The Eukaryotic UDP-N-Acetylglucosamine Pyrophosphorylases

GENE CLONING, PROTEIN EXPRESSION, AND CATALYTIC MECHANISM*

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A search of the yeast data base for a protein homologous to Escherichia coli UDP-N-acetylglucosamine pyrophosphorylase yielded UAPI (UDP-N-acetylglucosamine pyrophosphorylase), the Saccharomyces cerevisiae gene for UDP-N-acetylglucosamine pyrophosphorylase. The Candida albicans and human homologs were also cloned by screening a C. albicans genomic library and a human testis cDNA library, respectively. Sequence analysis revealed that the human UAPI cDNA was identical to previously reported AGX1. A null mutation of the S. cerevisiae UAPI (ScUAPI) gene was lethal, and when expressed under the control of ScUAPI promoter, both C. albicans and Homo sapiens UAPI (CaUAPI and HsUAPI) rescued the ScUAPI-deficient S. cerevisiae cells. All the recombinant ScUAPIp, CaUAPIp, and HsUAPIp possessed UDP-N-acetylglucosamine pyrophosphorylase activities in vitro. The yeast UAPI utilized N-acetylglucosamine-1-phosphate as the substrate, and together with Agm1p, it produced UDP-N-acetylglucosamine from N-acetylglucosamine-6-phosphate. These results demonstrate that the UAPI genes indeed specify eukaryotic UDP-GlcNAc pyrophosphorylase and that phosphomutase reaction precedes uridylyltransfer. Sequence comparison with other UDP-sugar pyrophosphorylases revealed that amino acid residues, Gly112, Gly114, Thr115, Arg116, Pro122, and Lys123 of ScUAPI are highly conserved in UDP-sugar pyrophosphorylases reported to date. Among these amino acids, alanine substitution for Gly112, Arg116, or Lys123 severely diminished the activity, suggesting that Gly112, Arg116, or Lys123 are possible catalytic residues of the enzyme.

UDP-N-acetylglucosamine (UDP-GlcNAc) is a ubiquitous and essential metabolite and plays important roles in several metabolic processes. In bacteria, it is known as a major cytoplasmic precursor of cell wall peptide glycan and the disaccharide moiety of lipid A (1–3). In eukaryotes, it serves as the substrate of chitin synthase, whose product is shown to be essential for fungal cell wall (4). It is also used in the GlcNAc moiety of N-linked glycosylation and the GPI-anchor of cellular proteins (5).

Biosynthesis of UDP-GlcNAc has been extensively studied in bacteria, and it requires the following enzymatic reactions: i) conversion of fructose-6-phosphate (Fru-6-P) into glucosamine-6-phosphate (GlcN-6-P) by glutamine:Fru-6-P amidotransferase; ii) conversion of GlcN-6-P into glucosamine-1-phosphate (GlcN-1-P) by glucosamine (GlcN) phosphate mutase; iii) acetylation of GlcN-1-P by GlcN-1-P acetylyltransferase to produce N-acetylglucosamine-1-phosphate (GlcNAc-1-P); and iv) synthesis of UDP-GlcNAc from GlcNAc-1-P and UTP by GlcNAc-1-P uridylyltransferase (also called UDP-GlcNAc pyrophosphorylase) (6–8). The Escherichia coli GlmS gene encodes glutamine:Fru-6-P amidotransferase (9–11). E. coli GlmU specifies a bifunctional protein with GlcN-1-P acetylyltransferase and UDP-GlcNAc pyrophosphorylase activities (12, 13).

