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Minireview

Structure-Based Insight on the Mechanism of N-Glycosylation Inhibition by Tunicamycin

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N-glycosylation, a common post-translational modification, is widely acknowledged to have a significant effect on protein stability and folding. N-glycosylation is a complex process that occurs in the endoplasmic reticulum (ER) and requires the participation of multiple enzymes. GlcNAc-1-P-transferase (GPT) is essential for initiating N-glycosylation in the ER. Tunicamycin is a natural product that inhibits N-glycosylation and produces ER stress, and thus it is utilized in research. The molecular mechanism by which GPT triggers N-glycosylation is discussed in this review based on the GPT structure. Based on the structure of the GPT-tunicamycin complex, we also discuss how tunicamycin reduces GPT activity, which prevents N-glycosylation. This review will be highly useful for understanding the role of GPT in the N-glycosylation of proteins, as well as presents a potential for considering tunicamycin as an antibiotic treatment.

Keywords: DPAGT1, GlcNAc-1-P transferase, GPT, N-glycosylation, tunicamycin

INTRODUCTION

Post-translational modification (PTM) refers to a biological event in which the characteristics of a protein are altered by various processes to form a newly produced protein. Through PTM, proteins are partially degraded by cleavage or attached to many functional groups (e.g., acetyl, phosphoryl, and methyl) to acquire diverse characteristics that are utilized for a variety of cellular processes (Ramazi et al., 2020). Glycosylation is an example of a PTM that frequently manifests in cells. Because glycosylation is a PTM present in proteins involved in cell adhesion and cell-to-cell contact, these processes rely heavily on glycosylation. Furthermore, glycosylation plays a crucial role in altering the characteristics of individual proteins, including their ability to fold, dissolve in solutions, and degrade. Finally, glycosylation may have an impact on typical protein secretion and intracellular protein transport. This demonstrates the importance of glycosylation of proteins in cells for a variety of purposes (Goulabchand et al., 2014; Haltiwanger and Lowe, 2004; Karve and Cheema, 2011; Ohtsubo and Marth, 2006). Furthermore, the connection between abnormal protein glycosylation and several diseases, including cancer, liver cirrhosis, and diabetes, is another illustration of the significance of glycosylation (Goulabchand et al., 2014; Karve and Cheema, 2011; Lauc et al., 2013).

Glycosylation involves a series of chemical events that are catalyzed by various glycosyltransferases to attach oligosaccharides to particular amino acid residues of proteins through covalent bonds. Although oligosaccharides attach to a wide range of amino acid residues, the two that experience the most glycosylation are Asn and Ser/Thr (Blom et al., 2004; Huang et al., 2019; Karve and Cheema, 2011; Ohtsubo and Marth, 2006). When an oligosaccharide binds to an Asn residue, the process is known as N-glycosylation, and when bound to a Ser/Thr residue, it is known as O-glycosylation.

Lipid-linked oligosaccharides (LLO) are first produced for N-glycosylation, which is common in prokaryotic cells, eu-

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karyotic cells, and archaea (Aebi, 2013). The oligosaccharide attached to an isopreneoid lipid to synthesize LLO is formed from nucleotide-based sugar. The lipid serves as a carrier to transport oligosaccharides. However, there is a distinction, prokaryotic cells use bactoprenol-based lipids while mammals and archaea, including humans, use dolichol as lipid carriers. All glycosyltransferases that catalyze the reactions in N-glycosylation employ the produced LLO as a substrate. GlcNAc, Mannose (Man), and glucose (Glc), which take the forms of UDP-GlcNAc, UDP-Man, and UDP-Glc, respectively, during LLO production, are the carbohydrates that attach to LLO utilized during N-glycosylation.

GlcNAc-1-P-transferase (GPT) is an enzyme that is part of the polyprenyl-phosphate N-acetylhexoamine 1-phosphate transferase (PNPT) family and catalyzes the initiation of LLO in human (Lehrman, 1991). The transfer of GlcNAc-1-P from UDP-GlcNAc to dolichol phosphate (DolP) is catalyzed by GPT. DolPP-GlcNAc, a byproduct of GPT, is anchored in the ER membrane to form the N-glycan necessary for N-glycosylation of the protein. The importance of this enzyme in N-glycosylation can be observed in CMS (congenital myasthenic syndrome) and CDG-Ij (congenital disorder of glycosylation type Ij), which are the two most representative diseases. A loss-of-function mutation in the *DPAGT1* gene, which codes for the GPT protein, is known to be the root cause of these two illnesses (Belaya et al., 2012; Wu et al., 2003; Wurde et al., 2012). Protein N-glycosylation is improper in these two disorders, leading to severe neurological dysfunction.

Owing to the recent discovery of the GPT structure, the molecular details of the GPT LLO production pathway are now known (Dong et al., 2018). Certain natural products, such as tunicamycin, which decreases N-glycosylation in cells to create ER stress, can also inhibit the function of GPT (Izumi et al., 2009; Keller et al., 1979; Lehle and Tanner, 1976; Takatsuki et al., 1971; Tkacz and Lampen, 1975; Wang et al., 1997). Additionally, the structure of the GPT-tunicamycin complex has been determined, allowing for molecular studies on how tunicamycin inhibits GPT (Dong et al., 2018; Yoo et al., 2018). In this review, we provide a detailed description of the mechanisms behind the activity of GPT and tunicamycin as inhibitors of GPT, based on the structures of the GPT and GPT-tunicamycin complex.

