Yeast gene *YOR137c* is involved in the activation of the yeast plasma membrane H^+-ATPase by glucose

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Abstract Glucose triggers transcriptional and post-transcriptional mechanisms that increase the level and activity of *Saccharomyces cerevisiae* plasma membrane H^+-ATPase. We have studied the post-transcriptional activation of the enzyme by glucose and have found that the *YOR137c* gene product is implicated in this activation. Deletion of *YOR137c* does not affect the level of Pma1 at the plasma membrane, but disturbs the glucose-triggered V_{max} increase of the enzyme. We propose that at least two independent mechanisms are involved in glucose activation of the H^+-ATPase.

Key words: H^+-ATPase; Plasma membrane; Glucose activation; *Saccharomyces cerevisiae*

1. Introduction

The plasma membrane H^+-ATPase of yeast is a proton pump that plays an essential role in the physiology of this organism [1–4]. The activity of yeast ATPase is under strict genetic and environmental control [5–7]; glucose is the external signal that has been most extensively investigated. The activity of the ATPase is controlled, at least, in two ways by the presence of glucose in the medium. First, glucose metabolism increases ATPase gene (*PMA1*) expression [8,9] and second, glucose induces ATPase activation [10]. This activation results from a combined effect on the kinetic parameters of the enzyme, including K_m decrease and V_{max} increase [10]. The molecular mechanism of glucose-induced activation is not known, but it is based on the elimination of an inhibitory interaction of the C-terminus with the active site of the enzyme [11–13], probably mediated by Ser/Thr phosphorylation [14] by an unknown protein kinase activity. This glucose activation of the enzyme seems to be prevented by a glucose starvation-dependent, YCK-mediated phosphorylation of Pma1 [15].

In an earlier study, we isolated mutations in seven genes affecting regulation of the enzyme [9]. In this report, we show that one of these genes corresponds to *YOR137c*. Deletion of *YOR137c* disturbs glucose activation of the ATPase by abolishing the V_{max} increase that occurs during glucose activation.

2. Materials and methods

2.1. Strains and growth conditions

Saccharomyces cerevisiae strains BWG1-7A (*MAT***a** *ade1-100 his4-519 leu2-3,112 ura3-52*) [16], H114 (BWG1-7A *apa7-1*) [9] and its derivatives were grown in medium with 2% glucose, 0.7% Yeast Nitrogen Base without amino acids (USBiologicals, Swampscott, MA) and the appropriate requirements [17]. When indicated, medium was buffered with 50 mM Mes adjusted to pH 6.0 with Tris (SD6.0), or with 50 mM succinic acid adjusted to pH 3.0 with Tris (SD3.0).

2.2. Cloning of the YOR137c gene

Yeast strain H114 carrying the *apa7-1* mutant allele was transformed [18] with 50 μg of DNA from a Ycp50-based library [19]. Approximately 9,000 transformants were selected in SD6.0 medium. Transformed cells were pooled and plated in SD3.0 medium. After four days at 30°C, two colonies grew on acidic medium and exhibited wild type ATPase levels. Plasmids were rescued from yeast [20] and amplified in *E. coli*. Restriction analysis and partial sequencing of these clones revealed that both plasmids contained the same insert (Fig. 1).

2.3. Deletion of YOR137c

The 3.3-kb *Xho*I-*Pst*I fragment (Fig. 1) was subcloned into a pSK(+) (Stratagene, La Jolla, CA) in which the *Hin*dIII site had been destroyed. The resulting plasmid was cut with *Stu*I and *Hin*dIII and ligated with a *Sma*I-*Hin*dIII fragment containing the *LEU2* gene obtained from the plasmid pJJ283 [21]. The resulting plasmid was cut with *Xho*I and *Nsi*I before transformation of an autodiploid constructed by transformation of strain BWG1-7A with the *HO* gene [22]. Integration of the Δyor137c::LEU2 construct at the *YOR137c* locus was confirmed by Southern blotting. The heterozygous diploid Δ*yor137c*::*LEU2*/*YOR137c* was sporulated, and tetrads were dissected.

