研究报告

Construction and Expression of *HXT7* Promoter Deletion Mutants in *Saccharomyces cerevisiae* *

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Abstract To understand the control of growth and glucose repression in *Saccharomyces cerevisiae* by glucose transport, a set of *S. cerevisiae* strains with variable expression of only one glucose transporter, Hxt7, the most abundantly expressed high-affinity transporter, was constructed. The strains were constructed by partial deletion of the *HXT7* promoter *in vitro* and integration of the gene at various copy numbers into the genome of an *hxt*Δ (*hxt1-hxt7 gal2* deletion) strain. The 149 bp DNA region − 495 to − 346 in the *HXT7* promoter plays an important role in *HXT7* expression. In the mutant strains with promoter length of more than 495 bp, the expression of *HXT7* at high glucose concentrations was much higher than that in the wildtype strain. The level was dependent on copy number and promoter length. Increased expression at low glucose was maintained in these mutants. Hxt7 in the *hxt null* strain displayed an incomplete glucose repression. The growth rate correlated with the level of *HXT7* expression at high glucose concentrations.

Key words glucose, transporter, Hxt7, promoter, deletion, yeast

Glucose, the most abundant monosaccharide in nature, is the primary fuel for yeast. Glucose is not only used as a nutrient for new cell material and source of energy, but is a prime factor for signaling and triggering different regulatory mechanisms involved in regulation of growth, metabolism and development[1~3]. After glucose has been taken up by the cell, it is converted via the common glycolysis into pyruvate and then catabolized either to ethanol (fermentation) or to CO_2 and H_2O (respiration). The flux through these steps determines the rates of fermentation and respiration. Glucose transport in yeast is mediated by proteins encoded by the *HXT* gene family, of which twenty members have been identified genetically or by sequence homology[4]. Individual hexose transporter proteins have distinct affinities for glucose which are used to adapt cells to an extremely broad range of conditions. Expression of *HXT7* is repressed by high glucose and induced by low glucose[5]. Under derepressed conditions *HXT7* is by far the most strongly expressed *HXT* gene in most cultures[6].

In the yeast *S. cerevisiae*, glucose metabolism is a complex network of reactions, catalyzed by numerous enzymes. Metabolic control analysis predicts that control of metabolic pathways is distributed amongst all steps of the pathway, but that some steps can have high proportions of the total pathway control[7,8]. In principle, every step in a pathway shares the control of that pathway; the sum of the control coefficients in a pathway is one. A large proportion of the control of the glycolytic pathway is thought to reside in the step of glucose transport in most growth conditions. To assess quantitatively the role of glucose transport in the control of glycolysis under various growth conditions at steady state, the level of glucose transport activity can be regulated by genetically manipulating the expression of hexose transporters, except other approaches[9,10]. The non-coding upstream region of many genes in yeast have regulatory sequences. Partial removal of these sequences from *HXT* genes may lead to alter hexose transporter expression levels by small amounts. The work presented here is construction of strains in which only one hexose transporter (Hxt7) is expressed to different levels. The goal is to understand better the control and regulation of glucose transport under defined growth conditions[11].

1 Materials and Methods

1.1 Strains and growth conditions

The *S. cerevisiae* strains used in this study are listed in Table 1. Yeast cells were grown in a rotary

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shaker at 30 ℃ in either SC-UM (containing 0.16% Yeast Nitrogen Base, 0.5% ammonium sulfate, 0.1% casamino acids, 0.01% tryptophan and 2% maltose), or YP medium (2% peptone, 1% yeast extract) containing either 2% maltose (YPM) or 1% glucose (YPD). Plasmid transformation of yeast cells was carried out by the lithium acetate method[12].

E. coli DH5α served as a host for all plasmids and was transformed by the $CaCl_2$ method as described[13]. Transformants were grown in Luria-Bertani medium containing 60 mg/L ampicillin at 37 ℃.

Table 1 *S. cerevisiae* strains

Strain	Genotype
MC996A	*MAT*α *ura3-52 his3-11, 15 leu2-3, 112 MAL2 SUC2 GAL MEL*
RE607B	*MAT*α *hxt1* Δ::*HIS3*:: Δ*hxt4 hxt5*::*LEU2 hxt2*Δ::*HIS3 hxt3*Δ::*LEU2*:: Δ*hxt6 ura3-52*
KY73 (the hxt null strain)	*MAT*α *hxt1* Δ::*HIS3*:: Δ*hxt4 hxt5*::*LEU2 hxt2*Δ::*HIS3 hxt3*Δ::*LEU2*:: Δ*hxt6 HXT7*::*HIS3 gal2*Δ *ura3-52*
LYY0~ LYY21	*MAT*α *hxt1* Δ::*HIS3*:: Δ*hxt4 hxt5*::*LEU2 hxt2*Δ::*HIS3 hxt3*Δ::*LEU2*:: Δ*hxt6 HXT7*::*HIS3 gal2*Δ *ura3-52*::(*HXT7* Δ*p-URA3*)

