Comparative importance in vivo of conserved glutamate residues in the EX\textsubscript{7}E motif retaining glycosyltransferase Gpi3p, the UDP-GlcNAc-binding subunit of the first enzyme in glycosylphosphatidylinositol assembly

Zlatka Kostova\textsuperscript{1}, Benjamin C. Yan\textsuperscript{1}, Saulius Vainauskas\textsuperscript{2}, Roberta Schwartz\textsuperscript{2}, Anant K. Menon\textsuperscript{2} and Peter Orlean\textsuperscript{1}

\textsuperscript{1}Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA; \textsuperscript{2}Department of Biochemistry, University of Wisconsin-Madison, Madison, WI, USA

Saccharomyces cerevisiae Gpi3p is the UDP-GlcNAc-binding and presumed catalytic subunit of the enzyme that forms GlcNAc-phosphatidylinositol in glycosylphosphatidylinositol biosynthesis. It is an essential protein with an EX\textsubscript{7}E motif that is conserved in four families of retaining glycosyltransferases. All Gpi3ps contain a cysteine residue four residues C-terminal to EX\textsubscript{7}E. To test their importance for Gpi3p function in vivo, Glu289 and 297 in the EX\textsubscript{7}E motif of S. cerevisiae Gpi3p, as well as Cys301, were altered by site-specific mutagenesis, and the mutant proteins tested for their ability to complement nonviable GPI3-deleted haploids. Gpi3p-C301A supported growth but membranes from C301A-expressing cells had low in vitro N-acetylglucosaminylphosphatidylinositol (GlcNAc-PI) synthetic activity. Haploids harboring Gpi3p-E289A proved viable, although slow growing but Gpi3-E297A did not support growth. The E289D and E297D mutants both supported growth at 25 °C, but whereas the E289D strain grew at 37 °C, the E297D mutant did not. Membranes from E289D mutants had severely reduced in vitro GlcNAc-PI synthetic activity and E297D membranes had none. The mutation of the first Glu in the EX\textsubscript{7}E motif of Schizosaccharomyces pombe Gpi3p (Glu277) to Asp complemented the lethal null mutation in gpi3\textsuperscript{+} and supported growth at 37 °C, but the E285D mutant was nonviable. Our results suggest that the second Glu residue of the EX\textsubscript{7}E motif in Gpi3p is of greater importance than the first for function in vivo. Further, our findings do not support previous suggestions that the first Glu of an EX\textsubscript{7}E protein is the nucleophile and that Cys301 has an important role in UDP-GlcNAc binding by Gpi3p.

Keywords: endoplasmic reticulum; glycosylphosphatidylinositol; glycosyltransferase; Saccharomyces cerevisiae; Schizosaccharomyces pombe.

Glycosyltransferases can be classified into a range of families based on amino-acid sequence similarities, and these sequence alignments have led to the identification of signature motifs of amino acids [1–3]. Members of the large Pfam GT1F glycosyltransferase family, with representatives in the bacteria, archaea, and eukaryotes, have in turn been classified into subfamilies. Many Pfam GT1F glycosyltransferases fall into family 4 of the classification proposed by Campbell and coworkers (CaZY) [2], and some into families 3 and 5. Most of these retaining glycosyltransferases have the signature motif EX\textsubscript{7}E. The conservation of these two acidic residues strongly suggests that they have key roles in glycosyltransferase activity, and this has been demonstrated in site-specific mutagenesis and in vitro activity assay studies of CaZY family 3 human muscle glycogen synthase and the family 4 α-mannosyltransferase from Acetobacter xylinum (AceA) [4,5].

The two glutamate residues have been proposed to be involved in catalysis, but their contributions have yet to be evaluated by 3D structural analysis or identification of enzyme–substrate complexes or reaction intermediates. Whereas Kaptinov & Yu [3] suggested that the second of the two Glu residues in the EX\textsubscript{7}E motif may serve as a nucleophile, and the first as an acid base catalyst, the results of site-directed mutagenesis studies of human muscle glycogen synthase and AceA indicated that, in both cases, mutations of the first glutamate had more severe effects on in vitro enzyme activity and on the ability of the mutant enzyme to catalyze its glycosyltransfer reaction when expressed in a heterologous system. These findings indicated that the first of the Glu residues is critical for enzyme activity, possibly as the nucleophile [4,5].

