Isolation and Characterization of Two Distinct myo-Inositol Transporter Genes of Saccharomyces cerevisiae*

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By the complementation of a yeast mutant defective in myo-inositol transport (Nikawa, J., Nagumo, T., and Yamashita, S. (1982) J. Bacteriol. 150, 441–446), we isolated two myo-inositol transporter genes, ITR1 and ITR2, from a yeast gene library. The ITR1 and ITR2 genes contained long open reading frames capable of encoding 584 and 612 amino acids with calculated relative molecular masses of 63,605 and 67,041, respectively. The sequence similarity between the ITR1 and ITR2 products was extremely high, suggesting that the two genes arose from a common ancestor. Both gene products show significant sequence homology with a superfamily of sugar transporters, including human HepG2 hepatoma/erythrocite glucose transporter and Escherichia coli xylose transporter. Hydropathy analysis indicated that the ITR1 and ITR2 products are both hydrophobic and contain 12 putative membrane-spanning regions. Thus, yeast myo-inositol transporters could be classified into the sugar transporter superfamily. Gene disruption and tetrad analysis showed that yeast cells contain two separate myo-inositol transporters. The ITR1 product was the major transporter and the ITR2 product the minor one in cells grown in minimum medium containing glucose. Northern blot analysis showed that ITR1 mRNA was much more abundant than ITR2 mRNA. The previously isolated myo-inositol transport mutant was determined to be defective in ITR1.

Inositol was first identified as a yeast growth factor present in the “bios” complex by Eastcott (1). Since then this polyol has been shown to be an almost ubiquitous cellular component in a wide variety of organisms. It normally occurs as a bound form, e.g. inositol phospholipids (2, 3). Phosphatidylinositol is the third most abundant phospholipid in most eukaryotic cells and plays a structural role in membranes. Bacteria, such as Aerobacter aerogenes (12) and Pseudomonas putida (13), possess inducible inositol transport systems. The incorporated inositol is utilized as a carbon source in these bacteria. A DNA fragment containing genes involved in inositol transport was cloned from a Pseudomonas species (14), but its nucleotide sequence is not known. Although inositol transport is widely found in nature, the genes encoding inositol transporters have not been well characterized.

The yeast Saccharomyces cerevisiae exhibits both inositol transport (15) and inositol synthesis (16). In the previous study (15), we isolated an inositol transport mutant with an activity level of less than 4% from UV-mutagenized wild-type cells. The defect was due to a single nuclear gene mutation. In the present study, we used this mutant to isolate an inositol transporter gene. Unexpectedly, we obtained two distinct genes, ITR1 and ITR2, both of which are capable of complementing the mutation. ITR1 was effective as a single copy, but ITR2 was only effective as multiple copies. Selective gene disruption and transcript analysis showed that they encoded separate inositol transport systems. The ITR1 product was the major inositol transporter and the ITR2 the minor one when cells were grown in minimum medium containing glucose. The ITR1 and ITR2 products showed very high sequence similarity, and they could be classified into the superfamily of sugar transporters including human glucose transporter and Escherichia coli xylose transporter.

EXPERIMENTAL PROCEDURES

Yeast Strains and Culture—The S. cerevisiae strains used in this study are listed in Table I. The composition of YPD medium and the routine genetic methods were as described by Sherman et al. (19). The compositions of the inositol-free minimum medium (M-i medium) and minimum media containing different concentrations of inositol were as described previously (20). When used, L-leucine, L-histidine, and uracil were added to the medium at the concentration of 20 µg/ml. Yeast cells were grown aerobically with shaking at 30 °C.

Bacterial Strains and Culture—E. coli K12 strain HB101 (21) was used for the amplification of plasmids. JM103 (22) was used to propagate M13 recombinant phages. The bacteria were cultured in LB broth (23) at 37 °C. Ampicillin was used at a concentration of 50 µg/ml.

