric regulation of the irreversible steps catalyzed by phosphofructokinase (1), pyruvate kinase (review in Ref. 2), and fructose-1,6-bisphosphatase (1) has been proposed, even though the overexpression of these key enzymes does not increase the glycolytic flux (3). Other mechanisms of control have been proposed such as futile cycle activity (4) and an inhibitory effect of ATP (5). Indeed, it seems likely that the regulation of glycolysis is a complex process involving different hierarchical events leading from gene expression to the metabolic fluxes via protein levels, enzyme activities, and metabolite effects (6, 7). Among these actors, the product of the TPS1 gene has been shown to play an essential role in the control of the glycolytic pathway by restricting the influx of glucose into glycolysis (8). TPS1 encodes trehalose 6-phosphate (Tre6P)3 synthase (9–12). This enzyme is part of a multimeric protein complex composed of at least three other subunits, i.e. Tre6P phosphatase encoded by TPS2 (13) and the redundant regulatory subunits Tps3 and Tsl1 (14).

A particularly intriguing finding is that tps1Δ mutants are defective not only for Tre6P synthesis but also for growth on glucose or related rapidly fermented sugars (8, 11, 15). This may be explained by an uncontrolled influx of glucose into the glycolytic pathway. This phenomenon is characterized by hyperaccumulation of glucose 6-phosphate, fructose 6-phosphate, and fructose 1,6-bisphosphate (Fru1,6bP) (8, 16–18) and depletion of ATP, P4, and all intermediates of glycolysis downstream of glyceraldehyde-3-phosphate dehydrogenase (19). Several mutations have been described that suppress the growth defect of tps1Δ mutants apparently by reducing sugar influx into glycolysis (16, 20) or by diverting the excess sugar phosphate into glycerol synthesis through overexpression of the GPD1-encoded NAD-dependent glycerol-3-phosphate dehydrogenase (17, 21). Reconstitution of ethanolic fermentation in permeabilized yeast spheroplasts indicated that in addition to Tre6P, the Tps1 protein itself also seems to play a role in restricting glucose influx into glycolysis (22).

Whatever the mechanism by which the multimeric complex involved in trehalose synthesis controls glycolytic flux in yeast, such a regulation is associated with modification of the cellular content of sugar phosphates. Moreover, in a recent paper, we have shown that in yeast, low physiological concentrations of glucose 6-phosphate and fructose 6-phosphate slightly (20%) stimulate the respiratory flux and that this effect was strongly antagonized by Fru1,6bP (18). On the other hand, Fru1,6bP by itself is able to inhibit mitochondrial respiration only in mitochondria isolated from a Crabtree-positive strain. Taken together, these results indicate that besides the thermodynamic link between glycolysis and mitochondrial respiration (i.e. the cytosolic ATP/ADP and NADH/NAD+ ratio), a kinetic control of oxidative phosphorylation activity is exerted by the level of glycolytic sugar phosphates (18, 23). This raises the question 3 The abbreviations used are: Tre6P, trehalose 6-phosphate; Fru1,6bP, fructose 1,6-bisphosphate; MOPS, 4-morpholinepropanesulfonic acid; CCCP, cyanide m-chlorophenylhydrazone; DW, dry weight.
of a possible direct or indirect regulation of oxidative phosphorylation by the trehalose synthesis pathway.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The *S. cerevisiae* strains used in this work were the following: W303-1A, *Mata leu2-3.112 uRA3-1 trp1-1 his3-11,15 ade2-1 can1-100 GAL SUC; *tps1Δ*, YSH648 (*Mata leu2-3.112 ura3-1 trp1-1 his3-11,15 ade2-1 can1-100 tsp1::TRP1 GAL SUC; tsp2Δ*, YSH672 (*Mata leu2-3.112 ura3-1 trp1-1 his3-11,15 ade2-1 can1-100 tsp2::LEU2 GAL SUC; tsp1Δ tsp2Δ*, YSH652 (*Mata leu2-3.112 ura3-1 trp1-1 his3-11,15 ade2-1 can1-100 tsp1::TRP1 tsp2::LEU2 GAL SUC; hxs2Δ*, YSH310 (*Mata leu2-3.112 ura3-1 trp1-1 his3-11,15 ade2-1 can1-100 hxs2Δ::LEU2 GAL SUC; tsp1Δ hxs2Δ*, YSH312 (*Mata leu2-3.112 ura3-1 trp1-1 his3-11,15 ade2-1 can1-100 tsp1::TRP1 hxs2Δ::LEU2 GAL SUC; and tsp2Δ hxs2Δ*, W303–1A (*Mata hxs2::LEU2 tsp2::HIS3*. The cells were grown in a shaking incubator at 28 °C for one night in complete medium (YP, pH 5.5), containing 1% yeast extract, 0.1% potassium phosphate, 0.12% ammonium sulfate supplemented with 2% galactose (YPGal2%), or 2% lactate (YPLac2%) as a carbon source. The cells were harvested in the middle of the logarithmic growth (YPGal 2%), or 2% lactate (YPLac2%) as a carbon source. The cells were harvested in the middle of the logarithmic growth phase at an optical density of 4.