OVERALL ARCHITECTURE OF GPT

It is necessary to understand the protein structure of GPT to

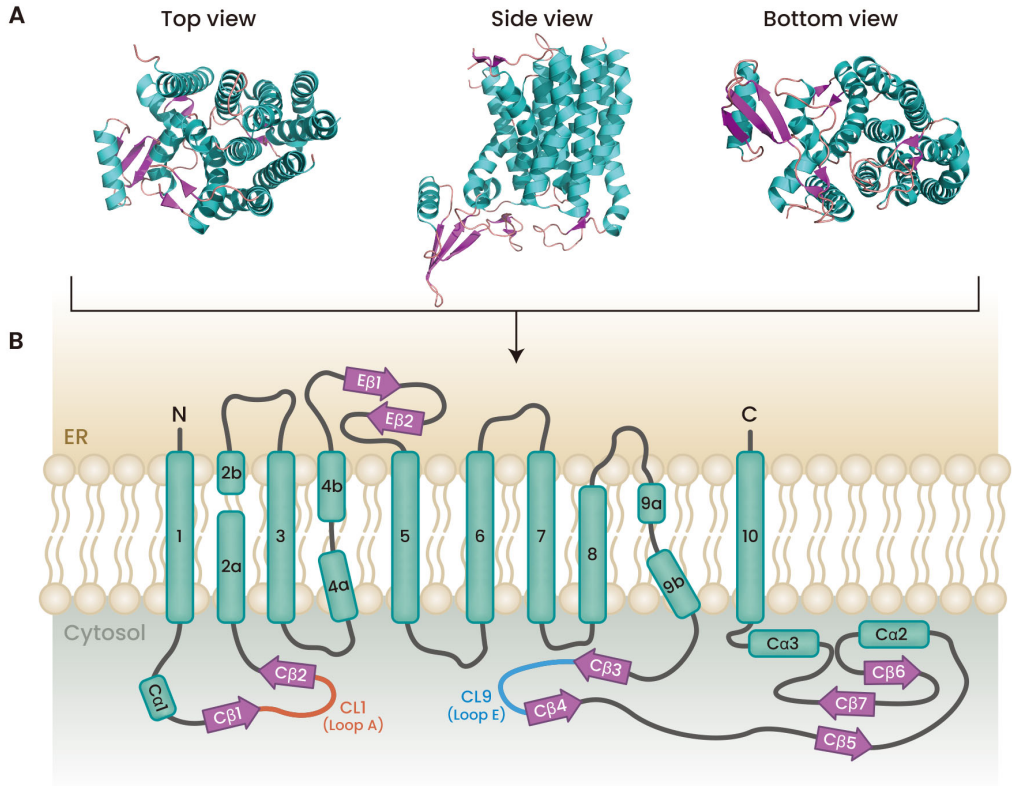

Fig. 1. Overall structure of monomeric GPT. (A) Structure of GPT from different views (PDB ID: 5LEV). Top and bottom view shows an arrangement of each TMH. The structural distinction between the cytoplasmic side and the extracellular side is clear in the side view. (B) Topology of GPT. Orange and cyan indicate CL1, also known as "Loop A," and CL9, also known as "Loop E" (Dong et al., 2018; Yoo et al., 2018). GPT, GlcNAc-1-P-transferase; TMH, transmembrane helices; ER, endoplasmic reticulum.

verify that it plays a role N-glycosylation. To date, X-ray crystallography has been used to determine both the apo-form (PDB:5LEV and 6FM9) and UDP-GlcNAc-bound structures of GPT (PDB:6FWZ) (Dong et al., 2018). Furthermore, the binding structure of tunicamycin and GPT has also been determined (PDB:5O5E, 6BW5, and 6BW6), which means that the inhibition mechanism of tunicamycin against GPT can also be explained at the molecular level (Dong et al., 2018; Yoo et al., 2018).

GPT consists of transmembrane helices (TMH) in the ER membrane (Fig. 1A). TMH are linked to each other by a total of five cytoplasmic loops (CLs). TMH1 and TMH2, in particular, as well as TMH9b and TMH10, have loops that are considerably more exposed to the ER lumen than other loops; Fig. 1B depicts these two loops as "Loop A" and "Loop E," respectively, and they are crucial for binding to the substrates like tunicamycin and UDP-GlcNAc (Dong et al., 2018; Yoo et al., 2018). In contrast, three loops in ER side connecting the TMH in the direction of the ER lumen are comparatively shorter than the CL. GPT has a motif between TMH9b and TMH10 (Fig. 1B). This motif consists of three β-sheets, two α-helices, and two β-hairpins. This structure is absent in bacterial PNPT enzymes, such as MraY, indicating that structural differences may exist between eukaryotic and prokaryotic PNPT enzymes (Chung et al., 2013; Dong et al., 2018; Yoo et al., 2018).

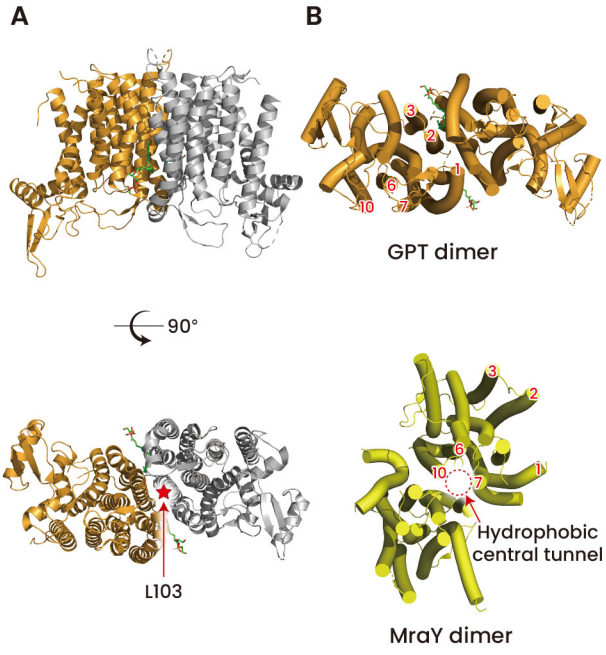

Fig. 2. Dimeric structure of GPT. (A) The view of dimeric structure of GPT. Orange and gray depict each protomer. In GPT, green denotes POPG (PDB ID: 6BW5). The red asterisk indicates the location of L103. (B) Comparison of a dimer interface between GPT (PDB ID: 6BW5) and MraY (PDB ID: 4J72). A POPG is attached to the dimer interface of the GPT. Red arrow points to the MraY's hydrophobic central tunnel. GPT, GlcNAc-1-P-transferase; POPG, 1-palmitoyl-2-oleoyl-sn-glycerol.

