Review

Mucin-Type *O*-Glycosylation in Invertebrates

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Abstract: *O*-Glycosylation is one of the most important posttranslational modifications of proteins. It takes part in protein conformation, protein sorting, developmental processes and the modulation of enzymatic activities. In vertebrates, the basics of the biosynthetic pathway of *O*-glycans are already well understood. However, the regulation of the processes and the molecular aspects of defects, especially in correlation with cancer or developmental abnormalities, are still under investigation. The knowledge of the correlating invertebrate systems and evolutionary aspects of these highly conserved biosynthetic events may help improve the understanding of the regulatory factors of this pathway. Invertebrates display a broad spectrum of glycosylation varieties, providing an enormous potential for glycan modifications which may be used for the design of new pharmaceutically active substances. Here, overviews of the present knowledge of invertebrate mucin-type *O*-glycan structures and the currently identified enzymes responsible for the biosynthesis of these oligosaccharides are presented, and the few data dealing with functional aspects of *O*-glycans are summarised.

Keywords: *O*-glycosylation; invertebrates; mucin-type; nematode; insect; helminth; mollusc

1. Introduction

Protein glycosylation plays an important role in several types of recognition processes ranging from fertilisation and development to pathological events and cell death. 30 years ago, the role of *O*-glycans was only seen in the influence of physical parameters, such as ensuring protein stability and tertiary structure, providing the basis for rigid conformations and slowing down proteolytic degradation of the peptide chain; most of specialised recognition events have been allocated to *N*-glycans. However, it is

now clear that different types of *O*-glycans are relevant for many more functions, such as modulating of enzyme activity (e.g., the reversible attachment of an *O*-linked GlcNAc (*N*-acetylglucosamine) residue to cytoplasmic and nuclear proteins), modulating pro-protein processing and acting as signal molecules or sorting determinants guiding the modified protein in the cell from the place of biosynthesis to its target location [1–4]. The functional and pathological consequences of altered *O*-glycans in human cancer have been determined [5,6]. *O*-glycans are essential in the von Willebrand factor [7], critical for vascular integrity [8], regulating secretion and secretory vesicle formation [9] and are essential during eukaryotic development [10].

In *N*-glycans, the linkage of a glycan to the protein is always formed by a GlcNAc-residue linked to an asparagine within the consensus sequence Asn-Xxx-Ser/Thr. Biosynthesis begins in the endoplasmic reticulum by forming a highly conserved oligosaccharide structure (two GlcNAc, nine Man (mannose), three Glc (glucose) residues) on a lipid precursor. Then, the glycan is transferred *en bloc* onto the growing peptide chain and is matured within Golgi vesicles. In *O*-glycans, in contrast, a number of amino acids (serine, threonine, tyrosine, hydroxylysine and hydroxyproline) can be connected with several involved sugar-residues (GalNAc (*N*-acetylgalactosamine), GlcNAc, Man, Glc, Gal (galactose), Ara (arabinose), Xyl (xylose), Fuc (fucose) and *N*-acetylfucosamine, with no fixed consensus sequence [11]. *O*-Glycan biosynthesis begins by the transfer of one sugar to the protein, followed by the addition of more monosaccharides one by one. Furthermore, the first step of biosynthesis is not restricted to a single organelle but may occur, depending on the linkage formed, in the endoplasmic reticulum, Golgi apparatus, cytosol or nucleus [1]. Most structural investigations on *O*-glycans have been carried out on mucin-type *O*-glycans (GalNAc-α-Ser/Thr), which frequently occur in higher eukaryotes. Mucin-type *O*-glycosylation is initiated by the transfer of a GalNAc residue from UDP-GalNAc to a Ser or Thr of a polypeptide chain (for enzyme classification see [12]). Eight core subtypes with different tissue expression patterns have been described in mammals (reviewed in [13]). Depending on the organism, tissue and developmental stage, these cores are elongated and modified by GlcNAc, Gal and Fuc, and are often sialylated and sometimes sulphated. The direct peptide-linkage of monosaccharides other than GalNAc monosaccharides is characteristic for other types of *O*-glycosylation. A GlcNAc-β-Ser/Thr without any elongation is a typical feature of signal recognition for nuclear and cytoskeletal proteins. In many cases, *O*-GlcNAcylation competes with phosphorylation [14]. A protein-linked α-Fuc residue with or without elongation has been found to be essential in epidermal growth factor domains, together with Glc-β-Ser [1]. The biosynthesis of chondroitin and heparan sulphates, large glycan chains with disaccharide repeating units, is initiated by *O*-xylosylation of the peptide. *O*-Mannosylation is a typical yeast feature, but it has also been found in some muscular dystrophies [15].

Particularly for invertebrates the knowledge regarding any kind of *O*-glycosylation is very rudimentary (Figure 1). Even when some structural aspects are solved, the corresponding biosynthetic active enzymes are often not identified or characterized. This limits the opportunities for determining structure-function relations. However, the few existing data lead to the suggestion that a broad spectrum of modifications and recognition events are still waiting to be discovered. This review aims to motivate further reading and stimulate the interest in invertebrates.

Here, the focus will be on the current knowledge of mucin-type *O*-glycosylation in invertebrates mainly covering structural aspects as well as the corresponding enzymes. Investigations on other types of *O*-glycosylation in invertebrates are currently restricted to a few model organisms

(*Drosophila melanogaster* and *Caenorhabditis elegans*) and have been discussed recently (for *O*-GlcNAc see [16], for *O*-Fuc see [17], for *O*-Glc see [18], for *O*-Man (mannose) see [19,20] and for xylosyltransferases initiating proteoglycans see [21]).
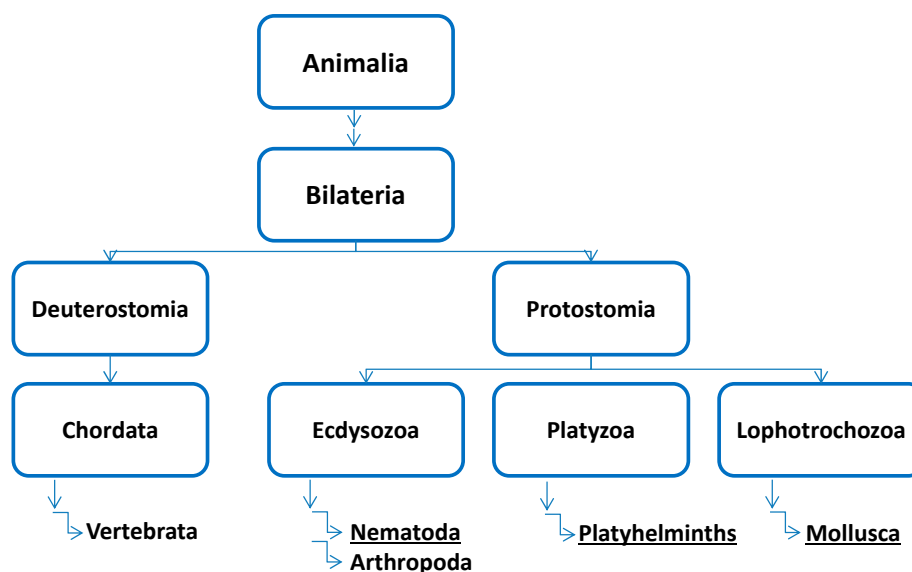

Figure 1. Evolution tree of the kingdom of *Animalia* (condensed). Only those phyla where at least some information about *O*-glycans is available are given. Underlined phyla are topic of this review.

2. Nematoda

Studies on *Toxocara* excretory-secretory larvae antigens determined for *T. canis* (a parasite of dogs and canid animals) 2-*O*-Me-Fucα1-2(4-*O*-Me)Galβ1-3GalNAc and 2-*O*-Me-Fucα1-2Galβ1-3GalNAc, both based on core 1 type structures, as major oligosaccharides (Figure 2), while in the corresponding parasite for cats, *T. cati*, only the di-*O*-methylated trisaccharide was present [22]. Both are targets of the strong immune response against *Toxocara* excretory-secretory antigens [23].

For *Trichinella spiralis* highly immunogenic characteristic tetra-antennary tyvelose containing *N*-glycans have been described [24]. An antibody reaction against an *O*-glycan/peptide was detected, but no detailed sugar structure was determined in this case [25]. No *O*-glycan structures were found in a detailed investigation of the intestinal parasitic nematode of cattle, *Cooperia oncophora* [26].

