Ultrastructural & Analytical Methods in Life Sciences

LS.6.P154
FRAP analysis of dystroglycan, a membrane receptor involved in congenital muscular dystrophies.

V. Palmieri[1], G. Maulucci[1], M. De Spirito[1], A. Maiorana[1], M. Papi[1], A. Brancaccio[2], M. Bozzi[3]
F. Sciandra[2]

[1]Università Cattolica del Sacro Cuore, Istituto di Fisica, Roma, Italy
[2]Istituto di Chimica del Riconoscimento Molecolare, Roma, Italy
[3] Università Cattolica del Sacro Cuore, Istituto di Biochimica e Biochimica Clinica, Roma, Italy

vplabcemi@gmail.com
Keywords: Dystroglycan, congenital muscular dystrophies, FRAP, confocal microscopy

Dystroglycan (DG) is a ubiquitous membrane-spanning protein that plays a crucial role in the assembly of several basement membranes, promoting the recruitment of laminins and other extracellular matrix molecules during morphogenesis, tissue remodelling, cell polarization and wound healing [1]. DG is formed by two subunits, the highly glycosylated and extracellular protein □-DG and the transmembrane protein □-DG. A non-covalent interaction between the two DG subunits anchors □-DG to the plasma membrane.

In skeletal muscle, DG is the central component of the dystrophin-glycoprotein complex (DGC), a multisubunit complex comprised of peripheral and integral membrane proteins, which links the cytoskeleton to the extracellular matrix. DGC ensures structural stability to the sarcolemma during the contraction–relaxation cycle. Primary and secondary mutations in the DGC components lead to distinct forms of muscular dystrophies, a diverse group of inherited disorders characterized by progressive muscle weakness and wasting [Cohn and Campbell, 2000]. In particular, genetic defects in the O-mannosylation pathways underline some congenital muscular dystrophies (CMD) characterized by a hypoglycosylated form of □-DG that displays a reduced affinity for the extracellular matrix proteins destabilizing the muscle fibers [3]. In CMD, the chronic damage of the muscle fibers leads to a continuous sealing of the sarcolemma and to a remodelling of DGC. The hypoglycosylation of □-DG, reducing the binding of □-DG to the surrounding extracellular matrix, may also influence the migration of DG through the plasmalemma influencing the membrane repair process. We analyzed, using confocal microscopy, GFP labelled wild-type DG and DG mutants with an altered glycosylation pattern in 293-EBNA cell line. In particular, cell tracking analysis was used to analyze the influence of the DG binding to extracellular matrix protein laminin on cells velocity and directionality. Moreover, using fluorescence recovery after photobleaching (FRAP) we have analyzed for the first time the DG mobility in plasma-membranes. FRAP allowed to measure diffusion and the mobile and immobile fraction of DG. Our study may give new insights in the cell trafficking of DG and its targeting and dynamics within the plasma membrane.

1. M. Bozzi, S. Morlacchi, M.G. Bigotti, F. Sciandra and A. Brancaccio, Matrix Biol. 28 (2009), 179-187.
2. R.D, Cohn and K.P. Campbell, Muscle Nerve 23 (2000), 1456-1471.
3. F. Sciandra, K.E. Gawlik, A. Brancaccio and M. Durbeej, Trends Biotech. 25 (2007), 262-268.