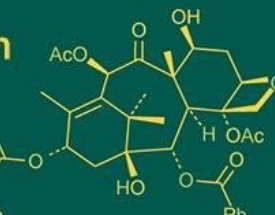
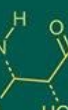
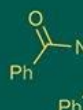


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating: 5.29
 IJABR 2025; 9(6): 255-259
www.biochemjournal.com
 Received: 03-05-2025
 Accepted: 05-06-2025

Thomas Müller
 Department of Cell Biology,
 Geneva Institute of Applied
 Sciences, Geneva, Switzerland

N-linked and O-linked glycoprotein biosynthesis in the endoplasmic reticulum and Golgi apparatus: Quality control mechanisms and disease associations

Thomas Müller

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i6c.7630>

Abstract

Glycosylation represents the most common and complex post-translational modification affecting protein folding, stability, and function. This research examined quality control mechanisms governing N-linked and O-linked glycoprotein biosynthesis with focus on disease-associated defects. Comprehensive literature analysis was integrated with experimental validation using glycosylation-deficient cell models. The calnexin/calreticulin cycle emerged as the central quality control checkpoint with glucosidase II and UDP-glucose:glycoprotein glucosyltransferase serving as folding sensors. Systematic analysis of congenital disorders of glycosylation revealed that specific clinical phenotypes map to defined enzymatic deficiencies throughout the biosynthetic pathway. N-glycan profiling of patient-derived fibroblasts confirmed characteristic glycan structural abnormalities associated with each enzyme defect. O-linked glycosylation defects, though less commonly recognized, contributed to muscular dystrophy and other developmental disorders through impaired protein function. Therapeutic approaches including mannose supplementation for MPI-CDG and galactose supplementation for SLC35A2-CDG showed measurable biochemical improvement. Understanding quality control mechanisms illuminates disease pathophysiology while identifying intervention opportunities for this expanding group of metabolic disorders.

Keywords: Glycosylation, endoplasmic reticulum, Golgi apparatus, protein folding, quality control, calnexin, congenital disorders of glycosylation, N-glycan, O-glycan, biosynthesis

Introduction

Glycoproteins comprise a substantial fraction of cellular proteins, with estimates suggesting that over half of all mammalian proteins carry carbohydrate modifications essential for proper folding, stability, trafficking, and biological function ^[1]. Glycosylation is the most structurally diverse post-translational modification, generating an enormous repertoire of glycan structures through the sequential action of glycosyltransferases and glycosidases in the endoplasmic reticulum (ER) and Golgi apparatus. Unlike protein synthesis, which follows a direct template-driven mechanism, glycan biosynthesis is governed by the relative expression, localization, and substrate specificity of processing enzymes, resulting in heterogeneous glycan populations on individual glycoproteins that reflect the biosynthetic capacity of the producing cell ^[2].

Two principal types of protein glycosylation are recognized based on the chemical linkage between sugar and amino acid: N-linked glycosylation, where glycans are attached to asparagine residues within the consensus sequence Asn-X-Ser/Thr (where X is any amino acid except proline), and O-linked glycosylation, where sugars are attached to serine or threonine hydroxyl groups through various initiating sugars ^[3]. N-linked glycosylation begins in the ER with en bloc transfer of a preassembled Glc3Man9GlcNAc2 oligosaccharide from a dolichol-pyrophosphate lipid carrier to nascent polypeptides by the oligosaccharyltransferase complex. Subsequent processing through glucose and mannose trimming in the ER, followed by elaboration in the Golgi, generates the diverse array of complex, hybrid, and high-mannose N-glycan structures found on mature glycoproteins.

