



Glycosyltransferase complexes in eukaryotes: long-known, prevalent but still unrecognized

Sakari Kellokumpu¹ · Antti Hassinen¹ · Tuomo Glumoff¹

Received: 9 June 2015 / Revised: 28 September 2015 / Accepted: 8 October 2015 / Published online: 17 October 2015
© Springer Basel 2015

Abstract Glycosylation is the most common and complex cellular modification of proteins and lipids. It is critical for multicellular life and its abrogation often leads to a devastating disease. Yet, the underlying mechanistic details of glycosylation in both health and disease remain unclear. Partly, this is due to the complexity and dynamics of glycan modifications, and the fact that not all the players are taken into account. Since late 1960s, a vast number of studies have demonstrated that glycosyltransferases typically form homomeric and heteromeric complexes with each other in yeast, plant and animal cells. To propagate their acceptance, we will summarize here accumulated data for their prevalence and potential functional importance for glycosylation focusing mainly on their mutual interactions, the protein domains mediating these interactions, and enzymatic activity changes that occur upon complex formation. Finally, we will highlight the few existing 3D structures of these enzyme complexes to pinpoint their individual nature and to emphasize that their lack is the main obstacle for more detailed understanding of how these enzyme complexes interact and function in a eukaryotic cell.

Keywords Golgi apparatus · Glycosylation · Glycosyltransferase complexes · Protein–protein interactions

Glycosyltransferase complexes: the first signs

In eukaryotes, the majority of glycans are synthesized in specialized organelles, the endoplasmic reticulum (ER) and the Golgi apparatus. Together they harbor dozens of functionally distinct glycosyltransferases (and glycosidases) that sequentially add (or remove) a single sugar residue at a time to (or from) the growing oligosaccharide chain [1, 2]. As an example, Fig. 1 shows the schematic representation of the N-glycan processing steps that take place in the ER and the Golgi apparatus of eukaryotic cells. This generally accepted view, however, fails to explain how this sequence of enzymatic reactions is orchestrated to guarantee faithful synthesis of thousands of different glycans without any template, in the presence of enzymes that compete for the same substrate and/acceptor protein and also localize in the same Golgi sub-compartment [3]. Preservation of fidelity is important, as even a single change in the linkage type can have a drastic effect on glycan's 3D structure and thus, also for its normal functions in a cellular context.

As of now, it turns out that one answer to that puzzle has been lying around for the last 50 years, but remained mainly unrecognized. Namely, several different studies carried out during the late 1960s and early 1970s already showed that glycosyltransferases tend to exist as enzyme complexes in the cells. One of the first examples of these was the observation that a soluble lactose synthase (LS, EC 2.4.1.22, for enzyme names and definitions, see Table 1) typically found in bovine milk consists of two

The term “complex” is used in this review to define a functional assembly of two or more similar (homomer) or dissimilar (heteromer) glycosyltransferases that interact with each other and in the case of the latter, typically act sequentially during glycan synthesis.

✉ Sakari Kellokumpu
sakari.kellokumpu@oulu.fi

¹ Faculty of Biochemistry and Molecular Medicine, University of Oulu, Aapistie 7, 90220 Oulu, Finland

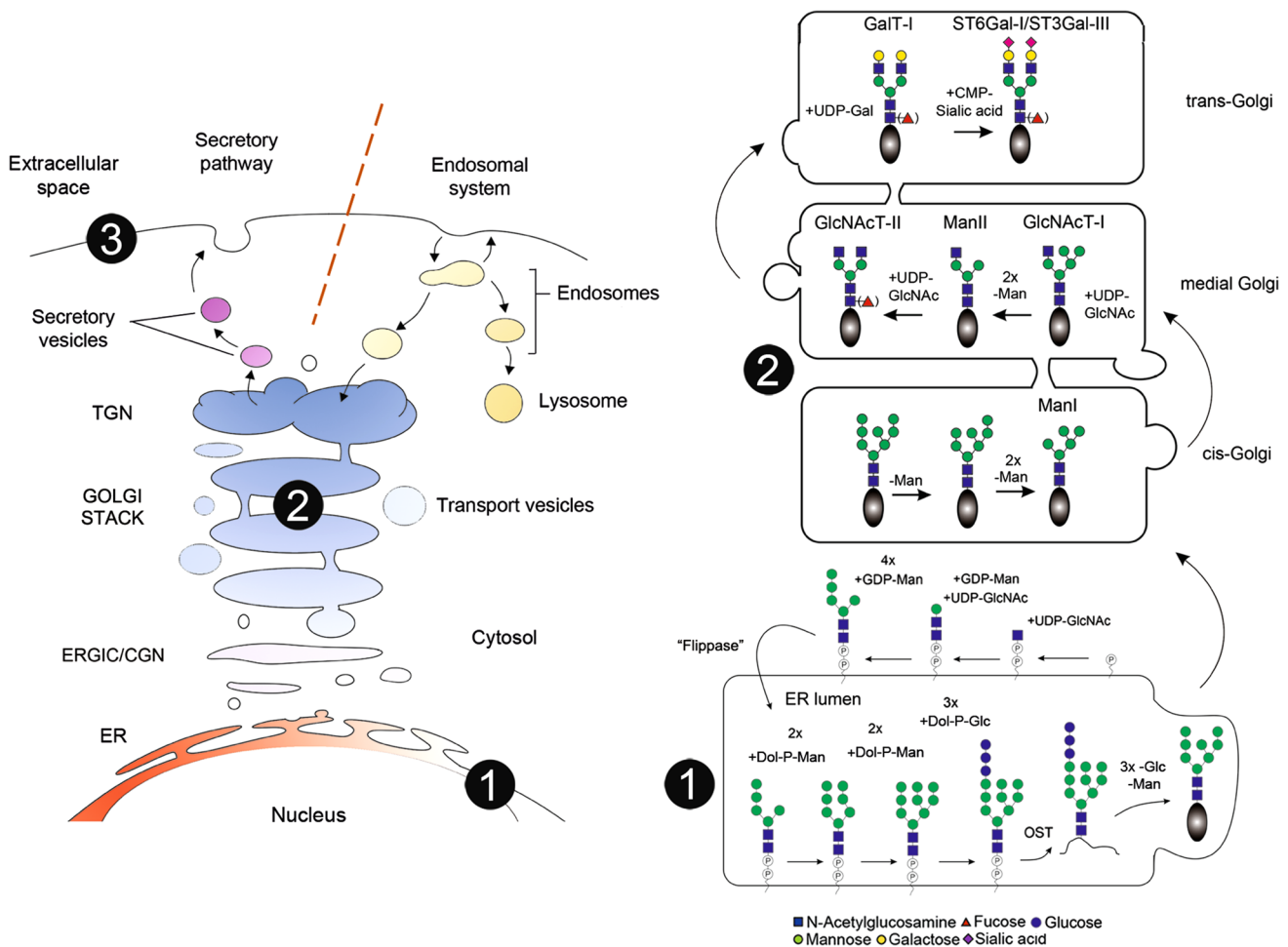


Fig. 1 *Left* schematic representation of the secretory pathway and the main organelles involved in glycan synthesis. *1* The endoplasmic reticulum (ER), *2* the Golgi apparatus and *3* the plasma membrane. Note that some glycosylation (addition of *N*-acetylglucosamine) takes place also in the cell's cytoplasm. *Right* a schematic cartoon showing

the sequential processing of *N*-glycans by glycosyltransferases in the ER and the Golgi. The enzymes (glycosyltransferases and glycosidases) involved are traditionally thought to function separately one after the other by adding or removing sugar residues one at the time in a specified order to and from the growing oligosaccharide chain

protein components, GalT-I (encoded by the B4GALT1 gene) and α -lactalbumin [4–8]. This interaction lowered the K_m of GalT-I transferase for glucose, enabling it to use glucose as an acceptor for lactose synthesis. Schwarz et al. [9, 10] were the first to identify complexes that consist of two dissimilar glycosyltransferases. By using immunoprecipitation, they showed that a xylosyltransferase (XylT) and a galactosyltransferase (GalT), enzymes that initiate the synthesis of chondroitin sulfate, interact directly with each other. Similar results were also obtained by Fishman [11] between *N*-acetylgalactosaminyl transferase (GalNAcT) and galactosyltransferase (GalT) that synthesize neural glycolipids. Although no direct interaction between the two enzymes was demonstrated in this study, it was found that the endogenous acceptor, i.e., the product of the GalNAcT, was a far better substrate for the GalT than an exogenously added glycolipid. It also had an order of

magnitude lower K_m than the latter. In addition, Ivatt [12] suggested that *N*-acetyllactosamine synthesizing enzymes (GlcNAcT, GalT) form a complex upon their co-adsorption into the same liposome (in contrast to separate liposomes), as the endogenously generated reaction intermediate was used as the preferential substrate for the reaction. These observations were suggested to be consistent with the facilitated passage of the intermediate glycoprotein within the complex itself and also beneficial for glycosylation, as complex formation likely provides a means to increase the fidelity of glycan synthesis by preventing intervention by competing enzymes.

Since then, a number of other biochemical and cell biological studies including recent live cell imaging methods have provided compelling evidence for the existence of glycosyltransferase complexes in all eukaryotes and also bacteria. The current data now shows that such

Table 1 Gene and enzyme names with their synonyms and definitions

Gene name/synonyms	EC number	Definition
LS, α -lactalbumin/B4GalT-I	EC 2.4.1.22	UDP-galactose:D-glucose 4-beta-D-galactosyltransferase
ALG7, TUR1	EC:2.7.8.15	UDP-N-acetylglucosamine-dolichyl-phosphate N-acetylglucosaminephosphotransferase
ALG13, YGL047 W	EC:2.4.1.141	Beta-1,4-N-acetylglucosaminyltransferase
ALG14, YBR070C	EC:2.4.1.141	Beta-1,4-N-acetylglucosaminyltransferase
ALG1, YBR110 W	EC:2.4.1.142	Beta-1,4-mannosyltransferase
ALG2, YGL065C	EC:2.4.1.257; 2.4.1.132	Alpha-1,3/alpha-1,6-mannosyltransferase
ALG11, YNL048 W	EC:2.4.1.131	Alpha-1,2-mannosyltransferase
OST1, NLT1, YJL002C	EC:2.4.99.18	Oligosaccharyltransferase complex subunit alpha (ribophorin I)
OST2, YOR103C	EC:2.4.99.18	Oligosaccharyltransferase complex subunit epsilon
OST3, YOR085 W	EC:2.4.99.18	Oligosaccharyltransferase complex subunit gamma
OST4, YDL232 W	EC:2.4.99.18	Oligosaccharyltransferase complex subunit OST4
OST5, YGL226C-A	EC:2.4.99.18	Oligosaccharyltransferase complex subunit OST5
OST6, YML019 W	EC:2.4.99.18	Oligosaccharyltransferase complex subunit gamma
WBP1, YEL002C	EC:2.4.99.18	Oligosaccharyltransferase complex subunit beta
STT3, YGL022 W	EC:2.4.99.18	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase
SWP1, YMR149 W	EC:2.4.99.18	Oligosaccharyltransferase complex subunit delta (ribophorin II)
MNN9, YPL050C	EC:2.4.1.-	Mannan polymerase complexes MNN9 subunit
VAN1, LDB13, VRG7, VRG8, YML115C	EC:2.4.1.-	Mannan polymerase I complex VAN1 subunit
MNN10, BED1, REC41, SLC2, YDR245 W	EC:2.4.1.-	Mannan polymerase II complex MNN10 subunit
MNN11, YJL183 W	EC:2.4.1.-	Mannan polymerase II complex MNN11 subunit
ANP1, GEM3, MNN8, YEL036C	EC:2.4.1.-	Mannan polymerase II complex ANP1 subunit
HOC1, YJR075 W	EC:2.4.1.-	Mannan polymerase II complex HOC1 subunit
PMT2, FUN25, YAL023C	EC:2.4.1.109	Dolichyl-phosphate-mannose-protein mannosyltransferase
PMT3, YOR321 W	EC:2.4.1.109	Dolichyl-phosphate-mannose-protein mannosyltransferase
PMT1, YDL095 W	EC:2.4.1.109	Dolichyl-phosphate-mannose-protein mannosyltransferase
PMT5, YDL093 W	EC:2.4.1.109	Dolichyl-phosphate-mannose-protein mannosyltransferase
GlcNAcT-1, MGAT1, GLCNAC-TI, GLCT1, GLYT1, GNT-1, GNT-I, MGAT	EC:2.4.1.101	Alpha-1,3-mannosyl-glycoprotein beta-1,2-N-acetylglucosaminyltransferase
GlcNAcT-2, MGAT2, CDG2A, CDGS2, GLCNACTII, GNT-II, GNT2	EC:2.4.1.143	Alpha-1,6-mannosyl-glycoprotein beta-1,2-N-acetylglucosaminyltransferase
GalT-I, B4GALT1, B4GAL-T1, CDG2D, GGTB2, GT1, GTB, beta4Gal-T1	EC:2.4.1.22; 2.4.1.90; 2.4.1.38	Beta-1,4-galactosyltransferase 1
ST6Gal-I, ST6GAL1, SIAT1, ST6GalI, ST6N	EC:2.4.99.1	Beta-galactoside alpha-2,6-sialyltransferase (sialyltransferase 1)
ST3Gal-III, ST3GAL3, EIEE15, MRT12, SIAT6, ST3GALII, ST3GalIII, ST3N	EC:2.4.99.6	N-acetyllactosaminide alpha-2,3-sialyltransferase (sialyltransferase 6)
B3GNT8, B3GALT7, BGALT15, beta3Gn-T8	EC:2.4.1.-	Beta-1,3-N-acetylglucosaminyltransferase 8
B3GNT2, B3GN-T2, B3GNT, B3GNT-2, B3GNT1, BETA3GNT, BGNT2, BGnT-2	EC:2.4.1.149	N-acetyllactosaminide beta-1,3-N-acetylglucosaminyltransferase
TPST1, TANGO13A	EC:2.8.2.20	Protein-tyrosine sulfotransferase
TPST2, TANGO13B	EC:2.8.2.20	Protein-tyrosine sulfotransferase
Mgat4d, 4933434I20Rik, GnT1IP, GnT1IP-L	EC:2.4.1.145	Alpha-1,3-mannosylglycoprotein beta-1,4-N-acetylglucosaminyltransferase A/B
GalNAcT-6, GALNT6, GALNAC-T6, GalNAcT6	EC:2.4.1.41	Polypeptide N-acetylgalactosaminyltransferase
C1GalT-I, C1GALT1, C1GALT, T-synthase	EC:2.4.1.122	Glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase

