Carbohydrates and glycosylation*

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Abstract

The *C. elegans* genome contains sequences similar to a large number of mammalian genes implicated in the assembly, processing, and modification of glycans. In recent years, spectacular progress has been made in developing and refining tools to obtain structural information with small amounts of material, increasing our understanding of glycan structural complexity in this organism. These approaches have revealed novel N- and O-glycan structures in *C. elegans*, as well as a high degree of conservation in glycosaminoglycan structure. In parallel, studies in which glycan structure is perturbed by genetic manipulation have begun to reveal the roles of specific carbohydrate moieties in developmental and physiological processes. This review summarizes recent work elucidating the fine structure of complex carbohydrates in *C. elegans* as well as genetic studies that have uncovered novel roles for complex carbohydrates in developmental processes.

*Edited by Patricia Kuwabara. Last revised October 17, 2006. Published December 18, 2006. This chapter should be cited as: Berninsone, P.M. Carbohydrates and glycosylation (December 18, 2006), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.125.1, http://www.wormbook.org.

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1. Introduction†

Complex carbohydrates are involved in multiple biological processes, from protein folding, oligomerization and stability, to the immune response and host-pathogen interactions (Varki, 1993). Glycoconjugates also play important roles in developmental processes, as revealed by the pathology of human diseases caused by abnormal glycosylation (Freeze and Aebi, 2005) and genetic studies in model organisms (Haltiwanger and Lowe, 2004).

The structural diversity of naturally occurring glycans is determined by complex biosynthetic pathways, which typically vary among species and appear to be modulated by factors that can differ greatly among cell types. Unlike nucleic acids and proteins, oligosaccharide chains are often assembled in non-linear, branched structures that are synthesized without the use of a template. In principle, each monosaccharide can generate an α or a β linkage to one of several positions on another monosaccharide or to another type of molecule, having the potential to produce an astounding molecular complexity. Moreover, individual sugar moieties within a chain can be modified by phosphorylation, sulfation, methylation, O-acetylation, or fatty acylation, further increasing the combinatorial possibilities for oligosaccharide diversity (Varki et al., 1999). In addition to the complexity of glycan moieties *per se*, it is not possible to predict with certainty whether a protein will be modified by oligosaccharides, even when glycosylation motifs have been observed for a number of glycopeptide bonds (Spiro, 2002). Another source of variation is microheterogeneity, in which the structure of the oligosaccharide attached to each glycosylation site may vary (Jones et al., 2005).The focus of this review will be on the contributions that genetic and biochemical analysis in *C. elegans* have made to our understanding of the role of glycans in development.

Abbreviations:

Cer:	ceramide
Fuc:	fucose
FucT:	fucosyltransferase
GAG:	glycosaminoglycan
Gal:	galactose
GalNAc:	N-acetylgalactosamine
GalNAcT:	N-acetylgalactosiminyltransferase
GalT:	galactosyltransferase
Glc:	glucose
GlcA:	glucuronic acid
GlcAT:	glucuronyltransferase
GlcNAc:	N-acetylglucosamine
GlcT:	glucosyltransferase
GnT:	N-acetylglucosamilyltransferase
IdoA:	iduronic acid
HS:	heparan sulfate
HSPG:	heparan sulfate proteoglycan
Man:	mannose
ManT:	mannosyltransferase
PCho:	phosphorylcholine
P-glu:	phosphoglucose
P-man:	phosphomannose
Xyl:	xylose
XylT:	xylosyltransferase

2. Glycosylation pathways in *C. elegans*

The *C. elegans* genome contains sequences similar to a large number of mammalian genes implicated in the assembly, processing and modification of a variety of glycans (Schachter, 2004; see Appendix 1). Although only a limited number of these genes have been functionally characterized, the degree of homology suggests that *C. elegans* possesses a repertoire of enzymes for the synthesis of a variety of complex carbohydrates. The occurrence of N-glycans [reviewed in Schachter (2004)], GalNAc-O-Ser/Thr glycans (Guerardel et al., 2001), glycosaminoglycans (Toyoda et al., 2000; Yamada et al., 1999), glycolipids (Gerdt et al., 1999; Gerdt et al., 1997) and chitin (Veronico et al., 2001) has been verified in *C. elegans* in structural or biochemical studies. Although *C. elegans* N- and O-glycans have common features with vertebrate glycans in terms of their core glycan biosynthesis, their terminal structures show significant differences. Among them, *C. elegans* glycans lack sialic acid (Bacic et al., 1990) and contain unusual fucose additions, O-methylated fucose and mannose and phosphorylcholine substitutions (Cipollo et al., 2002; Haslam and Dell, 2003). In recent years, spectacular progress has been made in developing and refining tools to define the structures of glycans. Our understanding of the role of glycoconjugates in the development of *C. elegans* to date is largely due to the identification of mutations in genes involved in their biosynthesis.

3. Glycosaminoglycans

Proteoglycans consist of a core protein and one or more covalently attached glycosaminoglycan (GAG) chains. GAG chains are attached to serine residues in core proteins by a common “tetrasaccharide linkage” region. Structural studies have confirmed that *C. elegans* synthesizes this ubiquitous linker region (Guerardel et al., 2001). GAGs are linear polysaccharides, composed of repeating disaccharide units consisting of an amino sugar (GlcNAc or GalNAc) and an uronic acid (GlcA and IdoA; see Figure 1). Different classes of GAGs are defined by the composition of the disaccharides: heparan sulfate (HS) has a repeating disaccharide of GlcNAc-α1,4-GlcA-β1,4, whereas chondroitin and chondroitin sulfate (CS) have GalNAc-α1,4-GlcAβ1,3. Monosaccharides within the GAG polymers can be modified in several ways: GlcNAc N-deacetylation and N-sulfation, uronic acid epimerization and O-sulfation at different positions. These modifications result in an enormous molecular complexity that increases the capacity of GAGs to interact with proteins through varied arrangements of sulfated sugar residues (Turnbull et al., 2001). *C. elegans* produces heparan sulfate containing all modifications previously described in other organisms (Toyoda et al., 2000; Yamada et al., 1999). In contrast, chondroitin appears not to be secondarily modified in *C. elegans* (Toyoda et al., 2000).

