Different Glycosylation Requirements for the Synthesis of Enzymatically Active Angiotensin-converting Enzyme in Mammalian Cells and Yeast*

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For facilitating crystallization and structural studies of the testicular isozyme of angiotensin-converting enzyme (ACE$_T$), we attempted the production of enzymatically active ACE$_T$ proteins which are unglycosylated or underglycosylated. Expression in Escherichia coli of the rabbit ACE$_T$ cDNA resulted in the synthesis of an unglycosylated but inactive protein. Similarly, unglycosylated ACE$_T$ synthesized in HeLa cells, by using a cDNA in which all five potential N-glycosylation sites had been mutated, was inactive and rapidly degraded. Several ACE$_T$ variants carrying mutations in one or more of the potential N-glycosylation sites were used to examine the role of glycosylation at specific sites on ACE$_T$ synthesis, transport to the cell surface, cleavage processing, and enzyme activity. These experiments demonstrated that allowing glycosylation only at the first or the second site, as counted from the NH$_2$ terminus, was sufficient for normal synthesis and processing of active ACE$_T$. In contrast, ACE$_T$ g3, which had only the third glycosylation site available, was unglycosylated, enzymatically inactive and rapidly degraded. N-Glycosylated ACE$_T$ could also be produced in yeast. Surprisingly, the mutant ACE$_T$ g3 was synthesized, N-glycosylated, and properly transported in yeast. Wild type and mutant ACE proteins were cleavage-secreted from yeast and enzymatically active.

Angiotensin-converting enzyme (ACE) (EC 3.4.15.1, dipeptidyl carboxypeptidase) plays a major role in blood pressure regulation and fluid and electrolyte homeostasis by acting on two major vasoactive peptides. It converts the inactive precursor angiotensin I to an active vasopressor peptide angiotensin II and inactivates the vasodepressor peptide bradykinin (1-3). ACE has two structurally related isozymic forms (4-6) encoded by two different mRNAs, which arise from the same gene by tissue-specific choice of the alternative transcription initiation sites (7-13). In rabbit, the smaller isozyme, testicular (T) ACE has 737 amino acid residues and the larger pulmonary (P) isozyme has 1309 residues (7, 9). The COOH-terminal 665 residues of the isozymes are identical, whereas their NH$_2$ termini are unique. They carry signal sequences at their NH$_2$ termini which are removed during their biosynthesis. Both isozymes are extensively glycosylated and expressed as a cell surface Type 1 ectoprotein anchored in the plasma membrane by a 17-residue-long hydrophobic transmembrane domain near the COOH terminus. There is ample evidence to suggest that the COOH-terminal 30 residues constitutes the cytoplasmic tail and the rest of the protein is extracellular. In tissue culture (14-18), as well as in vivo, the extracellular domain of ACE is released into the culture media or in body fluids, by a regulated proteolytic cleavage of the membrane anchoring domain. Indeed, a COOH-terminally truncated, soluble form of enzymatically active ACE is found in many body fluids, including serum (16, 18, 19). Thus, several important post-translational modifications occur during biosynthesis of ACE.

Rabbit ACE$_T$ has five potential N-glycosylation sites as indicated by the presence of the Asn-X-Ser/Thr motif in its primary structure. In addition, it has a cluster of threonine residues near the amino terminus that can potentially be O-glycosylated (7). ACE$_T$ isolated from tissues and transfected cell lines is heavily glycosylated carrying both N- and O-linked sugars. Using inhibitors of glycosylation and a mutant cell line defective in protein glycosylation, we have shown that complete blockage of glycosylation causes rapid intracellular degradation of ACE$_T$. However, ACE$_T$ synthesized without N-linked complex sugars and O-linked sugars is transported to the cell surface, cleavage-secreted, and enzymatically active (20). Similarly, Ehlers et al. (21) have shown that a mutant ACE$_T$ devoid of most of its O-linked sugars has enzymatic activity.

In our current study, we evaluated the contributions of each of the five potential N-glycosylation sites of ACE$_T$, toward its synthesis, glycosylation, intracellular transport, cleavage secretion, and enzymatic activity. This was accomplished by site-directed mutagenesis of these sites individually or in combinations. Our studies demonstrated interesting differences among the contributions made by the different sites. Moreover, we attempted the production of wild type and mutant ACE$_T$ proteins in bacteria and yeast so that large quantities of these proteins can be easily produced for structural studies. In the process, we revealed an unexpected difference between yeasts and mammalian cells in the utilization of a specific glycosylation site in ACE$_T$.

