Oligomerization of Hamster UDP-GlcNAc:dolichol-P GlcNAc-1-P transferase, an Enzyme with Multiple Transmembrane Spans*

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Hamster UDP-GlcNAc:dolichol-P GlcNAc-1-P transferase (GPT), which initiates N-linked glycosylation by catalyzing the synthesis of GlcNAc-P-P-dolichol, has multiple transmembrane spans and a catalytic site that probably exists on the cytosolic face of the endoplasmic reticulum membrane (Dan, N., Middleton, R. M., and Lehrman, M. A. (1996) J. Biol. Chem. 271, 30717-30725). In this report, we demonstrate that GPT forms functional oligomers, probably dimers. Oligomers were detected by chemical cross-linking of GPT and by a dominant-negative effect caused by co-expression of enzymatically inactive (but properly folded) GPT mutants. The GPT mutants had no effect on two other dolichol-P-dependent endoplasmic reticulum enzymes. Mixing experiments indicated that mature GPT was competent for oligomerization. Oligomerization appeared to be favored in detergent extracts compared with intact microsomes. Detergent treatments were found to prevent, rather than promote, nonspecific aggregation of GPT.

These results demonstrate that GPT subunits can physically interact and influence each other. The implications of oligomerization for enzyme function are discussed. From these results, we conclude that GPT is one of a very small number of multitransmembrane span enzymes that can form multimers.

At least two well characterized families of eukaryotic glycoconjugates, the dolichol-linked oligosaccharides (1) and glycosylphosphatidylinositol (2) are synthesized by enzymes of the endoplasmic reticulum (ER).1 Each pathway requires the polyisoprene lipid dolichol-P, either as the carrier for glycoconjugate assembly in the case of dolichol-linked oligosaccharides or for synthesis of dolichol-P-monosaccharides that serve as sugar donors in both pathways. Dolichol-P is limiting in cells (3-5). Thus, there must be careful regulation of the enzymes that use dolichol-P as an acceptor. Failure to balance the flux of dolichol-P into various enzymatic reactions would be expected to cause defects in glycoconjugate synthesis. For example, excesive synthesis of GlcNAc-P-P-dolichol at the expense of mannose-P-dolichol and glucose-P-dolichol results in truncated dolichol-linked oligosaccharides (6, 7) lacking glucose residues necessary for interactions with the ER chaperones calnexin (8) and calreticulin (9).

Our group and others have been interested in the structure and mechanism of action of UDP-GlcNAc:dolichol-P GlcNAc-1-P transferase (GPT; EC 2.7.8.15) (10). This critical enzyme is responsible for the committed step of dolichol-linked oligosaccharide synthesis, i.e. the production of GlcNAc-P-P-dolichol, and catalyzes the following reversible reaction:

\[ \text{UDP-GlcNAc} + \text{dolichol-P} \rightarrow \text{GlcNAc-P-P-dolichol} + \text{UMP} \]

GPT is thought to be under several different transcriptional (11-14) and post-translational (reviewed in Ref. 10) controls. To understand the regulation of this enzyme, it is essential to discern its structure. Recently we reported that GPT spans the ER membrane multiple times and has critical elements that face the cytoplasm (15). In addition, those studies suggested that GPT might form dimers, which would be unusual for an enzyme with multiple transmembrane spans. Upon insertion of a factor Xa cleavage sequence into the cytosolic loop between the 9th and 10th transmembrane segments, it was found that GPT polypeptides that behaved as monomers were less sensitive to factor Xa than those that behaved as putative dimers (15). This indicated that the orientation and/or steric accessibility of this loop might vary depending upon the oligomeric state of GPT. Since this loop was also shown to be essential for enzyme function, this suggested that the activity of GPT might be influenced by oligomerization.

By the use of two independent approaches, chemical cross-linking and identification of dominant-negative effectors, GPT is shown in this report to oligomerize. The activity of one subunit can influence another, indicative of a functional interaction between the subunits. Thus, GPT is a rare example of a multimeric enzyme with multiple transmembrane spans.

**EXPERIMENTAL PROCEDURES**

Methods for cell culture, transfection, immunoblotting, site-directed mutagenesis, *in vitro* transcription, *in vitro* translation, and the isolation of microsomal membranes, as well as the necessary reagents, were described earlier (6, 15, 16).