In the crystal structure, GPT was found to exist in a homodimer form that displayed two-fold symmetry (Dong et al., 2018; Yoo et al., 2018) (Fig. 2A). The fact that the area of the dimer interface of GPT is rather substantial, around 1,900 Å (16% in total area), demonstrates that the dimerization of GPT is of critical significance in terms of its structural organization. When the residue L103F mutation was generated in the dimer interface of GPT, it was discovered that dimerization of GPT was disrupted, and its stability was extremely low (Dong et al., 2018). Based on these findings, it can be deduced that the process of dimerization of GPT has an important bearing on its stability.

It is fascinating to note that the dimerized form of GPT is significantly distinct from that of MraY, a member of the PNPT superfamily and the bacteriological counterpart of GPT (Fig. 2B). TMH1, 7, and 10 form a dimer interface in MraY, in contrast to GPT, in which TMH1, 2, and 3 form a dimer interface (Chung et al., 2013; Dan and Lehrman, 1997; Yoo et al., 2018). Therefore, the overall array of dimers is laid out horizontally in the case of GPT, but in the case of MraY, it is laid out vertically based on the location of the dimer interface-containing TMH1, 2, and 3. This lends credence to the notion that proteins that are members of the PNPT superfamily share a characteristic known as dimer formation, despite the fact that the shapes of dimers may be highly diverse from one another.

Additionally, in the case of GPT, it was discovered that the ER membrane was in contact with the side of the dimer interface, and that 1-palmitoyl-2-oleoyl-sn-glycerol (POPG) was bound to it (Yoo et al., 2018) (Fig. 2). In contrast, in the case of MraY, there is a hydrophobic tunnel in the middle of the dimer interface, which is believed to represent a particular lipid-binding region (Chung et al., 2013) (Fig. 2B). Because of the structural differences between these two proteins, even though proteins that belong to the PNPT superfamily need lipid molecular modules when forming dimers, the positions at which these lipids bind can vary from protein to protein. In the case of GPT, the activity displayed was dependent on phospholipids (Plouhar and Bretthauer, 1982; 1983). This is assumed to be the case because phospholipids are thought to play a role in GPT dimers as well as govern the stability of GPTs. According to the evidence presented here, the production of dimers of GPT and MraY is controlled by phospholipids.

ACTIVE SITE OF GPT

The structure in which GPT binds to its substrate, UDP-GlcNAc, allows one to observe the active site of the enzyme very clearly (Dong et al., 2018) (Fig. 3). The active site of GPT is encircled by four CLs all the way around it. CL5 and CL7 surround the side of UDP-GlcNAc, and CL1 (also called 'Loop A') covers the upper part of the uridine ring of UDP-GlcNAc. CL9 (also called as 'Loop E') is located above the GlcNAc moiety of UDP-GlcNAc. When the unliganded form of GPT is compared to the form in which UDP-GlcNAc is bound, it is clear that there is no significant difference in the structure of the molecule as a whole. Loop E, which moves approximately 3Å

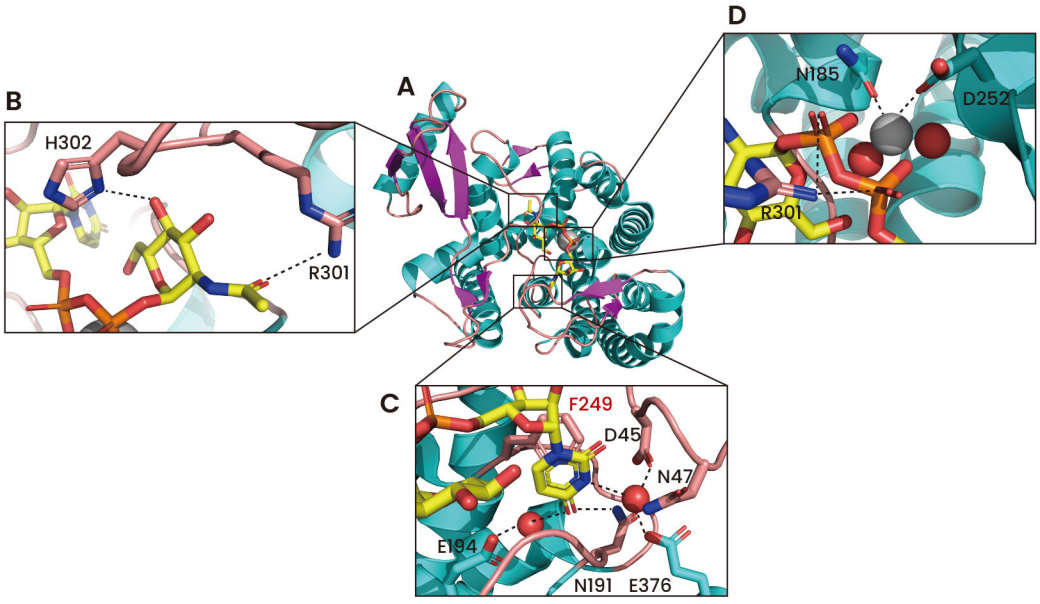

Fig. 3. The complex structure of GPT with UDP-GlcNAc. (A) The overall structure of GPT-UDP-GlcNAc complex (PDB ID: 6FWZ). (B) A magnification of the binding of the GlcNAc to UDP-GlcNAc and GPT. R301 and H302 are participating in the binding with the GlcNAc. Hydrogen bonds formed by R301 and H302 are indicated by black dotted lines. (C) The interaction between GPT and the uridine moiety of UDP-GlcNAc in detail. Red circles depict two water molecules. A black dot line is used to symbolize hydrogen bonds that are a part of the interaction between GPT and UDP-GlcNAc. F249, which makes a π-π stacking is labeled as red. (D) Interactions between GPT and the pyrophosphate part of UDP-GlcNAc. The black dotted line denotes hydrogen bonding, and the Mg^{2+} ions are indicated by a gray sphere. GPT, GlcNAc-1-P-transferase.

