Glycosyltransferases: Unraveling Molecular Insights and Biotechnological Implications

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Abstract: Glycosyltransferases (GTs) are present in almost all living organisms; plants, animals and microorganisms. GTs transfer sugar molecule from nucleotide sugars to a wide range of molecules including hormones, secondary metabolites, biotic and abiotic chemicals. When glycosyltransferases add a sugar moiety in any molecule, the hydrophilicity of that molecule changes and thus alter the chemical properties of the molecule. This phenomenon is vital for appropriate working of living organisms. For the first time, X-ray structure of bacteriophage T4-glucosyltransferase was reported in 1994. In bacteria, GTs play essential roles in various biological processes such as cell wall biosynthesis, surface glycosylation and virulence factor production. The point mutation as well as the domain-swapping has been reported in Bacteria. The sequence change as well as the whole cells has been engineered in bacteria too. GTs play very important role in survival, growth, development, metabolism, detoxification, insecticide resistance, chitin formation, chemosensation, defense and immunity, involved in various signaling pathways, etc. In plants, glycosyltransferase enzymes play essential role in biosynthesis of cell wall components, secondary metabolites, and signaling molecules. GTs are involved in the transfer of sugar moieties from activated donor molecules to specific acceptor molecules, leading to the formation of glycosidic bonds. GTs modify flavonoids, alkaloids and terpenoids, etc. with sugar residues and alter the solubility, stability, and bioactivity of these compounds and regulate the plant defense mechanism and interaction with insects, microorganisms and other organisms. GTs have direct impact on plant homeostasis. Site directed mutagenesis (SDM) in UGTs or GTs cause a change in substrate specificity and produce increased or total loss in the catalytic activity GTs. This kind of change demonstrated that a change in substrate specificity could cause better glycosylation and perked up anticancer activity of UGTs. GTs are also involved in glycosylaton of phytohormones and regulate their metabolism and signaling pathways. GTs are involved in the activity, stability, and transport of these hormones and influence the plant growth, development, and responses to various environmental stimuli. Four UGT families encoding 200 genes are reported in humans which regulate cell signaling, protein folding, immune response, growth and development, detoxification, metabolism and elimination of drugs, DNA methylation and histone modifications, transcriptional regulation, post-transcriptional regulation and post-translational regulation, synthesis of human blood group antigens A and B and recently GTs are also reported as linked with COVID-19-related loss of smell or taste. Various bioinformatics tools have been developed which would help analyse *in silico*, the structure of the GTs using any reference enzyme. The activity and the ordered structures along with various stability assays can be performed before to conduct *in vitro* analyses such as mutagenesis. Targeted mutagenesis have been reported through site

directed mutagenesis (SDM) or domain-swapping. Standard protocols of molecular biology i.e. transformation, protein expression, extraction and purification followed by mass spectrometric analysis has been described. This molecular technique would direct future endeavours to engineer more glycosyltransferases to augment their activity with different substrates and provide a basis for more exploration of GTs as an active compound for potential anti-cancer therapeutics. Additionally, the role of GTs in medicine, food industry, pharmaceutical industry and agriculture is discussed. More research work is needed for the better understanding of the biological processes and the mechanisms of glycosyltransferases involved in cancer, tumor, drug metabolism etc. New era of engineering is awaited to engineer these GT enzymes *in vitro* to get them boost in industry as well as to help cure cancer and other diseases as well.

Introduction

Glycosyltransferases (GTs) are present in almost all living organisms; plants, animals and microorganisms (Moremen et al. 2019; Rini et al. 2022; Andreu et al. 2023). They are involved in glycosylation reaction and change the hydrophilicity of molecules in living organisms and helps in detoxification and stabilization of natural products (Sakakibara, 2009; Bowles and Lim, 2010; Andreu et al. 2023). The glycosylation, in fact, is responsible for cell homeostasis and key mechanism catalyzed by glycosyltransferases to orchestrate bioactivity, metabolism and the position of molecules inside cells (Offen et al., 2006; Rini et al. 2022). GTs catalyze glycosyl group transfer with inversion or retention of the anomeric stereochemistry with respect to the donor sugar (Fig. 1)