1.2 Construction of *HXT7* promoter mutants by progressive deletion

Plasmid pBCY7 contains the promoter region and open reading frame of *HXT7*. Progressive deletion of the *HXT7* promoter was carried out by the procedure of Henikoff[14]. In order to minimize the proportion of nicked molecules in the starting DNA, high-quality pBCY7 plasmid DNA was prepared using the Plasmid Mini Kit (Qiagen). The closed circular DNA was digested with restriction enzyme *Xba* I (Roche) at 1 149 bp upstream of the *HXT7* start codon. Exonuclease III (New England Biolabs) was used to digest the linear DNA from the 5′ end, proceeding in two directions at 22 ℃. Samples were removed at 1 min intervals to tubes containing S_1 nuclease (Promega), which removed the single-stranded tails remaining after exonuclease III digestion. After neutralization and heat inactivation of the S_1 nuclease, Klenow enzyme (Roche) and T4 DNA ligase (Roche) were added to circularize the resulting DNA molecules. The ligation mixtures were used to transform DH5α competent cells. A number of subclones from each time point were then screened to identify plasmids with appropriate deletion endpoints. The names of the selected plasmids, pBCYΔ1 ~ pBCYΔ25, refer to the size of the undeleted promoter regions from large to small.

1.3 Sequence analysis of the *HXT7* promoter deletion series

Sequencing was performed by the dideoxy method using the Sequenase Version 2.0 DNA Sequencing Kit (Amersham) and [α-^{35}S]-dATP (Amersham) with the primers AK38 (5′ CTGCAATAGCAGCGTCTTG 3′), LY17 (5′ CTCTTCACCTTCACCATAAGC 3′) and PBCY7-3387 (5′ CCTCAGAAGAACACGCAGG 3′).

1.4 Construction of *HXT7* promoter deletion integrative plasmids and transformation into an *hxt* null strain

pBCYΔ1~ pBCYΔ25 were digested with *Eco*RI and pBCY7 was digested with *Eco*RI/*Stu* I respectively. *Eco*RI fragments from 5.16~ 2.95 kb in size, containing the *HXT7* open reading frame and (deleted) promoter, were isolated with the QIAquick Gel Extraction Kit (Qiagen) and ligated into the yeast-*E. coli* integrative shuttle vector YIplac211[15]. The resulting plasmids named pBCIΔ1~ pBCIΔ25 were transformed into *hxt* null strain KY73 by selection for uracil prototrophy on SC-UM plates. Targeted integration of the plasmids at the *URA3* locus was achieved by linearization of the plasmids with *Stu* I. The resulting strains were named LYY0~ LYY25 in order of increasing deletion of the *HXT7* promoter.

1.5 Southern blot analysis of *HXT7* promoter deletion DNA integrated into the yeast genome

Genomic DNA was isolated as described[16]. The genomic DNA was digested with *Pst* I and *Cla* I and separated by electrophoresis through a 1% agarose gel. The DNA fragments were transferred to nylon membrane (BioRad) by vacuum blotting, and cross-linked to the membrane with ultraviolet light (Stratalinker 2400, Stratagene). The blot was prehybridized in 5 ml prehybridization buffer (6 × SSC, 0.1% SDS, 5 × Denhardt's, 100 mg/L sheared, denatured salmon sperm DNA) at 45 ℃ for 1 h.

For detection of *HXT7* genomic DNA fragments, GB7, a 29 base oligonucleotide probe (5′ TTAAAAACGTATTTACTTTTCAAGATATC 3′), was designed by selecting a sequence about 90 bp 3′ of the *HXT7* stop codon. This region displays little similarity with the sequence of the HXT6 gene.

5 pmol of the *HXT7* probe were labeled with 740 Bq [γ-^{32}P] -ATP (Amersham) and 10 U T4 polynucleotide kinase (Roche), according to the manufacturer's instructions. Purified probe was hybridized with the blot at 45 ℃ for 4 h in a MICRO-4 Hybridization Oven (Hybaid). The blot was washed with 6 × SSC/0.1% SDS 2 × 5 min at 37 ℃, 1 × SSC/0.1% SDS 2 × 5 min at 37 ℃ and 2 × 5 min at 45 ℃.