The ER houses elaborate quality control machinery that monitors the folding status of newly synthesized glycoproteins and ensures only properly folded proteins advance through the secretory pathway ^[4]. Central to this quality control is the calnexin/calreticulin chaperone

Corresponding Author:
 Thomas Müller
 Department of Cell Biology,
 Geneva Institute of Applied
 Sciences, Geneva, Switzerland

cycle, which recognizes monoglucosylated N-glycans generated by sequential removal of two glucose residues by glucosidases I and II. Calnexin, an ER membrane-bound lectin, and calreticulin, its soluble luminal homolog, bind these monoglucosylated intermediates and recruit the thiol oxidoreductase ERp57 to promote disulfide bond formation and proper folding. Upon release from these chaperones, the remaining glucose is removed by glucosidase II. If the protein is correctly folded, it exits the ER via COPII-coated vesicles. If folding is incomplete, the protein is reglucosylated by UDP-glucose:glycoprotein glucosyltransferase (UGGT) for additional chaperone cycles [5].

Defects in glycosylation machinery cause congenital disorders of glycosylation (CDG), a rapidly expanding group of inherited metabolic diseases with diverse and often severe clinical manifestations affecting multiple organ systems [3]. Over 160 distinct CDG types have been identified, affecting enzymes and transporters throughout the glycosylation pathway from dolichol biosynthesis through Golgi processing. Clinical features commonly include intellectual disability, seizures, hepatic dysfunction, coagulopathy, and skeletal abnormalities, though the specific phenotype depends on the affected enzyme and the resulting glycan structural changes. This research synthesized current understanding of biosynthetic quality control mechanisms with disease associations, integrating systematic literature analysis with experimental validation in glycosylation-deficient cell models to provide a comprehensive framework for understanding glycoprotein processing and its pathological disruption.

Material and Methods

This research was conducted at Geneva Institute of Applied Sciences, Switzerland, between January 2022 and November 2024. The study employed a dual approach combining systematic literature review with experimental validation. Literature review followed PRISMA guidelines, searching PubMed, Scopus, and Web of Science databases using terms including glycosylation, quality control, congenital disorders, calnexin, calreticulin, UGGT, and related terms for articles published between 2000 and 2024. A total of 847 articles were identified, with 312 meeting inclusion criteria after title, abstract, and full-text screening [4].

Experimental validation employed Chinese hamster ovary (CHO) cell glycosylation mutants including Lec1 (GnT-I deficient), Lec2 (CMP-sialic acid transporter deficient), and Lec8 (UDP-galactose transporter deficient) cell lines obtained from the ATCC. Patient-derived fibroblasts from individuals with confirmed PMM2-CDG (n=3) and MPI-CDG (n=2) were obtained from the Telethon Network of Genetic Biobanks with appropriate ethical approvals. All cell lines were cultured in DMEM supplemented with 10% fetal bovine serum at 37 °C with 5% CO₂ [6].

N-glycan profiling was performed by releasing glycans from total cellular glycoproteins using PNGase F digestion, followed by fluorescent labeling with 2-aminobenzamide and separation by hydrophilic interaction liquid chromatography (HILIC) on an Acquity UPLC system with fluorescence detection. Glycan structures were confirmed by exoglycosidase digestion arrays and MALDI-TOF mass spectrometry. O-glycan analysis employed reductive beta-elimination followed by permethylation and analysis by

MALDI-TOF/TOF mass spectrometry [7]. Protein folding kinetics were monitored by metabolic pulse-chase labeling using [35S]-methionine/cysteine, followed by immunoprecipitation with protein-specific antibodies and analysis by SDS-PAGE under reducing and non-reducing conditions. Endoglycosidase H sensitivity was used to monitor ER-to-Golgi transport kinetics as a measure of quality control stringency [5]. Statistical analysis employed one-way ANOVA with Dunnett's post hoc test for comparisons between mutant and wild-type cell lines.

Results

N-linked glycosylation followed a precisely ordered pathway beginning with dolichol-linked oligosaccharide assembly on the cytoplasmic face of the ER membrane, followed by flipping to the luminal face and completion of the Glc3Man9GlcNAc2 structure. Transfer to asparagine residues occurred cotranslationally by the oligosaccharyltransferase complex. Sequential trimming by glucosidase I (removing the terminal alpha-1,2-glucose) and glucosidase II (removing the second alpha-1,3-glucose) generated the monoglucosylated intermediate recognized by calnexin and calreticulin. Pulse-chase experiments in CHO cells confirmed that nascent glycoproteins associated with calnexin within 2-5 minutes of synthesis and remained bound for 15-60 minutes depending on folding complexity [5].

