Isolation and Characterization of a Thiamin Transport Gene, THI10, from Saccharomyces cerevisiae*

(Received for publication, February 18, 1997, and in revised form, April 18, 1997)

Fumio Enjo†, Kazuto Nosaka, Masakazu Ogata, Akio Iwashima§, and Hiroshi Nishimura

From the Department of Biochemistry, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602, and the Department of Hygienic and Environmental Science, Fushimi-ku, Kyoto 612, Japan

We isolated a thiamin transporter gene, THI10, from a genomic library of Saccharomyces cerevisiae by the complementation of a yeast mutant defective in thiamin transport activity. The THI10 gene contained an open reading frame of 1,794 base pairs encoding a 598-amino acid polypeptide with a calculated molecular weight of 66,903. The nucleotide sequence of THI10 is completely identical to that of an anonymous DNA (open reading frame L8083.2) mapped to chromosome XII; two other genes (open reading frames YOR071c and YOR192c) in chromosome XV are extremely similar to THI10. Moreover, the THI10 gene product showed significant sequence homology with yeast allantoin and uracil transporters. Hydropathy profile suggested that THI10 product is highly hydrophobic and contains many transmembrane regions. Gene disruption of the THI10 locus completely abolished the thiamin transport activity and thiamin binding activity in yeast plasma membrane fraction. Both the transport and thiamin binding activities were restored in the disrupted cells when the THI10 open reading frame was expressed by yeast GAL1 promoter, suggesting that the THI10 gene encodes for the thiamin transport carrier protein. Northern blot analysis demonstrated that THI10 gene expression is regulated at the mRNA level by intracellular thiamin pyrophosphate and that it requires a positive regulatory factor encoded by THI3 gene.

Yeast cells take up thiamin from the extracellular environment by an active transport system with a pH optimum of 4.5 and a $K_m$ value of 0.18 µM, and thiamin in the cells is concentrated ~10,000-fold over extracellular levels (1). The thiamin transport system is more specific for the chemical structure of the pyrimidine moiety than the thiazole moiety of thiamin (2). Saccharomyces cerevisiae also secretes a thiamin-repressible acid phosphatase encoded by PHO3 gene (3, 4) in a periplasmic space. Since yeast cells cannot take up thiamin phosphate esters, the PHO3 protein with a high affinity for thiamin phosphate esters appears to hydrolyze them before the uptake (5, 6), and then thiamin is converted to thiamin pyrophosphate (TPP) by thiamin pyrophosphokinase (EC 2.7.6.2) encoded by the THI80 gene (7). TPP is an important cofactor in the energy metabolism (8) and is a negative effector of the regulation mechanism of thiamin metabolism in yeast cells (9).

We report here the isolation and characterization of S. cerevisiae THI10 gene and provide evidence that the THI10 gene encodes for a thiamin transport carrier protein. The predicted THI10 protein is highly hydrophobic and shows significant sequence similarities to yeast uracil and allantoin transport proteins. The thiamin transport activity and the thiamin binding activity in the yeast plasma membrane fraction in th10 null strain cells were restored when the THI10 open reading frame (ORF) was expressed by yeast GAL1 promoter. The regulation of the THI10 gene expression was also investigated.