DNA Preparation and Transformation—E. coli plasmids were prepared by the alkali lysis method. The rapid preparation of yeast genomic DNA was carried out as described by Sherman et al. (19). Yeast plasmids were prepared as described previously (24). The YEpM4 yeast gene library was constructed as described previously.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) D99352 and D99353.

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Digoxigenin labeling of probes and detection of hybridized DNA were carried out using reagents from Boehringer Mannheim according to the procedures suggested by the manufacturer. For Northern blot analysis, total RNA was isolated from wild-type yeast strain X2180-1B or strain D458-5A harboring pIT11, as described by Katoaka et al. (33). Samples were subjected to electrophoresis in a 1% agarose gel containing formaldehyde, blotted onto a Biodyne A membrane, and then hybridized with a 32P-labeled probe. Hybridization and detection were carried out according to the manufacturer’s manual.

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See “Experimental Procedures” for construction.

(25) except that strain SP1 was used as the source of genomic DNA instead of strain PS1-2. YepM4 is a 2-μm DNA-based vector containing a multicloning site, derived from pUC18, and LEU2 as a selectable marker. Yeast transformation was carried out by the lithium acetate method (26). The transformation of E. coli was performed by the standard method (23).

DNA Sequencing—The DNA sequence was determined by the dideoxy chain-termination method (22, 27) using a DNA sequencing kit (Takara Shuzo) after subcloning into the M13 vectors, mpl0, 11, 18, and 19 (28, 29). Both strands were sequenced, and the sequences determined in this laboratory were submitted to the EMBL database.

Inositol Transport Assay—Inositol transport activity was determined by measuring the uptake of [2-3H]inositol (Amersham Corp.) for 10 min at 30 °C, washed with saline, dried, and then subjected to autoradiography.

Yeast myo-Inositol Transporter Genes

<table>
<thead>
<tr>
<th>Table I</th>
<th>Strains used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>Genotype</td>
</tr>
</tbody>
</table>
| X2180-1B | a SUC2 mut gal2 CUP1 | Yeast Genetic Stock Center
| SYB02 | a leu2 his3 ino1 cys3 | S. Harashima
| D451-3 | a leu2 ura3 | (17)
| D452-2 | a leu2 ura3 his3 | D373-4B (18) x D448-2 (17)
| D452-3 | a leu2 ura3 his3 | D373-4B (18) x D448-2 (17)
| D453-2 | a leu2 ura3 ino1 | D302-3C (17) x SYB02
| D453-4 | a his3 ura3 ino1 | D302-3C (17) x SYB02
| D458-1A | his3 ura1 | D453-2 x 438-1B (15)
| D458-1B | a leu2 trl1 ino1 | D453-2 x 438-1B (15)
| D458-5A | a leu2 trl1 | D453-2 x 438-1B (15)
| 2027 | a leu2 ura3 his3 trl1::LEU2 | Derivative of D452-3
| 2028 | a leu2 ura3 his3 trl2::URA3 | Derivative of D452-2
| 2029 | a leu2 ura3 ino1 trl2::LEU2 | Derivative of D453-2
| 2030 | a leu2 ura3 ino1 trl2::URA3 | Derivative of D453-2
| 2033 | a leu2 ura3 his3 trl2::LEU2 | Derivative of D453-1
| D447-2A | a leu2 ura3 his3 trl1::LEU2 | 2027 x 2028
| D448-2 (17) | a ura1 his3 trl1::URA3 | Disruptant 2028 and 2029 were obtained, respectively.

The abbreviations used are: kbp, kilobase pairs; bp, base pairs; inositol, myo-inositol.

