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The Maltose Permease Encoded by the *MAL61* Gene of *Saccharomyces cerevisiae* Exhibits Both Sequence and Structural Homology to Other Sugar Transporters

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Manuscript received November 21, 1988
Accepted for publication August 10, 1989

ABSTRACT

The *MAL61* gene of *Saccharomyces cerevisiae* encodes maltose permease, a protein required for the transport of maltose across the plasma membrane. Here we report the nucleotide sequence of the cloned *MAL61* gene. A single 1842 bp open reading frame is present within this region encoding the 614 residue putative MAL61 protein. Hydropathy analysis suggests that the secondary structure consists of two blocks of six transmembrane domains separated by an approximately 71 residue intracellular region. The N-terminal and C-terminal domains of 100 and 67 residues in length, respectively, also appear to be intracellular. Significant sequence and structural homology is seen between the MAL61 protein and the Saccharomyces high-affinity glucose transporter encoded by the *SNF3* gene, the *Kluyveromyces lactis* lactose permease encoded by the *LAC12* gene, the human HepG2 glucose transporter and the *Escherichia coli* xylose and arabinose transporters encoded by the *xylE* and *araE* genes, indicating that all are members of a family of sugar transporters and are related either functionally or evolutionarily. A mechanism for glucose-induced inactivation of maltose transport activity is discussed.

MALTOSE fermentation in the Saccharomyces yeasts is initiated by the transport of the disaccharide across the plasma membrane. This transport is carried out by maltose permease and the process is the rate limiting step in fermentation. An understanding of the mechanisms controlling maltose transport is therefore fundamental to an understanding of the factors regulating maltose fermentation.

The Saccharomyces maltose uptake system is an inducible active transport system (HARRIS and THOMPSON 1961; OKADA and HALVORSON 1964; DE KROON and KONINGSBERGER 1970; SERRANO 1977). SERRANO (1977) reports that this transport is independent of intracellular ATP levels but is coupled to the electrochemical gradient of protons. That is, maltose transport occurs via a proton symport system. As has been seen in the glucose and galactose transport systems of Saccharomyces, the maltose transport system exists in both a high and a low affinity form (BISSON and FRAENKEL 1983a,b, 1984; RAMOS, SZKUTNICKA and CIRILLO 1989; BUSTURIA and LAGUNAS 1985). The basis of the difference between the two forms of these sugar transporters is not understood.

Saccharomyces strains able to ferment maltose carry any one of five *MAL* loci: *MAL1, MAL2, MAL3, MAL4,* and *MAL6* (reviewed by BARNETT 1976). The first indication that the gene encoding maltose permease mapped to any of the *MAL* loci came from the identification of a *MAL1*-linked temperature-sensitive maltose transport mutation (GOLDENTHAL, COHEN and MARMUR 1983). All of the *MAL* loci have been cloned and structurally and functionally compared (FEDEROFF *et al.* 1982; NEEDLEMAN and MICHELS 1983; CHARRON, DUBIN and MICHELS 1986; CHARRON and MICHELS 1987; CHARRON *et al.* 1989). The *MAL* loci are all highly sequence-homologous, exhibiting only a few restriction site polymorphisms. Each locus is a complex locus containing three genes required for maltose fermentation: GENEs 1, 2, and 3 (NEEDLEMAN *et al.* 1984). We have established a two digit numbering system in order to distinguish the GENE 1, 2 or 3 functions mapping to the different *MAL* loci. The first digit indicates the locus position and the second the GENE function (NEEDLEMAN *et al.* 1984; CHARRON and MICHELS 1987, 1988). Thus, the *MAL61* gene is the GENE 1 function mapping to the *MAL6* locus.

Transcription of GENEs 1 and 2 is induced by maltose and repressed by glucose (NEEDLEMAN *et al.* 1984). That GENE 2 encodes maltase is inferred from the identification of an allele of the *MAL12* gene (that is, GENE 2 of the *MAL1* locus) that encodes a temperature-sensitive maltase (DUBIN *et al.* 1985). GENE 1 encodes maltose permease. This conclusion is based on several lines of evidence reported by Y. S. CHANG,

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R. A. DUBIN, E. PERKINS, C. A. MICHELS and R. B. NEEDLEMAN (unpublished results). Point mutations in the *MAL61* gene as well as a deletion/disruption of the *MAL61* gene completely abolish maltose transport activity. Transformation of these mutant strains with high copy plasmids carrying the *MAL61* gene leads to up to a tenfold increase in maltose permease activity as compared to the single-copy parental strain. Most significantly, the integration of a fragment carrying the yeast *URA3* gene into the coding region of *MAL61* near the N-terminal end results in a low level constitutive transcription of *MAL61* and in a low level constitutive synthesis of maltose permease. GENE 3 encodes the MAL activator and the product of this gene is a cysteine-zinc finger protein (CHANG *et al.* 1988; KIM and MICHELS 1988; SOLLITI and MARMUR 1988).

This report presents the sequence of the *MAL61* gene. Analysis of the deduced amino acid sequence of the proposed MAL61 protein indicates that it is an integral membrane protein. Additionally, MAL61 protein shows significant homology to several other sugar transport proteins from yeast and other species. This homology is seen both on the level of the primary sequence and on the level of secondary structure.