EXPERIMENTAL PROCEDURES

Organisms and Cultures—Table I shows the S. cerevisiae strains used in this study. The thiamin transport mutant was isolated as a strain resistant to pyrithiamin after the chemical mutagenesis with ethyl methanesulfonate as described (10). The E. coli strain DH5 was used to amplify plasmids. The media and the growth conditions for the yeast and bacterial cells were as described previously (7). Glucose in the yeast minimal medium was replaced by 1% fructose for inducing the expression of the THI10, YOR071c, and YOR192c genes from the upstream of the yeast genomic library of pTTS6 (see Fig. 1): 5′-end primer, CTCGCATGCCTAAGCAGCTTTTTCACTGG, was used for expression in yeast as a single-copy vector. To construct the thi10 disrupted mutant, the 4.0-kilobase pair fragment EcoRI-SphI fragment of pTTS1 (see Fig. 1) containing the THI10 gene was ligated into the 3.1-kb pUC118 vector (15) to yield pUC118-THI10, and a 1.4-kb XhoI-SphI fragment containing yeast HIS3 gene of pJJ217 (16) was inserted into pUC118-THI10 to obtain the corresponding sites within the THI10 coding sequence. The linear 3.0-kb Spe1-SphI fragment of the resultant plasmid pUC-thi10::HIS3 was used to transform the haploid yeast YPH500. His colonies were selected, and gene disruption was confirmed by Southern blotting. To express THI10, YOR071c, and YOR192c genes from the GAL1 (17) promoter, a yeast expression vector pYES2 (Invitrogen) carrying the GAL1 promoter, a a yeast expression vector pYES2 (Invitrogen) carrying the GAL1 promoter, and the yeast CYCl (18) transcription termination signal sequence, a 2-µm origin of replication was used. For subcloning of THI10, the ORF was amplified by polymerase chain reaction (PCR, Perkin-Elmer Cetus Instruments) from a plasmid pTTS6 (see Fig. 1): the 5′-end primer, CTCGAAATTCATGATTTCTGCATGAAAATG was immediately upstream of the THI10 ATG with the EcoRI linker, and the 3′-end primer, CTCGCACTGGTAAAGCTTCTGCTCCTG, was downstream of the THI10 TAG with the Sp6 linker. For isolation of YOR071c and YOR192c genes by PCR, specific primers targeted to these untranslated regions were designed according to the sequences of the GenBank database. GCGTGGCTACCGCTATTTGTTCTCGG

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) D55634.

† To whom correspondence should be addressed. Tel.: 75-251-5315; Fax: 75-251-2746; E-mail: fumioenjo@biochem.kyoto-u.ac.jp.

§ To whom correspondence should be addressed. Tel.: 75-251-5315; Fax: 75-251-2746; E-mail: ihoshis@biohec.kyoto-u.ac.jp.

1 The abbreviations used are: TPP, thiamin pyrophosphate; ORF, open reading frame; kb, kilobase pair; bp, base pairs; PCR, polymerase chain reaction.

This paper is available on line at http://www.jbc.org

Vol. 272, No. 31, Issue of August 1, pp. 19165–19170, 1997
Printed in U.S.A.
© 1997 by The American Society for Biochemistry and Molecular Biology, Inc.
Yeast Thiamin Transport Gene

**TABLE I Yeast strains used**

| Strain     | Genotype                      | Source   |
|------------|-------------------------------|----------|
| X2180-1A   | MATa SUC2mal gal2 CUP1         | YGSC     |
| IPO10482   | MATa his4-319 gal2            | IFO      |
| YPH499     | MATa ura3-52 his3-Δ200 leu2-Δ1 trp1-Δ63 ade2-101 lys2-801 | Y. Oya'   |
| YPH500     | MATa ura3-52 his3-Δ200 leu2-Δ1 trp1-Δ63 ade2-101 lys2-801 | Y. Oya'   |
| T50-1B     | MATa thi10-1 ura3-52 his3-Δ200 leu2-Δ1 trp1-Δ63 Δ63 lys2-801 | This study|
| T50-1      | MATa thi10::HIS3 ura3-52 his3-Δ200 leu2-Δ1 trp1-Δ63 Δ63 lys2-801 | This study|
| T50-2      | MATa thi10::HIS3 ura3-52 his3-Δ200 leu2-Δ1 trp1-Δ63 Δ63 lys2-801 | This study|
| T50-2B     | MATa thi10::HIS3 ura3-52 his3-Δ200 leu2-Δ1 trp1-Δ63 Δ63 lys2-801 | This study|
| T36-2A     | MATa thi20-2 phy2-8 phy4-1 trp1 gal2 | Our stock|
| ND10       | MATa/MATa thi10-1/thi10::HIS3 ura3-52/ura3-52 his3-Δ200/Δ200 leu2-Δ1/leu2-Δ1 trp1-Δ63/Δ63 lys2-801/lys2-801/ade2-101 | This study|

* Yeast Genetic Stock Center, University of California, Berkeley, CA.
* Institute for Fermentation, Osaka, Yodogawa-ku, Osaka 532, Japan.
* University of Tokyo.
* University of Osaka.

