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Petri Net Representation and Analysis of Mannose Type O-Glycan Biosynthesis

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We provide a model and analysis of mannose type O-glycan biosynthesis. Synthesis of Core M1, M2 and M3 glycans is a complex biochemical pathway with numerous interdependent processes. We used Petri nets mathematical formalism to construct the synthesis and extension of Core M1, M2 and M3 glycans. Our analysis show that (Man)1 (Ser/Thr)1 is a critically important substrate for synthesis of all three types of glycans. Gene mutations in POMT1/POMT2 {1'} enzyme lead to muscular dystrophies type A, B and C, congenital muscular dystrophies (CMDs) and limb-girdle muscular dystrophy (LGMD). Core M1 [(Gal)1 (GlcNAc)1 (Man)1 (Ser/Thr)1] and Core M2 [(Gal)2 (GlcNAc)2 (Man)1 (Ser/Thr)1] glycans are also indispensable, as gene mutations in {3'} and {5'}, involved in Core M1 and M2 synthesis, bring forward congenital disorders of glycosylation (CDG) type II.

Key words: O-type glycosilation, Petri nets

Introduction

Biosynthesis of mammalian O-mannosyl glycans is initiated by the transfer of mannose from mannose-P-Dol to serine or threonine residue, followed by extensions with N-acetylglucosamine (GlcNAc) and galactose (Gal) to generate core M1, M2 and M3 glycans. Core M1 and M2 glycans can then be further attached by fucose residues, sialic acid and sulfated glucuroinc acid. Core M3 glycan is involved in the synthesis of alpha-dystroglycan, a heavily glycosylated protein found in muscle and brain tissues. Core M3 glycan contains a tandem repeat of ribitol 5-phosphate (Rbo5P) and -alpha3-GlcA-beta3-Xyl- repeating structures. Defects of genes encoding core glycans and modified core M3 glycans are associated with various congenital diseases, such as muscular dystrophies caused by reduced O-mannosylation of alpha-dystroglycan in skeletal muscles.

Computational systems biology, which is a sub-discipline of systems biology, has developed both as a tool supporting the processing of accumulated biological data and as a modeling discipline, building upon this data in order to predict biological behavior [7], [1]. Qualitative models of biochemical networks are a central component of modern systems biology. Building and managing these complex models is a major challenge that can benefit from the application of formal methods adopted from theoretical

computing science [3]. Petri nets provide graphical representation of the biochemical networks, from which it is possible to perform different kinds of analysis [12]. Petri nets are bipartite graphs with two types of nodes, one corresponding to molecules ('places'), the other corresponding to reactions ('transitions') [4]. The arcs (edges) connecting the nodes encode information about reaction, and involved substrates and products.

Material and Methods

The main source of pathway information was mannose type O-glycan biosynthesis pathway represented in KEGG/ENZYME database. Bipartite Petri graphs G = (V1, V2; E) were constructed from two disjoint sets of nodes, called places (V = P) and transitions (V = T), V1, $\in$ V2 =V, which are connected by edges e $\in$ E $\subseteq$ V. The input range I (x) of an element x $\in$ P $\in$ T of a Petri net is given by I (x) ={y|(y, x) $\in$ E}, the output range as O(x) ={y|(x, y) $\in$ E} [9] and [10].

Results

The mannose type O-glycan biosynthesis pathway starts with addition of dolichyl phosphate D-mannose to [Protein]-L-serine or [Protein]-L-threonine by dolichyl beta - D - mannosyl - phosphate: L - threonyl/L - seryl - [protein] O-D-mannosyltransferase {1'}, see **Fig.1.** Man-β-Dol is available through the N-glycan biosynthesis pathway. Deficiency of Dol-P-Man synthase subunit DPM3 bridges the congenital disorders of glycosylation with the dystroglycanopathies. Investigation of the Dol-P-Man-dependent glycosylation pathway in the ER reveal strongly reduced O-mannosylation of alpha-dystroglycan in a muscle biopsy, thereby explaining the clinical phenotype of muscular dystrophy [14].

