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Protein O-mannosylation in animal development and physiology: from human disorders to *Drosophila* phenotypes

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Abstract

Protein O-mannosylation has a profound effect on the development and physiology of mammalian organisms. Mutations in genes affecting O-mannosyl glycan biosynthesis result in congenital muscular dystrophies. The main pathological mechanism triggered by O-mannosylation defects is a compromised interaction of cells with the extracellular matrix due to abnormal glycosylation of α -dystroglycan. Hypoglycosylation of α -dystroglycan impairs its ligand-binding activity and results in muscle degeneration and failure of neuronal migration. Recent experiments revealed the existence of compensatory mechanisms that could ameliorate defects of O-mannosylation. However, these mechanisms remain poorly understood. O-mannosylation and dystroglycan pathway genes show remarkable evolutionary conservation in a wide range of metazoans. Mutations and downregulation of these genes in zebrafish and *Drosophila* result in muscle defects and degeneration, also causing neurological phenotypes, which suggests that O-mannosylation has similar functions in mammals and lower animals. Thus, future studies in genetically tractable model organisms, such as zebrafish and *Drosophila*, should help to reveal molecular and genetic mechanisms of mammalian O-mannosylation and its role in the regulation of dystroglycan function.

1. Introduction

The diversity and evolutionary conservation of glycans is underlain by a spectrum of their functions within cells and organisms, ranging from roles as structural components of cells and the extracellular matrix to direct involvement in various molecular and cell interactions [1]. While the structural complexity of glycosylation and intricate regulation of glycosylation pathways have been impeding the elucidation of biological functions of protein glycosylation in mammals, recent development of sensitive mass spectrometry approaches [2] along with the studies of evolutionarily conserved glycan functions in genetically tractable model organisms [3,4] have significantly contributed to our understanding of the molecular and genetic mechanisms of protein glycosylation.

In this review, we will discuss protein O-mannosylation, a type of glycosylation found in wide range of species, from prokaryotes to humans, while focusing on molecular and genetic features of this modification in metazoan organisms. In animals, functionally important O-

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mannosyl glycans appear to be present on a limited number of proteins, yet they are playing essential biological roles in muscles and the nervous system. Despite the recent progress in revealing biological functions of O-mannosylation, its molecular and genetic mechanisms still largely elude our understanding. We will discuss developmental and physiological abnormalities associated with defects in protein O-mannosylation in mammals and present an evolutionary perspective, reviewing known and predicted functions of O-mannosylation in zebrafish and *Drosophila*. Finally, we will consider the possibility of using zebrafish and *Drosophila* as model organisms to elucidate the evolutionarily conserved functions of O-mannosyl glycans and to shed light on the pathobiological mechanisms of human diseases associated with O-mannosylation defects.

2. Biosynthetic pathway of O-mannosylation in animals

2.1 Structure of O-mannosyl glycans

O-mannosylation was first discovered and characterized in yeast, where O-mannosylated proteins comprise the abundant component of the cell wall [5]. O-mannosyl glycans are built on serine (Ser) or threonine (Thr) residues of proteins, starting with O-linked mannose attached in α anomeric configuration to the hydroxyl groups of Ser/Thr. In yeast and fungi, the mannose attached to the protein backbone can be further extended by several α -linked mannoses, with predominant complete structure being a linear oligosaccharide composed of five mannose residues (reviewed in [6]). In mammals, O-mannosylation appears to be an uncommon modification, and so far it was found on a limited number of glycoproteins present in muscles and neural tissue, including a brain chondroitin sulfate proteoglycan [7], α -dystroglycan (α -DG) [8,9], and receptor protein tyrosine phosphatase β [10]. Mammalian O-mannosylation exists in several main forms (Fig. 1). Three of these forms are found on α -DG, with a linear tetrasaccharide being the most common structure of O-mannosyl glycans (reviewed in [11–13]).

2.2. Enzymes involved in O-mannosylation

2.2.1. Protein O-mannosyltransferases—In yeast, protein O-mannosyltransferases comprise three subfamilies of enzymes, PMT1, PMT2 and PMT4, with PMT1 and PMT2 subfamilies usually including multiple members in each species [6]. Only two O-mannosyltransferases are present in metazoan organisms, both vertebrates and invertebrates, with POMT1 and POMT2 being more closely related to PMT4 and PMT2 yeast enzymes, respectively (Fig.S1A) [13]. Similar to yeast PMTs, POMT1 and POMT2 proteins are localized to the ER compartment in mammalian and *Drosophila* cells, and they mediate the initial attachment of mannose to Ser/Thr of proteins [14–17]. POMT enzymes are integral membrane proteins with multiple (7–12) potential membrane-spanning regions [14,16,18,19]. The topology of human POMT1 proteins was modeled on a yeast homologue [13], which suggested that POMT1 has 7 transmembrane domains, with N- and C-termini located in the cytoplasmic and ER compartments, respectively (Fig. S2). All POMTs include a conserved PMT domain present in all protein O-mannosyltransferases and three MIR motifs shared with inositol triphosphate and ryanodine receptors. Research in yeast suggested that the ER-localized loops 1 and 5 are essential for POMT functioning, with loop 1 being likely involved in substrate binding and catalytic activity [6]. Loop 5, the largest ER-localized hydrophilic domain, shows significant homology to SDF2 chaperons [20] and thus may have a role in protein folding [21].

In the enzymatic reaction, protein O-mannosyltransferases use dolichyl phosphate-activated Man (Dol-P-Man) as a donor substrate to modify Ser/Thr residues of acceptors. *Drosophila* POMT enzymes can modify mammalian α -DG *in vitro* and *Drosophila* DG *in vitro* and *in vivo* [18,22], which illustrates evolutionary conservation of POMT1 and POMT2 functions

between invertebrates and mammals. Co-expression of POMT1 and POMT2 is a prerequisite for their enzymatic activity, which indicates that they probably function as a heterocomplex [15,18]. This conclusion is reinforced by co-immunoprecipitation of these proteins and their co-localization within the ER [23]. Furthermore, this hypothesis is supported by *in vivo* studies in *Drosophila* that revealed that *rt* (*rotated abdomen*) and *tw* (*twisted*), *Drosophila* POMT1 and POMT2 orthologues, respectively, have nonredundant functions and show mutually epistatic genetic interactions [17,22].

There were several attempts to elucidate substrate specificity of POMT enzymes. *In vitro* assays with short peptides corresponding to the mucin-type domain of mammalian α -DG found that 10- or 15-meric peptides cannot serve as substrates and suggested that POMTs recognize an 18-mer consensus sequence [15,24,25]. However, this substrate specificity was not confirmed by *in vivo* assays using recombinantly expressed α -DG peptides [24]. These experiments revealed the requirement for some distant structural determinants located upstream of the target sites within the mucin domain [24]. Yet, recent analysis of *in vivo* expressed extracellular domain of *Drosophila* DG identified sites that neither agree with a consensus sequence derived from *in vitro* assays nor conform to the preferences revealed by the *in vivo* approach [22]. Taken together, these results indicate that the substrate recognition of O-mannosyltransferases appears to be complex and not driven *in vivo* simply by a sequence consensus at modification sites but rather is mediated by structural elements in more distant flanking regions. This conclusion is consistent with the substrate recognition of yeast homologues [26], suggesting the evolutionary conservation of molecular mechanism of O-mannosylation among a broad family of eukaryotic O-mannosyltransferases.

