Biochemical and Biophysical Research Communications 425 (2012) 578–582

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

More than 40 years of glycobiology in Regensburg

1. Historical introduction and how we got to know Bill Lennarz

The 20th International Symposium on Glycoconjugates under the motto "Glycans: From Molecules to Structure to Therapeutics", held in December 2009 in Puerto Rico, USA, started with the session: "What we have learned about glycans and what lies ahead." We had the pleasure to contribute to this review of the past together with William J. Lennarz, Akira Kobata, Johannes F.G. Vliegenthart and Roland Schauer. At this occasion contributions to the field coming from the University of Regensburg were summarized (a modified and updated version will be presented here).

In the late sixties of the last century three papers had been published, which showed that polyisoprenol derivatives constituted the lipidic moiety of so-called lipid intermediates. One of these articles was from Bill Lennarz (together with Scher and Sweeley), who was trained to be a lipid expert in Konrad Bloch's laboratory. The lipid-intermediates were found to be involved in glycosyl transfer reactions in the biosynthesis of bacterial cell surface components [1–3]. We came from the field of sugar biochemistry [4,5] and were studying the biosynthesis of mannosylated polymers of the yeast cell wall. Influenced by the above mentioned publications, we started to look also into the lipid fraction and soon published the first paper describing a lipid intermediate from eukaryotes [6]. Together with papers coming from Leloir's group [7,8] this was the entry of dolichol-phosphate activated mono- and oligosaccharides into the field of glycoproteins.

It goes without saying that the small group of people working in this special area of biochemistry got to know each other very soon. Many met at the scientific symposium honoring Luis Leloir's Nobel Prize in Bariloche, Argentina, in 1971. While touring the United States by one of us (WT) in 1974, it was clearly a highlight to have met Bill Lennarz for the first time at John Hopkins in Baltimore and to be invited to a fancy restaurant for dinner. We stayed in loose but friendly contact ever since.

In 1970 we had moved from Munich to the newly founded University of Regensburg. Carbohydrate biochemistry, mainly the biosynthesis of glycoproteins with emphasis on the role of dolichols, remained of top interest for the 40 years to come. The obvious questions of the early days were: (i) what is the nature of the lipid moiety of the *eukaryotic* intermediate, and (ii) to which acceptors do the lipid-activated saccharides get transferred? To a certain extent the race of liver and hen oviduct against yeast had started.

For yeast we could show that external, chemically phosphorylated dolichol not only stimulated the lipid-linked mannose formation, but also that the endogenous mannosyl acceptor indeed was dolichol-phosphate, 14–18 isoprene units long [9]. The accepting molecules in animals and yeast were proteins [10,11]. In yeast, however, the major amount of [^{14}C]mannose transferred from Dol-P-[^{14}C]Man – not commercially available of course – to protein could be released by mild alkali treatment. This indicated a protein-(Ser/Thr)-O-Man linkage [11], whereas the animal glycoproteins were clearly Asn-linked ones [8,10]. The O-Man linkage, originally discovered by Sentandreu and Northcote was thought to be a fungal specialty and we concentrated on this type of protein glycosylation, not least because the reaction might be a promising target for a potential antifungal drug. Thus the cellular localization, the enzyme responsible for the mannosyl transfer to protein including the corresponding gene, as well as the functional importance of the mannosyl transfer from Dol-P-Man to Ser/Thr-residues of proteins was elucidated in our lab (see below).

In the late seventies, glycobiology in Regensburg gained considerably in momentum, when Manfred Sumper (joined by Felix Wieland) took over the chair of biochemistry. He and his group clarified essential dolichol-dependent steps in the biosynthesis of glycoproteins in archaea [12,13], but also started to study the role of glycoproteins and glycolipids in the development of the green algae *Volvox carteri* [14]. With a few colleagues of our University we applied for a "Collaborative Research Centre" (in German: Sonderforschungsbereich, SFB) at the German Granting Agency, DFG. We were successful, which meant, we got optimal support for our research, altogether for 24 years.

A special benefit of this funding was that we were able to organize international symposia and the first one was taking place in 1982. Among the invited speakers who accepted to visit us, were several guests from outside Germany: Stuart Kornfeld, Randy Schekman, Nathan Sharon, Ten Feizi, Peter Albersheim, Lennart Rodén and of course Bill Lennarz (Fig. 1). Bill talked about "Studies on the Role of Glycoproteins in Embryonic Development" taking sea urchins as a model organism [15]. With this topic he pursued a new direction of glycoprotein research, which in essence turned out to be a most fruitful one. This became obvious, when the various developmental dysfunctions in humans, known as "Congenital Disorders of Glycosylation" (CDG, see below) were uncovered.