Core M3 is synthesized from (Man)1 (Ser/Thr)1 {3} with the help of two enzymes: UDP-N-acetyl-alpha-D-glucosamine:alpha-D-mannosyl-threonyl-[protein] 4 - beta - N - acetyl - D - glucosaminyltransferase [[EC:2.4.1.312] and UDP-N - acetyl - alpha - D - galactosamine: N - acetyl - beta - D - glucosaminyl - (1->4) - alpha - D - mannosyl - threonyl - [protein]3 - beta - N-acetyl - D - galactosaminyltransferase [EC:2.4.1.313], see **Table 1.**

Core M3 is converted by ATP: O3 - [N-acetyl-beta-D-galactosaminyl-(1->3) - N-acetyl - beta - D-glucosaminyl - (1->4) - alpha - D - mannosyl] - L - threonyl/L-seryl-[protein] 6 - phosphotransferase {3.3'} to (GalNAc)1 (GlcNAc)1 (Man)1 (P)1 (Ser/Thr)1 {3.3}. This product and D-ribitol {3.6} are substrates for FKTN {3.5'}. Next, by a series of enzymes, Core M3 is extended to the final product of this branch of mannose type O-glycan biosynthesis, namely α-Dystroglycan.

Next the enzyme, beta-1,2-N-acetylglucosaminyltransferase {2'}, branches the pathway leading to synthesis of Core M1 and M2 glycans, see **Fig. 1.**

Synthesis of Core M1 glycans start from (ClcNAc)1(Man)1(Ser/Thr) [5] through action of B4GALT1 (beta-1,4-galactosyltransferase 1 [EC:2.4.1.22 2.4.1.90 2.4.1.38 2.4.1.-]), see **Table 2.**

Core M2 glycans are not obtained directly from (ClcNAc)1(Man)1(Ser/Thr) [4]. First (ClcNAc)1(Man)1(Ser/Thr) is transformed to (GlcNAc)2 (Man)1 (Ser/Thr)1 [13] by MGAT5B (mannosyl alpha-1,6-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase, isozyme B [EC:2.4.1.-]) {4'}. Core M2 are synthesized from (GlcNAc)2 (Man)1 (Ser/Thr)1 with the aid of B4GALT1 (beta-1,4-galactosyltransferase 1 [EC:2.4.1.22 2.4.1.90 2.4.1.38 2.4.1.-]), see **Table 3.**

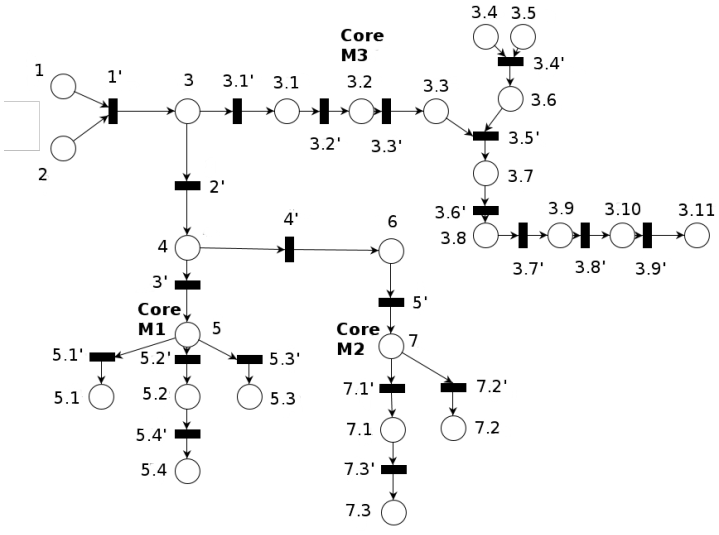

Fig. 1. Petri net representation of mannose type O-glycan biosynthesis pathway

Table 1. Reactions involved in synthesis and extension of Core M3 glycans. Note: Substrates and products are given in curly brackets. Enzymes are given in curly brackets, preceded and followed by '=>'. Numbers in curly brackets correspond to those in Fig. 1.