![Fig. 1. Restriction maps of ITR1 and ITR2 clones. A, ITR1 clones. B, ITR2 clones. Only the insert is shown for each clone. The right-hand BamHI site of pIT11 was formed as a result of ligation. The ability of each clone to complement the growth phenotype of strain D458-1B is indicated by + (complementation) or - (no complementation). Triangles indicate the sites modified on cleavage with BglII (pIT4AB1) or NcoI (pIT11AN), followed by treatment with Klenow fragment and religation. Each insert had a multicloning site at each end. The abbreviations used are: B, BamHI; Ba, BalI; Bg, BglII; D, DraII; E, EcoRI; H, HindIII; Nc, NcoI; P, PstI; Sc, ScaI; Sp, SphI; X, XhoI.](image-url)

![TABLE II](image-url)

Inositol transport activities of ITR1 and ITR2 transformants and an trl1 disruptant

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>Transport activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>X2180-1B</td>
<td>None</td>
<td>27.3</td>
</tr>
<tr>
<td>D418-5A</td>
<td>YepM4+</td>
<td>0.8</td>
</tr>
<tr>
<td>pIT4</td>
<td>34.8</td>
<td></td>
</tr>
<tr>
<td>pIT11</td>
<td>19.4</td>
<td></td>
</tr>
<tr>
<td>2027</td>
<td>None</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* 2-μm-based multicopy vector containing a multicloning site used for the construction of the gene library.
Yeast myo-Inositol Transporter Genes

RESULTS AND DISCUSSION

Construction and Properties of a Double Mutant Defective in Inositol Transport and Inositol Synthesis—In the previous study we isolated a mutant with reduced inositol transport activity by mutagenizing a wild-type yeast with ultraviolet light (15). Genetic analysis, combined with replica-printing assaying of the inositol transport in colonies, showed that this transport defect was due to a single gene mutation. This mutation by itself caused no apparent growth phenotype because yeast cells exhibit inositol-synthesizing activity (16). When inositol synthesis was blocked, as in the case of the inol mutant defective in inositol-1-phosphate synthase, the yeast required inositol for growth (34). Thus, we thought that a mutant carrying the inositol transport defect in combination with inol might not grow even if inositol was added to the medium.

To test this hypothesis, we tried to construct a double mutant by crossing the inositol transport mutant with an inol mutant (438–1B(15) × D453–2). An inositol auxotrophic strain (D458–1B) that only grew at a high inositol concentration (20 μg/ml) was obtained. This strain was thought to carry inol because it required inositol. But it differed from the parental inol mutant in failing to grow in the presence of a low inositol concentration (2 μg/ml). Direct assaying of inositol transport showed that the activity was greatly decreased in this mutant, showing that the mutant carried the inositol transport defect as well. Thus, contrary to our hypothesis, the double mutant could grow when a high concentration of inositol was present, suggesting that the remaining transport activity was sufficient for growth of the mutant. We decided to study the nature of the remaining transport activity by cloning inositol transporter genes.

Isolation of the Inositol Transporter Genes, ITR1 and ITR2—We used strain D458–1B obtained above as the cloning host to isolate inositol transporter genes. D458–1B was transformed with a yeast gene library constructed on a multicopy plasmid, YEpM4, and then the colonies that grew on minimum medium without inositol were selected. This screen- ing was designed to select Itr+ Ino− Leu+, but to exclude unwanted Itr− Ino+ Leu+. The selected transformants were assayed for inositol transport in colonies, showing that this transport defect was due to a single gene mutation. This mutant (438–1B(15)) to examine the restoration of the transport activity. Plasmids were isolated from the transformants. Their inserts ranged from 4.3 to 6.9 kbp in size. Based on their restriction maps, the obtained clones could be classified into two groups (Fig. 1). But no other types of plasmid were obtained. Each type contained a long open reading frame (see below), and thus the genes obtained here were named ITR1 and ITR2, respectively. Table II shows the inositol transport activity encoded by them. Transformants carrying pIT4 (ITR1) and pIT11 (ITR2) both exhibited restoration of the transport activity. But pIT4 was more effective than pIT11. Consistently, a single copy of the ITR1 clone was effective in complementing the mutant, but the ITR2 clone must be used as multiple copies.