MATERIALS AND METHODS

Sequencing: Figure 1 shows a restriction endonuclease map of the *MAL61* gene. Sequencing was done according to the method of SANGER, NICKLEN and COULSON (1977). The region was divided into three fragments: the *Pst*I-*Eco*RI fragment containing the *MAL61* upstream sequences and the 5′-end of the gene; the 1.7-kb *Eco*RI-*Sal*I fragment containing sequences internal to the *MAL61* gene; and the *Sal*I-*Hin*dIII fragment containing the 3′-end of the gene. Each of these was then sequenced by a combination of methods. Nested deletions within the *MAL61*-insert fragments were constructed with the fragment cloned into the M13 sequencing vector mp18 using exonuclease III and these were sequenced using the universal primer (MESSING 1983; HENIKOFF 1984). Gaps were filled by using oligonucleotide primers identical to known sequences. Nested deletions were also constructed using Bal31 to degrade the *MAL61*-insert fragment cloned in the plasmid vector pBR325. For sequencing, these deletions were subcloned into the M13 sequencing vectors. Sequencing of the second strand was carried out using the 3.6-kb *Bgl*II-*Hin*dIII fragment containing the entire *MAL61* gene cloned into the M13 vector mp19. This was sequenced with a series of oligonucleotide primers complementary to known *MAL61* sequence.

Computer analysis: Sequence data were analyzed using the programs of IntelliGenetics, Inc. of Palo Alto, California. Alignment of the MAL61 protein sequence with several other transport protein sequences was carried out using the GENALIGN program. GENALIGN is a copyrighted software product of IntelliGenetics, Inc.; the program was developed by HUGO MARTINEZ of the University of California at San Francisco. The hydropathy plots shown in Figure 4 comparing MAL61 and SNF3 proteins are the gift of JOHN CELENZA, LINDA MARSHALL-CARLSON and MARIAN CARLSON of the Department of Genetics and Development, Columbia University College of Physicians and Surgeons, New York; the profiles were made using the algorithm developed by KYTE and DOOLITTLE (1982) and utilized the values of EISENBERG (1984) with a 21-residue window.

RESULTS

Sequence of the *MAL61* gene and the proposed secondary structure of the deduced protein: Figure 2 presents the sequence of the DNA fragment containing the *MAL61* gene starting at the *Sca*I site shown in Figure 1 and extending to the right for 2000 basepairs. A single large open reading frame is observed with the AUG codon of the N-terminal methionine located 105 base pairs from the *Sca*I site. No other large open reading frames are observed in any of the five other reading frames. The orientation of this single 1842 basepair open reading frame is consistent with the size of the maltose inducible transcript of the *MAL61* gene (2.0 kbp) and with the direction of transcription of the *MAL61* gene as reported in NEEDLEMAN *et al.* (1984). Construction of a *MAL61-lacz* fusion at the *Eco*RI site near the N-terminal end of the coding region supports the conclusion that the AUG codon indicated as the translation initiation can function as such in Saccharomyces (J. LEVINE, L. TANOUYE and C. A. MICHELS, unpublished results). A consensus "TATA" sequence is located at position −89 to −94. The sequence of the open reading frame predicts a 67,174 dalton protein of 614 amino acid residues.

Figure 2 also depicts the positions of twelve postulated hydrophobic transmembrane domains. Each of the twelve postulated 21-residue transmembrane domains has an average hydrophobicity value of greater than 0.42. As in the SNF3 protein, no signal sequence is seen at the N-terminal end of the MAL61 protein and the first predicted transmembrane domain begins at residue 100, suggesting that the N-terminal 100 amino acid residues lie on the cytoplasmic face of the plasma membrane. The overall secondary structure of the MAL61 protein thus appears to consist of two blocks of six transmembrane domains separated by an approximately 71 residue intracellular region. Both the 100 N-terminal residues and the 67 C-terminal residues are predicted to lie on the cytoplasmic face of the plasma membrane. Although two potential N-linked glycosylation sites are found at Asn-15 and Asn-27, these may not be modified since they lie within the proposed cytoplasmic N-terminal region. This remains to be determined since it has been shown that tunicamycin inhibits the synthesis of the maltose transport system in Saccharomyces (LAGUNAS, DEJUAN and BENITO 1986). Work is now in progress in our laboratory to test the predicted membrane topology of the maltose permease.