2. Protein-O-mannosylation: from yeast to Drosophila to man

Glycosylated proteins of baker's yeast (*Saccharomyces cerevisiae*) contain to a large extent Ser/Thr linked α1,2- and α1,3-mannose oligosaccharides, predominantly one to four residues long. The first mannose gets transferred to the protein via Dol-P-Man, which happens in the lumen of the endoplasmic reticulum (ER), the following residues are coming from GDP-Man in the Golgi apparatus [16,17]. The function of the lipid intermediate as a transmembrane transport vehicle was demonstrated [18]. The purification of the enzyme that catalyzes the transfer of mannose from Dol-P-Man to accepting proteins was a major challenge and set the stage to clone the first Dol-P-Man:protein O-manosyltransferase [19]. The gene was called

0006-291X/$ - see front matter
http://dx.doi.org/10.1016/j.bbrc.2012.08.001

PMT1, for protein mannosyltransferase, and surprisingly several years later it was shown also to exist in *Drosophila* [20], mouse and man [21–23], designated there as *POMT*, but to be absent from plants and *Caenorhabditis elegans*. With this news, the potential antifungal target, at least for human pathogens, was lost.

Our subsequent work has revealed that the PMT protein family in yeast consists of at least 6 members, which are active as specific hetero- or homo-dimers and are specific concerning the target sites, to which mannose residues are transferred [24–26]. Creating pmt mutants we demonstrated that protein O-mannosylation in yeast is essential for viability and the pomt1 knock out in mice is embryonic lethal [23,27]. Finally, we obtained evidence that O-mannosylation precedes N-glycosylation in time and can therefore prevent N-glycosylation in cases where the mannose gets linked to the canonical OH-group of the sequon [28].

In 2004 Sabine Strahl budded off to the University of Heidelberg and since then the topic of protein-O-mannosylation is best in her hands. She and her team study the enzymatic mechanism [29] as well as the functional importance of the process in yeast, mammals and humans [30,31] (for further details see also Ref. [17,32], as well as below).

3. Protein N-glycosylation: universally conserved from yeast to man

Pioneering studies in the 1970/80s from several laboratories, including Luis Leloir, Stuart Kornfeld, Phil Robbins, Robert Spiro, Frank Hemming and Bill Lennarz [33,34] paved the foundations for the N-glycosylation pathway in the ER, which is called now the dolichol cycle. The unique lipid-linked core-oligosaccharide, $Glc_3Man_9GlcNAc_2$-PP-Dol (LLO), sequentially build up, is the glycosyl donor for all types of N-glycans irrespective of their final structure. Upon its transfer to the protein, the mature glycan chain is formed in further processing reactions on transit from the ER via the Golgi apparatus to the plasma membrane. An important landmark has been the isolation of temperature-sensitive alg mutants (for asparagine-linked glycosylation) by Tim Huffaker and Phil Robbins [35]. These mutants were defective in LLO-assembly and allowed to define the pathway and in particular to isolate the genes encoding the respective glycosyltransferases by complementing a particular phenotype characteristic for the mutant.

Our own studies mainly in yeast but also in plants dealt with the enzymology and the biochemical as well as the molecular characterization of various membrane-bound glycosyltransferases of the LLO-assembly and of the glycosyl transfer to the protein and thus demonstrating its conservation from yeast to man [36–40]. Among these studies also the mode of tunicamycin inhibition, a versatile inhibitor of N-glycosylation, could be elucidated. It blocks specifically the formation of $GlcNAc_2$-PP-Dol [41]. A surprising finding was the discovery that the second GlcNAc-transferase forming $GlcNAc_2$-PP-Dol is a bipartite glycosyltransferase consisting of a membrane-bound subunit, Alg14, which recruits the soluble Alg13 subunit to the cytosolic face of the ER to form the active enzyme [42]. The Alg2 enzyme turned out to be a bifunctional α1,3- and α1,6-mannosyltransferase converting $Man_1GlcNAc_2$-PP-Dol to $Man_3GlcNAc_2$-PP-Dol [43]; similarly the Alg11 α1,2-mannosyltransferase catalyzes two sequential glycosylation steps, thereby elongating $Man_3GlcNAc_2$-PP-Dol to $Man_5GlcNAc_2$-PP-Dol [44], before it is translocated to the luminal side, to be further elongated to the full length $Glc_3Man_9GlcNAc_2$-PP-Dol donor. To understand the mechanistic details of these reactions will be a future challenge and needs crystallization of the enzymes.

