YND1, a Homologue of GDA1, Encodes Membrane-bound Apyrase Required for Golgi N- and O-Glycosylation in Saccharomyces cerevisiae*

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The gene for the open reading frame YER005w that is homologous to yeast Golgi GDPase encoded by the GDA1 gene was cloned and named YND1. It encodes a 630-amino acid protein that contains a single transmembrane region near the carboxyl terminus. The overexpression of the YND1 gene in the gda1 null mutant caused a significant increase in microsomal membrane-bound nucleoside phosphate activity with a luminal orientation. The activity was equally high toward ADP/ATP, GDP/CTP, and UDP/UTP and ~50% less toward CDP/CTP and thiamine pyrophosphate, but there was no activity toward GMP, indicating that the Ynd1 protein belongs to the apyrase family. This substrate specificity is different from that of yeast GDPase, but similar to that of human Golgi UDPase. The Dyn1 mutant cells were defective in O- and N-linked glycosylation in the Golgi compartments. The overexpression of the YND1 gene complemented some glycosylation defects in Δgda1 disruptants, suggesting a partially redundant function of yeast apyrase and GDPase. From these results and the phenotype of the Δynd1Δgda1 double deletion showing a synthetic effect, we conclude that yeast apyrase is required for Golgi glycosylation and cell wall integrity, providing the first direct evidence for the in vivo function of intracellular apyrase in eukaryotic cells.

Apyrases (EC 3.6.1.5) are known as enzymes that hydrolyze both di- and triphosphate nucleotides as well as thiamine pyrophosphate. Apyrase family proteins are widely distributed in eukaryotic cells from yeast to mammals (1–3). Different from ATPases that specifically hydrolyze ATP, apyrases generally hydrolyze both ATP and ADP, although the rate of ATP and ADP hydrolysis differs depending on the sources. The main products of ATP hydrolysis by apyrase are AMP and two orthophosphate anions. Apyrases can be divided into ecto-apyrases with the catalytic domain outside the cell and endo-apyrases with the catalytic site inside the cell (2). It has been suggested that ecto-apyrases may participate in neurotransmission (4) and blood platelet aggregation and pressure regulation (5). However, less is known about the role of endo-apyrases. Although some roles of endo-apyrase have been suggested in protein glycosylation and sugar level control (3, 6) and in regulation of membrane integrity (7), there is no direct evidence for the in vivo function of endo-apyrase (2).

Specific oligosaccharide modification of glycoproteins in eukaryotic cells occurs mainly in the lumen of the Golgi apparatus. The substrates for these reactions are nucleotide sugars, which are synthesized in the cytosol. As shown by genetic and biochemical analysis (8, 9), nucleotide sugars are imported from the cytosol to the Golgi lumen by highly specific transporters, which may also antiport the corresponding nucleoside monophosphate from the Golgi lumen to the cytosol. NDPase hydrolyzes nucleoside diphosphate to nucleoside monophosphate, which is a putative antipporter of nucleotide sugars. This allows the entry of additional nucleotide sugars from the cytosol to the Golgi lumen. Thereby, the NDPase is considered to play a critical role in the translocation of these nucleotide sugars from the cytosol into the lumen (10).

In Saccharomyces cerevisiae, mannosylation of N- and O-linked oligosaccharides is mainly regulated by a GDPase that converts GDP to GMP. GDA1, the gene for GDPase, has been cloned and found to encode a type II membrane protein (8). Gda1p shows a high activity toward GDP, but a low activity toward UDP and no activity toward ATP and ADP (11). Deletion of GDA1 results in a marked reduction in Golgi mannosylation of proteins and lipids in vivo (8) and causes a 4-fold lower rate of GDP-mannose entry into Golgi vesicles in dolichol-P-Man synthase-deficient cells (12). Nevertheless, in Δgda1 cells, invertase is still heavily glycosylated, and in addition, a significant amount of GMP, as well as very low residual nonspecific nucleoside phosphatase activity unrelated to Gda1p, was detected in the Golgi membranes (8, 12). These observations suggested the importance of residual GDPase activity in the Golgi lumen of the Δgda1 mutant. Another possibility could be a direct supply of GMP from GDP-mannose by mannosyl-phosphatase transferase. The MN4 gene is known to encode a positive regulator of mannosyl phosphorylation (13, 14), but the analysis of mannosylation of invertase in Δgda1Δmn4 double disruptant cells showed no effect on mannosylation when compared with that of Δgda1 alone. This result strongly indicated that the remaining GDP did not arise from mannosyl phosphorylation in the Golgi lumen of Δgda1 cells and prompted us to investigate other GDPases involved in protein glycosylation. At that time, it was already known that the Saccharomyces Genome Database contains at least one homologue of Gda1p that possess ~20% amino acid identity to Gda1p and that also belongs to the apyrase superfamily, as determined by sequence alignment (15).