Structure and mechanism of glycosyltransferases

For the first time, X-ray structure of bacteriophage T4-glucosyltransferase was reported in 1994 (Vrielink *et al.,* 1994). Later, specific conserved domains were reviewed in glycosyltransferases which recognize the donor and acceptor molecules. In addition, the database for homologous sequences was also studied and the conserved amino acids were found (Kapitonov and Yu, 1999). The crystal structure and sequence-based classification of glycosyltransferases divided these enzymes into many families which represent the diversity of acceptor molecules used by these enzymes (Coutinho *et al.,* 2003). GTs catalyze the biosynthesis of glycosidic bond during which a nucleoside phosphate sugar is used as a donor molecule. Two structural folds of GTs have been reported, i.e. GT-A and GT-B; nucleotide sugar dependent and lipid phosphosugar-dependent folds, respectively (Breton *et al.,* 2006; Lairson *et al*., 2008). New folds were also discovered in bacterial sialytransferase and three dimensional databases were generated to gather the crystal structure of GTs (Breton *et al.,* 2006). GTs are involved in the biosynthesis of oligosaccharides, polysaccharides and glycoconjugates. According to sequence similarity, GTs are divided into 91 families (Kim et al., 2006). Coordinated action of number of glycosyltransferases catalyzes the transfer of sugar residue from donor molecule to acceptor molecule. Hansen *et al.* (2012) revealed that still many protein families in GTs have not been recognized and reviewed these unknown glycosyltransferases with their role in the biosynthesis of cell wall (Hansen *et al.,* 2012). Three-dimensional model was constructed using *Withania somnifera*, which showed that the GTs have some GT-B type folds (Jadhav *et al.,* 2012).

This enzyme group is very old and the research work related to structure, function and cellular mechanism of this class of enzymes has not been extensively performed yet. Moreover, subsequent kinetic studies have revealed important aspects in various mechanisms of glycosyltransferases (Breton *et al*., 2012). The detailed mechanism of molecular biology, gene expression and regulation for *in vivo* glycosylation is still inadequate for comprehensive understanding of glycosyltransferases (Kizuka *et al*., 2014).

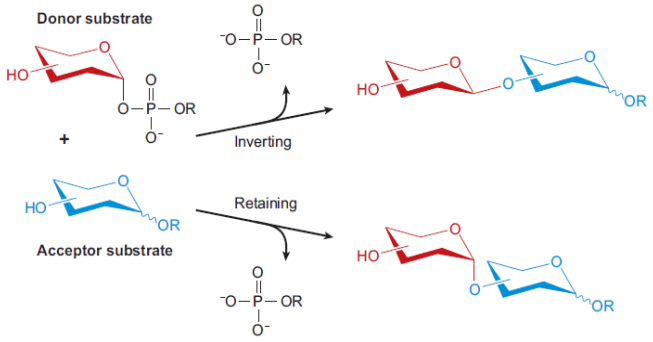

Figure 1: Glycosyltransferase and glycosyl transfer mechanism

Glycosyltransferases catalyze glycosyl group transfer with either inversion or retention of the anomeric stereochemistry with respect to the donor sugar (Taken from Coutinho et al., 2003)

1. Bacteria and glycosyltransferases

In prokaryotes, glycosylation occur in cytoplasm and periplasmic space, whereas in eukaryotes, it takes place in cytosol, golgi complex and the endoplasmic reticulum (Liang *et al*., 2015). In bacteria, GTs play essential roles in various biological processes such as cell wall biosynthesis, surface glycosylation and virulence factor production (Moremen and Haltiwanger, 2019). These enzymes are involved in the synthesis of polysaccharides, glycolipids, and glycoproteins, which are essential for bacterial survival, pathogenicity, and interaction with the host environment (Yakovlieva & Walvoort, 2019; Yakovlieva *et al*., 2021; Rini *et al*., 2022). GTs catalyze the transfer of sugar moieties from activated donor molecules to the specific acceptor molecules, leading to the formation of glycosidic bonds (Schmid *et al*., 2016; Andreu *et al*., 2023). In Gram-negative bacteria, GTs are involved in the assembly of complex carbohydrate structures, i.e. O-antigen of lipopolysaccharides. Similarly, other lipid-linked sugars serve as donor substrates for bacterial glycosyltransferases involved in the assembly of peptidoglycan in both Gram-negative and Gram-positive bacteria (Schmid *et al*., 2016).

The point mutation as well as the domain-swapping has been reported in Bacteria. In *Helicobacter pylori*, studies on molecular biology has been employed to generate twelve fucosyltransferase chimeras to better understand the regioselectivity of a-1,3/4-fucosyltransferases. It was found that 347-353 residues were the key region that regulated a-1,4 activity (Ma *et al*., 2003). Further, mutagenesis analyses of these seven amino acids revealed that Y350 was responsible for a-1,4 activity. Molecular studies confirmed the absolute need for an aromatic residue at this position along with the tyrosine hydroxyl group for optimal activity (Ma *et al*., 2005).