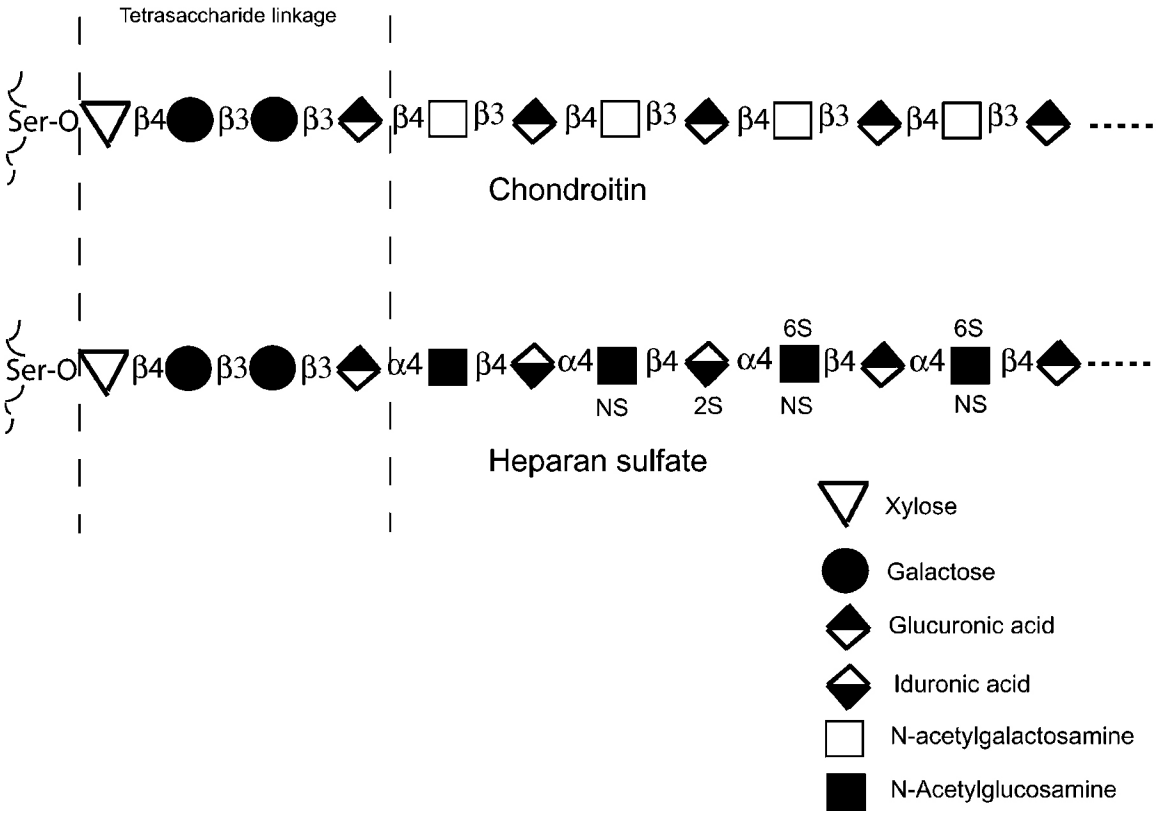

Figure 1. Structure of *C. elegans* chondroitin and heparan sulfate and their linkage tetrasaccharide to proteins. Considerable variations occur in the positions of heparan sulfate sulfations and epimerization (IdoA). [Based on the compositions described in Guerardel et al. (2001); Toyoda et al. (2000); Yamada et al. (1999).]

3.1. Chondroitin

A screen for *C. elegans* mutants defective in vulva morphogenesis identified 8 *sqv* (*sq*uashed *v*ulva) genes (Herman et al., 1999) required for various steps of chondroitin biosynthesis (see Figure 2). With the exception of *sqv-5*, the *sqv* genes control the biosynthesis of both heparan sulfate and chondroitin (Berninsone et al., 2001; Bulik et al., 2000; Hwang and Horvitz, 2002; Hwang and Horvitz, 2002; Hwang et al., 2003). SQV-5 is a bifunctional glycosyltransferase that functions in the initiation and elongation of chondroitin, but not heparan sulfate chains (Hwang et al., 2003; Mizuguchi et al., 2003), suggesting that defects in the biosynthesis of chondroitin account for the defects caused by mutations in all *sqv* genes.

In all the *sqv* mutants, the vulval extracellular space fails to expand during vulval morphogenesis (Herman et al., 1999). Strong *sqv* mutations also cause maternal-effect lethality: most progeny of homozygous mutants fail to initiate cytokinesis. These mutant eggs fail to form the fluid-filled extracellular space between the egg and the eggshell (Herman and Horvitz, 1999; Hwang and Horvitz, 2002). The ability of extracellular chondroitin to interact with water and generate osmotic pressure might be implicated in the expansion of the extracellular spaces in the *C. elegans* embryo and vulva; among other possibilities (Hwang et al., 2003).

3.2. Heparan sulfate

The EXT gene family members are glycosyltransferases required for the biosynthesis of heparan sulfate chains. Mutations in EXT1 and EXT2 lead to hereditary multiple exostoses. Only two homologs of the EXT family exist in *C. elegans*: *rib-1* and *rib-2*. *rib-2* is essential for embryonic development (Morio et al., 2003) and encodes a unique α1,4-N-acetylglucosaminyltransferase involved in both the biosynthetic initiation and elongation of heparan sulfate (Kitagawa et al., 2001). The biochemical activity of RIB-1 remains elusive.