An issue, to which also Bill Lennarz has substantially contributed [45,46], were investigations, dealing with the enzyme oligosaccharyltransferase (OST), sometimes called the gatekeeper of N-glycosylation. Earlier attempts to purify this enzyme failed, probably due to its instability upon solubilization and probably also due to the fact that, as it later turned out, it is a hetero-oligomeric membrane-bound complex consisting of several subunits [47–49]. Nevertheless, basic biochemical characteristics of this enzyme could be demonstrated using microsomal membranes or detergent-solubilized extracts. One of the interesting features of the enzyme certainly was that contradictory to the *in vivo* situation also transfer from $GlcNAc_2$-PP-Dol as the smallest oligosaccharide occurred at a similar rate as the full length donor and that the final addition of glucose residues lowered the K_m for the acceptor peptide [50]. The assay allowed also to study structural acceptor requirements for oligosaccharide transfer to the protein [51–53] and to verify the Asn-X-Ser/Thr consensus sequence (whereby X can be any amino acid except proline) postulated by R.D. Marshall.

When we succeeded in cooperation with the group of Markus Aebi to clone the first OST gene, *WBP1*, and realized that its overexpression did not result in an increased OST activity, we postulated that Wbp1p is a non-limiting component of a multi-component enzyme [54]. In the following genetic and biochemical studies have identified nine subunits. Wbp1, Swp1, Stt3, Ost1 and Ost2 are essential for viability of the cell, whereas Ost3, Ost4, Ost5 and Ost6 are not essential, but are required for maximal OST activity [48,55–58]. Apart from Stt3, identified as the catalytic subunit [59], the function of the various other components are largely enigmatic. The homologous Ost3 and Ost6 proteins are alternatively present in the OST and together with the other subunits form two distinct complexes [60], differing in their substrate-specific activities at the level of individual glycosylation sites [61]. The complexity of OST of eukaryotes is not fully understood, as also single subunit OSTs exist, such as in the trypanosomatid parasite *Leishmania* consisting of only Stt3. LmStt3 can substitute for the whole yeast OST complex with the glycosyl donor specificity of the parasite, i.e. broad specificity for Man-oligosaccharides. However, when incorporated into the yeast OST complex, substrate

Fig. 1. Left, Bill Lennarz together with Nathan Sharon; Middle, Bill Lennarz in front of the announcement of a "Bavarian Brotzeit"; Right, Kurt von Figura with Stuart Kornfeld.

specificity is altered and the common eukaryotic $Glc_3Man_9GlcNAc_2$-PP-Dol donor is transferred to the protein [62].

4. CDGs (Congenital disorders of glycosylation): demonstrating the fundamental role of sugars attached to proteins

For decades it was irritating that no general or vital role could be ascribed to such an evolutionary highly conserved protein modification like glycosylation. With the first clinical description of two patients with deficiencies in N-glycosylation by the Belgian pediatrician Jaak Jaeken in the 1980s [63], the gate has been opened for a fascinating research area dealing with glycoprotein function, but it took another 10 years before a biochemical defect could be assigned to a disease causing mutation [64]. Congenital Disorders of Glycosylation (CDG) in humans result in a severe, inherited multisystemic disease interfering with normal development of the brain and functions of the nerve-, liver-, intestinal- and muscle systems. They can be embryonic lethal depending on the glycosylation defect as revealed by respective mouse models [65]. The term CDG was originally used for defects in N-glycosylation. Defects in glycosylation steps occurring in the ER, such as in LLO-assembly or in the transfer of the core-oligosaccharide to the protein were grouped into CDG-I subtypes, whereas defects of N-glycan processing in the Golgi apparatus represent CDG-II subtypes. The field has expanded rapidly and also disorders of protein O-glycosylation, or combined of N- and O-glycosylation, as well lipid-glycosylation (GPI-anchor and glycosphingolipid glycosylation), nucleotide-sugar biosynthesis, or vesicular transport affecting glycosylation are considered as forms of CDG with the result that the CDG nomenclature has been changed using now the official gene symbol followed by the abbreviation CDG [66,67]. More than 50 disorders have been identified meanwhile and one can envision that probably any of the steps of the complex biosynthetic glycosylation pathway could result in a clinical picture.

Our experience using yeast as model to study basic principles of glycosylation led us in cooperation with clinicians into the field of CDG. Enzymatic radioactive assays for all the reactions of LLO-biosynthesis and protein-glycosylation in the ER that we have established in the yeast system could be successfully extended to human fibroblasts and helped to verify the presumptive biochemical defect in potential CDG patients [68–76]. Yeast made it also possible, to identify the corresponding human genes by searching for homologs to the yeast genes. Proof of concept and confirming the disease causing mutation was achieved by complementing the growth and LLO phenotype, the glycosyltransferase activity *in vitro*, as well as the underglycosylation of model glycoproteins of respective glycosylation defective mutants by the human gene, but not by the patient gene. It is indeed astonishing, how precisely the gene products of yeast and man have been preserved during evolution.