To confirm that the *HXT7* gene was integrated into the chromosome of KY73 at the *URA3* locus, a probe consisting of a 248 bp fragment of the *URA3* gene was prepared by digesting YIplac211 with *Eco*R V and *Stu* I. The genomic DNA of the *HXT7* promoter deletion mutant series was digested with *Eco*R V and *Hin*d III at 37 ℃ overnight. The *URA3* probe was labeled with α-^{32}P-dATP using the Prime-a-Gene Labeling Kit (Promega), according to the manufacturer's instructions. Hybridization and washing were carried out as described[13].

1.6 Measurement of growth and glucose consumption

Cells were grown in liquid YPD medium. Growth was monitored by measurement of the optical density (A_{600}) at various time points. Plotting the natural logarithm of A_{600} against time, the slope of the curve during the range of exponential phase is the growth rate. The residual glucose in the medium at indicated time points was determined by quenching an aliquot of the cultures in an equal volume of 5% trichloroacetic acid and measuring the glucose concentration enzymatically with hexokinase and glucose 6-phosphate dehydrogenase[17]. The absorbance change of NADH at 340 nm was measured with a COBAS auto-analyzer (Roche).

1.7 Northern blot analysis

Total RNA was isolated from yeast cells by acid-phenol extraction[18]. The RNA was dissolved in RNase-free water and formamide. RNA samples were separated by electrophoresis. Transfer to nylon membranes, prehybridization, hybridization with the GB7 or PDA1 (5′ GAATGAAGCAGCAAGCATTGGCAC 3′) probes, and washing were carried out as described above for DNA blots. The PDA1 probe was used as a loading control[19].

1.8 Western blot analysis

Cells were harvested by centrifugation, washed once in 1% KCl, and extracted by abrasion with glass beads in buffer A (50 mmol/L Tris-HCl pH 8, 10 mmol/L EDTA, 5% glycerol, plus protease inhibitors (1 mg/L leupeptin, 1 mg/L pepstatin A, 0.2 mmol/L AEBSF in DMSO)). The lysates were cleared by centrifugation at 1 300 × *g* for 2 min. The protein concentration was determined, and samples were diluted to 1 g·L^{-1} in buffer A and supplemented with 1/2 volume SDS-PAGE loading buffer. The samples were heated to 40 ℃ for 15 min and 10 μg of each protein sample were electrophoresed in a 10% SDS-PAGE minigel. The proteins were transferred to PVDF membrane using a mini trans-blot electrophoretic transfer cell (BioRad) with buffer B (48 mmol/L Tris, 39 mmol/L glycine, 5% methanol, 0.05% SDS pH 8.3) at 100V for 1.5 h at 4 ℃. Membranes were incubated with gentle agitation in phosphate buffered saline + 0.1% Tween-20 as follows: a. blocking for at least 1 h in 5% nonfat milk; b. incubation with anti-Hxt7 antibody (diluted 1∶500) for 16 h at room temperature in 1% nonfat milk, followed by 4 × 5 min washes; c. incubation with horseradish peroxidase-conjugated secondary antibody (diluted 1∶3000) for 1 h in 1% nonfat milk, followed by 3 × 10 min washes. The final wash was done without Tween-20 in the buffer. Detection was carried out by chemiluminescence using the Super Signal chemiluminescent substrate kit (Pierce).

1.9 Reagents

Anti-Hxt7 antibody was a kind gift of Dr. Boles (Heinrich-Heine-University, Germany). Horseradish peroxidase-conjugated goat anti-rabbit antibody was from BioRad. Yeast extract, peptone, tryptone, casamino acids and yeast nitrogen base were obtained from Difco. Chemicals were obtained from Sigma or Merck and were of reagent grade. Oligonucleotides were synthesized by Isogen.

2 Results

2.1 A promoter length of more than 346bp is required for high-level *HXT7* expression

A series of *HXT7* promoter mutants was constructed by progressive bi-directional deletion of the promoter DNA. The length of *HXT7* promoter region remaining in the mutants was determined by agarose gel electrophoresis (Fig. 1). The promoter length of *HXT7* in these mutants, derived from DNA sequence analysis, is presented in Fig. 2. The mutants are numbered in the order of the distance to the start codon from the proximal endpoint of deletion, from large to small. The mutant *HXT7* genes were cloned into an integrative plasmid to yield the pBCIΔ series of integrative plasmids. This series was transformed into the hxtΔ strain KY73 with integration at the URA3 locus.