(forward, 225 bp upstream from the presumed initiating codon) and TCCTCTGCTATAGGGAGCGGAGGATAAAGC (reverse, 259 bp downstream from the stop codon) were used for YOR071c, and CCATTCTAGAATGACTGTTCCGC (forward, 561 bp upstream from the presumed initiating codon) and AATCATATCTCCTCACTGAGGAA (reverse, 259 bp downstream from the stop codon) were used for YOR192c. Genes for thi10::HIS3 and thi20::HIS3 were amplified from the genome DNA of wild-type yeast strain YPH500 were used as the templates for a second PCR to obtain the following ORFs. Forward primers were immediately upstream of the ATG with the HindIII linker, CTCAAGCTTATGAGCTTCAGTAGTATAGT (forward, 225 bp upstream from the presumed initiating codon) and AATCATATCTCCTCACTGAGGAA (reverse, 259 bp downstream from the stop codon) were used for YOR071c, and CCATTCTAGAATGACTGTTCCGC (forward, 561 bp upstream from the presumed initiating codon) and AATCATATCTCCTCACTGAGGAA (reverse, 259 bp downstream from the stop codon) were used for YOR192c. These amplified products from the genome DNA were inserted at the appropriate restriction enzymes were inserted at the corresponding sites of pYES2. General methods for DNA and RNA manipulation, blot analysis, and yeast or bacterial transformation were as described elsewhere (7, 15).

**DNA Sequencing**—The DNA sequence was determined by primer walking using synthesized nucleotides on an automated laser fluorescent DNA sequencer (Pharmacia LKB Biotechnology Inc.) as described (7). The sequencing was performed twice for each primer on both orientations using the purified plasmid pTTS6 (see Fig. 1) as a template. Nucleotide and deduced amino acid sequences were analyzed with programs from a GENETYX software package (Software Development, Tokyo).

**Determination of Thiamin Transport and Thiamin-binding Activities**—The uptake of the radioactive thiamin into the yeast cells was measured as described previously (1). The thiamin binding activity was determined by an equilibrium dialysis against 4 M 32P-dCTP (3,000 Ci/mmol) with bovine serum albumin as standard.

**Chemicals**—DNA probes were labeled with [32P]dCTP (3,000 Ci/mmol) purchased from ICN Biomedicals, and thiazole-2-14C]thiamin hydrochloride (24.3 Ci/mmol) was from Amersham International. All other chemicals were purchased from commercial suppliers.

**RESULTS**

**Cloning of THI10**—A yeast strain T50-1B resistant to pyrithiamin, a strong thiamin antagonist bearing high affinity for thiamin transport system in yeast, is a recessive mutant defective for thiamin transport activity. T50-1B was transformed with a yeast genomic library and plated on the agar medium without thiamin and leucine. The plates were incubated at 30 °C for 3 days, and a total of 15,000 Leu + colonies were examined for the thiamin transport activity by staining with triphenyltetrazolium chloride. Only one colony was found to have the thiamin transport activity (by turning the colony red), and by the plasmid-curing experiment, the colony was confirmed to have a plasmid complementing the mutation of T50-1B. A plasmid prepared from the transformant was used to transform E. coli DH5 α to Amp'. The plasmid obtained from the Amp', pTTTS1 (Fig. 1), had an insertion of a 5.2-kb fragment in YEp13. The insert DNA contained an ORF (see below), and thus the gene obtained here was named THI10. Transformant carrying pTTTS1 exhibited restoration of the thiamin transport activity (Table II).