The biological significance of O-mannosylation in mammals was unraveled in studies of disorders belonging to the congenital dystroglycanopathies. α-Dystroglycan is the main carrier of O-Man glycans in vertebrates and these O-mannosyl glycans contribute to the binding of α-dystroglycan to the extracellular matrix proteins such as laminin [77,78]. Compared to CDG types of N-glycosylation, which are multi-organ diseases with neurological involvement, the clinical hallmarks of O-glycosylation defects are the pathology of muscle, eye and brain. Deficiencies in O-mannosyltransferases POMT1 and POMT2 [21,22] that were identified due to the homology to the Pmts from yeast and the study of their biochemistry, have been associated with one of the most severe forms of congenital muscular dystrophies, the Walker–Warburg syndrome [23,79]. Mutations in glycosyltransferases extending the Man-O-protein linkage result in Muscle–Eye–Brain, Fukuyama-type and Limb-Girdle muscular dystrophies [17,67]. Besides its role in muscle cell adhesion, α-dystroglycan is also involved in migration of neuronal cells during retinal development. Hence, in contrast to the multisystemic CDG forms caused by defects in N-glycosylation, the molecular details of faulty protein O-mannosylation are partially understood.

5. Outlook

In the field of protein glycosylation the cooperation between yeast and mammalian, respectively human molecular biology and biochemistry has been extremely fruitful, although the yeast work was started as purely basic investigation. This is another example arguing against short sighted political decisions to decrease support for research that is not applied or medically oriented to begin with.

Acknowledgment

We thank Sabine Strahl for reading our manuscript. All our own work mentioned in this review has been supported by the DFG, the Fonds der Chemischen Industrie and the Körber Stiftung.

References

[1] A. Wright, M. Dankert, P. Fennessey, P.W. Robbins, Characterization of a polyisoprenoid compound functional in O-antigen biosynthesis, Proc. Natl. Acad. Sci. USA 57 (1967) 1798–1803.
[2] Y. Higashi, J.L. Strominger, C.C. Sweeley, Structure of a lipid intermediate in cell wall peptidoglycan synthesis: a derivative of a C55 isoprenoid alcohol, Proc. Natl. Acad. Sci. USA 57 (1967) 1878–1884.
[3] M. Scher, W.J. Lennarz, C.C. Sweeley, The biosynthesis of mannosyl-1-phosphoryl-polyisoprenol in Micrococcus lysodeikticus and its role in mannan synthesis, Proc. Natl. Acad. Sci. USA 59 (1968) 1313–1320.
[4] W. Tanner, L. Lehle, O. Kandler, Myo-inositol, a cofactor in the biosynthesis of verbascose, Biochem. Biophys. Res. Commun. 29 (1967) 166–171.
[5] L. Lehle, W. Tanner, Synthesis of raffinose-type sugars, in: V. Ginsburg (Ed.), Meth. Enzymol., Academic Press, New York, 1972, pp. 522–530.
[6] W. Tanner, A lipid intermediate in mannan biosynthesis in yeast, Biochem. Biophys. Res. Commun. 35 (1969) 144–150.
[7] N.H. Behrens, L.F. Leloir, Dolichol monophosphate glucose: an intermediate in glucose transfer in liver, Proc. Natl. Acad. Sci. USA 66 (1970) 153–159.
[8] A.J. Parodi, N.H. Behrens, L.F. Leloir, H. Carminatti, The role of polyprenol-bound saccharides as intermediates in glycoprotein synthesis in liver, Proc. Natl. Acad. Sci. USA 69 (1972) 3268–3272.
[9] P. Jung, W. Tanner, Identification of the lipid intermediate in yeast mannan biosynthesis, Eur. J. Biochem. 37 (1973) 1–6.
[10] C.J. Waechter, J.J. Lucas, W.J. Lennarz, Membrane glycoproteins. I. Enzymatic synthesis of mannosyl phosphoryl polyisoprenol and its role as a mannosyl donor in glycoprotein synthesis, J. Biol. Chem. 248 (1973) 7570–7579.
[11] C.B. Sharma, P. Babczinski, L. Lehle, W. Tanner, The role of dolicholmonophosphate in glycoprotein biosynthesis in *Saccharomyces cerevisiae*, Eur. J. Biochem. 46 (1974) 35–41.
[12] J. Lechner, F. Wieland, M. Sumper, Transient methylation of dolichyl oligosaccharides is an obligatory step in halobacterial sulfated glycoprotein biosynthesis, J. Biol. Chem. 260 (1985) 8984–8989.
[13] M. Sumper, Halobacterial glycoprotein biosynthesis, Biochim. Biophys. Acta 906 (1987) 69–79.
[14] S. Wenzl, M. Sumper, A novel glycosphingolipid that may participate in embryo inversion in Volvox carteri, Cell 46 (1986) 633–639.
[15] D.D. Carson, W.J. Lennarz, Inhibition of polyisoprenoid and glycoprotein biosynthesis causes abnormal embryonic development, Proc. Natl. Acad. Sci. USA 76 (1979) 5709–5713.
[16] P. Babczinski, W. Tanner, Involvement of dolicholmonophosphate in the formation of specific mannosyl-linkages in yeast glycoproteins, Biochem. Biophys. Res. Commun. 54 (1973) 1119–1124.
[17] L. Lehle, S. Strahl, W. Tanner, Protein glycosylation, conserved from yeast to man: a model organism helps elucidate congenital human diseases, Angew. Chem. Int. Ed. Engl. 45 (2006) 6802–6818.
[18] A. Haselbeck, W. Tanner, Dolichyl phosphate-mediated mannosyl transfer through liposomal membranes, Proc. Natl. Acad. Sci. USA 79 (1982) 1520–1524.
[19] S. Strahl-Bolsinger, T. Immervoll, R. Deutzmann, W. Tanner, PMT1, the gene for a key enzyme of protein O-glycosylation in *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. USA 90 (1993) 8164–8168.
[20] E. Martin-Blanco, A. Garcia-Bellido, Mutations in the rotated abdomen locus affect muscle development and reveal an intrinsic asymmetry in Drosophila, Proc. Natl. Acad. Sci. USA 93 (1996) 6048–6052.
[21] L.A. Jurado, A. Coloma, J. Cruces, Identification of a human homolog of the Drosophila rotated abdomen gene (POMT1) encoding a putative protein O-