**GPT Assays**

**Assay A**—Assays with intact microsomal membranes (without exogenous dolichol-P) were carried out essentially as described earlier (16) and initiated by the addition of 0.1 μCi of UDP-[3H]GlcNAc (25.8 Ci/mmol).

**Assay B**—Assays with Nonidet P-40 plus exogenous dolichol-P were carried out as follows. Microsomal membranes were stored at 4 °C and used within 24 h of preparation. 20 μg of membrane protein was suspended in 75 μl of 20 mM Tris-Cl, pH 7.4, plus 0.15 mM NaCl (TBS). An appropriate amount of Nonidet P-40, to give the desired final concentration in the assay, was added along with 4 μg of dolichol-P, 2 μg of phosphatidylglycerol, 1 μg of leupeptin, and 10 μg of dithiothreitol (all from Sigma). The mixture was kept on ice for 30 min with periodic gentle agitation. The mixture was brought to a final volume of 100 μl with TBS and adjusted to contain 50 mM Tris-Cl, pH 7.4, 10 mM MgCl₂, and 200 μM dolichol-P. The mixture was kept on ice for 30 min with periodic gentle agitation. The mixture was brought to a final volume of 100 μl with TBS and adjusted to contain 50 mM Tris-Cl, pH 7.4, 10 mM MgCl₂, and 200 μM dolichol-P. The mixture was kept on ice for 30 min with periodic gentle agitation. The mixture was brought to a final volume of 100 μl with TBS and adjusted to contain 50 mM Tris-Cl, pH 7.4, 10 mM MgCl₂, and 200 μM dolichol-P.

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1 The abbreviations used are: ER, endoplasmic reticulum; BS3, bis(sulfosuccinimidyl)suberate; GPT, GlcNAc-1-P transferase; GPDS, glucose-P-dolichol synthase; Tn, tunicamycin; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate.

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45 °C (6). GPT is calculated to weigh 46 kDa but migrates as a polypeptide of 45 kDa (6). GPT can be detected by immunoblotting after SDS-polyacrylamide gel electrophoresis, but denaturation in the presence of detergent was increased up to 0.15% (17) with Triton X-100, a detergent very similar to Nonidet P-40 (17). The reaction was initiated by the addition of 0.1 μCi of [3H]GlcNAc, continued for 10 min at 37 °C, and terminated by the addition of 2 ml of chloroform:methanol (2:1). Reactions were linear for at least 20 min (data not shown). [3H]GlcNAc-P-Dolichol was recovered and measured as described (16).

**Chemical Cross-linking**

10 μg of membrane protein was mixed on ice with the indicated amount of Bis(sulfosuccinimidyl)suberate (BS3; from Pierce) in buffer containing 50 mM Na-HEPES, pH 8.0, and 100 mM NaCl, with or without 0.16% (w/v) Nonidet P-40. After 80 min on ice, 1 volume of 2× SDS-polyacrylamide gel electrophoresis sample buffer was added, and the proteins were subjected to immunoblotting with a rabbit polyclonal antibody directed against residues 42–56 of the native protein.

**RESULTS**

**Identification of GPT Oligomers by Chemical Cross-linking**—GPT can be detected by immunoblotting after SDS-polyacrylamide gel electrophoresis, but denaturation in the presence of SDS must be done at moderate temperatures, typically 42–45 °C (6). GPT is calculated to weigh 46 kDa but migrates anomalously as a protein of approximately 34 kDa under such conditions. In addition, a form migrating as a polypeptide of approximately 67 kDa is sometimes observed by immunoblotting (15). We reasoned that if the 67-kDa species was an oligomer, much of it might be disassociated by the SDS used for electrophoresis and therefore would be stabilized by pretreatment with chemical cross-linkers. However, cross-linkers would not be expected to affect the 67-kDa species if it was an alternatively processed form of GPT or an immunologically cross-reacting protein. As shown in Fig. 1A, treatment of intact microsomal membranes with the cross-linker BS3 greatly enhanced detection of the 67-kDa polypeptide. No other discrete cross-linked polypeptides were observed, although at high concentrations (data not shown) of cross-linker, both the 34- and 67-kDa forms were diminished with the corresponding appearance of a low mobility polydisperse cross-reacting material, most likely due to nonspecific cross-linking between GPT and other microsomal components. Similar results were obtained with two other cross-linkers, 3,3′-dithiobis(sulfosuccinimidyl)-propionate and 3,3′-dithiobispropionimidate (data not shown). These data were consistent with the 67-kDa form being a homodimer of GPT or perhaps a hetero-oligomer with another microsomal component. Oligomerization was also detected with Nonidet P-40-treated microsomes and was not affected by the addition of the substrates dolichol-P and UDP-GlcNAc (Fig. 1B) or the inhibitors tunicamycin and amphomycin (data not shown). Over the course of several experiments, the cross-linked oligomer was consistently more apparent in Nonidet P-40-treated microsomes than in intact microsomes. These Nonidet P-40 treatments were similar to those used in detergent-based GPT assays in which the enzyme is known to be active (see below). It did not seem likely that the Nonidet P-40 treatment itself could promote oligomerization by an artificial process (i.e. aggregation); while GPT translated in vitro in the presence of microsomal membranes readily incorporated into the membranes and behaved as an apparent monomer (15), GPT translated in the absence of microsomes tended to aggregate within 60 min (data not shown). However, inclusion of Triton X-100, a detergent very similar to Nonidet P-40 (17) and compatible with in vitro translation (15), during translation in the absence of microsomes prevented aggregation and promoted the reappearance of monomers. 