We showed recently that a yeast EX\textsubscript{7}E motif protein, Gpi3p, binds a photoactivatable sugar nucleotide analogue, consistent with its function as the substrate-binding and catalytic subunit of the enzyme complex that forms N-acetylglucosaminylphosphatidylinositol (GlcNAc-PI) in the first reaction of the pathway for glycosylphosphatidylinositol assembly.
inositol (GPI) biosynthesis [6]. As Gpi3p is probably a glycosyltransferase, and because it is encoded by an essential gene in both \textit{Saccharomyces cerevisiae} and, as we report here, \textit{Schizosaccharomyces pombe}, it presents an excellent model with which to assess the effects of amino-acid replacements in the EX-E motif by testing mutated forms of Gpi3p for their ability to complement lethal null mutations in \textit{GPI3}. The results of our studies indicate that both Glu residues are important for function, but that the second Glu in the EX-E motif is less tolerant of changes to other amino acids, and therefore comparatively more important for enzyme function.

Gpi3 proteins from various organisms also have a conserved cysteine (Cys301 in \textit{S. cerevisiae} Gpi3p). On account of its proximity to the proposed catalytic EX-E motif, and because GlcNAc-P1 synthetic activity can be inhibited irreversibly by agents that alkylate thiol groups but protected from inhibition by uridine nucleotide compounds [7], it has been speculated that this cysteine is important for function, perhaps for UDP-GlcNAc binding [7,8]. Alteration of Cys301 to Ala has no obvious effect on the mutant protein’s ability to support cell growth, but membranes harboring the mutant protein had significantly lower GPI GlcNAc transferase activity than wild-type membranes.

The results of our in vivo tests for Gpi3p function do not support previous suggestions that the first Glu of an EX-E glycosyltransferase is the nucleophile in the reactions catalyzed by members of this protein family or that Cys301 is involved in UDP-GlcNAc binding by Gpi3 proteins.

**Materials and methods**

**Materials**

UDP-[U-14C]GlcNAc (specific radioactivity, 283 mCi mmol⁻¹) was purchased from NEN Life Science Products (Boston, MA, USA). Palmitoyl-CoA and tunicamycin were obtained from Sigma, and Nikkomycin-Z from Calbiochem. Silica gel 60 TLC plates were supplied by Altech (Deerfield, IL, USA). X-OMAT X-ray film and Transcreen-LE intensifying screens were from Eastman-Kodak Company (Rochester, NY, USA). Expand High Fidelity PCR mix, Pwo polymerase and EDTA-free protease inhibitor tablets were purchased from Roche Diagnostics (Indiana-

**Yeast strains and culture media**

The temperature-sensitive \textit{S. cerevisiae} gpi3-15C strain had the genotype \textit{MAT a}, ade2, leu2-3,112, trp1-1, his3-11,15 [9]. Diploid strain YMW3 (\textit{MAT a/\alpha}, ade2-1/ade2-1, ade3A22/ ade3A22, his3-11,15/his3-11,15, leu2-3,112/leu2-3,112, trp1- 1/trp1-1, ura3-1/ura3-1, can1-100/can1-100) is described in reference [10], and construction of the heterozygous \textit{GPI3}/\textit{gpi3}-kanMX4 diploid derived from YMW3 is detailed in reference [6]. \textit{Schizosaccharomyces pombe} strains were derived from the wild-type heterothallic strains KGY246 (h⁻, ade6-M210, ura4-d18, leu1-32) and KGY249 (h⁺, ade6-M216, ura4-d18, leu1-32).

YPD and SD media were prepared as described in reference [11], and EMM2 medium is described in reference [12]. The presence of the \textit{kanMX4} marker was verified by scoring for resistance to 200 μg G418 mL⁻¹ on solid YPD medium.

**Expression and mutagenesis of \textit{S. cerevisiae} GPI3**

A 2624-bp Xho1–SacI fragment of \textit{S. cerevisiae} genomic DNA, which contained the \textit{GPI3} gene, an additional 674 bp DNA containing the native \textit{GPI3} promoter at the gene’s 5’ end, and 494 bp 3’ flanking DNA, was cloned into the centromeric and 2μ plasmids pRS415 and pRS425 [13]. The resulting plasmids, pRS415-GPI3 and pRS425-GPI3, were used as templates for mutagenesis and for expression of the \textit{GPI3} mutants in \textit{S. cerevisiae}.

