Glycoconjugate Journal 21, 3–7, 2004

Structure, function and pathology of *O*-mannosyl glycans

Tamao Endo

Glycobiology Research Group, Tokyo Metropolitan Institute of Gerontology, Foundation for Research on Aging and Promotion of Human Welfare, 35-2 Sakaecho, Itabashi-ku, Tokyo 173-0015, Japan

Animal cells contain many glycoproteins, *i.e.*, proteins with covalently liked sugar chains. The major glycans of glycoproteins can be classified into two groups, *N*-glycans and *O*-glycans, according to their glycan-peptide linkage regions. Development of sensitive methods for the analyses of glycan structures have revealed a new type of glycosidic linkage to the peptide portion, the *O*-mannosyl linkage, in mammals, which used to be considered specific to yeast. *O*-Mannosylation is present in a limited number of glycoproteins of brain, nerve, and skeletal muscle. Recently *O*-mannosylation has been shown to be important in muscle and brain development. Glycobiology of *O*-mannosyl glycans is expected to produce remarkable advances in the understanding and treatment of congenital muscular dystrophies. In this article, I describe the structure, biosynthesis, and pathology of *O*-mannosyl glycans.
Published in 2004.

***Keywords:* *O*-mannosylation, congenital muscular dystrophy, dystroglycan, glycosyltransferase**

Introduction

The major sugar chains of glycoproteins can be classified into two groups according to their sugar-peptide linkages. Those that are linked to asparagine (Asn) residues of proteins are termed *N*-glycans, while those that are linked to serine (Ser) or threonine (Thr) residues are called *O*-glycans. In *N*-glycans, the reducing terminal *N*-acetylglucosamine (GlcNAc) is linked to the amide group of Asn via an aspartylglycosylamine linkage. In *O*-glycans, the reducing terminal *N*-acetylgalactosamine (GalNAc) is attached to the hydroxyl group of Ser and Thr residues. In addition to the abundant *O*-GalNAc forms, several unique types of protein *O*-glycosylation have been found, such as *O*-linked fucose, glucose, GlcNAc, and mannose, which have been shown to mediate diverse physiological functions. For example, *O*-fucosylation has recently been implicated in Notch signaling [1–4] and *O*-mannosylation has been shown to be important in muscle and brain development.

Structure and occurrence

O-Mannosylation is known as a yeast-type modification, and *O*-mannosylated proteins are abundant in the yeast cell wall [5]. All *O*-mannosyl glycan structures elucidated so far in yeast are neutral linear glycans consisting of 1 to 7 mannose residues. *O*-Mannosylation of proteins is essential for viability in yeast, and its absence may affect cell wall structure and rigidity. Additionally, a deficiency in protein *O*-mannosylation in the fungal pathogen, *Candida albicans*, leads to defects in multiple cellular functions including expression of virulence [6]. In addition to fungi and yeast, clam worm has an *O*-mannosyl glycan (a glucuronosylα1-6mannosyl disaccharide) in skin collagen [7].

Mammalian *O*-mannosylation is an unusual type of protein modification that was first suggested in chondroitin sulfate proteoglycans of brain [8] and is present in a limited number of glycoproteins of brain, nerve, and skeletal muscle [9]. One of the best known *O*-mannosyl-modified glycoproteins is α-dystroglycan [9], which is a central component of the dystrophin-glycoprotein complex isolated from skeletal muscle membranes [10]. We previously found that the glycans of α-dystroglycan include *O*-mannosyl oligosaccharides, and that a sialyl *O*-mannosyl glycan, Siaα2-3Galβ1-4GlcNAcβ1-2Man, of α-dystroglycan is a laminin-binding ligand of α-dystroglycan [11,12]. Further, α-dystroglycan from sheep brain has a Galβ1-4(Fucα1-3)GlcNAcβ1-2Man structure [13] and mouse J1/tenascin contains the *O*-mannosyl glycans [14]. Additionally, an *O*-mannosyl glycan containing the HNK-1 epitope (HSO_3-3GlcAβ1-3Galβ1-4GlcNAcβ1-2Man) was detected in total brain glycopeptides [15]. It is noteworthy that these oligosaccharides have not only 2-substituted mannose but also 2,6-substituted mannose [16]. Therefore, it is likely