closer to GlcNAc as UDP-GlcNAc binds, is the primary contributor to the differences between the two structures (Fig. 3B). This structural change can be understood as a structural change in the form of an induced fit caused by the binding of the substrate.

The grooves created by CL5 and CL7 can serve as binding sites for the uridine moiety of UDP-GlcNAc (Dong et al., 2018). The uridine ring and the residue that surrounds it form numerous bonds in this groove (Fig. 3C). Five residues surrounding UDP-uridine GlcNAc form a hydrogen-bond network with the ring. The hydrogen bonds that form between the uridine ring and the surrounding residues are joined by two water molecules. The interaction between the uridine ring and F249 is by π-π stacking, which is present in both GPT and MraY (Yoo et al., 2018) (Fig. 3C). Given this, it is thought that for GPT to recognize UDP-GlcNAc, the hydrogen bond and stacking interaction formed between GPT and the uridine ring are essential.

The pyrophosphate of UDP-GlcNAc, which joins the uridine moiety and GlcNAc, also participates in binding with GPT (Dong et al., 2018) (Fig. 3D). Two hydrogen bonds were created between the two pyrophosphate oxygens and the side chain of R301 in Loop E. As already mentioned, the position of Loop E is brought closer to GlcNAc by the binding of GPT with UDP-GlcNAc, and the hydrogen bond between R301 and pyrophosphate also appears to be a factor in this structural change. The oxygen that binds to the Mg^{2+} ion is located across the oxygen that binds to R301 in pyrophosphate. This Mg^{2+} ion appears to mediate the binding of UDP-GlcNAc to GPT because it binds to N185 and D252 of GPT, in addition to the two oxygens of pyrophosphate (Fig. 3D). A stable bond configuration in the octahedral form is indicated by the simultaneous formation of the bond between the Mg^{2+} ion and two water molecules. Studies have revealed the structural similarity of MraY's coordination of the Mg^{2+} ion to GPT and the fact that this Mg^{2+} ion is crucial for enzyme activity of both MraY and GPT (Al-Dabbagh et al., 2008; Bouhss et al., 2008; Chung et al., 2013; Kaushal and Elbein, 1985). It seems that Mg^{2+} ions play a crucial role in catalyzing the chemical reaction of transferring sugar from UDP-sugar to the lipid substrate in enzymes that belong to the PNPT superfamily, in addition to maintaining stable binding to the substrate. Therefore, although the binding structures of GPT, UDP-GlcNAc, and DolP have not yet been determined, the active mechanism of GPT can be predicted based on the results of prior investigations. The pyrophosphate bridge linking UDP and GlcNAc is broken, and a new pyrophosphate bridge is generated between DolP and GlcNAc when the oxygen of DolP's phosphate in GPT contacts the β-phosphate of UDP-GlcNAc, another substrate, through a nucleophile attack. DolP-GlcNAc is produced as a product, and UMP is released from GPT (Dong et al., 2018).

In UDP-GlcNAc, CL5, and CL9 (Loop E) encircle GlcNAc (Dong et al., 2018) (Fig. 3B). H302 and R301 of Loop E form hydrogen bonds with the OH3 and OH4 of GlcNAc, respectively. These two residues work with GlcNAc to form Loop E in a position near GlcNAc, similar to the role of R301 mentioned earlier (Dong et al., 2018; Yoo et al., 2018). Intriguingly, Loop E binds to GlcNAc in GPT, but not in MraY, resulting in structural alterations. It is impossible to demonstrate