**Cellular Respiration Rate**—Oxygen consumption was measured at 28 °C in a 2-ml thermostatically controlled chamber equipped with a Clark oxygen electrode (Gilson) giving an online display of rate values.

The cells were incubated in the growing medium (YPGal2% or YPLac2%, spontaneous respiration) or the growing medium supplemented with 107 mM ethanol and 5 mM carboxyl cyanide m-chlorophenylhydrazone (uncoupled respiratory rate).

**Cytochrome Content Measurements**—Yeast cells were harvested, washed twice with distilled water, and concentrated to 20 mg of dry weight/ml. Cytochrome content was determined by using a dual beam spectrophotometer (Amico DW2000); a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 mM fH2O2) and a cell reduced state (in the presence of 1 MMA SEP 2000); a differential spectrum was performed between a cell oxidized state (in the presence of 1 μl of H2O2) and a cell reduced state (in the presence of dithionite). Calculations of cytochrome c + c1 and cytochrome b contents were performed using an extinction coefficient of 18,000 M−1 cm−1 for the wavelength pairs 550–540 and 561–575 nm, respectively (24). The calculation of cytochrome a + a1 contents was performed using an extinction coefficient of 12,000 M−1 cm−1 for the 603–630-nm interval (25).

**Treh6P and cAMP Determination**—Yeast cells grown on either YPGal2% or YPLac2% were harvested in exponential growth phase (optical density of 4). After the addition of 7% perchloric acid and glass beads, the cells were broken by mixing the test tube vigorously for 90 s on a vortex mixer. The yeast extract was transferred to a microcentrifuge tube, and the pH was adjusted to 6.8 with KOMO (0.3 M KOH, 2 M MOPS). Treh6P was determined in cell extracts using a modification of the protocol described in Ref. 13. Briefly, the samples were loaded onto a CarboPac PA-1 column equilibrated with a solution containing 50 mM NaAc and 100 mM NaOH. The samples were eluted by increasing NaAc concentrations up to 400 mM over 15 min at constant NaOH concentration. Determination of cAMP in cell extracts was performed as described previously (26) using a cAMP Assay kit including a standard (Amersham Biosciences).

**Statistical Analysis**—The results are expressed as the means ± S.E. Statistical analysis was carried out using nonparametric Mann-Whitney test for all tests. Prism software (GraphPad, San Diego, CA) was used for all tests. A P value of less than 0.05 was considered significant; n denotes the number of experiments.

