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Review

Molecular and structural basis for *N*-glycan-dependent determination of glycoprotein fates in cells ☆

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ABSTRACT

Background: *N*-linked oligosaccharides operate as tags for protein quality control, consigning glycoproteins to different fates, *i.e.* folding in the endoplasmic reticulum (ER), vesicular transport between the ER and the Golgi complex, and ER-associated degradation of glycoproteins, by interacting with a panel of intracellular lectins in the early secretory pathway.
Scope of review: This review summarizes the current state of knowledge regarding the molecular and structural basis for glycoprotein-fate determination in cells that is achieved through the actions of the intracellular lectins and its partner proteins.
Major conclusions: Cumulative frontal affinity chromatography (FAC) data demonstrated that the intracellular lectins exhibit distinct sugar-binding specificity profiles. The glycotopes recognized by these lectins as fate determinants are embedded in the triantennary structures of the high-mannose-type oligosaccharides and are exposed upon trimming of the outer glucose and mannose residues during the *N*-glycan processing pathway. Furthermore, recently emerged 3D structural data offer mechanistic insights into functional interplay between an intracellular lectin and its binding partner in the early secretory pathway.
General significance: Structural biology approaches in conjunction with FAC methods provide atomic pictures of the mechanisms behind the glycoprotein-fate determination in cells. This article is a part of a Special issue entitled: Glycoproteomics.

1. Introduction

Glycans displayed on proteins express diverse and variable biological messages for cell–cell communication and occasionally as targets of bacterial and viral infections. Glycans also serve as guides for their carrier protein for trafficking in intracellular environments as well as extracellular fluids. In most cases, glycan-encoded messages are detected and interpreted through interaction with lectins [1,2]. Growing evidence indicates that various intracellular lectins interact with *N*-linked oligosaccharides of glycoproteins, thereby governing their fates in the early secretory pathway [3–10] (Fig. 1).

In this pathway, a newly synthesized polypeptide is tagged with the $Glc_3Man_9GlcNAc_2$ (G3M9) oligosaccharide, a common precursor of *N*-glycans having D1, D2, and D3 arms (Fig. 2A), by the action of oligosaccharyl transferase, a hetero-oligomeric membrane protein complex, on the endoplasmic reticulum (ER) membrane [11–15]. The *N*-glycosylated polypeptide folds into its correct structure with the assistance of molecular chaperones and thiol-disulfide oxidoreductases in the ER lumen and is subsequently delivered to the Golgi complex through interaction with cargo receptors in the vesicular transport system [3–10]. If the glycoprotein is terminally misfolded, it is translocated from the ER to the cytosol and thereby subjected to deglycosylation that is catalyzed by peptide:*N*-glycanase (PNGase) and eventually the ubiquitin/proteasome-mediated degradation [4,5,7,16]. This process is called ER-associated degradation (ERAD) [17]. In all these processes, *N*-glycans function as quality control tags, which are recognized by the intracellular lectins operating as molecular chaperones, cargo receptors, or ERAD players (Fig. 1).

Abbreviations: ER, endoplasmic reticulum; ERAD, ER-associated degradation; GlcI, glucosidase I; GlcII, glucosidase II; UGGT, UDP-glucose:glycoprotein glucosyltransferase; CRT, calreticulin; CNX, calnexin; CRD, carbohydrate recognition domain; PDI, protein disulfide isomerase; MRH, mannose-6-phosphate receptor homology; MPR, mannose-6-phosphate receptor; ERGIC, ER–Golgi intermediate compartment; L-type, leguminous type; FV, blood coagulation factor V; FVIII, blood coagulation factor VIII; EDEM, ER degradation enhancing α-mannosidase-like protein; PNGase, peptide:*N*-glycanase; PUB, peptide:*N*-glycanase/ubiquitin-associated or ubiquitin regulatory X-containing proteins

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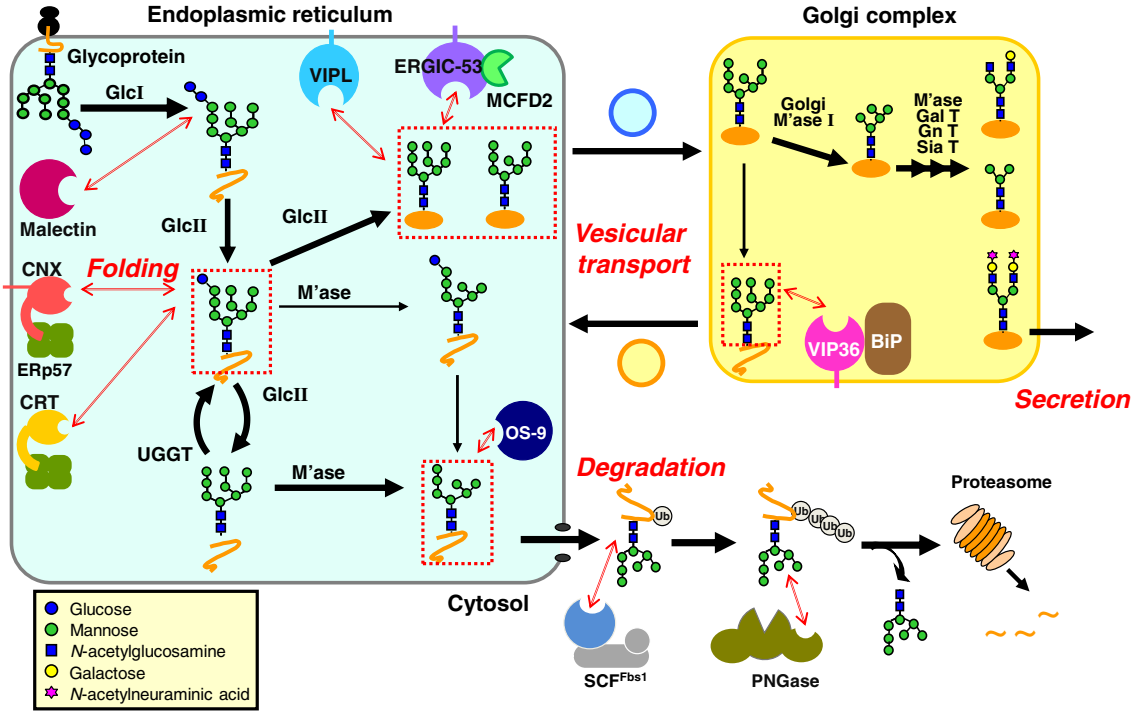