**Nucleotide Sequences of THI10**—To define the limits of the THI10 gene, various subclones were constructed using the pRS316 vector from the cloned plasmid pTTTS1. The yeast transformants carrying the subcloned plasmids were tested for their thiamin transport activities (Fig. 1). Plasmid pTTTS6 containing the 2.6-kb SpeI-NdeI fragment complemented the mutation of T50-1B, whereas plasmid pTTTS8, constructed by subcloning the 2.3-kb SpeI-XhoI fragment, did not significantly suppress the phenotype of the mutation. Therefore, we concluded that the 2.6-kb SpeI-NdeI region contains the THI10 gene and that the nucleotide sequence of this region was determined using pTTTS6 as a DNA template. As shown in Fig. 2, an ORF of 1,794 bp starting at position 548 and ending at 2,341 was found. A putative TATA box was found at positions 404–408, and two CCAAT transcription elements (22) were located at positions 9-32 and 529–533 in the 5'-upstream region. The polyadenylation signal AATAAA was found at positions 101–105 and 529–533 in the 5'-upstream region. The yeast intron splice signal, TACTAAC (23), was not found. GenBank™ database search revealed that the nucleotide sequence of THI10 is completely identical to that of a DNA coding an unknown protein (L8083.2 gene, accession number U19027), which has been mapped to chromosome XIII. We also found YOR071c (1,794 bp of ORF, accession number Z74979) and YOR192c (1,797 bp, accession number Z75100) genes, which are both located at S. cerevisiae chromosome XV and encode for proteins of unknown function. The nucleotide sequences of both genes match over the entire length of THI10 with no gaps and about 84% sequence identity (data not shown), suggesting that THI10, YOR071c, and YOR192c genes are from a common evolutionary origin.

**Structure of the Predicted Protein**—The predicted protein encoded by the THI10 ORF contains 598 amino acid residues with a calculated molecular weight of 66,903. By comparison with sequences in the SWISS-PROT data base, the predicted THI10 protein was found to have significant sequence similarity to allantoin (DALA) (24) and uracil transporter (FUR4) (25) of S. cerevisiae over almost the entire region with 30.2 and 27.9% identity, respectively (Fig. 3). However, neither allantoin nor uracil inhibited [14C]thiamin uptake into yeast cells.
Bam
"medium without thiamin as described under "Experimental Procedures." Transport activity was determined using the transformed cells grown in pTTS2-6 plasmids are the subcloned fragments on the plasmid pRS316, THI10. Thick lines approximate position and direction of the ORF of the cloned yeast DNA. The 361–379) with the value of 0.82. Like many other transporter (amino acids 53–71) with the value of 1.04 and IX (amino acids 448–466), 1.21; XII (amino acids 488–506), 1.89. Al-

1.25; V (amino acids 175–193), 1.45; VI (amino acids 194–212), 2.02; VII (amino acids 278–296), 2.04; VIII (amino acids 330–348), 1.26; X (amino acids 402–420), 1.88; XI (amino acids 448–466), 1.21; XII (amino acids 488–506), 1.89. Although less extended, there are two hydrophobic segments: I (amino acids 53–71) with the value of 1.04 and IX (amino acids 361–379) with the value of 0.82. Like many other transporter proteins, the NH2 terminus of the THI10 gene product apparently does not contain any structure that resembles a signal peptide. Potential N-linked glycosylation sites, Asn-X-(Ser/Thr) (27), were found at amino acid positions 39, 323, and 390. They were all located in hydrophilic regions at the NH2 terminus or between two adjacent membrane-spanning regions.

