ire-1-dependent Transcriptional Up-regulation of a Lumenal Uridine Diphosphatase from Caenorhabditis elegans*

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Luminal ecto-nucleoside tri- and di-phosphohydrolases (ENTPDases) of the secretory pathway of eukaryotes hydrolyze nucleoside diphosphates resulting from glycosyltransferase-mediated reactions, yielding nucleoside monophosphates. The latter are weaker inhibitors of glycosyltransferases than the former and are also antipor ters for the transport of nucleotide sugars from the cytosol to the endoplasmic reticulum (ER) and Golgi apparatus (GA) lumen. Here we describe the presence of two cation-dependent nucleotide phosphohydrolase activities in membranes of Caenorhabditis elegans: one, UDA-1, is a UDP/GDPase encoded by the gene uda-1, whereas the other is an apyrase encoded by the gene ntp-1. UDA-1 shares significant amino acid sequence similarity to yeast GA Gda1p and mammalian UDP/GDPases and has a luminal active site in vesicles displaying an intermediate density between those of the ER and GA when expressed in S. cerevisiae. NTP-1 expressed in COS-7 cells appeared to localize to the GA. The transcript of uda-1 but not those of two other C. elegans ENTPDase mRNAs (ntp-1 and mig-23) was induced up to 3.5-fold by high temperature, tunicamycin, and ethanol. The same effectors triggered the unfolded protein response as shown by the induction of expression of green fluorescent protein under the control of the BiP chaperone promoter and the UDP-glucose:glycoprotein glucosyltransferase. Up-regulation of uda-1 did not occur in ire-1-deficient mutants, demonstrating the role of this ER stress sensor in this event. We hypothesize that up-regulation of uda-1 favors hydrolysis of the glucosyltransferase inhibitory product UDP to UMP, and that the latter product then exits the lumen of the ER or pre-GA compartment in a coupled exchange with the entry of UDP-glucose, thereby further relieving ER stress by favoring protein re-glycosylation.

The occurrence of the eukaryotic endomembrane system has brought forth a need for a mechanism to coordinate the metabolic flux of nucleotides, nucleotide sugars, and nucleotide sul fate between the cytosol and the lumen of secretory organelles. E-type ATPases play an important role in this function. They have been conserved through evolution with their catalytic site in the eco-position, facing the outer surface of the plasma membrane or its topological equivalent, the lumen of intracellu lar organelles such as the endoplasmic reticulum (ER)1 Golgi apparatus (GA), and lysosomes/vacuoles. These enzymes, members of the eco-nucleoside triphosphate diphosphohydrolase (ENTPDase) family, hydrolyze nucleoside tri- and/or diphosphates in the presence of cations and share, at the amino acid sequence level, five conserved motifs called apyrase-conserved regions (ACRs) (1).

Extracellular nucleotides such as ATP and ADP are intercellular signaling molecules in virtually every tissue where, modulated by ecto-apyrases, they participate in a broad range of biological processes such as the regulation of immune responses (2) and modulation of signaling by neuronal cells (3). Specifically, mammalian ENTPDase1/CD39 is responsible for the inhibition of ADP-induced platelet aggregation (4), whereas ENTPDase2 participates in the specification of neural cell migration during brain development by directly modulating ATP receptor-mediated cell communication (5).

The physiological requirement for intracellular nucleotide diphosphatases in the lumen of the GA became apparent some years ago with the realization that nucleoside diphosphates (which are generated in this compartment as reaction products after glycosylation, sulfation, and phosphorylation of secretory and membrane proteins), proteoglycans, and lipids are inhibitors of glycosyltransferases. Therefore, nucleoside diphosphates, which do not cross the GA membrane, do not accumulate in the lumen of this organelle and are rapidly converted to nucleoside monophosphates by lumenal nucleotide diphosphatases. Nucleoside monophosphates, which inhibit glycosyltransferases to a much lesser extent, exit the lumen of the GA by means of a coupled exchange with entry from the cytosol of (additional) nucleotide sugars, nucleotide sulfate, or ATP, giving rise to a transport/export cycle present in every eukaryotic cell studied so far (6).