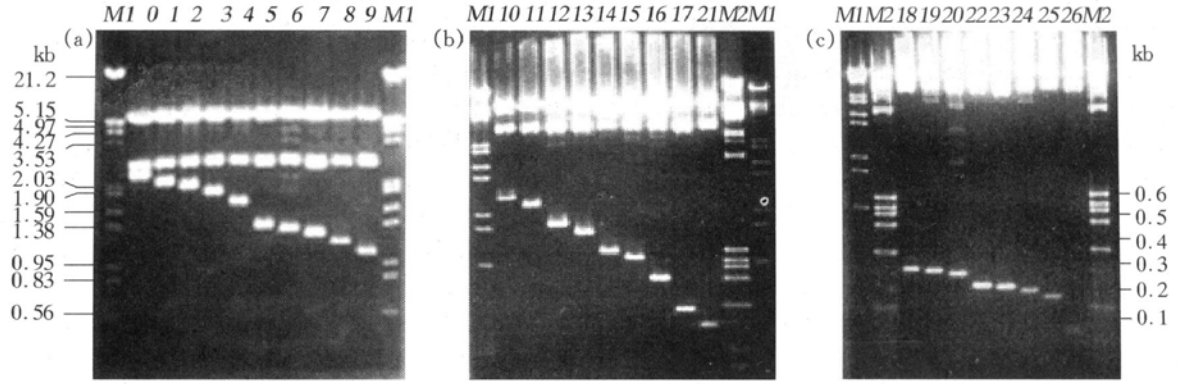

Fig. 1 *HXT7* promoter progressive deletion series

Plasmid pBCY7 was digested by *Xba* I. Progressive deletion of linear DNA was performed with *Exo* III and S_1. After ligation and transformation, the subclones from each time point were screened by *Hind* III/ *Cla* I digestion. (a) pBCY Δ1~ pBCY Δ9 in 1% agarose gel; (b) pBCY Δ10~ pBCY Δ17, pBCY Δ21 in 1.5% agarose gel; (c) pBCY Δ18~ pBCY Δ26 in 2% agarose gel. Molecular mass markers: M1, M2.

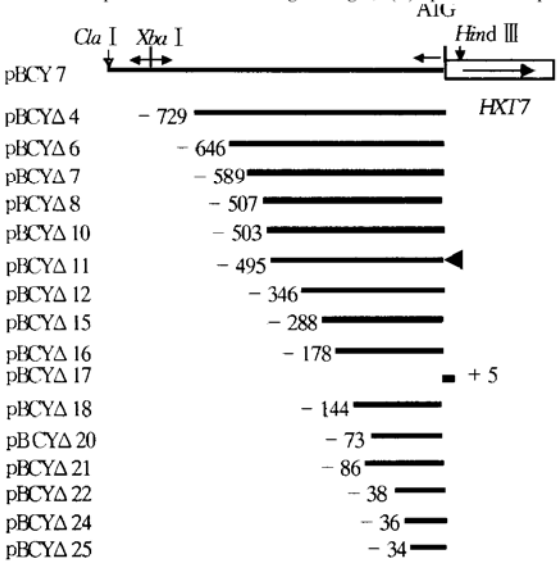

Fig. 2 *HXT7* promoter exonuclease III-generated 5′ deletion series

Based on sequence analysis, the 5′ deletion end-points relative to the ATG start codon (A is + 1) are indicated on the left side of each fragment.

The presence of the promoter deletion *HXT7* DNAs in the yeast genome was examined by Southern blotting with a *URA3* probe (Fig. 3a) and an *HXT7* probe (Fig. 3b), respectively. For hybridization with the *URA3* probe, genomic DNA was digested with the restriction enzymes *Eco*R V and *Hind* III which cut within the *URA3* locus on either side of the site of integration. RE607B is a strain, congenic to the wildtype strain MC996A, in which the *HXT1*~*HXT6* genes are inactivated[20]. The *HXT7* locus is intact and encodes an active glucose transporter. KY73 is a strain with null alleles in the *HXT1*~*HXT7* genes and the *GAL2* gene. The absence of high molecular mass bands in the parental strain and the variable size of one of the high molecular mass bands in the transformants demonstrate that the promoter deletion plasmids have been integrated correctly at the *URA3* locus.

Hybridization of *Pst* I-*Cla* I-digested genomic DNA with the *HXT7* probe labeled bands of different length, as expected from the extent of the deletion in the promoter region (Fig. 3b). The increase in molecular mass of the *HXT7* band between lanes *LYY10* and *LYY6* is due to elimination of the *Pst* I site in the region of plasmid pBCIΔ6 distal to the *Xba* I site and in plasmids with more extensive deletions (Fig. 3c). The varying intensities of the bands corresponding to the integrated *HXT7* genes reveal that in most isolates more than one copy of the plasmid was integrated into the chromosome. For example, strains LYY1, LYY4, LYY5 and LYY21

