* Do not include theses or articles with unknown journal

## Proposed EC/sub‐subclass

GCNT1, which encodes Beta‐1,3‐galactosyl‐O‐glycosyl‐glycoprotein beta‐1,6‐N‐acetylglucosaminyltransferase, is assigned to enzyme commission number EC 2.4.1.102, denoting it as a glycosyltransferase that transfers hexosyl groups from a nucleotide sugar donor to an acceptor substrate with inversion of configuration (taniguchi2014handbookofglycosyltransferases pages 143-146, stone2009glycosyltransferasefunctionin pages 13-13).

## Accepted name

The recommended, accepted name for this glycosyltransferase is Core 2 beta‐1,6‐N‐acetylglucosaminyltransferase. Alternative names include Core2 GlcNAc‐transferase, Core 2‐branching enzyme, and Leukocyte type core 2 beta‐1,6‐N‐acetylglucosaminyltransferase (taniguchi2014handbookofglycosyltransferases pages 143-146, taniguchi2014handbookofglycosyltransferases pages 149-151).

## Phylogeny

GCNT1 is a highly conserved enzyme with orthologs identified in multiple mammalian species, including mouse, rat, and cattle, which underscores its fundamental role in mucin‐type O‐glycosylation pathways (gupta2020asystematicreview pages 15-17, taniguchi2014handbookofglycosyltransferases pages 149-151). Comparative molecular analyses indicate that GCNT1 and its isoenzymes, such as Core2GlcNAcT‐III, share significant sequence identity – approximately 40–50% identity – reflecting preserved catalytic mechanisms and substrate specificities among core‐branching glycosyltransferases (taniguchi2014handbookofglycosyltransferases pages 146-148). Phylogenetic studies place GCNT1 within a group of glycosyltransferases dedicated to the elongation and branching of O‐glycans, and its evolutionary conservation across metazoans has been emphasized by its occurrence in both invertebrate and vertebrate systems (schjoldager2020globalviewof pages 6-6, taniguchi2014handbookofglycosyltransferases pages 149-151). Furthermore, evolutionary comparisons reveal that while closely related isoforms can display tissue‐specific expression patterns, the overall conservation of the catalytic domain suggests that these enzymes arose from a common ancestral gene and have subsequently diverged to fulfill specialized functions in different cell types (cheng2011mucinoglycanbranching pages 1-2, cheng2011mucinoglycanbranching pages 4-6).

## Glycosyltransferase family

GCNT1 is classified within the CAZy glycosyltransferase family GT-14. This family is characterized by members that share a GT-A fold architecture and are generally involved in the transfer of sugar moieties with inversion of configuration; in the case of GCNT1, it specifically transfers N-acetylglucosamine from UDP-GlcNAc onto acceptor substrates (cheng2011mucinoglycanbranching pages 2-4, cheng2011mucinoglycanbranching pages 4-6).

## Reaction Catalyzed

GCNT1 catalyzes the reaction in which an N-acetylglucosamine (GlcNAc) unit is transferred from the donor substrate UDP-N-acetylglucosamine (UDP-GlcNAc) to the 6-hydroxyl group of the galactose residue in a mucin-type core 1 O-glycan, which is typically arranged as Galβ1-3GalNAc-Ser/Thr. The reaction can be summarized as follows:  
  UDP-GlcNAc + Galβ1-3GalNAcα-Ser/Thr → UDP + Galβ1-3(GlcNAcβ1-6)GalNAcα-Ser/Thr.  
This branching reaction generates the core 2 structure that serves as a scaffold for further elaboration by additional glycosyltransferases, ultimately leading to the formation of complex O-glycan structures such as those presenting sialyl Lewis X epitopes (taniguchi2014handbookofglycosyltransferases pages 143-146, taniguchi2014handbookofglycosyltransferases pages 149-151, taniguchi2014handbookofglycosyltransferases pages 154-156).

## Cofactor Requirements

A distinctive feature of GCNT1 is that its catalytic mechanism is metal ion-independent; unlike many glycosyltransferases that require divalent metal ions (such as Mn²⁺ or Mg²⁺) for activity, GCNT1 does not require exogenous metal ion cofactors for its function (taniguchi2014handbookofglycosyltransferases pages 149-151, cheng2011mucinoglycanbranching pages 4-6). Instead, the enzyme relies solely on the binding of its nucleotide sugar donor, UDP-GlcNAc, and proper positioning of the acceptor substrate to facilitate the transfer reaction.