To whom correspondence should be addressed: Tamao Endo, Glycobiology Research Group, Tokyo Metropolitan Institute of Gerontology, Foundation for Research on Aging and Promotion of Human Welfare, 35-2 Sakaecho, Itabashi-ku, Tokyo 173-0015, Japan. Tel.: +81-3-3964-3241 (ext. 3080); Fax: +81-3-3579-4776; E-mail: endo@tmig.or.jp

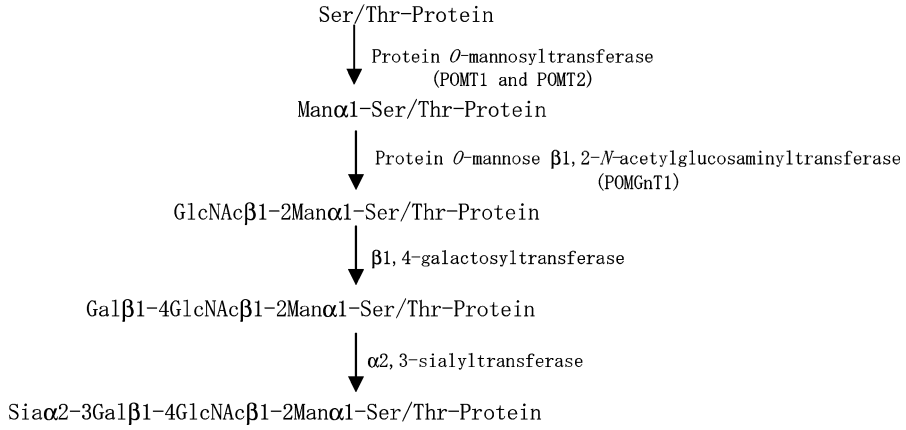

Figure 1. Biosynthetic pathway of mammalian *O*-mannosyl glycan. POMT1 and POMGnT1 are responsible for WWS and MEB, respectively. It is unclear whether others as yet characterized forms of muscular dystrophy are caused by defects in β1,4-galactosyltransferase and α2,3-sialyltransferase.

that a series of *O*-mannosyl glycans, with heterogeneity of mannose-branching and peripheral structures, is present in mammals. Further studies are needed to clarify the distribution of such *O*-mannosyl glycans in various tissues.

Biosynthesis

Identification and characterization of the enzymes involved in the biosynthesis of mammalian type *O*-mannosyl glycans will help to elucidate the function and regulation of these glycans (Figure 1).

Mammalian *O*-mannosyl glycans, unlike those of yeast, have the GlcNAcβ1-2Man linkage [9]. This linkage is assumed to be catalyzed by a glycosyltransferase, UDP-GlcNAc: protein *O*-mannose β1,2-*N*-acetylglucosaminyltransferase (POMGnT1). POMGnT1 catalyzes the transfer of GlcNAc from UDP-GlcNAc to *O*-mannosyl glycoproteins. Human *POMGnT1* gene has been cloned [17]. Based on its nucleotide sequence, human POMGnT1 is a 660-amino acid type II membrane protein. This topology was similar to the topologies of other Golgi glycosyltransferases [18]. *POMGnT1* was found to be expressed in all human tissues examined [17]. Careful examination of substrate specificity of POMGnT1 indicated that POMGnT1 did not have either UDP-GlcNAc: α-3-D-mannoside β-1,2-*N*-acetylglucosaminyltransferase I (GnT-I) or UDP-GlcNAc: α-6-D-mannoside β-1,2-*N*-acetylglucosaminyltransferase II (GnT-II) activity. On the other hand, GnT-I and GnT-II did not have any POMGnT1 activity [19]. Taken together, these results indicate that loss-of-function of POMGnT1 is not compensated by GnT-I and GnT-II.

As described above, mammalian *O*-mannosyl glycan has 2,6-substituted mannose [16]. A gene for encoding a 6-branching enzyme (GnT-IX, *i.e*, an enzyme that catalyzes the GlcNAcβ1-6Man linkage in *O*-mannosyl glycans) has recently been cloned [20]. Since GnT-IX is specifically expressed in the brain, identifying the functional roles of 2,6-branched *O*-mannosyl glycans in the brain is of great interest.