In yeast Saccharomyces cerevisiae, Fru-6-P is converted either into GlcN-6-P by glutamine:Fru-6-P amidotransferase or into mannose-6-phosphate by phosphomannose isomerase. GFA1 and PMI have been shown to be the genes for glutamine: Fru-6-P amidotransferase and phosphomannose isomerase, respectively (14, 15). Then, GlcN-6-P is N-acetylated by an acetylase to become GlcNAc-6-P, which is further converted into GlcNAc-1-P by GlcNAc phosphate mutase (16). S. cerevisiae harbors four different hexosephosphate mutase genes, PGM1 (17), PGM2 (18), SEC53 (19), and AGM1 (20). Among them, AGM1 is responsible for the interconversion of GlcNAc-6-P and GlcNAc-1-P (20). Interestingly, Agm1p has dual substrate specificity; it also converts glucose-6-phosphate to glucose-1-phosphate (Glc-1-P) (20). Finally, UDP-GlcNAc is produced from GlcNAc-1-P by UDP-GlcNAc pyrophosphorylase. However, the eukaryotic genes for GlcN-6-P acetylase and UDP-GlcNAc pyrophosphorylase remain unidentified.

On the other hand, there are three UDP-sugar pyrophosphorylase genes in S. cerevisiae reported to date. GAL7 (21) and UGP1 (22) encode UDP-galactose (UDP-Gal) pyrophosphorylase and UDP-glucose (UDP-Glc) pyrophosphorylase, respectively. Recently, VIG9 was identified as the GDP-mannose (GDP-Man) pyrophosphorylase gene by functional complementation using the glycosylation defective vig9-1 mutant (23), and the possible amino acid sequence motif for the active site of UDP-sugar pyrophosphorylase is proposed. Because all of these enzymes preserve substrate specificity to a certain type of sugar, there should be an enzyme specific to GlcNAc-1-P.

In an attempt to identify the gene for UDP-GlcNAc pyrophosphorylase, we searched the S. cerevisiae genome data base and found that the protein specified by YDL103C. The Candida albicans and human homologs were also isolated and characterized. From sequence comparison and mutation analysis, the probable catalytic residues of UDP-sugar pyrophosphorylases are proposed.
EXPERIMENTAL PROCEDURES

**Yeast Data Base Search and Screening of DNA Libraries**—An amino acid sequence motif of LXXGXXGTXXXXP at X where X represents any amino acid was obtained by comparing the amino acid sequences of E. coli UAP1 (originally designated YDL103C) was amplified by polymerase chain reaction using the S. cerevisiae genomic DNA extracted from strain A451 (MATa can1, aro7, can1, leu2, trp1, ura3) as a template, and cloned at the XbaI site of pUC18 or pYEur3 (Toyobo) generating pUC-ScUAP1 and pYEU-ScUAP1, respectively. Primers used for polymerase chain reaction were 5'-AGATCTGATAATGCATGCAAAACAGCT-3' and 5'-AGATCTGATTTTTTCTAATACGAC-3'.

The entire ORF of ScUAP1 were cloned by screening a C. albicans genomic DNA library and a human testis cDNA library using the 1.4-kilobase EcoRI-EcoRI fragment of ScUAP1 as a probe. Hybridization and washing of the filters were carried out under stringent conditions (20 mM sodium phosphate (pH 7.2), 5% SSC (1× SSC contains 150 mM NaCl and 15 mM sodium citrate), 5× Denhardt's solution, 0.1% SDS, 25% formamide at 42 °C for hybridization; 0.1× SSC and 0.1% SDS at 50 °C for washing). Bacterial cells and phages that were strongly hybridized with the probe DNA were collected. After the third screening, DNA was extracted from bacterial cells and phages, and the insert DNA was cloned at the Smal site of pUC19 for further plasmid construction. Radiolabeling of the probe DNA was performed by the random priming method using [α-32P]dCTP (24), and DNA sequencing was carried out as described elsewhere (24).

**Eukaryotic UDP-N-Acetylglucosamine Pyrophosphorylase**

Amino acid was obtained by comparing the amino acid sequences of human proteins as a fusion product with GST and purified by glutathione Sepharose CL-4B column chromatography, as described (26) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The primers used for amplifying the ScAGM1 open reading frame (ORF) were 5'-CGGGAATTCATAAGGT-3' and 5'-ATGATCTTCAAGCAGATGCCTT-3'.