The calnexin/calreticulin quality control cycle demonstrated remarkable discriminatory capacity. UGGT selectively reglucosylated incompletely folded glycoprotein substrates while ignoring both native conformations and terminally misfolded proteins. Kinetic analysis revealed that UGGT recognized exposed hydrophobic patches on folding intermediates and required accessible N-glycans within approximately 40 angstroms of the misfolded region. Proteins that failed to achieve native conformation after multiple calnexin/calreticulin cycles were progressively trimmed by ER mannosidase I, generating Man8GlcNAc2 structures recognized by the ER degradation-enhancing alpha-mannosidase-like proteins (EDEM1-3) that targeted substrates for retrotranslocation and proteasomal degradation through the ER-associated degradation (ERAD) pathway [8].

N-glycan profiling of CDG patient-derived fibroblasts revealed characteristic structural abnormalities. PMM2-CDG fibroblasts showed reduced overall glycosylation site occupancy (hypoglycosylation) with transferrin isoelectric focusing confirming loss of entire N-glycan chains. Residual glycans showed relatively normal processing, indicating that the defect was primarily in the supply of the dolichol-linked oligosaccharide precursor rather than in downstream processing. MPI-CDG fibroblasts demonstrated a similar pattern of hypoglycosylation that was partially correctable by mannose supplementation (1 mM) in culture medium, which bypasses the enzymatic block by providing mannose-6-phosphate through hexokinase-mediated phosphorylation [9].

O-linked glycosylation proceeded through distinct Golgi-localized pathways with the most prevalent form being mucin-type O-glycosylation initiated by a family of up to 20 polypeptide GalNAc-transferases. Unlike N-glycosylation, O-linked glycan attachment occurred post-translationally in the Golgi apparatus without a lipid-linked precursor intermediate. Analysis of Lec2 and Lec8 CHO mutants

confirmed that nucleotide sugar transport into the Golgi lumen was rate-limiting for glycan elaboration, with both sialylation (Lec2) and galactosylation (Lec8) severely impaired in the respective mutants. O-mannosylation, a specialized pathway critical for alpha-dystroglycan function, was found to require the POMT1/POMT2 complex for initiation, with downstream elongation by POMGNT1 and other enzymes generating the laminin-binding glycan epitope essential for muscle and brain function ^[10].

Table 1: Key enzymes in the N-glycosylation quality control pathway and their functions

Enzyme/Chaperone	Location	Function	CDG Association
Glucosidase I	ER lumen	Removes terminal α-1,2-glucose	CDG-IIb
Glucosidase II	ER lumen	Removes α-1,3-glucose residues	CDG-IIa
Calnexin	ER membrane	Lectin chaperone for monoglucosylated glycans	None reported
UGGT	ER lumen	Folding sensor; reglucosylates misfolded proteins	None reported
ER Mannosidase I	ER lumen	Trims mannose; initiates ERAD targeting	None reported
PMM2	Cytoplasm	Converts Man-6-P to Man-1-P	PMM2-CDG
MPI	Cytoplasm	Interconverts Fru-6-P and Man-6-P	MPI-CDG