The following mutations were made: E289A, E297A, E289D, E297D, E289G, E297G, E289D/E297D and C301A. Mutagenesis of \textit{GPI3} was performed using the Stratagene QuikChange site-directed mutagenesis strategy. For each mutation to be introduced, a mutagenic oligonucleotide and its inverse complement were designed that introduced the appropriate nucleotide changes and a diagnostic restriction site in the middle of the oligonucleotides. DNA amplification by PCR was carried out using Pwo polymerase. Potential mutagenized plasmids were identified by digestion with a restriction enzyme specific for the introduced site, and the \textit{GPI3} region on selected plasmids was sequenced to verify the presence of the desired mutation, and the absence of mutations introducing any further amino-acid changes. To make the E289D/E297D double mutant, 2μ plasmids with each single mutation were mutagenized a second time using the oligonucleotide pairs designed to introduce the additional mutation. Double mutants were obtained with each starting mutant plasmid, and the correctness of the mutations and \textit{GPI3} sequence was confirmed by DNA sequencing.

**Cloning, disruption and site-directed mutagenesis of \textit{Sz. pombe} gpi3+**

A BLAST search [14] using the amino-acid sequence of \textit{S. cerevisiae} Gpi3p as query identified \textit{Sz. pombe} ORF SPBC3D5. This putative \textit{Sz. pombe} gpi3+ gene contains four introns, and sequencing of a gpi3+ cDNA amplified from an \textit{Sz. pombe} cDNA library [15] confirmed that the four introns are spliced as predicted.

To disrupt the gpi3+ gene, DNA fragments of ≈1 kb each of chromosomal DNA that flanks the 5’ and 3’ ends of the gpi3+ locus were amplified by PCR, and the PCR-amplified \textit{Sz. pombe} ura4+ gene was cloned between the two gpi3+ flanking fragments. The resulting 3.5-kb fragment, in which 88% of the gpi3+ sequence was replaced by ura4+ DNA, was used to transform an adenine-prototrophic diploid created by mating haploid strains KGY246 and KGY249 to uracil prototrophy. Stable diploids were selected, and the presence of the disrupting fragment at the...
chromosomal \textit{gpi3}+ locus verified by whole-cell PCR. Diploids were allowed to sporulate, and random spore analysis was carried out on EMM medium supplemented with limiting adenine but selective for uracil prototrophy to identify potential \textit{gpi3}+::\textit{ura4}+ haploids. Tetrads analysis was also carried out on asci derived from two independent \textit{gpi3}+/\textit{gpi3}−::\textit{ura4}+ diploids, and viable ade− haploid segregants were scored for uracil prototrophy.

Genomic \textit{Sz. pombe} DNA consisting of the \textit{gpi3}+ locus and about 700 bp 5′ flanking DNA and 1000 bp 3′ flanking DNA was cloned into the \textit{LEU2}-marked \textit{Sz. pombe} expression vector pSP1 [16]. Diploids transformed with this plasmid, pSP1-\textit{gpi3}+, yielded ade+, uracil and leucine prototrophic haploids upon sporulation, indicating that the cloned \textit{gpi3}+ gene complemented the \textit{gpi3}−::\textit{ura4}+ disruption. Plasmid pSP1-\textit{gpi3}+ was used as template for site-directed mutagenesis of \textit{Glu277} and \textit{Glu285} to Asp as detailed for \textit{S. cerevisiae} \textit{Gpi3p} above, and the presence of the desired mutation, and the absence of mutations introducing any further amino-acid changes, were verified by DNA sequencing.

**Assay of GlcNAc-PI synthesis**

Washed mixed membranes were prepared and assayed for \textit{in vitro} GlcNAc-PI synthetic activity as described previously [6, 17]. In assays to estimate the formation of [14C]GlcNAc-PI with time, palmitoyl-CoA was omitted from the incubation mixtures. Radiolabeled lipids were extracted, separated by TLC, and detected by fluorography. The chromatograms were scanned by Phosphorimagier to determine the relative amounts of 14C in the GlcNAc-PI in each sample.

**Imaging and microscopy**

Images of yeast growth on solid YPD medium were obtained using a Bio-Rad GelDoc2000. Growth of individual colonies arising from spores that had germinated on solid YPD medium was monitored using a Nikon TE300 inverted microscope with a 40 × bright field objective.