To localize the ITR1 and ITR2 genes within the inserts, we constructed partial deletion mutants of the obtained clones and examined them as to their abilities to complement the mutant. As shown in Fig. 1A, the removal of the region to the right of the BamI site (pIT3BS) or the region between the two Scn1. With respect to the NspV and Sau3AI sites, only the used sites are shown. B, ITR1 sequence. The predicted amino acid sequence is shown, in a one-letter code, below the nucleotide sequence. Putative TATA boxes are underlined. The wavy underline indicates the putative poly(A)+ tail addition signal.
adjacent BglII sites (pIT3ΔB) from pIT3 caused the total loss of activity. But the deletion of the sequence to the left of the DraIII site had no effect. This localized ITR1 to the right of the DraIII site. This was also supported by the loss of complementation with pIT4ΔB, whose BglII site had been cleaved, filled in with Klenow fragment, and then religated, resulting in an insertion of 4 bp in the site. On the other hand, as shown in Fig. 1B, ITR2 was localized within a 2.5-kbp region by comparison of pIT9 and pIT11. Disruption of the NcoI site within this region abolished the complementation (plasmid pIT1ΔN).

Nucleotide Sequences of the ITR1 and ITR2 Sequences—Fig. 2 shows the nucleotide sequence of the 2.9-kbp DNA containing the entire ITR1 coding region and its flanking regions. The sequence was determined by the chain-termination method (22, 27) using the strategy shown in panel A. All regions were analyzed on both strands, and the sequences around the M13-cloning sites were confirmed using different M13 clones. As shown in panel B, a long open reading frame starting at position 928 and ending at position 2679 was found within the sequence. This could encode a protein consisting of 584 amino acids with a calculated relative molecular mass of 63,605. Putative TATA boxes (35) were found at positions 63,605. Putative Sp3 boxes (36) at positions 2,750-2,755. But there was no intron splice signal, TACTAAC (38). The yeast myo-inositol transporter, which mediates the facilitated diffusion of glucose across plasma membranes (39), the amino acid sequences predicted from the nucleotide sequence of at least 15 out of 20 bases. A high degree of sequence similarity was found between the ITR1 and ITR2 coding regions. But the 5'- and 3' flanking regions were divergent.

Comparison of the ITR1 and ITR2 Sequences—Fig. 4 shows the result of comparison of the ITR1 and ITR2 genes by means of dot matrix analysis. Each dot represents the match of at least 15 out of 20 bases. A high degree of sequence similarity was found between the ITR1 and ITR2 coding regions. But the 5'- and 3'-flanking regions were divergent. As shown in Fig. 5, the amino acid sequences predicted from the nucleotide sequences were accordingly very similar to each other, there being 455 identical amino acids out of 526 residues compared (86.5% identity). Their amino-terminal regions showed relatively low sequence similarity. These results suggest that the ITR1 and ITR2 genes arose from a common ancestor.

Homology to Sugar Transporters —A search of the National Biomedical Research Foundation protein data base revealed that the ITR1 and ITR2 products showed sequence similarities with various sugar transporters. Fig. 5 compares the ITR1 and ITR2 products with one of them, human HepG2 hepatoma/erythrocyte glucose transporter, which mediates the facilitated diffusion of glucose across plasma membranes (39). Gaps were introduced so that they could be aligned with maximal matching. Over a highly extended region, the human glucose transporter showed 27.6 and 26.8% sequence identities to the ITR1 and ITR2 products, respectively.

Table III shows a summary of the homology search. In addition to the human glucose transporter, a number of sugar transporters, such as the E. coli xylose (xyE) and arabinose (araE) permeases (40), the yeast Kluyveromyces lactis lactose
permease (LAC12)(41), the Saccharomyces carlsbergensis maltose permease (MAL67)(42), and the S. cerevisiae maltose (MAL61)2 (43), galactose (GAL2) (44, 45), and glucose (SNF3) (46) transporters, were found to show homology with both the ITR products and members of the human glucose transporter superfamily. Therefore, the ITR1 and ITR2 products could be classified into the human glucose transporter superfamily. This view was further supported by the results of hydropathy analysis.