Homology of MAL61 protein to other sugar transporters: Comparison of the deduced sequence of the

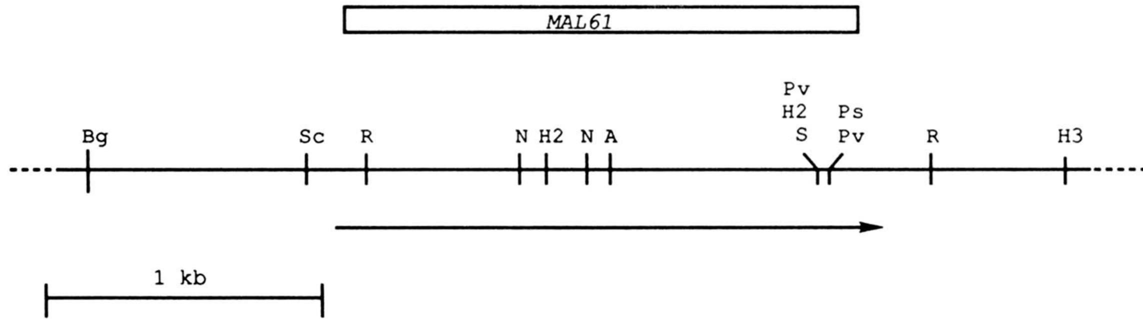

FIGURE 1.—Restriction endonuclease map of the *MAL61* gene of *S. cerevisiae*. The restriction endonuclease map of the 3.6-kb DNA fragment containing the *MAL61* gene and its flanking sequence is shown. The abbreviations used are: A, *Ava*I; Bg, *Bgl*II; H2, *Hind*II; H3, *Hind*III; N, *Nco*I; Ps, *Pst*I; Pv, *Pvu*II; R, *Eco*RI; Sc, *Sca*I.

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-105      AGTACTCAGCATATAAAGAGACACAATATACTCCATACTTGTTGTGAGTGGTTTTAGCGTATTCAGTATAACAATAAGAATTACATCCAAGACTATTAATTAACT

     Met Lys Gly Leu Ser Ser Leu Ile Asn Arg Lys Lys Asp Arg Asn Asp Ser His Leu Asp Glu Ile Glu Asn Gly Val Asn Ala Thr Glu   30
   1 ATG AAG GGA TTA TCC TCA TTA ATA AAC AGA AAA AAA GAC AGG AAC GAC TCA CAC TTA GAT GAG ATC GAG AAT GGC GTG AAC GCT ACC GAA

     Phe Asn Ser Ile Glu Met Glu Glu Gln Gly Lys Lys Ser Asp Phe Asp leu Ser His Leu Glu Tyr Gly Pro Gly Ser Leu Ile Pro Asn   60
  91 TTC AAC TCG ATA GAG ATG GAG GAG CAA GGT AAG AAA AGT GAT TTT GAT CTT TCC CAT CTT GAG TAC GGT CCA GGT TCA CTA ATA CCA AAC

     Asp Asn Asn Glu Glu Val Pro Asp Leu Leu Asp Glu Ala Met Gln Asp Ala Lys Glu Ala Asp Glu Ser Glu Arg Gly Met Pro Leu Met   90
 181 GAT AAT AAT GAA GAA GTC CCC GAC CTT CTC GAT GAA GCT ATG CAG GAC GCC AAA GAG GCA GAT GAA AGT GAG AGG GGA ATG CCA CTC ATG

     Thr Ala Leu Lys Thr Tyr Pro Lys Ala Ala Ala Trp Ser Leu Leu Val Ser Thr Thr Leu Ile Gln Glu Gly Tyr Asp Thr Ala Ile Leu  120
 271 ACA GCT TTG AAG ACA TAT CCA AAA GCT GCT GCT TGG TCA CTA TTA GTT TCC ACA ACA TTG ATT CAA GAG GGT TAT GAC ACA GCC ATT CTA

     Gly Ala Phe Tyr Ala Leu Pro Val Phe Gln Lys Lys Tyr Gly Ser Leu Asn Ser Asn Thr Gly Asp Tyr Glu Ile Ser Val Ser Trp Gln  150
 361 GGA GCT TTC TAT GCC CTG CCT GTT TTT CAA AAA AAA TAT GGT TCT TTG AAT AGC AAT ACA GGA GAT TAT GAA ATT TCA GTT TCC TGG CAA

     Ile Gly Leu Cys Leu Cys Tyr Met Ala Gly Glu Ile Val Gly Leu Gln Val Thr Gly Pro Ser Val Asp Tyr Met Gly Asn Arg Tyr Thr  180
 451 ATC GGT CTA TGT CTA TGC TAC ATG GCA GGT GAG ATT GTC GGT TTG CAA GTG ACT GGG CCT TCT GTA GAT TAC ATG GGC AAC CGT TAC ACT

     Leu Ile Met Ala Leu Phe Phe Leu Ala Ala Phe Ile Phe Ile Leu Tyr Phe Cys Lys Ser Leu Gly Met Ile Ala Val Gly Gln Ala Leu  210
 541 CTG ATC ATG GCG TTG TTC TTT TTA GCG GCT TTC ATT TTC ATT CTG TAT TTT TGC AAG AGT TTG GGT ATG ATT GCC GTG GGA CAG GCA TTG

     Cys Gly Met Pro Trp Gly Cys Phe Gln Cys Leu Thr Val Ser Tyr Ala Ser Glu Ile Cys Pro Leu Ala Leu Arg Tyr Tyr Leu Thr Thr  240
 631 TGT GGT ATG CCA TGG GGT TGT TTC CAA TGT TTG ACC GTT TCT TAT GCT TCT GAA ATT TGT CCT TTG GCC CTA AGA TAC TAT TTG ACG ACT