The hybrid human homologs of ScUAP1 were cloned by screening a C. albicans genomic DNA library and a human testis cDNA library using the 1.4-kilobase EcoRI-EcoRI fragment of ScUAP1 as a probe. Hybridization and washing of the filters were carried out under stringent conditions (20 mM sodium phosphate (pH 7.2), 5× SSC (1× SSC contains 150 mM NaCl and 15 mM sodium citrate), 5× Denhardt's solution, 0.1% SDS, 25% formamide at 42 °C for hybridization; 0.1× SSC and 0.1% SDS at 50 °C for washing). Bacterial cells and phages that were strongly hybridized with the probe DNA were collected. After the third screening, DNA was extracted from bacterial cells and phages, and the insert DNA was cloned at the Smal site of pUC19 for further plasmid construction. Radiolabeling of the probe DNA was performed by the random priming method using [α-32P]dCTP (24), and DNA sequencing was carried out as described elsewhere (24).

**Expression and Purification of the Recombinant Proteins**—The coding regions of ScUAP1, CaUAP1, HaUAP1, and ScAGM1 were cloned at the EcoRI (for ScUAP1 and ScAGM1) or Smal (for CaUAP1 and HaUAP1) site of pGEX2T (26), and the resulting plasmids were transfected into E. coli strain M109 to let them express recombinant human proteins as a fusion product with glutathione S-transferase (GST). Induction and expression of the recombinant UAP1 proteins was carried out with isopropyl β-D-thiogalactopyranoside as described (25, 26). After 4 h after the addition of isopropyl β-D-thiogalactopyranoside, the bacterial cells were harvested, suspended in a buffer containing 20 mM Tris-HCl (pH 7.5), 0.5 mM EDTA, 50 mM NaCl, 10 mM β-mercaptoethanol, 10% (v/v) glycerol, 1 mM phenylmethylsulfonyl fluoride, and lysed by sonication. After cell debris were removed by centrifugation at 15,000 × g at 4 °C for 30 min, GST-UAP1 and GST-AgM1 fusion proteins were purified by glutathione Sepharose CL-4B column chromatography, as described (26) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The primers used for amplifying the ScAGM1 open reading frame (ORF) were 5'-CGGGAATTCATAAGGT-3' and 5'-ATGATCTTCAAGCAGATGCCTT-3'.

**RESULTS**

**Cloning of the Yeast UDP-GlcNAc Pyrophosphorylase Gene**—Three distinct UDP-sugar pyrophosphorylase activities are present in yeast. In S. cerevisiae, the GAL7 (21), UGP1 (22), and VIG9 (23) genes have been shown to encode UDP-Gal pyrophosphorylase, UDP-Glc pyrophosphorylase, and GDP-Man pyrophosphorylase, respectively, but the gene for UDP-GlcNAc remains to be established. Comparison of the amino acid sequences between E. coli UDP-GlcNAc pyrophosphorylase GlnU (GlmUp) and S. cerevisiae UDP-GlcNAc pyrophosphorylase (Ugp1p) identified an amino acid sequence motif, L(,)Xaa where X represents any amino acid. In an attempt to identify the S. cerevisiae UDP-GlcNAc pyrophosphorylase gene, we searched the yeast data base and found that PS1A and YDL103C could encode proteins with a sequence similar to the above amino acid motif (Fig. 1). PS1A is identical to VIG9, which has been shown to be the GDP-Man pyrophosphorylase gene. Accordingly, we asked whether YDL103C specifies UDP-GlcNAc pyrophosphorylase. The Ydl103c protein was expressed in E. coli as a fusion protein with GST and purified by using column chromatography using glutathione-Sepharose CL-4B. The purified GST-Ydl103c fusion protein produced [32P]UDP-GlcNAc when incubated with GlcNAc-1-P and [α-32P]UTP, whereas GST alone did not (Fig. 2). The above result demonstrates that YDL103C is a gene for UDP-GlcNAc pyrophosphorylase, and, therefore, the gene was designated ScUAP1 (the S. cerevisiae UDP-GlcNAc pyrophosphorylase gene 1).