Hydropathy Profiles of the ITR1 and ITR2 Products—The hydropathy profiles of the ITR1 and ITR2 products were determined by Kyte and Doolittle's method (47) and are compared with that of human glucose transporter in Fig. 6. Twelve extended hydrophobic regions, likely to be membrane spanning, were discernible in both gene products. The amino- and carboxy-terminal regions were hydrophilic and are believed to reside on the cytoplasmic side of the membrane. The occurrence of 12 putative membrane-spanning regions has been proposed to be a common structural feature of the human glucose transporter superfamily. Furthermore, there were extended hydrophilic regions between hydrophobic peaks 6 and 7 for both the ITR1 and ITR2 product. The presence of an extended loop at this position has also been proposed to be a common structural feature of this superfamily (44). Like many other transporter proteins from mammals and microorganisms, the ITR1 and ITR2 gene products apparently lacked amino-terminal signal sequences.

Disruption of the ITR1 Gene—The above results suggested that the ITR1 and ITR2 products are transport proteins, but it was not determined whether they represent separate inositol transport systems or different subunits of the same transport system. To clarify this point, we conducted a gene disruption study. As shown in Fig. 7A, ITR1 was disrupted by inserting yeast LEU2 at the BglII site within the coding region. The in vitro disrupted itr1 gene was introduced into wild-type strains D452-3 and D453-4, and then Leu+ colonies were selected, disruptants 2027 and 2029 being obtained, respectively. The gene disruption was confirmed by Southern blot analysis of the genomic DNA from the disruptants (data not shown).

![Fig. 4. Dot matrix analysis of the nucleotide sequences of ITR1 and ITR2. Each dot represents the center of a stretch of nucleotide showing at least a 15-of-20 base matching.](image)

![Fig. 5. Comparison of the ITR1 gene product (ITR1), the ITR2 gene product (ITR2), and human HepG2 hepatoma/erythrocyte glucose transporter (HGT). The amino acid sequences are presented in a one-letter code. To maximize matching, breaks are introduced, as indicated by dashes. Identical amino acids are boxed.](image)

### Table III
Homology of the ITR1 and ITR2 products with sugar transporters

<table>
<thead>
<tr>
<th></th>
<th>ITR1</th>
<th>ITR2</th>
<th>HGT</th>
<th>SNF3</th>
<th>MAL</th>
<th>GAL2</th>
<th>LAC</th>
<th>XYL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARA</td>
<td>32.6</td>
<td>29.4</td>
<td>27.3</td>
<td>26.1</td>
<td>21.4</td>
<td>25.1</td>
<td>26.1</td>
<td>30.7</td>
</tr>
<tr>
<td>XYL</td>
<td>24.6</td>
<td>26.0</td>
<td>28.0</td>
<td>25.3</td>
<td>22.4</td>
<td>29.7</td>
<td>22.9</td>
<td></td>
</tr>
<tr>
<td>LAC</td>
<td>20.7</td>
<td>20.8</td>
<td>24.1</td>
<td>21.1</td>
<td>23.1</td>
<td>23.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAL</td>
<td>23.4</td>
<td>21.9</td>
<td>22.3</td>
<td>24.3</td>
<td></td>
<td></td>
<td></td>
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<td>25.1</td>
<td>25.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The deduced amino acid sequences of the S. carlsbergensis and S. cerevisiae maltose permeases are identical.*
ITR1 disruption, ITR2 disruption did not cause a drastic change in inositol transport activity. The activity of itr2 type, i.e. 90% of the wild-type level. This was confirmed by the original isolate was concluded to be consistent with the above notion that ITR1 encodes an inositol transporter.

Disruptant 2029 carrying both disrupted itr1 and inol showed a similar phenotype to the above-constructed double mutant containing the originally obtained inositol transport defect and inol mutation: it did not grow in the presence of 2 μg/ml inositol but grew in the presence of 20 μg/ml inositol. Hence, we examined whether or not ITR1 was the wild-type allele of the originally isolated mutation. D458-5A was crossed with 2029. The resulting diploid showed very low inositol transport activity, indicating that complementation did not occur. Thus, the original isolate was concluded to be an itr1 mutant.