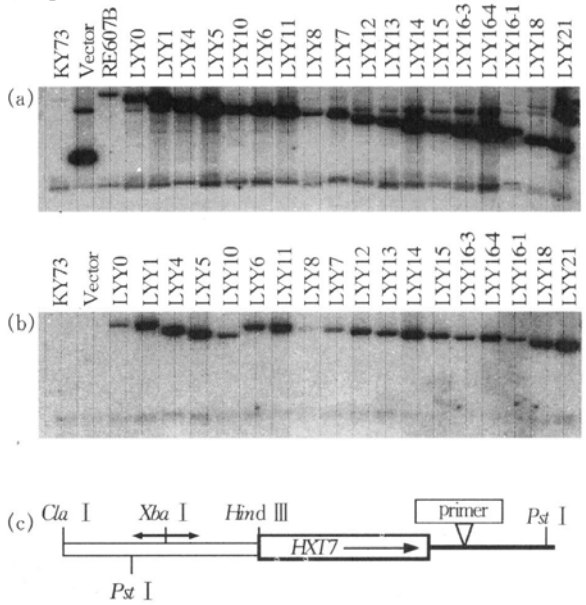

Fig. 3 Southern blot analysis of *HXT7* integration

(a) Genomic DNA of the indicated yeast strains was digested with *Eco*R V and *Hind* III, and the resulting DNA was blotted and hybridized with a *URA3* probe. (b) Genomic DNA of the indicated yeast strains was digested with *Pst* I and *Cla* I, and the resulting DNA was blotted and hybridized with an *HXT7* probe. (c) Restriction map of the *HXT7* locus. The *Xba* I site at which exonuclease deletion was initiated, and the binding site of the GB7 primer, is indicated.

have multiple copies of the plasmid; only strain LYY8 contains a single copy. By comparison of the relative band densities as measured with a Bio-RAD 1650 Scanning Densitometer the probable gene copy numbers were determined.

Growth on glucose was tested for a number of isolates from each transformation. The growth changed markedly between transformants containing pBCIΔ11 and pBCIΔ12. As shown in Fig. 4, representative strains LYY4 through LYY11 (containing pBCIΔ4 to pBCIΔ11) grew well on glucose, to approximately the same extent as observed for strains RE607B and LYY0 that contain *HXT7* with a full-length promoter. In contrast, representative strains LYY12 through LYY21 (containing pBCIΔ12 ~ pBCIΔ21) were unable to grow on glucose, as was also observed for isolates transformed with the empty vector YIplac211. The *HXT7* promoter is 149 bp shorter in pBCIΔ12 than pBCIΔ11.

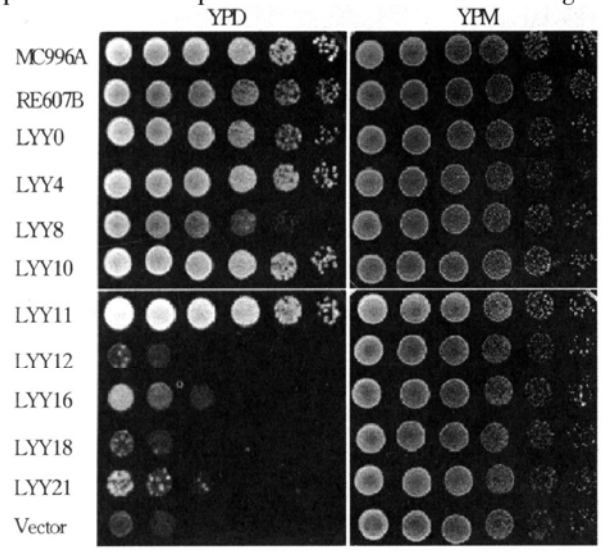

Fig. 4 Growth phenotypes of *HXT7* promoter mutants on YPD or YPM plates

Suspensions of 1.4×10^8 cells·ml^{-1} from each strain were serially ten-fold diluted and 5 μl of each dilution was spotted onto the solid medium. The plates were incubated for 3 d prior to photography.

2.2 *HXT7* expression is related to the promoter length and the gene copy number

Fig. 5 shows growth curves of 17 individual strains. All mutant strains as well as RE607B grew slower than the *HXT* wildtype strain MC996A. Most mutant strains with a promoter length of more than 346 bp (LYY1~ LYY11) grew faster than the RE607B strain. Since each of these contain multiple copies of *HXT7*, its enhanced growth relative to RE607B was probably due to over-expression of *HXT7*. This conclusion is reinforced by the growth phenotype of LYY8. This strain grew slower than RE607B and since both strains carry a single copy of *HXT7*, the difference in growth rate was probably due to the shortened *HXT7* promoter in LYY8. The mutant strains numbered LYY12 and higher (with promoter lengths less than 346 bp) did not grow significantly.