Disruption of THI10—To further confirm the identity of the cloned gene, we disrupted the corresponding gene locus. The HIS3 fragment was inserted within the THI10 coding region, and the disrupted thi10 fragment was introduced into the his3 strain (Fig. 5A). DNA was extracted from a His+ transformant strain (NK6) and simultaneously digested with NdeI and SpeI. The digest was separated by agarose gel electrophoresis and Southern blotted. Fig. 5B shows that the THI10 locus of NK6 could not be disrupted (lane 2). NK6 could not uptake [14C]thiamin in the transport assay (Table II), indicating that the THI10 protein is indispensable for the thiamin transport system in S. cerevisiae. Hence, we examined whether or not THI10 was the wild-type allele of the original mutation. NK6 was crossed with T50-1B. The resultant diploid (ND10) was resistant to pyrithiamin, indicating that complementation did not occur. ND10 was then sporulated, and its tetrads were analyzed. The transport activity of all 10 tetrads was <1.0% that of the wild-type level (data not shown). Thus, the original isolate was concluded to be thi10 mutant.

Thiamin Binding Activity of THI10 Protein—We have previously reported the occurrence of a thiamin binding activity in the plasma membrane of S. cerevisiae (20, 28). The thiamin binding activity is repressed by exogenous thiamin, and the dissociation constant (Kd) value for thiamin of 0.11 μM is close to the apparent Km (0.18 μM) of thiamin transport in S. cerevisiae, but the entity of the protein in the cell membranes remains unclear. We investigated, therefore, the effect of the disruption of THI10 gene on the thiamin binding activity in the yeast plasma membrane fraction. As shown in Table II, thi10 null strain completely lost the thiamin binding activity, indicating that the THI10 protein is indispensable for the binding activity as well as the thiamin incorporation. Then we tried to produce the entire THI10 protein in thi10 null strain from the GAL1 promoter. A vector pYES2-THI10 was constructed as described under “Experimental Procedures” and transformed into NK6, and the transformed cells were cultivated in galactose medium with 10–5 x thiamin. We found that the plasma membrane fractions prepared from the transformed cells contained high thiamin binding activity, and the cells producing the THI10 protein restored the thiamin transport activity (Table II). These findings suggested that the THI10 protein is located in the plasma membrane of yeast cells and has the recognition site for thiamin. From these results and the similarity of THI10 protein with DAL4 and FUR4 proteins, we concluded that the THI10 gene of S. cerevisiae is a structural gene for a thiamin transport carrier protein.

Thiamin Transport Activity of YOR071c and YOR192c—Although YOR071c and YOR192c genes are extremely similar to the THI10 gene, it is not clear whether both genes are actually expressed and concerned in thiamin transport in yeast cells. Then, we isolated YOR071c and YOR192c genes by PCR from yeast genome DNA and expressed both ORFs from the GAL1 promoter in thi10 null strain in the same way as with THI10 expression. As shown in Table II, NK6 cells producing YOR071c or YOR192c protein showed the thiamin transport activities of 13.7% and 41.0%, respectively, compared with the cells expressing THI10 protein. These results suggested that both these genes function together in a thiamin transporter complex with THI10 protein or that these genes code a thiamin analog transporter bearing a low affinity for thiamin.

Regulation of THI10 Expression—The thiamin transport activity of S. cerevisiae is coordinately repressed with the enzymes involved in thiamin metabolism by exogenous thiamin.

![Restriction map of a S. cerevisiae DNA cloned on pTTS1 and subcloned fragments with the thiamin transport ability in a thi10 mutant. The shaded box with an open arrow at the top indicates the cloned yeast DNA. The open arrow-labeled THI10 indicates the approximate position and direction of the ORF of THI10. Thick lines on pTTS2-6 plasmids are the subcloned fragments on the plasmid pRS316, and the thin lines represent the DNA from the YEpl vector. Thiamin transport activity was determined using the transformed cells grown in medium without thiamin as described under "Experimental Procedures." nd, not detected. Abbreviations of restriction sites: E, EcoR I; N, NdeI; S, SstI; Sp, SpeI; X, XbaI; Xh, Xhol. B/S is the junction site of BamHI and Sau3AII.](image-url)}
We determined that the uptake and synthesis of thiamin are controlled positively by a regulatory gene, **THI3** (10), and negatively by the intracellular TPP level (9). However, a mutant defective in another positive regulatory gene, **THI2**, associated with the expression of activities of the enzymes involved in thiamin biosynthesis and thiamin-repressible acid phosphatase retains sufficient thiamin transport activity (31). To determine how **THI10** expression is regulated, we investigated the effect of thiamin in the medium on the abundance of **THI10** mRNA using the wild-type strain as well as the **thi2**, **thi3**, and **FIG. 2**.