Intriguingly, a mouse POMT2 spliceform with an N-terminal extension is specifically expressed in testes where it localizes to the acrosome of sperm cells. This POMT2 variant appears not to be involved in O-mannosylation *in vivo* and probably has a novel non-enzymatic function [27].

2.2.2. POMGnT1 enzymes—POMGnT1, a β 1,2-*N*-acetylglucosaminyltransferase that elongates O-linked mannose with a β 1,2-linked GlcNAc residue [28,29], mediates the second step in the vertebrate pathway. This enzyme has a typical structure of a Golgi-resident glycosyltransferase, having a type II transmembrane topology, with a short N-terminal cytoplasmic tail, a stem region, and a C-terminal catalytic domain [30]. POMGnT1 proteins are related to β 1,2GlcNAc-transferases functioning in N-glycan biosynthesis, however they clearly represent a separate family of evolutionarily conserved enzymes (Fig.S1B) with unique substrate preferences and a specific role in O-mannosyl glycan biosynthesis [28,29]. A single orthologue of this enzyme is present in vertebrate species, while invertebrates, such as *Drosophila*, lack a functional counterpart of POMGnT1 [18]. Consistent with this conclusion, so far only non-elongated O-linked mannose has been identified in *Drosophila* [22,31] (Fig.1).

2.2.3. Other enzymes involved in O-mannosylation—Biosynthesis of branched O-mannose glycans is thought to be mediated by β 1,6-*N*-acetylglucosaminyltransferase VB (GlcNAcT-VB) that adds a β 1,6-linked GlcNAc residue to O-linked mannose (Fig. 1) [32]. This enzyme is highly expressed in the brain and testes [33,34]. GlcNAcT-VB is less specific for O-mannosylation pathway than POMGnT1 because it also participates in N-linked glycosylation. However, GlcNAcT-VB prefers O-linked mannose as an acceptor *in vitro* and thus probably plays a major role in producing branched O-mannosyl glycans [35].

There are three putative glycosyltransferases that are potentially involved in modification of O-mannosyl glycans: Fukutin, Fukutin-related protein (FKRP), and LARGE (see below) [36–38]. The biochemical activities of these proteins have not been characterized. Recent

study indicated that *LARGE* and *Fukutin* are involved in the biosynthesis of an unusual postphosphoryl modification of O-mannose that appears to be essential for α -DG ligand binding [39]. Putative orthologues of *FKRP* and *LARGE* genes are present in invertebrates, however the presence of *LARGE* is uneven between insect species (e.g., *LARGE* is absent in *Drosophila* but present in bees) [40,41].

There are several potential candidates for enzymes adding sialic acid, galactose, fucose and glucuronic acid to O-mannosyl glycans in mammals (Fig. 1). However, the exact identity of these glycosyltransferases is not yet known. It is likely that they are not specific for O-mannosylation, being also involved in biosynthesis of other glycans.

3. Biological functions of mammalian O-mannosylation

3.1. Dystrophin-glycoprotein complex and Dystroglycan

The best-studied prominent function of O-mannosylation is associated with the regulation of α -DG activity. Mammalian α -DG is a highly glycosylated cell adhesion protein crucial for the integrity of the muscle cells and neuronal migration during development (reviewed in [12,42]). α -DG is a central component of the dystrophin-glycoprotein complex (DGC) that provides structural stability to the sarcolemma during muscle contraction. α -DG binds to the extracellular matrix (ECM), and simultaneously, to β -DG, a transmembrane component of DGC, that, in turn, associates with dystrophin inside the cell. Thus, the DGC functions as an essential bridge between ECM and cytoskeleton (Fig. 2). Binding of the DGC to ECM is mediated via interactions between α -DG and the G-domains of a number of ECM ligands, such as laminin, agrin, perlecan, neurexin, and pikachurin ([43], reviewed in [42]). Abnormalities in the DGC function result in various congenital muscular dystrophies (CMDs) [44,45]. The pathobiological mechanism of muscle degeneration in these diseases is associated with the excessive fragility of muscle fibers and membrane microlesions induced by mechanical stress during muscle contractions [44].

3.2. Defective glycosylation causes muscular dystrophies

α -DG has a complex and still not fully characterized pattern of abundant glycosylation, including the presence of the O-mannose-linked glycans in its central mucin-type domain [12,42]. Mutations in glycosyltransferase genes implicated in O-mannosyl glycan biosynthesis result in CMDs with the phenotypes similar to those found in dystroglycan mutant tissue, such as dystrophic muscles and neuronal migration failure [29,46,47], supporting the idea that the deficiency of α -DG function is the main pathobiological mechanism of these dystrophies. In agreement with this conclusion, these CMDs show remarkable hypoglycosylation of α -DG and thus are classified as dystroglycanopathies. They include Walker Warburg syndrome (WWS) and Muscle-Eye-Brain disease (MEB) associated with mutations in *POMT1*, *POMT2*, *POMGnT1* [29,48,49] and other genes implicated in α -DG glycosylation (Fig.1, Table 1). WWS represents the most severe case of dystroglycanopathy, with the mean life span of less than a year, multiple brain defects and pronounced muscular degeneration. MEB clinical findings largely overlap with those of WWS, but the defects associated with MEB are less severe and have a more variable phenotype expressivity, with some patients surviving to adulthood (Table 1). Studies in mice revealed that *mPomt1* and *mPomt2* have elevated embryonic expression in developing muscles, the nervous system, the eye, and the mesenchyme, which correlates with the tissues affected in WWS [27,50,51]. Targeted disruption of *mPomt1* results in a defect of Reichert's membrane and E7.5–9.5 embryonic lethality [51], while similar phenotypes were observed in dystroglycan-null mice [52]. Unlike the *mPomt1* knockout, the targeted disruptions of *mPOMGnT1* produces viable mice which, however, have developmental abnormalities, including myopathy, neuronal migration defects, reduced cerebellum, and eye defects [53–

55]. The failure of neuronal migration in the cerebellum of these mice is probably caused by pial basement membrane breaches and the disruption of underlying glia limitans, which implies that *POMGnT1* has a cell-nonautonomous effect on neuron migration [55]. In addition, a role of *POMGnT1* in the maintenance of satellite cell proliferation and normal amount of muscle fibers *in vivo* was proposed [54].

Besides *POMT1* & 2 and *POMGnT1*, three other genes have been linked to dystroglycanopathies: *fukutin*, *fukutin-related protein (FKRP)*, and *LARGE* [36,37,40,56–58]. These genes encode putative glycosyltransferases, and their mutations result in significant hypoglycosylation of α -DG. However, it is not known whether their effect on α -DG glycosylation is direct, and the molecular activities of these genes' products remain obscure [47]. Although dystroglycanopathies show a general correlation between hypoglycosylation of α -DG and the pathology, and there is a tendency for genes at the top of O-mannosylation pathway to appear more frequently among causative mutations (e.g., *POMT1* & *POMT2* are more often mutated in dystroglycanopathies, followed by *POMGnT1* and other genes [57,59]), recent studies found no reliable genotype-phenotype correlation for these genes [59]. Mutations in any of the six dystroglycanopathy genes can result in a wide spectrum of clinical features, suggesting that all these genes work in the same functional pathway, while the severity of phenotype mostly depends on the strength of a particular allele and on a genetic background (Table 1) [59,60]. A significant correlation was found between the extent of hypoglycosylation and the severity of the pathology in the case of mutations in genes responsible for the core structure, *POMT1*, *POMT2* and *POMGnT1*. However, defects of *fukutin* and *FKRP* sometimes result in mild symptoms even in the absence or dramatically reduced immunoreactivity for the functionally glycosylated form of α -DG [60]. These data may be explained by the existence of additional compensatory factors that could restore the functionality of DGC, as long as α -DG has a minimally required modification with core O-mannosyl structures. Alternatively, these results may be associated with limitations of the detection method due to some masking of antibody epitopes.