The specificity and subcellular location of three different ENTPDases have been described until now in the mammalian secretory pathway. Two of these enzymes occur in the lumen of the ER and are UDP/GDPases; one of them is membrane-bound, and its activity depends solely upon Ca$^{2+}$ (7), whereas the other, ENTPD5, is soluble and is also active with Mg$^{2+}$ and Mn$^{2+}$ (8). The third mammalian ENTPDase is a GA luminal apyrase (9).

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1 The abbreviations used are: ER, endoplasmic reticulum; GA, Golgi apparatus; ENTPDase, ecto-nucleoside triphosphate diphosphohydrolase; ACR, apyrase-conserved regions; GT, glucosyltransferase; ORF, open reading frame; UPR, unfolded protein response.

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Saccharomyces cerevisiae and Schizosaccharomyces pombe each have two GA enzymes that degrade nucleoside diphosphates: ScGda1p (10) and SpGda1p (11), which are solely nucleotide diphosphatases and have preferred activity toward GDP and UDP; ScYnd1p (12, 13) and SpYnd1p (11) are apyrases and use nucleoside di- and tri- phosphates as substrates. Loss of ScGDA1 function results in a drastic reduction of GDP-mannose-dependent (as opposed to lipid-linked man- nose) mannosylation of proteins and lipids (10). The ynd1gda1 double mutant is synthetically lethal in S. pombe but not in S. cerevisiae, although the latter cells grow poorly and show severe cell-wall defects (11, 12).

Contrary to the GA, where numerous luminal nucleotide sugar-dependent glycosyltrasnsferases occur, the ER is the location of only two well described luminal nucleotide sugar-dependent glycosyltransferases: the glucuronosyltransferases that occur only in the liver of higher animals and the ubiquitous UDP-glucose:glycoprotein glucosyltransferase (GT) (14, 15) that, with the notable exception of S. cerevisiae, has been reported to occur in all cells studied so far. This enzyme plays a pivotal role in the quality control of glycoprotein folding, uses UDP-glucose as substrate, and is inhibited by the reaction product UDP. The fission yeast S. pombe has a well characterized glucosyltransferase that catalyzes folding of GT-derived vesicles containing UDP to the GA (11).

Several GA mutants in nucleotide sugar-transporter genes from mammals, yeast, Leishmania, Drosophila, and C. elegans have been identified and characterized. Most of these mutations are leaky, but all of them have serious physiological consequences (16).

C. elegans is an attractive model to study the relevance of intracellular ENTPDases in alleviating ER stress and regulating protein and lipid glycosylation. Its genome encodes at least three proteins with sequence similarity to the S. cerevisiae GA ENTPDase, Gda1p. A nucleotide diphosphatase functionally homologous to the yeast Ynd1p, encoded by the mig-23 gene, was recently described in C. elegans (17). Loss of mig-23 function results in altered gonad morphogenesis, demonstrating the importance of this enzyme (17). Here we report the presence of cation-dependent nucleotide di- and tri-phosphatase activities in membranes of C. elegans and the characterization of UDA-1 and NTP-1; the former is related, in substrate specificit,y to the yeast Gda1p, whereas the latter is an apyrase and is related to the yeast Ynd1p. Heterologous expression of the above two ENTPDases resulted in UDA-1 having its active site facing the lumen of vesicles of intermediate density between the ER and GA, whereas NTP-1 seems to colocalize with GA markers. Transcription of uda-1 but not ntp-1 and mig-23 was up-regulated by conditions causing ER stress and the accumulation of unfolded proteins such as tunicamycin, ethanol, and temperature. Up-regulation of uda-1 did not occur in ire-1 mutants, thus demonstrating the role of this pathway in this event. Our results show that metazoans, unlike yeast, can relieve ER stress not only by increasing the expression of GT as described previously, but also by simultaneously increasing transcription of a luminal UDPase that converts UDP to UMP. We hypothesize that increase of luminal UMP leads to the increase of UDP-glucose entry into the ER lumen under condi- tions of stress, thereby facilitating glycosylation and removal of improperly folded proteins in the ER lumen.