Because uridyltransfer to GlcNAc-1-P releases pyrophosphate from UDP, we developed a conventional high-flux assay by adding pyrophosphatase to the reaction mixture, which allows us to estimate the enzyme activity from the amounts of inorganic phosphates produced after the hydrolysis of pyro-
phosphates in the reaction mixture. By this assay, it was demonstrated that the GST-ScUap1 fusion protein converted GlcNAc-1-P to UDP-GlcNAc in a dose-dependent manner (see below). Moreover, UTP was essential for the production of UDP-GlcNAc by ScUap1p; none of ATP, GTP, and CTP were used as the substrate (not shown).

Because UDP-GlcNAc is an essential metabolite serving as a precursor of cell wall chitin, protein N-glycosylation, and GPI anchor in yeast (4, 5), ScUAP1 may be an essential gene for viability if it is the only UDP-GlcNAc pyrophosphorylase gene in S. cerevisiae. The S. cerevisiae uap1Δ null mutant strain in which the endogenous UAP1 gene was disrupted, but where episomal copies of UAP1 whose transcription was under the control of GAL1 promoter were maintained, grew on galactose plates but died on glucose plates. The cells of S. cerevisiae uap1Δ null mutant displayed an aberrant morphology; most of the yeast cells fully swelled and some were lysed, which is a phenotype quite similar to that caused by a null mutation of AGM1, the gene for GlcNAc phosphate mutase (Fig. 3). This is below).
suggestive that the \textit{ScUAPI} is a sole UDP-GlcNAc pyrophosphorylase gene in \textit{S. cerevisiae} and that the most apparent defect resulting from depletion of the functional \textit{UAP1} occurred in the cell wall.

\textbf{Identification of the UDP-GlcNAc Pyrophosphorylase Genes of Other Organisms—} To gain more insight into the characteristics of UDP-GlcNAc pyrophosphorylase, we intended to isolate the \textit{ScUAPI} homologs from the pathogenic fungus \textit{C. albicans} as well as from human. By screening a \textit{C. albicans} genomic DNA library and a human testis cDNA library with \textit{ScUAPI} DNA as a probe, \textit{CaUAPI}, or \textit{HsUAPI}. Transfectants were spread on agar plates containing galactose or glucose and incubated at 30 °C for 3 days.