The worm model organism *Caenorhabditis elegans* is rather well studied. A number of unusual mucin-type structures have been found in an extensive NMR-based study. Based on core 1 type (Galβ1-3GalNAc) or the "invertebrate core" type (Galβ1-6(Galβ1-3)GalNAc), a series of structures containing GlcA and β1-4- and/or β1-6-linked Glc residues were detected (Figure 2). The Glc residues on mucin-type *O*-glycans and the extension of GalNAc by three sugars are unique. Furthermore, a large branched structure with GlcNAc instead of GalNAc directly linked to the protein was identified. This structure contained α1–2 linked Fuc with and without an additional methyl group in terminal position (Figure 2) [27]. In 1996, an indication of the presence of phosphocholine on *O*-glycans of a nematode of *Onchocerca gibson*, a parasite of Australian cattle, was found but no detailed structure was determined [28].
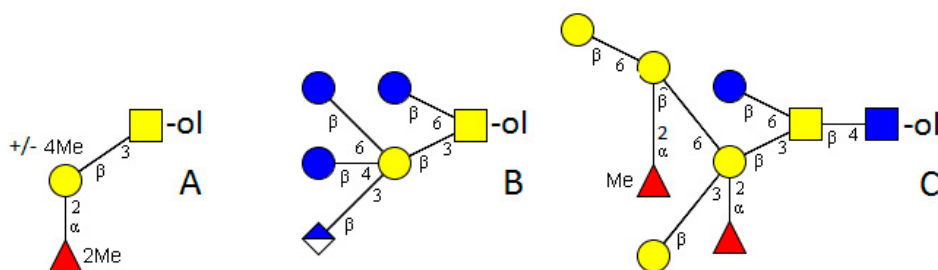
Figure 2. Examples of extended *O*-glycans in nematodes. (**A**) *Toxocara* [22]; (**B,C**) *Caenorhabditis elegans* [26]. The structure plots (Figures 2–6) were generated in the notation of the Consortium for Functional Glycomics (http://www.functional glycomics.org) using the visual editor of "GlycoWorkbench". This software application was developed as part of the EUROCarbDB project and is available online (http://www.ebi.ac.uk/pdbe-srv/eurocarbdb/). Yellow square = GalNAc, blue square = GlcNAc, yellow circle = Gal, blue circle = Glc, red triangle = fucose, upper blue diamond = GlcA (glucuronic acid).

At least nine distinct members of the UDP-GalNAc:polypeptide GalNAc transferase family [GalNAc-T, E.C. 2.4.1.41] have been identified in *C. elegans*. They are highly conserved type-2 membrane proteins with a 60%–80% sequence identity to their human counterparts [29]. They fit well into the phylogenetic subfamilies of the putative orthologous genes of mammals and flies according to their structure, their acceptor specificity and their kinetic data. Their expression patterns vary significantly in the different tissues of *C. elegans* [30]. These facts support the hypothesis that important biological functions are associated with specific isoforms [31]. The subsequent enzyme forming core 1 type *O*-glycan structures is a β1-3-galactosyltransferase [β1-3-Gal-T, T-synthase, E.C. 2.4.1.122], which transfers Gal from UDP-Gal to GalNAcα1-Ser/Thr. The *C. elegans* enzyme consists of 389 amino acids with an overall identity of 42.7% to human T-synthase. In contrast to the human enzyme, the worm enzyme contains four putative *N*-glycosylation sites and does not require Cosmc ("core 1 βGal-T specific molecular chaperone") for activity [32]. Furthermore, an α1-3-Fuc-T family [33] and an UDP-GalNAc:GlcNAcβ-R β1-4-*N*-acetylgalactosaminyltransferase [34] were cloned and characterized, but it is not completely clear whether they act *in vivo* as modifier of the termini of *N*- or of *O*-glycans, or both.

Thus far, the function of mucin-type *O*-glycosylation in *C. elegans* is not clear in detail. But RNAi studies of those genes involved in glycosylation pathways showed that there is an unique requirement for proteoglycan modification of the cell surface for the terminal phase of cytokinesis in early *C. elegans* embryos [35,36].

3. Arthropoda

3.1. Crustacea (Crustaceans)

A putative *O*-glycosylation site has been identified in the sequence of a catechol-*O*-methyltransferase from the shrimp *Penaeus monodon*, which seems to play a role in salinity stress tolerance under low salinity conditions [37]. In the hemocyanin of the crab *Carcinus aestuarii*, three *O*-glycosylated tripeptides were identified by mass spectrometry and subsequent exoglycosidase digests. Each glycopeptide consisted of two *O*-linked glycan chains, where the binding sites were separated by one amino acid. In the

first peptide, two *O*-GalNAc residues were linked to a Ser and a Thr, while all other glycans were linked exclusively to Ser (GalNAc-residues elongated by Gal and *N*-Acetyl-*O*-NeuAc or *N*-Acetyl-*O*-di-NeuAc) [38].

3.2. Hexapoda (Insects)

In relation to the enormous biodiversity of insects, our knowledge on insect glycosylation is rather limited as funding is often only available if analysis is somehow connected with human nutrition or health (*i.e.*, pathogens, allergens or poisons). Therefore, insect glycosylation has been investigated only for the model organism *Drosophila melanogaster*, some cell lines used for expression and some venoms which are relevant to allergies.

3.2.1. Drosophila Melanogaster

Drosophila melanogaster is the model organism for insects. Its genome is completely sequenced and the posttranslational modifications are well investigated [39]. Due to the well-established *Drosophila*-system in several laboratories, much is known about the function of its different structures and related enzymes. Core 1 type is the dominant *O*-glycan structure [40]. It can be modified by GlcNAc forming core 2 type structures (Figure 3). Further elongations by GlcA linked to GalNAc or a Man residue are possible. GlcA may be in a terminal or internal position and is attached to any type of core. Some cores with more than one GlcA are present (Figure 4) [41]. No modification by phosphoethanolamine or sulfation has been found [42]. A rather unusual trisaccharide with an *O*-linked Fuc extended by β1-3GlcNAc and β1-4GlcA comprised 11.3% of the total *O*-linked glycan profile in wild-type embryo powder, but its precursors *O*-Fuc or GlcNAcβ1-3Fuc were not determined [42]. Surprisingly, Fuc was not found in any other structure.
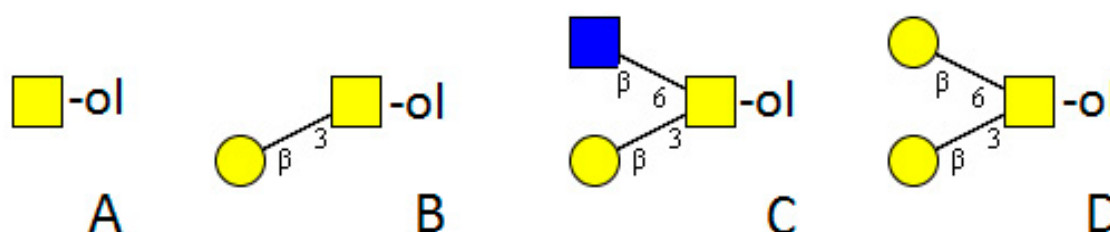
Figure 3. Basic core structures common in invertebrates. (**A**) Tn-antigen; (**B**) T-antigen; core 1 type structure; (**C**) core 2 type structure; (**D**) invertebrate core.

Corresponding to vertebrates in *Drosophila,* an evolutionary conserved family of GalNAc-Ts initiating the mucin-type *O*-glycosylation has been found (10 biochemically confirmed genes, four putative members of the family). The members are expressed in different tissues and temporal patterns during development and organogenesis and have been shown to be essential for viability [31,43–47]. The enzymes have high sequence identity to the mammalian enzymes and display similar acceptor preferences [48]. β1-3-Gal-Ts, necessary for forming the core 1 type structure (Figure 3), have also been identified, and one was confirmed as essential for neural development [49,50]. Overexpression of Fuc-TA, an α1-3-Fuc-T acting on *N*-glycans, affects Notch signalling. Presumably this is due to the

rendered *O*-fucosylation caused by imbalanced GDP-Fuc consumption [51]. Two specific Xyl-Ts transferring Xyl into α1,3-linkage towards *O*-linked Glc have been identified [52]. The rather striking variety of glycosylation in *Drosophila* seems to be controlled to some extent by a fragmentation of the Golgi into functional units with different enzyme equipment and transport opportunities [53].

Drosophila melanogaster is one of the few organisms in which the function of *O*-glycosylation has been investigated in detail. First, *O*-glycosylation-dependent changes in epithelial tube formation were detected [54], and later, the relatively new method of RNA interference allowed for the determination of several more functional and morphological changes. Reduced expression of individual GalNAc-T genes resulted in reduced secretion, altered epithelial cell adhesion and cytokinesis or changes in Golgi organisation, thus confirming the importance of *O*-glycosylation [55,56]. Normal muscle development requires *O*-mannosylation of *Drosophila dystro-glycan* homologue [20].