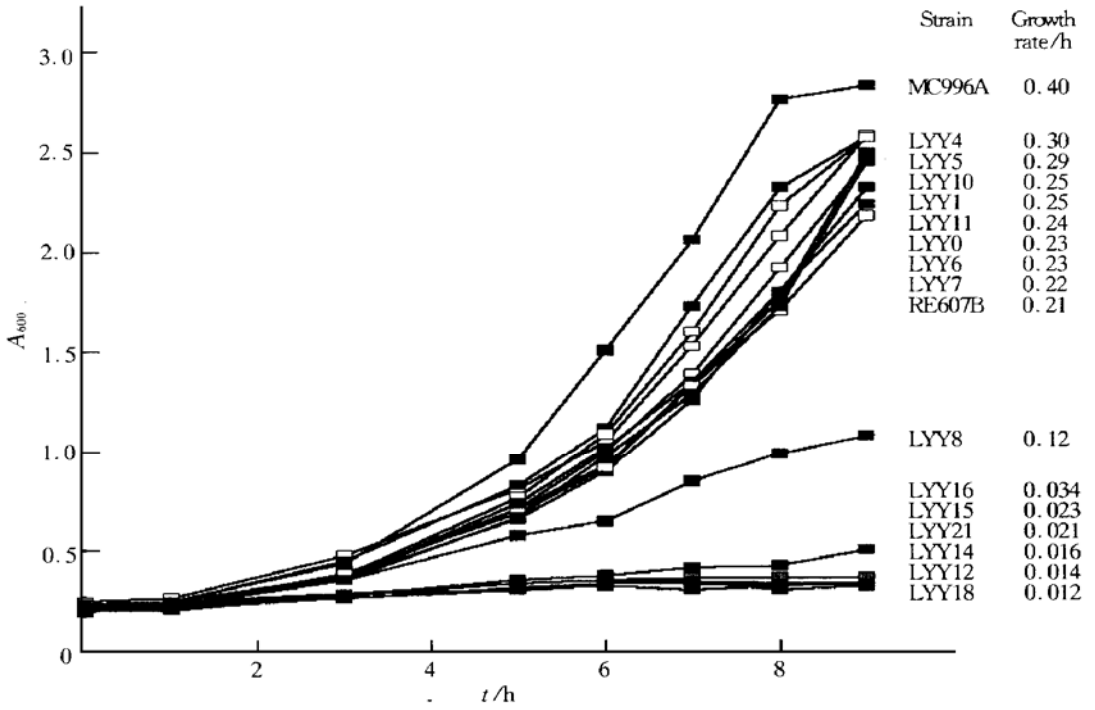

Fig. 5 Growth curves of *HXT7* promoter mutants

Each strain was inoculated from a single colony to YPM pre-cultures, and grown to early stationary phase. YPD medium was inoculated to an optical density (A_{600}) of approximately 0.2, and growth was monitored by measuring A_{600} for 9 h.

Analysis of *HXT7* mRNA levels confirmed that *HXT7* expression correlates with the promoter structures on the growth phenotypes of the strains (Fig. 6). LYY0~ LYY11 showed clear *HXT7* expression and the signal strength was roughly proportional with the gene copy number. Strains LYY12~ LYY21 had low levels of *HXT7* expression.

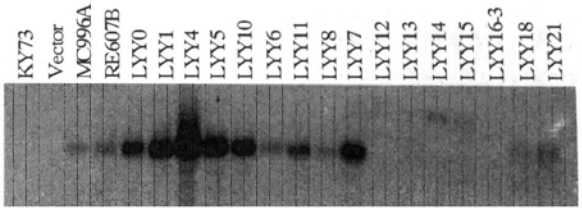

Fig. 6 Northern blot analysis

RNA was isolated from each strain after growth for 8 h on YPD medium. The resulting RNA blot was probed for *HXT7* mRNA as described in Materials and Methods.