\begin{figure}[h]
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\caption{Functional complementation of \textit{ScUAPI} by the \textit{C. albicans} and human \textit{UAPI}. \textit{S. cerevisiae uap1}Δ cells harboring pBEU-\textit{ScUAPI} were further transformed with pRS414–1 bearing \textit{ScUAPI}, \textit{CaUAPI}, or \textit{HsUAPI}. Transfectants were spread on agar plates containing galactose or glucose and incubated at 30 °C for 3 days.}
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\caption{Substrate specificity of \textit{ScUap1p}. A, approximately 0.1 µg of the purified GST-\textit{ScUap1p} was incubated with \textit{[α-32P]}UTP and 20 µM of each sugar nucleotide. The reaction products were separated by polyethyleneimine cellulose TLC and visualized by autoradiography. The positions of the UDP-GlcNAc, UDP-Glc, and UTP that were visualized under UV light are indicated. Lane 1, none; lane 2, GlcNAc-1-P; lane 3, GlcNAc-6-P; lane 4, GlcN-1-P; lane 5, Glc-1-P; lane 6, Gal-1-P; lane 7, GalNAc-1-P; lane 8, Man-1-P. B, the indicated amounts of the purified GST-\textit{ScAgm1p} were incubated with approximately 0.1 µg of the purified GST-\textit{ScUap1p} and UDP-Glc, and UTP that were visualized under UV light are indicated. It should be noted that UDP-Glc, which appeared in the presence of \textit{ScUap1p} and Glc-1,6-P\textsubscript{2}, was formed from a trace of Glc-1-P in the Glc-1,6-P\textsubscript{2}. B, the indicated amounts of the purified GST-\textit{ScAgm1p} were incubated with approximately 0.1 µg of the purified GST-\textit{ScUap1p}, GlcNAc-6-P, and UTP in the presence (○) or absence (●) of 20 mM Glc-1,6-P\textsubscript{2}. The amounts of the released inorganic phosphate that represent the enzyme activities were determined with malachite green and ammonium molybdate.}
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\caption{Production of UDP-GlcNAc from GlcNAc-6-P by \textit{ScUap1p} and \textit{ScAgm1p}. A, approximately 0.1 µg of the purified GST-\textit{ScUap1p} (\textit{ScUap1p}) was incubated with \textit{[α-32P]}UTP and 20 µM each of the indicated sugar nucleotides in the presence or absence of approximately 0.1 µg of the purified GST-\textit{ScAgm1p}. The reaction products were separated by polyethyleneimine cellulose TLC and visualized by autoradiography. The positions of the UDP-GlcNAc, UDP-Glc, and UTP that were visualized under UV light are indicated. It should be noted that UDP-Glc, which appeared in the presence of \textit{ScUap1p} and Glc-1,6-P\textsubscript{2}, was formed from a trace of Glc-1-P in the Glc-1,6-P\textsubscript{2}. B, the indicated amounts of the purified GST-\textit{ScAgm1p} were incubated with approximately 0.1 µg of the purified GST-\textit{ScUap1p}, GlcNAc-6-P, and UTP in the presence (○) or absence (●) of 20 mM Glc-1,6-P\textsubscript{2}. The amounts of the released inorganic phosphate that represent the enzyme activities were determined with malachite green and ammonium molybdate.}
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\textbf{Identification of the UDP-GlcNAc Pyrophosphorylase Genes of Other Organisms—} To gain more insight into the characterics of UDP-GlcNAc pyrophosphorylase, we intended to isolate the \textit{ScUAPI} homologs from the pathogenic fungus \textit{C. albicans} as well as from human. By screening a \textit{C. albicans} genomic DNA library and a human testis cDNA library with \textit{ScUAPI} DNA as a probe, \textit{CaUAPI}, and \textit{HsUAPI}. Transfectants were spread on agar plates containing galactose or glucose and incubated at 30 °C for 3 days. The predicted products of \textit{ScUAPI}, \textit{CaUAPI}, and
FIG. 7. Comparison of the amino acid sequences of UDP-sugar pyrophosphorylases. Amino acid sequences of ScUap1p are compared with those of other UDP-sugar pyrophosphorylases using the FASTA and BLAST programs. Identical amino acids among the proteins listed here are indicated by bold letters. Amino acids that were replaced by alanine are marked by *.

Substrate Specificity of UDP-GlcNAc Pyrophosphorylase—We next examined the substrate specificity of UDP-GlcNAc pyrophosphorylase using ScUap1p. ScUap1p reproducibly converted GlcNAc-1-P into UDP-GlcNAc in the presence of UTP but did not utilize GlcNAc-6-P, galactose-1-phosphate (Gal-1-P) or mannose-1-phosphate (Man-1-P) as a substrate (Fig. 5A). Unexpectedly, the enzyme generated a spot whose mobility corresponded to that of UDP-Glc from Glc-1-P, indicating the dual substrate utility of Uap1p. However, Glc-1-P was much less efficient as shown in Fig. 5B. Consequently, ScUap1p did not complement ScUAP1 (data not shown).