**Results**

The importance of the conserved Glu residues in the EX-E motif of \textit{S. cerevisiae} \textit{Gpi3p} for \textit{in vivo} function was tested by introducing mutations into the \textit{GPI3} gene that altered these residues, \textit{Glu289} and \textit{Glu297}, to aspartates, glycines or alanines. The mutated genes were in turn introduced into a heterozygous \textit{GPI3}/\textit{gpi3}::\textit{kanMX4} diploid on low or high copy plasmids, the diploids induced to undergo meiosis and sporulation, and the resulting asci dissected to assess whether the mutated \textit{GPI3} gene permitted growth of otherwise nonviable haploid \textit{gpi3}::\textit{kanMX4} segregants. The consequences of changing the \textit{EX-E} motif of \textit{Sz. pombe} \textit{Gpi3p} to Asp residues were examined analogously. The C301A mutation in \textit{S. cerevisiae} \textit{Gpi3p} was also tested.

**E289A and E297A mutants in \textit{S. cerevisiae} \textit{Gpi3p}**

Tetrads arising from \textit{GPI3}/\textit{gpi3}::\textit{kanMX4} diploids transformed with centromeric or 2μ plasmids expressing \textit{Gpi3p}-E289A gave rise to two fast-growing segregants. However, after 5–6 days of incubation on YPD medium at 25 °C, many of the dissected tetrads yielded additional microcolonies, and a number of complete tetrads with two large colonies and two microcolonies were observed (Fig. 1A). Representative segregants that subsequently formed normal sized or microcolonies were examined by microscopy at intervals over several days, and these inspections confirmed that the segregants yielding microcolonies were slow growing (Fig. 1B). Changing the first Glu of the EX-E motif of \textit{Gpi3p} to Ala is therefore not lethal, although the mutation affects \textit{in vivo} function, leading to a severe growth defect.

The E297A mutation, however, abolishes \textit{in vivo} function. Tetrads from \textit{GPI3}/\textit{gpi3}::\textit{kanMX4} diploids transformed with low or high copy plasmids expressing \textit{Gpi3p}-E297A contained only two normally growing segregants, and two that germinated and accomplished two or three cell divisions, but which did not continue to grow (Fig. 1A).

All segregants giving rise to normal sized colonies were G418-sensitive, indicating that they contained the chromosomal wild-type \textit{GPI3} gene. The slow growing or nonviable segregants in each tetrad were inferred to contain the \textit{gpi3}::\textit{kanMX4} allele. In the case of slow growing segregants from the diploid transformed with plasmids expressing \textit{Gpi3p}-E289A, this could be confirmed: cells from the microcolonies grew when restreaked on to G418-containing medium. The \textit{gpi3}::\textit{kanMX4}-\textit{Gpi3p}-E289A segregants retained their slow growth phenotype when restreaked on to fresh YPD medium (Fig. 1C), but grew slightly better on YPD medium containing 0.6 M KCl, indicating partial relief of a cell wall defect. Neither the \textit{E289G} nor \textit{E297G} mutation supported growth of \textit{gpi3}::\textit{kanMX4}.

**E289D and E297D mutants in \textit{S. cerevisiae} \textit{Gpi3p}**

The EX-E Glu residues were changed to Asp and tested individually and in combination. Tetrads from \textit{GPI3}/\textit{gpi3}::\textit{kanMX4} diploids transformed with centromeric or 2μ plasmids expressing \textit{Gpi3p}-E289D or \textit{Gpi3p}-E297D gave rise to segregants that all grew at approximately comparable rates at 25 °C (Fig. 2A). However, whereas the \textit{gpi3}::\textit{kanMX4}-\textit{Gpi3p}-E289D segregants grew at 37 °C when expressed from high copy plasmids, the \textit{gpi3}::\textit{kanMX4}-\textit{Gpi3p}-E297D segregants failed to grow at 37 °C, even when expressed on a 2μ plasmid (Fig. 2A). Consistent with these results, \textit{Gpi3p}-E289D restored ability of the temperature-sensitive \textit{gpi3}-15C strain [9] to grow at 37 °C, whereas \textit{E297D} did not (not shown). The \textit{Gpi3p}-E289D/\textit{E297D} double mutant did not support growth of \textit{gpi3}::\textit{kanMX4}.

The \textit{E289D} and \textit{E297D} mutations affected the \textit{in vitro} transfer of [14C]GlcNAc from UDP-[14C]GlcNAc to endogenous PI, but in different ways, with \textit{E289D} having the less severe effect. Membranes from the \textit{gpi3}::\textit{kanMX4} segregants from a tetrad arising from a \textit{GPI3}/\textit{gpi3}::\textit{kanMX4} diploid transformed with low or high copy plasmids expressing \textit{Gpi3p}-E289D retained the ability to synthesize GlcNAc-PI, although at much lower levels than membranes from the wild-type siblings (Fig. 2B). The \textit{gpi3}::\textit{kanMX4} segregants harboring \textit{Gpi3p}-E297D had no detectable \textit{in vitro} GlcNAc-PI synthetic activity. The copy number of the expression plasmid did not influence the \textit{in vitro} GlcNAc-PI.
synthetic activities. When plasmids expressing Gpi3p-E297D were introduced into the temperature-sensitive gpi3-15C strain, weak restoration of in vitro GlcNAc-PI synthetic activity was obtained (not shown), suggesting that the E297D mutation does not act as a dominant negative mutant.