Table 1 continued

Gene name/synonyms	EC number	Definition
C2GnT-1, GCNT1, C2GNT, C2GNT-L, G6NT, NACGT2, NAGCT2	EC:2.4.1.102	Beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,6- <i>N</i> -acetylglucosaminyltransferase
C3GnT-1, B3GNT6, B3Gn-T6, BGnT-6, C2GnT-3, beta-1,3-Gn-T6, beta3Gn-T6	EC:2.4.1.149	Acetylgalactosaminyl-O-glycosyl-glycoprotein beta-1,3- <i>N</i> -acetylglucosaminyltransferase
Cosmc, C1GALT1C1, C1GALT2, C38H2-L1, MST143, TNPS	EC:2.4.1.-	C1GALT1-specific chaperone 1
EXT1, EXT, LGCR, LGS, TRPS2, TTV	EC:2.4.1.225 2.4.1.224	Glucuronyl/ <i>N</i> -acetylglucosaminyl transferase EXT1
EXT2, SOTV	EC:2.4.1.225 2.4.1.224	Glucuronyl/ <i>N</i> -acetylglucosaminyl transferase EXT2
OST, UST, 2OST	EC:2.8.2.-	Dermatan/chondroitin sulfate uronyl 2- <i>O</i> -sulfotransferase UST
Epi, GLCE, HSEPI	EC:5.1.3.17	Heparosan- <i>N</i> -sulfate-glucuronate 5-epimerase
CHSY1, CHSY, CSS1, ChSy-1, TPBS	EC:2.4.1.175 2.4.1.226	Chondroitin sulfate synthase
CHSY2, CHSY3, ChSy-2, CSS3	EC:2.4.1.175 2.4.1.226	Chondroitin sulfate synthase
CHPF, CHSY2, CSS2	EC:2.4.1.175 2.4.1.226	Chondroitin-polymerizing factor
CSGLCA-T, CHPF2, CSGlcAT, ChSy-3, chPF-2	EC:2.4.1.226	Chondroitin-polymerizing factor 2
B4GALT6, B4Gal-T6, beta4Gal-T6	EC:2.4.1.274	Beta-1,4-galactosyltransferase 6
ST3GAL5, SATI, SIAT9, SIATGM3S, ST3GalV	EC:2.4.99.9	Lactosylceramide alpha-2,3-sialyltransferase (sialyltransferase 9)
SIAT8A, GD3 synthase, ST8SIA1, GD3S, SIAT8, SIAT8-A, ST8Sial	EC:2.4.99.8	Alpha- <i>N</i> -acetyl-neuraminate alpha-2,8-sialyltransferase (sialyltransferase 8A)
B4GALNT1, GM2 synthase, GALGT, GALNACT, GalNAc-T, SPG26	EC:2.4.1.92	(<i>N</i> -Acetylneuraminy)-galactosylglucosylceramide <i>N</i> -acetylgalactosaminyltransferase
B3GALT4, BETA3GALT4, GALT2, GALT4	EC:2.4.1.62	ganglioside galactosyltransferase
HAS1, HAS	EC:2.4.1.212	Hyaluronan synthase
HAS2	EC:2.4.1.212	Hyaluronan synthase
HAS3	EC:2.4.1.212	Hyaluronan synthase
POMT1, LGMD2K, MDDGA1, MDDGB1, MDDGC1, RT	EC:2.4.1.109	Dolichyl-phosphate-mannose-protein mannosyltransferase
POMT2, LGMD2N, MDDGA2, MDDGB2, MDDGC2	EC:2.4.1.109	Dolichyl-phosphate-mannose-protein mannosyltransferase
OGT, HRNT1, O-GLCNAC	EC:2.4.1.255	Protein <i>O</i> -GlcNAc transferase
NCOAT, MGEA5, MEA5, OGA	EC:3.2.1.169	Protein <i>O</i> -GlcNAcase/histone acetyltransferase
CESA1, AT4G32410	EC:2.4.1.12	Cellulose synthase A
CESA2, AT4G39350	EC:2.4.1.12	Cellulose synthase A
CESA3, AT5G05170, CEV1, ATCESA3, Ath-B	EC:2.4.1.12	Cellulose synthase A
CESA4, AT5G44030	EC:2.4.1.12	Cellulose synthase A
CESA5, AT5G09870	EC:2.4.1.12	Cellulose synthase A
CESA6, AT5G64740	EC:2.4.1.12	Cellulose synthase A
CESA7, AT5G17420, IRX3	EC:2.4.1.12	cellulose synthase A
CESA8, IRX1, AT4G18780	EC:2.4.1.12	Cellulose synthase A
CESA9, AT2G21770	EC:2.4.1.12	cellulose synthase A
CESA10, AT2G25540	EC:2.4.1.12	Cellulose synthase A
CHS3, YBR023C, CAL1, CSD2, DIT101, KTI2	EC:2.4.1.16	Chitin synthase
CHS4, SKT5, YBL061C, CAL2, CSD4	EC:2.4.1.16	Chitin synthase
GALT1, AT1G26810	EC:2.4.1.-	Beta-1,3-galactosyltransferase
GMII, AT5G14950	EC:3.2.1.114	Alpha-mannosidase II
GAUT1, AT3G61130	EC:2.4.1.43	Alpha-1,4-galacturonosyltransferase

Table 1 continued

Gene name/synonyms	EC number	Definition
GAUT7, AT2G38650	EC:2.4.1.43	Alpha-1,4-galacturonosyltransferase
XXT1, XT1, AT3G62720	EC:2.4.2.39	Xyloglucan 6-xylosyltransferase
XXT2, XT2, AT4G02500	EC:2.4.2.39	Xyloglucan 6-xylosyltransferase
XXT5, AT1G74380	EC:2.4.2.39	Xyloglucan 6-xylosyltransferase
CSLC4, AT3G28180, CSLC04	EC:2.4.1.-	Xyloglucan glycosyltransferase 4, Cellulose synthase-like protein C4
SSI, WSSI-2	EC: 2.4.1.21	Starch synthase 1, chloroplastic/amyloplastic
SSIIa, wSSII-A, wSs2a-2	EC: 2.4.1.21	Starch synthase 2a, chloroplastic/amyloplastic
SBEI	EC:2.4.1.18	Starch branching enzyme I, chloroplastic/amyloplastic
SBEIIa	EC: 2.4.1.18	Starch branching enzyme IIa
SBEIIb	EC: 2.4.1.18	Starch branching enzyme IIb
SP, Pho1	EC: 2.4.1.1	Starch phosphorylase
PMT4, YJR143C	EC:2.4.1.109	Dolichyl-phosphate-mannose-protein mannosyltransferase
GlcNAcT-V, GNT-V, MGAT5, GNT-VA	EC:2.4.1.155	Alpha-1,3(6)-mannosylglycoprotein beta-1,6- <i>N</i> -acetylglucosaminyltransferase
FUT1, FucT-I, H, HH, HSC	EC:2.4.1.69	Galactoside 2-L-fucosyltransferase 1/2
FUT3, CD174, FT3B, FucT-III, LE, Les	EC:2.4.1.65	Galactoside 3(4)-fucosyltransferase 3
FUT6, FCT3A, FT1A, Fuc-TVI, FucT-VI	EC:2.4.1.65	Galactoside alpha-1,3-fucosyltransferase 6
LH3, PLOD3	EC:1.14.11.4	Lysyl hydroxylase/galactosyltransferase/glucosyltransferase
GlcAT-I, B3GAT1, CD57, GLCATP, GLCUATP, HNK1, LEU7, NK-1, NK1	EC:2.4.1.135	Galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase 1
CHS-2	EC:2.4.1.16	Chitin synthase
MNS1, AT1G51590	EC:3.2.1.113	Mannosyl-oligosaccharide alpha-1,2-mannosidase
GnTI, AT4G38240, CGL1	EC:2.4.1.101	Alpha-1,3-mannosyl-glycoprotein 2-beta- <i>N</i> -acetylglucosaminyltransferase
GnTII, AT2G05320	EC:2.4.1.143	Beta-1,2- <i>N</i> -acetylglucosaminyltransferase II
XYLT, AT5G55500	EC:2.4.2.38	Beta-(1,2)-xylosyltransferase
ARAD1	–	Putative arabinosyltransferase
ARAD2	–	Putative arabinosyltransferase
XXT3, AT5G07720	EC:2.4.2.39	Xyloglucan xylosyltransferase 3
XXT4	EC:2.4.2.39	Xyloglucan xylosyltransferase 4
MUR3, AT2G20370, KATAMARI1	EC:2.4.1.-	Xyloglucan galactosyltransferase
XLT2	EC:2.4.1.-	Xyloglucan galactosyltransferase

complexes are prevalent and exist in most—if not all—glycosylation pathways found in eukaryotic cells. In the next paragraphs, we will review the data gathered from yeast, plant and animal cells on these glycosyltransferase complexes one pathway at the time. We will focus mainly on their mutual interactions, rather than to their complexes with other proteins, unless the interaction is critical for the activity of a given enzyme. In the latter part of the review, we will also discuss both structural and biochemical data on the few complexes whose enzymatic activities change upon complex formation, or whose 3D structures have already been determined, just to provide a first glimpse on how these enzymes interact and how complex formation regulates the enzymatic activity of the complex constituents. We apologize the bacterial community for not

including any bacterial data in this review, and also those whose data may have escaped our attention.

Complexes involved in N-linked glycans synthesis

Yeast N-glycosyltransferase complexes The yeast *N*-glycosylation pathway shows a high degree of pathway conservation with higher eukaryotes, involving similar oligosaccharide-dolichol precursor synthesis, glycan transfer to nascent proteins by an oligosaccharyltransferase (OST) complex, and removal in the endoplasmic reticulum (ER) of the three glucoses and the central-arm α 1,2-linked Man from the newly transferred $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ [1, 2, 13–15]. The first steps to form GlcNAc_2 -PP-dolichol

involves stepwise addition of two GlcNAcs by GlcNAc-1 phosphate transferase (ALGT7) and Alg13p/Alg14p UDP-GlcNAc-transferases (Fig. 1). These first three enzymes have been shown both by immunoprecipitation and gel filtration analyses [16] to form a hexameric complex with a stoichiometry of 2:2:2 and with native molecular weight of ~200 kDa. Recently, Alg14 was shown to be the central unit and able to organize the formation of this three-enzyme glycosyltransferase complex [17].

The next enzymes in the row, the ER mannosyltransferases Alg1, Alg2 and Alg11 that add three more mannose residues to form the $\text{Man}_5\text{GlcNAc}_2\text{-PP-dol}$ intermediate, have similarly been shown to form complexes with each other. Both genetic and biochemical evidence indicates that Alg1 interacts with itself, Alg2, or with Alg11 [18]. Thus, the two heteromeric Alg1-containing complexes differ from one another in that one complex contains Alg2 and the other contains Alg11. Both of these complexes were found to be functionally important, as missense mutations affecting the activity of Alg1, but not its assembly with Alg2 or Alg11, exhibited a dominant negative phenotype. Thus, dolichol-linked *N*-glycan precursor synthesis on the cytoplasmic side of the ER membrane appears to involve three main enzyme complexes, one formed by Alg7p/Alg13p/Alg14p and the other two either by Alg1/Alg2 or Alg1/Alg11. This arrangement likely ensures that each mannose residue will be linked correctly to the dolichol-linked precursor glycan structure even in the presence of several other competing mannosyltransferases on the cytosolic surface of the ER membrane.

After flipping to the ER lumen by the Rft1 protein [15], four additional mannose residues and three glucose residues are further added to $\text{Man}_5\text{GlcNAc}_2\text{-PP-dol}$ intermediate. Enzymes responsible for these additions involve the Alg3/Alg9/Alg12 mannosyltransferases [19], which add the four mannoses to form the $\text{Man}_9\text{GlcNAc}_2\text{-dolichol}$ structure. This is then followed by addition of three glucoses that are added by Alg6, Alg8 and Alg10 glucosyltransferases, but it is currently unclear whether these enzymes form complexes with each other as well. This core oligosaccharide is next attached to a specific asparagine residue (consensus motif Asn-X-Ser/Thr) in a polypeptide chain by the 8 subunit oligosaccharyl-transferase complex [20–24]. The $\text{Glc}_3\text{-Man}_9\text{GlcNAc}_2$ core structure is subject to trimming by α -glucosidases I and II, yielding $\text{Glc}_2\text{Man}_9\text{GlcNAc}_2$ and $\text{Glc}_1\text{Man}_9\text{GlcNAc}_2$ structures, the latter of which serves for ER quality control by ER chaperones Erp57, calnexin and calreticulin [25–27]. The glucose and the terminal mannose residue in the middle branch of the oligosaccharide chain are removed by α -glucosidase II and ER mannosidase I, respectively, before transport of the $\text{Man}_8\text{GlcNAc}_2$ to the Golgi.

Upon arrival in the yeast Golgi, up to 200 mannose residues are added to an outer chain of an *N*-glycan of secretory pathway proteins such as invertase, and the ‘mannan’ structural proteins of the cell wall. In contrast to mammalian *N*-glycan processing in the Golgi (Fig. 1), this ‘hypermannose’ structure consists of a long backbone of α 1,6-linked residues with α 1,2-linked branches which usually terminate in α 1,3-linked residues [28]. After Och1p has attached the first α 1,6-linked mannose to the core structure, two distinct polymerase complexes, M-Pol I (which consists of Van1p/Mnn9p) and M-Pol II (Hoc1p/Mnn11p/Mnn10p/Anp1p/Mnn9p) then synthesize the long mannan backbone [29], while the branching of this backbone is accomplished by the sequential actions of Mnn2p, Mnn5p and Mnn1p. Some of the branches also receive a phosphomannose that is added by Mnn4p and Mnn6p. All these proteins or their complexes were found to have mannosyltransferase activity *in vitro*.