In *Saccharomyces cerevisiae*, the family of protein *O*-mannosyltransferases catalyzes the transfer of a mannosyl residue from dolichyl phosphate mannose (Dol-P-Man) to Ser/Thr residues of certain proteins [5]. There is no obvious consensus sequence for attachment of *O*-mannosyl glycans, though *in vitro* studies with peptide acceptors show that the presence of a proline residue in the vicinity enhanced *O*-mannosylation of nearby Ser or Thr residues [5]. However, attempts to detect protein *O*-mannosyltransferase activity and to characterize the enzyme(s) responsible for the biosynthesis of *O*-mannosyl glycans in vertebrates have not been successful. Two human homologues, *POMT1* and *POMT*2, were found, but their gene products did not show any protein *O*-mannosyltransferase activity [21,22]. POMT1 and POMT2 share almost identical hydropathy profiles that predict both to be integral membrane proteins with multiple transmembrane domains. Recently, we developed a new method to detect the enzymatic activity of protein *O*-mannosyltransferase in mammalian cells and tissues [23]. Initially, we attempted to detect mannose transferase activity based on an assay for protein *O*-mannosyltransferase activity in yeast using several synthetic peptides and Triton X-100 as a detergent. However, we did not detect any activity in several mammalian tissues and cells, possibly due to the specificity of the acceptor peptide sequence. If the enzyme recognizes a specific amino acid sequence, α-dystroglycan may be a suitable acceptor because it has *O*-mannosyl glycans [9]. Therefore, we prepared a GST fusion protein of α-dystroglycan for a candidate acceptor. However, using the α-dystroglycan-GST as an acceptor and the Dol-P-Man as a donor substrate did not observe any enzymatic activity. Next, we examined the effect of detergent because yeast protein *O*-mannosyltransferases are integral membrane proteins and thus hydrophobic proteins. Because Triton X-100 is a non-ionic detergent, we examined many ionic and ampholytic detergents, including alkyl-glycosides. Finally, we found that the best detergent was *n*-octyl-β-D-thioglucoside. With these changes, we succeeded in detecting mammalian protein *O*-mannosyltransferase activity [23].

Using this new method, we demonstrated that human POMT1 and POMT2 have protein *O*-mannosyltransferase activity, but only when they are co-expressed [23]. This suggests that POMT1 and POMT2 form a hetero-complex to express enzymatic activity similar to the complex in yeast [24]. *POMT1* and *POMT2* are expressed in all human tissues, but *POMT1* is highly expressed in fetal brain, testis and skeletal muscle, and *POMT2* is predominantly expressed in testis [21,22]. *O*-Mannosylation seems to be uncommon in mammals and only a few *O*-mannosylated proteins have been identified. It will be of interest to determine the regulatory mechanisms for protein *O*-mannosylation in each tissue.

No enzymes for galactosylation, sialylation, fucosylation, glucuronylation, or sulfation of *O*-mannosyl glycans have been identified.

Pathology

Muscular dystrophies are genetic diseases that cause progressive muscle weakness and wasting [25]. Recent data suggest that aberrant *O*-mannosylation of α-dystroglycan is the primary cause of some forms of congenital muscular dystrophy (Table 1).

Muscle-eye-brain disease [MEB: OMIM 253280, OMIM = Online Mendelian Inheritance in Man (http://www.ncbi.nih.gov/)], is an autosomal recessive disorder characterized by congenital muscular dystrophy, ocular abnormalities and brain malformation (type II lissencephaly). After we screened the sequences of the *POMGnT1* gene for mutations in patients with MEB, we identified 13 independent disease-causing mutations in these patients [17,26]. We have not detected these 13 substitutions in any of 300 normal individuals, indicating that the *POMGnT1* gene is responsible for MEB. To confirm that the mutations observed in patients with MEB are responsible for the defects in the synthesis of *O*-mannosyl glycan, we expressed all of the mutant proteins and found that none of them had enzymatic activity [17,27]. These findings indicate that MEB is inherited as a loss-of-function of the *POMGnT1* gene. If POMGnT1 does not function, no peripheral structure can be formed on *O*-mannose residues. Because these structures are involved in adhesive processes, a defect of *O*-mannosyl glycan may severely affect cell migration and cell adhesion. Additionally, a selective deficiency of glycosylated α-dystroglycan in MEB patients was found [28]. This finding suggests that α-dystroglycan is a potential target of POMGnT1 and that hypoglycosylation of α-dystroglycan may be a pathomechanism of MEB. Thus, MEB muscle and brain phenotypes can be explained by abnormal *O*-mannosylation.