EXPERIMENTAL PROCEDURES

Strains, Cultures, and Reagents—C. elegans Bristol strain N2, used as the standard wild-type strain, and RE666 (ir-1) were cultured as described previously (18). The S. cerevisiae yeast strain used was G2–11 (MAT α, ura3-52, lys2–801 am, ade2–101 oc, trpl-1, his3–Δ200, leu2–1, gda1::LEU2) (19), described previously (10). Yeast cells were grown at 30 °C in yeast extract/petone/dextrose or S.D. medium supplemented with amino acids as needed. Transformations with plasmids were done by electroporation (19). Escherichia coli DH5α (Invitrogen) was grown in LB medium with 50 μg/ml ampicillin when needed. Reagents for yeast media were obtained from Difco Laboratories. Unless other- wise stated, all other reagents were from Sigma.

Characterization and Heterologous Expression of UDA-1 and NTP-1—One cDNA clone corresponding to the K08H10.4 transcript (yk400011) and another corresponding to the C33H5.14 open reading frame (ORF) (yk1565b1) were kindly provided by Dr. Kohara (National Institute of Genetics, Mishima, Japan). The former ORF was sequenced and subcloned into the plasmid p426 (20) to obtain p426-uda-1. This plasmid was then used for heterologous expression into yeast gda1 mutant cells. The cDNA sequence of yk400011 completely matched the predicted sequence of the ORP K08H10.4 which had been deposited in GenBank (21). The C33H5.14 ORF was sequenced and subcloned in the pCDNA3.1 (Invitrogen) to be transfected into COS-7 cells. The transfection was performed with LipofectAMINE (Invitrogen) according to the manufacturer’s instructions. The sequence obtained from the clone yk1565b1 completely matched the sequence found in the GenBank (22).

Preparation of Yeast Vesicle Fractions—Vesicle fractions were prepared from gda1-null mutant cells transformed with the plasmid p426-uda-1 or with the corresponding empty plasmid. Cells were grown and vesicles were obtained as described previously (22). Protein was deter- mined using the BCA method (Bio-Rad).

Preparation of Membrane Fractions from COS-7 Cells—C33H5.14 ORF was sequenced and subcloned in the pCDNA3.1 (Invitrogen) to be transfected into COS-7 cells. The transfection was performed with LipofectAMINE (Invitrogen) according to the manufacturer’s instructions. The sequence obtained from the clone yk1565b1 completely matched the sequence found in the GenBank (22).

Preparation of Membrane Fractions from C. elegans—Two ml of packed N2 mixed stage worms, grown in liquid culture, were suspended in 1 ml of PBS buffer supplemented with protease inhibitors mixture (Sigma) and disrupted four times (1 min each time) using a Mini- Beadbeater-8 cell disrupter (Biospec Products). One volume of this homogenate was diluted 20 times with PBS and centrifuged at 3000 rpm for 10 min. The resulting supernatant was centrifuged at 100,000 × g for 40 min, and the pellet was suspended in buffer containing 10 mm triethanolamine acetic acid, pH 7.2, 0.8 M sorbitol, 1 mm EDTA, 1 μg/ml leupeptin, 0.5 mm phenylmethylsulfonyl fluoride and stored in aliquots at −70 °C. For measurement of nucleotide phosphatase activity, hydrolisis of nucleotides was measured in membrane fractions as described previously (22).

Sucrose Gradient Fractionation—Yeast gda1 mutant cells, carrying the p426-uda-1 plasmid, were grown overnight until exponential phase. Subsequent workup and sucrose gradient fraction was done as de- scribed (10). n-1, 2-mannosyltransferase activity was used as the GA marker (24), whereas NADPH cytochrome c reductase activity was used as the ER marker (25).