N-glycosyltransferase complexes in mammals The processing of *N*-glycans in the Golgi apparatus of higher eukaryotes differs markedly from that of yeast. High mannose glycans synthesized in the ER are extensively processed to form hybrid and complex type *N*-glycans (Fig. 1) by a defined set of glycosyltransferases and glycosidases in each cell type (Stanley et al. Chap 8. In: [30]). Similar to the ER-localized glycosyltransferases, these Golgi enzymes also tend to form complexes with each other. The first example of these was GalT-I, which has been shown to form high molecular weight oligomers and/or homo(dimers) in isolated membrane preparations prepared from mammalian cells [31, 32]. Oligomerization was thought to have a role in Golgi retention [32]. Similar oligomers or homodimers have since been detected also with GlcNAcT-V, ST6Gal-I, FucT-I, FucT-III and FucT-VI [33–39]. By utilizing bimolecular fluorescence complementation (BiFC) and fluorescence resonance energy transfer (FRET) approaches, we have also recently confirmed that not only GalT-I but also the other main human Golgi *N*-glycosyltransferases tested (GlcNAcT-I, II, GalT-I, ST6Gal-I, ST3Gal-III) form homomers in live cells [40, 41].

The first heteromeric glycosyltransferase complexes were detected by using an ER-re-location assay developed by the Nilsson group [42, 43]. They showed that GlcNAcT-I, fused with a dibasic ER-retention signal derived from the cytoplasmic tail of the p33 invariant chain, was able to relocate endogenous mannosidase II or GlcNAcT-II (but not GalT-I) from the Golgi to the ER, suggesting a direct interaction between these medial-Golgi enzymes. Based on their data, the authors suggested the new Golgi “kin recognition” model for medial-Golgi enzymes, which serves their correct targeting to the Golgi. Later on, the interaction between GlcNAcT-I and GlcNAcT-II has also

been confirmed by using an immunoprecipitation approach [35]. Enzymes such as β 3GnT-8 and β 3GnT-2 involved in the elongation of specific branch structures of multi-antennary N-glycans with poly-lactosamine have also been shown to form a complex with each other [44, 45]. Moreover, cross-testing of all the potential interactions between the main N-glycosyltransferases by using BiFC and FRET approaches, we have shown that they all form heteromeric complexes with each other [40, 41]. The medial-Golgi enzymes GlcNAcT-I and GlcNAcT-II were found to form one such complex that may also contain other medial-Golgi GlcNAc transferases and mannosidase II. The other complexes consist of trans-Golgi enzymes, GalT-I and ST6Gal-I or GalT-I and ST3Gal-III. Cross-testing potential interactions between N- and O-glycosyltransferases [41] did not reveal any interacting partners, suggesting that complexes form only between sequentially acting enzymes within the same glycosylation pathway. Very recently, the BiFC approach was used to show interactions between tyrosylprotein sulfotransferases TPST1 and TPST2 [46] with the result that these enzymes formed complexes not only with themselves, but also with STGalT-I, producing either TPST1/ST6Gal-I or TPST2/ST6Gal-I heteromers. A different type of interaction takes place between GlcNAcT-I and GnT1IPL (Mgat4D) protein in that the latter has no known enzymatic activity and is expressed at high levels in the testicular germ cells [47]. The functional relevance of this inhibitory interaction has been shown to be in the down-regulation of the synthesis of hybrid and complex type N-glycans and thereby to increase attachment of developing sperm cells to nourishing Sertoli cells.

Plant N-glycosyltransferase complexes Complex type N-glycans in plants are essential for plant development and defense mechanisms. However, the glycans themselves are somewhat different from mammalian N-glycans. For example, the proximal core N-acetylglucosamine in plants is substituted by an α 1,3 fucose (instead of α 1,6 fucose in mammals) and the β -mannose of the core is substituted by a bisecting β 1,2 xylose (a bisecting β 1,4 N-acetylglucosamine in mammals). In addition, β 1,3 galactose and α 1,4 fucose linked to the terminal N-acetylglucosamine of plant N-glycans form the Lewis a (Le a) oligosaccharide structure instead of β 1,4 linked galactose followed by a sialic acid in mammals. Recently, it has been shown [48, 49] that a number of plant N-glycosyltransferases also form complexes with each other. By using the two-photon FRET-FLIM approach, it was shown that *Arabidopsis thaliana* Golgi α -mannosidase I, *Nicotiana tabacum* β 1,2-N-acetylglucosaminyltransferase I, *Arabidopsis* Golgi α -mannosidase II (ManII), and *Arabidopsis* β 1,2-xylosyltransferase, form both homodimers and heterodimers, whereas the late-acting *Arabidopsis* β 1,3-galactosyltransferase1 (GALT1) and *Arabidopsis* α 1,4-

fucosyltransferase, do not. However, GALT1 was found to interact with the medial-Golgi ManII. This observation may reflect slight differences in the organization of the Golgi cisternae between plants and mammalian cells, as the latter do not show any such interactions between medial- and trans-Golgi enzymes tested thus far [40, 41].

Collectively, these data show that most N-glycosyltransferases in yeast, plant and mammalian cells form both enzyme homomers and a variety of functionally relevant enzyme heteromers between sequentially acting glycosyltransferases in each glycosylation pathway (see Table 2 for a collected list the known enzyme complexes described in this review). Enzymes that function in different glycosylation pathways are expected to form distinct complexes with other enzymes in a pathway dependent manner. An important question that needs to be answered is whether heteromerization is an inherent property of all glycosyltransferases or only those that operate at critical points such as at initiation, branching, and termination of glycan chains.

Complexes involved in O-linked glycan synthesis

Mucin type O-GalNAc glycosylation O-glycosyltransferases catalyze addition of sugar residues to hydroxyl groups of serine or threonine amino acids in proteins. Most common O-linked glycans are mucins, and ~20 different GalNAcTs exist to initiate their synthesis using different protein acceptors. This is then followed by chain elongation to form 7 different core structures which are further processed to form a number of different glycan structures. Enzymes that form these core structures are well known and have also been tested for their ability to form complexes in live cells. As with N-glycosyltransferases, all the 9 core forming transferases and two sialyltransferases [41] were found to form homomeric complexes in the cells (see Table 2). This is consistent with the earlier notion that the core 2 β 1,6 N-acetylglucosaminyl-transferase (C2GNT-I) forms disulfide bonded dimers [50]. In addition, it has been shown that the active C1GalT-1 (T-synthase, Core 1 synthase, β 3-galactosyltransferase) is a dimer and does not bind its specific folding chaperone Cosmc, unlike the unfolded and inactive enzyme [51, 52]. Other potential homomeric and heteromeric interactions between O-glycosyltransferases themselves were recently tested by the dynamic FRET approach. Similarly to N-glycosyltransferases, it was found that all these 9 enzymes form enzyme homomers [41]. In addition, the initiating GalNAcT-6 and C1GalT required for the synthesis of the T(F)-antigen were shown to form a heteromeric complex with each other. The same GalNAcT6 was also found to interact with the core 3 and core 6 forming glycosyltransferases (C3GnT-I,

Table 2 Known glycosyltransferase complexes, their functions, species identified and references

	Function	Organism	References
Heteromeric enzyme complexes			
Lactose synthase (LS, GalT-I/ α -lactalbumin)	Lactose synthesis	<i>H. sapiens</i>	[4–8]
Alg7p/Alg13p/Alg14p	Synthesis of man-dolichol precursor	<i>S. cerevisiae</i>	[16]
Alg1/Alg2/Alg11	Synthesis of man-dolichol precursor	<i>S. cerevisiae</i>	[18]
Oligosaccharyltransferase complex (OST)	Glycan precursor transfer to the acceptor	<i>S. cerevisiae</i>	[20–24]
M-Pol I [Van1p/Mnn9p]	Early mannosyl addition in the Golgi	<i>S. cerevisiae</i>	[29]
M-Pol II [Hoc1p/Mnn11p/Mnn10p/Anp1p/Mnn9p]	Late mannosyl addition in the Golgi	<i>S. cerevisiae</i>	[29]
Pmt2p/Pmt3p, Pmt1p/Pmt2p, Pmt5p/Pmt3p	Initiation of <i>O</i> -mannosyl glycans	<i>S. cerevisiae</i>	[58]
GlcNAcT-I/GlcNAcT-II	N-glycan branching	<i>H. sapiens</i>	[35, 40, 41]
GalT-I/ST6Gal-I and GalT-I/ST3Gal-III	Termination of N-glycans	<i>H. sapiens</i>	[40, 41]
B3GnT-8/B3GnT-2	Synthesis of polylectosamine	<i>H. sapiens</i>	[44, 45]
TPST1/ST6Gal-I, TPST2/ST6Gal-I	Tyrosylprotein sulfation/sialylation	<i>H. sapiens</i>	[46]
GlcNAcT-I/GnT1IPL	Inhibition of N-glycan branching	<i>M. musculus</i>	[47]
GalNAcT-6/C1GalT-1, -/C3GnT-1, -/C2GnT-1	Synthesis of O-glycan cores 1, 3 and 6	<i>H. sapiens</i>	[41]
EXT1/EXT2, Epi/2OST	Heparan sulfate synthesis	<i>H. sapiens, M. musculus</i>	[72–74]
ChSy-1/ChSy-2, ChPF/CSGlcA-T	Chondroitin sulfate synthesis	<i>H. sapiens</i>	[75]
B4GalT6/SiaT9/SiaT8A, B4GalNT1/B3GalT4	Synthesis of ganglio-series GSLs	<i>H. sapiens</i>	[68–70]
SiaT8A/B4GALNT1	Synthesis of ganglio-series GSLs	<i>H. sapiens</i>	[67]
HAS1/HAS2, HAS1/HAS3, HAS2/HAS3	Hyaluronan synthesis	<i>H. sapiens</i>	[76]
POMT1/POMT2	<i>O</i> -mannosylation	<i>H. sapiens</i>	[56, 57]
CESA1/CESA2/CESA3/CESA6	Primary cell wall synthesis	<i>A. thaliana</i>	[87]
CESA4/CESA7/CESA8	Secondary cell wall synthesis	<i>A. thaliana</i>	[87]
Chs3p/Chs4p	Chitin synthesis	<i>S. cerevisiae</i>	[77]
GALT-1/GMII	Plant N-glycan branching	<i>A. thaliana</i>	[48, 49]
GAUT1/GAUT7	Homogalacturonan synthesis	<i>A. thaliana</i>	[85]
XXT2/XXT5, XXT1/XXT2, XXT5/CSLC4	Xyloglucan backbone synthesis	<i>A. thaliana</i>	[92]
SSI/SSIIa/SBEIIa or SBEIIb	Starch synthesis and branching	<i>T. aestivum/Z. mays</i>	[93, 94]
SP/SBEI, SP/SBEIIb	Starch branching and degradation	<i>Z. mays</i>	[95]
Homomeric enzyme complexes			
Pmt4p	Elongation of mannan	<i>S. cerevisiae</i>	[58]
GlcNAcT-I, GlcNAcT-II	N-glycan branching	<i>H. sapiens</i>	[35, 40, 41]
GlcNAcT-V	N-glycan branching	<i>H. sapiens</i>	[36]
FucT-I, FucT-III and FucT-VI	Modification of N-glycans	<i>H. sapiens</i>	[34, 38, 39]
GalT-I	Termination of N-glycans	<i>H. sapiens</i>	[30, 31, 39, 42]
ST6Gal-I	N-glycan termination	<i>H. sapiens/R. norvegicus</i>	[33, 37, 38]
C2GnT-1	Core 6 synthetase	<i>H. sapiens</i>	[50]
C1GalT-1	Core 1 synthetase	<i>H. sapiens</i>	[51, 52]
LH3	<i>O</i> -Glycosylation of collagens	<i>H. sapiens</i>	[61, 62]
GlcAT-1	GlcA transfer to proteoglycans	<i>H. sapiens</i>	[71]
B4GALNT1 and SiaT8a	Synthesis of ganglio-series GSLs	<i>H. sapiens</i>	[65, 66]
HAS2 and HAS3	Hyaluronan synthesis	<i>H. sapiens</i>	[76]
CESA1 to CESA10	Plant cell wall synthesis [cellulose]	<i>A. thaliana</i>	[84]
CHS-2	Chitin synthesis [cell wall]	<i>M. sexta</i>	[78]
MNS1, GMII, GnTI and GnTII	N-glycan branching	<i>A. thaliana/N. tabacum</i>	[48, 49]
XylT	Xyloylation	<i>A. thaliana</i>	[48]
ARAD1 and ARAD2	Synthesis of pectic arabinan	<i>N. benthamiana</i>	[90]
FUT1, CSLC4 and XXT1 to 5	Xyloglucan backbone synthesis	<i>A. thaliana</i>	[91, 92]
MUR3 and XLT2	Xyloglucan backbone synthesis	<i>A. thaliana</i>	[92]
SSI, SSIIa, and SBEIIa/b	Amylopectin synthesis and branching	<i>T. aestivum/Z. mays</i>	[93, 94]

C2GNT-I). Cross-testing of other potential interactions between the nine *O*-glycosyltransferases tested did not reveal any other enzyme heteromers in the cells [41].

Other *O*-linked glycans *Drosophila* and mammalian Fringe proteins possess a fucose-specific α 1,3 *N*-acetylglucosaminyltransferase activity that initiates elongation of *O*-linked fucose residues attached to epidermal growth factor-like sequence repeats of Notch. Although direct evidence is missing, it has been suggested that Fringe proteins function as dimers [53]. *O*-mannose containing proteins were originally identified in yeast already in the 1950s. Their typical tetra-saccharide structure consists of Sia α 2–3Gal β 1–4GlcNAc β 1–2ManSer/Thr. They are now known to constitute one-third of all *O*-linked glycans in the brain of higher organisms, but are also abundant in the muscle tissue, in which the protein α -dystroglycan is the main acceptor for *O*-linked mannose addition. A defect in its *O*-mannosylation/glycosylation is associated with Walker-Warburg syndrome, an autosomal recessive multisystem disorder characterized by complex eye and brain abnormalities with congenital muscular dystrophy [54, 55]. The enzymes responsible for adding the *O*-mannose and *N*-acetylglucosamine are catalyzed by *O*-mannosyltransferase 1 (POMT1) and 2 (POMT2). Neither POMT1 nor POMT2 have enzymatic activity alone, but when co-expressed, *O*-mannosyltransferase activity was recovered [56, 57]. This suggests that the activity of POMT1 and POMT2 requires physical interaction of these enzymes *in vivo*. It is not known whether POMGNT1, which adds β 1,2-linked *N*-acetylglucosamine to *O*-mannose-containing glycoproteins and glycopeptides forms complexes with itself, later enzymes in the pathway or with POMTs themselves. In addition to mammalian enzymes, yeast *O*-mannosyltransferases have also been shown to form complexes with each other. Members of the PMT1 subfamily Pmt1p and Pmt5p were found to interact with members of the PMT2 subfamily, forming complexes between Pmt2p/Pmt3p, Pmt1p/Pmt2p or Pmt5p/Pmt3p [58]. On the other hand, Pmt4p (a member of the PMT4 subfamily) was found to form and function as a homomer. Finally, Proteins with collagen domains are modified by a disaccharide, Glc α 1–2Gal β , which is assembled on hydroxylysine or hydroxyproline residues. The first step in the pathway is the obligate hydroxylation of either lysine or proline by lysyl- and prolylhydroxylases prior to addition of glucose (and galactose). Glycosylation takes place before the formation of the triple helix, and may control the rate of triple-helix formation and, thus, also the size of the collagen fibrils [59]. Mice lacking the lysylhydroxylase 3 isoform (LH3) fail to glycosylate collagen IV properly, causing embryonic lethality and deposition of misfolded collagen in the ER [60]. This single polypeptide-containing enzyme has all three enzyme activities needed for collagen glycosylation,

and has been shown to exist as a homodimer on the luminal side of the ER membrane [61, 62].