     Tyr Ser Asn Leu Cys Trp Thr Phe Gly Gln Leu Phe Ala Ala Gly Ile Met Lys Asn Ser Gln Asn Lys Tyr Ala Asn Ser Glu Leu Gly  270
 721 TAT TCT AAT TTA TGT TGG ACG TTC GGT CAA CTT TTC GCT GCT GGT ATT ATG AAA AAT TCC CAG AAC AAA TAT GCC AAC TCA GAA CTA GGA

     Tyr Lys Leu Pro Phe Ala Leu Gln Trp Ile Trp Pro Leu Pro Leu Ala Val Gly Ile Phe Leu Ala Pro Glu Ser Pro Trp Trp Leu Val  300
 811 TAT AAG CTA CCT TTT GCT TTG CAG TGG ATC TGG CCC CTT CCT TTG GCG GTA GGT ATT TTT TTG GCA CCA GAG TCT CCA TGG TGG CTG GTT

     Lys Lys Gly Arg Ile Asp Gln Ala Arg Arg Ser Leu Glu Arg Ile Leu Ser Gly Lys Gly Pro Glu Lys Glu Leu Leu Val Ser Met Glu  330
 901 AAA AAA GGA AGG ATT GAT CAG GCG AGG AGA TCA CTT GAA AGA ATA TTA AGT GGT AAA GGA CCC GAG AAA GAA TTA CTA GTG AGT ATG GAA

     Leu Asp Lys Ile Lys Thr Thr Ile Glu Lys Glu Gln Lys Met Ser Asp Glu Gly Thr Tyr Trp Asp Cys Val Lys Asp Gly Ile Asn Arg  360
 991 CTC GAT AAA ATC AAA ACT ACT ATA GAA AAG GAG CAG AAA ATG TCT GAT GAA GGA ACT TAC TGG GAT TGT GTG AAA GAT GGT ATT AAC AGG

     Arg Arg Thr Arg Ile Ala Cys Leu Cys Trp Ile Gly Gln Cys Ser Cys Gly Ala Ser Leu Ile Gly Tyr Ser Thr Tyr Phe Tyr Glu Lys  390
1081 AGA AGA ACG AGA ATA GCT TGT TTA TGT TGG ATC GGT CAA TGC TCC TGT GGT GCA TCA TTA ATT GGT TAT TCA ACT TAC TTT TAT GAA AAA

     Ala Gly Val Ser Thr Asp Thr Ala Phe Thr Phe Ser Ile Ile Gln Tyr Cys Leu Gly Ile Ala Ala Thr Phe Val Ser Trp Trp Ala Ser  420
1171 GCT GGT GTT AGC ACT GAT ACG GCT TTT ACT TTC AGT ATT ATC CAA TAT TGT CTT GGT ATT GCT GCA ACG TTT GTA TCC TGG TGG GCT TCA

     Lys Tyr Cys Gly Arg Phe Asp Leu Tyr Ala Phe Gly Leu Ala Phe Gln Ala Ile Met Phe Phe Ile Ile Gly Gly Leu Gly Cys Ser Asp  450
1261 AAA TAT TGT GGC AGA TTT GAC CTT TAT GCT TTT GGG CTG GCT TTT CAG GCT ATT ATG TTC TTC ATT ATC GGT GGT TTA GGA TGT TCA GAC

     Thr His Gly Ala Lys Met Gly Ser Gly Ala Leu Leu Met Val Val Ala Phe Phe Tyr Asn Leu Gly Ile Ala Pro Val Val Phe Cys Leu  480
1351 ACT CAT GGC GCT AAA ATG GGT AGT GGT GCT CTT CTA ATG GTT GTC GCG TTC TTT TAC AAC CTC GGT ATT GCA CCT GTT GTT TTT TGC TTA

     Val Ser Glu Met Pro Ser Ser Arg Leu Arg Thr Lys Thr Ile Ile Leu Ala Arg Asn Ala Tyr Asn Val Ile Gln Val Val Val Thr Val  510
1441 GTG TCT GAA ATG CCG TCT TCA AGG CTA AGA ACC AAA ACA ATT ATT TTG GCT CGT AAT GCT TAC AAT GTG ATC CAA GTT GTA GTT ACA GTT

     Leu Ile Met Tyr Gln Leu Asn Ser Glu Lys Trp Asn Trp Gly Ala Lys Ser Gly Phe Phe Trp Gly Gly Phe Cys Leu Ala Thr Leu Ala  540
1531 TTG ATC ATG TAC CAA TTG AAC TCA GAG AAA TGG AAT TGG GGT GCT AAA TCA GGC TTT TTC TGG GGA GGA TTT TGT CTG GCC ACT TTA GCT

     Trp Ala Val Val Asp Leu Pro Glu Thr Ala Gly Arg Thr Phe Ile Glu Ile Asn Glu Leu Phe Arg Leu Gly Val Pro Ala Arg Lys Phe  570
1621 TGG GCT GTT GTC GAT TTA CCA GAA ACC GCT GGC AGG ACT TTT ATT GAG ATA AAT GAA TTG TTT AGA CTT GGT GTT CCA GCA AGA AAG TTC