1'	Dolichyl phosphate D-mannose {1} + [Protein]-L-serine {2} => dolichyl-phosphate-mannose-protein mannosyltransferase [EC:2.4.1.109] {1'} => (Man)1 (Ser/Thr)1 {3} Dolichyl phosphate D-mannose {1} + [Protein]-L-threonine {2} => {1'} => (Man)1 (Ser/Thr)1 {3}
2'	UDP-N-acetyl-alpha-D-glucosamine + (Man)1 (Ser/Thr)1 {3} => protein O-mannose beta-1,4-N-acetylglucosaminyltransferase [EC:2.4.1.312] {3.1'} => UDP + (GlcNAc)1 (Man)1 (Ser/Thr)1 {3.1}
3'	UDP-N-acetyl-alpha-D-galactosamine + [(GlcNAc)1 (Man)1 (Ser/Thr)1] {3.1} => beta-1,3-N-acetylgalactosaminyltransferase 2 [EC:2.4.1.313] {3.2'} => UDP + (GalNAc)1 (GlcNAc)1 (Man)1 (Ser/Thr)1 {3.2} **[Core M3]**
4'	ATP + (GalNAc)1 (GlcNAc)1 (Man)1 (Ser/Thr)1 {3.2}=> glycoprotein-mannosyl O6-kinase [EC:2.7.1.183] {3.3'} => ADP + (GalNAc)1 (GlcNAc)1 (Man)1 (P)1 (Ser/Thr)1 {3.3}
5'	CTP {3.4} + D-ribitol 5-phosphate {3.5} => D-ribitol-5-phosphate cytidylyltransferase [EC:2.7.7.40] {3.4'} => diphosphate + CDP-ribitol {3.6}
6'	(GalNAc)1 (GlcNAc)1 (Man)1 (P)1 (Ser/Thr)1 {3.3} + CDP-ribitol {3.6} => fukutin [EC:2.7.8.-] {3.5'} => (GalNAc)1 (GlcNAc)1 (Man)1 (Rib-ol)1 (P)2 (Ser/Thr)1 {3.7}
7'	(GalNAc)1 (GlcNAc)1 (Man)1 (Rib-ol)1 (P)2 (Ser/Thr)1 {3.7} => fukutin-related protein [EC:2.7.8.-] {3.6'} => (GalNAc)1 (GlcNAc)1 (Man)1 (Rib-ol)2 (P)3 (Ser/Thr)1 {3.8}
8'	(GalNAc)1 (GlcNAc)1 (Man)1 (Rib-ol)2 (P)3 (Ser/Thr)1 {3.8} => ribitol beta-1,4-xylosyltransferase [EC:2.4.2.-] {3.7'} => (GalNAc)1 (GlcNAc)1 (Man)1 (Rib-ol)2 (Xyl)1 (P)3 (Ser/Thr)1 {3.9}
9'	(GalNAc)1 (GlcNAc)1 (Man)1 (Rib-ol)2 (Xyl)1 (P)3 (Ser/Thr)1 {3.9} => beta-1,4-glucuronyltransferase 1 [EC:2.4.1.-] {3.8'} => (GalNAc)1 (GlcA)1 (GlcNAc)1 (Man)1 (Rib-ol)2 (Xyl)1 (P)3 (Ser/Thr)1 {3.10}
10'	(GalNAc)1 (GlcA)1 (GlcNAc)1 (Man)1 (Rib-ol)2 (Xyl)1 (P)3 (Ser/Thr)1 {3.10} => glycosyltransferase-like protein LARGE [EC:2.4.2.- 2.4.1.-] {3.9'} => alpha-Dystroglycan {3.11}

Table 2. Reactions involved in synthesis and extension of Core M1 glycans. Note: Substrates and products are given in curly brackets. Enzymes are given in curly brackets, preceded and followed by '=>'. Numbers in curly brackets correspond to those in Fig. 1.