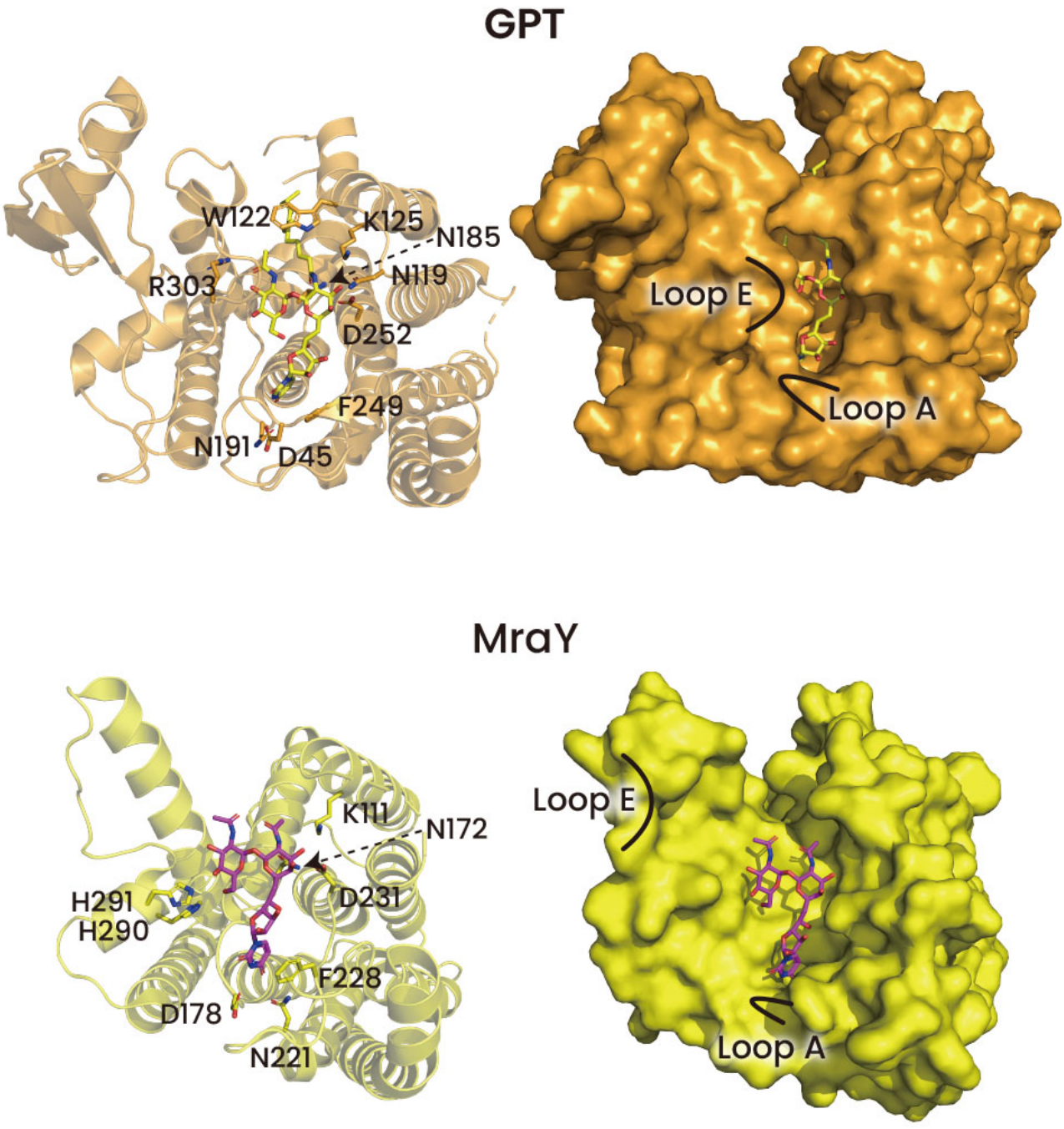

Fig. 4. The comparison of tunicamycin binding to GPT and MraY. A cartoon and surface model of tunicamycin binding to GPT and MraY were used to illustrate the binding (PDB ID: 6BW5 and 5JNQ). In GPT and MraY, yellow magenta denotes tunicamycin. The lipid tail of tunicamycin was not visible in the structure of MraY, which has a complex structure. The thick black line shows where Loops A and E are in GPT and MraY. GPT, GlcNAc-1-P-transferase

structural alterations induced by binding with GlcNAc in the instance of MraY because, unlike GPT, Loop E is relatively short. These variations result in the long Loop E in GPT not fully exposing the region to which GlcNAc binds, whereas MraY does. This indicates that GPT is more selective for sugary moieties than MraY because it has a lower spatial margin for them (Yoo et al., 2018).

INHIBITORY MECHANISM OF TUNICAMYCIN IN GPT

Inhibitors of enzymes in the PNPT superfamily include tunicamycin, which also inhibits the activity of GPT, prevents N-glycosylation, and induces ER stress in humans. Although the effect of tunicamycin has been known for some time, its mechanism of action remains unclear. However, now that the complex structure between GPT and tunicamycin has been discovered, it is possible to examine the molecular basis of the mechanism of action of tunicamycin (Dong et al., 2018; Yoo et al., 2018).

TMH4, 5, 6, and 8 comprise the binding region of tunicamycin, which is nearly identical to the region to which UDP-GlcNAc binds (Figs. 4 and 5). The uridine moiety of tunicamycin is covered with CL1 (Loop A), and the GlcNAc component is covered with Loop E, specifically like UDP-GlcNAc. The relationship between the uridine moiety of tunicamycin and GPT was comparable to that between GPT and UDP-GlcNAc. The hydrogen bond network in the uridine moiety of tunicamycin and the π-π stacking interaction with F249 were identical to those of UDP-GlcNAc (Fig. 4). However, UDP-GlcNAc and tunicamycin differ somewhat in terms of the GlcNAc moiety. While only R303 contributed to binding to the GlcNAc moiety of tunicamycin, both H302 and R303 did so for UDP-GlcNAc. However, because of the interaction between tunicamycin and R303, Loop E approaches GlcNAc in a manner identical to that of UDP-GlcNAc (Fig. 4).

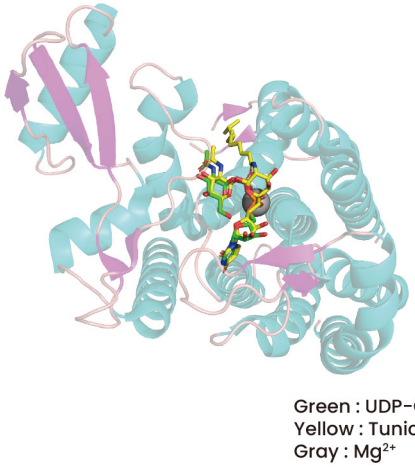

Fig. 5. The comparison of tunicamycin binding and UDP-GlcNAc binding to GPT. In the GPT structure, UDP-GlcNAc, Mg^{2+} and tunicamycin were superimposed. Green, gray, and yellow labels were used to identify each one. The figure demonstrates that the binding sites for Mg^{2+}, UDP-GlcNAc, and tunicamycin were consistent. GPT, GlcNAc-1-P-transferase.