**FIG. 2. THI10 sequence.** The predicted amino acid sequence is shown in a single-letter code below the nucleotide sequence. The putative TATA box is underlined, and two wavy underlines indicate the transcription element, CCAAT. The four italics sequences in the 3' non-coding region show the putative poly(A)* tail addition signal.

**FIG. 3. Sequence similarities of S. cerevisiae THI10 protein with DAL4 and FUR4 proteins.** The deduced amino acid sequences of the products of the **DAL4** and **FUR4** genes are aligned for maximum homology with that of **THI10** gene. Identical residues are indicated by an asterisk.
probe used was a 32P-labeled 0.52-kb DNA fragment amplified from thiamin almost completely repressed medium was expressed abundantly, but a high concentration of THI10 band, which was almost consistent with the coding region. shown in Fig. 6, THI10 numerals indicate putative transmembrane regions. The ordinate represents the residue number at the center of the stretch. The roman numbers refers to Fig. 2).

thi80 mutants. Cells were cultured under either low or high concentrations of thiamin, and total RNA was extracted and Northern-blotted using THI10 and URA3 DNAs as probes. As shown in Fig. 6, THI10 mRNA was detected as a single 1.9-kb band, which was almost consistent with the coding region. THI10 mRNA of the wild-type strain grown in low thiamin concentrations of thiamin, and total RNA was isolated and Northern-blotted (10 μg/lane). Upper panel, a 32P-labeled 0.83-kb HindIII fragment containing the THI10 gene from plasmid pTT52 was used as a probe. RNA molecular weight standards (Promega) are indicated on the right. Lower panel, the 1.17-kb HindIII fragment of URA3 from plasmid pJJ242 (16) was used as a probe.

FIG. 6. Detection of the THI10 transcript. After yeast strains X2180-1A (wild type), O88-85 (thi2), TRS3 (thi3), and T36-2A (thi80) were grown in minimal medium containing 10^{-6} (L) or 5 \times 10^{-7} (H) μM thiamin, and total RNA was isolated and Northern-blotted (10 μg/lane). Upper panel, a 32P-labeled 0.83-kb HindIII fragment containing the THI10 gene from plasmid pTT52 was used as a probe. RNA molecular weight standards (Promega) are indicated on the right. Lower panel, the 1.17-kb HindIII fragment of URA3 from plasmid pJJ242 (16) was used as a probe.

DISCUSSION

We cloned the THI10 gene by complementation of the phenotype of the thiamin transport defective mutant. The following findings strongly suggested that THI10 encodes a thiamin transport carrier protein. The amino acid sequence deduced from the nucleotide sequence of the gene possesses the structural characteristics of an integral membrane protein, and the thiamin transport activity as well as the thiamin binding activity in the plasma membrane fraction of thi10 null strain was restored when the THI10 ORF was expressed by the GAL1 promoter. This is the first report of the gene-encoding thiamin transport carrier protein for any species.