## Substrate Specificity

GCNT1 exhibits a high degree of substrate specificity for acceptor substrates that contain the mucin-type core 1 O-glycan motif, specifically the Galβ1-3GalNAc structure linked to serine or threonine residues on glycoproteins (taniguchi2014handbookofglycosyltransferases pages 143-146, taniguchi2014handbookofglycosyltransferases pages 149-151). The enzyme selectively recognizes and acts on these core 1 structures, transferring GlcNAc in a β1-6 linkage to yield core 2 branched O-glycans. In addition, its activity can also extend to glycolipid substrates; for instance, GCNT1 can transfer GlcNAc to GalGb4Cer globosides, contributing to the biosynthesis of stage-specific embryonic antigen 1 (SSEA-1) determinants (gupta2020asystematicreview pages 15-17, taniguchi2014handbookofglycosyltransferases pages 149-151). Notably, the presence of specific terminal modifications on the acceptor—such as α1,2-linked fucose or α2,3-linked sialic acid on the terminal galactose—impairs the enzyme’s ability to recognize the substrate, thereby inhibiting branching (taniguchi2014handbookofglycosyltransferases pages 143-146, hodgson2023theroleof pages 9-10).

## Structure

GCNT1 is organized as a type II transmembrane protein, a structural arrangement that is common among Golgi-resident glycosyltransferases. This organization comprises a short N-terminal cytosolic tail, a single transmembrane domain responsible for Golgi localization, a stem region that may contain N-linked glycosylation sites critical for protein stability and proper folding, and a large lumenal catalytic domain where substrate recognition and transfer occur (cheng2011mucinoglycanbranching pages 2-4, taniguchi2014handbookofglycosyltransferases pages 146-148). The catalytic domain adopts a GT-A fold, a structure typically characterized by a central mixed β-sheet flanked by α-helices, which forms a Rossmann-like nucleotide-binding motif. Despite lacking the conventional metal ion-binding DXD motif common to many GT-A glycosyltransferases, GCNT1 utilizes a set of highly conserved catalytic residues that govern donor and acceptor recognition. Key residues identified include a free cysteine residue (Cys217), which is critical for catalysis and must remain in a reduced state, as well as residues such as Glu320 that help activate the 6-hydroxyl group of the acceptor substrate, and additional residues including Arg-254, Lys-401, and other conserved amino acids that contribute to the formation of the donor binding pocket and overall catalytic efficiency (unknownauthors2010structuralandfunctional pages 25-30, cheng2011mucinoglycanbranching pages 4-6, pak2011structuralandmechanistic pages 11-12). In support of these observations, structural studies involving crystallization of a C217S mutant have provided high-resolution insights into the active site configuration and validated the metal ion-independent catalytic mechanism (unknownauthors2010structuralandfunctional pages 25-30, pak2011structuralandmechanistic pages 11-12). Moreover, AlphaFold predictions and comparative analyses with other members of the GT-14 family further corroborate the presence of a well-defined catalytic domain, with hypervariable loops that adjust to accommodate the core 1 acceptor substrates and facilitate the specific positioning of UDP-GlcNAc (cheng2011mucinoglycanbranching pages 2-4, cheng2011mucinoglycanbranching pages 4-6).

## Regulation

GCNT1 expression and activity are regulated via multiple mechanisms. Transcriptionally, the GCNT1 gene is subject to regulation by specific transcription factors such as Sp1, which has been shown to be critical for GCNT1 expression in leukocytes and epithelial cells (unknownauthors2010structuralandfunctional pages 30-36). In addition, cytokines including IL-2, IL-4, and IL-15 modulate GCNT1 expression in activated CD8⁺ T-cells, aligning its expression with immune cell activation and differentiation (unknownauthors2010structuralandfunctional pages 30-36). At the post-translational level, the enzyme’s localization to the cis/medial Golgi is mediated by signals present in its transmembrane and stem domains, ensuring that its catalytic activity occurs in the appropriate subcellular compartment (cheng2011mucinoglycanbranching pages 2-4, taniguchi2014handbookofglycosyltransferases pages 146-148). Moreover, N-linked glycosylation sites within the stem region contribute to the enzyme’s proper folding, stability, and retention within Golgi membranes (cheng2011mucinoglycanbranching pages 2-4, taniguchi2014handbookofglycosyltransferases pages 146-148). Regulation of GCNT1 activity is also influenced by the availability of its substrates and competition with other Golgi-resident enzymes, such as sialyltransferases and fucosyltransferases, which act on similar acceptor substrates and can modulate the extent of O-glycan branching (taniguchi2014handbookofglycosyltransferases pages 143-146, taniguchi2014handbookofglycosyltransferases pages 149-151). In certain cellular contexts, such as in T-cell activation and in various cancers, upregulation of GCNT1 has been observed and is associated with altered glycan structures on the cell surface, affecting cell adhesion and signaling pathways (gupta2020asystematicreview pages 14-15, taniguchi2014handbookofglycosyltransferases pages 154-156).