2.4. Biochemical methods

Yeast plasma membrane was purified from glucose-starved and glucose fermenting cells by differential and sucrose gradient centrifugation [10]. ATPase activity was assayed at pH 6.5 with ATP concentrations from 0.8 to 6 mM [23]. The apparent K_m and V_{max} were extrapolated from double-reciprocal plots fitted using a standard

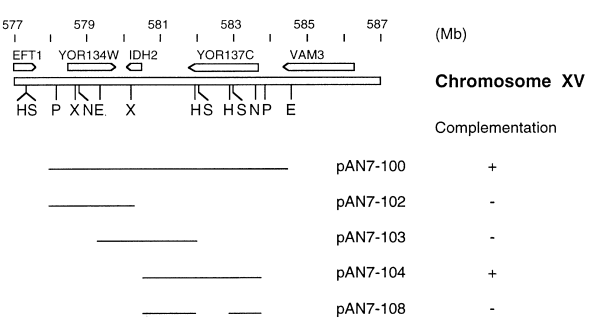

Fig. 1. Chromosomal location and subcloning of the *YOR137c* gene. Restriction map of part of chromosome XV [31]. Positions of the ORFs are indicated by arrows. DNA subclones used to test the ability to complement the *apa7* mutation are represented by thin horizontal lines. Restriction enzyme sites: E, *Eco*RI; H, *Hin*dIII; N, *Nsi*I; P, *Pst*I; X, *Xho*I.

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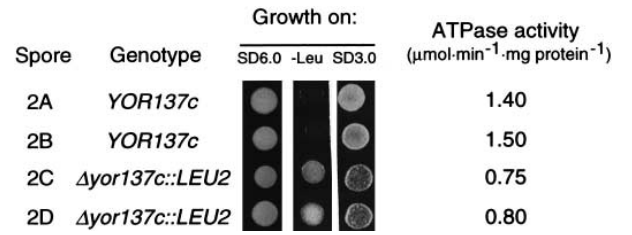

Fig. 2. Growth phenotype and ATPase activity of a representative tetrad from the heterozygous diploid Δ*yor137c*::*LEU2*/*YOR137c*. Cells were plated in media containing glucose media buffered at pH 6.0 (SD6.0), SD6.0 minus leucine (−Leu) or glucose media buffered at pH 3.0 (SD3.0) and incubated at 30°C for 36 h. ATPase activity was measured in purified plasma membrane from glucose fermenting cells. Values are the average of two different experiments. The specific activity differed by less than 10%.

least-squares method. Similar values (within 10%) were obtained with two different plasma membrane preparations isolated independently. Protein concentration was determined by the method of Bradford [24] using the Bio-Rad protein assay reagent and bovine IgG as standard. Plasma membrane proteins were separated by SDS-PAGE on 8% acrylamide using the Laemmli system [25]. The Mab16 monoclonal antibody against the yeast ATPase [26] was used in immunoblot. Western blot with second antibody conjugated to alkaline phosphatase (Bio-Rad) was as described [27].

3. Results and discussion

The *YOR137c* gene was cloned by its ability to suppress the phenotype of the *apa7-1* mutant strain when carried on a centromeric plasmid. This strain exhibits slow growth in SD3.0 medium, as a consequence of a diminished plasma membrane H^+-ATPase activity level [9]. After transformation of the mutant strain with a YCp50-based yeast genomic library, we recovered a plasmid (pAN7-100) containing a 6.5-kb insert corresponding to a segment of chromosome XV (Fig. 1). Subcloning experiments confined the complementary activity to a 3.3-kb *Bgl*II-*Pst*I fragment containing the *YOR137c* gene (Fig. 1). The *YOR137c* gene encodes a 71.8-kDa membrane protein of unknown function [28,29].

A *YOR137c* deletion was created by removal of a fragment of the protein coding sequence and replacement of this segment with the selectable marker *LEU2* (see Section 2). This Δyor137c::LEU2 construct was used to transform a diploid yeast strain. The transformant diploid was sporulated and tetrads dissected. In all 10 four-spore tetrads studied, the Leu^+ segregants exhibited slow growth in SD3.0 and reduced ATPase activity. An example of the growth phenotype and ATPase activity level of a representative tetrad is shown in Fig. 2. The possibility that *YOR137c* was an extragenic suppressor of *apa7-1* was discarded by crossing a Δ*yor137c*::*LEU2* haploid segregant with the strain H114 (*apa7-1*) and analyzing the segregation of the low pH sensitivity phenotype conferred by these mutations. In all cases (12 asci analyzed), the low pH-sensitive phenotype segregated at a ratio of 4:0, suggesting that the cloned gene corresponds to the *APA7* locus.

To study the biochemical phenotype of the Δ*yor137c*::*LEU2* allele, we first examined plasma membrane Pma1 levels by Western analysis of purified plasma membrane preparations from isogenic wild-type and Δ*yor137c*::*LEU2* strains using specific anti-ATPase antibody (Fig. 3). The level of Pma1 was similar in both strains. This suggests that the reduced ATPase activity of the Δ*yor137c*::*LEU2* mutant is not due to a defect in the synthesis or intracellular sorting of Pma1.