mannosyl-transferase, and assignment to human chromosome 9q34.1, Genomics 58 (1999) 171–180.
[22] T. Willer, W. Amselgruber, R. Deutzmann, S. Strahl, Characterization of POMT2, a novel member of the PMT protein O-mannosyltransferase family specifically localized to the acrosome of mammalian spermatids, Glycobiology 12 (2002) 771–783.
[23] T. Willer, B. Prados, J.M. Falcon-Perez, I. Renner-Muller, G.K. Przemeck, M. Lommel, A. Coloma, M.C. Valero, M.H. de Angelis, W. Tanner, E. Wolf, S. Strahl, J. Cruces, Targeted disruption of the Walker–Warburg syndrome gene Pomt1 in mouse results in embryonic lethality, Proc. Natl. Acad. Sci. USA 101 (2004) 14126–16131.
[24] V. Girrbach, S. Strahl, Members of the evolutionarily conserved PMT family of protein O-mannosyltransferases form distinct protein complexes among themselves, J. Biol. Chem. 278 (2003) 12554–12562.
[25] M. Gentzsch, W. Tanner, Protein-O-glycosylation in yeast: protein-specific mannosyltransferases, Glycobiology 7 (1997) 481–486.
[26] J. Hutzler, M. Schmid, T. Bernard, B. Henrissat, S. Strahl, Membrane association is a determinant for substrate recognition by PMT4 protein O-mannosyltransferases, Proc. Natl. Acad. Sci. USA 104 (2007) 7827–7832.
[27] M. Gentzsch, W. Tanner, The PMT gene family: protein O-glycosylation in *Saccharomyces cerevisiae* is vital, EMBO J. 15 (1996) 5752–5759.
[28] M. Ecker, V. Mrsa, I. Hagen, R. Deutzmann, S. Strahl, W. Tanner, O-mannosylation precedes and potentially controls the N-glycosylation of a yeast cell wall glycoprotein, EMBO Rep. 4 (2003) 628–632.
[29] M. Lommel, A. Schott, T. Jank, V. Hofmann, S. Strahl, A conserved acidic motif is crucial for enzymatic activity of protein O-mannosyltransferases, J. Biol. Chem. 286 (2011) 39768–39775.
[30] J. Arroyo, J. Hutzler, C. Bermejo, E. Ragni, J. Garcia-Cantalejo, P. Botias, H. Piberger, A. Schott, A.B. Sanz, S. Strahl, Functional and genomic analyses of blocked protein O-mannosylation in baker's yeast, Mol. Microbiol. 79 (2011) 1529–1546.
[31] M. Lommel, T. Willer, S. Strahl, POMT2, a key enzyme in Walker–Warburg syndrome: somatic sPOMT2, but not testis-specific tPOMT2, is crucial for mannosyltransferase activity in vivo, Glycobiology 18 (2008) 615–625.
[32] M. Lommel, S. Strahl, Protein O-mannosylation: conserved from bacteria to humans, Glycobiology 19 (2009) 816–828.
[33] C.J. Waechter, W.J. Lennarz, The role of polyprenol-linked sugars in glycoprotein synthesis, Annu. Rev. Biochem. 45 (1976) 95–112.
[34] R. Kornfeld, S. Kornfeld, Assembly of asparagine-linked oligosaccharides, Annu. Rev. Biochem. 54 (1985) 631–664.
[35] T.C. Huffaker, P.W. Robbins, Yeast mutants deficient in protein glycosylation, Proc. Natl. Acad. Sci. USA 80 (1983) 7466–7470.
[36] L. Lehle, Biosynthesis of the core region of yeast mannoproteins. Formation of a glucosylated dolichol-bound oligosaccharide precursor, its transfer to protein and subsequent modification, Eur. J. Biochem. 109 (1980) 589–601.
[37] G. Palamarczyk, L. Lehle, T. Mankowski, T. Chojnacki, W. Tanner, Specificity of solubilized yeast glycosyl transferases for polyprenyl derivatives, Eur. J. Biochem. 105 (1980) 517–523.
[38] C.B. Sharma, R. Knauer, L. Lehle, Biosynthesis of lipid-linked oligosaccharides in yeast: the ALG3 gene encodes the Dol-P-Man:Man5GlcNAc2-PP-Dol mannosyltransferase, Biol. Chem. 382 (2001) 321–328.
[39] S. te Heesen, L. Lehle, A. Weissmann, M. Aebi, Isolation of the ALG5 locus encoding the UDP-glucose:dolichyl-phosphate glucosyltransferase from *Saccharomyces cerevisiae*, Eur. J. Biochem. 224 (1994) 71–79.
[40] L. Lehle, Plant cells synthesize glucose-containing lipid-linked oligosaccharides similar to those found in animals and yeast, FEBS Lett. 123 (1981) 63–66.