## Function

The primary biological function of GCNT1 is to synthesize mucin-type core 2 O-glycans by transferring an N-acetylglucosamine residue to core 1 O-glycans, thereby introducing a branched structure that can serve as a scaffold for the attachment of additional sugars. These core 2 branches are fundamental for the biosynthesis of complex glycan structures, including the generation of selectin ligands such as sialyl Lewis X, which are essential for mediating leukocyte adhesion, rolling, and extravasation at sites of inflammation (taniguchi2014handbookofglycosyltransferases pages 154-156, taniguchi2014handbookofglycosyltransferases pages 149-151). Expression of GCNT1 is noted in various tissues with a pronounced presence in leukocytes, particularly in activated T-cells and myeloid cells, which underscores its role in immune cell function and inflammatory responses (pqac-b010a50a, unknownauthors2010structuralandfunctional pages 30-36). In addition to its role in protein O-glycosylation, GCNT1 can also act on glycolipid substrates, facilitating the formation of branched structures on globosides that are instrumental in the biosynthesis of stage-specific embryonic antigen 1 (SSEA-1) determinants (gupta2020asystematicreview pages 15-17, taniguchi2014handbookofglycosyltransferases pages 149-151). Through these glycosylation modifications, GCNT1 influences cell–cell interactions, modulates receptor functions, and impacts processes such as lymphocyte trafficking and tissue homeostasis. In oncological contexts, aberrant expression of GCNT1 has been linked to cancer progression and metastasis, where altered glycan structures may promote tumor cell migration, immune evasion, and enhanced adhesion to stromal components (gupta2020asystematicreview pages 14-15, dimitroff2019ibranchedcarbohydratesas pages 3-4). These changes in glycosylation patterns can affect both the architecture of cell surface glycoproteins such as mucins (for example, MUC1) and the overall signaling networks that govern cell proliferation and invasion (gupta2020asystematicreview pages 14-15).

## Inhibitors

To date, no specific inhibitors for GCNT1 have been extensively characterized in the literature; available studies have focused primarily on its enzymatic activity and functional role rather than the development of targeted inhibitors (no valid citation provided).

## Disease relevance

GCNT1 has been implicated in several pathological conditions owing to its critical role in glycan branching. In cancers such as colorectal, prostate, and lung carcinomas, increased expression of GCNT1 correlates with more aggressive tumor behavior, enhanced lymphatic and venous invasion, and worse patient prognosis (gupta2020asystematicreview pages 14-15, dimitroff2019ibranchedcarbohydratesas pages 3-4). This is potentially due to the alteration of the glycan structures on cell surfaces, which affect cell adhesion, migration, and interactions with immune cells. Overexpression of GCNT1 in transgenic mouse models has been linked to reduced adhesion between T-cells and their substrates, impairing immune responses and contributing to altered lymphocyte homing (taniguchi2014handbookofglycosyltransferases pages 154-156). Moreover, dysregulation of GCNT1-mediated glycosylation has been associated with immune-related syndromes, including immunodeficiency states such as AIDS and Wiskott-Aldrich syndrome, where improper glycan branching may affect the display of selectin ligands critical for leukocyte recruitment (taniguchi2014handbookofglycosyltransferases pages 154-156, taniguchi2014handbookofglycosyltransferases pages 149-151). Although specific disease-causing mutations within the GCNT1 gene have not been elaborated in the provided context, altered expression levels and mutations in glycosyltransferases, in general, have been linked to congenital disorders of glycosylation and complex diseases affecting glycoprotein function (pqac-9a1035ae, unknownauthors2010structuralandfunctional pages 30-36).

## Other Comments

GCNT1 is a versatile glycosyltransferase whose substrate flexibility extends beyond protein acceptors, as evidenced by its ability to utilize glycolipid substrates and oligosaccharide derivatives, thereby contributing to the structural diversification of glycoconjugates (cheng2011mucinoglycanbranching pages 1-2, hodgson2023theroleof pages 9-10). Recombinant expression strategies have been successfully employed in both mammalian (CHO, COS-1) and insect (Sf9 via baculovirus) systems, facilitating detailed biochemical analyses and structure–function studies (taniguchi2014handbookofglycosyltransferases pages 154-156, taniguchi2014handbookofglycosyltransferases pages 159-161, taniguchi2014handbookofglycosyltransferases pages 149-151). In addition, comparative studies have identified viral homologs with high sequence identity, such as those found in bovine herpesvirus 4, suggesting that the gene encoding GCNT1 may have been acquired or adapted in certain viral contexts to modulate host glycosylation pathways (taniguchi2014handbookofglycosyltransferases pages 154-156). The enzyme’s unique metal ion-independent catalytic mechanism distinguishes it from many other glycosyltransferases and underscores a specialized catalytic strategy within the GT-A fold that is pertinent to its biological roles (taniguchi2014handbookofglycosyltransferases pages 149-151, cheng2011mucinoglycanbranching pages 4-6). Finally, the role of GCNT1 in generating scaffolds for selectin ligand synthesis not only impacts immune cell trafficking but also indicates potential therapeutic significance in managing inflammatory diseases and cancer metastasis, making it a subject of continued research interest in glycobiology (pqac-b010a50a, pqac-8888cb0b, taniguchi2014handbookofglycosyltransferases pages 154-156).

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