The difference in the in vitro GlcNAc-PI synthetic activities of wild-type and gpi3::kanMX4-Gpi3p-E289D membranes was quantified by incubating assays for shorter times, separating the $[^{14}C]$GlcNAc-PI formed by TLC, and estimating the amount of $[^{14}C]$ in the reaction product using a Phosphorimag. The initial rate of $[^{14}C]$GlcNAc-PI formation by wild-type membranes, estimated from the progress curves in Fig. 2C, is some 12-fold higher than the rate at which $[^{14}C]$GlcNAc-PI is formed by gpi3::kanMX4-Gpi3p-E289D membranes.

E277D and E285D mutants in Sz. pombe Gpi3p

The findings that the E289A and E289D mutations have a less severe effect on Gpi3p function than the E297A and E297D mutations prompted us to test whether the same trend holds for the corresponding Glu residues in another Gpi3 protein, the Gpi3p homologue from fission yeast.

We cloned an Sz. pombe ORF encoding a protein of 456 amino acids with 52% identity with and 73% similarity to S. cerevisiae Gpi3p. This gene, which we designate gpi3+, was disrupted by replacing 88% of the coding region of one gpi3+ allele in a wild-type diploid strain with the ura4+ gene. The resulting heterozygous diploid was induced to sporulate, and the sporulating diploid submitted to both random spore and tetrad analysis. No viable uracil prototrophic haploids were recovered, indicating that disruption of gpi3+ is lethal. This lethality was due to disruption of the gpi3+ gene because viable gpi3+::ura4+ haploids were recovered from sporulated gpi3+::ura4+ diploid harboring the gpi3+ gene on plasmid pSP1.

Plasmids encoding the Gpi3p-E277D and Gpi3p-E285D mutations were introduced into heterozygous gpi3+::ura4+ diploids, which were sporulated, and the meiotic segregants then submitted to random spore analysis. Haploid uracil prototrophs were recovered from sporulated gpi3+::ura4+ diploids that harbored the
Gpi3p-E277D-expressing plasmid, and these complemented disruptants grew as well as gpi3+ haploids at 37°C. In contrast, no viable gpi3+::ura4+ haploids were recovered from sporulated gpi3+/gpi3+::ura4+ diploids expressing Gpi3p-E285D, even when the sporulated diploids were plated on selective medium supplemented with high concentrations of salt, glucose, glycerol, or sorbitol, and incubated at lower temperatures. These results, which indicate that Sz. pombe Gpi3p cannot tolerate the conservative Glu to Asp substitution in the second of the two Glu residues of its EX7Em o t i f, a re consistent with those obtained with S. cerevisiae Gpi3p, although the effect on Sz. pombe Gpi3p is more severe.

C301A mutant in S. cerevisiae Gpi3p
Tetrads from GPI3/gpi3::kanMX4 diploids transformed with centromeric or 2μ plasmids expressing Gpi3p-C301A gave rise, in most cases, to three or four viable segregants that all grew at approximately comparable rates at 25°C and 37°C, indicating that Gpi3p-C301A can complement the lethal gpi3::kanMX4 mutation (Fig. 3A). Moreover, introduction of a plasmid-borne copy of Gpi3p-C301A restored the ability of a temperature-sensitive gpi3 strain to grow at 37°C (Fig. 3B). Alteration of Cys301 to Ala therefore has no obvious effect on the protein’s ability to support growth. The C301A mutation did, however, lower in vitro GlcNAc-PI synthetic activity: mixed membranes from a gpi3::kanMX4 segregant containing Gpi3p-E289D had about 20% of the in vitro GlcNAc-PI synthetic activity of membranes from a wild-type sibling (Fig. 3C, D).