Northern Analyses—For Northern blotting, total RNA from mixed- stage animals (21) was resolved on a 1% formaldehyde-containing gel, transferred onto a nylon membrane, and hybridized with 32P-labeled cDNA probes using a random priming kit (Roche Applied Science). Bands were then quantitated by densitometry. The cDNA probes were obtained by amplification of full-length ORFs utilizing the expressed sequence tag clones yk400011 (K08H10.4, uda-1), yk1565b1 (C33H5.14, ntp-1), and yk5761a2 (mig-23). The UDP-glucose:glycoprotein glu- cosyltransferase probe was obtained by amplification of 500 bp at the 3’ end of expressed sequence tag clone yk525b10.3. All clones were kindly provided by Dr. Kohara and converted to plasmids with a Rapid Excisi- sion kit (Stratagen).

Preparation of Yeast Vesicle Fractions—Vesicle fractions were prepared from gda1-null mutant cells transformed with the plasmid p426-uda-1 or with the corresponding empty plasmid. Cells were grown and vesicles were obtained as described previously (22). Protein was deter- mined using the BCA method (Bio-Rad).

Preparation of Membrane Fractions from COS-7 Cells—Crude mem- branes of transfected COS-7 cells were prepared by the method of Coppi and Guidotti (23). Protein concentration was determined using the BCA method (Bio-Rad).

Preparation of Membrane Fractions from C. elegans—Two ml of packed N2 mixed stage worms, grown in liquid culture, were suspended in 1 ml of PBS buffer supplemented with protease inhibitors mixture (Sigma) and disrupted four times (1 min each time) using a Mini- Beadbeater-8 cell disrupter (Biospec Products). One volume of this homogenate was diluted 20 times with PBS and centrifuged at 3000 rpm for 10 min. The resulting supernatant was centrifuged at 100,000 × g for 40 min, and the pellet was suspended in buffer containing 10 mm triethanolamine acetic acid, pH 7.2, 0.8 M sorbitol, 1 mm EDTA, 1 μg/ml leupeptin, 0.5 mm phenylmethylsulfonyl fluoride and stored in aliquots at −70 °C. For measurement of nucleotide phosphatase activity, hydrolisis of nucleotides was measured in membrane fractions as described previously (22).

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Northern Analyses—Sucrose gradient fractions were loaded on SDS- PAGE and electoblotted onto a polyvinylidene difluoride membrane (Bio-Rad) in Towbin buffer at 100 V for 1 h. Antibody against Chs3p (kindly provided by Dr. H. Lucero, Boston University) was used at a 1:3000 dilution. Anti-Pep12 antibody (Molecular Probes) was used at a
Nucleoside diphosphates (2 mM) were incubated with crude membranes from *C. elegans* wild-type strain in the presence of 2 mM CaCl$_2$. A pool of nucleoside diphosphates was assayed in the presence of CaCl$_2$ or 0.2 mM EDTA. Values are the mean ± S.D. of three independent experiments.

1:1000 dilution. The chitinase detection was performed as described in Lopez-Avalos et al. (26); the chitinase antibody (kindly provided by Dr. C. Specht, Boston University) was used at a 1:3000 dilution. The FLAG antibody (Sigma), used to detect the NTP-1 in COS-7 cells, was diluted 1:1000. The secondary antibodies were anti-rabbit or anti-mouse IgG conjugated with peroxidase (Bio-Rad). The detection procedure was done with an ECL blot detection kit (Amersham Biosciences) according to the manufacturer’s instructions.