Overall, interactions between the tunicamine moiety of tunicamycin and GPT differ significantly from those between the uridine and GlcNAc moieties, which are comparable to those between UDP-GlcNAc and GlcNAc. As the pyrophosphate moiety of UDP-GlcNAc is replaced by the tunicamine moiety of tunicamycin, the interaction created in the tunicamine moiety of tunicamycin can only be distinct from that of UDP-GlcNAc (Fig. 5). This is because the structure of tunicamine is significantly different from that of pyrophosphate. First, the tunicamine moiety does not interact with R301, which interacts with the pyrophosphate of UDP-GlcNAc (Fig. 4). However, as was already mentioned above, since R303 interacts with the GlcNAc of tunicamycin, Loop E moves closer to GlcNAc even if R301 and the pyrophosphate in UDP-GlcNAc do not interact.

The Mg^{2+} ion implicated in the binding of GPT and UDP-GlcNAc was not observed in the binding of GPT and tunicamycin, which is the most notable change in the tunicamine moiety (Figs. 4 and 5). The position of the tunicamine moiety in the binding structure of GPT and tunicamycin corresponds to the location of the Mg^{2+} ion in the binding structure of UDP-GlcNAc and GPT (Fig. 5). In this instance, the tunicamine moiety interacted with N185 and D252 without Mg^{2+} as a mediator. Due to this structural change, dependence of tunicamycin on Mg^{2+} when associated with GPT is at odds with earlier predictions (Price and Momany, 2005; Wang et al., 1997; Xu et al., 2004). Contrary to GPT, MraY and tunicamycin interactions are interestingly Mg^{2+} ion-dependent. This was supported by the finding of a mutation in which the Asp residue in MraY, which interacts with the Mg^{2+} ion, was changed to Ala, resulting in a considerable reduction in tunicamycin binding affinity to MraY, but not to GPT (Yoo et al., 2018). These results suggest that Mg^{2+} plays a distinct role in GPT and MraY in tunicamycin binding.

Tunicamycin possesses a lipid tail that is comparable to the DolP structure. In the binding structure of GPT and tunicamycin, the lipid tail of tunicamycin is situated in the groove formed by GPT between TMH4, 5, and 9 (Dong et al., 2018; Yoo et al., 2018). To date, the binding structure of DolP and GPT has not been identified; therefore, it can be expected that DolP binds to the lipid tail of tunicamycin. Unlike the apo structure of GPT and the UDP-GlcNAc binding structure, where the side chain of W122 is facing outward, in the binding structure of GPT and tunicamycin, W122 faces the side chain toward the lipid tail which traps the lipid tail (Fig. 4). Therefore, W122 in GPT plays a critical role in the binding of tunicamycin to GPT, and it is anticipated that this W122 will also be present when DolP attaches to GPT. In the instance of MraY, it is interesting to note that Pro is situated where W122 of GPT is located. Given the structural difference between Trp and Pro and the variation in lipid substrates between GPT and MraY, it is reasonable to conclude that W122 contributes to selectivity of GPT for lipid substrates (Yoo et al., 2018).

Overall, GPT has a relatively long length of Loop A and Loop E compared to MraY (Fig. 4). Therefore, in GPT, the uridine and GlcNAc moieties of tunicamycin are largely ob-

scured by Loop A and Loop E, so that the compound is not exposed to the outside when bound to GPT. However, in the case of MraY, Loop A and Loop E are relatively shorter than GPT; therefore, it can be observed that most of the tunicamycin is completely exposed to the outside when bound to MraY. These two protein structural differences are thought to have a significant impact on the respective selectivity of GPT and MraY to their substrates, which is expected to provide clues to develop antibiotics that can specifically act on MraY (Hakulinen et al., 2017; Mashalidis and Lee, 2020; Yoo et al., 2018).

The intricate relationship between the structures of GPT and tunicamycin demonstrates that tunicamycin reduces GPT activity. The molecular structure of tunicamycin is highly similar to the shapes of UDP-GlcNAc and DolP, which are substrates of GPT; hence, it can bind to the active site of GPT like UDP-GlcNAc and DolP. Additionally, the structure in which tunicamycin is bound to GPT is strikingly comparable to that of UDP-GlcNAc. These protein structures demonstrate how tunicamycin competes with the GPT substrates UDP-GlcNAc and DolP to suppress GPT activity. The fact that tunicamycin can effectively limit the activity of GPT through competitive interactions with UDP-GlcNAc and DolP is supported by the fact that the Kd values of pure GPT protein and tunicamycin are approximately 6 nM (Yoo et al., 2018).

DISCUSSION

This review explains how GPT, an enzyme that is crucial in initiating intracellular N-glycosylation, is engaged in N-glycosylation based on its structure. In addition, through the binding structure of tunicamycin and GPT, the N-glycosylation inhibition mechanism of tunicamycin was identified at the molecular level.

Even though tunicamycin is a natural substance with alluring antibacterial effects, GPT works as an "off-target" of tunicamycin, which is why it has not yet been used in clinical practice. Therefore, it is essential to develop tunicamycin so that it can specifically act only on MraY and not GPT to establish it as a next-generation antibiotic. Tunicamycin derivatives with great selectivity solely for MraY are continuously being created based on the structures of GPT and MraY that have been previously found (Dong et al., 2018; Mashalidis and Lee, 2020). The creation of novel antibiotics is urgently required to address bacterial infections such as tuberculosis, which have high rates of transmission and mortality, as well as to combat the spread of antibiotic-resistant strains. To achieve these objectives, research should be conducted to comprehend the mechanism of action of natural products like tunicamycin through the structure of GPT and MraY and create novel antibiotics based on this knowledge (Chung et al., 2016; Mashalidis et al., 2019; Nakaya et al., 2022).