3.2.2. Venoms

The N-glycans of some insect venoms became of interest because of their involvement in allergic reactions [57], but no attention was paid to *O*-glycans. Just in the nests of *Vespula germanica*, Tn and T-antigen *O*-glycans were determined along with their derivatives modified by 2-aminoethyl phosphate (Figure 3) [58].

3.2.3. Insect Expression Systems

Insect cells are efficient tools for the expression of recombinant glycoproteins. There are several cell lines established from moth (*Lepidoptera*) origin. The most frequently used are derived from the fall armyworm *Spodoptera frugiperda* (Sf9, Sf21) and the cabbage looper *Trichoplusia ni* (High Five™); Others are from the silkworm *Bombyx mori*, the cabbage moth *Mamestra brassicae* or the Acrea moth *Estigmene acrea*. Besides technical requirements enabling large-scale protein production, such as growing of the cells, baculovirus infection rate, expression rate and downstream processing, the expression system is selected due to its ability to form the desired protein as similarly as possible to the mammalian model. Correct glycosylation is a main factor in this attempt. Therefore, the glycosylation potential of these cell lines was investigated to analyse their original abilities and to evaluate the engineering needs in order to mimic the glycosylation patterns of mammals.

All investigated cell lines have the ability to form mucin-type *O*-glycosylation. However, the structures differ with regard to cell line and medium. Tn-antigens were detected, the activities of more than one GalNAc-T activity could be determined, and the formation of core 1 type structures, including the activity of the responsible enzymes, was shown [59]. A further elongation of this core by an α1-4-linked Gal residue was observed, and the enzyme was characterised [60]. Mass spectrometric analysis and lectin detection revealed that the recombinant expressed glycans were generally much larger and more diverse than the native ones in a previous study. Large extensions containing several HexNAc and HexA residues were found. Fuc directly linked to the protein and extended by uronic acid and HexNAc (HexNAc-HexA-Fuc-ol) or as an extension of the core (HexA-(Fuc)-GalNAc-ol) was also detected (Figure 4) [61].
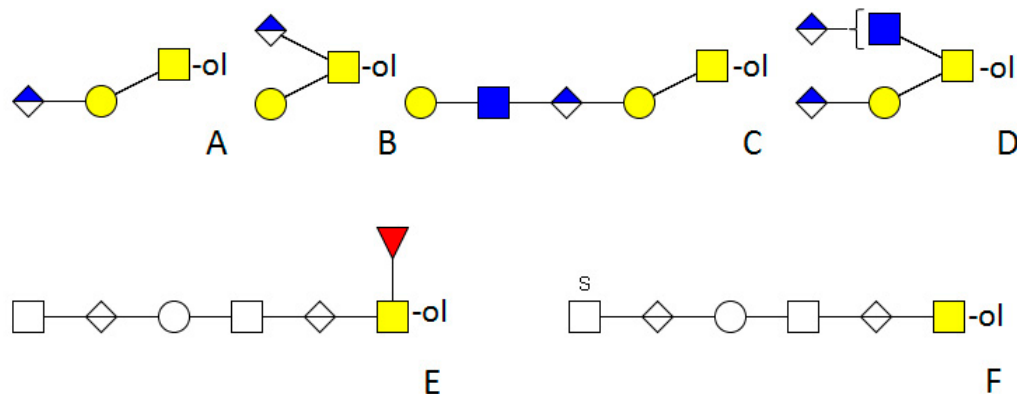
Figure 4. Examples of extended *O*-glycans in insects. (**A–D**) *Drosophila melanogaster* [41,42]; (**E**,**F**) were derived from a recombinant protein produced in High-Five™ cells [61]. Empty square = *N*-acetylhexosamine, empty circle = hexose, empty diamond = uronic acid, S = sulphate.

A fascinating issue is the question of sialylation in insects. There are a number of controversial studies regarding the ability of sialylation in insects. In *Drosophila, in situ* hybridization analysis of embryonic expression revealed a very restricted pattern of *Drosophila* sialyltransferase in a few cells of the developing central nervous system [40]. Furthermore, the cytosine-monophosphate-sialic acid synthetase which provides the donor substrate, as well as the sialyltransferase gene, were found in the central nervous system cells [62,63]. In lepidopteran insect cell lines, no sialyltransferase activity or terminal sialylation was detected [59]. The negative charge related to the *O*-glycans of recombinant expressed proteins generated in Sf9 and High Five™ cells was determined to be GlcA, GalA, sulphate and phosphocholine [61]. Sialylation in insects is thus restricted to few tissues and some developmental stages and is therefore not as common as in mammals.

Even when the moth expression systems and the model organism *Drosophila melanogaster*, in particular, are well investigated, we cannot claim to know much about insects. Thus far, about one million species are zoologically described, but about five times more are anticipated. Currently, we only have information from a handful of species regarding their glycosylation abilities.

4. Platyhelminthes

A number of helminth parasites show *O*-glycan structures and mucins. In particular, the Tn antigen is widespread. The parasitic flatworm *Fasciola hepatica*, a trematode which causes fasciolosis, a chronic disease in humans and domestic animals, expresses Tn (mainly in the testis) as along with sialyl-Tn-antigens (widely distributed in different tissues), which may play a role in the interaction with the host in terms of mimicry or influence on the immune system (Figure 3) [64]. The Tn antigen was also found in *Echinococcus granulosus*, the sheep-dog tapeworm [65]. In both *F. hepatica* and *E. granulosus*, high activity levels of the *O*-glycan initiating enzyme GalNAc-T were detected [66,67]. Similarly, on microtriches of the cestode *Mesocestoides vogae* (syn. Corti), Tn and sialyl-Tn antigens were found, as well as GalNAc-T activity [68]. Cestodes also contain the Tk-antigen (GlcNAcβ1-6(GlcNAcβ1-3)Gal), which was detected in *Taenia hydatigena*, *Mesocestoides vogae* (syn. Corti) and *Taenia crassiceps* by immunoblotting using the monoclonal antibody LM389 [69].

The only well-investigated representatives of platyhelminthes are trematodes of the genus *Schistosoma* (blood-fluke), which cause schistosomiasis, an acute and chronic disease. 249 million people were affected by and 42.1 million people were treated in 2012 for schistosomiasis [World Health Organization. Schistosomiasis. Fact Sheet No. 115. Geneva: available at: http://www.who.int/mediacentre/factsheets/ fs115/en/ (accessed January 2015)]. The five main species, *Schistosoma mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum* and *S. haematobium* are known to infect humans in south-Sahara Africa, South America, the Caribbean and the Middle and Far East. *S. mansoni*, which occurs mainly in Africa and South America, and *S. japonicum*, which occurs mainly in the Far East, were in the focus of analysis. The life-cycle of the worms takes place in different snails as intermediate hosts and is finalised in humans. Cercariae are released into water by infected snails, where they then penetrate the skin of the final host, migrate and develop into male and female adults, which mate and produce eggs. Passing from the vessels and penetrating the intestinal wall, the eggs are released with the faeces. The glycome of schistosomes is rather well investigated. Besides studies on *N*-glycans and glycosphingolipids, a number of *O*-glycan structures derived from different developmental stages are also known. In the late 1990s Khoo *et al.* identified core 1 and core 2 type *O*-glycans with further elongation by a high proportion of additional $(+/-Fuc_1)Hex_1HexNAc_1$ units in the egg glycoproteins of *S. mansoni* and *S. japonicum*. Further, the "invertebrate core" $Hex_2HexNAc$ was also determined (Figure 3). *S. mansoni* and *S. japonicum* differed clearly in their fucosylation. While HexNAc-$(+/-Fuc)$HexNAc were terminal decoration in both schistosomes, multifucosylated termini with $Fuc_{1,2}HexNAc-(Fuc_{1,2})HexNAc$ appeared only on *S. mansoni* (Figure 5) [70]. Both egg antigens showed LacNAc (Galβ1-4GlcNAc) as well as LacdiNAc (GalNAcβ1-4GlcNAc) type structures, including Lewis^x (Galβ1-4(Fucα1-3)GlcNAc), as terminal elongations. Further modifications were terminal $HexNAc_3$ and up to $HexNAc_4$-chains directly linked to the *O*-glycan cores [70,71]. A small but important sub-fraction of *S. mansoni* egg antigens contained large heavily fucosylated branched *O*-glycan structures carrying up to four HexNAcs per chain [72]. In a recent large study on the proteome (45 proteins) of *S. mansoni,* difucosylated LacdiNAc and Lewis^x structures on the egg shells were detected by antibodies [73]. However, no assignment to *N-* or *O*-glycan structures has been performed.

Multifucosylated terminal sequences were seen in cercariae on core 1 and core 2 type structures with Lewis^x, LacNAc units or single GlcNAc residues directly attached to the -3Galβ1-3GalNAc core or indirectly via a β1-6-linked Gal, forming biantennary structures (Figure 5) [74].