It was believed that the interconversion of GlcNAc-6-P and GlcNAc-1-P precedes the uridytransfer in vivo. This prompted us to add the yeast GlcNAc phosphate mutase (ScAgm1p) to the reaction mixture. As shown in Fig. 6A, together with ScAgm1p, ScUap1p produced UDP-GlcNAc from GlcNAc-6-P, whereas ScAgm1p alone did not. It is also demonstrated that hexose-phosphate mutases require Glc-1,6-P₂ as an activator or a cofactor for the catalytic reaction (30, 31). Therefore, we examined the effect of Glc-1,6-P₂ on the synthesis of UDP-GlcNAc from GlcNAc-6-P. The TLC analysis of the products indicated that Glc-1,6-P₂ was not essential for the interconversion of GlcNAc-6-P and GlcNAc-1-P, because UDP-GlcNAc was produced from GlcNAc-6-P by Agm1p and Uap1p even in the absence of Glc-1,6-P₂ (Fig. 6A).

Possible Active Sites of ScUap1p—Comparison of the amino acid sequences among UDP-sugar pyrophosphorylases revealed that the region between amino acid positions 111 and 123 of ScUap1p shares significant sequence identity with other UDP-sugar pyrophosphorylases (Fig. 7). To verify the importance of this region for the catalytic activity, the highly conserved amino acids in this region, Gly₁¹¹, Gly₁¹², Gly₁¹⁴, Thr₁¹⁵, Arg₁¹⁶, Leu₁¹⁷, Pro₁²², and Lys₁²³ were replaced by alanine. As was done for the wild type ScUap1p, all the mutant enzymes were expressed as a fusion with GST and purified with glutathione-Sepharose beads. A, approximately 1 µg of the wild type and the indicated mutant proteins were separated on a 10% SDS-polyacrylamide gel (PAGE) and stained with Coomassie Brilliant Blue. The position of GST-ScUap1p is indicated by the arrowhead. B, approximately 0.1 µg of the purified GST and the indicated mutant proteins were incubated with GlcNAc-1-P and UTP, and the amounts of the released inorganic phosphate that represented the enzyme activities were determined with malachite green and ammonium molybdate.

TABLE I

Characteristics of the mutant ScUap1p

| Mutant | Kₘ (µM) | kₘ (min⁻¹) | kₘ⁺ (µM⁻¹ min⁻¹) | Kₘ to UTP |
|--------|--------|------------|------------------|-----------|
| Wild type | 13.52 | 925.18 | 68.41 | 20.58 |
| G111A | 33.57 | 80.05 | 2.85 | 44.67 |
| G112A | 124.20 | 1.06 | 0.01 | 71.36 |
| G114A | 19.37 | 318.62 | 16.45 | 24.57 |
| T115A | 15.96 | 526.54 | 34.95 | 23.28 |
| R116A | 10.92 | 0.90 | 0.08 | 16.72 |
| L117A | 29.33 | 136.31 | 4.65 | 54.03 |
| P122A | 11.78 | 264.18 | 22.44 | 30.73 |
| K123A | 18.49 | 5.09 | 0.28 | 17.62 |

FIG. 8. Effects on ScUap1p activity of alanine substitution for the conserved amino acids. The ScUap1 mutant proteins harboring an alanine substitution for each of the amino acids that are highly conserved in UDP-sugar pyrophosphorylases were expressed as a fusion with GST and purified with glutathione-Sepharose beads. A, approximately 1 µg of the wild type and the indicated mutant proteins were separated on a 10% SDS-polyacrylamide gel (PAGE) and stained with Coomassie Brilliant Blue. The position of GST-ScUap1p is indicated by the arrowhead. B, approximately 0.1 µg of the purified GST and the indicated mutant proteins were incubated with GlcNAc-1-P and UTP, and the amounts of the released inorganic phosphate that represented the enzyme activities were determined with malachite green and ammonium molybdate.
In this paper, we have identified the eukaryotic UDP-GlcNAc pyrophosphorylase genes. The expected amino acid sequence of the yeast and human enzymes are well conserved, and both C. albicans and human enzymes functionally complement S. cerevisiae UAP1. Although the yeast enzyme catalyzed uridylation to Glc-1-P, ScUap1p displayed a reasonable substrate specificity to GlcNAc-1-P, because the enzyme utilized Glc-1-P much less efficiently than GlcNAc-1-P. In fact, overexpression of ScUAP1 did not overcome the lethal phenotype caused by a deletion of UGP1 in S. cerevisiae. Moreover, the enzyme did not recognize GlcNAc-6-P, but together with ScAgm1p, it produced UDP-GlcNAc from GlcNAc-6-P, demonstrating that the GlcNAc phosphate mutase reaction precedes uridylation in UDP-GlcNAc biosynthesis. However, we cannot rule out the possibility that the results of the TLC assays and the high flux assays may not be exactly the same, because in the high flux assay the reverse reaction was eliminated by pyrophosphatase.