In addition to antibiotics, several studies have revealed that tunicamycin has anticancer effects (Wang et al., 2020; Wu et al., 2018; Zhao et al., 2020). It was confirmed that tunicamycin showed anticancer effects in various cancers such as gastric cancer as well as head and neck cancer. Tunicamycin induces strong ER stress and exhibits anticancer effects because it effectively blocks N-glycosylation in cancer cells (Wang et al., 2020; Wu et al., 2018; Zhao et al., 2020). Hence, while it is currently difficult to use tunicamycin as an anticancer agent due to its toxicity, the possibility of its development as a new anticancer agent is substantial, as it has shown to have strong anticancer potential. As anticancer drugs that inhibit N-glycosylation and exhibit anticancer effects have not yet been used in cancer patients, it is worthwhile to investigate the anticancer effects of tunicamycin in patients with anticancer therapy resistance.

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AUTHOR CONTRIBUTIONS

J.S.C., Y.L., and J.Y. supervised the overall process and reviewed and edited the manuscript. D.Y., J.H.M., A.C., and H.B. wrote the original draft and designed the figure and tables.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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REFERENCES

Aebi, M. (2013). N-linked protein glycosylation in the ER. Biochim. Biophys. Acta *1833*, 2430-2437.

Al-Dabbagh, B., Henry, X., El Ghachi, M., Auger, G., Blanot, D., Parquet, C., Mengin-Lecreulx, D., and Bouhss, A. (2008). Active site mapping of MraY, a member of the polyprenyl-phosphate N-acetylhexosamine 1-phosphate transferase superfamily, catalyzing the first membrane step of peptidoglycan biosynthesis. Biochemistry *47*, 8919-8928.

Belaya, K., Finlayson, S., Slater, C.R., Cossins, J., Liu, W.W., Maxwell, S., McGowan, S.J., Maslau, S., Twigg, S.R., Walls, T.J., et al. (2012). Mutations in DPAGT1 cause a limb-girdle congenital myasthenic syndrome with tubular aggregates. Am. J. Hum. Genet. *91*, 193-201.

Blom, N., Sicheritz-Ponten, T., Gupta, R., Gammeltoft, S., and Brunak, S. (2004). Prediction of post-translational glycosylation and phosphorylation of proteins from the amino acid sequence. Proteomics *4*, 1633-1649.

Bouhss, A., Trunkfield, A.E., Bugg, T.D., and Mengin-Lecreulx, D. (2008). The biosynthesis of peptidoglycan lipid-linked intermediates. FEMS Microbiol. Rev. *32*, 208-233.

Chung, B.C., Mashalidis, E.H., Tanino, T., Kim, M., Matsuda, A., Hong, J., Ichikawa, S., and Lee, S.Y. (2016). Structural insights into inhibition of lipid I production in bacterial cell wall synthesis. Nature *533*, 557-560.

Chung, B.C., Zhao, J., Gillespie, R.A., Kwon, D.Y., Guan, Z., Hong, J., Zhou, P., and Lee, S.Y. (2013). Crystal structure of MraY, an essential membrane enzyme for bacterial cell wall synthesis. Science *341*, 1012-1016.

Dan, N. and Lehrman, M.A. (1997). Oligomerization of hamster UDP-

GlcNAc:dolichol-P GlcNAc-1-P transferase, an enzyme with multiple transmembrane spans. J. Biol. Chem. *272*, 14214-14219.

Dong, Y.Y., Wang, H., Pike, A.C.W., Cochrane, S.A., Hamedzadeh, S., Wyszynski, F.J., Bushell, S.R., Royer, S.F., Widdick, D.A., Sajid, A., et al. (2018). Structures of DPAGT1 explain glycosylation disease mechanisms and advance TB antibiotic design. Cell *175*, 1045-1058.e16.

Goulabchand, R., Vincent, T., Batteux, F., Eliaou, J.F., and Guilpain, P. (2014). Impact of autoantibody glycosylation in autoimmune diseases. Autoimmun. Rev. *13*, 742-750.

Hakulinen, J.K., Hering, J., Branden, G., Chen, H., Snijder, A., Ek, M., and Johansson, P. (2017). MraY-antibiotic complex reveals details of tunicamycin mode of action. Nat. Chem. Biol. *13*, 265-267.

Haltiwanger, R.S. and Lowe, J.B. (2004). Role of glycosylation in development. Annu. Rev. Biochem. *73*, 491-537.

Huang, K.Y., Lee, T.Y., Kao, H.J., Ma, C.T., Lee, C.C., Lin, T.H., Chang, W.C., and Huang, H.D. (2019). dbPTM in 2019: exploring disease association and cross-talk of post-translational modifications. Nucleic Acids Res. *47*(D1), D298-D308.

Izumi, M., Yuasa, H., and Hashimoto, H. (2009). Bisubstrate analogues as glycosyltransferase inhibitors. Curr. Top. Med. Chem. *9*, 87-105.

Karve, T.M. and Cheema, A.K. (2011). Small changes huge impact: the role of protein posttranslational modifications in cellular homeostasis and disease. J. Amino Acids *2011*, 207691.

Kaushal, G.P. and Elbein, A.D. (1985). Purification and properties of UDP-GlcNAc:dolichyl-phosphate GlcNAc-1-phosphate transferase. Activation and inhibition of the enzyme. J. Biol. Chem. *260*, 16303-16309.

Keller, R.K., Boon, D.Y., and Crum, F.C. (1979). N-Acetylglucosamine- 1 -phosphate transferase from hen oviduct: solubilization, characterization, and inhibition by tunicamycin. Biochemistry *18*, 3946-3952.