Other developmental stages, such as miracidia, primary sporocysts and adults, were analysed by specific antibodies for the location and structures of their glycan epitopes. The expression patterns were determined to be different in the course of the transformation process and seem to be regulated developmentally [75,76].

No genetic data exist for the corresponding enzymes; only some activity studies are available. Fuc-T activities were determined in three developmental stages (egg, cercariae and adult) of *S. mansoni,* showing a wide acceptor range. Non-sialylated core 1 type structures, core 2 type oligosaccharides with or without sialic acid or already fucosylated (such as Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc) were valuable acceptor substrates. Compared with the structural data, the total activity of Fuc-Ts in eggs was far higher (50 fold) than in the other investigated stages [77]. In cercariae of the schistosome *Trichobilharzia ocellata,* an α1-3-Fuc-T with broad acceptor tolerance for different variants of core 2 type oligosaccharides (unsubstituted, already α1-2-fucosylated or α1-3-sialylated) was determined. It

should be noted that *N*-ethylmaleimide, which is an inhibitor of many vertebrate Lewis^x–creating Fuc-Ts, did not inhibit the helminth enzyme. In addition, α1-2-Fuc-T activity forming difucose chains of the structure Fucα1-2Fucα1-3GlcNAc was detected [78].
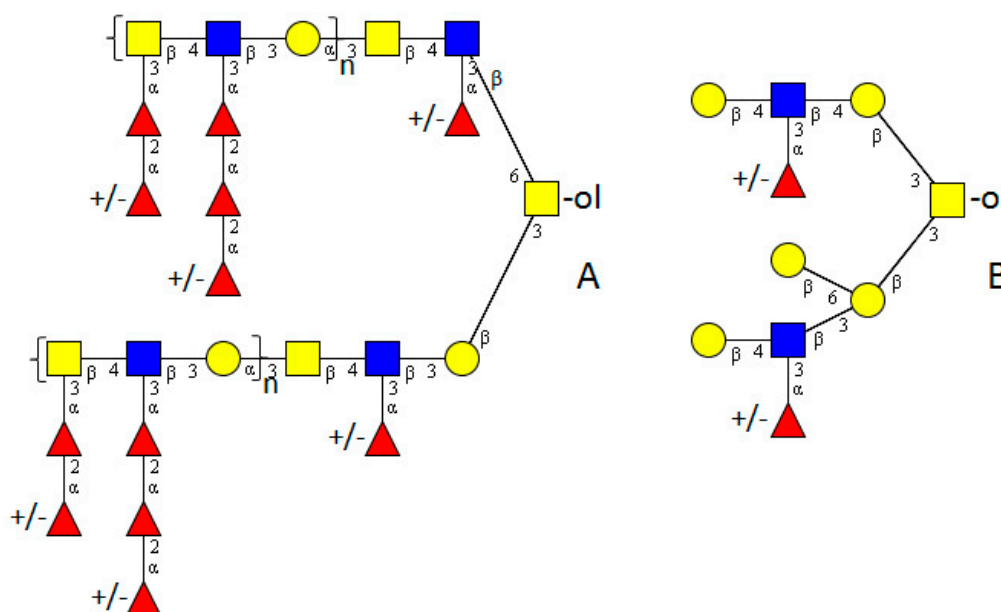
Figure 5. Examples of extended cercariae *O*-glycans of schistosomes. (**A**) from [70]; (**B**) from [74].

The presentation of surface glycans in the different stages caused different immune responses. While adult worms did not evoke a significant immune response of the host, the eggs were highly immunogenic, which is seen in elevated Th2 response. The importance and role of the schistosome glycome in the course of host parasite interactions has been reviewed extensively [79,80]. Investigations of the different stages during the life-cycle and possible targets of interruption of this cycle have been carried out to prevent the development of the parasite or to interfere in host parasite interaction. The most common characteristic features of schistosome *O*-glycans are the terminal multifucosylated motifs, which may be promising epitopes for diagnostic antibodies.

However, preventing parasitic infection, perhaps by immunization, is only one of the medically relevant aspects of platyhelminthes. Another is to benefit from the severe reaction of the vertebrate immune system on schistosome glycans. Extracts from helminth parasites have been shown to activate the human immune system by immunomodulatory glycan structures [81]. Parasites contain minimal mucin-type *O*-glycans (T, Tn, sialyl-Tn Tk-epitopes) similar to some cancer cells, which may induce an immune response against them. These findings were supported by a negative correlation between cancer development and parasite infection (reviewed in [82]) Other studies have indicated that the T-cell response is enhanced by soluble *S. mansoni* egg antigens in general [83,84]. However, studies on mice or analysis of data of human patients showed more differentiated patterns. It is clear that there are also some immune suppressive molecules that suppress the immune response of the mammalian host during infection (e.g., in *Schistosoma* egg extract). It may be the third fucose of the multifucosylated side-chain, present on *O*-glycans of the cercarial glycocalyx, which blocks the recognition by anti-LDN-F/LDN-DR MAbs [85]. These structures could be an interesting option for a possible treatment of inflammatory

diseases towards down-regulation of autoimmune or allergic responses [86], but we first must determine which of the structures correlates with which of the effects.

5. Mollusca

5.1. Cephalopoda

Two *O*-linked GalNAc residues and one *O*-linked Fuc were detected in octopus (*Octopus dofleini*) rhodopsin. *O*-glycosylation is conserved in this type of G-protein-coupled receptors; therefore, it may contribute in the maturation, transport or cell surface expression of this protein [87].

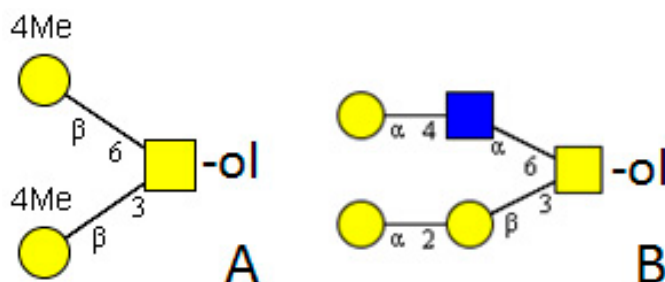### *5.2. Gastropoda*

5.2.1. Bivalves

While the *N*-glycome of *Crassostrea virginica* (Eastern oyster) is rather complicated, the *O*-glycans found in oyster haemocytes and plasma are simple core 1 or β1-6-GlcNAc extended core 1 type structures [88]. A lectin cytochemistry study of the sorting pathways of lysosomal enzymes in *Mytilus galloprovincialis* (Mediterranean mussel) digestive cells showed that *O*-glycosylation is already fully completed in the cis-Golgi network, which is in contrast to mammals where this takes longer [3]. Although the first complete genome of a mollusc, the pacific oyster *Crassostrea gigas*, is now sequenced [89], no detailed information on its glycosylation machinery is available so far.

5.2.2. Snails

Marine snails of the genus *Conus* are found in tropical areas near coral reefs. Their characteristics are beautiful shells and neurotoxic poisons which are intended for hunting and defence. Investigations of the poisons yielded the determination of several hundred, often *O*-glycosylated, peptides per *Conus* species, which may be of interest for pharmaceutical purposes (for a recent review see [90]). The focus was often on peptide analysis rather than glycans. Only a core 1 type structure was determined, while more complicated glycans were often given as Hex$_{2-4}$HexNAc$_2$, with no monosaccharide composition or linkage information. One detailed NMR study on *Conus consors* venom determined a remarkable new structure: Galα1-4GlcNAcα1-6(Galα1-2Galβ1-3)GalNAcα1-*O*-Ser (Figure 6) [91]. The unusual features of this structure are an α1-6-linked GlcNAc (usually β1-6) and terminally α-linked galactoses in L-configuration (usually β linked in D-configuration).

Megathura crenulata (keyhole limpet) as well as *Biomphalaria glabrata* share the structural features of their glycans with schistosomes. However, the carbohydrate epitopes have been assigned to *N*-glycans or glycolipids [92,93], or were not specified in detail [94]. A comparison of *O*-glycan structures is missing. Only for KLH 2-c, a functional unit of keyhole limpet hemocyanin, a positive binding to peanut agglutinin indicated mucin-type *O*-glycosylation [95]. Histochemical and lectin-histochemical studies on the pedal glandular system of the rayed Mediterranean limpet, *Patella caerulea*, revealed different expressions of Gal, GalNAc, α1-2- and α1-4-linked Fuc and carboxylated and/or sulphated structures, presumably in *O*-glycans, in the course of the annual cycle [96]. In some snail tissues, traces of sialic acids were identified but could not be assigned to specific glycans [97].