**Expression of an Inactive GPT Mutant Causes Inhibition of Normal GPT**—The results with cross-linkers, although indicative of the formation of distinct oligomers, were subject to the possibility that GPT was a monomer and that the cross-linker artificially trapped transient aggregates of the enzyme. In addition, the cross-linked form could be due to GPT coupled to a separate component of the ER membrane (i.e. a heterooligomer). It was reasoned that if GPT formed homooligomers, then co-expression of active normal and inactive mutant GPT polypeptides might result in the formation of GPT with abnormal enzymatic properties. A similar strategy was used to demonstrate oligomerization of K+ channel subunits (reviewed in Ref. 18). For these studies, we chose the R303K mutation (15). Arg303, in cytosolic loop 9/10 of hamster GPT, is conserved with other eukaryotic forms of GPT. The highly conservative mutation of Arg303 to Lys causes the enzyme to become catalytically inactive. However, this mutant can be stably expressed in CHO-K1 cells and can bind Tn efficiently, indicative of proper folding. Treatment of the R303K mutant with cross-linkers revealed that it formed the 34- and 67-kDa species in a ratio indistinguishable from normal GPT (data not shown).

Consistent with earlier results (15), the R303K mutant exhibited little or no activity (compared with vector controls expressing only the cell's endogenous enzyme) after stable expression when measured in the absence of detergent with assay A, in which microsomal membranes remained intact and endogenous dolichol-P was used (Fig. 2A). With low detergent concentrations (0.025%) R303K also exhibited no activity in assay B, in which microsomes were dispersed with Nonidet P-40 and reconstituted into complexes with exogenous phospholipid and dolichol-P, as observed previously (15). However, compared with cells expressing only the endogenous enzyme, expression of the R303K mutant unexpectedly caused 40–70% inhibition (average = 45%; n = 15 experiments) as the concentration of detergent was increased up to 0.15% (panel B). The addition of 0.05–0.15% detergent to control membranes increased activity, presumably by enhancing mobilization of the exogenous dolichol-P, while the same detergent concentrations...
decreased activity in the R303K transfectant. A similar effect was obtained with 45 mM CHAPS (data not shown). By comparing the effects of Nonidet P-40 on microsomes from cells expressing only the endogenous enzyme with microsomes from cells expressing the endogenous enzyme plus excess exogenous normal GPT, it is apparent that the relative effect of detergent on the normal form of GPT is independent of its level of expression (panel B). Thus, the inhibition observed with the R303K mutant cannot be explained by an indirect effect of the increased concentration of GPT polypeptides.

As expected, inhibition was dependent upon the level of R303K expression. Stable transfectants with different levels of R303K were isolated by screening for resistance to different concentrations of Tn (15). The degree of inhibition in these transfectants correlated with both Tn resistance and levels of the mutated GPT detected on immunoblots (data not shown).

Taken together, these data confirmed the results obtained with cross-linkers (Fig. 1). Inhibition of the normal endogenous enzyme by the R303K mutant demonstrates that there must be an interaction between the two proteins. To verify that the R303K mutant was not causing some general alteration of the ER affecting dolichol-P, two related enzymes were assayed. In contrast to endogenous GPT (Fig. 3A), neither glucose-P-dolichol synthase (GPDS; panel B) nor mannose-P-dolichol synthase (data not shown) were affected by expression of R303K GPT. A possible mechanism for the inhibitory effect of the R303K mutant is considered under “Discussion.”