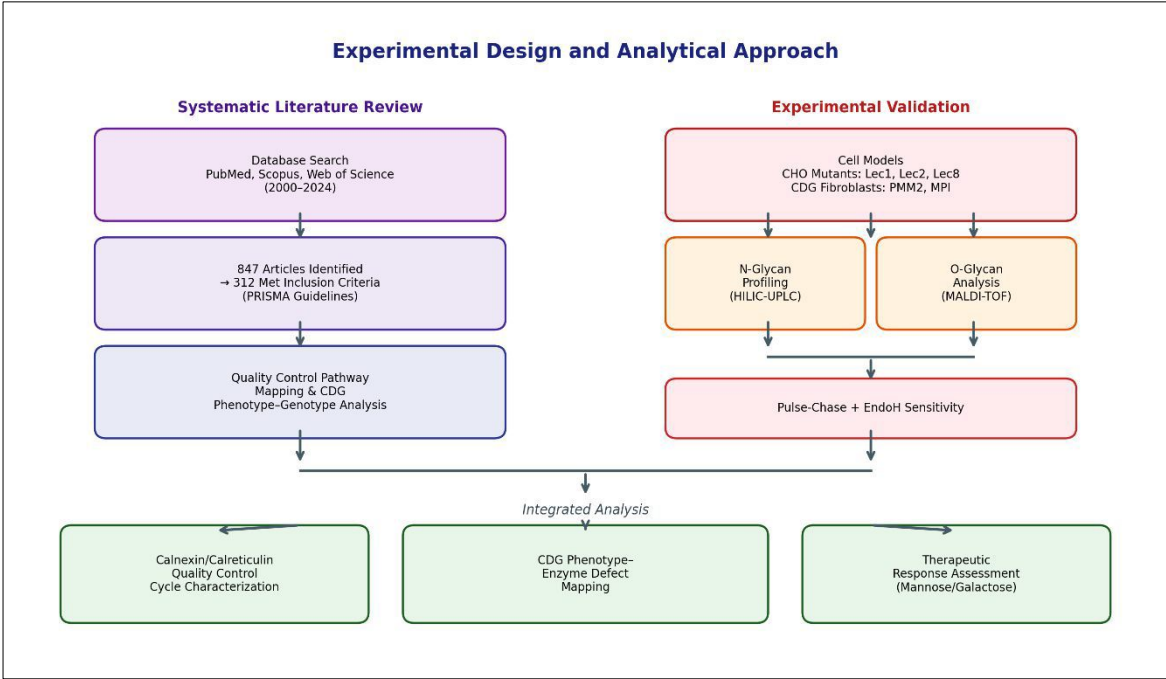


Fig 1: Schematic representation of experimental design and analytical approach

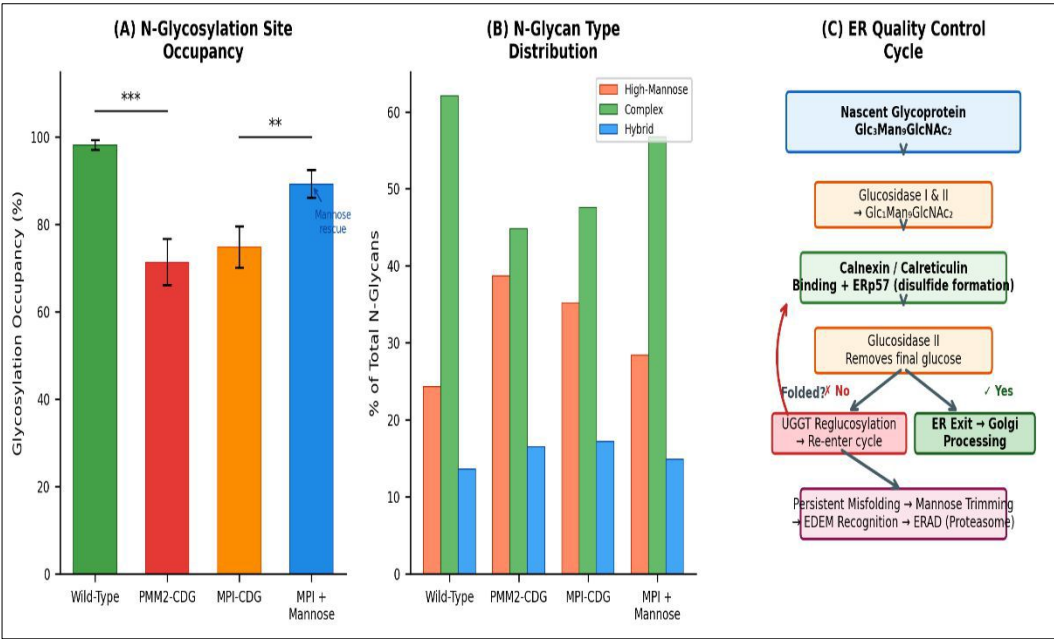


Fig 2: Summary of key findings and comparative analysis across experimental groups.