     Lys Ser Thr Lys Val Asp Pro Phe Ala Ala Ala Lys Ala Ala Ala Ala Glu Ile Asn Val Lys Asp Pro Lys Glu Asp Leu Glu Thr Ser  600
1711 AAG TCG ACT AAA GTC GAC CCT TTT GCA GCT GCC AAA GCA GCA GCT GCA GAA ATT AAT GTT AAA GAT CCG AAG GAA GAT TTG GAA ACT TCT

     Val Val Asp Glu Gly Arg Ser Thr Pro Ser Val Val Asn Lys ***        ***                                                 614
1801 GTG GTA GAT GAA GGG CGA AGC ACC CCA TCT GTT GTG AAC AAA TGATTTTTTTAGCCAGTAGGTAGATCGGCGTTATTTAATTTTATTTTATATAA
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FIGURE 2.—Nucleotide sequence of the *MAL61* gene and predicted amino acid sequence of the gene product. The nucleotide sequence of the *MAL61* gene is given starting at the upstream *Sca*I site. Nucleotide numbers are on the left with the first base of the initiation codon as nucleotide +1. The amino acid residue numbers are shown to the right. Asterisks indicate the termination codons. Putative 21 residue membrane-spanning regions are boxed and shaded. The location of these is based on the algorithm of KYTE and DOOLITTLE (1982) using the hydropathy parameters of EISENBERG (1984).

MAL61 protein to that of the SNF3 protein reveals an approximate 24% sequence homology (Figure 3). The *SNF3* gene encodes the high affinity glucose transporter of Saccharomyces or a component of this transport system (CELENZA, MARSHALL-CARLSON and CARLSON 1988). More impressive than the sequence