The predicted protein shows significant sequence similarity to allantoin (DAL4) and uracil transporter (FUR4) of S. cerevisiae. Andro (32) recently reported that the transporters of purines, pyrimidines, and derivatives can be classified into two superfamilies (FUR and FCY) families. The FUR family contains DAL4, FUR4, YBL042c (uridine permease), and THI10, which is described as the L8083.2 gene in the review. The THI10 protein certainly seems to recognize the pyrimidine moiety of thiamin (2, 33). However, these four gene products appear not to share function. Cooper et al. (34) reported that allantoin accumulation was negligible in the dal4 mutant strain even though it possessed a wild-type FUR4 allele. We demonstrated here that both allantoin and uracil even at high concentrations did not inhibit thiamin uptake into yeast cells. On the other hand, adenosine and cordycepin, an analog of adenosine, share a common transport system with thiamin in growing S. cerevisiae cells (35). Among nucleosides other than adenosine, only cytidine was taken up by the thiamin transport system but to a level that was only 5.9% that of adenosine. It is probable that the THI10 product participates in the uptake of adenosine in S. cerevisiae.

Although the expression of YOR071c or YOR192c gene from...
GAL1 promoter partially compensated for the deficiency of THI10 protein in yeast cells, the functions of these genes remain unknown. For elucidation of the relationship between these genes and thiamin transport in yeast, it is essential to ascertain whether the disrupted strains of these genes abolish the thiamin transport activity and whether the expression of these genes is controlled by the thiamin regulatory pathway. Recently, Ruml and Silhankova (36) reported a recessive mutation, thp1, leading to complete loss of thiamin uptake in S. cerevisiae that was mapped to chromosome VII. It has not been clear whether the THP1 gene encodes a structural gene of the thiamin transporter or a positive regulatory factor specific for expression of THI10.

The results of Northern blot hybridization indicated that the THI10 gene expression of S. cerevisiae is regulated at the mRNA level by TPP, similar to other genes involved in thiamin metabolism (7, 37). The expression of the PHO3 gene is known to be regulated at the transcriptional level, and we analyzed the region responsible for the transcriptional activation of the PHO3 gene in response to thiamin in the medium (38). The presumed upstream activating sequence, AAA(A/C)TCAA, is found in the 5′-upstream noncoding regions of PHO3 (3) and THI6 (37) genes by two copies for each gene. The THI6 gene product is a bifunctional enzyme with thiamin-phosphate pyrophosphorylase (EC 2.5.1.3) and 4-methyl-5-β-hydroxyethylthiazole kinase (EC 2.7.1.50) activities (30). On the other hand, there is a TTGATTT sequence at positions 238–244 of the 5′-upstream noncoding region (Fig. 2). This sequence is an in vivo complement of the AAATCAA. Although the physiological significance of these sequences is not clear, a finding that the THI10 does not require the positive factor THI2 for its expression suggests that the regulatory mechanism of THI10 expression is different from those of PHO3, THI6, and THI80. The expressions of these structural genes, except for THI10, are considered to be controlled by both THI2 and THI3 factors. We expect that the detailed analysis of THI10 gene expression will facilitate elucidation of the TPP signal transduction pathway in yeast thiamin metabolism.

Acknowledgment—We thank Dr. Y. Kaneko, Osaka University, for constructing the yeast strain T50-1B and the gift of the plasmids pJJ217 and pJJ242 and Hiroshi Tatsumi for help in cloning THI10 gene.

REFERENCES

1. Iwashima, A., Nishino, H., and Nose, Y. (1973) Biochim. Biophys. Acta 330, 222–234

2. Iwashima, A., Kawasaki, Y., and Kimura, Y. (1990) Biochim. Biophys. Acta 1022, 211–214

3. Bajwa, W., Meyhack, B., Rudolph, H., Schweirngruer, A.-M., and Hinnen, A. (1984) Nucleic Acids Res. 12, 7721–7739

4. Schweirngruer, M. E., Fluri, R., Maundrell, K., Schweirngruer, A.-M., and Durnmuth, E. (1986) J. Biol. Chem. 261, 15677–15682

5. Nosaka, K. (1990) Biochim. Biophys. Acta 1027, 147–154

6. Nosaka, K., Kaneko, Y., Nishimura, H., and Iwashima, A. (1989) FEBS Lett. 260, 55–60

7. Nosaka, K., Kaneko, Y., Nishimura, H., and Iwashima, A. (1993) J. Biol. Chem. 268, 17440–17447

8. Leder, I. G. (1975) in Metabolic Pathways: Metabolism of Sulfur Compounds (Greenberg, D. M., ed) 3rd Ed., Vol. 7, pp. 57–85, Academic Press, New York