EXPERIMENTAL PROCEDURES

Materials

Pichia pastoris GS115(His-4) was used for expression of ACE$_T$ and its mutants (the Pichia expression kit, Invitrogen Corp., San Diego, CA). Escherichia coli TOP10F' was used for all plasmid construction and propagation. The Muta-Gene phagemid in vitro mutagenesis kit and fluorescein-labeled anti-goat IgG were obtained from Bio-Rad and Vector Laboratories, respectively.
Expression of ACE$_T$ and Its Mutants in Yeast

Vector Construction and Transformation—P. pastoris, a methylotrophic yeast host, was chosen for this study, since its glycosylation pattern resembles more closely that of mammalian cells (24). Expression vector pHIL-S1 (Invitrogen Corp.) containing methanol-inducible alcohol dehydrogenase (AOX1) promoter sequence was digested to express ACE$_T$ and its various mutants. Briefly, a 2.4-kilobase EcoRI fragment containing the full coding sequence of ACE$_T$ was excised from the parent plasmid (pZIBU-ACE$_T$) and ligated to a EcoRI cleaved pHIL-S1 vector plasmid. Proper orientation of the insert was analyzed by restriction analysis using SfiI and XhoI. The resulting recombinant plasmid pHIL-S1-ACE$_T$-WT codes for a fusion protein containing signal sequences from both PHO1 gene and ACE$_T$. The recombinant plasmid pHIL-S1-ACE$_T$, WT was linearized with BglII and later transferred into Pichia yeast host through lithium acetate transformation method (25). Several transformants were cultured and screened for ACE expression. The purified recombinant deglycosylated ACE$_T$, from P. pastoris, migrated on SDS-PAGE at 70 kDa (Fig. 8). Hence, it is expected that both the signal sequences (PHO1 and ACE$_T$) were cleaved, and the resulting mature ACE$_T$ is a nonfusion protein and resembles the native enzyme from mammalian cells. Amino-terminal sequencing is in progress to further confirm the cleavage of both the signal sequences. Similarly, three other constructs, pHIL-S1-ACE$_g$2, pHIL-S1-ACE$_g$3, and pHIL-S1-ACE$_{g12345}$, with the coding sequences of the respective glycosylation mutants doned in the vector were also generated, introduced in the genome of P. pastoris, and expressed.

Yeast Media, Growth, and Expression of ACE$_T$ Protein—Cells were grown in 100 ml of BMGY medium (yeast extract, 10 g; peptone, 20 g; glycerol, 10 ml; biotin, 400 µg; yeast nitrogen base with ammonium sulfate, 13.4 g; and 100 ml of 1 M potassium phosphate buffer, pH 5.0, per liter) at 30°C. After 2 days, cells were harvested, resuspended in BMMY medium (same as BMGY media with the exception that 5 ml of glycerol/liter was added in place of glycerol) and incubated at 30°C for another 2 days to induce expression. Finally, the cultures were centrifuged, and the supernatant containing the secreted ACE$_T$ protein was concentrated, extensively dialyzed against 20 mM Hepes buffer, pH 7.0, containing 300 mM NaCl and 2 M CaCl$_2$, and analyzed by Western blot analysis using anti-ACE antibody to detect the presence of ACE$_T$ proteins as well as assayed for enzymatic activity.

Transient Expression of ACE$_T$ Proteins, Pulse-Chase Analysis, Immunoprecipitation, and Deglycosylation

ACE$_T$ proteins were expressed in HeLa cells using the vaccinia virus-T7 RNA polymerase expression system as described earlier (20). The transfected cells were pulse-labeled with $^{[35]S}$methionine for 30 min, and the label was chased for the indicated time. ACE-related proteins were immunoprecipitated from the cell extracts and media and analyzed by SDS-PAGE. For deglycosylation, immunoprecipitates were incubated with PNGase-F, 10 milliunits of neuraminidase, and 1 milliunit of O-glycosidase for total deglycosylation or with one or a combination of the enzymes as indicated in the figures.

Western Analysis and Enzyme Activity Measurements

Western analysis was carried out using either anti-rabbit lung ACE (anti-ACE) or anti-COOH-terminal peptide (antipeptide) antibody (23). ACE enzyme activity was measured (23) using hippuryl-L-histidyl-L-leucine (Hip-His-Leu) as substrate.