2'	UDP-GlcNAc + (Man)1(Ser/Thr) {3} => beta-1,2-N-acetylglucosaminyltransferase [EC:2.4.1.-] {2'} => UDP + (ClcNAc)1(Man)1(Ser/Thr) {4}
3'	(ClcNAc)1(Man)1(Ser/Thr) {4} => beta-1,4-galactosyltransferase 1 [EC:2.4.1.22 2.4.1.90 2.4.1.38 2.4.1.-] {3'} => (Gal)1 (GlcNAc)1 (Man)1 (Ser/Thr)1 **[Core M1]** {5}
5.1'	CMP-N-acetyl-beta-neuraminate + (Gal)1 (GlcNAc)1 (Man)1 (Ser/Thr)1 {5} => neolactotetraosylceramide alpha - 2,3 - sialyltransferase (sialyltransferase 6) [EC:2.4.99.6] {5.1'} => CMP + (Sia)1(Gal)1 (GlcNAc)1 (Man)1 (Ser/Thr)1 {5.1}
5.2'	UDP-alpha-D-glucuronate + (Gal)1 (GlcNAc)1 (Man)1 (Ser/Thr)1 {5} => galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase 1 [EC:2.4.1.135] {5.2'} => UDP + [protein]-3-O-(beta-D-GlcA-(1->3)-beta-D-Gal-(1->3)-beta-D-Gal-(1->4)-beta-D-Xyl)-L-serine {5.2}
5.3'	GDP-beta-L-fucose + (Gal)1 (GlcNAc)1 (Man)1 (Ser/Thr)1 {5} => 4-galactosyl-N-acetylglucosaminide 3-alpha-L-fucosyltransferase [EC:2.4.1.152] {5.3'} => GDP + (Gal)1 (GlcNAc)1 (LFuc)1 (Man)1 (Ser/Thr)1 {5.3}
5.4'	[protein]-3-O-(beta-D-GlcA-(1->3)-beta-D-Gal-(1->3)-beta-D-Gal-(1->4)-beta-D-Xyl)-L-serine {5.2} => carbohydrate 3-sulfotransferase 10 [EC:2.8.2.-] {5.4'} => (Gal)1 (GlcA)1 (GlcNAc)1 (Man)1 (S)1 (Ser/Thr)1 [5.4]

Table 3. Reactions involved in synthesis and extension of Core M2 glycans. Note: Substrates and products are given in curly brackets. Enzymes are given in curly brackets, preceded and followed by '=>'. Numbers in curly brackets correspond to those in Fig. 1.

4'	(ClcNAc)1(Man)1(Ser/Thr) {4} => mannosyl alpha-1,6-glycoprotein beta-1,6-N-acetylglucosaminyltransferase, isozyme B [EC:2.4.1.-] {4'} => (GlcNAc)2 (Man)1 (Ser/Thr)1 {6}
5'	(GlcNAc)2 (Man)1 (Ser/Thr)1 {6} => beta-1,4-galactosyltransferase 1 [EC:2.4.1.22 2.4.1.90 2.4.1.38 2.4.1.-] {5'} => (Gal)2 (GlcNAc)2 (Man)1 (Ser/Thr)1 {7} **[Core M2]**
7.1'	(Gal)2 (GlcNAc)2 (Man)1 (Ser/Thr)1 {7} **[Core M2]** => galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase 1 [EC:2.4.1.135] {7.1'} => (Gal)2 (GlcA)1 (GlcNAc)2 (Man)1 (Ser/Thr)1 {7.1}
7.2'	(Gal)2 (GlcNAc)2 (Man)1 (Ser/Thr)1 {7} **[Core M2]** => neolactotetraosylceramide alpha-2,3-sialyltransferase (sialyltransferase 6) [EC:2.4.99.6] {7.2'} => (Gal)2 (GlcNAc)2 (Man)1 (Neu5Ac)2 (Ser/Thr)1 {7.2}
7.3'	(Gal)2 (GlcA)1 (GlcNAc)2 (Man)1 (Ser/Thr)1 {7.1} => carbohydrate 3-sulfotransferase 10 [EC:2.8.2.-] {7.3'} => (Gal)2 (GlcA)1 (GlcNAc)2 (Man)1 (S)1 (Ser/Thr)1 {7.3}