Disruption of ITR2—ITR2 was disrupted by inserting URA3 into the NcoI site within the coding region (Fig. 7B). This was then transformed into wild-type strains D452-2 and D453-2, and Ura+ transformants were selected (strains 2026 and 2030, respectively). The gene disruption was confirmed by Southern blot analysis (data not shown). In contrast to ITR1 disruption, ITR2 disruption did not cause a drastic change in inositol transport activity. The activity of itr2 disruptant 2030 was only slightly lower than that of the wild type, i.e. 90% of the wild-type level. This was confirmed by constructing another itr2 disruptant, 2033, in which part of the ITR2 coding region (0.45 kbp) was replaced with the yeast LEU2 gene (Fig. 7C). Its inositol transport activity was 88% of that of the wild type. These results do not favor the view that ITR1 and ITR2 encode separate subunits of the same transport system. Instead, it is more likely that ITR1 and ITR2 encode separate inositol transport systems. The ITR2-encoded transport system was thought to play a minor part in the inositol transport in cells grown under the present conditions. This view was further supported by the determination of the abundances of ITR1 and ITR2 transcripts (see below).

Disruption of Both ITR1 and ITR2 Abolishes Inositol Transport Completely—We were interested in determining whether or not the ITR1- and ITR2-encoded transport systems can account for all the inositol transport activity of yeast cells. We constructed a double disruptant, itr1::LEU2 itr2::URA3, by crossing 2027 with 2028. We found that itr1 and itr2 were unlinked, and the desired double mutant could be easily obtained. The disruptant (D477-2A) did not display any abnormality in growth, but inositol transport activity was not detectable. Thus, ITR1 and ITR2 accounted for all of the inositol transport activity. We confirmed this further by constructing an itr1 itr2 itr2 inol triple mutant and testing of its viability. We expected that the combination of these three mutations would abolish all the inositol transport activity as well as inositol synthesis, and that the triple mutant would be nonviable. D477-2A was crossed with 2030, and the resulting diploid was allowed to sporulate. Spores were dissected and germinated on YPD medium containing 100 μg/ml inositol, and then their genotypes were examined. inol, itr1::LEU2, and itr2::URA3 were determined by the Ino-, Leu+, and Ura+ phenotypes, respectively. As shown in Table IV, itr2, itr1 itr2, and itr2 inol were obtained, but itr1 itr2 inol was not. Thus, itr1 itr2 inol was lethal. This supports the above notion that ITR1 and ITR2 accounted for all of the inositol transport activity.

Characterization of the ITR2-encoded Inositol Transport System—The above findings prompted us to study the properties of the ITR2-encoded inositol transport system. Although it was difficult to characterize the ITR2-encoded transport system using the itr1 disruptant because of its very low activity, itr1 mutant D458-5A harboring multiple copies of ITR2 (pIT11) overproduced the ITR2-encoded transporter, and therefore could be used for this purpose. As shown in

![Hydropathy profiles](image)

Table II shows the effect of ITR1 disruption on the inositol transport of the wild-type yeast. It can be seen that the activity was greatly reduced by the disruption. These data are consistent with the above notion that ITR1 encodes an inositol transporter.

![Disrupted itr1 (A) and itr2 (B and C) loci](image)
TABLE IV
Tetrad analysis of a cross of itr1 itr2 with itr2 ino1
D477-2A was crossed with 2030 and the resulting diploid was allowed to sporulate. Twenty-two tetrads were dissected. The genotypes of viable spores were determined from their auxotrophic requirements and those of nonviable spores were inferred from the genotypes of the viable sister spores. + denotes the wild-type and − the mutant.