Immunodetection of ACE$_T$ in Transfected HeLa Cells by Indirect Immunofluorescence

HeLa cells were grown on glass coverslips and transfected with ACE$_T$-WT or ACE$_T$-g3 cDNA as described. For labeling cell surface ACE$_T$, the transfected cells were fixed with 3.7% paraformaldehyde for 10 min at room temperature. To localize ACE$_T$ proteins in the intracellular compartment, the paraformaldehyde-fixed cells were permeabilized by treatment in 0.25% Triton X-100 for 5 min. After washing with 500 mM NaCl, 20 mM Tris-HCl, pH 7.5 (Tris-buffered saline), containing 2% glycerol, 10 ml; biotin, 400 µg; yeast nitrogen base with ammonium sulfate, 13.4 g; and 100 ml of 1 M potassium phosphate buffer, pH 5.0, per liter) at 30°C. After 2 days, cells were harvested, resuspended in BMMY medium (same as BMGY media with the exception that 5 ml of glycerol/liter was added in place of glycerol) and incubated at 30°C for another 2 days to induce expression. Finally, the cultures were centrifuged, and the supernatant containing the secreted ACE$_T$ protein was concentrated, extensively dialyzed against 20 mM Hepes buffer, pH 7.0, containing 300 mM NaCl and 2 M CaCl$_2$, and analyzed by Western blot analysis using anti-ACE antibody to detect the presence of ACE$_T$ proteins as well as assayed for enzymatic activity.

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fetal bovine serum, cells were incubated sequentially with anti-ACE antibody (1:3000) and fluorescein conjugated anti-goat IgG (1:100) for 30 min each, mounted, and photographed (Nikon Optiphot-2). Tris-buffered saline was used to wash cells in between each step.

RESULTS

Expression of ACE<sub>T</sub> in E. coli—We have observed previously that unglycosylated ACE<sub>T</sub>, synthesized in HeLa cells in the presence of tunicamycin, is rapidly degraded intracellularly (20). Since protein glycosylation does not occur in E. coli, we attempted the production of ACE<sub>T</sub> in bacteria as an alternative source of unglycosylated ACE. E. coli transformed with pET3a-ACE<sub>T</sub>, but not with pET3a alone, produced a 70-kDa protein upon induction with isopropylthiogalactopyranoside. This protein had a molecular weight similar to that predicted for unglycosylated truncated ACE<sub>T</sub>, and it reacted specifically with ACE<sub>T</sub> antibody (Fig. 1). Thus, unglycosylated ACE<sub>T</sub> could be produced in E. coli in ample quantities. This protein, however, was enzymatically inactive (data not shown). Attempts to de-nature and renature this presumably misfolded protein failed to produce an active enzyme. Thus, like many other biologically active mammalian glycoproteins, bacterially expressed ACE<sub>T</sub> is inactive.