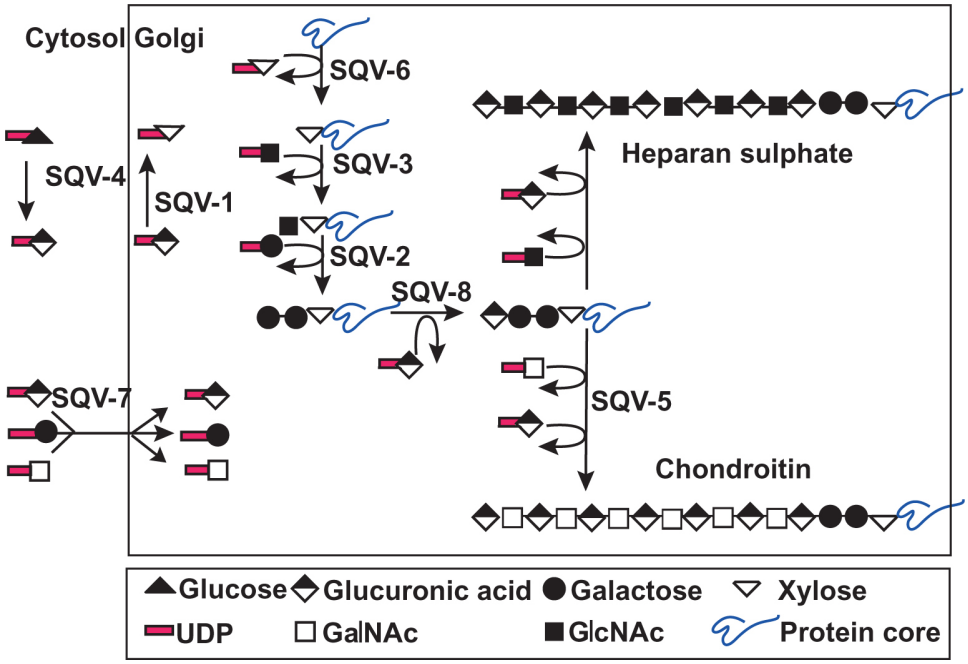

Figure 2. Model of the function of the SQV proteins in the biosynthesis of heparan and chondroitin chains. SQV-4 (UDP-glucose dehydrogenase) synthesizes UDP-glucuronic acid (Hwang and Horvitz, 2002) and SQV-1 catalyzes the synthesis of UDP-xylose by decarboxylation of UDP-glucuronic acid (Hwang and Horvitz, 2002). SQV-7 transports UDP-glucuronic acid, UDP-galactose and UDP-N-acetylgalactosamine into the Golgi lumen (Berninsone et al., 2001). *sqv-6*, *sqv-3*, *sqv-2* and *sqv-8* encode the xylosyltransferase, galactosyltransferase I, galactosyltransferase II and glucuronyltransferase I, respectively, involved in the synthesis of the common "core tetrasaccharide" linkage region (Bulik et al., 2000; Herman and Horvitz, 1999; Hwang et al., 2003). SQV-5 is required for the polymerization of chondroitin chains (Hwang et al., 2003; Mizuguchi et al., 2003). Reprinted by permission from Macmillan Publishers Ltd: Nature 423, 439–443, copyright (2003).

Contrasting with the essential nature of heparan sulfate chain synthesis (Morio et al., 2003), *C. elegans* mutants lacking three heparan sulfate modifying enzymes are viable and fertile. *hse-5*, *hst-6* and *hst-2* mutants (HSE-5: glucuronyl C5-epimerase, HST-6: heparan sulfate 6O-sulfotransferase and HST-2: 2O-sulfotransferase) exhibit distinct as well as overlapping axonal and cellular guidance defects in specific neurons (Bulow and Hobert, 2004). *hst-2* mutants also display cell migration defects (Kinnunen et al., 2005). These HS modifications are required for the function of the *slt-1/sax-3* signaling system in some, but not all cellular contexts (Bulow and Hobert, 2004).

Defects in heparan sulfate synthesis or specific modifications disrupt neuroblast migrations. C-5 epimerized and 6-O-sulfated heparan sulfate chains attached to the cell surface proteoglycans syndecan (SDN-1) and glypican (GPN-1) are required for the activity of KAL-1, the *C. elegans* anosmin ortholog, in embryonic neuroblast migration (Hudson et al., 2006). These *in vivo* analyses underscore the importance of molecular diversity encoded by HS chains and argue that each individual modification provides information required for axon patterning and cell migration.

3.3. Heparan sulfate and chondroitin modified proteins

The *C. elegans* genome encodes several putative heparan sulfate proteoglycan (HSPG) core proteins: the cell surface HSPGs SDN-1/syndecan (Minniti et al., 2004; Rhiner et al., 2005) and GPN-1/glypican (Hudson et al., 2006) and the basement membrane HSPGs UNC-52/perlecan (Rogalski et al., 1993) and CLE-1/collagen XVIII (Ackley et al., 2001). SDN-1 (Minniti et al., 2004) and GPN-1 (Hudson et al., 2006) have been shown to be modified by heparan sulfate chains.

Nine chondroitin proteoglycan core proteins (CPG-1 to CPG-9) were identified in *C. elegans* using a combination of biochemical purification and mass spectrometry (Olson et al., 2006). None of these proteins have homologs in vertebrates or other invertebrates, but all of them have sequence motifs required for initiation of glycosaminoglycan biosynthesis similar to those found in vertebrates. Simultaneous RNAi depletion of two of these core proteins (CPG-1/C07G2.1a and CPG-2/B0280.5) resulted in multinucleate single cell embryos (Olson et al., 2006), resembling the embryonic phenotype resulting from inhibiting the SQV-5 chondroitin synthase (Hwang et

al., 2003; Mizuguchi et al., 2003). This study shows that the chondroitin proteoglycans encoded by *cpg-1* and *cpg-2* play a crucial role in embryonic cytokinesis.

4. N-linked glycans

N-linked glycans are complex and diverse sugar chains attached to an asparagine residue of a polypeptide chain within the consensus peptide sequence Asn-X-Ser/Thr. The yeast and mammalian biosynthetic pathways have been elucidated in great detail (Herscovics, 1999; Kornfeld and Kornfeld, 1985). The initial steps of this pathway are conserved in most eukaryotes and involve the synthesis of a lipid-linked oligosaccharide precursor structure that is transferred *en bloc* to nascent proteins in the ER. Transfer of $Glc_3Man_9GlcNAc_2$ to Asn is followed by glucose trimming in the ER. Subsequent cycles of glucose re-addition and removal participate in quality control of protein-folding. The processed high-mannose $Man_5GlcNAc_2$-Asn N-glycan serves as a substrate for the diversification of N-glycans in the Golgi. In vertebrates, these reactions generate a large repertoire of hybrid and complex N-glycan subtypes (see Figure 3, top panel).