The most striking feature of snail glycosylation is the high degree of methylation of terminal hexose residues. 3-*O*-methyl Man, 3- and 4-*O*-methyl Gal have been identified and determined for several species of land and water snails [98,99]. The *O*-glycosylation patterns were determined for *Arion lusitanicus*, *Achatina fulica*, *Biomphalaria glabrata*, *Cepaea hortensis*, *Clea helena*, *Helix pomatia*, *Limax maximus* and *Planorbarius corneus*. The main structure was a di-methylated "invertebrate core" consisting of the protein bound GalNAc residue with two 4-*O*-methyl Gal residues in β1-3- and β1-6-position. Further elongations with Gal, Man, and Fuc in different positions were found in minor amounts (Figure 6) [100].

Figure 6. Examples of *O*-glycans from gastropod origin. (**A**) *Arion lusitanicus* [100] and (**B**) *Conus consors* [91].

Thus far only, one *O*-glycosylation initiating GalNAc-T of mollusc origin has been cloned. It was a type-2 membrane protein from *Biomphalaria glabrata,* containing all domains typical for GalNAc-Ts: a short transmembrane domain, a stem region, a Gal/GalNAc motif, a manganese binding site and a ricin-like lectin domain [101]. It is homologous to other group Ib enzymes (for a classification of GalNAc-Ts see [12]) and has similar substrate specificity [102].

In *Lymnaea stagnalis*, the intermediate host for the trematode *Moliniella anceps*, a β1-4-Glc-T activity, was detected, with a substrate preference towards GlcNAc β1-6-linked to Gal or GalNAc, which may occur in *N*- as well in *O*-glycans [103]. No corresponding glycans have yet been identified.

6. Conclusions

All generated data show that the general glycosylation machinery is similar for all organisms, even when special features are limited to specific phyla. While core 1 type and core 2 type *O*-glycan structures are frequent modifications of vertebrates and invertebrate proteins, the "invertebrate core" has been identified only in nematodes, schistosomes and molluscs. Fuc is a common feature in terminal position of all animals, but it seems to be more dominant in invertebrates; multifucosylated and further elongated Fuc residues are possible there. Methylation of monosaccharides has never been detected in vertebrates. In invertebrates, it may occur in different linkages on Fuc or hexose residues (Gal, Man) [104]. Methylated glycans are conserved targets of animal innate defence [105], but their benefit for the invertebrate itself is still not clear. Another critical feature of glycan structures is the presence of a negative charge. This often acts as a general sorting and recognition marker. While vertebrates commonly display sialic acids in terminal position of their glycans, in invertebrates, sialic acids are very limited and clearly restricted to few tissues and/or early developmental stages. These organisms gain their negative charge by modifications with sulphate, carboxyl groups, uronic acids, aminoethylphosphate or phosphocholine.

In general, the few identified and characterised glycosyltransferases show high similarity to their mammalian counterparts in terms of structure and specificity. However, there are some important differences, such as, the ability of T-synthases of flies and nematodes to transfer Gal without needing a chaperon. We know that glycosylation is an important factor in several biological recognition processes and we know that invertebrates are extremely successful in their survival strategies and their adaption abilities to changing environments. Therefore, their glycosylation abilities seem to be an interesting target for further research in order to learn more about the influence and regulation of external factors on biosynthetic pathways in general.

Acknowledgments

Conflicts of Interest

The author declares no conflict of interest.

References

1. Van den Steen, P.; Rudd, P.M.; Dwek, R.A.; Opdenakker, G. Concepts and principles of *O*-linked glycosylation. *Crit. Rev. Biochem. Mol. Biol.* **1998**, *33*, 151–208.
2. Proszynski, T.J.; Simons, K.; Bagnat, M. *O*-glycosylation as a sorting determinant for cell surface delivery in yeast. *Mol. Biol. Cell* **2004**, *15*, 1533–1543.
3. Robledo, Y.; Marigómez, I.; Angulo, E.; Cajaraville, M.P. Glycosylation and sorting pathways of lysosomal enzymes in mussel digestive cells. *Cell Tissue Res.* **2006**, *324*, 319–333.
4. Schjoldager, K.T.B.G.; Clausen, H. Site-specific protein *O*-glycosylation modulates proprotein processing—Decipering specific functions of the large polypeptide GalNAc-transferase gene family. *Biochim. Biophys. Acta* **2012**, *1820*, 2079–2094.
5. Gill, D.J.; Tham, K.M.; Chia, J.; Wang, S.C.; Steentoft, C.; Clausen, H.; Bard-Chapeau, E.A.; Bard, F.A. Initiation of GalNAc-type *O*-glycosylation in the endoplasmic reticulum promotes cancer cell invasiveness. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, E3152–E3161.
6. Radhakrishnan, P.; Dabelsteen, S.; Madsen, F.B.; Francavilla, C.; Kopp, K.L.; Steentoft, C.; Vakhrushev, S.Y.; Olsen, J.V.; Hansen, L.; Bennett, E.P.; *et al.* Immature truncated *O*-glycophenotype of cancer directly induces oncogenic features. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E4066–E4075.
7. Badirou, I.; Kurdi, M.; Legendre, P.; Rayes, J.; Bryckaert, M.; Casari, C.; Lenting, P.J.; Christophe, O.D.; Denis, C.V. *In vivo* analysis of the role of *O*-glycosylations of Von Willebrand factor. *PLoS ONE* **2012**, *7*, e37508.
8. Herzog, B.H.; Fu, J.; Xia, L. Mucin-type *O*-glycosylation is critical for vascular integrity. *Glycobiology* **2014**, *24*, 1237–1241.

9.   Zhang, L.; Syed, Z.A.; van Dijk Härd, I.; Lim, J.M.; Wells, L.; Ten Hagen, K.G. *O*-Glycosylation regulates polarized secretion by modulating Tango1 stability. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 7296–7301.

10. Tran, D.T.; Ten Hagen, K.G. Mucin-type *O*-glycosylation during development. *J. Biol. Chem.* **2013**, *288*, 6921–6929.

11. Spiro, R.G. Protein glycosylation: nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds. *Glycobiology* **2002**, *12*, 43R–56R.

12. Bennett, E.P.; Mandel, U.; Clausen, H.; Gerken, T.A.; Fritz, T.A.; Tabak, L.A. Control of mucin-type *O*-glycosylation: A classification of the polypeptide GalNAc-transferase gene family. *Glycobiology* **2012**, *22*, 736–756.

13. Patsos, G.; Corfield, A. *O*-Glycosylation: Structural Diversity and Functions. In *The Sugar Code*; Gabius, H.J., Ed.; Wiley-VCH: Weinheim, Germany, 2009; pp. 111–137.

14. Bullen, J.W.; Balsbaugh, J.L.; Chanda, D.; Shabanowitz, J.; Hunt, D.F.; Neumann, D.; Hart, G.W. Cross-talk between two essential nutrient-sensitive enzymes. *O*-GlcNAc transferase (OGT) and AMP-activated protein kinase (AMPK). *J. Biol. Chem.* **2014**, *289*, 10592–10606.

15. Live, D.; Wells, L.; Boons, G.J. Dissecting the molecular basis of the role of the *O*-mannosylation pathway in disease: α-Dystroglycan and forms of muscular dystrophy. *ChemBioChem* **2013**, *14*, 2392–2402.

16. Bektas, M.; Rubenstein, D.S. The role of intracellular protein *O*-glycosylation in cell adhesion and disease. *J. Biomed. Res.* **2011**, *25*, 227–236.

17. Ishio, A.; Sasamura, T.; Ayukawa, T.; Kuroda, J.; Ishikawa H.O.; Aoyama, N.; Matsumoto, K.; Gushiken, T.; Okyjima, T.; Yamakawa, T.; Matsuno, K. *O*-Fucose monosaccharde of *Drosophila* Notch has a temperature-sensitive function and cooperates with *O*-glucose glycan in Notch transport and Notch signalling activation. *J. Biol. Chem.* **2015**, *290*, 505–519.

18. Takeuchi, H.; Kantharia, J.; Sethi, M.K.; Bakker, H.; Haltiwanger, R.S. Site-specific *O*-glucosylation of the epidermal growth factor-like (EGF) repeats of Notch: efficiency of glycosylation is affected by proper folding and amino acid sequence of individual EGF repeats. *J. Biol. Chem.* **2012**, *287*, 33934–33944.

19. Xu, C.; Ng, D.T.W. *O*-mannosylation: The other glycan player of ER quality control. *Semin. Cell Dev. Biol.* **2015**, in press.

20. Nakamura, N.; Lyalin, D.; Panin, V.M. Protein *O*-mannosylation in animal development and physiology: From human disorders to *Drosophila* phenotypes. *Sem. Cell Dev. Biol.* **2010**, *21*, 622–630.