3.3. Molecular role of O-mannosylation in dystroglycan functioning

The presence of O-mannose-linked glycans in α -DG mucin-type domain appears to be particularly important for the ligand-binding activity of α -DG. This conclusion is supported by the accumulated biochemical and genetic evidence, including combined results of enzymatic and chemical deglycosylation, sugar competition binding experiments, and the blockade of α -DG–laminin interactions by antibodies specific for the glycosylated form of α -DG. The importance of O-mannosyl glycans is also highlighted by the correlation between mutations in glycosyltransferases involved in O-mannosylation, α -DG hypoglycosylation, and muscular dystrophy phenotypes (reviewed in [12,42]). However, it is still unclear whether α -DG–ligand binding is directly mediated by these glycans. Moreover, *in vitro* deglycosylation of α -DG suggested that known O-mannosyl glycans have no direct effect on α -DG ligand binding [61]. Thus, it is possible that O-mannosylation exerts the effect by helping α -DG to acquire its functional conformation and/or regulating some unknown types of functionally important modifications. The first possibility is indirectly supported by the structural analysis of α -DG that revealed its dumbbell-like architecture, with two globular domains connected by a stretched mucin-type region, a putative target of most extensive O-mannosylation [42,62]. By analogy with mucins, O-linked sugars potentially induce an extended conformation, which may be important for ligand-binding activity. The possibility that O-mannosylation regulates some other modifications that, in turn, provide functionality to α -DG, was recently supported by the discovery of α -DG O-mannosyl phosphorylation [39]. *LARGE*, a potential bi-functional glycosyltransferase mutated in the myodystrophy mouse model and human CMDs [38,63], was proposed to participate in postphosphoryl

glycosylation that is required for laminin binding [39] (Fig. 1). This is consistent with the fact that LARGE overexpression can stimulate the production of highly glycosylated form of α -DG with restored ligand-binding activity even in cells derived from patients with dystroglycanopathies, including FCMD, MEB, and WWS [64], and in POMGnT1 mutant mice [65]. LARGE2, a vertebrate paralogue of LARGE, appears to have a similar effect on α -DG [66,67]. LARGE-induced modification occurs within the mucin-type domain and requires direct interaction between LARGE and the N-terminal part of α -DG [39,64,68]. In the absence of O-mannosyl glycans, overexpressed LARGE may have a promiscuous activity, possibly inducing the modification of N-linked and mucin-type O-GalNAc-linked glycans of α -DG [69]. Thus, the molecular role of O-mannosylation potentially concentrates on setting a stage for further functional maturation of α -DG, including modifications induced by LARGE and some other factors/enzymes, such as Fukutin and FKR. P.

4. O-mannosylation in lower vertebrates and *Drosophila*

Despite notable progress in revealing the function of α -DG O-mannosylation (reviewed in [12,42,70]), the complexity of mammalian glycosylation pathways along with limitations of genetic approaches hamper the elucidation of molecular and genetic mechanisms of O-mannosylation and its involvement in human pathobiology, indicating that relevant genetically tractable models would provide helpful tools for these studies. Recent research has hinted at zebrafish and *Drosophila* as candidate organisms for developing such models [17,22,71–75]

4.1. Zebrafish

4.1.1. Conservation of dystroglycan and the DGC in zebrafish—Conservation of genes involved in DGC formation in zebrafish has been confirmed in several studies, while DGC components were found to be localized to the sarcolemma of adult muscle [76]. Morpholino-mediated knockdown and mutations of DGC genes, such as dystrophin and sarcoglycan, result in phenotypes reminiscent of human muscular dystrophies, including decreased activity, muscle attachment defects, muscle tearing, and progressive muscle degeneration [77–79]. A mutation in zebrafish *laminin α 2* (*lama2*, *candyfloss*), an orthologue of human *LAMA2* mutated in the most common form of CMD (MDC1A), results in the phenotypes superficially similar to that of dystrophin mutants [80]. However, unlike mutations in *dystrophin* that cause sarcolemmal rupture, the defect of *lama2* results in fiber detachments without compromised membrane integrity [80]. Similarly, removal of DG is largely dispensable for basement membrane and muscle formation in early zebrafish development, yet leading later to the loss of muscle integrity and muscle degeneration, along with the loss of sarcomere and sarcoplasmic reticulum organization, which indicates that DG plays crucial role in long-term muscle survival [81].

4.1.2. Zebrafish orthologues of dystroglycanopathy genes—Remarkably, the seven genes implicated in functional glycosylation of α -DG in mammals, *POMT1* & 2, *POMGnT1*, *Fukutin*, *FKRP*, *LARGE* and *LARGE2*, are conserved in zebrafish. Each of these zebrafish orthologues is present in a single copy and broadly expressed during early development in a pattern overlapping with that of *Dag1*, a zebrafish *dystroglycan* [72]. In late embryogenesis, their expression becomes elevated in the CNS, eyes, and muscles, the tissues predominantly affected in dystroglycanopathies. *LARGE2* shows most restricted expression that is present in developing muscles, lens, pronephric duct, and cerebellum. Moreover, IIH6 antibody against glycosylated mammalian α -DG can also recognize zebrafish DG, with IIH6 reactivity being lost in *LARGE2* morphants, which suggests that the functional glycosylation of DG, including its O-mannosylation, is conserved in zebrafish [72]. In agreement with this conclusion, recent experiments revealed that *FKRP* knockdown

results in muscle defects, such as alterations in somitic structure and muscle fiber organization, as well as neuronal and eye abnormalities, which are accompanied by a reduction in DG glycosylation and laminin binding [82]. So far, however, the glycosylation of zebrafish DG has not been directly analyzed, and the structure of O-mannosyl glycans in zebrafish is not yet known.

4.2. *Drosophila*

4.2.1. Conservation of dystroglycan and the DGC in *Drosophila*—*Drosophila* genome includes genes encoding all essential components of DGC, albeit having substantially decreased diversity of homologues as compared to vertebrates (Fig. 2) [83]. In embryogenesis, many of these genes, including *dystrophin* (*Dys*), *dystroglycan* (*Dg*), and *sarcoglycan* orthologues, are expressed in a partially overlapping and dynamic pattern in the nervous system and the body wall muscles [84]. *Drosophila*, unlike mammals, has three different DG isoforms, DG-A, -B, and -C, produced by alternative splicing [85]. Among these isoforms, only DG-C includes a predicted mucin-type domain with the potential for extensive O-glycosylation, sharing this feature with mammalian α -DG [85,86]. *Drosophila* *Dg* is required for planar polarity of the basal actin stress fibers in the follicle cells [85,87], however *Dg* mutants appear to develop normally, with the only conspicuous phenotype being a wing posterior crossvein defect [88]. In addition, *Dg* has been implicated in establishing epithelial polarity in follicle cells and anteroposterior polarity of the oocyte during oogenesis [85,86]. This effect, however, depends on energetic stress conditions, and *Dg* appears to be not required for cell polarity when the energy pathway is not compromised [87]. The maintenance of epithelial polarity under energetic stress involves DG interaction with Perlecan and induces two different pathways that impinge on cytoskeleton polarization, one of them acting through Myosin II, and another working through yet unknown mechanism [87].