Walker-Warburg syndrome (WWS: OMIM 236670) is another form of congenital muscular dystrophy that is characterized by severe brain malformation and eye anomalies. Patients with WWS are severely affected from birth and usually die within their first year. Recently, 20% of WWS patients (6 of 30 unrelated WWS cases) have been found to have mutations in *POMT1* [29], but none of the 30 cases studied had mutations in another homologue, *POMT2*. This suggests that other as yet unidentified genes are responsible for this syndrome.

In WWS patients, as in MEB patients, the glycosylated α-dystroglycan was selectively deficient in skeletal muscle. WWS and MEB are clinically similar disorders, but WWS is a more severe syndrome than MEB. The difference of severity between the two diseases may be explained as follows. If POMGnT1, which is responsible for the formation of the GlcNAcβ1-2Man linkage of *O*-mannosyl glycans (Figure 1), is non-functional, only *O*-mannose residues may be present on α-dystroglycan in MEB. On the other hand, *POMT1* mutations cause complete loss of *O*-mannosyl glycans in WWS. Thus, it is possible that attachment of a single mannose residue on α-dystroglycan in MEB is responsible for the difference in clinical severity of WWS and MEB.

Interestingly, defective myogenesis in the *Drosophila rt* mutant was found to be due to a mutation in a homologue of *POMT1* [22,30]. Although the *rt* gene product is not known to be a component involved in the initial step of *O*-mannosyl glycan biosynthesis, *O*-mannosylation is an evolutionarily conserved protein modification [9], and may be essential for muscle development in both vertebrates and invertebrates.

Table 1. Possible muscular dystrophies caused by abnormal glycosylation of α-dystroglycan

Condition	*Gene*	*Protein function*
Muscle-eye-brain disease (MEB)	*POMGnT1*	GlcNActransferase
Walker-Warburg syndrome (WWS)	*POMT1*	*O*-Mannosyltransferase
Fukuyama-type congenital muscular dystrophy (FCMD)	*Fukutin*	Putative glycosyltransferase
MDC1C	*FKRP*	Putative glycosyltransferase
Limb-girdle muscular dystrophy 2I (LGMD2I)	(Fukutin-related protein)	
MDC1D	*LARGE*	Putative glycosyltransferase
myd mouse	*large*	

In addition to MEB and WWS, other muscular dystrophies have been suggested to be caused by abnormal glycosylation of α-dystroglycan, *e.g.*, Fukuyama-type congenital muscular dystrophy (FCMD, which is caused by the *fukutin* gene: OMIM 253800), congenital muscular dystrophy type 1C (MDC1C, which is caused by the *FKRP* gene, fukutin-related protein: OMIM 606612) and its allelic disease called limb-girdle muscular dystrophy 2I (LGMD2I: OMIM 607155), congenital muscular dystrophy type 1D (MDC1D, which is caused by the *LARGE* gene), and the myodystrophy (*myd*) mouse, which is caused by the *large* gene (Table 1). Highly glycosylated α-dystroglycan was found to be selectively deficient in the skeletal muscle of these patients and the mouse, as it was in MEB and WWS patients, and the gene products were thought be putative glycosyltransferases [31]. However, it is still unclear whether these gene products are involved in the *O*-mannosyl glycan biosynthesis. Future studies may also reveal that presently uncharacterized forms of muscular dystrophy are caused by defects in other glycosyltransferases. Identification of these defects may provide new clues to the glycopathomechanism of muscular dystrophy.

Perspectives

O-Mannosylation is an uncommon protein modification in mammals, but it is important in muscle and brain development. Further studies are needed to clarify the distribution of *O*-mannosyl glycans in various tissues and to examine their changes during development and pathological conditions. A major challenge will be to integrate the forthcoming structural, cell biological, and genetic information to understand how α-dystroglycan *O*-mannosylation contributes to muscular dystrophy and brain development.

Acknowledgments

This work was carried out in collaboration with many scientists, whose names appear in the list of literature citations. The author gratefully acknowledges the financial support of Research Grants for Nervous and Mental Disorders (14B-4) and Research on Psychiatric and Neurological Diseases and Mental Health from the Ministry of Health, Labour and Welfare of Japan, and a Grant-in-Aid for Scientific Research on Priority Area (14082209) from the Ministry of Education, Culture, Sports, Science and Technology of Japan throughout this project.