Lauc, G., Huffman, J.E., Pucic, M., Zgaga, L., Adamczyk, B., Muzinic, A., Novokmet, M., Polasek, O., Gornik, O., Kristic, J., et al. (2013). Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers. PLoS Genet. *9*, e1003225.

Lehle, L. and Tanner, W. (1976). The specific site of tunicamycin inhibition in the formation of dolichol-bound N-acetylglucosamine derivatives. FEBS Lett. *72*, 167-170.

Lehrman, M.A. (1991). Biosynthesis of N-acetylglucosamine-P-P-dolichol, the committed step of asparagine-linked oligosaccharide assembly. Glycobiology *1*, 553-562.

Mashalidis, E.H., Kaeser, B., Terasawa, Y., Katsuyama, A., Kwon, D.Y., Lee, K., Hong, J., Ichikawa, S., and Lee, S.Y. (2019). Chemical logic of MraY inhibition by antibacterial nucleoside natural products. Nat. Commun. *10*, 2917.

Mashalidis, E.H. and Lee, S.Y. (2020). Structures of bacterial MraY and human GPT provide insights into rational antibiotic design. J. Mol. Biol. *432*, 4946-4963.

Nakaya, T., Yabe, M., Mashalidis, E.H., Sato, T., Yamamoto, K., Hikiji, Y., Katsuyama, A., Shinohara, M., Minato, Y., Takahashi, S., et al. (2022). Synthesis of macrocyclic nucleoside antibacterials and their interactions with MraY. Nat. Commun. *13*, 7575.

Ohtsubo, K. and Marth, J.D. (2006). Glycosylation in cellular mechanisms of health and disease. Cell *126*, 855-867.

Plouhar, P.L. and Bretthauer, R.K. (1982). A phospholipid requirement for dolichol pyrophosphate N-acetylglucosamine synthesis in phospholipase A2-treated rat lung microsomes. J. Biol. Chem. *257*, 8907-8911.

Plouhar, P.L. and Bretthauer, R.K. (1983). Restoration by phospholipids of dolichol pyrophosphate N-acetylglucosamine synthesis in delipidated rat lung microsomes. J. Biol. Chem. *258*, 12988-12993.

Price, N.P. and Momany, F.A. (2005). Modeling bacterial UDP-HexNAc: polyprenol-P HexNAc-1-P transferases. Glycobiology *15*, 29R-42R.

Ramazi, S., Allahverdi, A., and Zahiri, J. (2020). Evaluation of post-translational modifications in histone proteins: a review on histone modification defects in developmental and neurological disorders. J. Biosci. *45*, 135.

Takatsuki, A., Arima, K., and Tamura, G. (1971). Tunicamycin, a new antibiotic. I. Isolation and characterization of tunicamycin. J. Antibiot. (Tokyo) *24*, 215-223.

Tkacz, J.S. and Lampen, O. (1975). Tunicamycin inhibition of polyisoprenyl N-acetylglucosaminyl pyrophosphate formation in calf-liver microsomes. Biochem. Biophys. Res. Commun. *65*, 248-257.

Wang, R., Steensma, D.H., Takaoka, Y., Yun, J.W., Kajimoto, T., and Wong, C.H. (1997). A search for pyrophosphate mimics for the development of substrates and inhibitors of glycosyltransferases. Bioorg. Med. Chem. *5*, 661-672.

Wang, Y., Zhang, L., He, Z., Deng, J., Zhang, Z., Liu, L., Ye, W., and Liu, S. (2020). Tunicamycin induces ER stress and inhibits tumorigenesis of head and neck cancer cells by inhibiting N-glycosylation. Am. J. Transl. Res. *12*, 541-550.

Wu, J., Chen, S., Liu, H., Zhang, Z., Ni, Z., Chen, J., Yang, Z., Nie, Y., and Fan, D. (2018). Tunicamycin specifically aggravates ER stress and overcomes chemoresistance in multidrug-resistant gastric cancer cells by inhibiting N-glycosylation. J. Exp. Clin. Cancer Res. *37*, 272.

Wu, X., Rush, J.S., Karaoglu, D., Krasnewich, D., Lubinsky, M.S., Waechter, C.J., Gilmore, R., and Freeze, H.H. (2003). Deficiency of UDP-GlcNAc:Dolichol Phosphate N-Acetylglucosamine-1 Phosphate Transferase (DPAGT1) causes a novel congenital disorder of Glycosylation Type Ij. Hum. Mutat. *22*, 144-150.

Wurde, A.E., Reunert, J., Rust, S., Hertzberg, C., Haverkamper, S., Nurnberg, G., Nurnberg, P., Lehle, L., Rossi, R., and Marquardt, T. (2012). Congenital disorder of glycosylation type Ij (CDG-Ij, DPAGT1-CDG): extending the clinical and molecular spectrum of a rare disease. Mol. Genet. Metab. *105*, 634-641.

Xu, L., Appell, M., Kennedy, S., Momany, F.A., and Price, N.P. (2004). Conformational analysis of chirally deuterated tunicamycin as an active site probe of UDP-N-acetylhexosamine:polyprenol-P N-acetylhexosamine-1-P translocases. Biochemistry *43*, 13248-13255.

Yoo, J., Mashalidis, E.H., Kuk, A.C.Y., Yamamoto, K., Kaeser, B., Ichikawa, S., and Lee, S.Y. (2018). GlcNAc-1-P-transferase-tunicamycin complex structure reveals basis for inhibition of N-glycosylation. Nat. Struct. Mol. Biol. *25*, 217-224.

Zhao, G., Kang, J., Xu, G., Wei, J., Wang, X., Jing, X., Zhang, L., Yang, A., Wang, K., Wang, J., et al. (2020). Tunicamycin promotes metastasis through upregulating endoplasmic reticulum stress induced GRP78 expression in thyroid carcinoma. Cell Biosci. *10*, 115.