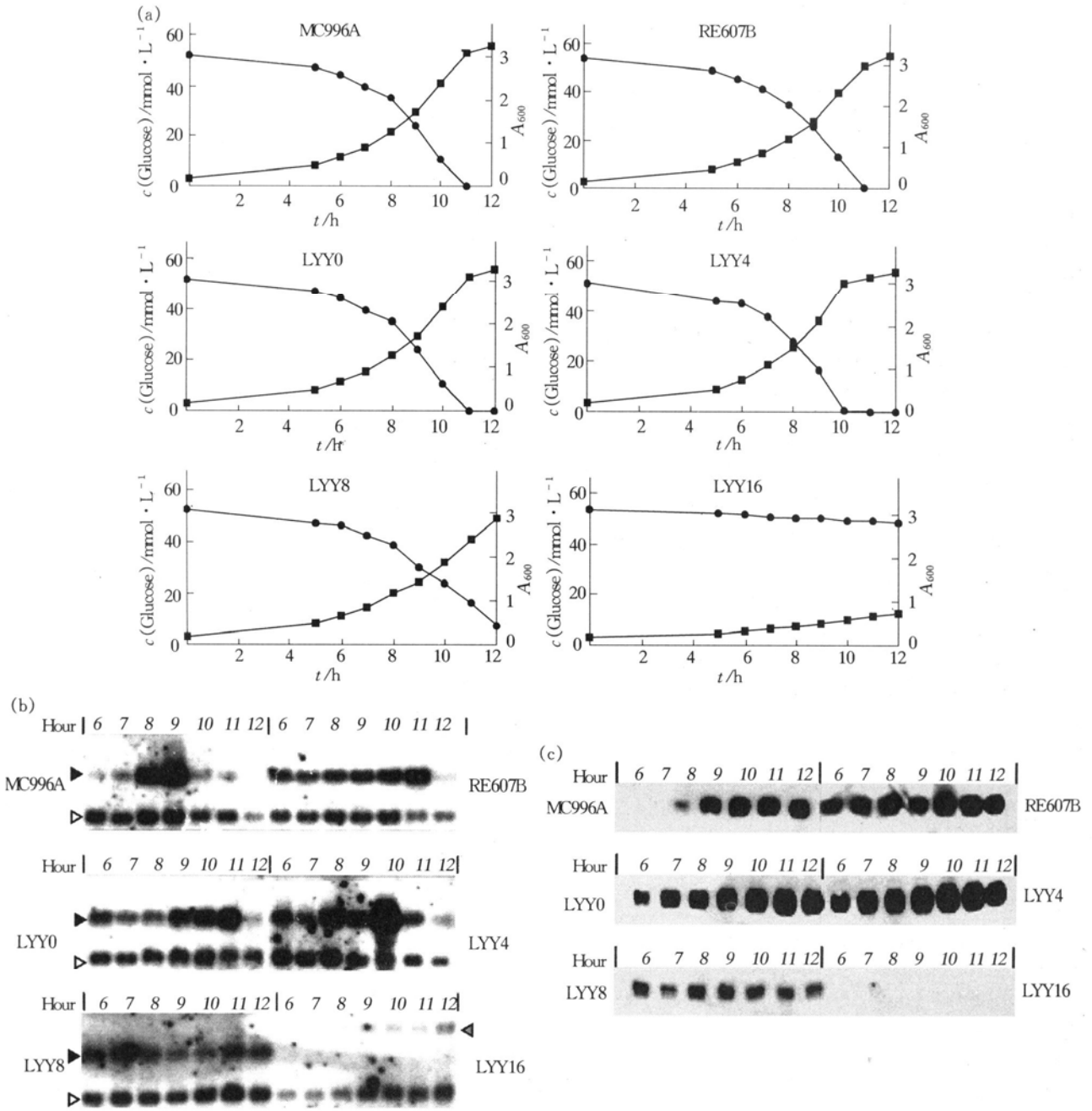

Fig. 7 Growth (■), glucose consumption (●), and *HXT7* expression

Each strain was inoculated from a single colony to YPM pre-cultures, and grown to early stationary phase. YPD medium was inoculated to an optical density (A_{600}) of approximately 0.2. (a) Over a 12 h period growth was monitored by measuring A_{600} and the glucose concentration was measured as described in Materials and Methods. At the same timepoints *HXT7* mRNA (b) and Hxt7 protein (c) were detected by Northern blot and Western blot analysis, respectively, on culture samples harvested by brief centrifugation at 4℃ and frozen in liquid nitrogen. (b) *HXT7* mRNA, filled arrows; *PDA1* mRNA, open arrows; gray arrow, anomalously large *HXT7* mRNA.

2.3 *HXT7* is highly expressed at low concentrations of glucose

The expression pattern of *HXT7* in the promoter deletion strains was compared with the wildtype strain. For six strains growing in medium containing 1% glucose the growth curve, glucose consumption pattern, and *HXT7* mRNA and protein levels were measured simultaneously. The wildtype strain MC996A grew faster than all other strains. *HXT7* mRNA was abundant in this strain at 8 h (residual glucose, 18 mmol/L), and reached a maximum level at 9 h (residual glucose, 3 mmol/L). After 10 h, residual glucose in the medium was undetectable and MC996A stopped growing exponentially (Fig. 7a). The level of *HXT7* mRNA declined rapidly after glucose exhaustion (Fig. 7b). As expected, the appearance of Hxt7 protein was delayed compared to the appearance of the mRNA, and the protein level remained high after glucose exhaustion (Fig. 7c).