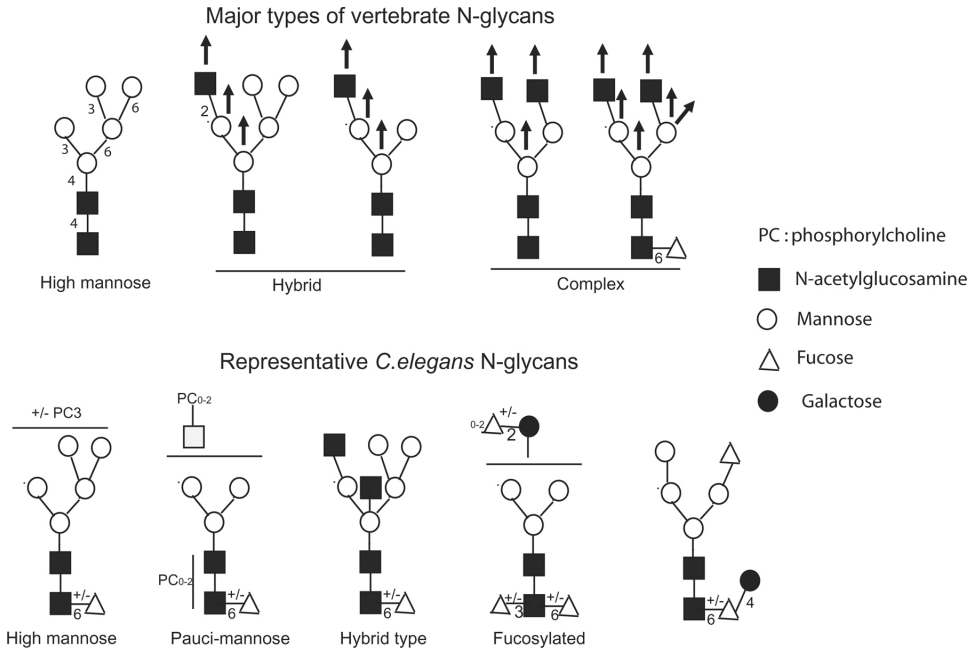

Figure 3. Comparative overview of the major types of vertebrate N-glycan subtypes and some representative *C. elegans* N-glycans. Top panel: Vertebrate diversification in the Golgi apparatus generates high-mannose, hybrid and complex N-glycan subtypes. Most cell surface and secreted N-glycans are of the complex subtype. Vertical arrows indicate locations of branch formation in diversification, not all of which occur on a single N-glycan. (Adapted from (Varki et al., 1999) Bottom panel: The main classes of *C. elegans* N-glycans include high-mannose (up to Man9GlcNAc2), pauci-mannosidic and hybrid type. Note that this scheme only includes some of the documented structures.

4.1. Diversity of N-glycan structures in *C. elegans*

Whereas the N-glycan biosynthetic pathway appears to be fairly well conserved in *C. elegans* (see Appendix 1), the resulting N-glycan structures are qualitatively and quantitatively different than those found in mammals, as shown by structural analysis of N-glycans enzymatically released from *C. elegans* glycoproteins (see Figure 3, reviewed in Haslam and Dell, 2003; Schachter, 2004). *C. elegans* predominantly contains oligomannose $Man_{5-9}GlcNAc_2$-Asn glycans (which are present in vertebrates) and large amounts of paucimannose $Man_{3-4}GlcNAc_2$-Asn glycans (which are not usually present in vertebrates; Altmann et al., 2001; Cipollo et al., 2002; Haslam and Dell, 2003; Haslam et al., 2002; Natsuka et al., 2002; Schachter et al., 2002; Zhang et al., 2003; see Figure 3, bottom panel). Complex and hybrid N-glycans, which are very abundant in vertebrates, are either absent or present in low levels in *C. elegans* (Cipollo et al., 2002; Haslam et al., 2002; Natsuka et al., 2002), although this organism has at least some of the biosynthetic components required for the synthesis of high mannose and abbreviated mammalian-type complex glycans. GnTI (N-acetylglucosaminyltransferase I) initiates and is essential for the conversion of $Man_5GlcNAc_2$-Asn to hybrid and complex N-glycans. Three genes encoding enzymatically active GnTI (*gly-12*, *gly-13* and *gly-14*) exist in *C. elegans* (Chen et al., 1999). GnTII (*gly-20*; Chen et al., 2002) and

GnTV (*gly-2*; Warren et al., 2002; Warren et al., 2002), which initiate the formation of the N-glycan "antennae", are also expressed in *C. elegans*. A model to explain this apparent discrepancy has been proposed, based on the expression in *C. elegans* of a N-acetylglucosaminidase, which removes GnTI-added GlcNAc, thus preventing the formation of complex glycan and resulting in the formation of large amounts of "paucimannose" N-glycans (Zhang et al., 2003).

Unusually modified N-glycans have been detected in *C. elegans*. Fuc-rich glycans, which carry terminal Fuc on the antennae as well as the core, sometimes contain O-methylated Fuc and O-methylated Man. Phosphorylcholine (PCho)-substituted N-glycans have been identified in a high mannose structure carrying three PCho groups (Cipollo et al., 2002) and on complex type glycans where PCho is attached at GlcNAc (Haslam and Dell, 2003). This last structure has been previously found in several parasitic nematodes (Haslam et al., 1999; Haslam et al., 1997; Morelle et al., 2000).

The structural studies summarized above identified N-glycans extracted from mixed stage *C. elegans* cultures. These preparations are expected to be enriched in glycans from the largest and most represented developmental stages. A recent study (Cipollo et al., 2005) has shown that the N-glycan profile of each developmental stage appears to be unique. The general trend of the number and abundance of N-glycans was Dauer~L1>Adult~L4>L3~L2. Dauer larvae contain complex glycans with higher molecular masses than those of other stages, and a higher abundance of high molecular weight PCho-containing glycans was observed in L1 and Dauer larvae. This study also documents the structures of novel N-glycans, e.g., difucosyl PCho glycans in which PCho substitutes both core and terminally linked GlcNAc.

4.2. N-glycosylated proteins in *C. elegans*

The rather strict consensus peptide sequence Asn-X-Ser/Thr for N-glycosylation has been supported by numerous structural, mutagenic and *in vitro* approaches (Spiro, 2002). Although this sequence occurs frequently in proteins, N-linked glycosylation does not occur at every potential glycosylation site, most probably due to conformational factors (Apweiler et al., 1999). Thus, it is not possible to predict with certainty if a protein is in fact N-glycosylated. Microheterogeneity in N-glycan chains can also occur among different molecules of the same protein (Jones et al., 2005).

Recently developed methods have allowed the identification of 304 *C. elegans* proteins containing N-glycans and the simultaneous determination of the glycosylation sites (Fan et al., 2004; Fan et al., 2005; Kaji et al., 2003). These studies employed lectin affinity chromatography to isolate glycopeptides generated by tryptic digestion of *C. elegans* protein fractions, followed by a variety of mass spectrometry approaches. All but four of the N-glycosylation sites identified correspond to the conventional Asn-X-Ser/Thr. Three peptides contain Asn-X-Cys (Fan et al., 2005; Kaji et al., 2003). The identified N-glycoproteins comprise soluble and hydrophobic proteins, and many of them are extracellular matrix components which have been implicated in cell adhesion or are components of basement membranes. Among them, PAT-2, INA-1 (the two *C. elegans* integrin α subunits), PAT-3 (the integrin β subunit), T22A3.8, EPI-1, LAM-1, C54D1.5 (the four laminin subunit gene products), NID-1 (nidogen) and DGN-1 (dystroglycan) contain at least one N-linked glycan [see Fan et al. (2005); Kaji et al., (2003) for complete lists].