References

1 Bruckner K, Perez L, Clausen H, Cohen S, Glycosyltransferase activity of Fringe modulates Notch-Delta interactions, *Nature* **406**, 411–5 (2000).
2 Moloney DJ, Panin VM, Johnston SH, Chen J, Shao L, Wilson R, Wang Y, Stanley P, Irvine KD, Haltiwanger RS, Vogt TF, Fringe is a glycosyltransferase that modifies Notch, *Nature* **406**, 369–75 (2000).
3 Okajima T, Irvine KD, Regulation of notch signaling by O-linked fucose, *Cell* **111**, 893–904 (2002).
4 Shi S, Stanley P, Protein *O*-fucosyltransferase 1 is an essential component of Notch signaling pathways, *Proc Natl Acad Sci USA* **100**, 5234–9 (2003).
5 Strahl-Bolsinger S, Gentzsch M, Tanner W, Protein *O*-mannosylation, *Biochim Biophys Acta* **1426**, 297–307 (1999).
6 Timpel C, Strahl-Bolsinger S, Ziegelbauer K, Ernst JF, Multiple functions of Pmt1p-mediated protein *O*-mannosylation in the fungal pathogen *Candida albicans*, *J Biol Chem* **273**, 20837–46 (1998).
7 Spiro RG, Bhoyroo VD, Studies on the carbohydrate of collagens. Characterization of a glucuronic acid-mannose disaccharide unit from *Nereis* cuticle collagen, *J Biol Chem* **255**, 5347–54 (1980).
8 Finne J, Krusius T, Margolis RK, Margolis RU, Novel mannitol-containing oligosaccharides obtained by mild alkaline borohydride treatment of a chondroitin sulfate proteoglycan from brain, *J Biol Chem* **254**, 10295–300 (1979).
9 Endo T, *O*-Mannosyl glycans in mammals, *Biochim Biophys Acta* **1473**, 237–46 (1999).
10 Winder SJ, The complexities of dystroglycan, *Trends Biochem Sci* **26**, 118–24 (2001).
11 Chiba A, Matsumura K, Yamada H, Inazu T, Shimizu T, Kusunoki S, Kanazawa I, Kobata A, Endo T, Structures of sialylated *O*-linked oligosaccharides of bovine peripheral nerve α-dystroglycan. The role of a novel *O*-mannosyl-type oligosaccharide in the binding of α-dystroglycan with laminin, *J Biol Chem* **272**, 2156–62 (1997).
12 Sasaki T, Yamada H, Matsumura K, Shimizu T, Kobata A, Endo T, Detection of *O*-mannosyl glycans in rabbit skeletal muscle α-dystroglycan, *Biochim Biophys Acta* **1425**, 599–606 (1998).
13 Smalheiser NR, Haslam SM, Sutton-Smith M, Morris HR, Dell A, Structural analysis of sequences *O*-linked to mannose reveals a novel Lewis X structure in cranin (dystroglycan) purified from sheep brain, *J Biol Chem* **273**, 23698–703 (1998).
14 Wing DR, Rademacher TW, Schmitz B, Schachner M, Dwek RA, Comparative glycosylation in neural adhesion molecules, *Biochem Soc Trans* **20**, 386–90 (1992).
15 Yuen CT, Chai W, Loveless RW, Lawson AM, Margolis RU, Feizi T, Brain contains HNK-1 immunoreactive *O*-glycans of the sulfoglucuronyl lactosamine series that terminate in 2-linked or 2,6-linked hexose (mannose), *J Biol Chem* **272**, 8924–31 (1997).
16 Chai W, Yuen CT, Kogelberg H, Carruthers RA, Margolis RU, Feizi T, Lawson AM, High prevalence of 2-mono- and 2,6-di-substituted manol-terminating sequences among *O*-glycans released from brain glycopeptides by reductive alkaline hydrolysis, *Eur J Biochem* **263**, 879–88 (1999).
17 Yoshida A, Kobayashi K, Manya H, Taniguchi K, Kano H, Mizuno M, Inazu T, Mitsuhashi H, Takahashi S, Takeuchi M, Herrmann R, Straub V, Talim B, Voit T, Topaloglu H, Toda T, Endo T, Muscular dystrophy and neuronal migration disorder caused by mutations in a glycosyltransferase, POMGnT1, *Dev Cell* **1**, 717–24 (2001).
18 Taniguchi N, Honke K, Fukuda M, *Handbook of Glycosyltransferases and Related Genes* (Springer-Verlag, Tokyo, 2002).
19 Takahashi S, Sasaki T, Manya H, Chiba Y, Yoshida A, Mizuno M, Ishida H, Ito F, Inazu T, Kotani N, Takasaki S, Takeuchi M, Endo T, A new β-1,2-*N*-acetylglucosaminyltransferase that may play a role in the biosynthesis of mammalian *O*-mannosyl glycans, *Glycobiology* **11**, 37–45 (2001).