Expression of Underglycosylated ACE<sub>T</sub> in HeLa Cells—Since we failed to produce enzymatically active unglycosylated ACE<sub>T</sub> in either HeLa cells or E. coli, we turned our attention to producing underglycosylated ACE<sub>T</sub> in mammalian cells. For this purpose, glycosylation at one or more of the five potential N-glycosylation sites, present in ACE<sub>T</sub> at residues 108, 126, 145, 373, and 622, was prevented. This was achieved by mutating the Asn residues, present at these sites, to Gln. Such a mutation does not alter the local net charge or hydrophobicity but prevents glycosylation. A panel of 19 ACE<sub>T</sub> mutants carrying various combinations of the five glycosylation site mutations was generated (Table I). These mutants were named by indicating the sites, as counted from the amino terminus, still available for N-glycosylation. Thus, the mutant ACE<sub>T</sub>g2345 had only the first site mutated. The mutant proteins were expressed in HeLa cells and their synthesis, modification, processing, and cleavage secretion to the culture medium were monitored using published procedures (17, 20, 23). As expected, ACE<sub>T</sub>g0, which had all five potential sites mutated, produced a 76-kDa protein similar to the one produced by ACE<sub>T</sub> WT in the presence of tunicamycin (Fig. 2). This protein was rapidly degraded intracellularly, and no ACE<sub>T</sub>g0 was secreted to the medium. The 76-kDa ACE<sub>T</sub>g0 protein was unglycosylated, as judged by endo-β-N-acetylglucosaminidase H digestion and enzymatically inactive (data not shown). In the next series of experiments, synthesis and processing of ACE<sub>T</sub> mutants with four, three, or two available N-glycosylation sites were analyzed (Fig. 3, A–C). All of these mutants were synthesized, glycosylated, and secreted to the medium. Among the mutants carrying single mutations, only ACE<sub>T</sub>g1234 had a molecular weight similar to that of ACE<sub>T</sub> WT; others had lower molecular weights (Fig. 3A). This result suggests that ACE<sub>T</sub> WT is probably minimally glycosylated at site 5, but all other sites are used. For all mutants, like the wild type protein, the secreted form was slightly smaller than the cell-bound forms. Among the double mutants (Fig. 3B), there were noticeable differences with regard to the molecular weight, the rate of synthesis, and the rate of secretion. For example, ACE<sub>T</sub>g234 (lane 4, Fig. 3B) was synthesized much less than ACE<sub>T</sub>g124 (lane 2, Fig. 3B). ACE<sub>T</sub>g123 (lane 6) had the lowest molecular weight, and the cleavage secretion was much more efficient for ACE<sub>T</sub>g134 (lane 5) than for ACE<sub>T</sub>g135 (lane 3). Three triple mutants were tested (Fig. 3C). All of them were synthesized and cleavage-secreted normally. Taken together, these results suggest that the specific carbohydrate structures at different glycosylation sites in ACE<sub>T</sub> may be different, thus the different molecular weights of different species. They also suggest that glycosylation at different sites differentially affect the rate of synthesis and the rate of cleavage secretion of the corresponding protein.

Expression of ACE<sub>T</sub> Mutants with Single Available N-glycosylation Sites—Since ACE<sub>T</sub>g12, ACE<sub>T</sub>g13, and ACE<sub>T</sub>g23 proteins were normally synthesized and secreted (Fig. 3C), we decided to examine the corresponding quadruple mutants from this set. ACE<sub>T</sub>g1, ACE<sub>T</sub>g2, and ACE<sub>T</sub>g3 had, respectively, only site 1, 2, or 3 available for glycosylation. ACE<sub>T</sub>g1 gave rise to a mature protein of 98 kDa (Fig. 4A, lane 4 and Table II), which was cleavage-secreted normally (Fig. 4A, lane 8). ACE<sub>T</sub>g2 was slightly bigger and processed normally (Fig. 4A, lanes 2 and 6; Table II). In contrast, we failed to detect any cell-bound or secreted ACE<sub>T</sub>g3 in this experiment (Fig. 4A, lanes 3 and 7). To explore this further, a detailed kinetic analysis of the synthesis and processing of these proteins was carried out in the experiment shown in Fig. 4B. ACE<sub>T</sub> WT, ACE<sub>T</sub>g2, and ACE<sub>T</sub>g1 had identical kinetics of synthesis and maturation. The conversion of the partially glycosylated form to the fully mature form was virtually completed within 4 h for all three proteins. ACE<sub>T</sub>g3 was synthesized in lower quantities. The protein had a molecular mass of 76 kDa (Table II), and it was never converted to...
the high molecular weight form. Moreover, like ACE<sub>1</sub>g0 (Fig. 2), ACE<sub>1</sub>g3 was rapidly degraded intracellularly.

The glycosylation status of these mutant proteins was directly tested by measuring their sensitivity to glycosidases (Fig. 5). As expected, ACE<sub>1</sub>WT was both N- and O-glycosylated. The same was true for both ACE<sub>1</sub>g2 and ACE<sub>1</sub>g1, although the extent of their N-glycosylation was less than that of ACE<sub>1</sub>WT. The 76-kDa ACE<sub>1</sub>g3 protein, on the other hand, was neither O-glycosylated nor N-glycosylated. It should be noted that for this analysis of ACE<sub>1</sub>g3, we used a relatively larger quantity of an extract of cells that had been pulse-labeled but not chased. Enough of the 76-kDa protein was available for analysis only under these conditions. The lack of glycosylation of ACE<sub>1</sub>g3 was further confirmed by the failure to react with an anti-sugar antibody (data not shown). Thus, ACE<sub>1</sub>g3, although synthesized, remained unglycosylated in the HeLa cells. As a consequence, it was rapidly degraded and not transported to the cell surface. The above conclusions drawn from the metabolic labeling experiments (Figs. 4 and 5) were confirmed by immunofluorescence studies (Fig. 6). ACE<sub>1</sub>WT was present both on the surface and inside of the transformed cells. In contrast, a small amount of ACE<sub>1</sub>g3 was present inside the cells, but none was displayed on the cell surface. The pattern of intracellular distribution of ACE<sub>1</sub>g3 suggests that the protein is arrested in the endoplasmic reticulum. Taken together, these experiments demonstrate that all glycosylation sites in ACE<sub>1</sub> are not equivalent. Site 3, by itself, is not sufficient for glycosylation of the protein, but sites 1 and 2 are.