**RESULTS**

**Mutants in the Trehalose Pathway Exhibit Modifications in the Respiratory Chain Content**—We assessed spontaneous respiratory rate as well as substrate-related respiratory rate in wild type, *tps1Δ*, *tps2Δ*, and *tps1Δ tsp2Δ* cells (Table 1). The spontaneous respiratory rate is decreased in the *tps1Δ* cells, the *tps2Δ* cells exhibit a respiratory rate higher than the wild type, whereas in the double mutant *tps1Δ tsp2Δ*, the respiratory rate is comparable with that in the *tps1Δ* cells. Although glucose addition has only a very slight effect on the *tps1Δ* respiratory rate, it drastically inhibits oxygen consumption in both the *tps1Δ* and *tps1Δ tsp2Δ* cells. In this condition, ethanol addition did not restore the respiratory rate in these mutants, confirming that glucose induced inhibition of respiratory rate is due to a lack of substrate availability. For all four strains, respiratory rate with ethanol alone as substrate is slightly increased compared with spontaneous respiratory rate (Table 1). The maximal respiratory rate (i.e. in the presence of ethanol and uncoupler) is lower in *tps1Δ* cells and higher in *tps2Δ* cells compared with the wild type, whereas in the double mutant the maximal velocity is almost identical to the wild type one.

The cellular uncoupled respiratory rate is generally considered as an assessment of the total amount of respiratory chain components within the cell (27). To confirm that the amount of respiratory chain components was modified in the three mutant strains studied, we measured the cytochrome content of cells grown on galactose. Table 2 shows that cytochrome content in the four strains varies like the maximal respiratory rate, i.e. it is lower in *tps1Δ* cells and higher in *tps2Δ* cells compared with the wild type, whereas in the double mutant, it is almost identical to that in the wild type. Furthermore, Fig. 1 shows that there is a linear relationship between the amount of any of the cytochromes of the respiratory chain and the maximal respiratory rate in the different strains.

**TABLE 1**

**Cellular oxygen consumption rate of wild type and mutant strains**

The respiratory rates of wild type and the three mutant strains were measured in growth conditions on YPGal2% when the optical density was ~4 (spontaneous respiration). When added, glucose was 15 mM, ethanol was 107 mM, and CCCP was 5 μM. The values are the means ± S.E. of 10 measurements. nat O, nanomols of oxygen.

| Respiratory rate | Wild type | *tps1Δ* | *tps2Δ* | *tps1Δ tsp2Δ* |
|------------------|-----------|---------|---------|---------------|
| Ethanol + glucose | 166 ± 10  | 36 ± 4  | 174 ± 9 | 34 ± 3        |
| Ethanol | 167 ± 9  | 126 ± 10 | 187 ± 8 | 132 ± 7        |
| Ethanol + CCCP | 267 ± 16 | 193 ± 14 | 341 ± 24 | 251 ± 9        |

* The differences between wild type and mutant strains are significant at p < 0.05.
* The differences between wild type and mutant strains are significant at p < 0.001.
The results presented above concern cells grown on galactose medium, which induces a respiro-fermentative metabolism. To further study the influence of the trehalose pathway on mitochondrial metabolism in conditions that avoid any effect of these mutations on glycolysis, we switched to a purely respiratory metabolic growth substrate, i.e., lactate. As expected and compared with that on galactose (Table 1), the spontaneous respiratory rate is higher except for the \( \text{tps2}\Delta \) mutant, for which the rate is comparable with the one measured with galactose (Table 3). The spontaneous respiratory rate is pretty much the same for all four strains because the difference between the double mutant and the other strains is not significant. In contrast, the uncoupled respiratory rate is strongly increased in the \( \text{tps2}\Delta \) strain, whereas the other strains exhibit comparable rates. In accordance with this result, the cytochrome content is increased in the \( \text{tps2}\Delta \) strain, whereas it is comparable in the other strains. For each strain, it is worth noting that the cytochrome content is increased when cells are grown on lactate (compare Tables 2 and 3). However, whatever the carbon source and the strain, we observed a good correlation between the amount of each of the cytochromes and the uncoupled respiratory rate (Fig. 2). The differences observed show that in the absence of Tps1 there is a decrease in the amount of respiratory chain components (on galactose medium) within the cells, whereas in the absence of Tps2 there is an increase in the cytochrome content of the respiratory chain (on both media).