In individual studies, some *C. elegans* proteins have been demonstrated to contain N-linked oligosaccharides. GLP-1, a member of the LIN-12/Notch family of receptor proteins essential for distal tip cell (DTC) control of germline proliferation (Austin and Kimble, 1987) is a glycoprotein (Crittenden et al., 1994).

MIG-17, a member of the ADAM (a disintegrin and metalloprotease) family, regulates directional migration of the distal tip cell (DTC) (Nishiwaki et al., 2000). Sensitivity to N-glycanase indicated that MIG-17 is N-glycosylated (Nishiwaki et al., 2004). A mutation in *mig-23* causes defective DTC migration and genetically interacts with *mig-17*. MIG-23 is a membrane-bound nucleoside diphosphatase (NDPase) required for glycosylation and proper localization of MIG-17 (Nishiwaki et al., 2004). NDPase activities in the lumen of the Golgi apparatus generate nucleoside monophosphates required for translocation of nucleotide sugars from the cytosol into the Golgi lumen (Berninsone et al., 1994). Nucleotide sugars are the activated donors for glycosylation reactions in the lumen of the endoplasmic reticulum and Golgi apparatus (Hirschberg et al., 1998). Genetic studies in *S.cerevisiae* showed that guanosine diphosphatase, an NDPase, is required for protein and sphingolipid glycosylation in the Golgi lumen (Abeijon et al., 1993). Hence, MIG-23 likely affects DTC migration through its role in the transport of nucleotide sugars into the lumen of the Golgi apparatus. Although the *mig-23* mutation may affect the glycosylation of other proteins, MIG-23 appears to regulate DTC migration through glycosylation and correct localization of MIG-17.

5. O-linked glycans

5.1. O-GalNAc linked (mucin-type) glycans

The GalNAc α-Ser/Thr linkage has been considered a hallmark of mucins where it occurs in clusters. However, this linkage has also been found in a wide variety of other proteins (Hang and Bertozzi, 2005). No primary amino acid consensus sequence has emerged for mucin-type O-linked glycosylation. In general, glycosylation of Thr is preferred over Ser (Elhammer et al., 1993) and the linkage is found in clusters of Ser/Thr residues with a βturn near Pro and at a distance from charged amino acids (Hang and Bertozzi, 2005).

C. elegans expresses a family of functional polypeptide N-acetylgalactosaminyltransferases (ppGalNacT) involved in the initiation of mucin-type O-glycosylation (Hagen and Nehrke, 1998; see Appendix 1). A *C. elegans* core 1 β3-GalT or T-synthase has been recently characterized (Ju et al., 2006). This enzyme adds Gal to GalNAcα1-Ser/Thr to generate the core 1 structure Galβ1-3GalNAcα1-Ser/Thr. This is the most common of O-glycan structures in vertebrates and is a precursor to more complex O-glycans. Two families of unusual mucin-type O-glycans were identified in *C. elegans* (Guerardel et al., 2001). Both families were characterized by the presence of multiple β-Glc residues in non-reducing terminal positions, a type of substitution without precedent in O-glycans. One of these families presented either type-1 core Galβ1,3GalNAc or one of three novel core types: Galβ1,6[Galβ1,3]GalNAc, Glcβ1-6[Galβ1,3]GalNAc and [Glcβ1-6(Glcβ1,4)Galβ1-3)]GalNAc. Some of the O-glycans contain the GlcAβ1,3 Galβ1,3GalNAc trisaccharide sequence. The second mucin type O-glycan characterized is unusual in that it presented a GlcNAc-ol in the terminal position. This suggests that it would be linked to the protein through a GlcNAc and not a GalNAc. The unusual features of these structures suggest the expression in *C. elegans* of novel glycosyltransferase activities. For example, a novel β1,6GlcT acting on the GalNAc residue of the core has been cloned and expressed (Warren et al., 2002). The enzyme is encoded by the *gly-1* gene and is homologous to the vertebrate β1,6GlNAcT that acts on the GalNAc residue of O-glycan core 1 (Galβ1,3GlcNAc) to make O-glycan core 2.

The large amounts of O-glycans purified in this study suggest that *C. elegans* expresses an abundance of highly O-glycosylated proteins. Studies on the parasitic nematode *Toxocara canis* have shown that it secretes a family of highly O-glycosylated mucin type glycoproteins (Loukas et al., 2000; Dell et al., 1999; Khoo et al., 1991). In *C. elegans*, *let-653* encodes a mucin-type glycoprotein (Jones and Baillie, 1995). Mutations in *let-65*3 result in larval death and vacuole formation anterior to the pharyngeal bulb, suggesting malfunction or deformation of the excretory/secretory apparatus. Biochemical characterization of a *C. elegans* L1-specific surface antigen suggested that it contains O-linked carbohydrates (Hemmer et al., 1991). This antigen is not detected on the surface of L1 animals carrying mutations in the *srf-3* gene, which encodes a Golgi UDP-Gal/UDP-GlcNAc transporter implicated in glycosylation of surface components (Hoflich et al., 2004). Loss of SRF-3 activity affects both N-linked and O-linked glycosylation (Cipollo et al., 2004).

5.2. O-GlcNAc linked

O-linked GlcNAc at Ser and Thr residues is an evolutionary conserved modification emerging as a key regulator of nuclear and cytoplasmic protein activity (Love and Hanover, 2005). The GlcNAc transferase (OGT) responsible for this modification was the first glycopeptide-forming enzyme to be localized outside the secretory apparatus (Lubas et al., 1997). O-GlcNAc addition may compete with O-phosphorylation for certain Ser/Thr target sites, suggesting a potential regulatory cycle in which cytosolic β-N-acetylglucosaminidase plays a key role (Comer and Hart, 2001). O-GlcNAc addition is driven in part by the levels of UDP-GlcNAc derived from the hexosamine biosynthetic pathway, a nutrient-sensing pathway implicated in cellular signaling (Hanover, 2001). In mammals, the OGT gene is essential for embryonic and stem cell development and produces multiple transcripts (Hanover et al., 2003; O'Donnell et al., 2004; Shafi et al., 2000). O-GlcNAc addition has been implicated in mammalian insulin resistance (Hanover, 2001; McClain et al., 2002; Vosseller et al., 2002; Wells et al., 2001) histone remodeling, transcription, proliferation, apoptosis and proteosomal degradation.