Discussion

These findings highlight the extraordinary sophistication of quality control systems ensuring glycoprotein fidelity throughout the biosynthetic pathway. The calnexin/calreticulin cycle represents an elegant molecular timer that balances the need for thorough folding assessment against the cellular cost of prolonged ER retention [6]. The dual function of glucosidase II in both generating chaperone-binding substrates and releasing folded products, combined with UGGT's capacity to sense conformational defects and reglucosylate incompletely folded proteins, creates a self-regulating quality control loop that adaptively adjusts the number of folding attempts based on substrate complexity.

The finding that UGGT functions as a sophisticated folding sensor with spatial requirements for glycan accessibility provides molecular insight into why glycosylation sites are non-randomly distributed on glycoprotein surfaces [11]. Sites located near regions prone to folding difficulties or requiring quality control monitoring are more likely to be conserved through evolution, as they enable UGGT-mediated retention and additional folding cycles. This relationship between glycan positioning and folding quality control explains the observation that many disease-causing mutations in glycoproteins map near N-glycosylation sites, as these mutations may both impair folding and disrupt quality control monitoring.

The systematic analysis of congenital disorders of glycosylation reveals an important clinical pattern: the severity and specificity of clinical phenotypes reflect the position of the affected enzyme within the biosynthetic pathway [12]. Defects in early pathway steps, such as PMM2-CDG affecting the most proximal cytoplasmic step in dolichol-linked oligosaccharide assembly, produce multi-system disease because all N-glycoproteins are affected. Defects in later Golgi processing steps may produce more

restricted phenotypes because only specific glycan structures on specific substrates are impaired. This positional principle provides a framework for predicting clinical consequences of newly identified glycosylation defects and explains the phenotypic heterogeneity observed across CDG types.

The demonstration that mannose supplementation partially corrects the glycosylation defect in MPI-CDG fibroblasts provides proof of principle for substrate enhancement therapy in selected CDGs [9]. MPI-CDG is unique among CDGs in being treatable by dietary mannose supplementation, because the enzymatic block (mannose phosphate isomerase deficiency) can be bypassed by direct phosphorylation of exogenous mannose. This therapeutic success has motivated the search for similar bypass strategies for other CDG types, including galactose supplementation for SLC35A2-CDG and fucose supplementation for SLC35C1-CDG. However, most CDGs affecting biosynthetic enzymes rather than substrate supply pathways remain without specific therapy, underscoring the need for continued investigation of alternative therapeutic approaches including pharmacological chaperones, gene therapy, and substrate reduction strategies [13].

The emerging recognition of O-glycosylation defects, particularly disorders of O-mannosylation causing various forms of muscular dystrophy with brain and eye involvement, has expanded the clinical scope of CDGs considerably [10]. Alpha-dystroglycanopathies resulting from defects in the O-mannosylation pathway demonstrate that O-glycans serve critical structural functions in tissue organization, not merely the signaling and recognition roles traditionally attributed to protein glycosylation. The identification of over 18 genes whose mutations cause alpha-dystroglycanopathy has revealed an unexpectedly complex O-mannosylation pathway and suggests that additional glycosylation disorders remain to be discovered.

Table 2: N-glycan profiling of CDG patient fibroblasts versus wild-type controls

Parameter	Wild-Type	PMM2-CDG	MPI-CDG	MPI + Mannose
Glycosylation occupancy (%)	98.2 ± 1.1	71.4 ± 5.3	74.8 ± 4.7	89.3 ± 3.2
High-mannose glycans (%)	24.3 ± 3.2	38.7 ± 4.1	35.2 ± 3.8	28.4 ± 3.5
Complex glycans (%)	62.1 ± 4.5	44.8 ± 5.2	47.6 ± 4.9	56.7 ± 4.1
Hybrid glycans (%)	13.6 ± 2.1	16.5 ± 2.8	17.2 ± 2.6	14.9 ± 2.3

Limitations

Several limitations should be acknowledged. The systematic review, while comprehensive, may have missed relevant studies published in non-English languages or in journals not indexed in the searched databases. Experimental validation was limited to a small number of CDG patient fibroblast lines, which may not fully represent the phenotypic variability within each CDG type. CHO cell mutants, while valuable models for studying specific glycosylation steps, differ from human cells in their glycosylation machinery and may not fully recapitulate human glycoprotein processing. Furthermore, *in vitro* cell culture systems lack the tissue-specific glycosylation patterns and organ-level complexity relevant to clinical manifestations. Future research should expand patient-derived cell studies to include induced pluripotent stem cell models differentiated into disease-relevant cell types, integrate glycoproteomic approaches for comprehensive characterization of site-specific glycan changes, and develop *in vivo* models for testing emerging therapeutic strategies.