Complexes involved in glycolipid synthesis

Glycosphingolipids form the main class of glycolipids. Their synthesis starts in the ER, and is completed by distinct glycolipid glycosyltransferases operating in the Golgi apparatus [30, 63, 64]. The first step in this series of reactions involves addition of either galactose or glucose to ceramide to form galactosyl- or glucosylceramide (GalCer, GlcCer). GalCer and other galactolipids are seldom extended to larger glycans. In more complex vertebrate glycosphingolipids, the GlcCer is typically extended first with β -linked galactose to give lactosylceramide (Gal β 1-4Glc β Cer) which is then further extended to generate a series of neutral “core” structures classified as ganglio-series (Gal β 1-3GalNAc β 1-4Gal β 1-4Glc β Cer), neolacto-series (Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc β Cer), lacto-series (Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc β Cer), globo-series (Gal α 1-4Gal β 1-4Glc β Cer), and isoglobo-series (Gal α 1-3Gal β 1-4Glc β Cer). Of these, the ganglio-series are the most common, being highly concentrated in the brain and being rich in sialic acids, making them negatively charged and thus important in the processes of neural signaling [63]. The neolacto-series in turn are most common in the hematopoietic system; lacto-series are prominent in secretory organs while the globo-series are most abundant in erythrocytes [30].

The synthesis of ganglio-series glycosphingolipids involves first B4GalT6, which adds the β 1,4-linked galactose to ceramide yielding lactosylceramide (LacCer i.e. Gal β 1-4Glc β Cer). This is then further modified mainly (with the exception of neutral GA2 “core” structure by B4GALNT1) to acidic gangliosides by SiaT9 and SiaT8A to form mono-, di- or tri-sialylated gangliosides (GM3, GD3 and GT3 gangliosides, respectively). Their synthesis is further elaborated to a-, b- and c-series by B4GALNT1 *N*-acetylgalactosaminyltransferase and by B3GalT4 galactosyltransferase. Several of these enzymes have been shown to form complexes with each other. For example, both GM2 and GD3 synthases have been shown to form homodimers [65, 66]. Enzyme activity measurements performed by Bieberich et al. [67] suggested an enzyme complex also between ST2 (SiaT8A, also named ST8SIA1, Table 1) and GalNAcT (B4GALNT1) but not with ST1 (SiaT9). Complex formation between SiaT8A and B4GALNT1 was confirmed both by co-immunoprecipitation and by FRET measurements. Similarly, by using co-immunoprecipitation and FRET microscopy, Maccioni et al. were able to show that the GM3, GD3, and GT3 synthases (B4GalT6, SiaT9 and SiaT8A, respectively)

form a multi-enzyme complex in the proximal Golgi [68–70], whereas the GM2, GD2 and GT2 synthase (B4GALNT1 and B3GalT4) form a distinct complex in the distal Golgi (TGN). Currently, it is not known whether similar complexes form also between enzymes that extend these sugar structures with sialic acid, fucose or GlcA. Some of these enzymes (e.g., Siat7, Siat4) may also operate in other glycosylation pathways as well.

Yeast and plant glycolipids Although lactosylceramide is the most common glycosphingolipid in vertebrates, it is rare in other eukaryotes. For example, it is substituted by Man β 1-4Glc-Cer and GlcNAc β 1-4Glc-Cer in invertebrates by inositol-1-*O*-phosphorylceramide in yeast, and by GlcNAc α 1-4GlcA α 1-2-*myo*-inositol-1-*O*-phosphorylceramide in plants. No direct evidence exists at the moment if the enzymes that synthesize these glycolipids form similar complexes to those described above for mammalian glycolipid synthesizing enzymes.

Complexes involved in proteoglycan synthesis

The Golgi apparatus is also responsible for the synthesis of cell surface and extracellular matrix proteoglycans. The polysaccharide side chains (glycosaminoglycan) added to various proteoglycans include keratan sulfate, chondroitin sulfate, dermatan sulfate and heparin or heparan sulfate [30]. They all consist of disaccharide repeats made of either glucuronic acid (GlcA), iduronic acid (IdoA), GalNAc, GlcNAc or Gal. They are also variably sulfated, linear, and often up to 100 sugar residues long attached to serine residues of various proteins via a common tetrasaccharide primer (Xyl-Gal-Gal-GlcA). An exception is keratansulfate, which utilizes *N*- (KS I) or *O*-linked (KS II) sulfated poly-*N*-acetylglucosamine structures ([Gal β 1-4GlcNAc β 1-3] $_n$) that are otherwise identical to those found on *N*- and *O*-linked glycoproteins. Thus, the enzymes responsible for keratan sulfate synthesis, synthesize other glycans as well.

By using electrophoretic mobility assays and cross-linking studies of the membrane-bound GlcAT-1, Ouzzine et al. [71] have shown that the membrane-bound GlcAT-I, an enzyme that adds glucuronic acid residue onto the above trisaccharide primer, forms enzymatically active disulfide-linked homodimers. In addition, McCormick et al. [72] have shown that EXT1 and EXT2 (both of which have GlcA- and GlcNAc-transferase activities and contribute to heparan sulfate biosynthesis [73]) form not only enzyme homomers but also heteromers both in yeast and mammalian cells. Heteromerization also augmented their enzymatic activities in cell lysates. Moreover, of the five heparan sulfate biosynthetic enzymes, galactosyltransferase I and glucuronosyltransferase I (required for the

formation of the linkage region), GlcNAc *N*-deacetylase/*N*-sulfotransferase 1, uronosyl 5-epimerase, and uronosyl 2-*O*-sulfotransferase), Pinhal et al. [74] were able to show that the last two of these (uronosyl 5-epimerase and uronosyl 2-*O*-sulfotransferase) interact with each other based on the ER relocation assay developed by Nilsson's group [43]. Izumikawa et al. [75] also demonstrated multiple interactions between chondroitin synthase-1 (ChSy-1), ChSy-2 (chondroitin sulfate synthase 3, CSS3), and also between chondroitin-polymerizing factor (ChPF) and glucuronyltransferase II (CSGlcA-T).

Hyaluronan, a glycosaminoglycan defined by the disaccharide unit (GlcNAc β 1-4GlcA β 1-3) $_n$ that is neither sulfated nor covalently linked to any protein, is rather exceptionally synthesized at the plasma membrane in various cell types by transmembrane enzymes called hyaluronan synthases. By using co-immunoprecipitation, FRET measurements and the proximity ligation assay, Tammi et al. [76] also recently were able to show that each of the three different hyaluronan synthases (HAS1, HAS2, HAS3) interact either with itself or with each other, forming heteromeric HAS1/HAS2, HAS1/HAS3 and HAS2/HAS3 complexes.

A vast array of other proteins is known to be modified with various glycans such as *N*-acetylglucosamine (GlcNAcacylation) and C-linked mannose. These modifications are in many cases also accomplished by glycosyltransferases complexed with either soluble or membrane proteins, which however, do not possess known transferase activity, and therefore, do not represent the main focus of this review.

Complexes involved in cell wall glycan and starch synthesis

Chitin synthesis In *S. cerevisiae*, the β 1,4-linked *N*-acetylglucosamine polymer, chitin, is synthesized by a family of 3 specialized chitin synthases encoded by *CHS1*, *CHS2* and *CHS3* genes. Each of these have special role in the synthesis of septum, lateral cell walls and the bud neck. Whether all these enzymes form oligomers is currently mostly unclear, but it has been shown that at least the Chs3p isoform forms a complex with Chs4p/Skt5p protein [77]. In the tobacco hornworm (*Manduca sexta*), the CHS-2 has a molecular weight of 520 kDa, thrice the calculated molecular weight of the monomer, suggesting it exists as a trimer [78].

Cellulose synthesis Plant cell walls are composed mainly of high-molecular-weight polysaccharides, proteins, and lignins. Cellulose is the most abundant linear polysaccharide present and consists of up to thousands of β 1,4-linked D-glucose units. These are synthesized in plants

by a plasma membrane-localized cellulose synthase complex that forms 25–30 nm diameter symmetrical rosettes each with six subunits [79]. The core components of this complex in higher plants are a family of ten CESA proteins [80–82]. Genetic and biochemical evidence has shown that three unique CESA isoforms are required for both primary cell wall and secondary cell wall cellulose synthesis. In *Arabidopsis thaliana*, these are CESA1, CESA3, and CESA6 and CESA4, CESA7, and CESA8, respectively [83, 84]. The remaining CESAs (CESA2, CESA5, CESA9, and CESA10) are likely involved in tissue-specific processes and are partially redundant with CESA6 [85]. Intriguingly, these enzymes were also shown to homodimerize prior to formation of larger CESA oligomers [84], similar to *N*-glycosyltransferases found in mammalian cells [86]. Very recently, a split-ubiquitin membrane yeast two-hybrid system demonstrated interactions between the four primary CESAs (CESA1, CESA2, CESA3, CESA6) and three secondary CESAs (CESA4, CESA7, CESA8) but also between the primary CESAs and secondary CESAs in a limited fashion. Further functional analysis of transgenic lines showed that CESA1 could partially rescue *irx1* (*cesa8*) null mutants, resulting in complementation of the plant growth defect and cellulose content deficiency [87]. The interactions between these enzymes were shown to utilize both disulfide bonds and non-covalent interactions [88].

Pectin and pectic arabinan synthesis Plant cell wall pectic polysaccharides are complex carbohydrates that are synthesized by low-abundance, Golgi membrane-bound biosynthetic enzymes. *Arabidopsis* galacturonosyltransferase (GAUT) 1 is an α 1,4-galacturonosyltransferase (GalAT) that synthesizes homogalacturonan, the most abundant pectic polysaccharide. It has been shown that GAUT1 functions in a protein complex with the homologous GAUT7 protein [89]. In addition, Golgi localization of GAUT1 is dependent on the formation of the GAUT1/GAUT7 complex. Other yet unknown protein components were also found to co-immunoprecipitate with the GAUT1/GAUT7, but their functional relevance in pectin synthesis remains obscure. Similarly, pectic arabinan, consisting of α 1,5-linked *L*-arabinofuranosyl residues to which other *L*-arabinofuranosyl residues are attached via α 1,3- and α 1,2-linkages, form a comb-like arrangement, and are synthesized by ARAD1 and its close homolog ARAD2. By using bimolecular fluorescence complementation, FRET and non-reducing gel electrophoresis, Harholt et al. [90] showed that ARAD1 and ARAD2 are localized in the same Golgi compartment and form intermolecular homo- and heterodimers when expressed transiently in *Nicotiana benthamiana*.

Xyloglucan synthesis Xyloglucan is the major hemicellulosic polysaccharide in the primary cell walls of dicotyledonous plants and has important structural and

physiological functions in plant growth and development. In *Arabidopsis*, the Golgi-localized 1,4- β -glucan synthase, Cellulose Synthase-Like C4 (CSLC4), and three xylosyltransferases, XXT1, XXT2, and XXT5, are responsible for the synthesis of the xyloglucan backbone. By using bimolecular fluorescence, complementation and in vitro pull-down assays Chou et al. [91] have shown that at least two of these enzymes CSLC4 and XXT2 form homomeric complexes. Heteromeric complexes were also detected between XXT2/XXT5, XXT1/XXT2, and XXT5/CSLC4. The same authors also showed very recently that three additional enzymes (MUR3, XLT2 and FUT1) involved in the xyloglycan synthesis form complexes between FUT1 and MUR3, XLT2, XXT2 or XXT5. XLT2 also interacts with XXT5, but MUR3 does not [92]. They also showed that FUT1, XXTs and CSLC4 also form disulfide-linked enzyme homomers, while the formation of the heteromers does not involve covalent interactions. In vitro pull-down assays indicated that in the FUT1/MUR3 and FUT1/XXT2 interactions are mediated by the catalytic domains of these enzymes.

Starch synthesis Amylose is a major polysaccharide of starch, making up approximately 20–30 % of the structure. The other main component of starch is amylopectin, which is branched and makes up to 70–80 % of the structure. Previous studies have indicated that starch synthases form high molecular weight complexes [93–95]. Co-immunoprecipitation experiments and affinity chromatography assays with recombinant proteins showed that starch synthase I (SSI), SSIIa, and SBEIIb (starch branching enzyme) form complexes with each other. All interactions were enhanced by ATP and broken by alkaline phosphatase, indicating a role for protein phosphorylation in their assembly. The authors proposed that, during amylopectin biosynthesis, SSI and SSIIa form the core of a phosphorylation-dependent glucan-synthesizing protein complex, which then recruits SBEIIb. Differences in stromal protein complexes mirrored the complement of the starch synthesizing enzymes detected in the starch granules, suggesting that the complexes have a functional role in starch biosynthesis.