The Effect of the Mutations in the Trehalose Synthesis Pathway on Mitochondrial Enzymatic Content Is Mediated by the cAMP Level—We have previously shown that the mitochondrial enzymatic content is modulated through the activity of the Ras/cAMP-dependent protein kinase/cAMP pathway in such a way that overactivation of this pathway increases, whereas underactivation of this pathway decreases the mitochondrial enzymatic content (28–30). We thus investigated whether this pathway was involved in the presently observed regulation. Because the activity of cAMP-dependent protein kinase is largely dependent on the cAMP level in the cell (31), we assessed cAMP content under our different experimental con-
Trehalose Pathway Regulates Respiratory Chain Content

FIGURE 3. Relationship between cytochrome a + a3 and cAMP content. Cytochrome a + a3 and cAMP content were assessed as described under “Experimental Procedures.” The strains used are indicated on the figure. □, cells cultured in YPGal2%; □, cells cultured in YPLac2%. The values are the means of at least three measurements made with different cultures. WT, wild type.

Role of Hxk2 on the modulation of respiratory rates and cytochrome content in the Tps mutants

The respiratory rate of cells of the wild type and the three mutant strains was measured during growth on YPGal2% at an optical density of 4 (spontaneous respiration). When added, as indicated in the table (uncoupled [O2], the ethanol concentration was 107 mM, and CCCP was 5 µM. Cytochrome and cAMP contents were measured as described under “Experimental Procedures.” The values are the means ± S.E. of five measurements made with different cultures. natO, nanomoles of oxygen.

TABLE 4

| Parameters | Wild type | tps1∆ | tps2∆ | tps1∆ hxs2∆ | tps2∆ hxs2∆ |
|------------|-----------|-------|-------|-------------|-------------|
| Spontaneous [O2] (natO/min/mg DW) | 139 ± 11 | 156 ± 7 | 153 ± 6 | 155 ± 9 |
| Uncoupled [O2] (natO/min/mg DW) | 282 ± 24 | 339 ± 18 | 340 ± 25 | 356 ± 24 |
| cAMP (pmol/mg DW) | 2.2 ± 0.1 | 2.4 ± 0.1 | 2.6 ± 0.1 | 2.4 ± 0.2 |
| c + c1 (pmol/mg DW) | 63 ± 5 | 78 ± 5 | 77 ± 6 | 74 ± 4 |
| b (pmol/mg DW) | 32 ± 3 | 42 ± 4 | 42 ± 4 | 39 ± 3 |
| a + a3 (pmol/mg DW) | 7.7 ± 0.7 | 9.0 ± 1.0 | 8.8 ± 0.9 | 9.5 ± 1.1 |

Effect of high temperature on respiratory rate and cytochrome content in tps2∆ strains

The respiratory rate of cells of the wild type and the three mutant strains was measured during growth on YPGal2% at 35°C at an optical density of 4 (spontaneous respiration). When added, as indicated in the table (uncoupled [O2], the ethanol concentration was 107 mM, and CCCP was 5 µM. The cytochrome, Tre6P, and cAMP contents were measured as described under “Experimental Procedures.” The values are the means ± S.E. of seven measurements made with different cultures. natO, nanomoles of oxygen.

TABLE 5

| Parameters | Wild type | tps2∆ | tps2∆ hxs2∆ | tps1∆ |
|------------|-----------|-------|-------------|-------|
| Spontaneous [O2] (natO/min/mg DW) | 187 ± 10 | 207 ± 9 | 118 ± 7 | 131 ± 11 |
| Uncoupled [O2] (natO/min/mg DW) | 257 ± 16 | 364 ± 12 | 206 ± 8 | 202 ± 27 |
| cAMP (pmol/mg DW) | 1.9 ± 0.2 | 4.6 ± 0.2 | 1.5 ± 0.2 | 1.4 ± 0.4 |
| c + c1 (pmol/mg DW) | 44 ± 2 | 53 ± 5 | 41 ± 2 | 40 ± 4 |
| b (pmol/mg DW) | 27 ± 3 | 31 ± 4 | 25 ± 2 | 24 ± 3 |
| a + a3 (pmol/mg DW) | 6.0 ± 0.7 | 11.1 ± 3 | 4.2 ± 0.5 | 5 ± 1.8 |
| Tre6P (nmol/mg DW) | 3.1 ± 0.5 | 22.2 ± 7.3 | 3.3 ± 0.4 | 0.8 ± 0.4 |

The differences between wild type and mutant strains were significant at p < 0.001.

