Genomics 92 (2008) 159–167

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Genes required for functional glycosylation of dystroglycan are conserved in zebrafish ☆

Christopher J. Moore, Huey Tse Goh, Jane E. Hewitt *

Institute of Genetics, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH, UK

ARTICLE INFO

Article history:
Received 15 March 2007
Accepted 14 May 2008
Available online 15 July 2008

Keywords:
Dystroglycan
Glycosylation
Zebrafish
Comparative mapping
Glycosyltransferase
Dystroglycanopathy
Muscular dystrophy

ABSTRACT

Mutations in human genes encoding proteins involved in α-dystroglycan glycosylation result in dystroglycanopathies: severe congenital muscular dystrophy phenotypes often accompanied by CNS abnormalities and ocular defects. We have identified the zebrafish orthologues of the seven known genes in this pathway and examined their expression during embryonic development. Zebrafish *Large, POMT1, POMT2, POMGnT1, Fukutin,* and *FKRP* show in situ hybridization patterns similar to those of dystroglycan, with broad expression throughout early development. By 30 h postfertilization (hpf), transcripts of all these genes are most prominent in the CNS, eye, and muscle, tissues that are predominantly affected in the dystroglycanopathies. In contrast, *Large2* expression is more restricted and by 30 hpf is confined to the lens, cerebellum, and pronephric duct. We show that the monoclonal antibody IIH6, which recognizes a glycoform of dystroglycan, also detects the zebrafish protein. Injection of morpholino oligonucleotides against zebrafish *Large2* resulted in loss of IIH6 immunostaining. These data indicate that the dystroglycan glycosylation pathway is conserved in zebrafish and suggest this organism is likely to be a useful model system for functional studies.

Glycosylation is an important but poorly understood posttranslation modification implicated in many biological processes, including development, immunology, and cell recognition. It has been estimated that 1% of human genes encode enzymes involved in oligosaccharide synthesis and function [1]. Mutations in six human genes encoding proteins known or predicted to be involved in glycosylation processes (*POMT1, POMT2, POMGnT1, Fukutin, FKRP,* and *LARGE*) have been shown to produce severe congenital muscular dystrophy (CMD) phenotypes [2,3]. These dystrophies are often accompanied by neuronal migration abnormalities, cardiomyopathy, and ocular defects [2,3]. Mutation of the mouse orthologue of *Large* results in a muscular and neuronal phenotype (the Large^{myd} mutant) that shows many similarities to these CMDs [4,5].

These forms of muscular dystrophy are collectively termed "dystroglycanopathies" as they are all associated with hypoglycosylation of α-dystroglycan (α-DG), a key component of the dystrophin-associated glycoprotein complex (DGC). In skeletal muscle the DGC links cytoskeletal actin to laminin in the extracellular matrix [6]. However, the DGC is also found in many other tissues and cell types, including brain, peripheral nerve, and kidney, where it contributes to processes such as neuronal migration and basement membrane formation [7]. In mice, null mutations for dystroglycan (*Dag1*) display early embryonic lethality [8].

In the dystroglycanopathies, a hypoglycosylated form of α-DG is produced that is deficient in binding to extracellular matrix ligands such as laminin and agrin [5,9]. A key role for α-DG in the pathological mechanism of these disorders is supported by gene knockout studies in mice. Selective deletion of dystroglycan in the CNS produces brain abnormalities similar to those seen in the human disorders and the Large^{myd} mouse [10], while deficiency in skeletal muscle results in a progressive muscular dystrophy [11].