In *Pasteurella multocida*, engineering through activity knock-out on hyaluronan synthase was used to synthesize glycosaminoglycans (Jing & DeAngelis, 2004). Hyaluronan synthase possesses two catalytic domains (b-1,3-N-acetylglucosaminyltransferase and b-1,4-glucuronosyltransferase activities, respectively) and mutation in one domain caused loss of activity and produced monofunctional GTs. These mutant GTs could be immobilized and used in alternation for the production glycosaminoglycans (Jing & DeAngelis, 2003). In *E. coli*, engineering of GTs have been reported to synthesize oligosaccharide moieties of gangliosides GM3, GM2 (Fort *et al*., 2005). Similarly, mutation at Gln189 in a-galactosyltransferase from *Neisseria meningitides* also resulted in reduced activity of transferase (Lairson *et al*., 2004). Interestingly, beside sequence change, the whole cells were also engineered by introducing genes for carbohydrate-processing enzymes which showed valuable biosynthetic capabilities of GTs to generate large scale production of oligosaccharides. Herein, the whole cells overexpress the genes encoding glycosidases, GTs and sugar–nucleotide biosynthetic machinery (Hancock *et al*., 2006).

2. Insects and glycosyltransferases

The first ever evidence of glycosyltransferase activity in insects was obtained from feces of a locust, *Locusta migratoria* (Myers and Smith, 1954).

Glycosyltransferases play very important role in survival, development, metabolism and immunity. GTs are involved in the biosynthesis of complex carbohydrates, glycoproteins, and glycolipids which are essential for insect growth, reproduction, and response to environmental challenges (Nagare *et al*., 2021). GT-mediated detoxification in most of the insects is a defense strategy against plant allelochemicals and xenobiotic compounds. It has been reported that insects evolved a physiological modulation to feed on plants and hence; thereby, glyco-conjugation of lipophilic molecules by GTs convert them into water-soluble products which are then excreted out of the body (Winde and Wittstock, 2011). GTs are also thought to interact in detoxification with other enzymes such as glutathione-S-transferases (GST), phosphotransferases, sulfotransferases, aminotransferases and glycosidases (Berenbaum and Johnson, 2015). It is worth mentioning here that many of these enzyme families are an outcome of Horizontal Gene Transfer (HGT) between prokaryotic organisms and arthropod genome (Wybouw et al., 2016).

GTs are expressed in multiple tissues including fat bodies, haemolymph, antennae, midgut, legs, wings and gonads (Bozzolan *et al*., 2014). In *Bombyx mori*, GTs are expressed in various tissues i.e. testis, ovary, head, integument, fat body, midgut, haemocyte, malpighian tubules and silk glands (Huang *et al*., 2008). Tissue specific expression of GTs have been reported in the gut in *Athetis lepigone* moth involved in degrading plant allelochemicals and detoxification of insecticide (Zhang *et al*., 2017). In some lepidopteran insects i.e. *Helicoverpa armigera*, *Helicoverpa zea* and *Helicoverpa assulta*; GTs detoxify capsaicin by glycosylation and help excrete then the inactivated toxin in the form of capsaicin glucoside (Ahn *et al*., 2012). In *Myzus persicae nicotianae*, RNAi-mediated silencing revealed that four highly expressed UGT genes of UGT330A3, UGT344D5, UGT348A3 and UGT349A3 are required in the detoxification of nicotine (Pan *et al*., 2019). Few conserved UDP Glycosyltransferases (UGTs) such as UGT50A1 are expressed throughout the insect body and also have orthologs in humans (UGT8A1) or other higher eukaryotes. The conserved and ubiquitous expression of GTs might be involved in glycosylation of cell membrane lipid moieties and play an important role in the cellular homeostasis (Ahn et al., 2012).

GTs perform crucial functions in developmental processes of insects such as eye development, epithelial development, ommatidia development, embryonic development (Chen *et al*., 2007; Hagen *et al*., 2009), tissue differentiation, chitin synthesis for cuticle formation, cuticular tanning and body pigmentation, UV shielding; homeostasis by regulating diverse metabolic pathways, neuronal differentiation, chemosensation, odorant detection, communication, mate recognition, and defense against desiccation as well as various external and internal threats such as predator attacks, physiological dysfunctions etc. GTs play significant role in regulating developmental processes like organogenesis, metamorphosis and gametogenesis in insects (Walski *et al*., 2017). Signaling pathways like Notch signaling, Hedgehog (Hh) and Decapentaplegic (Dpp) in *Drosophila* are also reported to be regulated by GTs (Fig. 2) (Ahn *et al*., 2021; Nagare *et al*., 2021). GTs are reported to regulate the mechanism for pesticide cross-resistance (Chen *et al*., 2019). GTs are reported constitutively overexpressed in DDT-resistant *Drosophila melanogaster*, Carbamate-resistant *Myzus persicae* and Neonicotinoid-resistant *Bemisia tabaci* (Pedra *et al*., 2004). Permethrin resistance in *Anopheles gambiae* and Abamectin resistance in *Tetranychus cinnabarinus* is mediated via GTs (Wang *et al*., 2018; Nagare *et al*., 2021). In Colorado potato beetle (CPB), *Leptinotarsa decemlineata*, UGT2 has been identified as a putative enzyme involved in Imidacloprid resistance (Kaplanoglu *et al*., 2017). It has been suggested that the indispensability of GTs could make them a potential target in insect pest control strategy via RNAi as a genetic tool through introduction of dsRNA (Lopez *et al*., 2019).