21. Brunner, A.; Kolarich, D.; Voglmeir, J.; Paschinger, K.; Wilson, I.B.H. Comparative characterisation of recombinant invertebrate and vertebrate peptide *O*-xylosyltransferases. *Glycoconj. J.* **2006**, *23*, 543–554.

22. Khoo, K.H.; Maizels, R.M.; Page, A.P.; Taylor, G.W.; Rendell, N.B.; Dell, A. Characterization of nematode glycoproteins: the major *O*-glycans of *Toxocara* excretory-secretory antigens are *O*-methylated trisaccharides. *Glycobiology* **1991**, *2*, 163–171.

23. Schabussova, I.; Amer, H.; van Die, I.; Kosma, P.; Maizels, R.M. *O*-Methylated glycans from *Toxocara* are specific targets for antibody binding in human and animal infections. *Int. J. Parasitol.* **2007**, *37*, 97–109.

24. Ellis, L.A.; McVay, C.S.; Probert, M.A.; Zhang, J.; Bundle, D.R.; Appleton, J.A. Terminal β-linked tyvelose creates unique epitopes in *Trichinella spiralis* glycan antigens. *Glycobiology* **1997**, *7*, 383–390.

25. Romarís, F.; Escalante, M.; Lorenzo, S.; Bonay, P.; Gárate, T.; Leiro, J. Ubeira, F.M. Monoclonal antibodies raised in Btk xid mice reveal new antigenic relationships and molecular interactions among gp53 and other *Trichinella* glycoproteins. *Mol. Biochem. Parasitol.* **2002**, *125*, 173–183.

26. Borloo, J.; de Graef, J.; Peelaers, I.; Nguyen, D.L.; Mitreva, M.; Devereese, B.; Hokke, C.H.; Vercruysse, J.; Claerebout, E.; Geldhof, P. In-depth proteomic and glycomic analysis of the adult-stage *Cooperia oncophora* excretome/secretome. *J. Proteome Res.* **2013**, *12*, 3900–3911.

27. Guérardel, Y.; Balanzino, L.; Maes, E.; Leroy, Y.; Coddeville, B.; Oriol, R.; Strecker, G. The nematode *Caenorhabditis elegans* synthesizes unusual *O*-linked glycans: Identification of glucose-substituted mucin-type *O*-glycans and short chondroitin-like oligosaccharides. *Biochem. J.* **2001**, *357*, 167–182.

28. MacDonald, M.; Copeman, D.B.; Harnett, W. Do excretory-secretory products of *Onchocerca gibsoni* contain phosphorylcholine attached to *O*-type glycans? *Int. J. Parasitol.* **1996**, *26*, 1075–1080.

29. Hagen, F.K.; Nehrke, K. cDNA cloning and expression of a family of UDP-*N*-acetyl-D-galactosamine:polypeptide *N*-acetylgalactosaminyltransferase sequence homologs from *Caenorhabditis elegans*. *J. Biol. Chem.* **1998**, *273*, 8268–8277.

30. Hagen, F.; Layden, M.; Nehrke, K.; Gentile, K.; Berbach, K.; Tsao, C.C.; Forsythe, M. Mucin-type *O*-glycosylation in *C. elegans* is initiated by a family of glycosyltransferases. *TIGG* **2001**, *13*, 463–479.

31. Schwientek, T.; Bennett, E.P.; Flores, C.; Thacker, J.; Hollmann, M.; Reis, C.A.; Behrens, J.; Mandel, U.; Keck, B.; Schäfer, M.A.; *et al.* Functional conservation of subfamilies of putative UDP-*N*-acetylgalactosamine:polypeptide *N*-acetylgalactosaminyltransferases in *Drosophila*, *Caenorhabditis elegans*, and mammals: one subfamily composed of 1(2)35aa is essential in *Drosophila*. *J. Biol. Chem.* **2002**, *277*, 22623–22638.

32. Ju, T.; Zheng, Q.; Cummings, R.D. Identification of core 1 *O*-glycan T-synthase from *Caenorhabditis elegans*. *Glycobiology* **2006**, *16*, 947–958.

33. Nguyen, K.; van Die, I.; Grundahl, K.M.; Kawar, Z.S.; Cummings, R.D. Molecular cloning and characterization of the *Caenorhabditis elegans* α1-3-fucosyltransferase family. *Glycobiology* **2007**, *17*, 586–599.

34. Kawar, Z.S.; van Die, I.; Cummings, R.D. Molecular cloning and enzymatic characterization of a UDP-GalNAc:GlcNAcβR β1-4-*N*-acetygalactosaminyltransferase from *Caenorhabditis elegans*. *J. Biol. Chem.* **2002**, *277*, 34924–34932.

35. Hwang, H.Y, Olson, S.K.; Esko, J.D.; Horvitz, H.R. *Caenorhabditis elegans* early embryogenesis and vulval morphogenesis require chondroitin biosynthesis. *Nature* **2003**, *423*, 439–443.

36. Wang, H.; Spang, A.; Sullivan, M.A.; Hryhorenko, J.; Hagen, F.K. The terminal phase of cytokinesis in the *Caenorhabditis elegans* early embryo requires protein glycosylation. *Mol. Biol. Cell* **2005**, *16*, 4202–4213.

37. Rajesh, S.; Kiruthika, J.; Ponniah, A.G.; Shekhar, M.S. Identification, cloning and expression of Catechol-*O*-methyltransferase (COMT) gene from shrimp, *Penaeus monodon* and its relevance to salinity stress. *Fish Shellfish Immunol.* **2012**, *32*, 693–699.

38. Dolashka-Angelova, P.; Beltramini, M.; Dolashki, A.; Salvato, B.; Hristova, R.; Voelter, W. Carbohydrate composition of *Carcinus aestuarii* hemocyanin. *Arch. Biochem. Biophys.* **2001**, *389*, 153–158.

39. Katoh, T.; Tiemeyer, M. The N's and O's of *Drosophila* glycoprotein glycobiology. *Glycoconj. J.* **2013**, *30*, 57–66.

40. North, S.J.; Koles, K.; Hembd, C.; Morris, H.R.; Dell, A.; Panin, V.M.; Haslam, S.M. Glycomic studies of *Drosophila melanogaster* embryos. *Glycoconj. J.* **2006**, *23*, 345–354.

41. Aoki, K.; Tiemeyer, M. The glycomics of glycan glucuronylation in *Drosophila melanogaster*. *Meth. Enzymol.* **2010**, *480*, 297–321.

42. Aoki, K.; Porterfield, M.; Lee, S.S.; Dong, B.; Nguyen, K.; McGlamry, K.H.; Tiemeyer, M. The diversity of *O*-linked glycans expressed during *Drosophila melanogaster* development reflects stage-and tissue-specific requirements for cell signalling. *J. Biol. Chem.* **2008**, *283*, 30385–30400.

43. Ten Hagen, K.G.; Tran, D.T. A UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase is essential for viability in *Drosophila melanogaster*. *J. Biol. Chem.* **2002**, *277*, 22616–22622.

44. Ten Hagen, K.G.; Tran, D.T.; Gerken, T.A.; Stein, D.S.; Zhang, Z. Functional characterization and expression analysis of members of the UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase family from *Drosophila melanogaster*. *J. Biol. Chem.* **2003**, *278*, 35039–35048.

45. Tian, E.; Ten Hagen, K.G. Expression of the UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase family is spatially and temporally regulated during *Drosophila* development. *Glycobiology* **2006**, *16*, 83–95.

46. Tian, E.; Ten Hagen, K.G. *O*-linked glycan expression during *Drosophila* development. *Glycobiology* **2007**, *17*, 820–827.

47. Tran, D.T.; Zhang, L.; Zhang, Y.; Tian, E.; Earl, L.A.; Ten Hagen, K.G. Multiple members of the UDP-GalNAc: polypeptide *N*-acetylgalactosaminyltransferase family are essential for viability in *Drosophila*. *J. Biol. Chem.* **2011**, *287*, 5243–5252.

48. Gerken, T.A.; Ten Hagen, K.G.; Jamison, O. Conservation of peptide acceptor preferences between *Drosophila* and mammalian polypeptide-GalNAc transferase ortholog pairs. *Glycobiology* **2008**, *18*, 861–870.

49. Müller, R.; Hülsmeier, A.J.; Altmann, F.; Ten Hagen, K.; Tiemeyer, M.; Hennet, T. Characterization of mucin-type core-1 beta1-3 galactosyltransferase homologous enzymes in *Drosophila melanogaster*. *FEBS J.* **2005**, *272*, 4295–4305.

50. Lin, Y.R.; Reddy, B.V.V.G.; Irvine, K.D. Requirement for a core 1 galactosyltransferase in the *Drosophila* nervous system. *Dev. Dyn.* **2008**, *237*, 3703–3714.