Both UDP-GlcNAc pyrophosphorylase and GlcN-1-P acetyltransferase activities are authentic in E. coli GltUmp (9). It has also been demonstrated that the N-terminal region is responsible for the uridylation, and acetylase activity resides in the C-terminal half of GltUmp (13). Unlike bacterial UDP-GlcNAc pyrophosphorylase, the eukaryotic enzymes seem not to be bifunctional, because ScUap1p did not utilize Glc-1-P as the substrate and the C-terminal portion of GltUmp showed no significant sequence homology to any UDP-GlcNAc pyrophosphorylase. Thus, it is likely that in yeast, GlcN-6-P is first acetylated by an as yet unidentified enzyme and then the mutase reaction generates GlcNAc-1-P.

Phosphomannomutase and phosphoglucomutase require a sugar diphosphate as a cofactor, which serves as a phosphate donor necessary to activate the enzyme by phosphorylation (30, 31). In this study, ScAgm1p was able to produce GlcNAc-1-P even in the absence of cofactor, Glk1,6-P, if a sufficient amount of ScAgm1p was present. One possible explanation for this discrepancy is that a small portion of the recombinant ScAgm1p was already phosphorylated and thereby activated. However, this hypothesis is inconsistent with the recent report by Oesterhelt et al. (31) that the plant and yeast enzymes utilize a sugar diphosphate as a co-substrate.

Sequence comparisons of the UDP-sugar transferases revealed that there is a region where the amino acid sequence is highly conserved among most of the known UDP-sugar pyrophosphorylases. Alanine substitution for Gly112, Arg116, or Lys123 severely diminished the enzyme activity and ability to complement the wild type ScUAP1 gene, strongly suggesting that these amino acids are catalytic residues. Among these three amino acids, Gly112 was shown to be a possible binding site to GlcNAc-1-P, because G112A displayed an increased $K_m$ value. In human UDP-Glc pyrophosphorylase, it was demonstrated that a single mutation of Gly112 to Asp drastically impaired the enzyme activity and caused cellular UDP-Glc deficiency (32). Sequence comparison of the Uap and Ugp proteins reveals that Gly115 of the human Ugp1p corresponds to Gly112 of the yeast Ugp1p. Thus, it is likely that Gly115 of the human Ugp1p also serves as a Glc-1-P binding site. Curiously, Gal7p, which is known as UDP-Gal pyrophosphorylase, shares no significant sequence homology to known UDP-sugar pyrophosphorylases, and the conserved amino acids essential for the catalytic activity of ScUap1p are not found in Gal7p (21). This may imply that the catalytic mechanism of Gal7p differs from those of other UDP-sugar pyrophosphorylases.

The human UDP-GlcNAc pyrophosphorylase cDNA turned to be identical to the AGX1 cDNA. Although the physiological function of AGX1 remains to be established, it encodes an unknown antigen expressed in infertile males and is implicated in antibody-mediated human infertility (29). AGX1 is abundantly expressed in testes, and only low levels of AGX1 mRNA were detected in placenta, muscle, and liver (29). The reason why testes expresses a higher level of AGX1 mRNA and how UDP-GlcNAc pyrophosphorylase causes human male infertility awaits further study. In addition, there is an additional AGX cDNA, termed AGX2, which differs from AGX1 by a 48-base pair insertion in the ORF. The level of AGX2 mRNA was not remarkably increased in testis; low but similar levels of AGX2 mRNA were detected in testes, placenta, muscle, and liver (29). Therefore, it may also be of interest to study how the internal 48-base pair insertion affects the UDP-GlcNAc pyrophosphorylase activity.