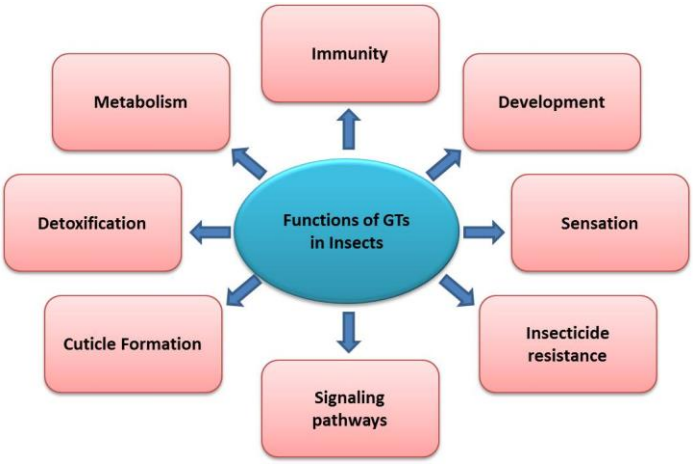

Figure 2: A summary of the role of GTs in insects

3. Plants and glycosyltransferases

In plants, glycosyltransferase enzymes play essential role in various biological processes, including the biosynthesis of cell wall components, secondary metabolites, and signaling molecules (He *at al*., 2022; Al-Khayri *et al*., 2023). GTs contribute significantly in the biosynthesis of cellulose, hemicellulose, and pectins which thereby, impart to overall structure, function and the integrity of the cell wall (Guerriero *et al*., 2018; Al-Khayri *et al*., 2023). UDP glycosyltransferases (UGTs) belongs to family 1 of glycosyltransferases and involved in glycosylation of wide range of acceptor molecules; hormones, phenylparanoids, flavonoids, betalains, coumarins, terpenoids, steroids and glucosinolates in plants. UGTs have very extensive substrate specificity for sugar acceptors, so their biochemical analyses helped a lot to understand their functions in Plants. UGTs are tremendously useful for *in vitro* manipulation of UDP sugars. UGTs catalyze the biosynthesis of polysaccharides of cell wall and addition of N-linked glycans to glycoproteins. In grape alone, more than 200 different glucosides have been identified (Sefton *et al*., 1994). In *Arabidopsis thaliana*, 120 UGT encoding genes have been identified, whereas in 244 UGTs are reported in *Oryza sativa* (Al-Khayri *et al*., 2023). The crystal structures of plant UGTs have also provided the structural basis for understanding the catalytic mechanism and the substrate specificity. The crystal-based 3D structures of four plant UGTs have recently been published. *Arabidopsis thaliana* is the first plant whose complete genome has been sequenced and served as a model plant for research work; out of 120 genes, known functions of many genes are completely documented (Sakakibara, 2009). GTs perform major function in plants to modify small molecules and secondary metabolites i.e. alkaloids flavonoids and terpenoids with sugar molecules. The glycosylation thereby regulate the solubility, bioavailability, stability, and bioactivity of the aforementioned compounds. This process affects the plant defense mechanism and the interaction with other organism like insects and animals (Al-Khayri *et al*., 2023). GTs involved in glycosylation of plant secondary metabolites has a conserved motif of 40 amino acids towards the C-terminus, known as the plant secondary product glycosyltransferases (PSPG) box. This PSPG box in GTs renders them characterisitic functioning in plants (Fig. 3) (Guerriero *et al*., 2018; Al-Khayri et al. 2023).

It has been recently reported that GTs contribute significantly to the mechanism of plant disease resistance i.e. tobacco mosaic virus (TVM) and *Pseudomonas syringae* inoculation in *Nicotiana tabacum* and CaUGT1 in *Capsicum annuum.* A detailed study has been conducted by Majeed to elucidate the activity of UGTs in *Arabidopsis thaliana* in order to find novel functions and the potential role of putative residues to combat Cancer in future (Majeed *et al*., 2015). Subsequently, UGT73B3 and UGT73B5 in *Arabidopsis thaliana* also revealed the role of GTs for resistance against bacterial infection (Campos *et al*., 2019).

In plants, GTs are also involved in the metabolism and signaling pathways of hormones by glycosylating phytohormones such as auxins, cytokinins, and gibberellins; and can modulate the activity, stability, and transport of these hormones and influence the plant growth, development, and

responses to various environmental stimuli. Various *in vitro* studies have facilitated more knowledge about the multigene family of glycosyltransferases (Bowles and Lim, 2010; Majeed *et al*., 2015). The blended knowledge of biochemistry, proteomics, genomics, molecular biology and computer analysis would provide splendid advancement in plant glycosyltransferases (Majeed *et al*., 2015; He *at al*., 2022; Al-Khayri *et al*., 2023).