51. Rendić, D.; Sharrow, M.; Katoh, T.; Overcarsh, B.; Nguyen, K.; Kapurch, J.; Aoki, K.; Wilson, I.B.H.; Tiemeyer, M. Neural specific α3-fucosylation of *N*-linked glycans in the *Drosophila* embryo requires fucosyltransferase A and influences developmental signalling associated with *O*-glycosylation. *Glycobiology* **2010**, *20*, 1353–1365.

52. Sethi, M.K.; Buettner, F.F.R.; Krylov, V.B.; Takeuchi, H.; Nifantiev, N.E.; Haltiwanger, R.S.; Gerardy-Schahn, R.; Bakker, H. Identification of glycosyltransferase 8 family members as xylosyltransferases acting on *O*-glucosylated Notch epidermal growth factor repeats. *J. Biol. Chem.* **2010**, *285*, 1582–1586.

53. Yano, H.; Yamamoto-Hino, M.; Abe, M.; Kuwahara, R.; Haraguchi, S.; Kusaka, I.; Awano, W.; Konoshita-Toyoda, A.; Toyoda, H.; Goto, S. Distinct functional units of the Golgi complex in *Drosophila* cells. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 13467–13472.

54. Tian, E.; Ten Hagen, K.G. A UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase is required for epithelial tube formation, *J. Biol. Chem.* **2007**, *282*, 606–614.

55. Zhang, L.; Zhang, Y.; Ten Hagen, K.G. A mucin-type *O*-glycosyltransferase modulates cell adhesion during *Drosophila* development. *J. Biol. Chem.* **2008**, *283*, 34076–34086.

56. Zhang, L.; Ten Hagen, K.G. Dissecting the biological role of mucin-type *O*-glycosylation using RNA interference in *Drosophila* cell culture. *J. Biol. Chem.* **2010**, *285*, 34477–34484.

57. Hemmer, W.; Focke, M.; Kolarich, D.; Wilson, I.B.H.; Altmann, F.; Wöhrl, S.; Götz, M.; Jarisch, R. Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. *J. Allergy Clin. Immunol.* **2001**, *108*, 1045–1052.

58. Maes, E.; Garénaux, E.; Strecker, G.; Leroy, Y.; Wieruszeski, J.M.; Brassart, C.; Guérardel, Y. Major *O*-glycans from the nest of *Vespula germanica* contain phospho-ethanolamin. *Carbohydr. Res.* **2005**, *340*, 1852–1858.

59. Lopez, M.; Tetaert, D.; Julinat, S.; Gazon, M.; Cerutti, M.; Verbert, A.; Delannoy, P. *O*-Glycosylation potential of lepidopteran insect cell lines. *Biochim. Biophys. Acta* **1999**, *1427*, 49–61.

60. Lopez, M.; Gazon, M.; Juliant, S.; Plancke, Y.; Leroy, Y.; Strecker, G.; Cartron, M.P.; Bailly, P.; Cerutti, M.; Verbert, A.; Delannoy, P. Characterization of a UDP-Gal:Galβ1,3GalNAc α1,4-galactosyltransferase activity in a *Mamestra brassicae* cell line. *J. Biol. Chem.* **1998**, *273*, 33644–33651.

61. Gaunitz, S.; Jin, C.; Nilsson, A.; Liu, J.; Karlsson, N.G.; Holgersson, J. Mucin-type proteins produced in the *Trichoplusia ni* and *Spodoptera frugiperda* insect cell lines carry novel *O*-glycans with phosphocholine and sulfate substitutions. *Glycobiology* **2013**, *23*, 778–796.

62. Islam, R.; Nakamura, M.; Scott, H.; Repnikova, E.; Carnahan, M.; Pandey, D.; Caster, C.; Khan, S.; Zimmermann, T.; Zoran, M.J.; Panin, V.M. The role of *Drosophila* cytidine monophosphate-sialic acid synthetase in the nervous system. *J. Neurosci.* **2013**, *33*, 12306–12315.

63. Koles, K.; Irvine, K.D.; Panin, V.M. Functional characterization of *Drosophila* sialyltransferase. *J. Biol. Chem.* **2004**, *279*, 4346–4357.

64. Freire, T.; Casaravilla, C.; Carmona, C.; Osinaga, E. Mucin-type *O*-glycosylation in *Fasciola hepatica*: Characterisation of carcinoma-associated Tn and sialyl-Tn antigens and evaluation of UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase activity. *Int. J. Parasitol.* **2003**, *33*, 47–56.

65. Errico, D.A.; Medeiros, A.; Míguez, M.; Casaravilla, C.; Malgor, R.; Carmona, C.; Nieto, A.; Osinaga, E. *O*-Glycosylation in *Echinococcus granulosus*: Identification and characterization of the carcinoma-associated Tn-antigen. *Exp. Parasitol.* **2001**, *98*, 100–109.

66. Casaravilla, C.; Freire, T.; Malgor, R.; Medeiros, A.; Osinaga, E.; Carmona, C. Mucin-type *O*-glycosylation in helminth parasites from major taxonomic groups: Evidence for widespread distribution of the Tn antigen (GalNAc-Ser/Thr) and identification of UDP-GalNAc:poloypeptide *N*-acetylgalactosaminyltransferase activity. *J. Parasitol.* **2003**, *89*, 709–715.

67. Freire, T.; Fernández, C.; Chalar, C.; Maizels, R.M.; Alzari, P.; Osinaga, E.; Robello, C. Characterization of a UDP-*N*-acetyl-D-galactosamine:polypeptide *N*-acetylgalactosaminyltransferase with an unusual lectin domain from the platyhelminth parasite *Echinococcus granulosus*. *Biochem. J.* **2004**, *382*, 501–510.

68. Medeiros, A.; Chiribao, M.L.; Ubillos, L.; Festari, M.F.; Saldaña, J.; Tobello, C.; Domínguez, L.; Calvete, J.J.; Osinaga, E. Mucin-type *O*-glycosylation in *Mesocestoides vogae* (syn. Corti). *Int. J. Parasitol.* **2008**, *38*, 265–276.

69. Ubillos, L.; Medeiros, A.; Cancela, M.; Casaravilla, C.; Saldaña, J.; Domínguez, L.; Carmona, C.; le Pendu, J.; Osinaga, E. Characterization of the carcinoma-associated Tk antigen in helminth parasites. *Exp. Parasitol.* **2007**, *116*, 129–136.

70. Khoo, K.H.; Sarda, S.; Xu, X.; Caufield, J.P.; Mcneil, M.R.; Homans, S.W.; Morris, H.R.; Dell, A. A unique multifucosylated -3GalNAcβ1-4GlcNAcβ1-3Galα1-motif constitutes the repeating unit of the complex *O*-glycans derived from the cercarial glycocalyx of *Schistosoma mansoni*. *J. Biol. Chem.* **1995**, *270*, 17114–17123.

71. Khoo, K.H.; Chatterjee, D.; Caulfield, J.P.; Morris, H.R.; Dell, A. Structural mapping of the glycans from the egg glycoproteins of *Schistosoma mansoni* and *Schistosoma japonicum*: identification of novel core structures and terminal sequences. *Glycobiology* **1997**, *7*, 663–677.

72. Robijn, M.L.M.; Koeleman, C.A.M.; Wuhrer, M.; Royle, L.; Geyer, R.; Dwek, R.A.; Rudd, P.M.; Deelder, A.M.; Hokke, C.H. Targeted identification of a unique glycan epitope of *Schistosoma mansoni* egg antigens using a diagnostic antibody. *Mol. Biochem. Parasitol.* **2007**, *151*, 148–161.

73. De Walick, S.; Bexkens, M.L.; van Balkom, B.W.M.; Wu, Y.P.; Smit, C.H.; Hokke, C.H.; de Groot, P.G.; Heck, A.J.R.; Tielens, A.G.M.; van Hellemond, J.J. The proteome of the insoluble *Schistosoma mansoni* eggshell skeleton. *J. Parasitol.* **2013**, *41*, 523–532.

74. Huang, H.H.; Tsai, P.L.; Khoo, K.H. Selective expression of different fucosylated epitopes on two distinct sets of *Schistosoma mansoni* cercarial *O*-glycans: Identification of a novel core type and Lewis X structure. *Glycobiology* **2001**, *11*, 395–406.

75. Peterson, N.A.; Hokke, C.H.; Deelder, A.M.; Yoshino, T.P. Glycotope analysis in miracidia and primary sporocysts of *Schistosoma mansoni*: Differential expression during the miracidium-to-sporocyst transformation. *Int. J. Parasitol.* **2009**, *39*, 1331–1344.

76. Van Roon, A.-M.M.; Aguilera, B.; Cuenca, F.; van Remoortere, A.; van der Marel G.A.; Deelder, A.M.; Overkleeft, H.S.; Hokke, C.H. Synthesis and antibody-binding studies of a series of parasite fuco-oligosaccharides. *Bioorg. Med. Chem.* **2005**, *13*, 3553–3564.