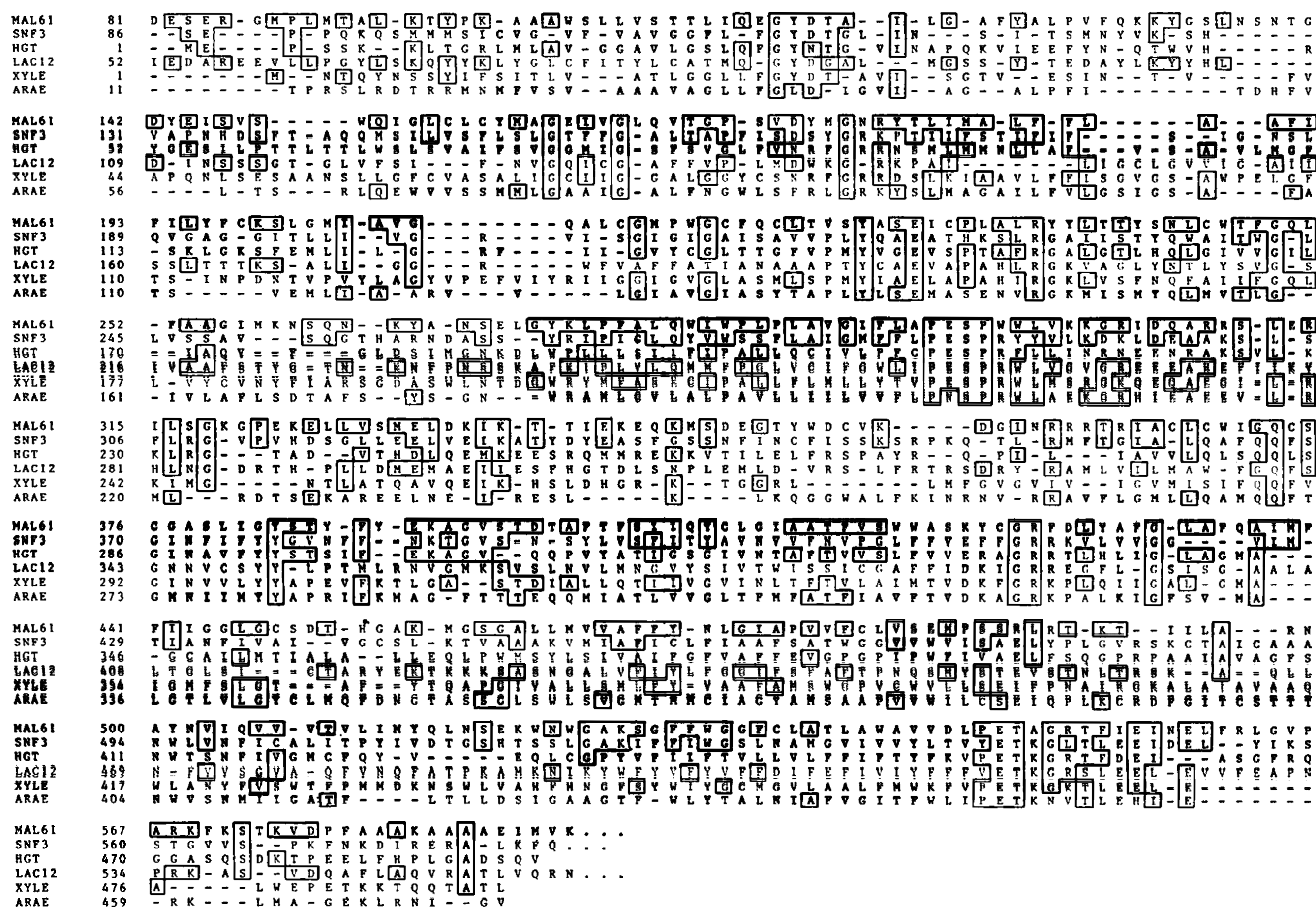

FIGURE 3.—Sequence and structural homology among MAL61 protein and other sugar transporters. Amino acid sequences of the MAL61 protein, the high affinity glucose transporter of Saccharomyces encoded by *SNF3* gene (CELENZA, MARSHALL-CARLSON and CARLSON 1988), the lactose permease of *K. lactis* encoded by the *LAC12* gene (CHANG and DICKSON 1988), human HepG2 glucose transporter (MUECKLER *et al.* 1985) and the *Escherichia coli* xylose and arabinose transporter (MAIDEN *et al.* 1987) are shown using standard single-letter amino acid symbols. The proteins are aligned so as to maximize identity to the MAL61 protein sequence. Gaps (indicated by dashes) are introduced to optimize the alignment. Identities with the MAL61 protein are boxed. Shaded regions indicate the putative transmembrane regions in the MAL61, SNF3 (CELENZA, MARSHALL-CARLSON and CARLSON 1988), human HepG2 (MUECKLER *et al.* 1985), and AraE (MAIDEN *et al.* 1987) protein sequences. Amino acids are numbered on the left.

homology is the structural homology between these two proteins. Figure 4 depicts the hydropathy plots of both MAL61 and SNF3 proteins. One clearly sees the twelve proposed transmembrane domains organized into two blocks of six each. The spatial distribution of these domains is so similar that the plots are nearly perfectly superimposable. Both proteins contain an hydrophilic N-terminal domain of similar size. While the MAL61 protein, like the SNF3 protein, contains an hydrophilic C-terminal domain, this domain is significantly smaller in the MAL61 protein.

Equivalent sequence and structural homology is seen to other sugar transport proteins from other species. Figure 3 aligns the amino acid sequence of the MAL61 protein with those of the SNF3 protein, the human HepG2 glucose transporter, the *Kluyveromyces lactis* lactose permease encoded by the *LAC12* gene, and the *Escherichia coli* xylose and arabinose transporters (MUECKLER *et al.* 1985; MAIDEN *et al.* 1987; CELENZA, MARSHALL-CARLSON and CARLSON 1988; CHANG and DICKSON 1988). Homologous sequences are seen in both hydrophobic membrane spanning domains as well as in hydrophilic regions. The proposed secondary structure of these sugar transporters is also remarkably similar and this is illustrated in Figure 3. Comparison of the MAL61 protein to the *E. coli* lactose permease and to the yeast plasma membrane ATPase (encoded by the *PMA1* gene) reveals little, if any, sequence homology even though all are proton transporters (SERRANO 1977; KABACK 1983; SERRANO, KIELLAND-BRANDT and FINK 1986).

DISCUSSION

The results presented here offer additional strong evidence that the *MAL61* gene encodes the maltose permease. Transcription of the *MAL61* gene is maltose induced and glucose repressed (NEEDLEMAN *et al.*