Here we report that the residual GDPase activity in the Δgda1 mutant results from this homologue of Gda1p. It turned out to be a typical apyrase that can hydrolyze not only nucleoside diphosphates, as Gda1p does, but also nucleoside triphosphates. We also demonstrate that additional expression of this
yeast apyrase can complement the lack of GDPase activity of Gda1p and plays an important role in controlling the protein glycosylation in Golgi compartments. For the above reason, we named the GDA1 homologue YND1 (yeast nucleoside diphosphatase) to indicate a possible redundancy between the Ynd1p and Nmn1p homologues. Wild-type cells (G2-9) containing the YEpGAP plasmid were grown in YPAD medium (1% yeast extract, 2% peptone, 100 mg/ml adenine sulfate, and 2% dextrose), in YP medium (1% yeast extract and 2% peptone) with 0.2% sucrose (in experiments requiring invertebrate induction), or in SD medium that contained 0.67% yeast nitrogen base and 2% dextrose supplemented with the relevant amino acids (16). Hygromycin B sensitivity and vanadate resistance were tested on YPAD plates supplemented with 50 μg/ml hygromycin B (Sigma) or 5 mM sodium orthovanadate (Sigma) (17). Standard procedures were used for sporulation of diploids and dissection of tetrads (18).

Construction of Plasmids and Strains—The construction of the YEpGAP and YEpGAP-YND1 plasmids for YND1 gene expression was carried out as follows. The HindIII site of pKT10 (19) was converted to a BamHI site using BamHI linkers, and a 0.7-kb BamHI fragment containing the TDH3 promoter and terminator was obtained by digesting the modified vector with BamHI. Subsequently, multilinking sites between two PvuII sites of YEp352 (20) were deleted using the PvuII enzyme, and a BamHI site was inserted at the PvuII site using BamHI linkers. The 0.7-kb BamHI fragment prepared as described above was then inserted into the BamHI sites of this modified YEp352 vector and renamed as YEpGAP. A 2.2-kb fragment containing the YND1 open reading frame was amplified by PCR using the primers 5′-CCGAGATCTGGCCCTGCGCTCCCGGTGAGATGTC-3′ and 5′-CCGCGTCAATGCATGTTTACTAAGTCTGCTGAAGT-3′ and directly cloned into the pCRII vector (pCRII-YND1) (Invitrogen). The EcoRI fragment with the entire YND1 open reading frame sequence from pCR-YND1S was then inserted into YEpGAP in the correct orientation.

The S. cerevisae strains studied in this use were derived from G2-9 and G2-10 backgrounds (8). Wild-type cells (G2-9) containing the YEpGAP plasmid were grown in YPAD medium (1% yeast extract, 2% peptone, 100 mg/ml adenine sulfate, and 2% dextrose), in YP medium (1% yeast extract and 2% peptone) with 0.2% sucrose (in experiments requiring invertebrate induction), or in SD medium that contained 0.67% yeast nitrogen base and 2% dextrose supplemented with the relevant amino acids (16). Hygromycin B sensitivity and vanadate resistance were tested on YPAD plates supplemented with 50 μg/ml hygromycin B (Sigma) or 5 mM sodium orthovanadate (Sigma) (17). Standard procedures were used for sporulation of diploids and dissection of tetrads (18).

RESULTS

Identification of Ynd1p as a Membrane Protein—The YND1 gene (GenBank™/EMBL accession number U18778 (30)) was cloned by PCR using S. cerevisiae genomic DNA as a template. The predicted Ynd1p protein consists of 630 amino acids with a calculated molecular mass of 71.8 kDa. It contains four conserved motifs typical for the apyrase superfamily and two putative N-linked glycosylation sites. Hydrophobicity analysis predicted that Ynd1p has a single transmembrane region near the C terminus, suggesting a type 1 membrane protein.