Dg also plays a role in the nervous system, where it is required for photoreceptor axon pathfinding, while at larval neuromuscular junctions (NMJs) *Dg* affects glutamate receptor subunit composition, the concentration of synaptic Laminin and Dystrophin, and the efficacy of synaptic transmission [73,89]. RNAi-mediated downregulation of DG results in postdevelopmental phenotypes reminiscent of human CMDs, with decreased mobility of flies and age-dependent muscle degeneration [90]. Crossvein and muscle degeneration phenotypes of *Dg* closely resemble those of *Dys*, while *Dys* is also required for normal NMJ synaptic transmission [73,88–92]. All these results further support the notion that DGC function is evolutionarily conserved between *Drosophila* and vertebrates.

4.2.2. O-mannosylation of *Drosophila* dystroglycan—It was recently demonstrated that RT and TW, the two *Drosophila* protein O-mannosyltransferases, modify the extracellular domain of DG-C *in vitro* and *in vivo*, and that RT-TW concurrent activity is required for the generation of a highly glycosylated form of DG *in vivo* [22], which mirrors the activity of mammalian POMT1-POMT2 toward α -DG in mammals [15,49]. The region of DG-C mucin-type domain is most extensively O-mannosylated. However, O-mannose is also present far outside of this region, in the part of the protein shared by all three DG isoforms, therefore all of them may be to some degree modified by O-mannosylation. Ectopic expression experiments suggested that RT-TW can modulate the activity of DG *in vivo*, which further supports the hypothesis that the function of O-mannosylation is conserved in *Drosophila*. Interestingly, O-mannosylation appears to compete with mucin-type O-GalNAc modification of *Drosophila*. Interestingly, despite the presence of numerous potential sites for O-mannosylation on DG-C extracellular domain, various levels of RT-TW activity seem to produce the same distinct glycoform, suggesting “all-or-nothing” mechanism without intermediate forms [22].

4.2.3. Function of *Drosophila* O-mannosyltransferases—*Drosophila* orthologues of *POMT1* and *POMT2*, *rt* and *tw*, encode proteins with significant structural homology to their mammalian counterparts (Fig.S1A) [16–19]. Embryonic expression of *rt* and *tw* becomes more prominent during active muscle differentiation, being pronounced in the epidermal cells of segmental borders, including muscle attachments, as well as in the developing trachea, pharynx and hindgut [17]. The expression of *rt* and *tw* shows largely overlapping pattern throughout all embryonic and larval stages ([17], S. Roosendaal and V. Panin, unpublished). Mutations in *rt* were among the first *Drosophila* alleles isolated in Thomas Morgan's laboratory [93]. The lack of either *rt* or *tw* activity results in essentially identical developmental defects of the abdomen, with abdominal segments being misaligned and rotated clockwise relative to each other, so-called “rotated abdomen” and “twisted” phenotypes [17–19]. Although it was initially proposed that this abdomen rotation phenotype was caused by muscle defects [19], *rt* and *tw* mutants were reported to have only low-frequency defects of larval general muscle morphology (including some abnormal attachments and a few missing or split muscles), as well as an abnormal sarcomere size [74], which does not explain the conspicuous misalignment of adult abdominal segments. Thus, the developmental mechanism of the abdomen rotation phenotype in *rt/tw* mutants remains unknown. Intriguingly, the muscle phenotypes of *Dg* downregulation was found to be similar to that of *rt* or *tw* mutants, while *rt/tw* and *Dg* also showed genetic interactions in these experiments, suggesting their involvement in the same functional pathway [74]. However, except for the sarcomere phenotype, the larval muscle abnormalities were analyzed in *Dg* mutants with a concomitant defect in mitochondria function [74,87], which complicates the interpretation of the results and opens up a possibility that these muscle defects were associated with energetic stress conditions. This possibility is consistent with the fact that independent experiments were unable to confirm abnormalities in general muscles morphology in *rt* and *tw* mutants (D. Lyalin, unpublished). Further experiments are necessary to elucidate a possible connection between O-mannosylation pathway and energy homeostasis.

The functional link between O-mannosylation and *Dg*-mediated pathways was also revealed by the analysis of NMJs in *rt* and *Dg* mutants. These experiments showed that *rt* and *Dg* mutations result in similar defects of synaptic transmission and the composition of glutamate receptor subunits [73]. *Dg* and *rt* mutations synergistically interact in producing synaptic phenotypes, indicating that *rt* and *Dg* collaborate within the same pathway [73]. Moreover, the level of Dystrophin was reduced in *rt* mutant muscles and NMJs, and thus *rt* may affect DGC stability [73]. Defects of NMJ function and development were also found in mouse models of dystroglycanopathies [94–96]. Thus, the synaptic role of DG glycosylation may be conserved between *Drosophila* and mammals. Interestingly, although rescue experiments indicated that *rt* is required for synaptic transmission on both sides of NMJs, in neurons and muscles [73], *Dys* and *Dg* appear to function at NMJs postsynaptically, exerting a cell-nonautonomous effect on presynaptic neurotransmitter release [89,92]. Hence, besides DG, other proteins involved in neural transmission may carry functionally important O-mannosylation (Fig.S3). Consistent with this idea, receptor tyrosine phosphatase β (RPTP β), a nervous system-specific signaling molecule implicated in regulation of neural cell adhesion and migration, was recently found to be regulated by O-mannosyl glycans in mammalian cells [10]. Intriguingly, *Drosophila* has six RPTP homologues, most of which are expressed in the nervous system and involved in axonal guidance [Supplemental Ref. 23]. However, their glycosylation has not been analyzed, and so far DG is the only known target of O-mannosylation in *Drosophila*.

5. Conclusions

Recent studies indicated that protein O-mannosylation pathway is evolutionarily conserved in a wide range of metazoan organisms, from flies to man. Mammalian O-mannosylation plays essential role in muscle physiology and neural development. Perturbations in O-mannosylation pathway result in congenital dystroglycanopathies characterized by hypoglycosylation and compromised ligand-binding activity of α -DG. Recent research has revealed the existence of compensatory mechanisms that can restore functional defects of α -DG glycosylation, indicating the importance of understanding the molecular and genetic mechanisms of α -DG O-mannosylation for developing therapeutic approaches. However the complexity of α -DG glycosylation, genetic redundancy and limited amenability to genetic manipulations hinder the research on α -DG glycosylation in mammals. On the other hand, evolutionary conservation of the genes involved in O-mannosylation and DG pathways suggests that genetically tractable model organisms should provide useful tools for the research. This notion has been supported by recent studies that revealed significant conservation of these genes' functions in *Drosophila* and zebrafish. Thus, it seems plausible that these model systems will help to uncover novel evolutionarily conserved factors regulating α -DG glycosylation, elucidate the molecular and genetic mechanisms of O-mannosylation and its involvement in dystroglycan-mediated processes, and reveal new important targets of O-mannosylation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Varki, A.; Cummings, RD.; Esko, JD., et al., editors. Essentials of Glycobiology. 2nd Edition ed.. Cold Spring Harbor, NY: ColdSpring Harbor Laboratory Press; 2009. p. 653
2. North SJ, Hitchen PG, Haslam SM, et al. Mass spectrometry in the analysis of N-linked and O-linked glycans. *Curr Opin Struct Biol* 2009;19:498–506. [PubMed: 19577919]
3. Ten Hagen KG, Zhang L, Tian E, et al. Glycobiology on the fly: developmental and mechanistic insights from *Drosophila*. *Glycobiology* 2009;19:102–111. [PubMed: 18824561]
4. Haltiwanger RS, Lowe JB. Role of glycosylation in development. *Annu Rev Biochem* 2004;73:491–537. [PubMed: 15189151]
5. Sentandreu R, Northcote DH. The structure of a glycopeptide isolated from the yeast cell wall. *Biochem J* 1968;109:419–432. [PubMed: 5685868]
6. Lommel M, Strahl S. Protein O-mannosylation: conserved from bacteria to humans. *Glycobiology* 2009;19:816–828. [PubMed: 19429925]
7. Finne J, Krusius T, Margolis RK, et al. Novel mannitol-containing oligosaccharides obtained by mild alkaline borohydride treatment of a chondroitin sulfate proteoglycan from brain. *J Biol Chem* 1979;254:10295–10300. [PubMed: 39937]
8. Chiba A, Matsumura K, Yamada H, et al. Structures of sialylated O-linked oligosaccharides of bovine peripheral nerve alpha-dystroglycan. The role of a novel O-mannosyl-type oligosaccharide in the binding of alpha-dystroglycan with laminin. *J Biol Chem* 1997;272:2156–2162. [PubMed: 8999917]
9. Smalheiser NR, Haslam SM, Sutton-Smith M, et al. Structural analysis of sequences O-linked to mannose reveals a novel Lewis X structure in cranin (dystroglycan) purified from sheep brain. *J Biol Chem* 1998;273:23698–23703. [PubMed: 9726975]