Fig. 1. Schemes for *N*-glycan-dependent glycoprotein-fate determination in cells. *N*-glycan processing coupled with glycoprotein-fate determination through interaction with the intracellular lectins and their partner proteins. Key processing intermediates are boxed with dashed lines. Abbreviations: Glc, glucosidase; M'ase, mannosidase; UGGT, UDP-glucose: glycoprotein glucosyltransferase; Gal T, galactosyl transferase; Gn T, *N*-acetylglucosaminyl transferase; Sia T, sialyl transferase; CRT, calreticulin; CNX, calnexin; VIP36, vesicular integral protein of 36 kDa; VIPL, VIP36-like; ERGIC-53, ER–Golgi intermediate compartment protein of 53 kDa; ERp57, ER resident protein of 57 kDa; BiP, immunoglobulin heavy chain binding protein; MCFD2, multiple coagulation factor deficiency protein 2; OS-9, osteosarcoma amplified-9 protein; SCF^{Fbs1}, Skp1-Cul1-F-box protein (the superscript denotes the F-box protein); Ub, ubiquitin.

Recently accumulating 3D structural data, in conjunction with frontal affinity chromatographic (FAC) data, provide a structural and molecular basis for sugar recognition by these intracellular lectins [7,18–26]. In this review, we discuss recent topics regarding *N*-glycan-dependent glycoprotein fate-determination in cells, predominantly focusing on the intracellular lectins that are functional in the early secretory pathway of mammalian systems.

2. Sugar-binding specificities of the intracellular lectins

The *N*-linked oligosaccharides attached to proteins are trimmed by glucosidases and mannosidases in the ER, thereby giving rise to a series of processing intermediates (Fig. 2A) [3,9,27]. Glucosidase I (GlcI) is responsible for the trimming of the outermost glucose residue at the D1 arm [28,29]. Thereafter, glucosidase II (GlcII) trims the second and third glucose residues [28,30], while UDP-glucose: glycoprotein glucosyltransferase (UGGT) catalyzes re-glucosylation, thereby reproducing monoglucosylated glycoforms [31–37]. The mannose residues are trimmed in the ER and subsequently in the Golgi complex, where extensively trimmed *N*-glycans undergo *N*-acetylglucosaminylation, galactosylation, sialylation, and/or sulfation [38,39].

The processing intermediates of *N*-glycans are recognition targets of the intracellular lectins (Fig. 2). For example, the ER chaperones calnexin (CNX) and calreticulin (CRT) specifically interact with the monoglucosylated *N*-glycans and thereby assist the folding of the carrier proteins [40]. Detailed characterizations of the sugar-binding specificities of the intracellular lectins were performed by comprehensive FAC analyses using a sugar library [7]. In the FAC analyses, pyridylaminated derivatives of the high-mannose-type oligosaccharides are subjected to an immobilized lectin column and their affinities for the lectin are estimated by inspecting their retardation in elution compared with that in a control analyte without any interaction [7,18,20,21,23,24,41–44]. This method enables a quantitative analysis of weak carbohydrate–protein interactions. Fig. 2B summarizes the lectin-binding constants of a series of the high-mannose-type oligosaccharide-processing intermediates for the ER chaperones CRT and CNX, the ERAD lectin OS-9, and cargo receptors ERGIC-53 and VIP36 (and its paralog VIPL) as well as the carbohydrate recognition domains (CRDs) of GlcII and PNGase. FAC data revealed that each intracellular lectin exhibits a characteristic profile of sugar-binding specificities; this indicates that they recognize distinct glycotopes that occupy different positions of the triantennary structures and become exposed upon glycan trimming (Fig. 2C). The processes can be roughly outlined as follows. Removal of the terminal glucose residue in the D1 arm results in its separation from the ER chaperones [7], while the lectins operating as cargo receptors capture the exposed trimannosyl glycotope for vesicular transport [18,20]. On the other hand, the Manα1–6 glycotope exposed after the mannose trimming at the D3 branch is recognized by ERAD lectins such as OS-9 [23,44]. Thus, the *N*-glycan processing pathway is tightly coupled with glycoprotein-fate determination in the early secretory pathway. In the following sections, we will review the underlying mechanisms for the molecular functions of the intracellular lectins on the basis of recently reported crystal structures.

Fig. 2. Sugar-binding specificity profiles of lectins involved in the folding, degradation, and vesicular transport of glycoproteins in cells. (A) Schematic representation of $GlcMan_9GlcNAc_2$ showing the nomenclature of sugar residues and branches, based on the convention stated by Vliegenthart et al. [141], and a list of the codes and structures of the individual high-mannose-type oligosaccharides used in FAC analyses. (B) K_a values for CRT, luminal domain of CNX, and CRD domains of OS-9, GlcII-β, PNGase, VIPL, VIP36, and ERGIC-53. n.d. = not detected. (C) The fate-determinants recognized by the intracellular lectins are displayed on the $Glc_3Man_9GlcNAc_2$ glycoform with the cleavage site of PNGase.