In *C. elegans*, *ogt-1* and *oga-1* encode the single orthologs of OGT (Lubas et al., 1997) and O-GlcNAcase (Forsythe et al., 2006), respectively. Putative null alleles of *ogt-1* and *oga-1* are viable and fertile, allowing the examination of the role of O-GlcNAc in this organism. Nuclear pore complexes in the *ogt-1* mutant strain lack O-GlcNAc but are capable of transporting transcription factors into the nucleus at normal rates. Conversely, *oga-1* null mutants accumulate O-GlcNAc on nuclear pores and other proteins. A dramatic elevation in the levels of trehalose and glycogen was detected in *ogt-1* and *oga-1* mutants with a concomitant decrease in triglyceride storage (Hanover et al., 2005). The *ogt-1* deletion allele suppresses dauer formation induced by the partial loss of *daf-2*,

encoding an insulin-like receptor (Riddle, 1977). In comparable conditions, the *oga-1* null mutant enhances dauer formation of the *daf-2* mutant. Thus, the nematode hexosamine-signaling pathway interacts with the insulin-like-signaling pathway, as suggested in mammals. The insulin-like-signaling pathway modulates lipid and carbohydrate storage in *C. elegans* (Kimura et al., 1997). These studies implicate O-GlcNAc cycling by OGT-1 and OGA-1 in modulating insulin-like signaling and/or parallel pathways regulating dauer formation in *C. elegans*.

6. Glycolipids

A glycosphingolipid consists of an oligosaccharide usually attached via glucose or galactose to the terminal primary hydroxyl group of the lipid moiety ceramide. Most mammalian glycosphingolipids contain a Gal-β1,4-Glc-β1-ceramide core, which is extended by the stepwise addition of further monosaccharides giving rise to several defined core structures (Varki et al., 1999).

C. elegans glycosphingolipids belong to the invertebrate-specific arthro-series, which contain the invertebrate-specific Man-β1,4-Glc-β1-ceramide core, extended by addition of neutral sugars. They share structural homology with the equivalent structures from the porcine parasitic nematode, *Ascaris suum* (Gerdt et al., 1997; Gerdt et al., 1999; Lochnit et al., 1997). Some of these glycosphingolipids are modified by phosphorylcholine (PCho), an antigenic determinant frequently detected in parasitic nematodes (Lochnit et al., 1998; Maizels et al., 1987).

A role for glycosphingolipids as receptors for the *Bacillus thuringensis* (Bt) crystal toxin was revealed by genetic studies in *C. elegans*. The crystal (Cry) proteins produced by Bt are pore forming toxins that are lethal to insects and nematodes but generally innocuous to vertebrates. The potential problem associated with long-term use of transgenic crops expressing Cry proteins to control insect pests is the development of resistance among target populations. Mutations in the *bre* (Bt resistant) genes cause Bt resistance in *C. elegans* (Griffitts et al., 2003; Griffitts et al., 2001; Marroquin et al., 2000). The *bre* genes encode four glycosyltransferases that act in a single pathway and are required for the uptake of toxin into intestinal cells. (Griffitts et al., 2003; Griffitts et al., 2001). BRE-2 and BRE-4 synthesize the Galβ1,3GlcNAc and GalNAcβ1,4GlcNAc moieties, respectively, which are commonly found on glycolipids and glycoproteins. *bre-3* and *bre-5* are the *C. elegans* homologs of the *Drosophila* genes *egghead* and *brainiac*, respectively, which encode consecutive glycosyltransferases involved in the synthesis of the carbohydrate chains on glycosphingolipids (Griffitts et al., 2003). Lipid and glycoprotein analysis of *bre* mutant animals demonstrated that the BRE enzymes are required to synthesize the carbohydrate chains of glycolipids and that Bt toxin specifically binds glycolipids (Griffitts et al., 2005).

Mutations in *bre-5* and other genes involved in glycosphingolipid biosynthesis also suppress the egg-laying defect associated with elevated *lin-12* activity (Katic et al., 2005). Brainiac modulates Notch signaling in *Drosophila* (Goode et al., 1996; Goode and Perrimon, 1997) suggesting that the importance of glycosphingolipids for proper LIN-12/Notch signaling might be functionally conserved. Genetic evidence suggests that *bre-5* functions prior to LIN-12 activation by ligand-induced ectodomain shedding (Katic et al., 2005).

7. Chitin

Chitin, a linear polymer of β1,4 linked GlcNAc, is one of the main components of the fungal cell wall and the exoskeletons of arthropods. The eggshells of many free-living and parasitic nematodes contain chitin (Brydon et al., 1987; Wharton, 1980). In addition, this polysaccharide has been detected in the feeding apparatus of the strongyloid nematode *Oesophagostomum dentatun* (Neuhaus et al., 1997). In *C. elegans*, chitin is detected in the eggshell and in elaborate patterns in the pharyngeal lumen walls of adult and larvae (Zhang et al., 2005). Two genes encoding chitin synthases, *chs-1* and *chs-2*, exhibit different temporal expression patterns: *chs-1* is expressed in later larval stages and adults, while *chs-2* expression is restricted to a short period before each molt, but not in adults (Veronico et al., 2001). Maternal *chs-1* gene activity is likely involved in the production of eggshell chitin, as shown by RNAi experiments and analysis of *chs-1* null mutants. *chs-2* is expressed in the pharynx and inactivation of *chs-2* by RNAi revealed visible defects in the grinder (Zhang et al., 2005). Hence, *chs-1* and *chs-2* play different and non-overlapping roles in development.

8. Conclusions

Assignment of biological functions to glycans and glycan modified acceptors in any multicellular organism is a difficult task. The extreme complexity of glycan structures is compounded by the fact that many yet unidentified

acceptors might be modified by the same type of glycan. In addition, very little is known about how the expression of these molecules is regulated during development and differentiation. *C. elegans* and small model organisms are likely to provide platforms where perturbations of glycan structure can be analyzed in the context of development and differentiation. Sensitive techniques for glycan analysis can be applied to mutants to obtain qualitative and quantitative information about their glycosylation phenotypes. Identification of the specific acceptor(s) carrying the abnormal glycan structures represents an additional challenge, yet these studies may help understand the molecular mechanisms linking glycosylation defects with specific phenotypes. Despite the enormous difference in complexity with mammals, the recent genetic and structural studies suggest that *C. elegans* is a promising model system for investigating basic roles of glycans in development.

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A. Appendix 1

This Appendix includes putative glycosylation genes based on homology to genes from other species. Activity refers to presumed biochemical function, except those entries indicated with *, for which the *C. elegans* gene has been expressed and its biochemical activity has been demonstrated. Adapted from Schachter (2004).