10. Abbott KL, Matthews RT, Pierce M. Receptor tyrosine phosphatase beta (RPTPbeta) activity and signaling are attenuated by glycosylation and subsequent cell surface galectin-1 binding. *J Biol Chem* 2008;283:33026–33035. [PubMed: 18838383]
11. Endo T. O-mannosyl glycans in mammals. *Biochim Biophys Acta* 1999;1473:237–246. [PubMed: 10580142]
12. Martin PT. Mechanisms of disease:congenital muscular dystrophies-glycosylation takes center stage. *Nat Clin Pract Neurol* 2006;2:222–230. [PubMed: 16932553]
13. Willer T, Valero MC, Tanner W, et al. O-mannosyl glycans: from yeast to novel associations with human disease. *Curr Opin Struct Biol* 2003;13:621–630. [PubMed: 14568618]
14. Jurado LA, Coloma A, Cruces J. Identification of a human homolog of the *Drosophila* rotated abdomen gene (POMT1) encoding a putative protein O-mannosyl-transferase, and assignment to human chromosome 9q34.1. *Genomics* 1999;58:171–180. [PubMed: 10366449]
15. Many H, Chiba A, Yoshida A, et al. Demonstration of mammalian protein O-mannosyltransferase activity: coexpression of POMT1 and POMT2 required for enzymatic activity. *Proc Natl Acad Sci U S A* 2004;101:500–505. [PubMed: 14699049]
16. Willer T, Amselgruber W, Deutzmann R, et al. Characterization of POMT2, a novel member of the PMT protein O-mannosyltransferase family specifically localized to the acrosome of mammalian spermatids. *Glycobiology* 2002;12:771–783. [PubMed: 12460945]
17. Lyalin D, Koles K, Roosendaal SD, et al. The twisted gene encodes *Drosophila* protein O-mannosyltransferase 2 and genetically interacts with the rotated abdomen gene encoding *Drosophila* protein O-mannosyltransferase 1. *Genetics* 2006;172:343–353. [PubMed: 16219785]
18. Ichimiya T, Many H, Ohmae Y, et al. The twisted abdomen phenotype of *Drosophila* POMT1 and POMT2 mutants coincides with their heterophilic protein O-mannosyltransferase activity. *J Biol Chem* 2004;279:42638–42647. [PubMed: 15271988]
19. Martin-Blanco E, Garcia-Bellido A. Mutations in the rotated abdomen locus affect muscle development and reveal an intrinsic asymmetry in *Drosophila*. *Proc Natl Acad Sci U S A* 1996;93:6048–6052. [PubMed: 8650217]
20. Hamada T, Tashiro K, Tada H, et al. Isolation and characterization of a novel secretory protein, stromal cell-derived factor-2 (SDF-2) using the signal sequence trap method. *Gene* 1996;176:211–214. [PubMed: 8918255]
21. Meunier L, Usherwood YK, Chung KT, et al. A subset of chaperones and folding enzymes form multiprotein complexes in endoplasmic reticulum to bind nascent proteins. *Mol Biol Cell* 2002;13:4456–4469. [PubMed: 12475965]
22. Nakamura N, Stalnakier SH, Lyalin D, et al. *Drosophila* Dystroglycan is a target of O-mannosyltransferase activity of two protein O-mannosyltransferases, Rotated Abdomen and Twisted. *Glycobiology* 2010;20:381–394. [PubMed: 19969597]
23. Akasaka-Many H, Many H, Nakajima A, et al. Physical and functional association of human protein o-mannosyltransferases 1 and 2. *J Biol Chem* 2006;281:19339–19345. [PubMed: 16698797]
24. Breloy I, Schwientek T, Gries B, et al. Initiation of mammalian O-mannosylation in vivo is independent of a consensus sequence and controlled by peptide regions within and upstream of the alpha-dystroglycan mucin domain. *J Biol Chem* 2008;283:18832–18840. [PubMed: 18456664]
25. Many H, Suzuki T, Akasaka-Many H, et al. Regulation of mammalian protein O-mannosylation: preferential amino acid sequence for O-mannose modification. *J Biol Chem* 2007;282:20200–20206. [PubMed: 17502374]
26. Hutzler J, Schmid M, Bernard T, et al. Membrane association is a determinant for substrate recognition by PMT4 protein O-mannosyltransferases. *Proc Natl Acad Sci U S A* 2007;104:7827–7832. [PubMed: 17470820]
27. Lommel M, Willer T, Strahl S. POMT2, a key enzyme in Walker-Warburg syndrome: somatic sPOMT2, but not testis-specific tPOMT2, is crucial for mannosyltransferase activity in vivo. *Glycobiology* 2008;18:615–625. [PubMed: 18490429]
28. Zhang W, Betel D, Schachter H. Cloning and expression of a novel UDP-GlcNAc:alpha-D-mannoside beta1,2-N-acetylglucosaminyltransferase homologous to UDP-GlcNAc:alpha-3-D-