Protein domains involved in glycosyltransferase interactions

The vast majority of Golgi localized glycosyltransferases are type II membrane proteins (Fig. 2), and thus, have a short N-terminal cytoplasmic domain, a single-pass transmembrane domain (TMD), a stem domain and a C-terminal catalytic domain facing the Golgi lumen [30, 96, 97]. Based on current evidence, all these domains have been shown to link glycosyltransferases together. For example,

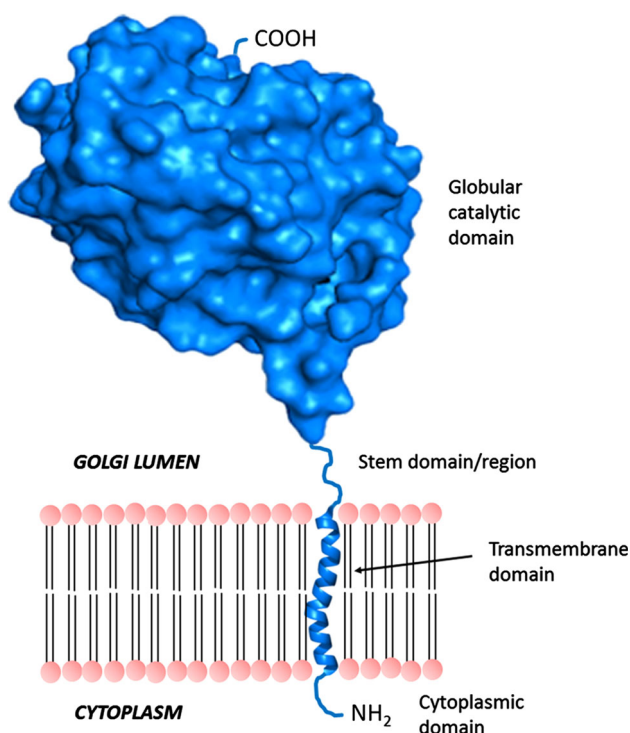


Fig. 2 Most of the Golgi localized glycosyltransferases are type II membrane proteins with a short N-terminal cytoplasmic domain, a ~20 amino acid α -helical TM-domain, a stem domain and a C-terminal globular catalytic domain in the lumen of the secretory pathway

homodimerization of many enzymes such as GalT-I and ST6Gal-I has been shown to be mediated by disulphide-bonds located in their transmembrane domains [32, 33, 37, 50, 71, 98]. Qian et al. [37] showed that a conserved Cys24 in the transmembrane region of rat ST6Gal-I is required for dimerization, as a Cys24Ala variant remained monomeric on SDS-PAGE. This does not, however, exclude the presence of other non-covalent interactions involved in homodimer formation. In fact, our domain swapping experiments combined with FRET measurements have shown that Cys24 deletion mutants still interact in the FRET assay via their catalytic domains [99]. Other Cys variants made (9 total) probably reflect misfolding rather than breakage of monomer–monomer contacts, given that the mutants localize into the ER (unpublished observations) or are used as sialyl motifs L and S within the catalytic domain of each ST6Gal-I monomer [100, 101]. Some plant glycosyltransferases also seem to utilize the same system for complex formation [48, 49, 90]. Similarly, glycolipid-synthesizing enzymes GalNAcT and GalT2 have also been shown to interact via their N-terminal cytosolic and transmembrane domains [68, 102].

In addition to disulphide bonds, both the stem and catalytic domains of glycosyltransferases have been shown to be responsible for the heteromeric interactions between

mammalian medial-Golgi enzymes GlcNAcT-I and ManII [35, 103]. Our previous domain swapping experiments showed that nearly all the main human *N*- and *O*-glycosyltransferase interactions are mediated by interactions between the catalytic domains of these enzymes [41], the only exception was GalT-I whose interactions either with itself or with ST6Gal-I seemed to involve mainly the cytoplasmic, transmembrane and/or the stem domain (120 N-terminal amino acids). Moreover, *Arabidopsis* cis- and medial-Golgi enzymes [48] seem also to utilize amino-terminal cytoplasmic-transmembrane-stem regions (CTS) for complex formation. Atmodjo et al. [89] in turn showed that *Arabidopsis* Golgi galacturonosyltransferases form heteromers via both covalent and non-covalent forces between their catalytic domains. By using BiFC and pull down assays, Chou et al. [92] have provided data recently to show that xyloglucan xylosyltransferases (XXT) not only form both homo- and heteromeric complexes via covalent and non-covalent bonds between the catalytic domains, but that the interaction surfaces are likely different depending on the XXT isoforms in the complex. The observed difference in the pH sensitivity of the *N*- and *O*-glycosyltransferase heteromers, relative to enzyme homomers [41], also suggests that the interaction surfaces needed for the self and non-self-binding are likely different.

Collectively, the observation that all different domains are utilized for complex formation depending on the enzyme(s) in question does not necessarily reflect contradictory findings, but rather, high sequence diversity of this class of enzymes and the use of distinct interaction surfaces in each case to link enzymes together. This would guarantee sufficient specificity for the interactions so that only relevant enzymes have a chance to interact. Sequence diversity at the amino acid level among glycosyltransferases may thus be an evolutionally driven phenomenon that likely has helped to organize glycosyltransferases into distinct and functionally relevant units.

Functional relevance of the glycosyltransferase complexes

Complex formation has been thought to be beneficial for glycosylation in several respects. Firstly, it has the potential to regulate the enzymatic activity of the complex constituents. Previous studies have shown that although enzymes can be active as monomers, homomers and heteromers [104], yet there is clear evidence that some enzymes also become activated upon complex formation. The best-known example of this is the heparin sulfate synthesis by EXT1 and EXT2 glycosyltransferases. When expressed alone, these enzymes localize predominantly to

Table 3 Enzymatic activity of known glycosyltransferase homomers and heteromers

Enzyme	Monomer/homomer	Heteromer	Activity increase ^a	References
EXT1/EXT2	Yes (low)	Yes	3–7	[72, 73]
β3GnT-2/β3GnT-8	Yes (low)	Yes	10	[45]
GalT-I/ST6Gal-I	Yes (moderate)	Yes	2.5	[41]
Sial-T1/Sial-T2	Yes (moderate)	Yes	2.5	[106, 107]

^a Heteromer activity vs. monomer/homomer

the ER and have only moderate activity [72]. However, when co-expressed, they localize to the Golgi and form more active enzyme heterodimers, as demonstrated by the increased levels of cell-associated GlcA and GlcNAc. Similarly, studies both in yeast and Cos-7 cells showed that while both EXT1 and EXT2 enzymes have GlcAT and GlcNAcT activity, their activities were markedly (3–7 fold, Table 3) augmented upon their co-expression [73]. The increase of glycosyltransferase activities was demonstrated also using only EXT1 and EXT2 catalytic domains, suggesting that they are sufficient to generate the heteromeric functional enzyme. Yet, there is still some controversy that relates to the exact functional role of EXT2, as it has very low activity. One possibility is that it may help folding and/or transport of EXT1 to the Golgi [105]. In addition, co-expression of poly-lactosamine synthesizing enzymes β3GnT-2 and β3GnT-8 has also been shown to increase the activity of both of these two enzymes in vitro [45]. Recent data from our laboratory has also shown that GalT-I and the ST6Gal-I activities are both increased by roughly 2.5 fold (relative to homodimers) upon their co-expression [41]. It is notable that in the case of GalT-I, the observed increase was not due to any trivial change in the amount of the enzyme protein itself nor of acceptor substrates, but rather to its interaction with the later enzyme in the same pathway. Furthermore, synthesis of GM3 and GD3 glycolipids by sialyltransferases Sial-T1 and Sial-T2 has been shown to result in near 2.5-fold higher Sial-T1 activity upon their co-transfection in CHO-K1 cells, relative to single transfected cells [106, 107]. This activity increase was also not found to be due to the appearance of Sial-T1 gene transcription activators or the stabilization of the Sial-T1 protein, but rather, to the activation of the Sial-T1 enzyme itself due to the formation of Gal-T1/Sial-T1/Sial-T2 multi-enzyme complex.

Complex formation may also modulate the enzymatic activity of glycosyltransferases in a different manner. For example, ganglioside GM2 synthase [98] was found to be more active as a homodimer than as a monomer, whereas ST6Gal-I [33] monomer was more active than the homodimer. Unfortunately, however, it remained unclear to what extent, if any, heteromerization alters the activity

of these enzymes (relative to enzyme homomers and monomers) in vivo and in vitro. Another example of an inhibitory interaction is the recently identified glycosyltransferase-like protein GnT1IP-L [47], which is known to interact specifically with the medial-Golgi enzyme GlcNAcT-I. Even though the GnT1IP-L protein does not seem to possess any measurable glycosyltransferase activity, its binding causes almost complete inhibition of GlcNAcT-I, which in turn down-regulates the synthesis of complex and hybrid N-glycans and helps developing sperm cells to remain attached to Sertoli cells and further differentiate into mature sperm cells. Similar interactions of glycosyltransferases with proteins that do not possess any enzymatic activity can modulate the enzymatic activity also by other means. As an example of this type of effect is the C1GalT-1 (T-synthase) and Cosmc, a specific folding chaperone that helps the enzyme to fold correctly [51, 52]. Another example is the DPM synthase, an enzyme that is needed for the synthesis of dolichol-phosphate-mannose (DPM), and consists of three non-homologous proteins, DPM1, DPM2 and DPM3. Of these, DPM2 and DPM3 are needed to keep the catalytically active DPM1 bound to the ER membrane and active, as otherwise it would move to the plasma membrane [108–112]. In addition, DPM2 may enhance the binding of the acceptor dolichol-phosphate and thereby keep DPM1 more active [109]. Moreover, cross-talk between DPM synthase and *N*-acetylglucosaminyl 1-phosphate transferase has been shown to increase the activity of both enzymes [111]. Complex formation between GPI-*N*-acetylglucosaminyltransferase and DPM2 has also been found to increase the transferase activity of the former by threefold [112]. Collectively, these observations suggest that the activity of the glycosyltransferases in many cases is modulated by complex formation. An increase in enzyme activity likely reflects co-operative functioning of the interacting enzymes in glycan synthesis and may involve substrate channeling through the complex and/or changes in the affinity of donor or acceptor substrates. Both of these are necessary prerequisites for faster processing and synthesis of a glycan chain. Complex formation may also be inhibitory to allow down-regulation of some glycosylation reactions for regulatory purposes.

Other surprises are likely to come when more work are accomplished with other glycosyltransferase complexes.

Secondly, complex formation likely helps to preserve fidelity during glycan synthesis. Although direct evidence is still lacking, complex formation is generally thought to provide a means to prevent intervention by potentially competing enzymes, which otherwise would be able to use the same sugar as an acceptor for glycan extension. Thus, by linking relevant (sequentially acting) enzymes together, complex formation would guarantee that correct sugar and linkage type would be used to extend the glycan chain. In this scenario, enzyme complexes could act as a kind of “templates” for each specific glycosylation step. The mucin-type core *O*-glycosylation represents an example of this. It involves several core structure forming enzyme complexes that can potentially compete with other enzymes that use the same GalNAc as an acceptor, such as sialylation of the initiating GalNAc to form the Tn-antigen. Normally, the presence of a preformed heteromeric core complex such as that between ppGalNAcT-6 and C1GalT-I is expected to prevent such competing reactions from occurring, thus favoring the synthesis of a complex-specific core structure (e.g. T-antigen, the product of the ppGalNAcT-6/C1GalT-I complex). In the absence of such a complex, such as in cancer cells [41], competing enzymes such as the ST6GalNAc-I can function and prematurely sialylate the initiating *O*-linked GalNAc. Consistent with this scenario, cancer cells typically express both Tn- and sialyl-Tn antigen at much higher levels than non-malignant cells (see [41] and references therein). Moreover, we showed that a Golgi pH increase inhibits the formation of this heteromeric ppGalNAcT-6/C1GalT-I complex and also the normal mucin type *O*-glycosylation likely by affecting both the core 1 synthesis and its extension to more complex *O*-glycans. Nevertheless, direct proof is needed and requires the construction and/or testing mutant enzymes which preserve their activity but cannot interact with their relevant partner.

Thirdly, complex formation may also modulate glycan synthesis by altering enzyme’s substrate or acceptor specificity, or its polymerizing capacity. Lactose synthase complex is a good example for the first case. Lactalbumin binding to GalT-I enables the enzyme to use glucose as an alternate acceptor [113]. On the other hand, chondroitin sulfate polymerizing glucuronyltransferase (chondroitin synthase-3) is an example of the case where the polymerizing capacity of an enzyme is changed upon complex formation. This enzyme has been shown to form multiple enzyme complexes that consist of distinct chondroitin synthase family members [75]. Depending on the complex, distinct sized chondroitin sulfate chains were produced. In addition, they exhibited distinct, but overlapping acceptor substrate specificities towards two synthetic acceptor

substrates. The same situation may also be true with hyaluronan synthesis where different complexes between HAS1, HAS2 and HAS3 have been shown to exist recently [76].

Fourthly, the observed pH sensitivity of the enzyme heteromers, but not of homomers [41], is important to notice, and reflects their formation in different cellular compartments. The latter were shown to form in the ER whereas the former are now known to assemble in the acidic Golgi environment [86]. This result emphasizes that homomers and heteromers are not competing enzyme species, but rather represent inter-dependent membrane constituents that undergo constant organelle micro-environment-dependent transitions between two physical states during their suggested recycling between the early secretory compartments (Fig. 3). Such transitions indeed take place between the ER and the Golgi [86], and provide a simple means to localize the most active enzyme species (heteromers) in the Golgi where these enzymes are known to operate, and thereby can increase the glycosylation potential of the Golgi. The absence of enzyme heteromers in acidification and glycosylation -defective cancer cells is in accordance with this view. Nevertheless, an important question that remains is whether divalent cations needed for the enzymatic activity of several glycosyltransferases have any role in complex formation. It needs also to be emphasized that not all enzymes have been shown to form

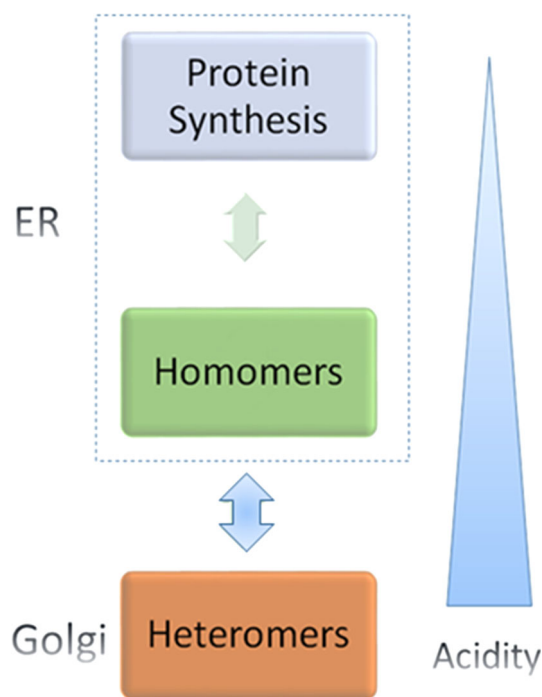


Fig. 3 A schematic model of the dynamic transitions between glycosyltransferase homomers and heteromers and their dependence on Golgi acidity (for details, see text)

such heteromers. Thus, the possibility that some enzymes may exist and also function as monomers and/or homomers cannot be excluded at this point.