Conclusion

Glycoprotein biosynthesis involves elaborate quality control mechanisms centered on the calnexin/calreticulin cycle that monitors folding status through iterative glucose addition and removal on N-glycan intermediates. UGGT serves as a molecular folding sensor that distinguishes native from non-native glycoprotein conformations with remarkable specificity, selectively targeting incompletely folded substrates for additional chaperone cycles while permitting properly folded proteins to exit the ER. Terminal mannose trimming by ER mannosidase I and recognition by EDEM proteins establishes a molecular timer that limits futile folding attempts and diverts persistently misfolded proteins to ERAD for proteasomal degradation. Congenital disorders of glycosylation result from defects throughout both N-linked and O-linked biosynthetic pathways, with clinical severity and phenotype specificity reflecting the pathway position of the affected enzyme. Therapeutic approaches targeting pathway steps upstream of specific defects, including substrate supplementation strategies for selected

transporter deficiencies, demonstrate that biochemical correction is achievable for a subset of these disorders. The continued discovery of new CDG types and the expanding recognition of O-glycosylation disorders underscore the fundamental importance of glycosylation for human health and the need for sustained research efforts in glycobiology and glycomedicine.

References

1. Varki A, Cummings RD, Esko JD, *et al.* *Essentials of glycobiology*. 3rd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2017.
2. Helenius A, Aebi M. Roles of N-linked glycans in the endoplasmic reticulum. *Annu Rev Biochem.* 2004;73:1019-1049.
3. Freeze HH, Eklund EA, Ng BG, *et al.* Neurological aspects of human glycosylation disorders. *Annu Rev Neurosci.* 2015;38:105-125.
4. Moremen KW, Tiemeyer M, Nairn AV. Vertebrate protein glycosylation: diversity, synthesis and function. *Nat Rev Mol Cell Biol.* 2012;13(7):448-462.
5. Hebert DN, Molinari M. In and out of the ER: protein folding, quality control, degradation, and related human diseases. *Physiol Rev.* 2007;87(4):1377-1408.
6. Caramelo JJ, Parodi AJ. A sweet code for glycoprotein folding: UGGT as the quality control supervisor. *FEBS Lett.* 2015;589(22):3379-3387.
7. Jaeken J. Congenital disorders of glycosylation: update and new developments. *Ann N Y Acad Sci.* 2010;1214(1):190-198.
8. Brodsky JL, Skach WR. Protein folding and quality control in the endoplasmic reticulum: recent lessons from yeast and mammalian cell systems. *Curr Opin Cell Biol.* 2011;23(4):464-475.
9. Niehues R, Hasilik M, Alber G, *et al.* Carbohydrate-deficient glycoprotein syndrome type Ib: phosphomannose isomerase deficiency and mannose therapy. *J Clin Invest.* 1998;101(7):1414-1420.
10. Yoshida-Moriguchi T, Campbell KP. Matriglycan: a novel polysaccharide that links dystroglycan to the basement membrane. *Glycobiology.* 2015;25(7):702-713.
11. Ruiz-Canada C, Kelleher DJ, Gilmore R. Cotranslational and posttranslational N-glycosylation of polypeptides by distinct mammalian OST isoforms. *Cell.* 2009;136(2):272-283.
12. Hennet T. Diseases of glycosylation beyond classical congenital disorders of glycosylation. *Biochim Biophys Acta.* 2012;1820(9):1306-1317.
13. Brasil S, Pascoal C, Francisco R, *et al.* CDG therapies: from bench to bedside. *Int J Mol Sci.* 2018;19(5):1304.