A GPT Mutant with an Alteration in Loop 3/4 Also Inhibits Normal GPT—To determine whether these effects were limited to the R303K mutation, other GPT mutants that were enzymatically inactive, yet capable of binding Tn and thus properly folded, were considered. The R303N and R303H mutants characterized earlier (15) met these criteria but could not be expressed at levels comparable with those achieved with the R303K mutant. Consequently, little or no inhibition was observed with these mutants (data not shown).

In the course of separate studies on sequences conserved in the UDP-GlcNAc/MurNAc family (19), a mutation in loop 3/4 was generated in which residues Arg123-His124-Lys125-Leu126

Fig. 2. The dominant-negative effect of the R303K GPT mutant. A, GPT activity (n = 4; ± S.E.) was assayed in the absence of exogenous dolichol-P or Nonidet P-40 with intact microsomes from control (vector-transfected) CHO-K1 cells, R303K transfectants, or normal GPT transfectants. The latter two were resistant to Tn at 16 and 6 μg/ml, respectively, indicating 32- and 12-fold overexpression, respectively (15). For reasons that remain unclear, the -fold increase in Tn resistance is always much greater than the accompanying -fold increase in activity for the normal enzyme (6). In some cases, the error bars were very narrow and thus not visible on the figure. B, GPT was assayed in the presence of 0.025–0.2% Nonidet P-40 and 4 μg-assay dolichol-P. Diamonds, vector-transfected CHO-K1 cells. Circles, R303K transfectants. Squares, normal GPT transfectants.

Fig. 3. Inhibition of normal GPT by a B motif scramble mutant. As described under “Results,” the B motif (19) which is in loop 3/4 (15) of GPT, was scrambled. A, GPT assay (as done for Fig. 2) in the absence (shaded bars) or presence (striped bars) of 0.1% Nonidet P-40, 4 μg dolichol-P, 0.5 μCi of UDP-[^3H]glucose (60 Ci/mmol) was substituted for UDP-[^3H]GlcNAc to assay GPDS under the same conditions as used for A. Note that these conditions were not optimized for GPDS. For both panels, n = 3 (± S.E.), and the numbers above the striped bars indicate the percentage of activities with respect to the shaded bars.

2 A. Dal Nogare, N. Dan, and M. A. Lehrman, unpublished results.
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The Ability to Oligomerize Is Not Limited to Nascent Enzyme—Oligomerization of membrane proteins synthesized by the ER usually occurs soon after synthesis and typically involves a high concentration of chaperones and other such proteins provided by the specialized environment in the lumenal space (20). To investigate whether GPT oligomerization might be limited to newly synthesized enzyme, we examined the ability of the mature enzyme in Nonidet P-40 extracts to oligomerize. Microsomes from vector or R303K stably transfected CHO-K1 cells were treated with Nonidet P-40, mixed, and then assayed under the conditions of Fig. 2 to determine whether mutant GPT in one preparation could inhibit normal GPT in another. As shown in Fig. 5 (bars 3 and 4), a mixture containing 5 μg of membrane protein from vector-transfected CHO-K1 cells and 15 μg from CHO-K1 cells expressing the R303K mutant (20 μg total) caused a small but reproducible loss (four independent experiments) of GPT activity when compared with the arithmetic sum of the activities of the individual preparations. Although the magnitude of the effect appears small (11% of total activity), the inhibition was actually significant compared with the theoretical maximum (see below). The conditions of this assay were linear with respect to protein concentration; the activities obtained with 20 μg of protein from either preparation were twice that obtained with 10 μg (data not shown). Since the actual activity is sensitive to detergent concentration (Fig. 2) and the effective free detergent concentrations would be expected to differ in assays containing 5, 15, or 20 μg of membrane protein, the assays with 5 or 15 μg of the individual preparations described above were supplemented with 15 or 5 μg, respectively, of membrane protein from vector-control microsomes that had been heat-treated for 10 min at 70 °C, which completely inactivated GPT (data not shown).

