第３回
山川民夫賞
受賞講演

Tamio Yamakawa Award

Dec.7 09:45 - 10:30

Kevin P. Campbell

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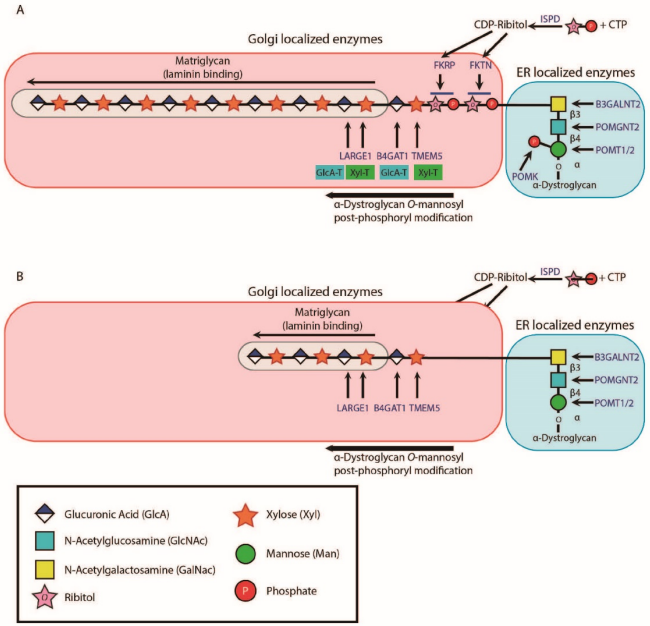
Kevin P. Campbell, Ph.D., is an investigator with the Howard Hughes Medical Institute, Director of the Senator Paul D. Wellstone Muscular Dystrophy Specialized Research Center, and Chair, Department of Molecular Physiology and Biophysics at the Carver College of Medicine, University of Iowa. He is widely recognized for his fundamental contributions to neuromuscular disease research. His work has led to the identification of the molecular and genetic basis of several forms of muscular dystrophy and has provided a clearer understanding of muscular dystrophy disease processes. Campbell discovered dystroglycan and elucidated its function as an extracellular matrix receptor. He established that dystroglycan is involved in a variety of physiological and developmental processes, including maintenance of skeletal muscle function as well as formation and function of the central nervous system. He showed that complex post-translational processing of dystroglycan, including extensive glycosylation, is required for its ability to function as an extracellular matrix receptor and that abnormal glycosylation results in a variety of congenital and limb-girdle muscular dystrophies with or without associated defects in brain development and function. Campbell's findings have already greatly improved the diagnosis of muscular dystrophy, and they point to strategies for developing therapies for these devastating inherited neuromuscular diseases. ~450 publications; Google h-index = 154; i10-index = 463

Structural Basis of LARGE1-Mediated Elongation of Matriglycan on Dystroglycan

Dystroglycan is a widely expressed plasma membrane protein that requires extensive post-translational processing and glycosylation to function as a receptor for laminin-G-like (LG) domain extracellular matrix (ECM) proteins including laminin, agrin and perlecan. Matriglycan is a linear repeating disaccharide of alternating xylose and glucuronic acid that is synthesized by the like-acetylglucosaminyltransferase-1 (LARGE1) on α-dystroglycan and binds LG domain ECM proteins. Addition of matriglycan enables α-dystroglycan to serve as the predominant ECM receptor in skeletal muscle and brain. Abnormalities in the post-translational processing of α-dystroglycan that result in an absence or reduction of matriglycan cause various forms of muscular dystrophy, known as dystroglycanopathies, with or without brain and eye involvement.

We have used a multidisciplinary approach to provide an atomic-level insight into the structure that is essential for the binding of matriglycan to LG domain ECM proteins. We used purified LARGE1 to synthesize matriglycan chains of defined lengths in sufficient quantities to perform both biochemical and structural studies. Using NMR spectroscopy, we found that matriglycan bound to laminin-α2 LG4,5 with high affinity (KD=0.23 μM) and that this binding was calcium dependent. Next, we initiated a collaboration with Erhard Hohenester, who had previously crystallized laminin-α2 LG4,5. Dr. Hohenester soaked matriglycan into crystals of LG4,5 to produce a high-resolution crystal structure of LG4,5 bound to matriglycan. This analysis revealed that the LG4 domain is a Ca^{2+}-dependent lectin with specificity for GlcA-β1,3-Xyl disaccharides, and that a single GlcA-β1,3-Xyl disaccharide repeat straddles a Ca^{2+} ion in the LG4 domain, with oxygen atoms from both sugars replacing Ca^{2+}-bound water molecules. This chelating binding mode is unprecedented among animal lectins and accounts for the high affinity of this protein-carbohydrate interaction. The multiple tandem repeats in matriglycan are predicted to increase the apparent affinity of the protein for LG domains by favoring rapid rebinding after dissociation. These results shed light on the mechanism of protein-carbohydrate interactions underlying dystroglycanopathies.



Model of Full-Length and Non-extended Matriglycan Synthesis.
A) Mature matriglycan is a long polysaccharide that is synthesized by LARGE1.
B) In the absence of the core M3 phosphate added by POMK, LARGE1 generates a shorter, non-extended form of matriglycan.

During skeletal muscle differentiation, LARGE1 elongates matriglycan to its full length for normal skeletal muscle function. However, little is known about the mechanisms that control matriglycan elongation. Protein O-Mannose Kinase (POMK) is a novel dystroglycanopathy gene that phosphorylates mannose of the core M3 trisaccharide (GalNAc-β1,3-GlcNAc-β1,4-Man) on α-dystroglycan during synthesis of the O-mannose-linked polysaccharide ending in matriglycan. We show that the absence of POMK activity does not preclude addition of matriglycan. Instead, in the absence of core M3 phosphorylation by POMK, LARGE1 synthesizes a very short, non-extended form of matriglycan on α-dystroglycan (~90-100 kDa). However, in order to generate full-length mature matriglycan on α-dystroglycan (~150 kDa), LARGE1 requires phosphorylation of core M3 by POMK. Solution NMR spectroscopy studies demonstrate that LARGE1 directly interacts with core M3 and binds preferentially to the phosphorylated form. These results suggest that phosphorylated core M3 anchors the LARGE1-dystroglycan enzyme-substrate complex for full-length matriglycan synthesis. Collectively, our work provides the first insights into the pathogenic mechanism behind POMK-deficient muscular dystrophy and better elucidates how full-length matriglycan is synthesized so it can act as a scaffold for ECM proteins, thereby preventing muscular dystrophy.

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