The growth defect in the presence of glucose in the tps1∆ mutant is suggested to be linked to the absence of Tre6P inhibition of hexokinase. To further understand how the deletions in TPS1 and TPS2 are linked to changes in the amount of respiratory chain components within the cells, we investigated whether there was a relationship between the amount of cytochromes and the amount of Tre6P, the concentration of which is known to be affected in these mutants. To evaluate this hypothesis, we modulated the amount of Tre6P in the tps2∆ mutant by increasing the growth temperature. It is well known that in this condition the Tre6P concentration increases very strongly (33). Table 5 shows that, in a tps2∆ mutant, an increase in temperature induces a clear increase in Tre6P, cAMP, and also mitochondrial enzymatic content. These increases are abolished when HXX2 is deleted in the tps2∆ strain. As expected, there is almost no Tre6P in the tps1∆ strain, and mitochondrial enzymatic content is related to the cAMP content. However, it can be hypothesized that Tre6P is the intermediary between the deletions (tps1∆ and tps2∆) and the influence of hexokinase2 on the cAMP concentration. Furthermore, Tre6P was assessed in most of our experimental conditions, and even though in a tps2∆ strain there is an increase in Tre6P concomitant with an increase in mitochondrial enzymatic content, there is no relationship between mitochondrial enzymatic content and Tre6P concentration in the other strains (data not shown). This rules out a possible direct regulation of mitochondrial enzymatic content by Tre6P.

In yeast, the cAMP intracellular concentration depends on the respective activities of Cyr1 (adenylate cyclase) and of the phosphodiesterases Pde1 and Pde2. Pde2 is the high affinity phosphodiesterase. When the PDE2 gene was overexpressed, the cAMP content in the wild type strain was slightly decreased, as were cytochrome content and respiratory activities (Table 6).
Moreover, the increase in cAMP and cytochrome content as well as the respiratory rate in tps2Δ cells was completely suppressed by overexpression of PDE2, indicating that the effects of this mutation on the electron transport chain machinery and activity go through the CAMP content.

**DISCUSSION**

The main goal of this work was to investigate whether the trehalose synthesis pathway is involved in the regulation of oxidative phosphorylation activity in yeast mitochondria. It is now well established that Tps1 plays a major role in the control of glycolytic flux by restricting glucose influx at the level of glucose phosphorylation. Thus, in a tps1Δ mutant that is not able to grow on glucose (8, 11, 15), glucose addition to cells growing on galactose or on nonfermentable carbon sources induces a large increase in sugar phosphates and particularly in fructose 1,6-bisphosphate (8, 16–18). Because fructose 1,6-bisphosphate is a potent inhibitor of respiration in Crabtree-positive yeast strains, such a huge increase in this compound must induce a strong inhibition of mitochondrial oxidative phosphorylation (18). Thus, through the control of glycolytic intermediate content, the trehalose synthesis pathway may be involved in the kinetic regulation of oxidative phosphorylation. This made the question arise of whether Tps1 would participate in the regulation of respiration also under conditions permissive for its growth and thus in a way independent of the dramatic metabolic changes associated with the specific growth defect of the tps1Δ mutant on rapidly fermented sugars like sucrose, fructose, and glucose.