Activity	*C. elegans* gene	Mutant phenotype	RNAi phenotype	Reference(s)
Genes involved in synthesis and modification of GAG (Xyl-Ser O-glycans)				
UDP-GlcA decarboxylase *	D2096.4 (*sqv-1*)	Squashed vulva, hermaphrodite sterile	WT	Hwang and Horvitz, 2002
βgal β1,3-GalT II *	Y110A2AL.14 (*sqv-2*)	Squashed vulva, hermaphrodite sterile	Clr, Cyk, Egl, Sma	Hwang et al., 2003; Wang et al., 2005
Xyl-prot β1,4-GalT *	R10E11.4 (*sqv-3*)	Squashed vulva, hermaphrodite sterile		Bulik et al., 2000
UDP-Glc dehydrogenase *	F29F11.1 (*sqv-4*)	Squashed vulva, hermaphrodite sterile	Emb	Hwang and Horvitz, 2002
β1,4GalNAcT I/II (chondroitin synthase) *	T24D11.1a,b (*sqv-5*)	Squashed vulva, hermaphrodite sterile		Hwang et al., 2003; Mizuguchi et al., 2003
Polypeptide β-XylT *	Y50D4C.4 (*sqv-6*)	Squashed vulva, hermaphrodite sterile	Cyk	Hwang et al., 2003
Gal-Gal-Xyl-S β1,3 GlcAT *	ZK1307.5 (*sqv-8*)	Squashed vulva, hermaphrodite sterile		Bulik et al., 2000
Heparan sulfate synthesis	F12F6.3 (*rib-1*)	Abnormal	WT	Kitagawa et al., 2001
α1,4-GnT I/II (heparan sulfate synthesis) *	K01G5.6 (*rib-2*)	Abnormal	WT	Kitagawa et al., 2001
GlcAT/GlcT/GalT?	B0310.5		WT	
	F08G5.5		WT	
D-GlcA C5-epimerase	B0285.5 (*hse-5*)	Axon, cell guidance defects	WT	Bulow and Hobert, 2004
Heparan sulfate N-deacetylase/N-sulfotransferase	F08B4.6 (*hst-1*)	Abnormal	WT/Emb, Ste	
Heparan sulfate 2O-sulfotransferase	C34F6.4 (*hst-2*)	Axon, cell guidance defects	Mig, Egl	Bulow and Hobert, 2004; Kinnunen et al., 2005
Heparan sulfate 6O-sulfotransferase	Y34B4A.3 (*hst-6*)	Axon, cell guidance defects	WT	Bulow and Hobert, 2004
Genes involved in N-glycan synthesis				
Synthesis of the lipid linked precursor				
ALG7	T08D2.2		WT	

Activity	*C. elegans* gene	Mutant phenotype	RNAi phenotype	Reference(s)
	Y60A3A.13		WT	
ALG1	T26A5.4		WT/Emb,Ste	
ALG2	T23F2.1		WT/Abn	
ALG3	K09E4.2		WT	
ALG6	C08B11.8		WT	
ALG8	C08H9.3		WT/Emb, Lva	
ALG9	C14A4.3		WT	
ALG10	T24D1.4		WT	
Lec35/Monosaccharide-P-dolichol utilization	F38E1.9		Let, Gro, Bmd, Lva	
Oligosaccharyltransferase complex				
OST1/ribophorin	T22D1.4		WT/ Abn	Wang et al., 2005
Ribophorin II	M01A10.3		WT/Abn	
OST3	ZK686.3		WT/Abn	
WBP1/DDOST/Ost48	T09A5.11		Emb, Gro, Lon, Lvl, Unc	
OST2/DAD-1	F57B10.10 (*dad-1*)		Emb, Gro, Lva, Unc	DAD-1 suppresses programmed cell death (Sugimoto et al., 1995)
STT3	T12A2.2		Emb, Lva, Lon, Ste	
Processing glycosydases				
α1,2 glycosidase I	F13H10.4		WT/Abn	
α1,3 glycosidase II	F40F9.6a,b		Emb, Lva	
α 1,2 mannosidase I	C47E12.3		WT	
	T03G11.4		WT	
	ZC410.3		WT/Emb	
	ZC506.1		WT	
α 3,6 mannosidase II	F48C1.1a,b		WT	
Quality control glycosyltransferases				
Glycoprotein GlcT	F26H9.8		WT	
	F48E3.3		WT	
N-acetylglycosaminyltransferases specific for the synthesis of complex N-glycans				
β 1,2 GnT I *	B0416.6 (*gly-13*)	WT	WT	Chen et al., 1999
	F48E3.1a,b (*gly-12*)	WT	Abn	
	M01F1.1 (*gly-14*)	WT	WT	
β 1,2 GnT II *	C03E10.4 (*gly-20*)	WT	WT	Chen et al., 2002
β 1,6 GnT V *	C55B7.2 (*gly-2*)	WT	WT/Emb	Warren et al., 2002

Activity	*C. elegans* gene	Mutant phenotype	RNAi phenotype	Reference(s)
N-Glycan core fucosyltransferase				
Gn-Asn-X α 1,6 FucT	C10F3.6 (*fut-8*)		WT	Paschinger et al., 2005
Gal(NAc)β1,4Gn (Fuc to Gn) α1,3FucT *	K08F8.3 (*fut-1*)	WT	WT	Paschinger et al., 2004
Genes specific for GalNAc Ser/Thr O-glycan synthesis				
Polypeptide GalNAcT *	ZK688.8 (*gly-3*)		WT	Hagen and Nehrke, 1998
	Y116F11B.12.a,b (*gly-4*)		WT	Wang et al., 2005
	Y39E4B.12a,b,c (*gly-5*)		WT	
	H38K22.5a (*gly-6*)		WT	
	Y46H3A.6 (*gly-7*)		WT	
	Y66A7A.6 (*gly-8*)		WT	
	Y47D3A.23(*gly-9*)		WT	
	Y45F10D.3 (*gly-10*)		WT	
	Y75B8A.9 (*gly-11*)		WT	
GalNAc α 1-O-S/T β 1,3GalT (O-glycan core 1)				
Core 1 β3-Gal-T or T-synthase*	C38H2.2		WT	Ju et al., 2006
	C02H6.1		WT	
	C16D9.6		WT	
	T22B11.2		WT	
	Y38C1AB.1		WT	
	Y38C1AB.5		WT	
	ZC250.2		WT	
	C17A2.3		WT	
Homology to O-glycan core 1 β1,3 GalT/core 2 β1,6 GnT				
	T09E11.6		WT	
Genes for GalNAc Ser/Thr O-glycan synthesis and possibly other pathways				
Gal β1,3GalNAca1-O-S/T (Gn to GalNAc) β1,6-GnT (O-glycan core 2); homologous to β1,6 IGnT				Warren et al., 2001
	F44F4.6 (*gly-1*)	WT	WT	Warren et al., 2002; *gly-1* does not encode core 2 β1,6 GnT, but rather a β1,6 GlcT
	C54C8.11 (*gly-15*)		WT	
	T15D6.2 (*gly-16*)	WT	WT	
	T15D6.3 (*gly-17*)		WT	
	F22D6.11 (*gly-18*)	WT	WT	
	F22D6.12 (*gly-19*)		WT	