In the Western blot analysis, a distinct band of His + transformants was selected, and replacement of the YND1 and MNN1 genes was confirmed by PCR (data not shown).

Preparation of Microsomal Membranes—To measure the enzyme activity, membrane orientation, and substrate specificity of Ynd1p, the intact Golgi-rich microsomal vesicles were isolated according to the procedures described previously (23). Final membrane pellets after the 100,000 x g centrifugation (P2 fraction) were resuspended in 0.2 mM imidazole buffer (pH 7.6) with 0.8 M sorbitol (buffer A) to preserve vesicle integrity.

Membrane Association of Ynd1p—To determine the nature of membrane association of Ynd1p, -1 mg of microsomal fraction was resuspended in 0.4 M buffer A, 0.6 M NaCl, and 0.1 mM Na2CO3 (pH 11) or of buffer A containing either 1.5% Triton X-100 or 0.5% SDS. These mixtures were incubated for 15 min on ice and then subjected to centrifugation at 100,000 x g for 30 min at 4 °C. The resulting membrane pellets were resuspended in 200 μl of Tris-HCl buffer (pH 8.6). Supernatant and pellet fractions were used for SDS-PAGE and analyzed by immunoblotting. Protein concentration was determined using the BCA protein assay reagent (Pierce) according to the manufacturer's instructions.

Measurement of Nucleoside Phosphatase Activity—The NDP and NTP hydrolyzing activity of the membrane fraction was essentially the same as described by Abeijon et al. (8). Briefly, the incubation mixture in a final volume of 100 μl contained microsomal vesicles (3 μg of P2 fraction protein per μl volume; see "Preparation of Microsomal Membranes") 0.2 mM imidazole buffer (pH 7.6), 10 mM MCl3, 0.1% Triton X-100, and 2 μM NDPTNP (if not mentioned in the figure legends). Incubation was done for 5 min at 30 °C. The reaction was stopped by adding 80 μl of 60% (v/v) perchloric acid. Released inorganic phosphate was determined with an Iatron P kit (Diatron) according to the manufacturer's instructions. The absorbance was measured at 540 nm, and the amount of inorganic phosphate released was calculated from a calibration curve prepared using KH2PO4 as a standard. One unit of activity is defined as 1 μmol of inorganic phosphate released per min under standard assay conditions. The specific activity was calculated as units/mg of protein. The latency of GDPase was calculated as described previously (23). Concentrations of saponin (24) and digitonin (25) for permeabilization of intact membranes were as described.

Preparation of Antiserum and Immunoblot Analysis—A polyclonal antibody was raised against the putative luminal region of Ynd1p (from amino acids 52 to 499) fused to glutathione S-transferase (GST). Expression of the GST-YND1 fusion gene was induced as described (26). A New Zealand white rabbit was subsequently immunized with the purified fusion protein, and the antiserum was obtained. Immunoblots were prepared using the method described previously (26) or the CDP-Star chemiluminescent substrate (New England Biolabs Inc.). Mobility of Invertase and Chitinase on SDS-PAGE—Preparation of the invertase extracts, gel electrophoresis, and activity staining of invertase were performed as described (27, 28). Secreted chitinase was isolated as described without any overexpression of the chitinase gene (CTSI) (28, 29). Samples were analyzed on 7.5% SDS-polyacrylamide gel stained with Coomassie Blue.

HPLC Analysis of O-Linked Oligosaccharides—O-Linked oligosaccharides were prepared from chitinase-bound chitin by hydrazinolysis using Hydrachlor S-204 (Honen Corp.). The sample was hydrolyzed by hydrazine at 65 °C for 6 h, followed by N-acetylation according to the manufacturer's protocol. The dried sample was labeled using a kit from Honen Corp. The ABEEL-labeled sample was dissolved in water, extracted with chloroform, and further analyzed on an ASAHIPAK NH2P-50 column (0.46 × 25 cm, Showa Denko) at a rate of 1.0 ml/min with solvent A (acetonitrile) and solvent B (200 mM acetic acid/triethylamine (pH 7.3)). After the sample injection, the proportion of solvent B was increased linearly from 10 to 60% for 60 min.