RESULTS

**Nucleotide Hydrolyzing Activities of *C. elegans* Membranes**—We were interested in determining the occurrence of ENTPDases and their activities in *C. elegans* as an initial step toward the elucidation of their role in luminal ER and/or GA posttranslational modifications of proteins. Crude membrane preparations of mixed stage, wild-type nematodes were assayed for nucleotide mono-, di-, and tri-phosphatase activities, substrate specificities, and ionic requirements. In the presence of Ca$^{2+}$, *C. elegans* membranes showed high UDPase and GD$$\text{P}$$ase activities, while CDP and ADP were only marginally hydrolyzed (Fig. 1). When a mixture of nucleoside diphosphates was assayed as substrates, GDPase and UDPase activities were not additive (Fig. 1), suggesting that both activities may be catalyzed by a single polypeptide, as reported for yeast and mammalian GDP/UDP$$\text{P}$$ases (8, 9, 10, 26, 27). The nucleotide diphosphatase activity was strictly cation-dependent, as shown by the negligible hydrolysis in the presence of EDTA (Fig. 1). In contrast, a significant portion of the nucleoside triphosphate hydrolysis was cation-independent (Fig. 1).

Members of the nucleotide phosphatase family share a high degree of amino acid sequence similarity, particularly at their ACRs. To identify nematode proteins responsible for the GDP/UDP$$\text{P}$$ase activities, the *C. elegans* genome database was searched for homologs of *S. cerevisiae* Gda1p, the principal yeast GDP/UDP$$\text{P}$$ase. This search resulted in the identification of two *C. elegans* ORFs, C33H5.14 and K08H10.4, in addition to the recently reported mig-23 (17). The K08H10.4 ORF has a high similarity to *S. cerevisiae* and *S. pombe* GTA1 and to a gene product encoding a rat ER UDP$$\text{P}$$ase (14), ENTPD5 (Fig. 2). This *C. elegans* ORF will be subsequently referred to as *uda-1* and its product, which contains the 5 ACRs that are the hallmark of the ENTPD family, as UDA-1 (Fig. 2). The Kyte-Doolittle hydrophobicity plot of this other *C. elegans* ORF (Fig. 3B) suggests the presence of one C-terminal transmembrane domain between residues 442 and 459.

**Substrate Specificity of UDA-1**—The substrate specificity and other biochemical properties of UDA-1 were studied after expression in *S. cerevisiae* gda1-null mutants because of their low background in nucleotide phosphatase activities. Nucleotide diphosphatase activities were significantly increased in membranes from *gda1* mutant cells transformed with the vector containing *uda-1* cDNA versus those transformed with the empty vector. Expression of *uda-1* resulted in a 6-fold increase in UDP$$\text{P}$$ase activity and a 3-fold increase in GDP$$\text{P}$$ase activity (Fig. 4). A small increase in CDP hydrolysis was also observed, whereas no significant changes were detected when either nucleoside mono- or tri-phosphates were used as substrates (Fig. 4). The catalytic activity of UDA-1 toward UDP was highest when the pH of the reaction was 8.0 (data not shown), whereas for GDP the maximum was at pH 7.5 (data not shown). The $K_m$ for UDP at pH 8 in the presence of 10 mM Ca$^{2+}$ was 180 ± 7 μM (mean ± S.E., n = 3). UDP$$\text{P}$$ase and GDP$$\text{P}$$ase activities were optimally and similarly stimulated at 10 mM Ca$^{2+}$, Mg$^{2+}$, or Mn$^{2+}$; however, some cation selectivity was detected at 2 mM, where Mg$^{2+}$ ≥ Mn$^{2+}$ = Ca$^{2+}$. No enzymatic activity could be measured in the presence of EDTA (data not shown). The substrate specificity profile determined for the recombinant UDA-1 (Fig. 4) is almost identical to the pattern of nucleotide diphosphate hydrolysis produced by a total membrane fraction obtained from mixed-stage nematodes (Fig. 1), suggesting that UDA-1 may be the major membrane-bound enzyme responsible for hydrolyzing UDP and GDP in *C. elegans* as Gda1p is in *S. cerevisiae* (10).

To determine whether the catalytic domain of UDA-1 was oriented toward the lumen of an organelle, we measured the UDP$$\text{P}$$ase activity in intact and permeabilized vesicles from *gda1* yeast cells expressing the enzyme. Only vesicles in which membrane permeability had been disrupted with either detergents or the ionophore alamethicin showed 2- to 6-fold higher activity compared with intact ones (Fig. 5). This result suggests that the active site of UDA-1 is luminal. Vesicles prepared from *gda1* yeast cells, carrying an empty vector, showed significantly lower activity when permeabilized with TX-100 than with alamethicin or digitonin, which is consistent with the previous observation that Ynd1p, responsible for the background activity in *gda1* mutant cells, is inhibited by TX-100 (13).