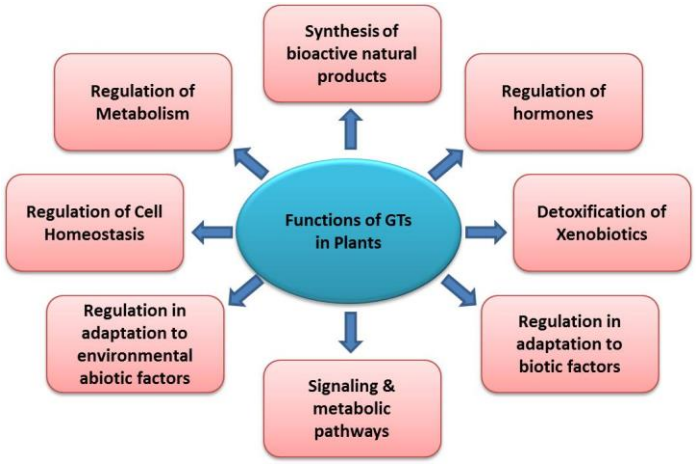

Figure 3: A summary of the role of GTs in Plants

4. Humans and glycosyltransferases

UDP-glycosyltransferases (UGTs) are phase II metaboloism enzymes of xenobiotics and use UDP-glucuronic acid as donar sugar in vertebrates (Dong *et al*., 2012; Buchheit *et al*., 2011). In humans, there are four UGT families (*UGT1*, *UGT2*, *UGT3* and *UGT8*) and each member of these families is unique in substrate selection (Hu *et al*., 2022). More than 200 glycosyltransferases are reported which regulate the enzymatic addition of various carbohydrate molecules in human cell (Shimma *et al*., 2006; Narimatsu *et al*., 2019). Out of 22 UGTs reported in humans, 19 UGTs have very distinct substrate specificity. For instance, UDP glucuronic acid is mostly accepted donor sugar for human UGTs (Hu *et al*., 2022).

GTs in humans are involved in numerous biological processes such as cell signaling, protein folding, immune response and the development (Schmid *et al*., 2016; Rini *et al*., 2022). Aberrant protein glycosylation leads to cancer, autoimmune disorders and/ or congenital disorders of glycosylation (Meech *et al*., 2015). GTs biosynthesize glycoproteins, glycolipids and glycosphingolipids which help regulate cell signaling, cell-cell recognition and communication, and adhesion (Ryckman *et al*., 2020; Jaroentomeechai *et al*., 2022). GTs are also reported to biosynthesize mucins which lubricate and protect mucosal surfaces (Grondin *et al*., 2020). UGTs regulate the metabolism and elimination of human hydrophilic drugs and chemical substances. UGTs conjugate lipophilic compounds to sugars i.e. glucuronide, galactose, glycosyl, or galacto; with substrates such as cancer-causing substances, medications, corticosteroids, triglycerides, fatty acid oxidation and bile salts etc. (Meech *et al*., 2015). Through glucuronidation process, UGTs weaken the biological activity of these drugs or chemical substances and increases their water solubility; hence, driving them to be eliminated in bile, urine and feces (Liu *et al*., 2023). Human UGTs are expressed in a wide range of organs and tissues. Mostly prominent in the liver, kidney and intestine; hence, reflecting their role in detoxification (Mazerska *et al*. 2016; Liu *et al*., 2023). UGTs are also involved in epigenetic modifications such as DNA methylation and histone modifications, transcriptional regulation, post-transcriptional regulation (miRNA), and post-translational regulation i.e. structural and functional modifications, and protein-protein interactions (Yasar et al. 2013; Hu *et al*., 2014; Hu *et al*., 2019).

GTs are responsible for the synthesis of human blood group antigens A and B (a-1,3-N-acetylgalactosaminyltransferase and a-1,3-galactosyltransferase, respectively); which are shown to differ by only four amino acids (R/G176, G/S235, L/M266 and G/A268). Glycosyltransferase adds an immunodominant carbohydrate to the H antigen. Blood group A (A phenotype) results from the 3-α-*N*-acetylgalactosaminyltransferase which adds

GalNAc to the H antigen; whereas, Blood group B (B phenotype) is results from the 3-α-galactosaminyltransferase that adds galactose (Gal) to the H antigen. Blood group AB (AB phenotype) has both the enzyme activities. Blood group O (O phenotype, recessive) has no functional enzyme due to a premature stop codon in the gene (Delaney, 2013). GTs has been recently reported to be linked with COVID-19-related loss of smell or taste (Shelton *et al*., 2022). Understanding the role of GTs needs extensive molecular biology and biotechnological work in order to elucidating various processes and disease mechanisms.