RESULTS

Identification of Ynd1p as a Membrane Protein—The YND1 gene (GenBank™/EMBL accession number U18778 (30)) was cloned by PCR using S. cerevisiae genomic DNA as a template. The predicted Ynd1p protein consists of 630 amino acids with a calculated molecular mass of 71.8 kDa. It contains four conserved motifs typical for the apyrase superfamily and two putative N-linked glycosylation sites. Hydrophobicity analysis predicted that Ynd1p has a single transmembrane region near the C terminus, suggesting a type 1 membrane protein.
Ynd1p is not only NDPase activity, but also NTPase activity. The activities toward GDP, GTP, and ADP were higher than those toward UDP, UTP, and ATP, and ~50% of these activities were observed toward CDP, CTP, and thiamine pyrophosphate (TPP), whereas no activity was detected toward GMP (Fig. 2B). The order of the divalent cation requirement for these substrates was the same as that described for GDP (data not shown). This substrate specificity was completely different from that of Gda1p, which has only 31% activity toward UDP and no activity toward ADP relative to the activity toward GDP in the presence of 10 mM Mn²⁺ (11). Recently, NDP and NTP hydrolyzing activities similar to that of Ynd1p was reported for human brain apyrase; however, it has very low activities toward ADP and ATP and an apparent preference for Ca²⁺ (3).

Ynd1p and Gda1p Are Partially Redundant in Function—Given the sequence homology and our observation that Ynd1p and Gda1p indeed have GDPase activity, we examined whether these two genes may have some redundant function with regard to each other. To address this point, we overexpressed the YND1 gene in Δgda1 cells. Invertase is a highly glycosylated protein that contains 14 potential N-glycosylation sites (27),
and chitinase is known as a secretory protein that exclusively contains O-linked oligosaccharides (29). The overexpression of the YND1 gene completely suppressed the faster migration of invertase in Δgda1 cells and partially (~50%) recovered the mobility shift of chitinase observed in Δgda1 cells compared with the wild-type cells (Fig. 3). Partial complementation of chitinase mobility is most likely due to the loss of plasmids containing the YND1 gene in Δgda1 cells during the cultivation of this strain in nonselective YPAD medium. These results indicate that the overexpression of the YND1 gene complements the glycosylation defect of Δgda1 cells, demonstrating some functional overlap between Ynd1p and Gda1p.

Disruption of Both Genes Causes a Synthetic Defect in Cell Growth and Cell Shape—To further investigate the genetic interactions between YND1 and GDA1, a double disruption of the YND1 and GDA1 genes was performed. The Δynd1::URA3 haploid (XGY4) was crossed with the Δgda1::LEU2 haploid (G2-11), and the resultant diploid was subjected to sporulation and tetrad dissection. The growth of double disruptant cells (KAI1), whose phenotype was confirmed by their growth after a transfer to a Ura- and Leu- plate, was slow relative to that of the single mutant and wild-type strain even after 5 days of incubation on a YPAD plate at 30 °C (Fig. 4A). However, once the cells were transferred to the fresh medium, the vegetative cell growth was less impaired than the growth during germination, and the doubling time was two times slower than that of the single mutant or wild-type cells. The Δynd1 Δgda1 double disruptant cells are in clumps and frequently bigger and more round in shape (Fig. 4C) than the isogenic Δynd1 cells (Fig. 4B) or Δgda1 cells (data not shown). Many dead or lysed cells with a ragged and abnormal shape were also observed in double disruptants during the saturated liquid culture period (>24 h), as shown by strong staining with propidium iodide (Fig. 4D). Some cells contained several nuclei as shown by 4,6-diamidino-2-phenylindole staining (data not shown). Essentially the same results were obtained with the yeast strain W303 and different selectable markers. Many features of the double disruptant cells were similar to those of some pmt mutants, where the observed phenotypes were interpreted as a consequence of cell wall defects because a significant decrease in the relative ratio of glucan to mannan was found (32). We therefore concluded that the double disruption of the YND1 and GDA1 genes results in a synthetic phenotype showing a severe defect in germination and cell wall integrity and a less severe but significant defect in vegetative cell growth.