<table>
<thead>
<tr>
<th>Genotype of spores</th>
<th>No. of spores</th>
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</thead>
<tbody>
<tr>
<td>INO1</td>
<td>ITR1</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Table V
Energy requirement of the inositol transport system encoded by ITR2
Strain D458-5A containing plasmid pIT11 was grown in M-1 medium and then used for determination of inositol transport activity.

<table>
<thead>
<tr>
<th>Assay mixture</th>
<th>Activity (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>8.6</td>
</tr>
<tr>
<td>− Glucose</td>
<td>2.4</td>
</tr>
<tr>
<td>− Glucose + 2% 2-deoxyglucose</td>
<td>2.0</td>
</tr>
<tr>
<td>+ 20 mM NaN3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

**Fig. 8. Lineweaver-Burk plots of inositol transport activity versus inositol concentration for the ITR1- and ITR2-encoded transport systems.** The transport activity (v) of strain D458-5A harboring pIT4 (○) or pIT11 (●) was determined with varying concentrations of inositol, as described under "Experimental Procedures."

Table V, omission of glucose from the incubation mixture significantly decreased the transport activity. 2-Deoxyglucose could not replace glucose. Sodium azide abolished the transport, hence the ITR2-encoded inositol transport was energy-dependent. Sodium ions had no effect (data not shown).

Using the same strain, we measured the apparent $K_m$ of the ITR2-encoded transport system for inositol and compared it with that of the ITR1-encoded transport system in Fig. 8. The value for the ITR2-encoded system was 0.14 mM, i.e. slightly higher than that for the ITR1-encoded system (0.1 mM).

**Transcript Analysis**—We determined the abundances of ITR1 and ITR2 transcripts by Northern blot analysis. Wild-type cells were grown in minimum medium (M-i), and then their total RNA was analyzed using the probes prepared from the ITR1 and ITR2 coding regions. As shown in Fig. 9 (lanes 1 and 3), the ITR1 transcript was clearly detectable, but the ITR2 transcript was hardly observed. The ITR1 transcript was approximately 2 kilobases in size, consistent with its coding region. When total RNA from D458-5A carrying multiple copies of ITR2 was used, bands hybridizing to the ITR2 probe were detected (lane 4). The major band was 2.3 kilobases in size, i.e. consistent with the size of the ITR2 open reading frame. The fast-moving, minor band could be that of a partially degraded ITR2 transcript. Lane 2 contained the transcript from itr1 in D458-5A. These data clearly show that the ITR1 transcript is much more abundant than the ITR2 transcript, consistent with the above notion that ITR1-encoded transporter is the major inositol transporter in yeast cells grown under the present culture conditions.

At present the roles of the two inositol transport systems encoded by ITR1 and ITR2 are not known. They might have different functions because they are controlled in different ways: the abundance of ITR1 mRNA decreased considerably in response to the addition of inositol and choline to the culture, but that of ITR2 mRNA did not. It has been well documented that many of the phospholipid-synthesizing enzymes in yeast are repressed by inositol and choline, which are constituents of major phospholipids, phosphatidylinositol and phosphatidylcholine (for review, see Ref. 48). Thus, the ITR1-encoded inositol transport system is likely to be coordinately controlled with phospholipid-synthesizing enzymes. This strongly suggests that inositol transport through the ITR1 system is principally linked to phospholipid synthesis. The ITR2 system might be involved only in some other function.

**Fig. 9. ITR1 and ITR2 transcripts.** Total RNA was isolated from strains X2180-1B (lanes 1 and 3) and D458-5A harboring pIT11 (lanes 2 and 4), and then subjected to Northern blot analysis, with 2 μg/lane, as described under "Experimental Procedures." The 32P-labeled 1.5-kbp HindIII/NheI fragment of ITR1 (lanes 1 and 2) and 1.1-kbp EcoRI fragment of ITR2 (lanes 3 and 4) were used as probes. Yeast 25 S and 18 S rRNAs are used as size markers (3.36 and 1.71 kilobases, respectively). Their locations are indicated at the right.

3 J. Nikawa, unpublished observation.
REFERENCES