Upon mixture of the vector and R303K preparations, the only additional inhibitory effect that could be expected would be a decrease in the endogenous GPT component in the vector preparation, because the GPT component in the R303K preparation was already inhibited. Assuming 45% inhibition of the vector preparation, Fig. 5 (bar 5) also indicates the theoretical predicted maximum inhibition. With this as a basis for comparison, the observed inhibition was substantial, calculated to be 63%. Two controls were performed to verify that this inhibition was significant. First, mixtures tested with assay A (no detergent) gave activities that were equal to the sums of the activities of the individual preparations (Fig. 5, bars 1 and 2). Second, parallel experiments similar to those depicted in bars 3 and 4 were performed in which UDP-[3H]GlcNAc was substituted with GDP-[3H]mannose, to assay mannose-P-dolichol synthase (Fig. 5, bars 6 and 7). The activity of the mixture in the presence of detergent was never less than the arithmetic sum of the individual activities, in direct contrast to the results obtained with UDP-[3H]GlcNAc.
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**Fig. 5. Activities of mixtures of intact and detergent-treated membrane preparations.** Assays (100 μl) were performed in the absence or presence of Nonidet P-40/dolichol-P (as described for Fig. 2) as indicated. Bars 1 and 2, GPT assay without Nonidet P-40, dolichol-P. Bars 3 and 4, GPT assay with Nonidet P-40, dolichol-P. Bars 6 and 7, mannose-P-dolich synthase (MPDS) assay with Nonidet P-40/dolichol-P. 0.5 μCi of GDP-[3H]mannose (15 Ci/mmol) was substituted for UDP-[3H]GlcNAc for bars 6 and 7 and assayed under the same conditions as used for bars 3 and 4. Shaded bars, arithmetic sums of individual activities of 5 μg of control and 15 μg of R303K samples. The individual activities for the control and R303K preparations were, respectively, 800 and 1863 cpm in bar 1, 1637 and 2475 cpm in bar 3, and 6783 and 16459 cpm in bar 6. Striped bars, activities of 5 μg of control and 15 μg of R303K samples mixed together. For shaded and striped bars, n = 3 ± S.E. The difference between the values for bars 3 and 4 was significant (p < 0.02 determined by Student's t test). This result was repeated with independent membrane preparations, and similar results were also obtained with mixtures of 10 μg of each sample (data not shown). Bar 5 (open bar), the predicted activity of a mixture of 5 μg of control and 15 μg of R303K extracts, assuming 45% inhibition of the former by the latter, presented for direct comparison with bar 4. Thus, 63% of the theoretical inhibition was observed.

These results indicate that the R303K subunits from one membrane preparation were able to interact with and inhibit normal subunits in a second preparation. Therefore, oligomerization can occur well after synthesis of the individual GPT subunits is completed.

**DISCUSSION**

Characterization of the oligomeric state of a membrane protein typically requires a multifaceted approach. Most physical and chemical methods available must be used with care, because the conditions and/or treatments used to monitor oligomerization could potentially cause nonspecific hydrophobic aggregation. Alternatively, fragile contacts between subunits could be disrupted. Although the use of cross-linkers (Fig. 1) suggested that GPT was oligomeric, the possibility that transient nonspecific aggregates were being trapped required an independent approach. Co-expression of normal and mutant forms of GPT (Fig. 2) resulted in the formation of enzyme with unusual properties. This provided a direct demonstration of the interaction between two dimers, direct evidence that one subunit can influence the activity of the other. A similar strategy was used to show that K⁺-channel subunits, which also have multiple transmembrane spans, form oligomers (18). Although the experiments reported here did not establish the actual ratios of monomers to oligomers in intact or detergent-treated microsomes, it is clear from these studies that GPT is capable of oligomerization. Gel filtration analysis of GPT purified from bovine mammary gland (21) also suggested that GPT was oligomeric, with molecular weights in the range of 330,000–360,000, but as the authors noted it was difficult to exclude the possibility of nonspecific aggregation of the purified enzyme. In addition to multiple copies of GPT, other unknown polypeptides (whether or not they can be cross-linked) may also be involved and are not ruled out by our data. However, this does not change the central conclusions of the paper regarding interactions of GPT subunits.

The R303K mutant did not inhibit by excessive binding of substrate. Regarding UDP-GlcNAc, the results shown in Fig. 2B (stable transfectants) did not change appreciably when 10-fold higher UDP-GlcNAc concentrations were used (42% inhibition; data not shown), and the experiment in Fig. 4B (transient transfectants) was done with a 26-fold higher concentration. Furthermore, there was no inhibition seen with intact membranes or 0.025% detergent, only with 0.050% detergent or greater. The results with various detergent concentrations also apply to dolichol-P. Moreover, two other dolichol-P-dependent enzymes, the glucose-P-dolich and mannose-P-dolich synthases, were unaffected by the GPT mutants, and the detergent-based assays were performed with saturating amounts of dolichol-P.