[41] L. Lehle, W. Tanner, The specific site of tunicamycin inhibition in the formation of dolichol-bound N-acetylglucosamine derivatives, FEBS Lett. 72 (1976) 167–170.
[42] T. Bickel, L. Lehle, M. Schwarz, M. Aebi, C.A. Jakob, Biosynthesis of lipid-linked oligosaccharides in *Saccharomyces cerevisiae*: Alg13p and Alg14p form a complex required for the formation of GlcNAc(2)-PP-dolichol, J. Biol. Biochem. 280 (2005) 34500–34506.
[43] M. Kampf, B. Absmanner, M. Schwarz, L. Lehle, Biochemical characterization and membrane topology of Alg2 from *Saccharomyces cerevisiae* as a bifunctional alpha1,3- and 1,6-mannosyltransferase involved in lipid-linked oligosaccharide biosynthesis, J. Biol. Chem. 284 (2009) 11900–11912.
[44] B. Absmanner, V. Schmeiser, M. Kampf, L. Lehle, Biochemical characterization, membrane association and identification of amino acids essential for the function of Alg11 from *Saccharomyces cerevisiae*, an alpha1,2-mannosyltransferase catalysing two sequential glycosylation steps in the formation of the lipid-linked core oligosaccharide, Biochem. J. 426 (2010) 205–217.
[45] A. Yan, W.J. Lennarz, Unraveling the mechanism of protein N-glycosylation, J. Biol. Chem. 280 (2005) 3121–3124.
[46] H. Li, M. Chavan, H. Schindelin, W.J. Lennarz, H. Li, Structure of the oligosaccharyl transferase complex at 12 A resolution, Structure 16 (2008) 432–440.
[47] R. Knauer, L. Lehle, The N-oligosaccharyltransferase complex from yeast, FEBS Lett. 344 (1994) 83–86.
[48] R. Knauer, L. Lehle, The oligosaccharyltransferase complex from yeast, Biochim. Biophys. Acta 1426 (1999) 259–273.
[49] D.J. Kelleher, R. Gilmore, The *Saccharomyces cerevisiae* oligosaccharyltransferase is a protein complex composed of Wbp1p, Swp1p, and four additional polypeptides, J. Biol. Chem. 269 (1994) 12908–12917.
[50] C.B. Sharma, L. Lehle, W. Tanner, N-Glycosylation of yeast proteins. Characterization of the solubilized oligosaccharyl transferase, Eur. J. Biochem. 116 (1981) 101–108.
[51] L. Lehle, E. Bause, Primary structural requirements for N- and O-glycosylation in yeast, Biochim. Biophys. Acta 799 (1984) 246–251.
[52] E. Bause, L. Lehle, Enzymatic N-glycosylation and O-glycosylation of synthetic peptide acceptors by dolichol-linked sugar derivatives in yeast, Eur. J. Biochem. 101 (1979) 531–540.
[53] T. Roitsch, L. Lehle, Structural requirements for protein N-glycosylation. Influence of acceptor peptides on cotranslational glycosylation of yeast invertase and site-directed mutagenesis around a sequon sequence, Eur. J. Biochem. 181 (1989) 525–529.
[54] S. te Heesen, B. Janetzky, L. Lehle, M. Aebi, The yeast WBP1 is essential for oligosaccharyl transferase activity in vivo and in vitro, EMBO J. 11 (1992) 2071–2075.
[55] S. te Heesen, R. Knauer, L. Lehle, M. Aebi, Yeast Wbp1p and Swp1p form a protein complex essential for oligosaccharyl transferase activity, EMBO J. 12 (1993) 279–284.
[56] R. Zufferey, R. Knauer, P. Burda, I. Stagljar, S. te Heesen, L. Lehle, M. Aebi, STT3, a highly conserved protein required for yeast oligosaccharyl transferase activity in vivo, EMBO J. 14 (1995) 4949–4960.
[57] R. Knauer, L. Lehle, The oligosaccharyltransferase complex from *Saccharomyces cerevisiae*. Isolation of the OST6 gene, its synthetic interaction with OST3, and analysis of the native complex, J. Biol. Chem. 274 (1999) 17249–17256.
[58] S. Silberstein, R. Gilmore, Biochemistry, molecular biology, and genetics of the oligosaccharyltransferase, FASEB J. 10 (1996) 849–858.
[59] C. Lizak, S. Gerber, S. Numao, M. Aebi, K.P. Locher, X-ray structure of a bacterial oligosaccharyltransferase, Nature 474 (2011) 350–355.