Finally, complex formation has been suggested to have a role also in the correct targeting of glycosyltransferases to the Golgi and to its various sub-compartments. This possibility was raised in the early 90s by showing that oligomerization correlated with the Golgi localization of various membrane proteins including coronavirus m1 protein and various glycosyltransferases [35, 42, 43, 114, 115] along with the “kin recognition” hypothesis [43]. These observations raised an idea that Golgi enzymes form complexes large enough not to fit into the forming transport carriers, leading to their retention in the organelle. However, the now generally accepted “cisternal maturation” model is not in accord with this oligomerization-mediated Golgi retention hypothesis, as it assumes that the Golgi enzymes continuously recycle between Golgi cisternae and the ER. In fact, it has been shown by using the FRAP (fluorescence recovery after photobleaching) approach that glycosyltransferases in general [116] as well as their homomers and heteromers [86] remain as mobile Golgi membrane constituents that recycle between the first two secretory compartments. Thus, even though some enzymes are mislocalized after experimental Golgi pH increase [99], it is currently not clear whether complex formation itself is a determinant for Golgi retention or retrieval. Further studies with explicitly monomeric and oligomeric glycosyltransferases will help answer to this pertinent question.

Structural aspects of glycosyltransferases and their complexes

There are approximately 250 known glycosyltransferases which, according to their DNA sequence, are categorized into 97 distinct families. Classification is based on amino acid sequence comparisons and differences in activated donor and specific acceptor molecules in forming glycosidic bonds. Crystal structures exist for glycosyltransferases representing 41 families including 11 families with at least one human enzyme structure solved. Yet, nearly all the existing crystallographic structures represent only the globular catalytic domains of enzyme monomers. They all also fall into one of the three main fold types (GT-A, GT-B and Cst II fold), depending on the relative positions of α -helices and a central β -sheet in each fold [97]. The main impact of all these structures is that they have helped to resolve both donor and acceptor glycan binding modes as well as their catalytic mechanisms (for excellent reviews, see [117–121]). Here, instead, we focus on the few enzymes whose structures either appear in the crystals, or have been resolved, as complexes.

Volkers et al. [122] recently published apo- and ligand-bound crystal structures of human ST8SiaIII

sialyltransferase active in polysialylation. The two enzyme monomers in the asymmetric unit are related by near two-fold symmetry (Fig. 4). Analysis by using the PISA server [123] showed that the dimer interface area $\approx 968 \text{ \AA}^2$ is well in the range typically observed for functionally relevant protein complexes and the presence of such a dimer was also experimentally detected in solution. ST8SiaIII structure represents a prime example of a glycosyltransferase homodimer with well plausible assembly: the dimer formation places both active sites on the same side of the dimer and within a distance from each other, which makes various functional scenarios possible.

The structure of human $\alpha 1,6$ -fucosyltransferase, FUT8 [124], is another glycosyltransferase crystal structure exemplifying a homodimer. The FUT8 catalytic domain resembles the GT-B fold, but has an embedded SH3 domain (a common interaction domain) in the C-terminus, which does not take part in the binding interface, however. The crystallized protein structure contains also a large part of the stem domain, which is disordered in the crystal structure. The beginning of the catalytic domain forms two long α -helices (Fig. 4), which serve as the dimerization interface forming a compact four-helix bundle with a buried area of 3195 \AA^2 . In this structure the catalytic centers of the two FUT8 monomers are also placed on the same side of the dimer, but not facing the membrane, and also on opposite ends of the dimer. Vicinity of the N-terminal ends of the catalytic domains would enable a scenario where the stem and the transmembrane domains contribute to dimer formation.

The other homodimeric complexes of glycosyltransferases present in crystal structures represent variations of the two structures discussed above. There are differences in the interaction surfaces, both in their volume and the structural details that characterize these interactions. Bovine $\beta 1,4$ -galactosyltransferase (GalT-I) [125] appears a dimer with the two catalytic domains making contact through eight hydrogen bonds within a total contact area of 424 \AA^2 [126]. The interaction area is sufficiently large to represent a functionally relevant interaction. Two structures of an engineered dual-specificity blood group A and B antigen glycosyltransferase AA(Gly)B by [127] were solved using two different crystals with different molecular packing (crystal contacts) and having either one or two molecules per asymmetric unit (Fig. 4). Despite these differences, the homodimer is assembled in either case exactly in the same way, indicating that the assembly represents a functionally relevant dimer possibly formed prior to exposing the protein sample to the crystallization screening. Human $\beta 1,3$ -Glucuronyltransferase I (GlcAT-I, Fig. 4) [128] and GlcAT-P [129] both represent physiologically relevant homodimers with 16 % of the total surface area being buried in the dimer

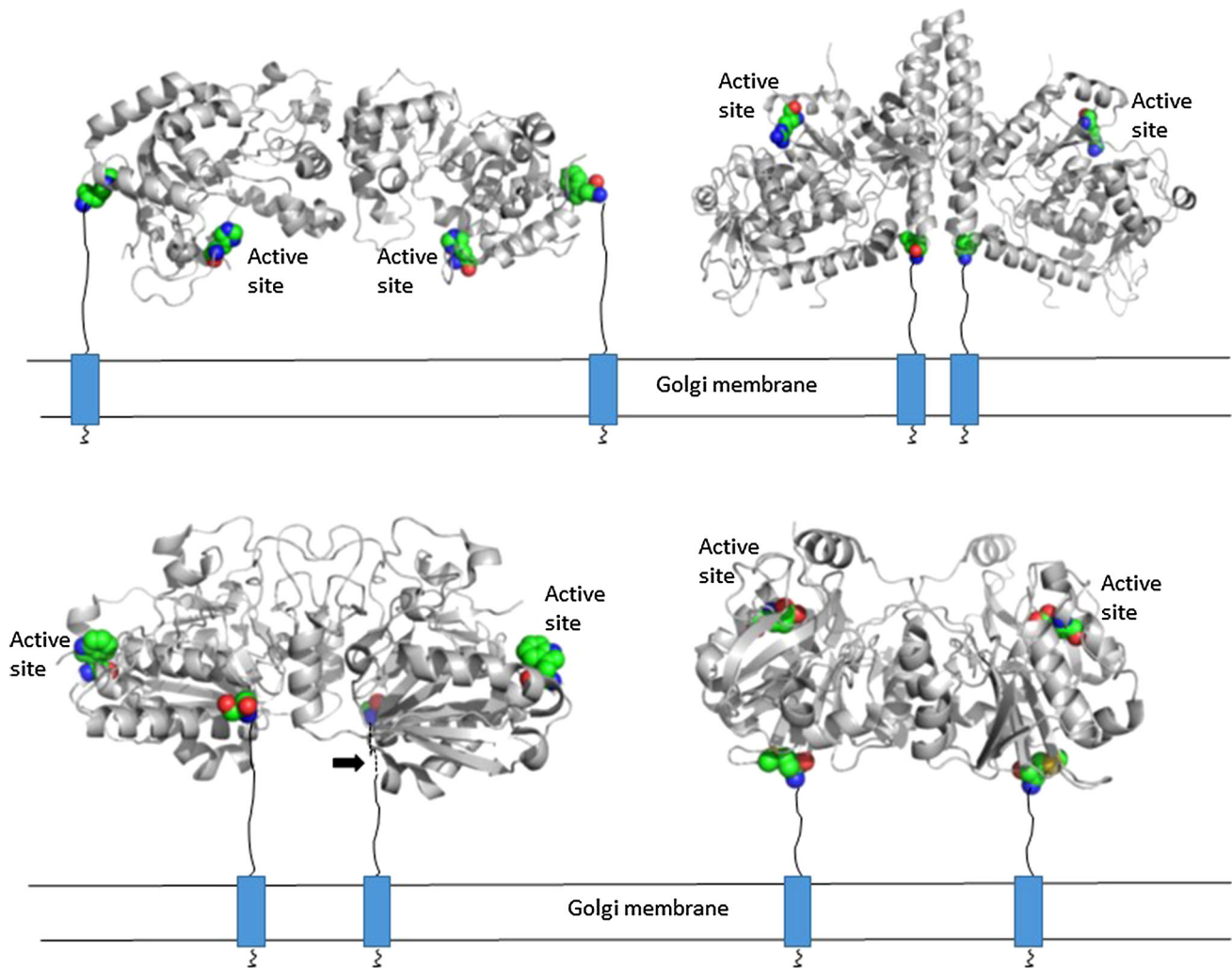


Fig. 4 Homomeric glycosyltransferase complex structures representing functionally relevant case studies. *Top left* ST8SiaIII sialyltransferase homodimer (gray cartoon model) [122] shows how the active sites come relatively close to each other and face the Golgi membrane. N-termini of the crystallized catalytic domains are situated on both ends of the dimer with the first amino acid residue (Trp90) indicated with space filling atoms and completed to a type II membrane protein model with schematic stem- and membrane spanning domains (black line and blue rectangle) included. Positions of the active sites are indicated with a catalytic residue His354. *Top right* The structure of α 1,6-fucosyltransferase FUT8 [124] is shown. The first residues in the crystallized construct, Leu108, come close to each other suggesting a possible assembly of the stem- and membrane spanning domains. Active sites are indicated by catalytic residues Arg365. *Bottom left* Engineered dual-specificity blood group A and B

antigen glycosyltransferase AA(Gly)B homodimer [127] also shows the active sites (represented by Trp181) on the ends of the dimer, but interestingly the N-termini are located on opposite sides of the dimer: the *left one* (Ser65) towards the viewer and the *right one* (Val64) on the back (indicated by a short dashed line and an arrow). *Bottom right* Human β 1,3-Glucuronyltransferase I (GlcAT-I [129] and GlcAT-P appear to have their active sites (Gln281) buried deeper in the end of each monomer and N-termini (Met75) located parallel with them, perpendicular to the membrane. Protein Data Bank entries 5BO6, 2DE0, 3ZGF and 1FGG were used, respectively, to draw the figure. It should be noted that N-termini in these figures are those made visible in the structures, and that the stem- and transmembrane regions can be assembled in different ways, and are represented only schematically in the pictures

interface (GlcAT-I) and nearly identical residues forming the interface also in the GlcAT-P homodimer. Interestingly, the homodimer assembly is highly different from that of the blood group A and B antigen glycosyltransferase AA(Gly)B complex [127]. While the latter has the active sites on opposite ends of the dimer, in GlcAT-I and GlcAT-P dimers they are next to each other on the same side of the homodimer.

The few cases discussed here show how little is known in structural detail on the formation and nature of glycosyltransferase complexes. From this data, it is apparent that such complexes can form in various ways and there is no one or two models that fit all. It is to be noted that all glycosyltransferase structures represent the catalytic domains with either complete or partial omission of the stem region from the crystallized constructs. Therefore the involvement of the

stem region and possibly also the transmembrane domain in taking part in the dimerization cannot be excluded. These observations emphasize that structure determinations of also functionally important heterodimers at atomic detail are necessary for detailed understanding on how the complexes interact and how they co-operatively function in glycan synthesis. It is not clear if the catalytic domains—for which the structures already exist—are sufficient in each case to establish stable homo- or heterodimers. Attempts to resolve these issues are thus necessary and should take into account the following important considerations. Firstly, crystallization conditions may fail to simulate the conditions of the Golgi lumen important for heteromer formation. This is especially important for the pH sensitive interactions of some of the glycosyltransferase heteromers, though they have been shown to resist solubilization but only under proper experimental conditions [41, 86]. Secondly, it is clear that the homomeric and heteromeric complexes are transient and dynamic owing to their constant and microenvironment dependent transitions between less active homomers and more active heteromers during their recycling within the early secretory compartments. This may in fact be behind the difficulties in defining functionally relevant or irrelevant interactions. Thirdly, the trials in which the complex constituents are combined in a test tube does not necessarily guarantee that a complex will form. Rather, they may require conditions that exist only in the living cell. Fourthly, structures or structural models of any glycosyltransferase dimers or higher complexes must fulfill the expectation that the active sites are not buried within the interaction surface, and also that the active sites are situated in such a manner that the binding of donor and acceptor molecules is feasible.

Future directions

A wealth of information now has accumulated and shows that glycosyltransferases in general tend to form functionally relevant complexes in live cells, as in some cases, it has already been shown that the formation of such complexes, especially between sequentially acting enzymes, either augments or diminishes the enzymatic activity of the complex constituents. Yet, the main impact of their existence in most glycosylation pathways in all eukaryotes is that they expose a new level of regulation of glycan synthesis. They also seem to provide the “molecular template” for critical points in glycan synthesis by linking relevant enzymes together and thereby, preventing competing reactions from occurring. Evidence already exist that their absence in diseases such as cancers alters glycosylation of cell surface glycans, a phenomenon that often has fatal consequences on human wellbeing. Due to the individual nature of these complexes, future work should focus on

detailed molecular characterization of the complexes, clarification of the interaction surfaces by mutagenesis and on resolving their 3D structures as well as functional consequences on glycan synthesis. This would help understand better how their interactions are regulated by organelle micro-environmental factors (such as pH) in both normal cells and cancerous cells in which such complexes are rare. Clarifying these issues will help uncover important cell and glycobiological questions related to the Golgi apparatus and its’ functioning as a glycosylation device and as a central station for protein delivery to post-Golgi compartments and beyond.

Acknowledgments The authors would like to thank Emil Aaltonen Foundation for financial support. We would also like to give our warmest thanks to an experienced Japanese female researcher who shared her early findings on glycosyltransferase complexes with us, but whose name we unfortunately cannot track anymore.