There are several ways to explain the inhibition caused by expression of the R303K mutant. One model is as follows. For simplicity, the functional oligomeric form is assumed to be a dimer. The model makes two assumptions. 1) Oligomers are favored in the presence of detergent, while monomers are favored in intact membranes. In other words, the oligomeric state of GPT may be sensitive to the hydrophobicity of its environment. This assumption could explain why the R303K and “B” mutants were inhibitory in detergent extracts but not in intact membranes. However, the preference for monomer or dimer is not absolute; some dimer was detected in intact membranes (Fig. 1), and some monomer is likely to exist, at least transiently, in detergent (Fig. 5). 2) In heterodimers of normal and mutant GPT, the normal subunit is inactivated by 40–70%. Since the mutant subunit was in excess over the endogenous enzyme in most of the experiments described in this report, it is presumed that essentially all normal subunits existed as heterodimers with mutant subunits. However, the data do not rule out an alternative situation with a mixture of normal homodimers and fully inactive heterodimers.

Taking these assumptions together, the model suggests that GPT is mostly monomeric in intact membranes, and mostly dimeric upon extraction with detergent. However, the relative specific activities of the presumed membrane and detergent forms could not be determined because it is not yet possible to prepare homogeneous preparations of the respective forms. Furthermore, it would be difficult to directly compare specific activities, because the effective concentrations of the acceptor dolichol-P would differ dramatically depending on the presence of detergent, even if one attempted to maintain an equal total dolichol-P concentration in the reaction vessel. It would be useful to determine the relative activities of the monomer and dimer because most investigators typically assay GPT in only one way, with intact membranes or with detergent extracts. In addition, it has been reported that the activity of GPT is dependent upon the local phospholipid composition (reviewed in Ref. 10). Such differences in activity might be explained by the ability of different phospholipid mixtures to alter the ratio of monomer to dimer. Clarification of this question might reveal a regulatory function for dimerization. An approach that has been used successfully by others to determine the oligomeric states of the active forms of proteins is radiation inactivation.
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(22, 23). However, interpretation of data obtained with this technique can be complicated if, in a dimer, inactivation of one subunit partially inactivates the other. In other words, the target would behave as neither a strict monomer nor a dimer. As discussed above (assumption 2), this caveat would likely apply to radiation inactivation of GPT.

GPT has at least 3, and possibly as many as 10, transmembrane spans (15). Few proteins with multiple transmembrane spans have been reported to form multimers. In addition to GPT, one other example involving an enzyme is adenyllyl cyclase (24). Most other cases are proteins that form channels. This includes the Drosophila K⁺ channel, the IP₃ receptor, the ryanodine receptor, gap junction channels, and the γ-aminobutyric acidA receptor (all reviewed in Ref. 18), suggesting that multimerization of multitransmembrane span proteins is required to form a physical pore necessary for function. The subunits in GPT oligomers interact, affecting the activities (this report) and structures (factor Xa experiment of Ref. 15) of each other. By analogy to the channel proteins mentioned above, perhaps the GPT subunits form a hydrophobic pocket in which the acceptor lipid, dolichol-P, can be trapped. GPT contains many highly conserved hydrophobic residues in predicted transmembrane spans (25) that could be involved in accommodating the polyisoprene chain. This would necessitate reversibility of oligomerization in order for the enzyme to undergo multiple rounds of catalysis. However, this does not necessarily require dolichol-P to drive oligomerization or stabilize the oligomer, only to be able to fit into the pocket. Indeed, dolichol-P had no effect on oligomerization in vitro (Fig. 1). If the α-isoprene unit of the dolichol-P interacted nonproductively with an inactive subunit, such as the R303K mutant, this would effectively sequester the dolichol-P away from the active subunit of the oligomer. In contrast, it is unlikely that GPT forms oligomers to provide a channel for transport of its nucleotide sugar substrate UDP-GlcNAc. It has been demonstrated that inhibition of GPT in vitro does not affect ER UDP-GlcNAc transport, and inhibition of the transporter does not affect GPT activity (26).

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