Expression of ACE<sub>1</sub> in Yeast—In the next series of experiments, we attempted the expression of ACE<sub>1</sub> in yeast. The methylotropic strain P. pastoris was chosen because of the ease of induced production of a larger quantity of a foreign protein. ACE<sub>1</sub>WT, ACE<sub>1</sub>g13, ACE<sub>1</sub>g2, and ACE<sub>1</sub>g3 were cloned in the appropriate yeast expression vector. After transformation and growth, the introduced genes were induced, and the expression of ACE<sub>1</sub> was monitored. For these experiments, instead of detection by metabolic labeling and immunoprecipitation, ACE<sub>1</sub> proteins secreted in the culture medium were detected by Western blotting. As shown in Fig. 7, ACE<sub>1</sub>WT was efficiently synthesized and secreted by P. pastoris. The secreted protein had a molecular mass of 90 kDa and was enzymatically active (Table II). Thus, unlike E. coli, P. pastoris was able to synthesize an active form of ACE<sub>1</sub>. ACE<sub>1</sub>g13 and ACE<sub>1</sub>g2 were similarly synthesized in yeast (Fig. 7). As in HeLa cells, ACE<sub>1</sub>g2 was slightly bigger than ACE<sub>1</sub>g13. Surprisingly, ACE<sub>1</sub>g3 was also synthesized and secreted by yeast. This protein had the lowest molecular mass (70 kDa) and was enzymatically active (Table II). Thus, unlike E. coli, P. pastoris was able to synthesize an active form of ACE<sub>1</sub>. ACE<sub>1</sub>g13 and ACE<sub>1</sub>g2 were similarly synthesized in yeast (Fig. 7). As in HeLa cells, these proteins were N-glycosylated, although to different extents, but insensitive to O-glycosidase treatment. It appears that the third glycosylation site, present at residue 145

![Figure 3](https://example.com/fig3.png)

**Expression of underglycosylated ACE<sub>1</sub> mutants.** HeLa cells were transfected with wild type or mutant ACE<sub>1</sub> cDNAs, pulse-labeled with [35S]methionine, and the label was chased for 15 h. Detergent lysates of cells (C) and the culture medium (M) were immunoprecipitated and analyzed. A, single-site mutants. B, double-site mutants. C, triple-site mutants.

![Figure 4](https://example.com/fig4.png)

**Expression of ACE<sub>1</sub> mutants with single N-glycosylation sites.** A, HeLa cells were transfected with wild type or mutant cDNAs, and pulse-chase (15 h) analysis was performed as described in the legend of Fig. 3. Immunoprecipitated cell lysates (C) and media (M) from cells transfected with ACE<sub>1</sub>WT (lanes 1 and 5), ACE<sub>1</sub>g2 (lanes 2 and 6), ACE<sub>1</sub>g3 (lanes 3 and 7), and ACE<sub>1</sub>g1 (lanes 4 and 8) were analyzed. B, kinetics of biosynthesis: transfected HeLa cells were pulse-labeled and chased for 0, 2, 4, 8, and 15 h as indicated. Immunoprecipitated lysates of cells transfected with ACE<sub>1</sub>WT (lanes 1-5), ACE<sub>1</sub>g2 (lanes 6-10), ACE<sub>1</sub>g1 (lanes 11-15), and ACE<sub>1</sub>g3 (lanes 16-20) cDNA were analyzed.