Discussion

Muscular dystrophies due to reduced glycosylation of alpha-dystroglycan are a common group of conditions, referred to as dystroglycanopathies. The most severe clinical spectrum (type A) are characterized by congenital muscular dystrophy with severe structural brain and eye abnormalities. Muscular dystrophy-dystroglycanopathy type B (MDDGB) is less severe and is characterized by early onset of muscle weakness, mental retardation, and mild brain anomalies. The mildest form (type C) are limb-girdle muscular dystrophy. Gene mutations of O-D-mannosyltransferase {1'} lead to muscular dystrophy-dystroglycanopathy type A [8], B [4] and C [2]. Another class of diseases connected with mannose type O-glycan biosynthesis are congenital muscular dystrophies (CMDs). They are a heterogeneous group of inherited disorders characterized by muscle weakness from birth and variable clinical manifestations of the eye and central nervous system. Defects in genes encoding for POMT1/POMT2 {1'} is a cause for CMDs [6]. Limb-girdle muscular dystrophy (LGMD) is also associated with disruption of the O-glycan biosynthesis pathway. It is a heterogeneous group of inherited disorders characterized by progressive muscle weakness that begins from the proximal limb muscles. Gene mutations of O-D-mannosyltransferase {1'} lead to LGMD [11]. This points out the role of (Man)1 (Ser/Thr)1, which is a product of O-D-mannosyltransferase, and starting point for synthesis of Core M1, M2 and M3 glycans.

Gene mutations in beta-1,4-N-acetylglucosaminyltransferase {3.1'} and beta-1,3-N-acetylgalactosaminyltransferase 2 {3.2'} lead to muscular dystrophy-dystroglycanopathy type A. In the first case beta - 1,4 - N - acetylglucosaminyltransferase obtain (Man)1 (Ser/Thr)1 [3], as we suppose in this case that O-D-mannosyltransferase is functioning normally. Muscular dystrophy-dystroglycanopathy type A is manifested if either the substrate of beta-1,3-N-acetylgalactosaminyltransferase 2 or its product are absent. So [(GlcNAc)1 (Man)1 (Ser/Thr)1] {3.1} or (GalNAc)1 (GlcNAc)1 (Man)1 (Ser/Thr)1 {3.2} [Core M3] are necessary to avoid manifestations of this disease.

Gene mutations in beta-1,2-N-acetylglucosaminyltransferase [EC:2.4.1.-] {2'} lead to same diseases, as mutations in O-D-mannosyltransferase gene, pointing to the role of (ClcNAc)1(Man)1(Ser/Thr) {4}. It appears that ClcNAc attached to (Man)1(Ser/Thr) is necessary to prevent above mentioned diseases. (ClcNAc)1(Man)1(Ser/Thr) is also a branching point for synthesis of Core M1 and Core M2 glycans.

Gene mutations in {3'} and {5'}, involved in Core M1 and M2 synthesis, bring forward a new disorder: congenital disorders of glycosylation (CDG) type II [6]. Multiple subtypes have been identified. In contrast to type I, the type II patients show a more severe psychomotor retardation, no peripheral neuropathy and a cerebellar hypoplasia. Consequently, both Core M1 [((Gal)1 (GlcNAc)1 (Man)1 (Ser/Thr)1)] and Core M2 [((Gal)2 (GlcNAc)2 (Man)1 (Ser/Thr)1)] are essential for prevention of this congenital disorder. Although not mentioned in KEGG database, gene mutations in {1'} and {2'} should also disrupt synthesis of Core M1 an M2 glycans, and consequently have the same pathological manifestation as mutations in {3'} and {5'}. But obviously presence of Man and/or ClcNAc attached to (Ser/Thr)1 are essential for prevention of muscular dystrophy-dystroglycanopathy type A, B and C, congenital muscular dystrophies (CMD/MDC) and limb-girdle muscular dystrophy (LGMD), but not congenital disorders of glycosylation (CDG) type II.

Conclusions:

Petri nets can be used to establish connections between pathology and chemical structure. In this way (Man)1 (Ser/Thr)1 is essential for prevention of muscular dystrophy-dystroglycanopathy types A, B and C. Both Core M1 [((Gal)1 (GlcNAc)1 (Man)1 (Ser/Thr)1)] and Core M2 [((Gal)2 (GlcNAc)2 (Man)1 (Ser/Thr)1)] glycans are essential for prevention of congenital disorders of glycosylation (CDG) type II. In summary, the presence of Man and/or ClcNAc attached to (Ser/Thr)1 are essential for prevention of muscular dystrophy-dystroglycanopathy type A, B and C, congenital muscular dystrophies (CMD/MDC) and limb-girdle muscular dystrophy (LGMD), but not congenital disorders of glycosylation (CDG) type II.

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