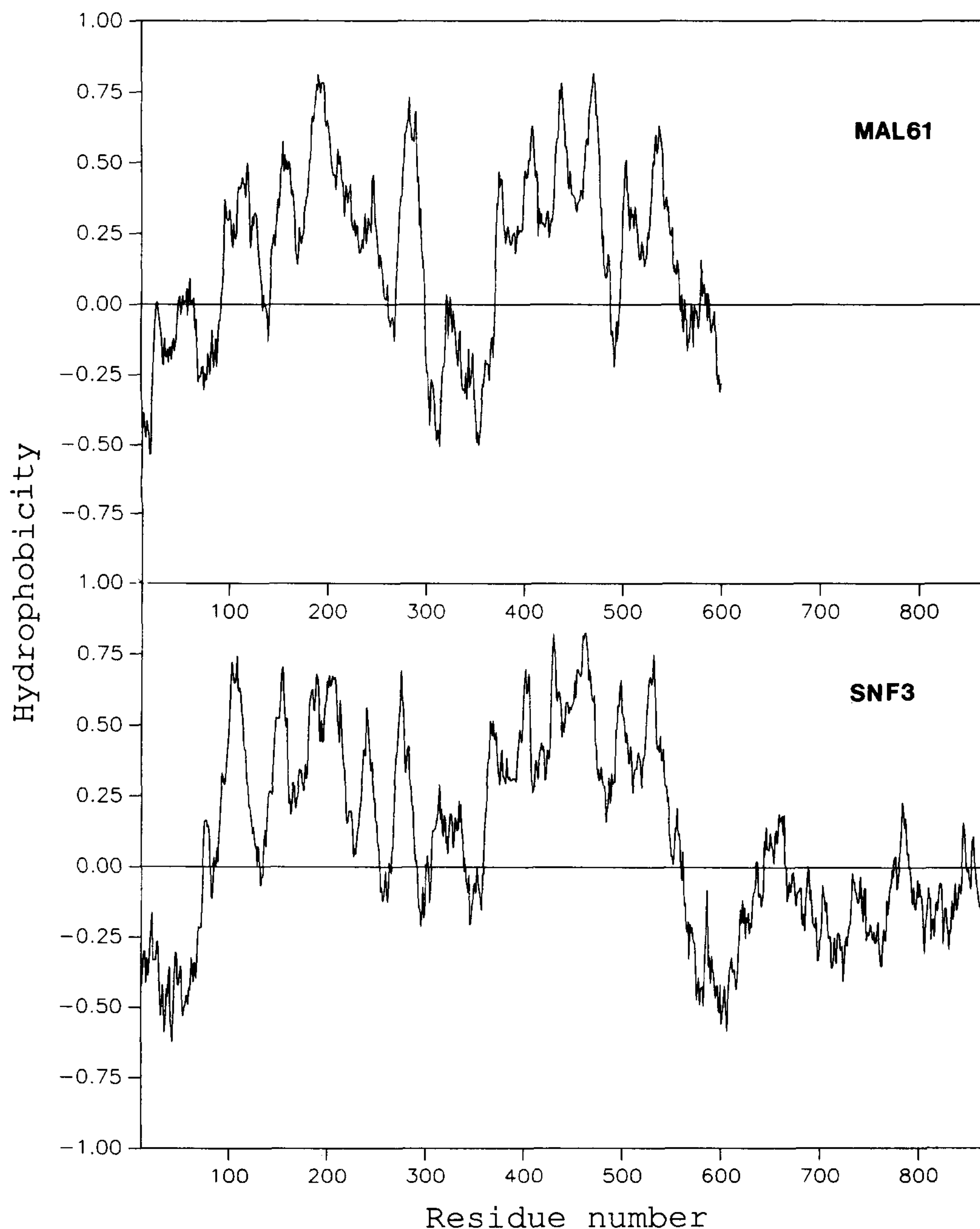

FIGURE 4.—Hydrophobicity profile of the predicted MAL61 protein and SNF3 proteins. The profiles were determined as described in MATERIALS AND METHODS and in Figure 2.

1984). Genetic evidence indicates that *MAL61* is required for maltose transport in *MAL6* strains (Y. S. CHANG, R. A. DUBIN, E. PERKINS, C. A. MICHELS and R. B. NEEDLEMAN, unpublished results). Our results clearly demonstrate that the MAL61 protein is homologous both in sequence and in secondary structure to other sugar transporters, particularly the Saccharomyces high affinity glucose transporter encoded by the *SNF3* gene. While the results presented here do not demonstrate that the MAL61 protein is a plasma membrane protein, they support this conclusion. The primary sequence of the MAL61 protein exhibits twelve highly hydrophobic regions approximately 21-residues in length and such structure is consistent with that of an integral membrane protein (KYTE and DOOLITTLE 1982; EISENBERG 1984). In another study (to be reported elsewhere), analysis of a series of *MAL61-phoA* fusions selected in *E. coli* by transposition of a Tn5-derivative carrying a truncated copy of the *E. coli phoA* gene into a plasmid carrying the *MAL61* gene also supports the localization of the MAL61 protein to the plasma membrane (MANOIL, BOYD and BECKWITH 1988; C. A. MICHELS and L. SEECCOOMER, unpublished results). *E. coli* strains carrying these fusion plasmids express alkaline phosphatase activity in whole cells and preliminary mapping localizes the

fusion junction sites to the region of the second group of six transmembrane domains.

The homology demonstrated here among the Saccharomyces maltose permease, the Saccharomyces high affinity glucose transporter, the human HepG2 glucose transporter, the *K. lactis* lactose permease and the *E. coli* xylose and arabinose transporters is remarkable. These proteins appear to be members of a family of related sugar transporters, even though their mechanisms of transport differ. Some are active transporters utilizing proton symport and others function by facilitated diffusion. The Saccharomyces galactose transporter, which transports by facilitated diffusion, also is reported to be a member of this family of sugar transporters (SZKUTNICKA *et al.* 1989). An evolutionary relationship is strongly implied among all of these proteins. However, homology resulting from a common ancestry is difficult to distinguish from convergent evolution. Sequence convergence could result from the fact that all are sugar transporters with similar functional constraints placed upon their structures.

Transmembrane domains 1, 2, 4, 5, 7, 8 and 11 of MAL61 protein contain several polar and negatively charged residues (serine, threonine, asparagine, glutamine, aspartate and glutamate). Particularly noteworthy is domain 1, which contains seven polar residues (4 threonine, 2 serine, 1 glutamine) and two charged residues (1 aspartate, 1 glutamate) and has an average hydrophobicity value that just exceeds the 0.42 minimum proposed by EISENBERG (1984). Graphic analysis of the membrane-spanning domains indicates that the polar and charged residues contained in these domains would largely be localized to the same face of the proposed alpha-helical structure in the case of domains 2, 4, 5, 8 and 11. MUECKLER *et al.* (1985) suggest that the hydroxyl and amide side chains in an amphipathic α-helix could line a transmembrane channel and function in the transport of the sugar. A similar structural organization may exist in the maltose permease. Additionally, since the transport of maltose in Saccharomyces is a proton symport system, it is possible that the charged residues located in the transmembrane domains of the MAL61 protein function in proton transport in a fashion similar to that seen in the lactose permease of *E. coli* (HERZLINGER, CARRASCO and KABACK 1985; CARRASCO *et al.* 1986; SERRANO 1977).