77. Marques, E.T.A. Jr.; Ichikawa, Y.; Strand, M.; August, J.T.; Hart, G.W.; Schnaar, R.L. Fucosyltranferases in *Schistosoma mansoni* development. *Glycobiology* **2001**, *11*, 249–259.

78. Hokke, C.H.; Neeleman, A.P.; Koelman, C.A.M.; van den Eijneden, D.H. Identification of an α3-fucosyltransferase and a novel α2-fucosyltransferase in cercariae of the schistosome *Trichobilharzia ocellata*: Biosynthesis of the Fucα1-2Fucα1-3[Gal(NAc)β1-4]GlcNAc sequence. *Glycobiology* **1998**, *8*, 393–406.

79. Hokke, C.H.; Yazdanbakhsh, M. Schistosome glycans and innate immunity. *Parasite Immunol.* **2005**, *27*, 257–264.

80. Hokke, C.H.; Fitzpatrick, J.M.; Hoffmann, K.F. Integrating transcriptome, proteome and glycome analyses of *Schistosoma* biology. *Trends Parasitol.* **2007**, *23*, 165–174.

81. Thomas, P.G.; Harn, D.A., Jr. Immune biasing by helminth glycans. *Cell. Microbiol.* **2004**, *6*, 13–22.

82. Osinaga, E. Expression of cancer-associated simple mucin-type *O*-glycosylated antigens in parasites. *IUBMB Life* **2007**, *59*, 269–273.

83. Bui, C.T.; Shollenberger, L.M.; Paterson, Y.; Harn, D.A. *Schistosoma mansoni* soluble egg antigens enhance T cell responses to a newly identified HIV-1 Gag H-2[b] epitope. *Clin. Vaccine Immunol.* **2015**, *22*, 193–199.

84. Bui, C.T.; Shollenberger, L.M.; Paterson, Y.; Harn, D.A. *Schistosoma mansoni* soluble egg antigens enhance *Listeria* vector HIV-1 vaccine induction of cytotoxic T cells. *Clin. Vaccine Immunol.* **2014**, *21*, 1232–1239.

85. Van Remoortere, A.; Hokke, C.H.; van Dam, G.J.; van Die, I.; Deelder, A.M.; van den Eijnden, D.H. Various stages of Schistosoma express Lewisx, LacdiNAc, GalNAcβ1-4(Fucα1-3)GlcNAc and GalNAcβ1-4(Fucα1-2Fucα1-3)GlcNAc carbohydrate epitopes: detection with monoclonal antibodies that are charcterized by enzymatically synthesized neoglycoproteins. *Glycobiology* **2000**, *10*, 601–609.

86. Wammes, L.J.; Mpairwe, H.; Elliott, A.M.; Yazdanbakhsh, M. Helminth therapy or elimination: epidemiological, immunological, and clinical considerations. *Lancet Infect. Dis.* **2014**, *14*, 1150–1162.

87. Nakagawa, M.; Miyamoto, T.; Kusakabe, R.; Takasaki, S.; Takao, T.; Shichida, Y.; Tsuda, M. *O*-Glycosylation of G-protein-coupled receptor, octopus rhodopsin. Direct analysis by FAB mass spectrometry. *FEBS Lett.* **2001**, *496*, 19–24.

88. Kurz, S.; Jin, C.; Hykollari, A.; Gregorich, D.; Giomarelli, B.; Vasta, G.R.; Wilson I.B.H.; Paschinger, K. Hemocytes and plasma of the Eastern oyster (*Crassostrea virginica*) display a diverse repertoire of sulfated and blood group A-modified *N*-glycans. *J. Biol. Chem.* **2013**, *288*, 24410–24428.

89. Zhang, G.; Fang, X.; Guo, X.; Li, L.; Luo, R.; Xu, F.; Yang, P.; Zhang, L.; Wang, X.; Qi, H.; *et al.* The oyster genome reveals stress adaptation and complexity of shell formation. *Nature* **2012**, *490*, 49–54.

90. Gerwig, G.J.; Hocking, H.G.; Stöcklin, R.; Kamerling, J.P.; Boelens, R. Glycosylation of conotoxins. *Mar. Drugs* **2013**, *11*, 623–642.

91. Hocking, H.G.; Gerwig, G.J.; Dutertre, S.; Violette, A.; Favreau, P.; Stöcklin, R.; Kamerling, J.P.; Boelens, R. Structure of the *O*-glycosylated conopeptide CcTx from *Conus consors* venom. *Chem. Eur. J.* **2013**, *19*, 870–879.

92. Geyer, H.; Wuhrer, M.; Kurokawa, T.; Geyer, R. Charcterization of keyhole limpet hemocyanin (KLH) glycans sharing a carbohydrate epitope with *Schistosoma mansoni* glycoconjugates. *Micron* **2004**, *35*, 105–106.

93. Lehr, T.; Frank, S.; Natsuka, S.; Geyer, H.; Beuerlein, K.; Doenhoff, M.J.; Hase, S.; Geyer, R. *N*-Glycosylation patterns of hemolymph glycoproteins from *Biomphalaria glabrata* strains expressing different susceptibility to *Schistosma mansoni* infection. *Exp. Parasitol.* **2010**, *126*, 592–602.

94. Yoshino, T.P.; Wu, X.J.; Gonzalez, L.A.; Hokke, C.H. Circulating *Biomphalaria glabrata* hemocyte subpopulations possess shared schistosome glycans and receptors capable of binding larval glycoconjugates. *Exp. Parasitol.* **2013**, *133*, 28–36.

95. Stoeva, S.; Schütz, J.; Gebauer, W.; Hundsdörfer, T.; Manz, C.; Markl, J.; Voelter, W. Primary structure and unusual arbohydrate moiety of functional unit 2-c of keyhole limpet hemocyanin (KLH). *Biochim. Biophys. Acta* **1999**, *1435*, 94–109.

96. Petraccioli, A.; Maio, N.; Guarino, F.M.; Scillitani, G. Seasonal variation in glycoconjugates of the pedal glandular system of the rayed Mediterranean limpet, *Patella caerulea* (Gastropoda: Patellidae). *Zoology* **2013**, *116*, 186–196.

97. Bürgmayr, S.; Grabher-Meier, H.; Staudacher, E. Sialic acids in gastropods. *FEBS Lett.* **2001**, *508*, 95–98.

98. Stepan, H.; Bleckmann, C.; Geyer, H.; Geyer, R.; Staudacher, E. Determination of 3-*O*- and 4-*O*-methylated monosaccharide constituents in snail glycans. *Carbohydr. Res.* **2010**, *345*, 1504–1507.

99. Stepan, H.; Staudacher, E. Optimization of monosaccharide determination using anthranilic acid and 1-phenyl-3-methyl-5-pyrazolone for gastropod analysis. *Anal. Biochem.* **2011**, *418*, 24–29.

100. Stepan, H.; Pabst, M.; Altmann, F.; Geyer, H.; Geyer, R.; Staudacher, E. *O*-Glycosylation of snails. *Glycoconj. J.* **2012**, *29*, 189–198.

101. Taus, C.; Lucini, C.; Sato, T.; Furukawa, K.; Grabherr, R.; Staudacher, E. Expression and characterization of the first snail-derived UDP-*N*-acetyl-α-D-galactosamine:polypeptide *N*-acetylgalactosaminyltransferase. *Glycoconj. J.* **2013**, *30*, 825–833.

102. Taus, C.; Windwarder, M.; Altmann, F.; Grabherr, R.; Staudacher, E. UDP-*N*-acetyl-α-D-galactosamine:polypeptide *N*-acetylgalactosaminyl-transferase from the snail *Biomphalaria glabrata*—Substrate specificity and preference of glycosylation sites. *Glycoconj. J.* **2014**, *31*, 661–670.

103. Van Die, I.; Cummings, R.D.; van Tetering, A.; Hokke, C.H.; Koeleman, C.A.M.; Van den Eijnden, D.H. Identification of a novel UDP-Glc:GlcNAc β1-4-glucosyltransferase in *Lymnaea stagnalis* that may be involved in the synthesis of complex-type oligosaccharide chains. *Glycobiology* **2000**, *10*, 263–271.

104. Staudacher, E. Methylation—An uncommon modification of glycans. *Biol. Chem.* **2012**, *393*, 675–685.

105. Wohlschlager, T.; Butschi, A.; Grassi, P.; Sutov, G.; Gauss, R.; Hauck, D.; Schmieder, S.S.; Knobel, M.; Titz, A.; Dell, A.; *et al.* Methylated glycans as conserved targets of animal and fungal innate defense. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E2787–E2796.