Deletion of YND1 Results in a Protein Glycosylation Defect—To assess the function of the YND1 gene in vivo, the phenotypes of Δynd1 cells were further examined. Several glycosylation-defective mutants are known to be sensitive to hygromycin B and resistant to vanadate (17, 33, 34). As shown in Fig. 5A, hygromycin B inhibited the growth of Δynd1 cells as well as Δgda1 cells at a concentration of 50 μg/ml, although the growth of the isogenic wild-type cells and Δmnn1 cells that are defective in α,1,3-linked mannosylation (35, 36) was not affected by this drug. In contrast, the Δynd1 cells showed a vanadate-resistant phenotype at a concentration of 5 mM, whereas the other isogenic strains, including Δgda1 cells, showed a vanadate-sensitive phenotype (Fig. 5A). These results suggest that Δynd1 cells behave differently from Δgda1 cells in their drug sensitivity.

To check the extent of N-linked glycosylation, invertase prepared from Δynd1 cells was subjected to SDS-PAGE and then detected by activity staining. As shown in Fig. 5B, invertase produced by Δynd1 cells migrated faster, as a smear, compared with the isogenic wild-type cells and at a similar position to that of the Δgda1 cells (8). This result clearly demonstrates that the YND1 gene is required for N-linked glycosylation. Chitinase was purified from the culture medium of Δynd1 cells by chitin binding, and its mobility was compared with that of wild-type and Δgda1 cells. Chitinase prepared from Δynd1 cells showed an intermediate mobility between those of wild-type and Δgda1 cells (Fig. 5B). The results indicate a defect in O-glycosylation in Δynd1 cells and suggest some functional differences in O-glycosylation between Ynd1p and Gda1p. Taken together, these results demonstrate that the YND1 gene functions in both N- and O-linked glycosylation.

Disruption of the YND1 Gene Inhibits the Formation of Mannotetraose in O-Linked Oligosaccharides—To examine the glycosylation defect of Δynd1 cells in more depth, O-linked oligosaccharides from chitinase were analyzed by HPLC. We found that formation of mannolactose (M4) and mannotetraose (M5) was partially inhibited in Δynd1 cells (Fig. 6). These analyses also confirmed the previous data (8) that Δgda1 cells (G2-12 strain) accumulate mannose (M1) and mannobiose (M2), but our double deletion mutant showed a significant increase in mannose and decrease in mannobiose as compared with the isogenic Δgda1 cells (Fig. 6). These results are consistent with the relative positions of chitinase bands of different mutants on SDS-PAGE (Fig. 5B) and provide evidence that the changes in chitinase mobility are due to a defect in sugar chain elongation.
Since the addition of the fourth and fifth mannososes of O-linked oligosaccharides is catalyzed mostly by α1,3-mannosyltransferase encoded by the MNN1 gene (35, 36), O-linked oligosaccharides prepared from Δmann1 cells were analyzed and compared with those from Δynd1 cells. As expected, the Δmann1 cells showed a dramatic decrease in mannnotetraose and mannotriose and a significant accumulation of mannotriose (Fig. 5). Gda1p is related to potato apyrase (15) and belongs to the apyrase family of enzymes (1, 2).

Fig. 5. The Δynd1 disruptant cells are defective in Golgi glycosylation. A, comparison of drug sensitivity among various strains. Isogenic strains, wild-type (act; G2-9), Δgda1 (G2-12), Δynd1 (XGY4), and Δmann1 (XGY5), were streaked out on YPAD plates containing either 50 μg/ml hygromycin B or 5 mM vanadate and incubated at 30 °C for 2–3 days. The Δynd1 cells showed a hygromycin B-sensitive and vanadate-resistant phenotype. B, electrophoretic mobilities of invertase and chitinase prepared from various strains. After a derepression of SUC2 gene expression by sucrose, samples were subjected to SDS-PAGE and stained for invertase activity. Secreted chitinase from various strains was purified from cultures by chitin binding. Samples were analyzed by SDS-PAGE and then stained with Coomassie Blue. The Δynd1 cells are defective in N- and O-glycosylation.