**Table II**

Properties of ACE<sub>1</sub> and its mutants produced in HeLa cells, yeast, and E. coli

| Properties | HeLa | Yeast | E. coli (WT) |
|------------|------|-------|-------------|
| Molecular mass (kDa) | WT g13 g1 g2 g3 | g13 g2 g3 | ND ND ND ND |
| Secreted | 104 94 92 96 | 90 77 79 70 | 70 |
| N-Linked | Y Y Y Y | N N N N | N N N N |
| O-Linked | Y Y Y Y | N N N N | N N N N |
| Cleavage secretion | Y Y Y Y | N N N N | N N N N |
| Enzyme activity | Y Y Y Y | N N N N | N N N N |
of ACE-g3, was recognized and used in yeast. As a result, the ACE-g3 protein synthesized in yeast was stable and secreted. These results demonstrated a remarkable difference between yeasts and mammalian cells with respect to the use of specific potential glycosylation sites of a protein.

Finally, the mode of secretion of ACEWT and ACEg3 from yeast was examined. We have shown previously that secretion of ACEWT from mammalian cell surface is accomplished by proteolytic cleavage of the ectodomain. As a result, the secreted form of ACEWT does not contain the membrane-anchoring domain and the intracellular domain present in the cell-bound form of ACEWT. In the experiment shown in Fig. 9, we examined if the ACEWT secreted by yeast also lacks these domains. For this purpose, we used a COOH-terminal-specific antibody, which reacts with HeLa cell-bound ACEWT, but not with secreted ACEWT (23). This antibody also failed to react with ACEWT and ACEg3 secreted by yeast (Fig. 9), thus indicating that ACEWT is also cleavage-secreted by P. pastoris.

**DISCUSSION**

We have established transfected mammalian cell lines that are high producers of enzymatically active ACEWT (14). We purified large quantities of ACEWT from the culture medium of these cells and attempted its crystallization without any success. We reasoned that the failure to crystallize could be due to an inherent heterogeneity of the purified protein which, in turn, is caused by its high level of glycosylation. We, therefore, sought out means to produce unglycosylated and underglycosylated ACEWT. There are reports in the literature that the potential glycosylation sites of a protein.

![Image](http://www.jbc.org/)

**Fig. 5.** N- and O-glycosylation status of mutants with single N-glycosylation sites. HeLa cells were transfected with ACEWT (lanes 1-4), ACE-g2 (lanes 5-8), ACE-g1 (lanes 9-12), and ACE-g3 (lanes 13-16) cDNAs. After pulse-labeling, the label was chased for 0 h (ACE-g3-transfected cells) or 15 h (all other cells). Cell lysates were immunoprecipitated, boiled with SDS, and left untreated (-) or treated with (+) deglycosylating enzymes as indicated. For the analysis of ACE-g3 (lanes 13-16), five times more extract was used than all other lanes.

![Image](http://www.jbc.org/)

**Fig. 6.** Absence of ACE-g3 on the cell surface: detection of ACEWT and ACE-g3 proteins by indirect immunofluorescence. HeLa cells grown on coverslips were transfected with ACEWT or ACE-g3 cDNA. Transfected cells were processed for indirect immunofluorescence as described under “Experimental Procedures” using anti-ACE antibody and fluorescence-conjugated rabbit anti-goat IgG, to detect ACE proteins expressed intracellularly (internal) or on the cell surface (surface).

![Image](http://www.jbc.org/)
be devoid of them.

ACE<sub>T</sub> produced in P. pastoris was cleavage-secreted (Fig. 9), since the secreted ACE<sub>T</sub> protein was devoid of the cytoplasmic tail. The exact cleavage site has not yet been determined and compared with the site used in mammalian cells. The cleavage secretion process in the mammalian cells is regulated by phor- bol esters and the responsible plasma membrane-associated proteolytic activity has the characteristics of a specific class of metalloprotease (28). It remains to be determined if the cleavage secretion process in yeast has similar characteristics. If so, it can serve as a model for the mammalian activity, and the responsible protease may be more easily identifiable in the yeast system.

The observed differential behaviors of ACE<sub>T</sub,g3 in HeLa cells and P. pastoris were unexpected. We are unaware of any other examples of a mammalian glycoprotein that fails to be glyco- sylated in the endoplasmic reticulum of a mammalian cell, but not in yeast. ACE<sub>T</sub,g3 can, thus, serve as a useful tool for identifying key differences in the glycosylation apparatus of the two cell types. Since yeasts are widely used for studying the process involved in eukaryotic protein trafficking and post- translational modifications, the differences noted here may serve as a warning that all the steps may not be equivalent in yeast and higher eukaryotes.

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