20 Inamori KI, Endo T, Gu J, Matsuo I, Ito Y, Fujii S, Iwasaki H, Narimatsu H, Miyoshi E, Honke K, Taniguchi N, N-Acetylglucosaminyltransferase IX acts on the GlcNAcβ1,2-Manα1-Ser/Thr moiety, forming a 2,6-branched structure in brain *O*-mannosyl glycan, *J Biol Chem* **279**, 2337–40 (2004).

21 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* **58**, 171–80 (1999).

22 Willer T, Amselgruber W, Deutzmann R, Strahl S, Characterization of POMT2, a novel member of the *PMT* protein *O*-mannosyltransferase family specifically localized to the acrosome of mammalian spermatids, *Glycobiology* **12**, 771–83 (2002).

23 Manya H, Chiba A, Yoshida A, Wang X, Chiba Y, Jigami Y, Margolis RU, Endo T, Demonstration of mammalian protein *O*-mannosyltransferase activity: Coexpression of POMT1 and POMT2 required for enzymatic activity, *Proc Natl Acad Sci USA* **101**, 500–5 (2004).

24 Girrbach V, Strahl S, Members of the evolutionarily conserved PMT family of protein *O*-mannosyltransferases form distinct protein complexes among themselves, *J Biol Chem* **278**, 12554–62 (2003).

25 Burton EA, Davies KE, Muscular dystrophy–reason for optimism? *Cell* **108**, 5–8 (2002).

26 Taniguchi K, Kobayashi K, Saito K, Yamanouchi H, Ohnuma A, Hayashi YK, Manya H, Jin DK, Lee M, Parano E, Falsaperla R, Pavone P, Van Coster R, Talim B, Steinbrecher A, Straub V, Nishino I, Topaloglu H, Voit T, Endo T, Toda T, Worldwide distribution and broader clinical spectrum of muscle-eye-brain disease, *Hum Mol Genet* **12**, 527–34 (2003).

27 Manya H, Sakai K, Kobayashi K, Taniguchi K, Kawakita M, Toda T, Endo T, Loss-of-function of an *N*-acetylglucosaminyltransferase, POMGnT1, in muscle-eye-brain disease, *Biochem Biophys Res Commun* **306**, 93–7 (2003).

28 Kano H, Kobayashi K, Herrmann R, Tachikawa M, Manya H, Nishino I, Nonaka I, Straub V, Talim B, Voit T, Topaloglu H, Endo T, Yoshikawa H, Toda T, Deficiency of α-dystroglycan in muscle-eye-brain disease, *Biochem Biophys Res Commun* **291**, 1283–6 (2002).

29 Beltrán-Valero de Bernabé D, Currier S, Steinbrecher A, Celli J, van Beusekom E, van der Zwaag B, Kayserili H, Merlini L, Chitayat D, Dobyns WB, Cormand B, Lehesjoki AE, Cruces J, Voit T, Walsh CA, van Bokhoven H, Brunner HG, Mutations in the *O*-mannosyltransferase gene *POMT1* give rise to the severe neuronal migration disorder Walker-Warburg syndrome, *Am J Hum Genet* **71**, 1033–43 (2002).

30 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 USA* **93**, 6048–52 (1996).

31 Michele DE, Campbell KP, Dystrophin-glycoprotein complex: post-translational processing and dystroglycan function, *J Biol Chem* **278**, 15457–60 (2003).

Received 14 April 2004; accepted 7 May 2004