DISCUSSION

Ynd1 Protein as a Member of the Apyrase Family—In this study, we have reported the characterization of a novel gene, YND1, that encodes an apyrase family protein in S. cerevisiae. The Ynd1 protein was confirmed as a distinct band of 72 kDa in the microsomal membrane fractions of wild-type yeast cells by Western blot analysis using a polyclonal anti-Ynd1p antibody (Fig. 1). The extraction of cell lysates with various reagents (Fig. 1) showed that Ynd1p behaves as an integral membrane protein. Triton X-100 (1.5%) released only part of Ynd1p into the soluble fraction. Ynd1p showed a broad substrate specificity not only toward nucleoside diphosphates, but also toward nucleoside triphosphates (Fig. 2B); these activities were more effectively stimulated by Mn2+ than by Ca2+ (Fig. 2A). These properties of Ynd1p demonstrated that Ynd1p belongs to the apyrase family of enzymes (1, 2).

Recently, human Golgi apyrase cDNA was cloned, and its gene product that has 51% amino acid similarity to Ynd1p was analyzed by heterologous expression in COS-7 cells (3). The activity was higher toward UDP than toward GDP, CDP, and UTP, and this activity was stimulated by divalent cations in the order of Ca2+ > Mg2+ > Mn2+. Interestingly, almost no activity was detected toward ADP and ATP. Based on the Golgi luminal localization and the highest activity toward UDP, this endo-apyrase was concluded to act as UDPase, suggesting its function as the antiporter (UMP) generator during the UDP-sugar nucleotide transport into Golgi vesicles (3). However, no direct in vivo evidence is known for the proposed function of this apyrase.

Sequence alignment of apyrase superfamily members based on evolutionary relatedness suggests they are classified into four subgroups (3). Gda1p is related to potato apyrase (15) and...
pea NTPase, whereas Ynd1p is related to human Golgi UDPase (3) and two nematode hypothetical proteins (C33H5.14 and R07E4.4). In contrast to Ynd1p, Gda1p shows only 11–31% activity toward UDP and <2% or null activity toward other NDPs and NTPs relative to full activity toward GDP (11). Although the substrate specificity of potato apyrase and pea NTPase has not been carefully examined, Gda1p may have evolved from some ancestor apyrase to develop its GDPase function that is essential for protein and lipid glycosylation in the yeast Golgi apparatus. However, the null mutant of Gda1p is viable (8). Therefore, Wang and Guidotti (3) stressed their interest in analyzing the functions of the yeast hypothetical apyrase (Ynd1p) and its effect on the viability of yda1 null mutants because there are only two copies of the apyrase superfamily gene, GDA1 and YND1, in the genome of S. cerevisiae.

In Vivo Function of Ynd1p endo-Apyrase—The phenotypic studies of Δynd1 cells described in this paper provide the answer for the above question and present the first direct evidence for the in vivo function of endo-apyrase. It is widely known that the inhibition of glycosylation or cell wall biosynthesis in yeast cells leads to a marked increase in their sensitivity to the aminoglycoside hygromycin B (33, 34) and resistance to sodium orthovanadate (17). The Δynd1 cells are hygromycin B-sensitive at 50 μg/ml, similar to the Δgda1 cells, although the isogenic wild-type and Δmnn1 cells are not affected by this drug (Fig. 5A). This result suggests that a defect in the glycosylation and/or cell wall of Δynd1 cells may be more severe than that of Δmnn1 cells, which lack terminal α1,3-linked mannosides. As for vanadate resistance, the Δynd1 cells exhibited cell growth at a concentration of 5 mM, whereas the wild-type, Δmnn1, and Δgda1 cells showed no cell growth under the same condition (Fig. 5A), indicating that Ynd1p and Gda1p may have some functional differences in Golgi glycosylation. This property of the Δynd1 cells toward antibiotics is similar to that of vir4 (vanadate-resistant glycosylation) cells (17), which are defective in GDP-mannose transport in the Golgi membranes (38, 39). The faster migration of invertase and chitinase prepared from Δynd1 cells on SDS-polyacrylamide gel indicated that the disruption of the YND1 gene affected both N- and O-linked glycosylation in the Golgi compartments (Fig. 5B). Furthermore, HPLC analysis of O-linked oligosaccharides purified from various strains (Fig. 6) provided clear evidence that Ynd1p plays an important role in Golgi glycosylation.