In Silico modeling of glycosyltransferases through Bioinformatics tools

Valuable information on enzyme-substrate interactions is available, which is obtained from crystal structures of GTs in complex with different acceptor- or donor-substrates or analogs (McArthur and Chen, 2016). However, bioinformatics tools for accurate prediction of sequence features important for defining the substrate specificity of a particular GTs have been devised (Majeed, 2014; Kuhlman and Bradley, 2019). In bacteria, a program was developed using available biochemical and crystal structure data to predict acceptors for GTs that glycosylate antibiotics (Kamra *et al*., 2005). Higher amino acid sequence identity between the query sequence and the template; as well as substantial amounts of biochemical data are essential elements to achieve reliable prediction (Jabeen *et al*., 2019; Kuhlman and Bradley, 2019; Jumper *et al*., 2021; Sasidharan & Saudagar, 2022). The 3D glycosyltransferase database holds crystal structures of 53 different GTs (http://www.cermav.cnrs.fr/glyco3d/). Furthermore, few other crystal structures can be found at the RCSB protein data bank (http://www.rcsb.org/pdb).

In silico prediction modeling approaches can be categorized as template-based modeling (TBM) or template-free modeling (TFM). Normally, best templates are searched out from the Protein Data Bank (PDB) library using TBM method (Pearce *et al*., 2021). Thus, before to perform targeted mutation or site directed mutagenesis (SDM); these prediction models help predict the potential mutation site (Majeed, 2014). The template search with Blast performed against SWISS-MODEL template library (SMTL) (Bienert *et al*., 2017). The target sequence can be searched with BLAST against the primary amino acid sequence contained in the SMTL (Altschul *et al*., 1997; Majeed, 2014). The predicted highest quality template has been identified and, subsequently selected for model building using UCSF Chimera (Meng *et al*., 2006). Further, the superimposition of UGT with reference enzyme i.e. VvGT1 was performed to reveal the target mutation site (Majeed, 2014). Mostly, the disordered probability for all the Amino Acids has been analyzed through PrDOS (http://prdos.hgc.jp/cgi-bin/top.cgi). Ramachandran plot, introduced by an Indian Physicist G. N. Ramachandran is reported to visualize the back bone of polypeptide chain, to calculate the phi and psi angles and also for structural validation (Ramachandran and Sasisekharan 1968; Singh, 2012). The mutated or target amino acid falls in allowed region; meaning thereby an indication of stability in protein structure which can be worked out for further molecular biology and biotechnological engineering (Singh, 2012).

Molecular Biology and Engineering of Glycosyltransferases

Biochemical characterization of the wide range substrate specificity of the GTs is a major task to gain a thorough understanding of the specificity of individual glycosyltransferase enzyme. When the substrate profile for a GT has been determined, mutational analyses may provide valuable information regarding the role of single amino acid residues with respect to sugar acceptor and donor specificity and catalytic efficiency (Ramakrishnan *et al*., 2008). Glycosyltransferases have specific domains which regulates the process of glycosylation in reactant molecules. The addition of a sugar molecule from donor sugar to acceptor molecule by glycosyltransferases during glycosylation reaction ultimately changes the hydrophilicity and bioactivity of acceptor molecules (Offen *et al*., 2006). Hence, GTs engineering would help to understand the domain specificity for their functionality in a given biological process (He *et al*., 2022; Rini *et al*., 2022; Andreu *et al*., 2023; Liu *et al*., 2023).

The structure-based UGT engineering can alter substrate specificity; compromise or enhance catalytic efficiency; and confer reversibility to the glycosylation reaction. It not only changes the substrate specificity but also increases or decreases their catalytic activity and may lead to totally inactive enzyme or the enzyme with enhanced activity (Wang, 2009).

Enzymatic engineering of original enzymes either by SDM or domain swapping is an

authoritative tool for determination of actual amino acids present in the catalytic site and it is also helpful in modifying enzyme action (Katoh *et al*., 2004; Majeed *et al*., 2015). Site directed mutagenesis in highly conserved Serine 134 to Leucine of UGT74B1 of *Arabidopsis* showed very mild morphological and metabolic changes. Nevertheless, the mutated Serine showed altered affinity for substrate, UDP-glucose (Kopycki *et al*., 2013). A mutational study of the *A. cordata* UGT78A2 identified a single amino acid residue responsible for determining UDP-galactose versus dual UDP-galactose and UDP-glucose specificity (Kubo *et al*., 2004). Sulfolobus solfataricus β-glycosidase has been modified by alteration of two residues which are involved in substrate recognition and this modification helped to accept different substrates in transglycosylation reactions (Hancock *et al*., 2006). In case of UGT85B1 of *Sorgham bicolor*, attempts to convert glucosyltransferases to glucuronosyltransferases by mutating the corresponding residues was not successful which showed that UGTs may require multiple amino acids to recognize sugars although single residues may play a decisive role (Osmani *et al*., 2008). Subsequently, the whole N-terminal domain of UGT74F2 was fused to the C-terminal domain of UGT74F1 and the chimera displays UGT74F2-like kinetic parameters and regiospecificity toward the quercetin acceptor. This domain swapping approach identified an amino acid distal to the active site which is important for determining the regiospecificity of UGT74F1 (Cartwright *et al*., 2008).