Dystroglycan is synthesized as a precursor protein that is posttranslationally cleaved into α and β subunits [6]. α-DG has an apparent molecular mass varying from 120 kDa in brain and peripheral nerve to >150 kDa in skeletal and cardiac muscle, due to tissue-specific differences in the extent and type of glycosylation [6]. The protein contains a central mucin-like domain that undergoes extensive O-glycosylation, including the addition of unusual *O*-mannosyl-type oligosaccharide structures [12]. O-mannosylation is rare in mammals and has been identified in only a limited number of glycoproteins (including dystroglycan) in brain, nerve, and skeletal muscle [12]. Several of the genes implicated by genetic studies to be required for functional dystroglycan glycosylation have biochemical activities that are involved in biosynthesis of *O*-mannose glycans. *POMT1* and *POMT2* encode protein *O*-mannosyltransferases, appearing to function as a complex [13]. POMGnT1 encodes the protein O-linked mannose β1,2-*N*-acetylglucosaminyltransferase, which transfers *N*-acetylglucosamine from UDP-GlcNAc to *O*-mannosyl glycoproteins, thus acting

☆ Sequence data from this article have been deposited with the GenBank Data Library under Accession Nos. DQ826745 (*Fukutin*), DQ826746 (*FKRP*), DQ826747 (*POMGnT1*), DQ826748 (*POMT1*), and DQ826749 (*POMT2*).

* Corresponding author. Fax: +44 115 823130.
E-mail address: jane.hewitt@nottingham.ac.uk (J.E. Hewitt).

0888-7543/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygeno.2008.05.008

Table 1
Dystroglycanopathy-associated genes

Gene	Organism	Gene size (kb)	Genomic location (Mb)
FKRP	Human	2	Chr 19q13.32 (51.9)
	Zebrafish	2	Chr 15 (26.6)
Fukutin	Human	60	Chr 9q31.2 (107.4)
	Zebrafish	12	Chr 5 (78.5)
POMGnT1	Human	9	Chr 1p34.1 (46.4)
	Zebrafish	17	Chr 6 (69.0)
POMT1	Human	19	Chr 9q34.13 (134.4)
	Zebrafish	20	Chr 5 (56.3)
POMT2	Human	43	Chr 14q24.3 (76.8)
	Zebrafish	15	Chr 14 (39.2)

The gene sizes and genomic locations of the human and zebrafish dystroglycanopathy-associated genes are presented. Zebrafish gene locations are according to Ensembl version 40.

downstream of POMT1. However, the biochemical functions of Fukutin, FKRP, and Large are currently unknown.

The use of mice for genetic dissection of this glycosylation pathway is both time consuming and expensive. Furthermore, because *Fukutin, Dag1,* and *POMT1* null mutations in mice all result in embryonic lethality [8,14,15], it has proved difficult to study the roles of α-DG glycosylation in muscular dystrophies through this approach. The zebrafish is an attractive alternative model system for investigation of the functional relationships between these genes, especially during early development. An advantage over the mouse is that in zebrafish, basement membrane formation is not a crucial step until organogenesis is under way [16], therefore early embryonic development is not as severely affected by loss of basement membrane as it is in mice. Muscle structure and embryonic studies are also well suited to zebrafish due to their translucent early developmental stages and the high proportion of muscle tissue in embryos [17]. DGC has been well characterized in zebrafish [18–20] and morpholino oligonucleotide (MO) knockdowns or mutants have been produced for dystrophin [21], dystroglycan [16], and laminins β1 and γ1 [22,23]. In addition, this organism is particularly suited to large-scale screens for modifier genes and testing of potential therapeutic reagents [24].

We have previously shown that *Large* and its vertebrate paralogue *Large2* are conserved in zebrafish [25]. Here we show that the other known members of this pathway are also conserved in this species, indicating that the zebrafish is likely to be a useful model organism for future functional studies of these genes.

Results

Identification of dystroglycanopathy genes in zebrafish

Genes were identified by TBLASTN searches of the zebrafish genome assembly and EST databases with the human amino acid sequence followed by detailed analysis of genomic hits. Single orthologues were identified for all of the genes investigated (*POMT1, POMT2, POMGnT1, Fukutin,* and *FKRP;* Table 1). We carried out a phylogenetic analysis of the POMT1 and POMT2 proteins to confirm the gene assignments as they are very closely related. An unrooted neighbor-joining tree is shown in Supplemental Fig. 1. The POMT1 and POMT2 proteins form two distinct groups that are well separated in the tree by a long branch, confirming the correct classification of the zebrafish genes.