[60] M. Schwarz, R. Knauer, L. Lehle, Yeast oligosaccharyltransferase consists of two functionally distinct sub-complexes, specified by either the Ost3p or Ost6p subunit, FEBS Lett. 579 (2005) 6564–6568.
[61] B.L. Schulz, M. Aebi, Analysis of glycosylation site occupancy reveals a role for Ost3p and Ost6p in site-specific N-glycosylation efficiency, Mol. Cell. Proteomics 8 (2009) 357–364.
[62] K. Hese, C. Otto, F.H. Routier, L. Lehle, The yeast oligosaccharyltransferase complex can be replaced by STT3 from Leishmania major, Glycobiology 19 (2009) 160–171.
[63] J. Jaeken, H.G. van Eijk, C. van der Heul, L. Corbeel, R. Eeckels, E. Eggermont, Sialic acid-deficient serum and cerebrospinal fluid transferrin in a newly recognized genetic syndrome, Clin. Chim. Acta 144 (1984) 245–247.
[64] E. van Schaftingen, J. Jaeken, Phosphomannomutase deficiency is a cause of carbohydrate-deficient glycoprotein syndrome type I, FEBS Lett. 377 (1995) 318–320.
[65] K.W. Marek, I.K. Vijay, J.D. Marth, A recessive deletion in the GlcNAc-1-phosphotransferase gene results in peri-implantation embryonic lethality, Glycobiology 9 (1999) 1263–1271.
[66] J. Jaeken, T. Hennet, G. Matthijs, H.H. Freeze, CDG nomenclature: time for a change!, Biochim Biophys. Acta 1792 (2009) 825–826.
[67] T. Hennet, Diseases of glycosylation beyond classical congenital disorders of glycosylation, Biochim. Biophys. Acta 2012 (1820) 1306–1317.
[68] R. Knauer, L. Lehle, F. Hanefeld, K. von Figura, Normal N-oligosaccharyltransferase activity in fibroblasts from patients with carbohydrate-deficient glycoprotein syndrome, J. Inherit. Metab. Dis. 17 (1994) 541–544.
[69] C. Korner, R. Knauer, U. Holzbach, F. Hanefeld, L. Lehle, K. von Figura, Carbohydrate-deficient glycoprotein syndrome type V: deficiency of dolichyl-P-Glc:Man9GlcNAc2-PP-dolichyl glucosyltransferase, Proc. Natl. Acad. Sci. USA 95 (1998) 13200–13205.
[70] C. Korner, R. Knauer, U. Stephani, T. Marquardt, L. Lehle, K. von Figura, Carbohydrate deficient glycoprotein syndrome type IV: deficiency of dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase, EMBO J. 18 (1999) 6816–6822.
[71] C. Thiel, M. Schwarz, M. Hasilik, U. Grieben, F. Hanefeld, L. Lehle, K. von Figura, C. Korner, Deficiency of dolichyl-P-Man:Man7GlcNAc2-PP-dolichyl mannosyltransferase causes congenital disorder of glycosylation type Ig, Biochem. J. 367 (2002) 195–201.
[72] C. Thiel, M. Schwarz, J. Peng, M. Grzmil, M. Hasilik, T. Braulke, A. Kohlschutter, K. von Figura, L. Lehle, C. Korner, A new type of congenital disorders of glycosylation (CDG-Ii) provides new insights into the early steps of dolichol-linked oligosaccharide biosynthesis, J. Biol. Chem. 278 (2003) 22498–22505.
[73] M. Schwarz, C. Thiel, J. Lubbehusen, B. Dorland, T. de Koning, K. von Figura, L. Lehle, C. Korner, Deficiency of GDP-Man:GlcNAc2-PP-dolichol mannosyltransferase causes congenital disorder of glycosylation type Ik, Am. J. Hum. Genet. 74 (2004) 472–481.
[74] N. Rind, V. Schmeiser, C. Thiel, B. Absmanner, J. Lubbehusen, J. Hocks, N. Apeshiotis, E. Wilichowski, L. Lehle, C. Korner, A severe human metabolic disease caused by deficiency of the endoplasmatic mannosyltransferase hALG11 leads to congenital disorder of glycosylation-Ip, Hum. Mol. Genet. 19 (2010) 1413–1424.
[75] V. Cantagrel, D.J. Lefeber, B.G. Ng, Z. Guan, J.L. Silhavy, S.L. Bielas, L. Lehle, H. Hombauer, M. Adamowicz, E. Swiezewska, A.P. De Brouwer, P. Blumel, J. Sykut-Cegielska, S. Houliston, D. Swistun, B.R. Ali, W.B. Dobyns, D. Babovic-Vuksanovic, H. van Bokhoven, R.A. Wevers, C.R. Raetz, H.H. Freeze, E. Morava, L. Al-Gazali, J.G. Gleeson, SRD5A3 is required for converting polyprenol to dolichol and is mutated in a congenital glycosylation disorder, Cell 142 (2010) 203–217.