When growing on galactose or on a nonfermentable carbon source (i.e. lactate), the different mutants in the trehalose pathway (tps1Δ, tps2Δ, and tps1Δ tps2Δ) are not much affected in their growth characteristics compared with the wild type strain (34). However, as evidenced by cytochrome content, these mutations do modulate the quantity of respiratory chain components also under these permissive growth conditions. The quantity decreased or increased in the absence of Tps1 or Tps2, respectively. There is a good correlation between maximal respiratory capacity (measured in the presence of ethanol and uncoupler) and the cytochrome content whatever the strain considered (Fig. 2). The Ras-cAMP cascade in yeast has been extensively studied (35–37). By using different mutants in the RAS/cAMP signaling pathway, we have shown that underactivation or overactivation of this signaling pathway leads to a decrease or an increase in the content of all the cytochromes and in maximal respiratory capacity, respectively (28). As in the present study, these changes in cytochrome content correlated with the variation in respiratory capacity of the cells under different conditions. A direct involvement of CAMP in the regulation of cellular mitochondrial content has been shown by making use of the OL556 strain (38), in which the high affinity cAMP phosphodiesterase (Pde2) and Cdc25 are inactivated. Consequently, this strain is sensitive to exogenous cAMP, and addition of cAMP in the culture medium induced all the mitochondrial characteristics typically seen in mutants with an overactive RAS-cAMP protein kinase A pathway (29). Moreover, we showed that the yeast protein kinase Tpk3p is specifically involved in the regulation of mitochondrial enzymatic content during the transition phase to a reduced cell growth rate in response to substrate limitation (30).

The results presented here are in agreement with previous data indicating a major role for the Ras-cAMP protein kinase A pathway in the control of mitochondrial respiratory content in yeast cells. One of the main interesting results is the strong linear relationship between mitochondrial cytochromes and CAMP content observed in all the mutants studied (Fig. 3 and Tables 4 and 6). A strong correlation is also present between cytochrome content and respiratory capacity and of course as a consequence respiratory capacity and CAMP content. Thus, all the effects of the mutations in the trehalose synthesis pathway on the mitochondrial respiratory content and its activity go through modulation of the cAMP content. Hence, this paper reveals the existence of a new signaling pathway, leading from the trehalose synthesis pathway to the mitochondrial composition. We have clearly shown that the TPS1 gene product modulates cAMP concentration through hexokinase 2. It is worth noting that Tre6P synthase, Tps1, is involved in both the regulation of glycolytic flux and control of mitochondrial respiratory content through hexokinase 2. Glycolysis and mitochondrial activity through oxidative phosphorylation are the cellular “energetic” pathways that convert redox potential in phosphate potential. It thus makes great physiological sense that both pathways have common regulatory mechanisms. Glycolysis control by Tps1 is well documented, and this work shows that Tps1 is also involved in the regulation of mitochondrial biogenesis through Hxk2 and cAMP in the yeast S. cerevisiae. There is a great rationale to this regulation: when the glycolysis flux is inhibited through hexokinase 2/tsp1 interaction, mitochondrial biogenesis is increased, thus allowing a balance in ATP synthesis between glycolysis and oxidative phosphorylation.