References

1. Dunphy WG, Brands R, Rothman JE (1985) Attachment of terminal *N*-acetylglucosamine to asparagine-linked oligosaccharides occurs in central cisternae of the Golgi stack. *Cell* 40:463–472
2. Kornfeld R, Kornfeld S (1985) Assembly of asparagine-linked oligosaccharides. *54*: 631–664
3. Rabouille C, Hui N, Hunte F, Kieckbusch R, Berger EG, Warren G et al (1995) Mapping the distribution of Golgi enzymes involved in the construction of complex oligosaccharides. *J Cell Sci* 108:1617–1627
4. Brodbeck U, Ebner KE (1966) Resolution of a soluble lactose synthetase into two protein components and solubilization of microsomal lactose synthetase. *J Biol Chem* 241:762–764
5. Brodbeck U, Denton WL, Tanahashi N, Ebner KE (1967) The isolation and identification of the B protein of lactose synthetase as alpha-lactalbumin. *J Biol Chem* 242:1391–1397
6. Brew K, Vanaman TC, Hill RL (1968) The role of alpha-lactalbumin and the A protein in lactose synthetase: a unique mechanism for the control of a biological reaction. *Proc Natl Acad Sci* 59:491–497
7. Klee WA, Klee CB (1970) The role of alpha-lactalbumin in lactose synthetase. *Biochem Biophys Res Commun* 39:833–841
8. Klee WA, Klee CB (1972) The interaction of -lactalbumin and the A protein of lactose synthetase. *J Biol Chem* 247:2336–2344
9. Schwartz NB, Roden L, Dorfman A (1974) Biosynthesis of chondroitin sulfate: interaction between xylosyltransferase and galactosyltransferase. *Biochem Biophys Res Commun* 56:717–724
10. Schwartz NB (1975) Biosynthesis of chondroitin sulfate: immunoprecipitation of interacting xylosyltransferase and galactosyltransferase. *FEBS Lett* 49:342–345
11. Fishman PH (1974) Normal and abnormal biosynthesis of gangliosides. *Chem Phys Lipids* 13:305–326
12. Ivatt RJ (1981) Regulation of glycoprotein biosynthesis by formation of specific glycosyltransferase complexes. *Proc Natl Acad Sci* 78:4021–4025
13. Parodi AJ (2000) Role of *N*-oligosaccharide endoplasmic reticulum processing reactions in glycoprotein folding and degradation. *Biochem J* 348(Pt 1):1–13
14. Roth J (2002) Protein *N*-glycosylation along the secretory pathway: relationship to organelle topography and function,

- protein quality control, and cell interactions. *Chem Rev* 102:285–303
15. Helenius J, Ng DT, Marolda CL, Walter P, Valvano MA, Aebi M (2002) Translocation of lipid-linked oligosaccharides across the ER membrane requires Rft1 protein. *Nature* 415:447–450
 16. Noffz C, Keppler-Ross S, Dean N (2009) Hetero-oligomeric interactions between early glycosyltransferases of the dolichol cycle. *Glycobiology* 19:472–478
 17. Lu J, Takahashi T, Ohoka A, Nakajima K, Hashimoto R, Miura N et al (2012) Alg14 organizes the formation of a multiglycosyltransferase complex involved in initiation of lipid-linked oligosaccharide biosynthesis. *Glycobiology* 22:504–516
 18. Gao XD, Nishikawa A, Dean N (2004) Physical interactions between the Alg1, Alg2, and Alg11 mannosyltransferases of the endoplasmic reticulum. *Glycobiology* 14:559–570
 19. Burda P, Aebi M (1999) The dolichol pathway of N-linked glycosylation. *Biochim Biophys Acta* 1426:239–257
 20. Zufferey R, Knauer R, Burda P, Stagljar I, te Heesen S, Lehle L et al (1995) STT3, a highly conserved protein required for yeast oligosaccharyl transferase activity in vivo. *EMBO J* 14:4949–4960
 21. Yan Q, Lennarz WJ (1999) Oligosaccharyltransferase: a complex multisubunit enzyme of the endoplasmic reticulum. *Biochem Biophys Res Commun* 266:684–689
 22. Chavan M, Chen Z, Li G, Schindelin H, Lennarz WJ, Li H (2006) Dimeric organization of the yeast oligosaccharyl transferase complex. *Proc Natl Acad Sci* 103:8947–8952
 23. Pfeiffer S, Dudek J, Gogala M, Schorr S, Linxweiler J, Lang S et al (2014) Structure of the mammalian oligosaccharyl-transferase complex in the native ER protein translocon. *Nat Commun* 5:3072
 24. Mohorko E, Owen RL, Malojcic G, Brozzo MS, Aebi M, Glockshuber R (2014) Structural basis of substrate specificity of human oligosaccharyl transferase subunit N33/Tusc3 and its role in regulating protein N-glycosylation. *Structure* 22:590–601
 25. Cabral CM, Liu Y, Sifers RN (2001) Dissecting glycoprotein quality control in the secretory pathway. *Trends Biochem Sci* 26:619–624
 26. Ellgaard L, Helenius A (2003) Quality control in the endoplasmic reticulum. *Nat Rev Mol Cell Biol* 4:181–191
 27. Ruddock LW, Molinari M (2006) N-glycan processing in ER quality control. *J Cell Sci* 119:4373–4380
 28. Herscovics A, Orlean P (1993) Glycoprotein biosynthesis in yeast. *FASEB J* 7:540–550
 29. Jungmann J, Munro S (1998) Multi-protein complexes in the cis Golgi of *Saccharomyces cerevisiae* with α -1, 6-mannosyltransferase activity. *EMBO J* 17:423–434
 30. Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, et al (eds). *Essentials of glycobiology*. 2nd ed. La Jolla, California: The Consortium of Glycobiology Editors; 2009
 31. Teasdale RD, Matheson F, Gleeson PA (1994) Post-translational modifications distinguish cell surface from Golgi-retained beta 1,4 galactosyltransferase molecules. Golgi localization involves active retention. *Glycobiology* 4:917–928
 32. Yamaguchi N, Fukuda MN (1995) Golgi retention mechanism of beta-1,4-galactosyltransferase. Membrane-spanning domain-dependent homodimerization and association with alpha- and beta-tubulins. *J Biol Chem* 270:12170–12176
 33. Ma J, Colley KJ (1996) A disulfide-bonded dimer of the Golgi beta-galactoside alpha2,6-sialyltransferase is catalytically inactive yet still retains the ability to bind galactose. *J Biol Chem* 271:7758–7766
 34. Borsig L, Katopodis AG, Bowen BR, Berger EG (1998) Trafficking and localization studies of recombinant alpha1, 3-fucosyltransferase VI stably expressed in CHO cells. *Glycobiology* 8:259–268
 35. Opat AS, Houghton F, Gleeson PA (2000) Medial Golgi but not late Golgi glycosyltransferases exist as high molecular weight complexes. Role of luminal domain in complex formation and localization. *J Biol Chem* 275:11836–11845
 36. Sasai K, Ikeda Y, Tsuda T, Ihara H, Korekane H, Shiota K et al (2001) The critical role of the stem region as a functional domain responsible for the oligomerization and Golgi localization of N-acetylglucosaminyltransferase V. The involvement of a domain homophilic interaction. *J Biol Chem* 276:759–765
 37. Qian R, Chen C, Colley KJ (2001) Location and mechanism of alpha 2,6-sialyltransferase dimer formation. Role of cysteine residues in enzyme dimerization, localization, activity, and processing. *J Biol Chem* 276:28641–28649
 38. El-Battari A, Prorok M, Angata K, Mathieu S, Zerfaoui M, Ong E et al (2003) Different glycosyltransferases are differentially processed for secretion, dimerization, and autoglycosylation. *Glycobiology* 13:941–953
 39. Sousa VL, Brito C, Costa T, Lanoix J, Nilsson T, Costa J (2003) Importance of Cys, Gln, and Tyr from the transmembrane domain of human alpha 3/4 fucosyltransferase III for its localization and sorting in the Golgi of baby hamster kidney cells. *J Biol Chem* 278:7624–7629
 40. Hassinen A, Rivinoja A, Kauppila A, Kellokumpu S (2010) Golgi N-glycosyltransferases form both homo- and heterodimeric enzyme complexes in live cells. *J Biol Chem* 285:17771–17777
 41. Hassinen A, Pujol FM, Kokkonen N, Pieters C, Kihlstrom M, Korhonen K et al (2011) Functional organization of the Golgi N- and O-glycosylation pathways involves pH-dependent complex formation that is impaired in cancer cells. *J Biol Chem* 286:38329–38340
 42. Nilsson T, Slusarewicz P, Hoe MH, Warren G (1993) Kin recognition. A model for the retention of Golgi enzymes. *FEBS Lett* 330:1–4
 43. Nilsson T, Hoe MH, Slusarewicz P, Rabouille C, Watson R, Hunte F et al (1994) Kin recognition between medial Golgi enzymes in HeLa cells. *EMBO J* 13:562–574
 44. Seko A, Yamashita K (2005) Characterization of a novel galactose beta1,3-N-acetylglucosaminyltransferase (beta3Gn-T8): the complex formation of beta3Gn-T2 and beta3Gn-T8 enhances enzymatic activity. *Glycobiology* 15:943–951
 45. Seko A, Yamashita K (2008) Activation of beta1,3-N-acetylglucosaminyltransferase-2 (beta3Gn-T2) by beta3Gn-T8. Possible involvement of beta3Gn-T8 in increasing poly-N-acetyllactosamine chains in differentiated HL-60 cells. *J Biol Chem* 283:33094–33100
 46. Hartmann-Fatu C, Trusch F, Moll CN, Michin I, Hassinen A, Kellokumpu S et al (2015) Heterodimers of Tyrosylprotein Sulfotransferases suggest existence of a higher organization level of transferases in the membrane of the trans-Golgi apparatus. *J Mol Biol* 427:1404–1412
 47. Huang HH, Stanley P (2010) A testis-specific regulator of complex and hybrid N-glycan synthesis. *J Cell Biol* 190:893–910
 48. Schoberer J, Liebminger E, Botchway SW, Strasser R, Hawes C (2013) Time-resolved fluorescence imaging reveals differential interactions of N-glycan processing enzymes across the Golgi stack in planta. *Plant Physiol* 161:1737–1754
 49. Oikawa A, Lund CH, Sakuragi Y, Scheller HV (2013) Golgi-localized enzyme complexes for plant cell wall biosynthesis. *Trends Plant Sci* 18:49–58
 50. Yen TY, Macher BA, Bryson S, Chang X, Tvaroška I, Tse R et al (2003) Highly conserved cysteines of mouse core 2 β 1, 6-N-acetylglucosaminyltransferase I form a network of disulfide bonds and include a thiol that affects enzyme activity. *J Biol Chem* 278:45864