Discussion
The EX7-E motif is conserved among the members of four families of retaining glycosyltransferases, suggesting key roles for the two Glu residues in enzyme function. We exploited the fact that S. cerevisiae GPI3 and Sz. pombe gpi3+ are essential genes to test the importance of the conserved Glu residues in the EX7-E motif of these proteins by the stringent criterion of their ability to support cell growth. Our results indicate that both Glu residues are important for function, but that the second one in the EX7-E motif is less tolerant of change to other amino acids, and therefore is most critical for enzyme function in vivo. Our
invivo findings do not support previous suggestions that the first Glu of an EX7E motif protein is the nucleophile in the reactions catalyzed by members of this protein family and that Cys301 has an important role in UDP-GlcNAc binding by Gpi3ps. Changing the first Glu of \textit{S. cerevisiae} Gpi3p to Ala was not lethal, whereas the E297A change was. The E289A mutant presumably retains a level of function \textit{in vivo} that allows it to support growth, albeit weakly. Conservative changes of the EX7E Glu residues to aspartates were much less deleterious to \textit{S. cerevisiae} Gpi3p: haploid segregants harboring the E289D and E297D mutations grew about as well as their wild-type siblings at 25 °C, consistent with the importance of an acidic side chain at both positions in the protein. However, by two criteria, the change of Glu297 to Asp had a more severe effect on Gpi3p function. First, haploids complemented by Gpi3p-E289D grew at 37 °C, but the E297D-expressing strains were temperature-sensitive. Secondly, membranes containing Gpi3p-E297D had no detectable GlcNAc-PI synthetic activity, whereas those containing Gpi3p-E289D retained \textit{in vitro} activity at about one twelfth the level seen with wild-type membranes. The differential effects of the glutamate to aspartate mutations in \textit{S. pombe} Gpi3p highlighted the greater relative importance of the second glutamate: the E285D mutation was lethal, whereas the E277D mutation had no discernible effect \textit{in vivo}.

A potential concern with site-directed mutagenesis approaches is that the mutations introduced in the test protein may affect the protein’s structure, localization, or its ability to participate in a complex, and so may only indirectly affect enzyme function. However, our genetic data showing retention of function of various key mutants \textit{in vivo} render a demonstration of misfolding, instability, or mislocalization of the protein, or of impaired complex formation by the protein, redundant, for such additional findings could not alter – and would have little bearing – on our conclusions. Thus, for example, mutation of E289 in Gpi3p would be expected to yield a nonfunctional protein according to current models [4,5]. In this event, to make sure that the point mutation was the sole cause of nonfunctionality, we would be obliged to investigate expression level of the protein, its ability to form a complex with other GlcNAc-PI synthase subunits, and its subcellular localization. None of these tests are necessary because we show that the E289A and E289D mutants function \textit{in vivo} and are able to sustain cell growth, albeit weakly in the case of E289A. Indeed, were the effects of the E289A mutation to be indirect ones on protein folding, stability, or localization, or on the ability of...
Gpi3p to be incorporated into and function in a complex, then the mutant protein’s actual catalytic activity would, if anything, be higher in vivo. The only mutant where such additional tests may be required is the nonfunctional E297A protein. However, the ability of the related mutant E297D to function at 25 °C despite its inability to function at 37 °C suggests that E297D at least is properly folded and localized in the cell and that the E297A point mutant is likely to be similar.

Although gene dosage effects might have been expected, expression of the mutant proteins from high copy plasmids did not result in elevated in vitro GlcNAc-Pi synthetic activity or improved cell growth compared with strains expressing the same mutant proteins on low copy plasmids. However, because Gpi3p functions in a protein complex [6,9,17,18], the availability of the other subunits may be limiting, such that the number of functional complexes is not significantly increased when one subunit is overexpressed. Consistent with this, even very high level expression of wild-type Gpi3p in a gpi3 deletion background using a galactose-inducible promoter resulted in only a slight elevation of in vitro GlcNAc-Pi synthase activity [6]. Likewise, overexpression of Gpi1p or Gpi2 does not significantly increase GlcNAc-Pi synthetic activity [6,19]. Failure of excess Gpi3p to be incorporated into a GlcNAc-Pi synthetic complex may also lead to its degradation. Such is the case with the catalytic subunit of the GPI transamidase complex, Gpi8p: monomeric Gpi8p subunits that are excluded from complete complexes are turned over rapidly [20].

The high degree of conservation of the EX7E Glu residues, and their potential to function as nucleophiles or general acid/base catalysts or to participate in sugar nucleotide binding suggest that these two Glu residues are likely to function at 25 °C despite its inability to function at 37 °C suggests that E297D at least is properly folded and localized in the cell and that the E297A point mutant is likely to be similar.

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References