The addition of glucose to maltose-induced fermenting cultures not only leads to the cessation of synthesis of maltose permease but also leads to the loss of any existing maltose permease activity by an as yet undefined process referred to as glucose-induced inactivation (GORTS 1969; BUSTURIA and LAGUNAS 1985). Glucose-induced inactivation also affects the activity of several other enzymes in Saccharomyces (reviewed in HOLZER 1984; JONES 1984; ACHSTETTER and WOLF 1985). These include enzymes of the gluconeogenic pathway (fructose-1,6-bisphosphatase, cytoplasmic malate dehydrogenase and phosphoenolpyruvate carboxykinase), aminopeptidase I, uridine nucleosidase, the high affinity glucose transporter (SNF3 protein) and galactose permease (WITT, KRONAU and HOLYER 1966; FERGUSON, BOLL and HOLYER 1967; GANCEDO 1971; HAARASILTA and OURA 1975; MATERN and HOLZER 1977; MAGNI *et al.* 1977; FREY and ROHM 1979; BISSON and FRAENKEL 1984; RAMOS, SZKUTNICKA and CIRILLO 1988). Maltose uptake is almost completely inactivated within 90 minutes following the addition of glucose to the culture medium (GORTS 1969; BUSTURIA and LAGUNAS 1985). The most recent results indicate that this deadaptation inactivates both the high and low affinity uptake systems but earlier studies report that only the high affinity transport is affected. Glucose specifically initiates the inactivation since the transfer of a maltose fermenting culture to a noninducing medium containing ethanol does not lead to the rapid loss of maltose transport activity. Recovery from glucose inhibition requires that the cells be returned to inducing medium and recovery does not occur if *de novo* protein synthesis is inhibited. These results imply that glucose-induced inactivation irreversibly destroys the maltose transport protein.

The mechanism of this irreversible inactivation is unknown. Studies on aminopeptidase I and the gluconeogenic enzymes fructose-1,6-bisphosphatase, cytoplasmic malate dehydrogenase and phosphoenolpyruvate carboxykinase from Saccharomyces indicate that the glucose-induced inactivation of these enzyme activities is paralleled by a decrease in the amount of cross-reacting material suggesting that inactivation results from their selective proteolysis (NEEFF *et al.* 1978; FREY and ROHM 1979; FUNA-YAMA, GANCEDO and GANCEDO 1980; MULLER, MULLER and HOLZER 1981; TORTORA *et al.* 1981). While maltose permease is an integral membrane protein and these enzymes are cytosolic proteins, it is nevertheless tempting to propose that the irreversible inactivation of maltose permease is the result of proteolytic degradation. In a survey of several proteins from different eukaryotic organisms, RECHSTEINER and coworkers have found a correlation between short half-life and the presence of sequences rich in proline, glutamate, serine and threonine (so-called PEST-regions) which they propose target proteins for proteolysis (ROGERS, WELL and RECHSTEINER 1986; RECHSTEINER 1987; RECHSTEINER, ROGERS and ROTE 1987). A search of the MAL61 and SNF3 proteins reveals potential PEST sequences located in the N-terminal cytoplasmic regions. These are found at residues 49–78 of MAL61 protein (score of 0.64) and at residues 1-13 (score of

7.85) and 63–91 (score of 1.66) of SNF3 protein. ROGERS, WELL and RECHSTEINER (1986) also suggest that a protein containing a region with a low positive PEST score could be subject to degradation only under certain physiological conditions, such as when that region was phosphorylated, thereby increasing the negative charge of the region. In such cases, the degradation of a protein could be regulated, as is seen in glucose-induced inactivation of the gluconeogenic enzymes. It is worth noting that the N-terminal peptide of Saccharomyces fructose-1,6-bisphosphatase contains a PEST-region (score of 1.7). Whether or not PEST regions are relevant in the Saccharomyces system remains to be determined, but the possibility that a common mechanism exists for the glucose-induced inactivation of all of these proteins even though they are located in different cellular compartments and lack any obvious sequence similarities is an interesting one to pursue.

The authors wish to thank JOHN CELENZA, LINDA MARSHALL-CARLSON and MARIAN CARLSON for providing the hydropathy plots of MAL61 and SNF3 protein shown in Figure 4, and MARTIN RECHSTEINER for providing the program used to perform the PEST-search analysis. The authors are indebted to HOWARD SHUMAN for many hours of enlightened discussion. They also wish to thank EVAN READ for help in the preparation of the figures.

This work was supported by a National Science Foundation Visiting Professorship for Women Award (RII-8700006) to C. A. M., by a National Institute of Health Research Award (GM28216) to C. A. M. and by a Board of Higher Education-Professional Staff Congress Research Award.

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Communicating editor: E. W. JONES