We then studied, by sucrose gradient fractionation, the subcellular localization of UDA-1 expressed in *S. cerevisiae* gda1 mutants. Migration of UDA-1 was detected by its UDP$$\text{P}$$ase activity and localized to a compartment having a density in between that of the yeast ER and GA enzymatic markers, Pep12p, which is implicated in vesicular protein transport, or digitonin, which coincides with markers of the endosomal compartment such as Pep12p, which is implicated in vesicular protein transport, or the catalytic subunit of chitin synthase complex III, Chs3p (data not shown).

The glycosylation defects present in *gda1* yeast mutants are the result of a severe reduction in GDP hydrolysis in the Golgi apparatus. Any phenotypic correction detected upon expression of UDA-1 in this yeast strain would indicate that at least some of the *C. elegans* protein reached the GA. We analyzed the O-glycosylation profile of chitinase secreted by *gda1* cells because its under-glycosylation results in a faster migration on SDS-PAGE than chitinase secreted by wild-type yeast. No difference in the migration of chitinase secreted by *gda1* cells transformed with the plasmid-containing *uda-1* or the vector alone were seen,

![Graph](image-url)
indicating no correction of the O-glycosylation defects and presumably no GA localization (Fig. 6). The global cell wall alteration present in gda1 mutants (as revealed by sensitivity to /H92521,3-glucanase-induced lysis; Ref. 26) also could not be suppressed by the expression of uda-1 (data not shown). Presumably, the nematode enzyme did not reach the GA of yeast.

Because a heterologous expression system was used, the specific organelle where the UDPase resides in C. elegans can only be inferred. Nevertheless, two additional lines of evidence suggest an intracellular location for UDA-1: (i) the enzymatic activity is latent as shown above (Fig. 5), and (ii) the substrate specificity is very similar to that of intracellular ENTPDases, most of which are devoid of ATPase activity. Representative examples of the latter group are mammalian ER-UDPase (ENTPD6, Ref. 8), mammalian GA-UDPase (ENTPD5, Ref. 9), and yeast Golgi GDP/UDPases (S. cerevisiae Gda1p, Ref. 10).

Substrate Specificity of NTP-1—To determine enzymatic activity of NTP-1, we first attempted heterologous expression in yeast; this resulted in no significant increase in enzymatic activity of the transformants. Transient transfection into COS-7 cells was then performed. Western blots against the epitope tag localized at the C terminus of the ORF showed expression of a protein of 110 kDa (data not shown). The substrate specificity of NTP-1 was then measured in crude lysates of the transfected COS-7 cells.

Fig. 2. Sequence comparison of C. elegans K08H10.14 (UDA-1) and C33H5.14 (NTP-1) with related UDP/GDPases. Sequence alignment was performed using the BOXSHADE program. Underlined residues represent the five ACR (apyrase conserved region) domains. The GenBank accession numbers of the sequences are given in parentheses: Gda1p, Saccharomyces cerevisiae (U18799); MIG-23, Caenorhabditis elegans (U39652); ENTPDase6, Rattus norvegicus (AJ238636); K08H10.4, Caenorhabditis elegans (Z83113); ENTPDase5, Homo sapiens (AF016032); C33H5.14, Caenorhabditis elegans (U41007).
membranes of COS-7 cells transfected transiently with the heterologous cDNA and compared with the activity of cells transfected with the empty vector. Expression of ntp-1 resulted in a significant increase in calcium-dependent hydrolysis of nucleoside triphosphates and a marginal increase in the hydrolysis of nucleoside diphosphates (Fig. 7). Immunostaining of the expressed protein showed a perinuclear distribution with partial overlap, with fluorescent staining with an anti-GOS28, a Golgi apparatus protein, suggesting perhaps a GA localization for NTP-1 (data not shown).