**REFERENCES**

1. Estévez, A. M., Heinisch, J. J., and Aragón, J. J. (1995) FEBS Lett. 374, 100–104
2. Gancedo, C., and Serrano, R. (1989) in The Yeasts, pp. 205–260, Academic Press, London
3. Schaffr., I., Heinisch, J., and Zimmermann, F. K. (1989) Yeast 5, 285–290
4. Navas, M. A., Cerdañ, S., and Gancedo, J. M. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 1290–1294
5. Larsson, C., Nilsson, A., Blomberg, A., and Gustafsson, L. (1997) J. Bacte...
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6. Rossell, S., van der Weijden, C. C., Kruckeberg, A. L., Bakker, B. M., and Westerhoff, H. V. (2005) *FEMS Yeast Res.* 5, 611–619
7. Daran-Lapujade, P., Rossell, S., van Gulik, W. M., Luttik, M. A., de Groot, M. J., Sliper, M., Heck, A. J., Daran, J. M., de Winde, J. H., Westerhoff, H. V., Pronk, J. T., and Bakker, B. M. (2007) *Proc. Natl. Acad. Sci. U.S.A.* 104, 15753–15758
8. Thevelein, J. M., and Hohmann, S. (1995) *Trends Biochem. Sci.* 20, 3–10
9. Van Aelst, L., Hohmann, S., Bulaya, B., de Koning, W., Sierkstra, L., Neves, M. J., Luyten, K., Alijo, R., Ramos, J., and Coccetti, P. (1993) *Mol. Microbiol.* 8, 927–943
10. Vuorio, O. E., Kalkkinen, N., and Londesborough, J. (1993) *Eur. J. Biochem.* 216, 849–861
11. González, M. I., Stucka, R., Blázquez, M. A., Feldmann, H., and Gancedo, C. (1992) *Yeast* 8, 183–192
12. Bell, W., Klaasen, P., Ohnacker, M., Boller, T., Herweijer, M., Schoppink, P., Van der Zee, P., and Wiemken, A. (1992) *Eur. J. Biochem.* 209, 951–959
13. De Virgilio, C., Bürckert, N., Bell, W., Jenö, P., Boller, T., and Wiemken, A. (1993) *Eur. J. Biochem.* 212, 315–323
14. Bell, W., Sun, W., Hohmann, S., Wera, S., Reinders, A., De Virgilio, C., Wiemken, A., and Thevelein, J. M. (1998) *J. Biol. Chem.* 273, 33311–33319
15. Navon, G., Shulman, R. G., Yamane, T., Eccleshall, T. R., Lam, K. B., Bar-onofsky, I. J., and Marmur, J. (1979) *Biochemistry* 18, 4487–4499
16. Hohmann, S., Neves, M. J., de Koning, W., Alijo, R., Ramos, J., and Thevelein, J. M. (1993) *Curr. Genet.* 23, 281–289
17. Van Aelst, L., Hohmann, S., Zimmermann, F. K., Jans, A. W., and Thevelein, J. M. (1991) *EMBO J.* 10, 2095–2104
18. Díaz-Ruiz, R., Aveñet, N., Araiza, D., Pinson, B., Uribe-Carvajal, S., Devin, A., and Rigoulet, M. (2000) *Biochim. Biophys. Acta* 1457, 45–56
19. Dejean, L., Beauvoit, B., Guérin, B., and Rigoulet, M. (2000) *Biochim. Biophys. Acta* 1554, 159–169
20. Chevtzoff, C., Vallortigara, J., Avéret, N., Rigoulet, M., and Devin, A. (2005) *Biochim. Biophys. Acta* 1706, 117–125
21. Portela, P., Van Dijck, P., Thevelein, J. M., and Moreno, S. (2003) *FEMS Yeast Res.* 3, 119–126
22. Bonini, B. M., Van Dijck, P., and Thevelein, J. M. (2003) *Biochim. Biophys. Acta* 1606, 83–93
23. van Vaeck, C., Wera, S., van Dijck, P., and Thevelein, J. M. (2001) *Biochem. J.* 353, 157–162
24. Devin, A., Dejean, L., Beauvoit, B., Chevtzoff, C., Avéret, N., Bunoust, O., and Rigoulet, M. (2006) *J. Biol. Chem.* 281, 26779–26784
25. Toda, T., Uno, I., Ishikawa, T., Powers, S., Kataoka, T., Broek, D., Cameron, S., Broach, J., Matsumoto, K., and Wigler, M. (1985) *Cell* 40, 27–36
26. Toda, T., Cameron, S., Sass, P., Zoller, M., Scott, J. D., McMullen, B., Hurwitz, M., Krebs, E. G., and Wigler, M. (1987) *Mol. Cell. Biol.* 7, 1371–1377
27. Toda, T., Cameron, S., Sass, P., Zoller, M., and Wigler, M. (1987) *Cell* 50, 277–287
28. Boy-Marcotte, E., Tadi, D., Perrot, M., Boucherie, H., and Jacquet, M. (1996) *Microbiology* 142, 459–467

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