HPLC analysis of O-linked oligosaccharides also provided some grounds for speculations about the localization of Ynd1p. Formation of mannotetraose (M4) and mannopentaoe (M5) was partially inhibited in Δynd1 cells compared with wild-type cells (Fig. 6). The addition of the fourth and fifth mannosides of O-linked oligosaccharides is mainly catalyzed by Mnn1p, an α1,3-mannosyltransferase, so it is likely that Ynd1p at least partially colocalizes with Mnn1p in the Golgi compartments. On the other hand, since the formation of mannosiose (M2) was more inhibited in Δynd1Δgda1 cells than in Δgda1 cells (Fig. 6), Ynd1p is likely to colocalize with Krb2p/Mnt1p, Krl1p, and Krf3p, the α1,2-mannosyltransferases that together add most of the second and third O-linked mannosides (40–42). Taken together, Ynd1p is most likely to function in different Golgi compartments, but confirmation of this hypothesis requires further study.

Complementation of the glycosylation defect of invertase and chitinase in Δgda1 cells by the overexpression of the YND1 gene (Fig. 3) suggested that the Ynd1p function is partially redundant with Gda1p. Either of the YND1 and GDA1 single mutants can maintain mostly normal growth due to remaining GDPase activity. Single disruptive cells (Δynd1) are sensitive to hygromycin and resistant to vanadate (Fig. 5A), whereas the double disrupting cells (Δynd1Δgda1) are severely impaired in their germination (Fig. 4A) and are in clumps with a ragged and abnormal shape (Fig. 4C). This result demonstrates that the double deletion causes a synthetic effect on cell growth and cell shape, providing additional evidence for the in vivo function of yeast apyrase (Ynd1p) in cell wall integrity. Two reasons can be considered for the synthetic phenotype of the double mutant. One reason is due to a complete loss of GDP hydrolysis in the double mutant. This would indicate that the GDP-mannose/GMP antiport system in Golgi membranes (10), which is regulated by the hydrolysis of GDP to GMP, is essential for cell wall mannan synthesis. The other reason involves a synergistic defect in both GDP and NDP or NTP hydrolysis. This suggests that, together with GDPase activity, the UDPase or ADPase/ATPase activity of Ynd1p may have an important function in cell wall integrity, for instance, in the regulation of ATP transport that is needed for phosphorylation of luminal proteins or in the UDP-glucose transport required for β1,6-glucan synthesis in the Golgi lumen. To understand the precise relationship among Ynd1p, Gda1p, and glycosylation in the Golgi, we will now characterize the growth conditions, mutations, and multicity suppressor genes that can rescue the growth defect of the double deletion mutant. Another attractive direction is the construction of mutant variants of Ynd1p with separate substrate specificities.

In mammalian cells, the nucleotide sugars utilized for glycosylation in the Golgi are both UDP-sugars, such as UDP-GlcNAc and UDP-Gal, and GDP-sugars such as GDP-Fuc. This diversity of nucleotide sugar usage provides a good reason for the presence of an NDPase (apyrase) that possesses a broad substrate specificity. It has been commonly believed that in S. cerevisiae, GDP-mannose is the sole nucleotide sugar in Golgi compartments (37, 43) and that no other NDPase activity except GDPase is required for glycosylation. This raises the question of why a broad specificity NDPase (apyrase) would be required in S. cerevisiae. In fact, there is recent evidence that suggests that UDP-sugars may also function in the Golgi. Although a UDP-glucose transporter has not yet been identified in yeast Golgi vesicles, β1,6-glucan polymer is thought to be elaborated in Golgi compartments and depends on two Golgi proteins, Kre6p and Skn1p (44). Presumably, this would require the presence of luminal UDP-Glu in the Golgi. In support of a UDPase function, we have also identified the presence of UDP-galactose transport activity in S. cerevisiae (23). Whether Ynd1p functions as UDPase or ADPase/ATPase in the yeast Golgi complex in vivo is unknown. However, it will be interesting to investigate the connection between Ynd1p function and UDP-glucose or UDP-galactose transport in yeast and to address another apyrase function in the Golgi apparatus, especially its relation to GDP-mannose and ATP transport into the Golgi lumen.

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