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Review

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.

Contents

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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-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 protein O-mannosyltransferases (POMTs) 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]. POMTs 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 chaperones [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. Coexpression 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 the molecular mechanism of O-mannosylation among a broad family of eukaryotic O-mannosyltransferases.

Intriguingly, a mouse POMT2 splicoform 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 β1,2-N-acetylglucosaminyltransferase that elongates O-linked mannose with a β1,2-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