[76] D.J. Lefeber, J. Schonberger, E. Morava, M. Guillard, K.M. Huyben, K. Verrijp, O. Grafakou, A. Evangeliou, F.W. Preijers, P. Manta, J. Yildiz, S. Grunewald, M. Spilioti, C. van den Elzen, D. Klein, D. Hess, H. Ashida, J. Hofsteenge, Y. Maeda, L. van den Heuvel, M. Lammens, L. Lehle, R.A. Wevers, Deficiency of Dol-P-Man synthase subunit DPM3 bridges the congenital disorders of glycosylation with the dystroglycanopathies, Am. J. Hum. Genet. 85 (2009) 76–86.
[77] A. Chiba, K. Matsumura, H. Yamada, T. Inazu, T. Shimizu, S. Kusunoki, I. Kanazawa, A. Kobata, T. Endo, Structures of sialylated O-linked oligosaccharides of bovine peripheral nerve alpha-dystroglycan. The role of a novel O-mannosyl-type oligosaccharide in the binding of alpha-dystroglycan with laminin, J. Biol. Chem. 272 (1997) 2156–2162.
[78] R. Barresi, K.P. Campbell, Dystroglycan: from biosynthesis to pathogenesis of human disease, J. Cell Sci. 119 (2006) 199–207.
[79] D. Beltran-Valero de Bernabe, S. Currier, A. Steinbrecher, J. Celli, E. van Beusekom, B. van der Zwaag, H. Kayserili, L. Merlini, D. Chitayat, W.B. Dobyns, B. Cormand, A.E. Lehesjoki, J. Cruces, T. Voit, C.A. Walsh, H. van Bokhoven, H.G. Brunner, Mutations in the O-mannosyltransferase gene POMT1 give rise to the severe neuronal migration disorder Walker–Warburg syndrome, Am. J. Hum. Genet. 71 (2002) 1033–1043.

Widmar Tanner
Ludwig Lehle
Lehrstuhl für Zellbiologie und Pflanzenbiochemie, Universität Regensburg, Universitätsstraße 31, 93053 Regensburg, Germany
Fax: +49 941 943 3352.
E-mail addresses: widmar.tanner@biologie.uni-regensburg.de (W. Tanner), ludwig.lehle@biologie.uni-regensburg.de (L. Lehle).