The rates of growth and glucose consumption in cultures of RE607B, LYY0 and LYY4 were slower than in the MC996A culture (Fig. 7a). However, considerable expression of *HXT7* mRNA and Hxt7 protein was observed at 6 h, and expression remained high until 12 h (i. e. 2~ 3 h after glucose exhaustion) (Fig. 7b, c). *HXT7* expression in wildtype strain MC996A at high glucose concentrations (more than 30 mmol/L, at 6 and 7 h) was lower than that in Hxt7 only strains (RE607B, LYY0, LYY4, LYY8). Strain LYY8, which contains only a single copy of promoter-deleted *HXT7*, grew more slowly and had not consumed all of the available glucose after 12 h of growth. This strain also had a lower level of *HXT7* mRNA and Hxt7 protein; the level of expression was approximately the same between 6 and 12 h of growth.

3 Discussion

Expression of the *HXT7* gene was examined with respect to the extracellular glucose concentration during batch growth on glucose, and to the size of the promoter region and the copy number of *HXT7* genes in the genome. High glucose concentrations repressed *HXT7* expression, but at lower glucose concentrations *HXT7* was expressed at a high level. This expression was transient, and the level of *HXT7* mRNA declined rapidly as glucose was exhausted from the medium. However, the Hxt7 protein was stable for at least 2 h after glucose exhaustion. This means that the degradation rate of Hxt7 protein is slower than its synthesis rate.

Expression of *HXT7* in the wildtype strain reached a maximum when the glucose concentration fell below 5 mmol/L glucose. High glucose concentrations repressed *HXT7* expression. But in the absence of glucose or at too low concentrations of glucose, *HXT7* transcription was also repressed, and the *HXT7* mRNA pool turned over rapidly. The Hxt7 protein, however, was stable under these conditions. In contrast, strains expressing only *HXT7* with a promoter longer than the critical length, partly derepressed the gene at higher glucose concentrations, and relied solely upon *HXT7* for glucose consumption. While the sequence between − 495 and − 346 seems responsible for expression to occur, the increased expression at low glucose concentrations may be due to this same region.

The effect of mutating the *HXT7* promoter by progressive deletion was analyzed with respect to growth and *HXT7* expression. Shorter deletions had only small effects on transcription; however, deletion of a critical 149 bp region drastically reduced the *HXT7* mRNA and protein levels and the ability of these *HXT7* alleles to support growth on glucose. What kind of activator sequence is it? To find the transcriptional activation element in this special 149 bp DNA region will be an interesting and exciting work.

Based on this set of *HXT7* promoter progressive deletion strains, the consequences for various physiological properties of varying the glucose transport capacity were examined in the separate experiments[11]. We found the glucose transport capacity increased in strains with higher levels of *HXT7* expression. The control coefficient of glucose transport with respect to growth rate was 0.54. The results demonstrate that glucose transport exerts a high level of control over growth and glucose transport affects glucose repression.

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酵母己糖转运蛋白 Hxt7 启动子突变体的构建与表达*

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摘要 葡萄糖在酵母细胞中的转运是通过一个庞大的己糖转运子家族实现的. Hxt7 为一种高亲和性转运蛋白, 在这个家族中的表达丰度最高. 它的表达为低浓度葡萄糖所诱导. 为了研究葡萄糖转运对细胞生长和葡萄糖阻遏的控制作用, 提高对糖酵解通路和糖代谢过程的可调控性, 该研究对 *HXT*7 启动子进行逐步删除, 并将含启动子区域长短不同的 *HXT*7 基因整合转入酿酒酵母己糖转运子缺失菌株 *hxt*Δ (*hxt*1~ *hxt*7, *gal*2 缺失) 的基因组, 构建一组仅含 *HXT*7 且表达水平不一的酵母启动子突变株. 将 *HXT*7 在突变株中的表达与在野生菌株 (MC996A) 中的表达进行比较, 对它们在含 50 mmol/L 葡萄糖培养基中的生长速率、葡萄糖消耗性能、*HXT*7 mRNA 及蛋白质表达水平等进行了实时测定. 结果表明, 当葡萄糖浓度低于 5 mmol/L 时, *HXT*7 的表达量最高. 高浓度葡萄糖环境下, *HXT*7 在突变株中的表达高于在野生株中的表达, 显示出不完全性葡萄糖阻遏效应. *HXT*7 的表达水平与启动子长短和基因拷贝数有关. 位于- 495 至- 346 之间的 149 bp *HXT*7 启动子区域对 *HXT*7 的表达至关重要. 启动子短于 346 bp, *HXT*7 基本不表达.

关键词 葡萄糖, 转运子, Hxt7, 启动子, 删除, 酵母
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