Since Δ*yor137c*::*LEU2* does not affect the ATPase protein level, we explored the effect of the mutation on enzyme activation by glucose (Fig. 4). After 15 min incubation with glucose, a six-fold increase in ATPase activity was observed in the wild-type strain, while under the same conditions, glucose caused a three-fold activation of the mutant strain.

To further characterize the effect of the Δ*yor137c*::*LEU2* mutation on the enzyme activation by glucose, we determined the kinetic properties of the enzyme in purified plasma membrane of the wild-type and mutant strains obtained from glucose-starved and glucose fermenting cells (Fig. 5). In mutant enzyme, the typical V_{max} increase caused by glucose was not observed, whereas the K_m decrease was not significantly different from that of the enzyme from wild-type cells. This result suggests that the glucose-triggered V_{max} increase in this enzyme is mediated by the *YOR137c* gene product.

Recent findings suggest that the K_m decrease produced by glucose is a ubiquitin-dependent process [30]. Here we show

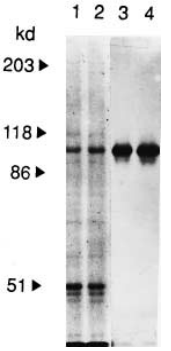

Fig. 3. Analysis of the Pma1 level in *YOR137c* and Δ*yor137c*::*LEU2* strains plasma membranes. SDS-polyacrylamide gel electrophoresis (lanes 1 and 2) and Western blot analysis (lanes 3 and 4) of purified plasma membrane from *YOR137c* (lanes 1 and 3) and Δ*yor137c*::*LEU2* (lanes 2 and 4) strains. Each lane contains 5 μg protein. The position of pre-stained molecular weight standards is indicated.

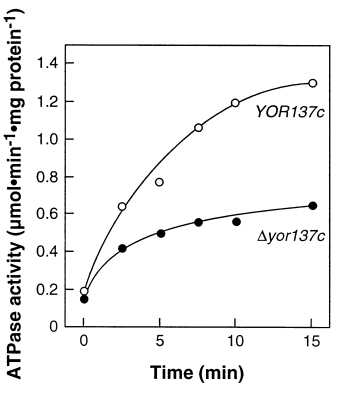

Fig. 4. Glucose activation of ATPase from wild-type and Δ*yor137c*::*LEU2* mutant strains. At time zero, glucose was added to wild-type (○) or to the Δ*yor137c*::*LEU2* mutant strain (●); samples were taken at the times indicated and plasma membrane purified. The ATPase activity of the purified membrane fraction was determined at pH 6.5 with 2 mM ATP. Similar values (within 10%) were obtained in two independent experiments.

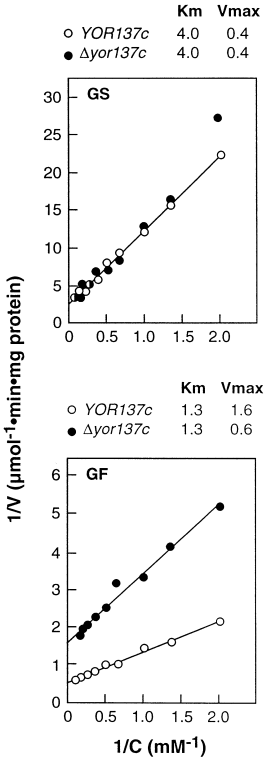

Fig. 5. Effect of glucose on kinetics of plasma membrane ATPase from wild-type and Δ*yor137c*::*LEU2* mutant strain. Wild-type (○) and Δ*yor137c*::*LEU2* (●) were incubated with (GF) or without glucose (GS) before homogenization. The ATPase activity of purified plasma membrane was assayed at pH 6.5 with the indicated concentration of ATP. The apparent K_m (mM) and the V_{max} (μmol/min/mg protein) calculated from the figure are indicated. Similar values (within 10%) were obtained with two independently obtained plasma membrane preparations.

that the glucose-induced V_{max} increase is mediated by an apparently unrelated gene; it thus seems that two independent mechanisms are needed to achieve glucose activation of the ATPase. The fact that deletion of *YOR137c* abolishes the glucose-triggered V_{max} increase suggests that *YOR137c* probably has an upregulating role in the V_{max} increase produced by glucose addition to yeast cells. Although the specific function of *YOR137c* is unknown, this study provides a starting point for further characterization of H^+-ATPase activation.

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