- mannoside beta1,2-N-acetylglucosaminyltransferase I. *Biochem J* 2002;361:153–162. [PubMed: 11742540]
29. Yoshida A, Kobayashi K, Manya H, et al. Muscular dystrophy and neuronal migration disorder caused by mutations in a glycosyltransferase, POMGnT1. *Dev Cell* 2001;1:717–724. [PubMed: 11709191]
 30. Akasaka-Manya K, Manya H, Kobayashi K, et al. Structure-function analysis of human protein O-linked mannoside beta1,2-N-acetylglucosaminyltransferase 1, POMGnT1. *Biochem Biophys Res Commun* 2004;320:39–44. [PubMed: 15207699]
 31. Aoki K, Porterfield M, Lee SS, et al. The diversity of O-linked glycans expressed during *Drosophila melanogaster* development reflects stage- and tissue-specific requirements for cell signaling. *J Biol Chem* 2008;283:30385–30400. [PubMed: 18725413]
 32. Inamori K, Endo T, Gu J, et al. N-Acetylglucosaminyltransferase IX acts on the GlcNAc beta 1,2-Man alpha 1-Ser/Thr moiety, forming a 2,6-branched structure in brain O-mannosyl glycan. *J Biol Chem* 2004;279:2337–2340. [PubMed: 14617637]
 33. Inamori K, Endo T, Ide Y, et al. Molecular cloning and characterization of human GnT-IX, a novel beta1,6-N-acetylglucosaminyltransferase that is specifically expressed in the brain. *J Biol Chem* 2003;278:43102–43109. [PubMed: 12941944]
 34. Kaneko M, Alvarez-Manilla G, Kamar M, et al. A novel beta(1,6)-N-acetylglucosaminyltransferase V (GnT-VB)(1). *FEBS Lett* 2003;554:515–519. [PubMed: 14623122]
 35. Alvarez-Manilla G, Troupe K, Fleming M, et al. Comparison of the substrate specificities and catalytic properties of the sister N-acetylglucosaminyltransferases, GnT-V and GnT-Vb (IX). *Glycobiology*. 2009
 36. Brockington M, Yuva Y, Prandini P, et al. Mutations in the fukutin-related protein gene (FKRP) identify limb girdle muscular dystrophy 2I as a milder allelic variant of congenital muscular dystrophy MDC1C. *Hum Mol Genet* 2001;10:2851–2859. [PubMed: 11741828]
 37. Kobayashi K, Nakahori Y, Miyake M, et al. An ancient retrotransposal insertion causes Fukuyama-type congenital muscular dystrophy. *Nature* 1998;394:388–392. [PubMed: 9690476]
 38. Grewal PK, Holzfeind PJ, Bittner RE, et al. Mutant glycosyltransferase and altered glycosylation of alpha-dystroglycan in the myodystrophy mouse. *Nat Genet* 2001;28:151–154. [PubMed: 11381262]
 39. Yoshida-Moriguchi T, Yu L, Stalnaker SH, et al. O-mannosyl phosphorylation of alpha-dystroglycan is required for laminin binding. *Science* 2010;327:88–92. [PubMed: 20044576]
 40. Brockington M, Blake DJ, Prandini P, et al. Mutations in the fukutin-related protein gene (FKRP) cause a form of congenital muscular dystrophy with secondary laminin alpha2 deficiency and abnormal glycosylation of alpha-dystroglycan. *Am J Hum Genet* 2001;69:1198–1209. Epub 2001 Oct 1198. [PubMed: 11592034]
 41. Grewal PK, McLaughlan JM, Moore CJ, et al. Characterization of the LARGE family of putative glycosyltransferases associated with dystroglycanopathies. *Glycobiology* 2005;15:912–923. [PubMed: 15958417]
 42. Barresi R, Campbell KP. Dystroglycan: from biosynthesis to pathogenesis of human disease. *J Cell Sci* 2006;119:199–207. [PubMed: 16410545]
 43. Sato S, Omori Y, Katoh K, et al. Pikachurin, a dystroglycan ligand, is essential for photoreceptor ribbon synapse formation. *Nat Neurosci* 2008;11:923–931. [PubMed: 18641643]
 44. Blake DJ, Weir A, Newey SE, et al. Function and genetics of dystrophin and dystrophin-related proteins in muscle. *Physiol Rev* 2002;82:291–329. [PubMed: 11917091]
 45. Dalkilic I, Kunkel LM. Muscular dystrophies: genes to pathogenesis. *Curr Opin Genet Dev* 2003;13:231–238. [PubMed: 12787784]
 46. Satz JS, Barresi R, Durbeej M, et al. Brain and eye malformations resembling Walker-Warburg syndrome are recapitulated in mice by dystroglycan deletion in the epiblast. *J Neurosci* 2008;28:10567–10575. [PubMed: 18923033]
 47. Martin PT. Congenital muscular dystrophies involving the O-mannose pathway. *Curr Mol Med* 2007;7:417–425. [PubMed: 17584082]

48. van Reeuwijk J, Janssen M, van den Elzen C, et al. POMT2 mutations cause alpha-dystroglycan hypoglycosylation and Walker-Warburg syndrome. *J Med Genet* 2005;42:907–912. [PubMed: 15894594]
49. Beltran-Valero de Bernabe D, Currier S, Steinbrecher A, et al. Mutations in the O-mannosyltransferase gene POMT1 give rise to the severe neuronal migration disorder Walker-Warburg syndrome. *Am J Hum Genet* 2002;71:1033–1043. [PubMed: 12369018]
50. Prados B, Pena A, Cotarelo RP, et al. Expression of the murine *Pomt1* gene in both the developing brain and adult muscle tissues and its relationship with clinical aspects of Walker-Warburg syndrome. *Am J Pathol* 2007;170:1659–1668. [PubMed: 17456771]
51. Willer T, Prados B, Falcon-Perez JM, et al. Targeted disruption of the Walker-Warburg syndrome gene *Pomt1* in mouse results in embryonic lethality. *Proc Natl Acad Sci U S A* 2004;101:14126–14131. [PubMed: 15383666]
52. Williamson RA, Henry MD, Daniels KJ, et al. Dystroglycan is essential for early embryonic development: disruption of Reichert's membrane in *Dag1*-null mice. *Hum Mol Genet* 1997;6:831–841. [PubMed: 9175728]
53. Liu J, Ball SL, Yang Y, et al. A genetic model for muscle-eye-brain disease in mice lacking protein O-mannose 1,2-N-acetylglucosaminyltransferase (POMGnT1). *Mech Dev* 2006;123:228–240. [PubMed: 16458488]
54. Miyagoe-Suzuki Y, Masubuchi N, Miyamoto K, et al. Reduced proliferative activity of primary POMGnT1-null myoblasts in vitro. *Mech Dev* 2009;126:107–116. [PubMed: 19114101]
55. Li X, Zhang P, Yang Y, et al. Differentiation and developmental origin of cerebellar granule neuron ectopia in protein O-mannose UDP-N-acetylglucosaminyl transferase 1 knockout mice. *Neuroscience* 2008;152:391–406. [PubMed: 18295407]
56. Beltran-Valero de Bernabe D, Voit T, Longman C, et al. Mutations in the FKRP gene can cause muscle-eye-brain disease and Walker-Warburg syndrome. *J Med Genet* 2004;41:e61. [PubMed: 15121789]
57. Godfrey C, Clement E, Mein R, et al. Refining genotype phenotype correlations in muscular dystrophies with defective glycosylation of dystroglycan. *Brain* 2007;130:2725–2735. [PubMed: 17878207]
58. Longman C, Brockington M, Torelli S, et al. Mutations in the human *LARGE* gene cause MDC1D, a novel form of congenital muscular dystrophy with severe mental retardation and abnormal glycosylation of alpha-dystroglycan. *Hum Mol Genet* 2003;12:2853–2861. [PubMed: 12966029]
59. Mercuri E, Messina S, Bruno C, et al. Congenital muscular dystrophies with defective glycosylation of dystroglycan: a population study. *Neurology* 2009;72:1802–1809. [PubMed: 19299310]
60. Jimenez-Mallebrera C, Torelli S, Feng L, et al. A Comparative Study of alpha-Dystroglycan Glycosylation in Dystroglycanopathies Suggests that the Hypoglycosylation of alpha-Dystroglycan Does Not Consistently Correlate with Clinical Severity. *Brain Pathol.* 2008 PMID: 18691338 [epub ahead of print].
61. Combs AC, Ervasti JM. Enhanced laminin binding by alpha-dystroglycan after enzymatic deglycosylation. *Biochem J* 2005;390:303–309. [PubMed: 15865602]
62. Brancaccio A, Schulthess T, Gesemann M, et al. Electron microscopic evidence for a mucin-like region in chick muscle alpha-dystroglycan. *FEBS Lett* 1995;368:139–142. [PubMed: 7615068]
63. van Reeuwijk J, Grewal PK, Salih MA, et al. Intragenic deletion in the *LARGE* gene causes Walker-Warburg syndrome. *Hum Genet* 2007;121:685–690. [PubMed: 17436019]
64. Barresi R, Michele DE, Kanagawa M, et al. *LARGE* can functionally bypass alpha-dystroglycan glycosylation defects in distinct congenital muscular dystrophies. *Nat Med* 2004;10:696–703. [PubMed: 15184894]
65. Kanagawa M, Nishimoto A, Chiyonobu T, et al. Residual laminin-binding activity and enhanced dystroglycan glycosylation by *LARGE* in novel model mice to dystroglycanopathy. *Hum Mol Genet* 2009;18:621–631. [PubMed: 19017726]
66. Brockington M, Torelli S, Prandini P, et al. Localization and functional analysis of the *LARGE* family of glycosyltransferases: significance for muscular dystrophy. *Hum Mol Genet* 2005;14:657–665. [PubMed: 15661757]