51. Aryal RP, Ju T, Cummings RD (2010) The endoplasmic reticulum chaperone Cosmc directly promotes in vitro folding of T-synthase. *J Biol Chem* 285:2456–2462
52. Aryal RP, Ju T, Cummings RD (2012) Tight complex formation between Cosmc chaperone and its specific client non-native T-synthase leads to enzyme activity and client-driven dissociation. *J Biol Chem* 287:15317–15329
53. Correia T, Papayannopoulos V, Panin V, Woronoff P, Jiang J, Vogt TF et al (2003) Molecular genetic analysis of the glycosyltransferase Fringe in *Drosophila*. *Proc Natl Acad Sci* 100:6404–6409
54. Buysse K, Riemersma M, Powell G, van Reeuwijk J, Chitayat D, Roscioli T et al (2013) Missense mutations in beta-1,3-N-acetylglucosaminyltransferase 1 (B3GNT1) cause Walker-Warburg syndrome. *Hum Mol Genet* 22:1746–1754
55. Roscioli T, Kamsteeg EJ, Buysse K, Maystadt I, van Reeuwijk J, van den Elzen C et al (2012) Mutations in ISPD cause Walker-Warburg syndrome and defective glycosylation of alpha-dysglycan. *Nat Genet* 44:581–585
56. Akasaka-Manyu K, Manyu H, Endo T (2004) Mutations of the POMT1 gene found in patients with Walker-Warburg syndrome lead to a defect of protein O-mannosylation. *Biochem Biophys Res Commun* 325:75–79
57. Manyu H, Chiba A, Yoshida A, Wang X, Chiba Y, Jigami Y et al (2004) Demonstration of mammalian protein O-mannosyltransferase activity: coexpression of POMT1 and POMT2 required for enzymatic activity. *Proc Natl Acad Sci* 101:500–505. doi:10.1073/pnas.0307228101
58. Girschbach V, Strahl S (2003) Members of the evolutionarily conserved PMT family of protein O-mannosyltransferases form distinct protein complexes among themselves. *J Biol Chem* 278:12554–12562
59. Sipila L, Ruotsalainen H, Sormunen R, Baker NL, Lamande SR, Vapola M et al (2007) Secretion and assembly of type IV and VI collagens depend on glycosylation of hydroxylysines. *J Biol Chem* 282:33381–33388
60. Risteli M, Ruotsalainen H, Salo AM, Sormunen R, Sipila L, Baker NL et al (2009) Reduction of lysyl hydroxylase 3 causes deleterious changes in the deposition and organization of extracellular matrix. *J Biol Chem* 284:28204–28211
61. Wang C, Risteli M, Heikkinen J, Hussa AK, Uitto L, Myllyla R (2002) Identification of amino acids important for the catalytic activity of the collagen glucosyltransferase associated with the multifunctional lysyl hydroxylase 3 (LH3). *J Biol Chem* 277:18568–18573
62. Heikkinen J, Risteli M, Lampela O, Alavesu P, Karppinen M, Juffer AH et al (2011) Dimerization of human lysyl hydroxylase 3 (LH3) is mediated by the amino acids 541–547. *Matrix Biol* 30:27–33
63. Hakomori S (2003) Structure, organization, and function of glycosphingolipids in membrane. *Curr Opin Hematol* 10:16–24
64. Maccioni HJ, Quiroga R, Ferrari ML (2011) Cellular and molecular biology of glycosphingolipid glycosylation. *J Neurochem* 117:589–602
65. Bieberich E, MacKinnon S, Silva J, Li DD, Tencomnao T, Irwin L et al (2002) Regulation of ganglioside biosynthesis by enzyme complex formation of glycosyltransferases. *Biochemistry* 41:11479–11487
66. Giraudo CG, Daniotti JL, Maccioni HJF (2001) Physical and functional association of glycolipid N-acetyl-galactosaminyl and galactosyl transferases in the Golgi apparatus. 98:1625
67. Giraudo CG, Maccioni HJF (2003) Ganglioside glycosyltransferases organize in distinct multi-enzyme complexes in CHO-K1 cells. *J Biol Chem* 278:40262–40271
68. Maccioni HJ (2007) Glycosylation of glycolipids in the Golgi complex. *J Neurochem* 103(Suppl 1):81–90
69. Li J, Rancour DM, Allende ML, Worth CA, Darling DS, Gilbert JB et al (2001) The DXD motif is required for GM2 synthase activity but is not critical for nucleotide binding. *Glycobiology* 11:217–229
70. Daniotti JL, Martina JA, Giraudo CG, Zurita AR, Maccioni HJ (2000) GM3 alpha2,8-sialyltransferase (GD3 synthase): protein characterization and sub-golgi location in CHO-K1 cells. *J Neurochem* 74:1711–1720
71. Ouzzine M, Gulberti S, Netter P, Magdalou J, Fournel-Gigleux S (2000) Structure/function of the human Galbeta1,3-galacturonosyltransferase. Dimerization and functional activity are mediated by two crucial cysteine residues. *J Biol Chem* 275:28254–28260
72. McCormick C, Duncan G, Goutsos KT, Tufaro F (2000) The putative tumor suppressors EXT1 and EXT2 form a stable complex that accumulates in the Golgi apparatus and catalyzes the synthesis of heparan sulfate. *Proc Natl Acad Sci* 97:668–673
73. Senay C, Lind T, Muguruma K, Tone Y, Kitagawa H, Sugahara K et al (2000) The EXT1/EXT2 tumor suppressors: catalytic activities and role in heparan sulfate biosynthesis. *EMBO Rep* 1:282–286
74. Pinhal MA, Smith B, Olson S, Aikawa J, Kimata K, Esko JD (2001) Enzyme interactions in heparan sulfate biosynthesis: uronosyl 5-epimerase and 2-O-sulfotransferase interact in vivo. *Proc Natl Acad Sci* 98:12984–12989
75. Izumikawa T, Koike T, Shiozawa S, Sugahara K, Tamura J, Kitagawa H (2008) Identification of chondroitin sulfate glucuronyltransferase as chondroitin synthase-3 involved in chondroitin polymerization: chondroitin polymerization is achieved by multiple enzyme complexes consisting of chondroitin synthase family members. *J Biol Chem* 283:11396–11406
76. Bart G, Vico NO, Hassinen A, Pujol FM, Deen AJ, Ruusala A et al (2015) Fluorescence Resonance Energy Transfer (FRET) and Proximity Ligation Assays Reveal Functionally Relevant Homo- and Heteromeric Complexes among Hyaluronan Synthases HAS1, HAS2 and HAS3. *J Biol Chem* 290:11479–11490
77. Lesage G, Shapiro J, Specht CA, Sdicu AM, Menard P, Hussein S et al (2005) An interactional network of genes involved in chitin synthesis in *Saccharomyces cerevisiae*. *BMC Genet* 6:8
78. Maue L, Meissner D, Merzendorfer H (2009) Purification of an active, oligomeric chitin synthase complex from the midgut of the tobacco hornworm. *Insect Biochem Mol Biol* 39:654–659
79. Mueller SC, Brown RM Jr (1980) Evidence for an intramembrane component associated with a cellulose microfibril-synthesizing complex in higher plants. *J Cell Biol* 84:315–326
80. Arioli T, Peng L, Betzner AS, Burn J, Wittke W, Herth W et al (1998) Molecular analysis of cellulose biosynthesis in *Arabidopsis*. *Science* 279:717–720
81. Kimura S, Laosinchai W, Itoh T, Cui X, Linder CR, Brown RM Jr (1999) Immunogold labeling of rosette terminal cellulose-synthesizing complexes in the vascular plant *Vigna angularis*. *Plant Cell* 11:2075–2086
82. Richmond TA, Somerville CR (2000) The cellulose synthase superfamily. *Plant Physiol* 124:495–498
83. Persson S, Paredez A, Carroll A, Palsdottir H, Doblin M, Poindexter P et al (2007) Genetic evidence for three unique components in primary cell-wall cellulose synthase complexes in *Arabidopsis*. *Proc Natl Acad Sci* 104:15566–15571
84. Wang J, Elliott JE, Williamson RE (2008) Features of the primary wall CESA complex in wild type and cellulose-deficient mutants of *Arabidopsis thaliana*. *J Exp Bot* 59:2627–2637
85. Taylor NG, Howells RM, Huttly AK, Vickers K, Turner SR (2003) Interactions among three distinct Cesa proteins essential for cellulose synthesis. *Proc Natl Acad Sci* 100:1450–1455

86. Hassinen A, Kellokumpu S (2014) Organizational interplay of Golgi *N*-glycosyltransferases involves organelle microenvironment-dependent transitions between enzyme homo- and heteromers. *J Biol Chem* 289:26937–26948
87. Li S, Lei L, Gu Y (2013) Functional analysis of complexes with mixed primary and secondary cellulose synthases. *Plant Signal Behav* 8:e23179
88. Atanassov II, Pittman JK, Turner SR (2009) Elucidating the mechanisms of assembly and subunit interaction of the cellulose synthase complex of *Arabidopsis* secondary cell walls. *J Biol Chem* 284:3833–3841
89. Atmodjo MA, Sakuragi Y, Zhu X, Burrell AJ, Mohanty SS, Atwood JA et al (2011) Galacturonosyltransferase (GAUT)1 and GAUT7 are the core of a plant cell wall pectin biosynthetic homogalacturonan:galacturonosyltransferase complex. *Proc Natl Acad Sci* 108:20225–20230
90. Harholt J, Jensen JK, Verhertbruggen Y, Sogaard C, Bernard S, Nafisi M et al (2012) ARAD proteins associated with pectic Arabinan biosynthesis form complexes when transiently overexpressed in planta. *Planta* 236:115–128
91. Chou YH, Pogorelko G, Zabolina OA (2012) Xyloglucan xylosyltransferases XXT1, XXT2, and XXT5 and the glucan synthase CSLC4 form Golgi-localized multiprotein complexes. *Plant Physiol* 159:1355–1366
92. Chou YH, Pogorelko G, Young ZT, Zabolina OA (2015) Protein-protein interactions among xyloglucan-synthesizing enzymes and formation of Golgi-localized multiprotein complexes. *Plant Cell Physiol* 56:255–267
93. Tetlow IJ, Beisel KG, Cameron S, Makhmoudova A, Liu F, Bresolin NS et al (2008) Analysis of protein complexes in wheat amyloplasts reveals functional interactions among starch biosynthetic enzymes. *Plant Physiol* 146:1878–1891
94. Liu F, Ahmed Z, Lee EA, Donner E, Liu Q, Ahmed R et al (2012) Allelic variants of the amylose extender mutation of maize demonstrate phenotypic variation in starch structure resulting from modified protein-protein interactions. *J Exp Bot* 63:1167–1183
95. Subasinghe RM, Liu F, Polack UC, Lee EA, Emes MJ, Tetlow IJ (2014) Multimeric states of starch phosphorylase determine protein-protein interactions with starch biosynthetic enzymes in amyloplasts. *Plant Physiol Biochem* 83:168–179
96. Colley KJ (1997) Golgi localization of glycosyltransferases: more questions than answers. *Glycobiology* 7:1–13
97. Breton C, Snajdrova L, Jeanneau C, Koca J, Imbert A (2006) Structures and mechanisms of glycosyltransferases. *Glycobiology* 16:29R–37R
98. Li J, Yen TY, Allende ML, Joshi RK, Cai J, Pierce WM et al (2000) Disulfide bonds of GM2 synthase homodimers. *J Biol Chem* 275:41476–41486
99. Rivinoja A, Hassinen A, Kokkonen N, Kauppila A, Kellokumpu S (2009) Elevated Golgi pH impairs terminal *N*-glycosylation by inducing mislocalization of Golgi glycosyltransferases. *J Cell Physiol* 220:144–154
100. Kuhn B, Benz J, Greif M, Engel AM, Sobek H, Rudolph MG (2013) The structure of human alpha-2,6-sialyltransferase reveals the binding mode of complex glycans. *Acta Cryst D* 69:1826–1838
101. Meng L, Forouhar F, Thieker D, Gao Z, Ramiah A, Moniz H et al (2013) Enzymatic basis for *N*-glycan sialylation: structure of rat alpha2,6-sialyltransferase (ST6GAL1) reveals conserved and unique features for glycan sialylation. *J Biol Chem* 288:34680–34698
102. Ferrari ML, Gomez GA, Maccioni HJ (2012) Spatial organization and stoichiometry of *N*-terminal domain-mediated glycosyltransferase complexes in Golgi membranes determined by fret microscopy. *Neurochem Res* 37:1325–1334
103. Nilsson T, Rabouille C, Hui N, Watson R, Warren G (1996) The role of the membrane-spanning domain and stalk region of *N*-acetylglucosaminyltransferase I in retention, kin recognition and structural maintenance of the Golgi apparatus in HeLa cells. *J Cell Sci* 109(Pt 7):1975–1989
104. Ju T, Cummings RD (2002) A unique molecular chaperone Cosmc required for activity of the mammalian core 1 β -galactosyltransferase. 99:16613–16618
105. Busse-Wicher M, Wicher KB, Kusche-Gullberg M (2014) The exostosin family: proteins with many functions. *Matrix Biol* 35:25–33
106. Maccioni HJ, Quiroga R, Spessott W (2011) Organization of the synthesis of glycolipid oligosaccharides in the Golgi complex. *FEBS Lett* 585:1691–1698
107. Spessott W, Crespo PM, Daniotti JL, Maccioni HJ (2012) Glycosyltransferase complexes improve glycolipid synthesis. *FEBS Lett* 586:2346–2350
108. Tomita S, Inoue N, Maeda Y, Ohishi K, Takeda J, Kinoshita T (1998) A homologue of *Saccharomyces cerevisiae* Dpm1p is not sufficient for synthesis of dolichol-phosphate-mannose in mammalian cells. *J Biol Chem* 273:9249–9254
109. Maeda Y, Tomita S, Watanabe R, Ohishi K, Kinoshita T (1998) DPM2 regulates biosynthesis of dolichol phosphate-mannose in mammalian cells: correct subcellular localization and stabilization of DPM1, and binding of dolichol phosphate. *EMBO J* 17:4920–4929
110. Maeda Y, Tanaka S, Hino J, Kangawa K, Kinoshita T (2000) Human dolichol-phosphate-mannose synthase consists of three subunits, DPM1, DPM2 and DPM3. *EMBO J* 19:2475–2482
111. Banerjee DK (2012) *N*-glycans in cell survival and death: cross-talk between glycosyltransferases. 1820:1338–1346
112. Watanabe R, Murakami Y, Marmor MD, Inoue N, Maeda Y, Hino J et al (2000) Initial enzyme for glycosylphosphatidylinositol biosynthesis requires PIG-P and is regulated by DPM2. *EMBO J* 19:4402–4411
113. Ramakrishnan B, Shah PS, Qasba PK (2001) alpha-Lactalbumin (LA) stimulates milk beta-1,4-galactosyltransferase I (beta 4Gal-T1) to transfer glucose from UDP-glucose to *N*-acetylglucosamine. Crystal structure of beta 4Gal-T1 x LA complex with UDP-Glc. *J Biol Chem* 276:37665–37671
114. Swift AM, Machamer CE (1991) A Golgi retention signal in a membrane-spanning domain of coronavirus E1 protein. *J Cell Biol* 115:19–30
115. Weisz OA, Swift AM, Machamer CE (1993) Oligomerization of a membrane protein correlates with its retention in the Golgi complex. *J Cell Biol* 122:1185–1196
116. Cole NB, Smith CL, Sciaky N, Terasaki M, Edidin M, Lippincott-Schwartz J (1996) Diffusional mobility of Golgi proteins in membranes of living cells. *Science* 273:797–801
117. Lairson LL, Henrissat B, Davies GJ, Withers SG (2008) Glycosyltransferases: structures, functions, and mechanisms. *Annu Rev Biochem* 77:521–555
118. Rao R, Rich JR, Rakic B, Buddai S, Schwartz MF, Johnson K et al (2009) Structural insight into mammalian sialyltransferases. *Nat Struct Biol* 16:1186–1188
119. Breton C, Fournel-Gigleux S, Palcic MM (2012) Recent structures, evolution and mechanisms of glycosyltransferases. *Curr Opin Struct Biol* 22:540–549
120. Hurtado-Guerrero R, Davies GJ (2012) Recent structural and mechanistic insights into post-translational enzymatic glycosylation. *Curr Opin Chem Biol* 16:479–487
121. Albesa-Jove D, Giganti D, Jackson M, Alzari PM, Guerin ME (2014) Structure-function relationships of membrane-associated GT-B glycosyltransferases. *Glycobiology* 24:108–124
122. Volkens G, Worrall LJ, Kwan DH, Yu CC, Baumann L, Lameignere E et al (2015) Structure of human ST8SiaII

- sialyltransferase provides insight into cell-surface polysialylation. *Nat Struct Mol Biol* 22:627–635
123. Krissinel E, Henrick K (2007) Inference of macromolecular assemblies from crystalline state. *J Mol Biol* 372:774–797
124. Ihara H, Ikeda Y, Toma S, Wang X, Suzuki T, Gu J et al (2007) Crystal structure of mammalian alpha1,6-fucosyltransferase, FUT8. *Glycobiology* 17:455–466
125. Ramakrishnan B, Balaji PV, Qasba PK (2002) Crystal structure of beta1,4-galactosyltransferase complex with UDP-Gal reveals an oligosaccharide acceptor binding site. *J Mol Biol* 318:491–502
126. Carugo O, Argos P (1997) Protein-protein crystal-packing contacts. *Protein Sci* 6:2261–2263
127. Jorgensen R, Batot G, Mannerstedt K, Imberty A, Breton C, Hindsgaul O et al (2014) Structures of a human blood group glycosyltransferase in complex with a photo-activatable UDP-Gal derivative reveal two different binding conformations. *Acta Crystallogr F Struct Biol Commun* 70:1015–1021
128. Pedersen LC, Tsuchida K, Kitagawa H, Sugahara K, Darden TA, Negishi M (2000) Heparan/chondroitin sulfate biosynthesis. Structure and mechanism of human glucuronyltransferase I. *J Biol Chem* 275:34580–34585
129. Kakuda S, Shiba T, Ishiguro M, Tagawa H, Oka S, Kajihara Y et al (2004) Structural basis for acceptor substrate recognition of a human glucuronyltransferase, GlcAT-P, an enzyme critical in the biosynthesis of the carbohydrate epitope HNK-1. *J Biol Chem* 279:22693–22703