**uda-1 Is Transcriptionally Up-regulated in an ire-1-Dependent Manner**—Accumulation of unfolded/misfolded proteins within the ER lumen is deleterious for cell survival. A signaling pathway between the ER and the nucleus, the unfolded protein response (UPR), has evolved in all eukaryotes to mitigate such stress. A mechanism has also been described for retaining not yet properly folded N-glycan-bearing proteins and for increasing their folding efficiency in the ER (14). Monoglucosylated oligosaccharides, which originated as trimming intermediates, mediate the interactions of folding glycoproteins with the ER resident lectin/chaperones, calnexin and calreticulin. Deglucosylation by glucosidase II releases glycoproteins from their chaperone anchors. GT, which uses UDP-glucose as a substrate, is an ER sensor, together with lectin chaperones, of glycoprotein conformations, giving rise to monoglucosylated N-glycans only if the oligosaccharides are linked to completely folded glycopeptides. Conditions that promote accumulation of unfolded proteins in the ER have been reported to significantly induce synthesis of GT mRNAs in S. pombe and mammals (14).

It has been hypothesized that a rat Ca\(^{2+}\)/H\(_2\)UDPase, ENTDP6, which remains soluble in the lumen of the ER and is devoid of transmembrane domains and KDEL-related ER localization sequences (8), promotes reglucosylation reactions involved in glycoprotein folding and quality control by hydrolyzing UDP, which is an inhibitory product of the UDP-glucose:glycoprotein glucosyltransferase (8, 29). This ER UDPase may also facilitate reglucosylation by generating the antiporter, UMP, which is required for entry into the ER lumen of the substrate, UDP-glucose. In mammals, this nucleotide sugar enters the lumen of the ER by means of a specific transporter in a coupled, equimolar exchange with UMP (30). No in vivo supporting evidence for this hypothesis has yet been provided.

The amino acid sequence and predicted topology for UDA-1 is very similar to that experimentally demonstrated for the above Ca\(^{2+}\) UDPase. If indeed UDA-1 participates in glycoprotein reglucosylation reactions, then stress conditions that may promote the accumulation of misfolded proteins, such as high temperature, addition of tunicamycin or ethanol, should result in an increased mRNA encoding for this enzyme. To investigate this point, RNA from mixed stage, wild-type nematodes, before and after treatment of the animals with the above effectors,
was extracted and analyzed by Northern blotting. A 6-h shift from 16 to 25 °C resulted in a 3- to 4-fold induction of the expression of *uda-1* mRNA (Fig. 8). A similar effect was observed upon the addition of either tunicamycin or ethanol (Fig. 8). No modification in the expression of an unrelated mRNA, actin, was observed (Fig. 8).

The *C. elegans* genome predicts two proteins that are homologous to all canonical GTs, both in term of size and amino acid sequence conservation. The probe utilized here was derived from the 3′ region, which contained the terminal 300 amino acids where the identity is highest and would, therefore, recognize both GT homologs, which differ by 300 bases in length. Similar, up-regulation of the mRNAs of *uda-1* and GT was observed upon exposure of nematodes to tunicamycin or ethanol (Fig. 8); the latter treatment, as well as high temperature, served upon the addition of either tunicamycin or ethanol (Fig. 8). No modification in the expression of an unrelated mRNA, actin, was observed (Fig. 8).