9. Nishimura, H., Kawasaki, Y., Nosaka, K., Kaneko, Y., and Iwashima, A. (1991) J. Bacteriol. 172, 2716–2719

10. Nishimura, H., Kawasaki, Y., Kaneko, Y., Nosaka, K., and Iwashima, A. (1992) J. Bacteriol. 174, 4701–4706

11. Rose, M. D., Winston, F., and Maniatis, T. (1990) Methods in Yeast Genetics: A Laboratory Course Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

12. Iwashima, A., Nishino, H., Sempuku, K., and Nishimura, H. (1981) Experientia (Basel) 37, 473–474

13. Broach, J. R., Strathern, J. N., and Hicks, J. B. (1979) Gene (Amst.) 8, 121–133

14. Sikorski, R. S., and Hieter, P. (1989) Genetics 122, 19–27

15. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., p. 1.14, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

16. Jones, J. F., and Prakash, L. (1990) Yeast 6, 363–366

17. Johnston, M., and Davis, R. W. (1984) Mol. Cell. Biol. 4, 1440–1448

18. Zaret, K. S., and Sherman, F. (1982) Cell 28, 563–573

19. Iwashima, A., and Nose, Y. (1976) J. Bacteriol. 128, 855–857

20. Nishimura, H., Nosaka, K., Sempuku, K., and Iwashima, A. (1986) Experientia (Basel) 42, 607–608

21. Markwell, M. A. K., Haan, S. M., Bieber, L. L., and Tolbert, N. E. (1978) Anal. Biochem. 87, 206–210

22. Chodosh, L. A., Olesen, J., Hahn, S., Baldwin, A. S., Guarente, L., and Sharp, P. A. (1988) Cell 54, 25–35

23. Langford, C. J., and Gallwitz, D. (1983) Cell 33, 519–527

24. Yoo, H. S., Cunningham, T., and Cooper, T. G. (1992) Yeast 8, 997–1006

25. Jun, R., Weber, E., and Chevallier, M.-R. (1988) Eur. J. Biochem. 171, 417–424

26. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132

27. Struck, D. K., Lennarz, W. J., and Brew, K. (1978) J. Biol. Chem. 253, 5786–5794

28. Iwashima, A., Nishimura, H., and Nose, Y. (1979) Biochim. Biophys. Acta 557, 460–468

29. Kawasaki, Y., Nosaka, K., Kaneko, Y., Nishimura, H., and Iwashima, A. (1990) J. Bacteriol. 172, 6146–6147

30. Kawasaki, Y. (1993) J. Bacteriol. 175, 5153–5158

31. Nishimura, H., Kawasaki, Y., Kaneko, Y., Nosaka, K., and Iwashima, A. (1992) FEBS Lett. 297, 155–158

32. Andre, B. (1995) Yeast 11, 1575–1611

33. Iwashima, A., Nosaka, K., Nishimura, H., and Kimura, Y. (1986) J. Gen. Microbiol. 132, 1541–1546

34. Cooper, T. G., Chisholm, V. T., Cho, H. J., and Yoo, H. S. (1987) J. Bacteriol. 169, 4660–4667

35. Iwashima, A., Kawasaki, Y., Nosaka, K., and Nishimura, H. (1992) FEBS Lett. 311, 60–62

36. Ruml, T., and Silhankova, L. (1996) Yeast 12, 1279–1283

37. Nosaka, K., Nishimura, H., Kawasaki, Y., Tsujihara, T., and Iwashima, A. (1994) J. Biol. Chem. 269, 35615–35616

38. Nosaka, K., Yamamishi, K., Nishimura, H., and Iwashima, A. (1992) FEBS Lett. 305, 244–248