GT engineering causes the change of color of flowers; elevate the production of bioactive glycosides and higher tolerance in plants against any stress (Bowles *et al*., 2005 and 2006). Enzymatic engineered curumin glycosyltransferase (CaUGT2) by domain swapping and SDM found to improve the catalytic activity of CaUGT2. The CaUGT2 mutants with functionally important Cys377 site also showed similar Km value as the wild type CaUGT2 (Masada *et al*., 2010). The engineered AtUGT78D2 and AtUGT78D3 from *Arabidopsis thaliana* through domain swapping made these enzymes catalytically more efficient with extended sugar selectivity (Kim *et al*., 2013).

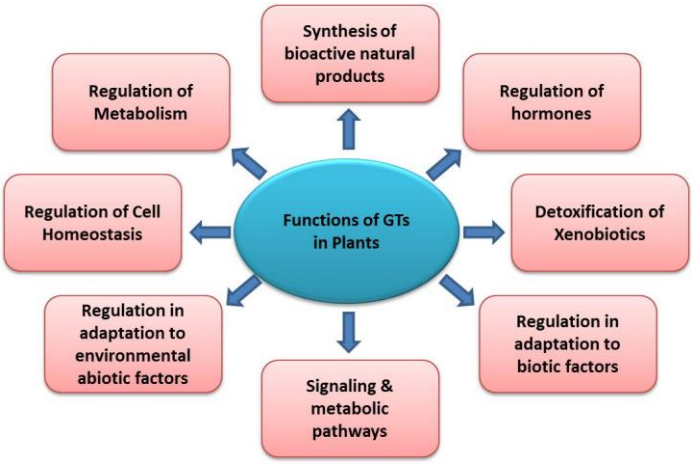

Figure 4: Schematic diagram of various steps in Modeling, Molecular Biology and Engineering of Glycosyltransferases

Upper panel: *In silico* modeling and stability assay for glycosyltransferases
Lower Left panel: Transformation for glycosyltransferases by heat shock method
Lower Middle panel: Protein Expression, Extraction and Purification for glycosyltransferases
Lower right panel: Mutagenesis protocol in glycosyltransferases

The development of new UGTs through enzymatic engineering also alters the regiospecificity of UGTs and pattern of glycosylation. In short, by playing with UGTs in this way improves not only the efficiency of the enzyme but also changes the whole cell bioactivity (Lim *et al*., 2005; Lim, 2005). In pigs, a point mutation in α-1,3-galactosyltransferase leads to failure in the synthesis of Gala-1,3-Gal epitope; which is responsible of hyperacute rejection in pig-to primate xeno-grafts (Phelps *et al*., 2003). Similarly, chimeric GTs were created using mouse-derived retroviral vectors and this Gala-1,3-

Gal epitope was reduced as it was not recognized by the human immune system (Hansen *et al*., 2005)..

Importance and Applications of Glycosyltransferases

Glycosyltransferases and cancer treatment

Cancer is one of the major causes of death these days. The rise in population has laid more global burden of cancer which is continuously increasing due to the adoption of cancer-associated lifestyle such as smoking, decreased physical activity and choice of diets. As GTs are involved in the secondary metabolism and remove toxins, drugs and dangerous chemicals including carcinogens from the body through glycosylation; hence, the exacerbated glycosylation contributes towards incidence of cancer (Gupta *et al*., 2020; Hu *et al*., 2022; Pucci *et al*., 2022; Liu *et al*., 2023). There is an inhibitory metabolic effect of N-acetylglucosamine (GlcNAc) on human prostate cancer and this was an illustration towards drug development by targeting GTs (Nishimura *et al*., 2012). The Cancer Genome Atlas (TCGA) data revealed that glycosylation changes thereby the expression pattern of glycosyltransferases are linked with Cancer (Pucci *et al*., 2022). Conventional therapies i.e., hormonal therapy, surgery, immunotherapy and anti-angiogenesis therapy are deficit in actual effectiveness (Hu and Fu, 2012). The most promising feature reported for cancer is anomalous glycosylation which changed the expression of glycosyltransferases (Meany and Chan, 2011). Hence, due to crucial role of glycosyltransferases in biological system, GTs have drawn extraordinary attention of researchers to develop new drugs by using these enzymes (Gupta *et al*., 2020; Pucci *et al*., 2022). The development of glycoproteomic technology is indispensible because of its higher sensitivity and specificity. More importantly, this technology has been considered as reproducible without any reservation (He *et al*., 2024). These metabolic inhibitors of glycosyltransferases have probability to be focused in drug discovery. Thus, advanced research in glycomics would unveil the pathological role of glycosyltransferases as potential biomarkers and novel targets would lead to therapeutic use of GTs (Gupta *et al*., 2020; Pucci *et al*., 2022; He *et al*., 2024).