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REFERENCES
1. Holte, J. V., and Schwartz, U. (1985) in Molecular Cytology of Escherichia coli. (Nanninga, N., ed) pp. 77–119, Academic Press, Inc., New York
2. Park, J. T. (1987) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) Vol. 1, pp. 663–671, American Society for Microbiology, Washington, D. C.
3. Raetz, C. R. H. (1987) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) Vol. 1, pp. 498–503, American Society for Microbiology, Washington, D. C.
4. Cahm, E., Roberts, R., and Bowers, B. (1982) Annu. Rev. Biochem. 51, 763–793
5. Herscovics, A., and Orlean, P. (1993) FASEB J. 7, 540–550
6. Dobrogosz, W. J. (1968) J. Bacteriol. 95, 578–584
7. White, R. J. (1989) Biochem. J. 266, 847–858
8. Freese, E. R., Cole, R. M., Klofac, W., and Freese, E. (1970) J. Bacteriol. 101, 1046–1062
9. Wu, H. C., and Wu, T. C. (1971) J. Bacteriol. 105, 455–466
10. Walker, J. R., Gay, N. J., Saraste, M., and Ebere, A. N. (1984) Biochem. J. 224, 799–815
11. Dutka-Malen, S., Mazodier, P., and Badet, B. (1988) Biochimie (Paris) 70, 287–290
12. Smith, D. B., and Johnson, K. S. (1988) Gene 72, 95–102
13. Mengin-Lecreulx, D., and Heijenoort, J. (1993) J. Bacteriol. 175, 6150–6157
14. Watzele, G., and Tanner, W. (1989) J. Bacterial 176, 5788–5795
15. Gotoh, T., and Nisonoff, A. (1989) J. Bacterial 168, 1415–1422
16. Boles, E., Liebetrau, W., Hofmann, M., and Zimmermann, F. K. (1994) Eur. J. Biochem. 220, 83–96
17. Kepes, F., and Schekman, R. (1988) J. Biochem. 263, 9155–9161
18. Hofmann, M., Boles, E., and Zimmermann, F. K. (1994) Eur. J. Biochem. 221, 741–747
19. Tajima, M., Nogi, Y., and Fukasawa, T. (1985) Yeast 1, 67–77
20. Daron, M. J., Dallies, N., Thines-Sempoux, D., Paquet, V., and Ferrandis, J. (1995) Eur. J. Biochem. 233, 520–530
21. Hofmann, M., Boles, E., and Zimmermann, F. K. (1994) Eur. J. Biochem. 220, 6308–6314
22. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
23. Yamada-Okae, T., Shimmi, O., Dei, R., Mizumoto, K., Arisawa, M., and Yamada-Okae, H. (1996) Microbiology 142, 2515–2523
24. Smith, D. B., and Johnson, K. S. (1988) Gene (Amst.) 67, 31–40
25. Diefman, A. B., and Goldberg, E. (1994) Biochem. Biophys. Res. Commun. 201, 1087–1093
26. Guha, S. K., and Rose, Z. B. (1985) Arch. Biochem. Biophys. 243, 168–173
27. Oesterhelt, D., Schnarrenberger, C., and Gross, W. (1997) FEBS Lett. 401, 265–272
28. Flores-Diaz, M., Alape-Giron, A., Persson, B., Pollesello, P., Moos, M., von Eichel-Streiber, C., Thelestam, M., and Florin, I. (1997) J. Biol. Chem. 272, 20764–20771
29. Peng, H. L., and Chang, H. Y. (1993) FEBS Lett. 329, 153–158
30. Weisborn, A. C., Liu, Q., Rumley, M. K., and Kennedy, E. P. (1994) J. Bacteriol. 176, 2611–2618