67. Fujimura K, Sawaki H, Sakai T, et al. LARGE2 facilitates the maturation of alpha-dystroglycan more effectively than LARGE. *Biochem Biophys Res Commun* 2005;329:1162–1171. [PubMed: 15752776]
68. Kanagawa M, Saito F, Kunz S, et al. Molecular recognition by LARGE is essential for expression of functional dystroglycan. *Cell* 2004;117:953–964. [PubMed: 15210115]
69. Patnaik SK, Stanley P. Mouse large can modify complex N- and mucin O-glycans on alpha-dystroglycan to induce laminin binding. *J Biol Chem* 2005;280:20851–20859. [PubMed: 15788414]
70. Hewitt JE. Abnormal glycosylation of dystroglycan in human genetic disease. *Biochim Biophys Acta* 2009;1792:853–861. [PubMed: 19539754]
71. Kunkel LM, Bachrach E, Bennett RR, et al. Diagnosis and cell-based therapy for Duchenne muscular dystrophy in humans, mice, and zebrafish. *J Hum Genet* 2006;51:397–406. [PubMed: 16583129]
72. Moore CJ, Goh HT, Hewitt JE. Genes required for functional glycosylation of dystroglycan are conserved in zebrafish. *Genomics* 2008;92:159–167. [PubMed: 18632251]
73. Wairkar YP, Fradkin LG, Noordermeer JN, et al. Synaptic defects in a *Drosophila* model of congenital muscular dystrophy. *J Neurosci* 2008;28:3781–3789. [PubMed: 18385336]
74. Haines N, Seabrooke S, Stewart BA. Dystroglycan and protein O-mannosyltransferases 1 and 2 are required to maintain integrity of *Drosophila* larval muscles. *Mol Biol Cell* 2007;18:4721–4730. [PubMed: 17881734]
75. Bassett DI, Currie PD. The zebrafish as a model for muscular dystrophy and congenital myopathy. *Hum Mol Genet* 2003;12 Spec No 2:R265–R270. [PubMed: 14504264]
76. Steffen LS, Guyon JR, Vogel ED, et al. Zebrafish orthologs of human muscular dystrophy genes. *BMC Genomics* 2007;8:79. [PubMed: 17374169]
77. Guyon JR, Goswami J, Jun SJ, et al. Genetic isolation and characterization of a splicing mutant of zebrafish dystrophin. *Hum Mol Genet* 2009;18:202–211. [PubMed: 18957474]
78. Guyon JR, Mosley AN, Jun SJ, et al. Delta-sarcoglycan is required for early zebrafish muscle organization. *Exp Cell Res* 2005;304:105–115. [PubMed: 15707578]
79. Bassett DI, Bryson-Richardson RJ, Daggett DF, et al. Dystrophin is required for the formation of stable muscle attachments in the zebrafish embryo. *Development* 2003;130:5851–5860. [PubMed: 14573513]
80. Hall TE, Bryson-Richardson RJ, Berger S, et al. The zebrafish candyfloss mutant implicates extracellular matrix adhesion failure in laminin alpha2-deficient congenital muscular dystrophy. *Proc Natl Acad Sci U S A* 2007;104:7092–7097. [PubMed: 17438294]
81. Parsons MJ, Campos I, Hirst EM, et al. Removal of dystroglycan causes severe muscular dystrophy in zebrafish embryos. *Development* 2002;129:3505–3512. [PubMed: 12091319]
82. Thornhill P, Bassett D, Lochmuller H, et al. Developmental defects in a zebrafish model for muscular dystrophies associated with the loss of fukutin-related protein (FKRP). *Brain* 2008;131:1551–1561. [PubMed: 18477595]
83. Greener MJ, Roberts RG. Conservation of components of the dystrophin complex in *Drosophila*. *FEBS Lett* 2000;482:13–18. [PubMed: 11018515]
84. Dekkers LC, van der Plas MC, van Loenen PB, et al. Embryonic expression patterns of the *Drosophila* dystrophin-associated glycoprotein complex orthologs. *Gene Expr Patterns* 2004;4:153–159. [PubMed: 15161095]
85. Deng WM, Schneider M, Frock R, et al. Dystroglycan is required for polarizing the epithelial cells and the oocyte in *Drosophila*. *Development* 2003;130:173–184. [PubMed: 12441301]
86. Schneider M, Khalil AA, Poulton J, et al. Perlecan and Dystroglycan act at the basal side of the *Drosophila* follicular epithelium to maintain epithelial organization. *Development* 2006;133:3805–3815. [PubMed: 16943280]
87. Mirouse V, Christoforou CP, Fritsch C, et al. Dystroglycan and perlecan provide a basal cue required for epithelial polarity during energetic stress. *Dev Cell* 2009;16:83–92. [PubMed: 19154720]
88. Christoforou CP, Greer CE, Challoner BR, et al. The detached locus encodes *Drosophila* Dystrophin, which acts with other components of the Dystrophin Associated Protein Complex to

- influence intercellular signalling in developing wing veins. *Dev Biol* 2008;313:519–532. [PubMed: 18093579]
89. Bogdanik L, Framery B, Frolich A, et al. Muscle dystroglycan organizes the postsynapse and regulates presynaptic neurotransmitter release at the *Drosophila* neuromuscular junction. *PLoS ONE* 2008;3:e2084. [PubMed: 18446215]
90. Shcherbata HR, Yatsenko AS, Patterson L, et al. Dissecting muscle and neuronal disorders in a *Drosophila* model of muscular dystrophy. *Embo J* 2007;26:481–493. [PubMed: 17215867]
91. van der Plas MC, Pilgram GS, de Jong AW, et al. *Drosophila* Dystrophin is required for integrity of the musculature. *Mech Dev* 2007;124:617–630. [PubMed: 17543506]
92. van der Plas MC, Pilgram GS, Plomp JJ, et al. Dystrophin is required for appropriate retrograde control of neurotransmitter release at the *Drosophila* neuromuscular junction. *J Neurosci* 2006;26:333–344. [PubMed: 16399704]
93. Bridges CB, Morgan TH. The third-chromosome group of mutant characters of *Drosophila melanogaster*. *Publs Carnegie Instn* 1923;327:1–251.
94. Taniguchi M, Kurahashi H, Noguchi S, et al. Aberrant neuromuscular junctions and delayed terminal muscle fiber maturation in alpha-dystroglycanopathies. *Hum Mol Genet* 2006;15:1279–1289. [PubMed: 16531417]
95. Herbst R, Iskratsch T, Unger E, et al. Aberrant development of neuromuscular junctions in glycosylation-defective Large(myd) mice. *Neuromuscul Disord* 2009;19:366–378. [PubMed: 19346129]
96. Levedakou EN, Chen XJ, Soliven B, et al. Disruption of the mouse Large gene in the enr and myd mutants results in nerve, muscle, and neuromuscular junction defects. *Mol Cell Neurosci* 2005;28:757–769. [PubMed: 15797722]