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**DISCUSSION**

In this study, we have shown the occurrence of cation-dependent nucleotide di- and tri-phosphatase activities in membranes of *C. elegans*. We cloned and expressed the genes for two novel ENTPases, *uda-1* and *ntp-1*. Although UDA-1 causes hydrolysis of UDP and GDP and, to a much lesser extent, CDP, it does not hydrolyze other nucleoside diphosphates, triphosphates, or monophosphates. This finding is in contrast to the behavior of NTP-1, which hydrolyzes both nucleoside di- and tri-phosphate.
tri-phosphates. The substrate specificity of UDA-1 is very similar to the first purified and cloned ecto-nucleoside diphosphatase, Gda1p of *S. cerevisiae*, which, we have shown previously in *in vivo* and *in vitro* experiments (6), plays a pivotal role in the nucleotide sugar transport/antiport cycle. Recently a different ENTPDase of *C. elegans*, MIG-23, was described (17). Although this protein has activity toward UDP and GDP, it also hydrolyzes ADP, which UDA-1 does not; whether or not MIG-23 hydrolyzes nucleoside tri- or mono-phosphates is not known. Gene expression data\(^2\) indicate that the transcript for *uda-1* (K08H10.4), being high in the embryos, can be detected in all developmental stages of the nematode; it decreases during the first two larval stages and then rises to maximum in larva 4 stage and adult hermaphrodites.

We and others have previously demonstrated (6, 12, 13) that UDP/GDPases play crucial roles in protein and lipid glycosylation reactions that occur in the lumen of the GA. The sugar donors for these reactions, nucleotide sugars, must first be transported by a specific transporter from the cytosol, their site of synthesis, into the lumen of the GA, where sugars are transferred by specific transferases to proteins and lipids (6, 33, 37). These transporters have recently been described in *C. elegans*, where SQV-7 is involved in vulva invagination and early embryonic development (38, 39). Several GA lumenal glycosyltransferases, all of which yield nucleoside diphosphates as products (40–45), have also been identified in *C. elegans*. These can only exit the GA lumen after hydrolysis to nucleoside monophosphates. MIG-23 is such a lumenal nucleotide diphosphatase; it affects gonad morphogenesis through glycosylation of the MIG-17 ADAM protease (17). It is also likely that other ENTPDases from *C. elegans* will have similar functional roles.

\(^2\) Available on the World Wide Web at www.wormbase.org.
in generating nucleoside monophosphates necessary for the nucleotide sugar transport/antiporter cycle and glycosylation of proteins and lipids.

When C. elegans was exposed to elevated temperature, tunicamycin, or ethanol, conditions known to cause stress, transcriptional up-regulation of uda-1 but not of ntp-1 and mig-23 occurred. This specific transcriptional up-regulation is mediated by the ire-1 pathway, as mutants unable to signal by the ER stress sensor Ire-1 failed to elicit such up-regulation. We postulate that the principal role of transcriptional up-regulation of uda-1 is to increase the availability of UDP-glucose in the lumen of the ER or pre-GA (see below) under conditions of ER stress, thereby increasing the glycosylation of improperly folded proteins. Up-regulation of GT transcription under ER stress has been reported previously in S. pombe (46) and human cells (47) and seems to occur together with that of UDA-1 (Fig. 8). Synergy between GT and UDPase activities is postulated to bring fast relief of stress to the ER lumen: the increased rate of glycosylation of misfolded glycoproteins, as more GT becomes available, would then promote their recognition together with lectin chaperones which, in turn, provide them with additional opportunities to acquire a mature conformation and exit the ER. A direct consequence of increased GT activity could be the accumulation of its inhibitory product, UDP, in the lumen of the ER. Increase in UDA-1 would generate UMP, which can exit the ER or pre-GA as antiporter, thus promoting entry of additional UDP-glucose, the substrate of GT, from the cytosol.

Several lines of evidence support our hypothesis, although important questions discussed further below remain to be answered. UDA-1 is a nucleotide diphosphatase with its active site facing the lumen of an organelle having a density in between that of the classical ER and GA markers when expressed per se. UDA-1 is a nucleotide diphosphatase with its active site facing the lumen of an organelle having a density in between that of the classical ER and GA markers when expressed per se. UDA-1 is a nucleotide diphosphatase with its active site facing the lumen of an organelle having a density in between that of the classical ER and GA markers when expressed per se. UDA-1 is a nucleotide diphosphatase with its active site facing the lumen of an organelle having a density in between that of the classical ER and GA markers when expressed per se.

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