Activity	*C. elegans* gene	Mutant phenotype	RNAi phenotype	Reference(s)
	T09E11.9		WT	
	R07B7.6		WT	
	F30A10.4		WT	
	F35H8.2			
Other galactosyltransferases				
βGn β1,3-GalT	F14B6.6		WT/Gro	
	K08D12.5		WT	
	C47F8.3		WT	
	C47F8.6		WT	
	T09E11.11		WT	
	T09F5.1		WT	
	E03H4.11		WT	
	T15D6.5		WT	
	F14B6.4		WT	
	T09E11.10 (β1,3-GnT?)		WT	
	C47F8.5 (β1,3-GnT?)		WT	
	C54C8.3 (β1,3-GnT?)		WT	
βGn β1,4-GalT	W02B12.11		WT	
Other N-acetylhexosaminyltransferases				
Chitin Gn-T	T25G3.2 (*chi-1*)	Emb	Emb. Gro,Ste	Veronico et al., 2001; Zhang et al., 2005
	F48A11.1 (*chs-2*)		Bmd, Emb, Gro, Unc	Veronico et al., 2001; Zhang et al., 2005
Galβ1,4Gnb β1,3-GnT	C18G1.3		WT	
O-Gn transferase *	K04G7.3 (*ogt-1*)	Viable; suppressed constitutive dauer formation		Hanover et al., 2005
Galβ1,4GlcCer β1,3-GnT	F48F7.3		WT	
Protein O-Man β1,2-GnT1	M70.4a,b		WT	
Genes involved in the biosynthesis of glycosphingolipids				
βGn β1,3-GalT	Y39E4B.9 (*bre-2*)	Bt resistant	WT	Griffitts et al., 2005
Glcb1-Cer β1,4-Man-T	B0464.3 (*bre-3*)	Bt resistant		Griffitts et al., 2005
GnbR β1,4-GalNAcT	Y73E7A.7 (*bre-4*)	Bt resistant		Griffitts et al., 2005; Kawar et al., 2002

Activity	*C. elegans* gene	Mutant phenotype	RNAi phenotype	Reference(s)
Manβ1,4GlcR β1,3-GnT	T12G3.8 (*bre-5*)	Bt resistant Suppresses Egl defect associated with elevated lin-12 activity.		Griffitts et al., 2005; Katic et al., 2005
Cer GlcT	F59G1.1a,b,c,d		WT	
	F20B4.6		WT	
Other fucosyltransferases				
GalβRα1,2-FucT				
	EGAP9.2 (*fut-2*)	WT	WT	Zheng et al., 2002
	B0205.4			
	C06E1.7		WT	
	C14C6.3		WT	
	C17A2.4		WT	
	C18G1.8		WT	
	EGAP9.3		WT	
	F08A8.5		WT	
	F11A5.5		WT	
	F17B5.4		WT	
	F31F4.11		WT	
	F31F4.17		WT	
	K06H6.6		WT	
	T26E4.3		WT	
	T26E4.4		WT	
	T26E4.5		WT	
	T26H5.8		WT	
	T28A8.2		WT	
	T28F2.1		WT	
	W07G4.2		WT	
	Y5H2B.1		WT	
	F41D3.6 (galectin?)		WT	
Galβ1,4/3Gn (Fuc to Gn) α1,3/4-FucT (Lewis x or a)				
	F59E12.13 (*fut-3*)	WT	WT	
	K12H6.3 (*fut-4*)	WT	WT	
	T05A7.10 (*fut-5*)			
	T05A7.5 (*fut-6*)	WT	WT	
Protein O-FucT	C15C7.7		WT	Wang et al., 2005
	K10G9.3 (*pad-2*)		Egl, Bmd, Let,Evl	Menzel et al., 2004

Activity	*C. elegans* gene	Mutant phenotype	RNAi phenotype	Reference(s)
Nucleotide sugar transport / antiporter cycle				
UDP-GlcA/UDP-Gal/UDP-GalNAc transporter *	C52E12.3 (*sqv-7*)	Squashed vulva	WT/Emb	Berninsone et al., 2001
UDP-Gal/UDP-GlcNAc transporter *	M02B1.1 (*srf-3*)	Abnormal surface reactivity		Hoflich et al., 2004
GDP-Fuc transporter *	C50F4.14		WT	Luhn et al., 2001
Putative nucleotide sugar transporter	F15B10.1		WT	
Putative nucleotide sugar transporter	ZC250.3		WT/Abn	
Putative nucleotide sugar transporter	K02E10.5		WT	
UDP-GlcNAc/UDP-GalNAc transporter*	C03H5.2		WT/Ste	Caffaro et al., 2006
Putative nucleotide sugar transporter	ZK896.9		WT	
E-NTPDase *	R07E4.4 (*mig-23*)	Defective DTC migration	WT	Nishiwaki et al., 2004
E-NTPDase *	K08H10.4 (*uda-1*)		WT	Uccelletti et al., 2004
E-NTPDase *	C33H5.14		WT	Uccelletti et al., 2004
E-NTPDase	F08C6.6		WT	
Nucleotide sugar synthesis				
UDP-Gn 2 epimerase	D2007.5		WT	
GDP-Man pyrophosphorylase	C15F1.4 (*ppp-1*)		Abn	
	Y47D9A.1a,b		WT	
P-Man mutase 2	F52B11.2		WT/Gro	
P-Glc mutase/P-Man mutase	F21D5.1		Emb, Osm	
	R05F9.6		WT/Emb	
	Y43F4B.5a,b		WT/Emb/Gro	
P-Man isomerase	C05C8.7		WT/Emb, Lva, Sck, Ste	
	ZK632.4		WT	
UDP-Glu/UDP-Gal 4-epimerase	C47B2.6		WT	
Gal-1-phosphate uridyltransferase	Y55D5A.2			
	ZK1058.3		WT	
GDP-Man 4,6-dehydratase	F56H6.5		WT/ Abn	
UDP-galactopyranose mutase	H04M03.4			
Other glycosidases				
α-mannosidase	F55D10.1		WT	
Endo-βN-acetylglucosaminidase *	F01F1.10a,b		WT	
O-Gn selective β-Gnase	T20B5.3 (*oga-1*)		WT	Forsythe et al., 2006