Glycosyltransferases and Industrial applications

Glycosylation process serves as an important utility in food industry and the pharmaceutical industry as well (He *et al*., 2022). Glycosylation helps in stabilization of various natural compounds, i.e. Vitamin C (L-ascorbic acid), an essential nutrient for humans and certain other animal species. It has many biological functions such as collagen synthesis, antioxidation, and intestinal absorption of iron. It is widely used in medicine as an antioxidant and also as an additive in food industry (Kumar et al. 2016). Because GTs precisely modify the protein structures which is an hallmark for stability and the biological activity; and hence, are also reported having been used in the cosmetics, skin care and pharmaceutical industry (Schwab *et al*., 2015). Interestingly, GTs are also involved in deglycosylation, which makes these enzymes helpful in biosynthesis of many activated sugars (Rini *et al*., 2022; Andreu *et al*., 2023). GTs are reported with their use with certain food items and beverages to improve the taste as sweeteners and flavor (He *et al*., 2022; Andreu *et al*., 2023). An environmental friendly and more advantageous use of GTs is also reported for biofuel production (Greene *et al*., 2015; Schwab *et al*., 2015). In nutshell, glycosyltransferase enzymes are versatile enzymes which confer significant contribution across various industries.

Glycosyltransferases and Agriculture

Glycosyltransferase has been reported as ubiquitous in plants and used to enhance the plant physiology, adaptation and to improve the stress tolerance in plants. Moreover, GTs also reported to help improve the crop protection (Gharabli *et al*., 2023). Numerous bioactive compounds are being produced using glycosylation and biotechnological engineering (He *et al*., 2022; Andreu *et al*., 2023). Thanks to the insect cell lines, recombinant glycoproteins in insect cell expression systems have been produced (Geisler *et al*., 2015). To devise a best control strategy for integrated control of insects, understanding of the molecular biology of GT is essential. Thus, the genetic control strategy for insect pest management via RNAi has been employed **(**Lopez *et al*., 2019). Additionally, the insecticide resistance reported in various insects have been managed which could serve as a tool to be exploited further in other agricultural crops (Kaplanoglu *et al*., 2017; Wang *et al*., 2018; Chen *et al*., 2019; Nagare *et al*., 2021).

Conclusions & Future Perspectives

Glycosyltransferase engineering has been attempted in both prokaryotic and eukaryotic organisms. GTs are involved in a number of biological processes and connected to various diseases that shows the functional diversity and the versatility of GTs. In prokaryotes, GTs regulate cell wall biosynthesis, surface glycosylation and virulence, survival, pathogenicity, and interaction with the host environment. Whereas, in multicellular organism, i.e. insect; GTs being ubiquitously expressed in multiple tissues; paly significant role in survival, growth and development, metabolism and immunity, reproduction, detoxification, insecticide resistance, cuticle formation, homeostasis, chemosensation, odorant detection, communication, mate recognition, metamorphosis and gametogenesis, signaling pathways, etc. In plants,

GTs are component of cell wall and help regulate metabolisms through sysnthesis of secondary metabolites and signaling molecules. GTs are involved in glycosylation of wide range of acceptor molecules and show specificity for sugar acceptors. GTs contribute towards plant disease resistance, plant growth, development, and responses to various environmental stimuli. More than 200 glycosyltransferases are reported in humans which regulate the enzymatic addition of various carbohydrate molecules and regulate numerous biological processes such as cell signaling, protein folding, immune response and the development, cell signaling, detoxification, epigenetic modifications such as DNA methylation and histone modifications, transcriptional regulation, post-transcriptional regulation, synthesis of human blood group antigens, etc and recently known to be liked with COVID-19-related loss of smell or taste. The use of bioinformatics tools for modeling, and *in silico* stability assays are suggested as best approach before to perform *in vivo* GT analyses and engineering.

More research work is needed for the better understanding of the biological processes and the mechanisms of glycosyltransferases involved in cancer, tumor, drug metabolism etc. New era of engineering is awaited to engineer these GT enzymes *in vitro* to get them boost in industry as well as to help cure cancer and other diseases as well.

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