EST analysis and RT-PCR were used to confirm the exon structure and the coding sequence of each gene. The structures of the zebrafish genes are compared to those of their human orthologues in Fig. 1. The coding exon size and splice site locations are generally well conserved between human and zebrafish. However, there is very little conservation of intron sizes and UTRs; for example, zebrafish *Fukutin*(*fktn*) and *POMT2* both have much smaller 3′UTRs than their corresponding human genes. We confirmed that the zebrafish 3′UTR spans at least 700 bp by RT-PCR (primer sequences are provided in Table 2). As there are three potential poly(A) adenylation sites within this 700-bp region, there may be alternative 3′ ends of the mRNA. This predicted 3′ UTR also contains a short interspersed nuclear element and an ANGEL transposon element.

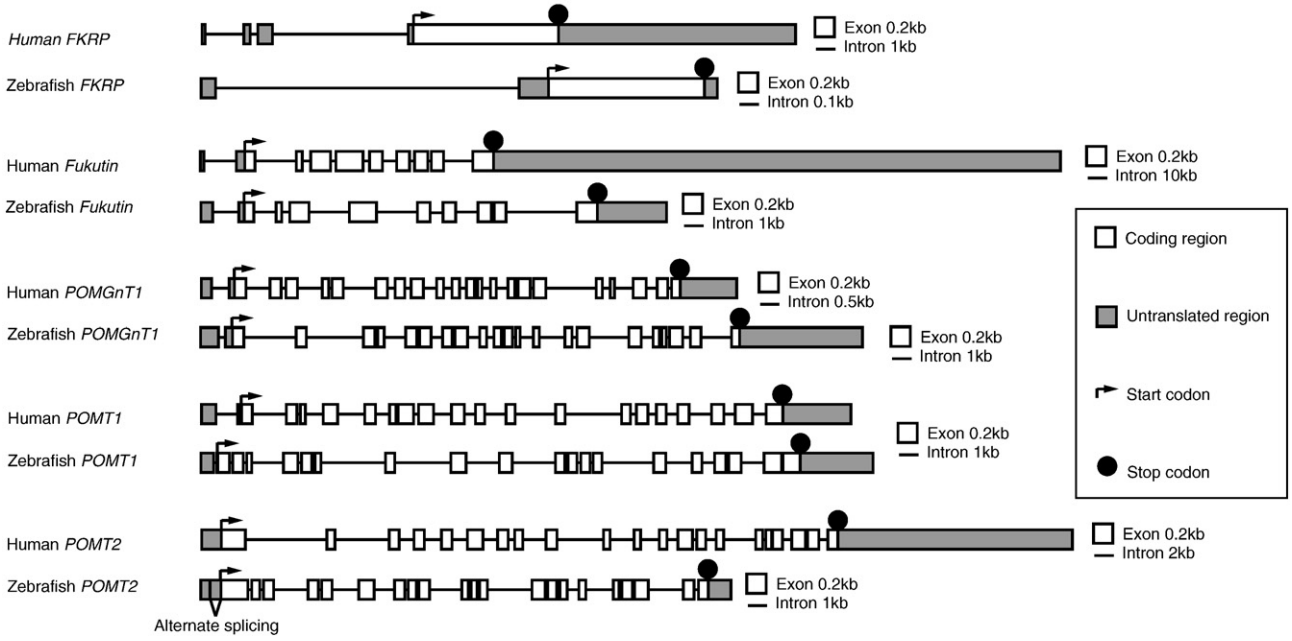

Fig. 1. Zebrafish and human gene structures. Stick and block representations of the exon/intron structures of human and zebrafish *FKRP, Fukutin, POMGnT1, POMT1,* and *POMT2* genes. UTRs and start and stop codons are marked on the genes, scale bars are shown to the right of each gene. For *POMT1* both the human and the zebrafish use the same scale. Two alternative splice products for the 5′UTR of zebrafish *POMT2* were identified; however, these do not alter the amino acid sequence of the protein.