Figure 1. Structures and biosynthesis of O-mannosyl glycans in animals

Arrows indicate enzymatic steps mediated by the enzymes shown next to the arrows: POMT1/POMT2, protein O-mannosyltransferase 1 & 2; POMGnT1, protein O-mannose β1,2-N-acetylglucosaminyltransferase; GlcNAcT-Vb (aka GnT-IX, Mgat-5b), β1,6-N-acetylglucosaminyltransferase; GalT, β1,4-galactosyltransferase; SiaT, α2,3-sialyltransferase; FucT, α1,3-fucosyltransferase; GlcAT, β1,3-glucuronyltransferase; HNK-1ST, carbohydrate sulfotransferase. *, structures identified on mammalian α-Dystroglycan. **, the structure found on *Drosophila* Dystroglycan. ?, unknown/potential steps or enzymes.

Figure 2. Evolutionary conservation of the dystrophin-glycoprotein complex between vertebrates and *Drosophila*

In vertebrates, Dystroglycan interacts with basal lamina via binding ECM ligands, including Laminin, Neurexin, Agrin, Perlecan, and Pikachurin. β -Dystroglycan spans the cell membrane and links extracellular (α -Dystroglycan) and intracellular (Dystrophin) DGC components. *Dystrophin* mediates connection between the DGC and actin cytoskeleton. Other membrane and intracellular DGC components include sarcoglycans, dystrobrevins, syntrophin, and NOS. *Drosophila* has all essential components of the DGC along with Dystroglycan ligands, including Laminin (LanA), Perlecan (Trol), Neurexin and a putative Pikachurin (CG8403). Unlike in vertebrates, Dystroglycan in *Drosophila* appears not to be processed into separate α and β subunits, and no extension of O-linked mannose has been identified in *Drosophila* so far. Note that besides O-mannosylation Dystroglycan has also other types of glycosylation, such as O-linked mucin-type and N-linked glycans, which are not shown here. Schematic is based on database searches and published data [83,85,86].

Table 1
Dystroglycanopathies, associated mutations and clinical findings

WWS, Walker-Warburg syndrome; MEB, Muscle-Eye-Brain disease; FCMD, Fukuyama congenital muscular dystrophy; MDC1C, muscular dystrophy, congenital, type 1C; MDC1D, muscular dystrophy, congenital, type 1D; LGMD2K, Muscular dystrophy, limb-girdle, type 2K; LGMD2I, muscular dystrophy, limb-girdle, type 2I. Hypoglycosylation of α -dystroglycan was assessed by antibodies specific for functional glycosylated form of α -dystroglycan (IIH6 and VIA4-1). CK, creatine kinase. S14–S21, Supplemental references.

Disorder (OMIM)	Gene	Clinical findings		[Refs.]
		Mobility and muscle	Nervous system	
WWS (236670)	<i>POMT1</i> <i>POMT2</i> <i>POMGnT1</i> <i>Fukutin</i> <i>FKRP</i> <i>LARGE</i>	Severe-impaired mobility, muscle weakness, reduced muscle bulk, dystrophic muscle biopsy, elevated serum CK level, hypoglycosylation of α -dystroglycan	Severe structural brain abnormalities including; Encephalocele, severe hydrocephalus, type II lissencephaly/agyria, absence of corpus callosum, fusion of cerebral hemispheres. Severe atrophy of the cerebellar vermis. Eye abnormalities: unilateral or bilateral microphthalmia, buphthalmus, hypoplastic or absent optic nerves, retinal detachment, common anterior chamber malformations (cataracts, iris malformation, glaucoma).	[45,56,57,59, S14–S16]
MEB (253280)	<i>POMT1</i> <i>POMT2</i> <i>POMGnT1</i> <i>FKRP</i> <i>LARGE</i>	Profound muscle hypotonia, impaired mobility, can sit unsupported, speak a few words, muscle degeneration, fibrosis elevated serum CK level, hypoglycosylation of α -dystroglycan	Brain abnormality less severe than WWS including: cerebellar hypoplasia, cortical dysplasia, pachygyria/polymicrogyria/agyria, common epilepsy seizures, flattening of the pons and brainstem. Eye abnormalities: high myopia, retinal atrophy, retinal dysplasia, glaucoma, cataracts, retinal detachment. In rare cases: severe autistic features, tonic-clonic seizures.	[26,56,57,59 S14, S15]
FCMD (253800)	<i>Fukutin</i>	Profound muscle hypotonia, impaired mobility, dilated cardiomyopathy, enlargement of calves, quadriceps, and tongue, scoliosis, elevated serum CK level, hypoglycosylation of α -dystroglycan	Brain abnormality less severe than WWS including; micropolygyria, cerebellar hypoplasia, cortical dysplasia, verrucous dysplasia, unlayered polymicrogyria, cerebellar cystic lesions, type II lissencephaly, flattening of the pons and brainstem. Eye abnormalities: in rare case, retinal detachment.	[37,59, S15–S18]
MDC1C (606612)	<i>FKRP</i>	Severe case; inability to walk Mild case; muscle weakness and hypotonia, limited mobility and movements, impairment of cardiac left-ventricular, hypotrophy, elevated serum CK level, hypoglycosylation of α -dystroglycan	Weak phenotype: normal brain structures and intelligence. Mild phenotype: mild mental retardation, mild structural changes of the cerebellum, cerebellar cysts, normal brain stem and eyes Severe phenotype: severe mental retardation, pontocerebellar hypoplasia, cerebellar cysts. Retinal changes with abnormal pigmentation, myopia.	[S14, S19]
MDC1D (608840)	<i>LARGE</i>	Muscle weakness, muscle spasticity, hypertrophy, reduced immunolabelling of α -dystroglycan, elevated serum CK levels	Profound mental retardation, white matter changes, structural abnormalities, abnormal neuronal migration.	[58]
LGMD2K (609308)	<i>POMT1</i>	Muscle weakness, muscle hypertrophy, hypoglycosylation of α -dystroglycan, elevated serum CK levels	Mild mental retardation, microcephaly, normal brain structure by cranial imaging.	[S20]
LGMD2I (607155)	<i>FKRP</i>	Mild cases: ability to walk, muscle weakness, elevated serum CK levels,	Brain structures judged by MRI are normal, normal intelligence.	[S15, S21]

Disorder (OMIM)	Gene	Clinical findings		[Refs.]
		Mobility and muscle	Nervous system	
		hypoglycosylation of α -dystroglycan Severe cases: inability to walk, elevated serum CK levels, dilated cardiomyopathy, muscle hypertrophy, hypoglycosylation of α -dystroglycan		