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Z-disc formation along myogenic differentiation: the role of α-actinin

S. Burattini[1], V. Baldassarri[1], S. Salucci[1], D. Curzi[1], E. Falcieri[1,2]

[1]Urbino University "Carlo Bo", DiSTeVA, Urbino, Italy
[2]Rizzoli Orthopaedic Institute, IGM,CNR, Urbino, Italy

sabrina.burattini@uniurb.it
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α-Actinin belongs to the spectrin superfamily of proteins and exists in, at least, six different isoforms [1]. The cytoskeletal isoforms (α-actinin-1 and -4) can be observed along microfilament bundles and in adherent junctions, where they are involved in binding actin to the plasma membrane. The muscle-specific isoforms (α-actinin-2 and -3) are necessary for actin filament attachment to the Z-discs in skeletal muscle fibers and to the analogous dense bodies in smooth muscle cells [2]. In the Z-discs, actin filaments from adjacent sarcomeres overlap and are held together to form an highly stable structure, composed by a variable number of α-actinin cross-links [3]. Furthermore sarcomeric α-actinin links several other proteins, providing membrane integrity during muscle contractions, and it is also able to modulate membrane receptors and channels and to interact with metabolic proteins [4]; not least, it serves as a scaffold to connect the sarcomere to several signaling pathways [5]. Thus, α-actinin is a multitasking protein that contributes to proper muscle physiology. Moreover, it has been shown to be dysregulated in several myopathies involving aberrant accumulation of myofilaments, called Z-discopathies [6].

The aim of this work was to investigate *in vitro* the Z-disc formation, which is closely related to α-actinin behavior. For this purpose, murine C2C12 skeletal muscle cells were analyzed at three time points of differentiation: at undifferentiated stage (T_0), after 4 days of differentiation (T_1) and after 7 days of differentiation (T_2) [7]. Finally, murine skeletal muscle tissue was used for the comparison between the last stage of cell differentiation and the adult skeletal muscle. Confocal laser scanner microscopy (CLSM) and transmission electron microscopy (TEM), with the immunogold technique, were utilized to carry out the immunolocalization of the protein [8].

Our results reveal that in T_0 myoblasts α-actinin is uniformly distributed throughout the cytoplasm: spot-like α-actinin Z-bodies can be observed, but Z-discs are not yet visible. T_1 myoblasts become spindle-shaped and fuse together to form early myotubes with few nuclei. They show filamentous α-actinin molecules, probably as a result of the lateral fusion of Z-bodies, and the labeling is evident especially beneath the plasma membrane and at the cell ends. T_2 myotubes appear intensely labeled: α-actinin is arranged into longitudinal arrays across the cytoplasm and almost all myotubes show evident Z-discs (Figure 1). Immunogold labeling, observed at TEM, confirmed α-actinin localization in all differentiation steps. The initial diffuse cytoplasmic labeling of α-actinin is followed by its progressive association with actin bundles, and gradual appearance of Z-discs (Figure 2).

So, when differentiation is induced, α-actinin links at first membrane-associated proteins, such as vinculin and integrins [9], then it aligns longitudinally throughout the cytoplasm and finally binds actin, giving rise to the Z-discs.

1. B. Sjöblom *et al*., Cell Mol Life Sci 65 (2008), p. 2688-2701.
2. N. Ichinoseki-Sekine *et al*., Biochem Biophys Res Commun 419 (2012), p. 401-404.
3. D.S. Courson and R.S. Rock, J Biol Chem 285 (2010), p. 26350-26357.
4. M. Lek, K.G. Quinlan and K.N. North, Bioessays 32 (2009), p. 17-25.
5. C.A. Otey and O. Carpen, Cell Motil Cytoskeleton 58 (2004), p. 104-111.
6. R. Knöll, B. Buyandelger and M. Lab, J Biomed Biotechnol (2011), ID 569628.
7. L. Casadei *et al*., Eur J Histochem 53 (2009), p. 261-268.
8. F. Luchetti *et al*., Apoptosis 2 (2002), 143-152.
9. J.M. Lewis and M.A. Schwartz, Mol Biol Cell 6 (1995), p.151-160.

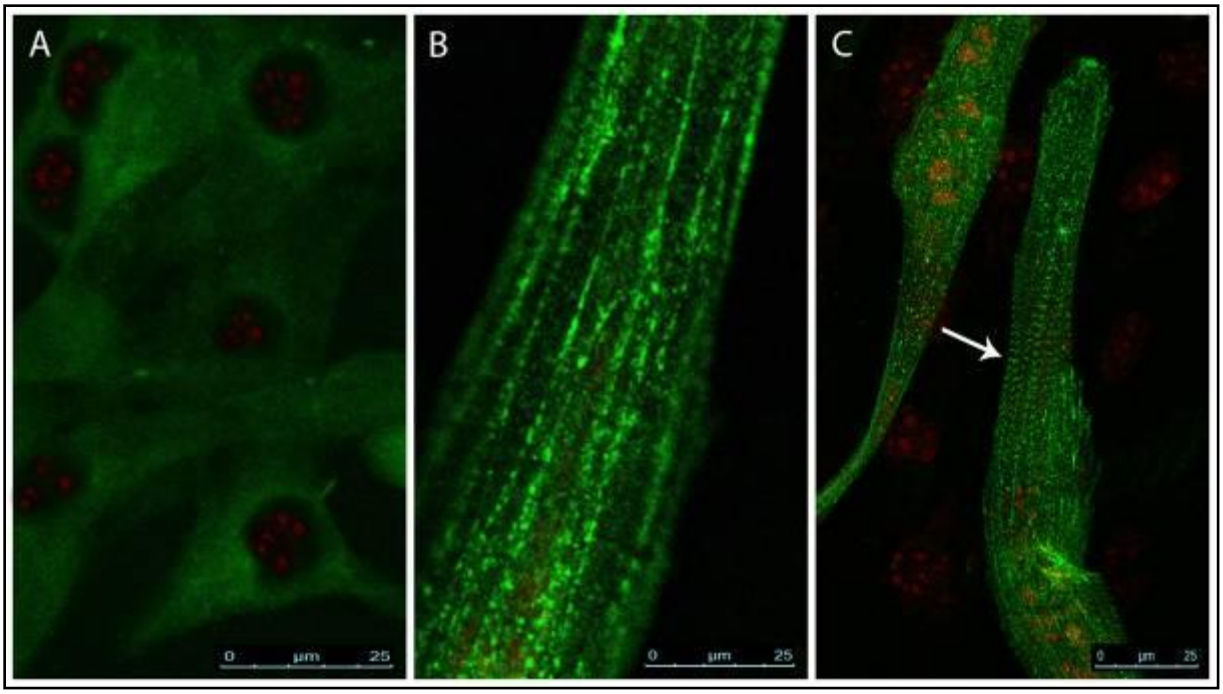

Figure 1. C2C12 cells, at three time points of differentiation, have been labeled for α-actinin; nuclei have been evidenced using propidium iodide. Myoblasts at T_0 (A); cells at T_1 (B); myotubes at T_2: Z-discs are clearly visible (arrow) (C). Bar scale = 25 µM.

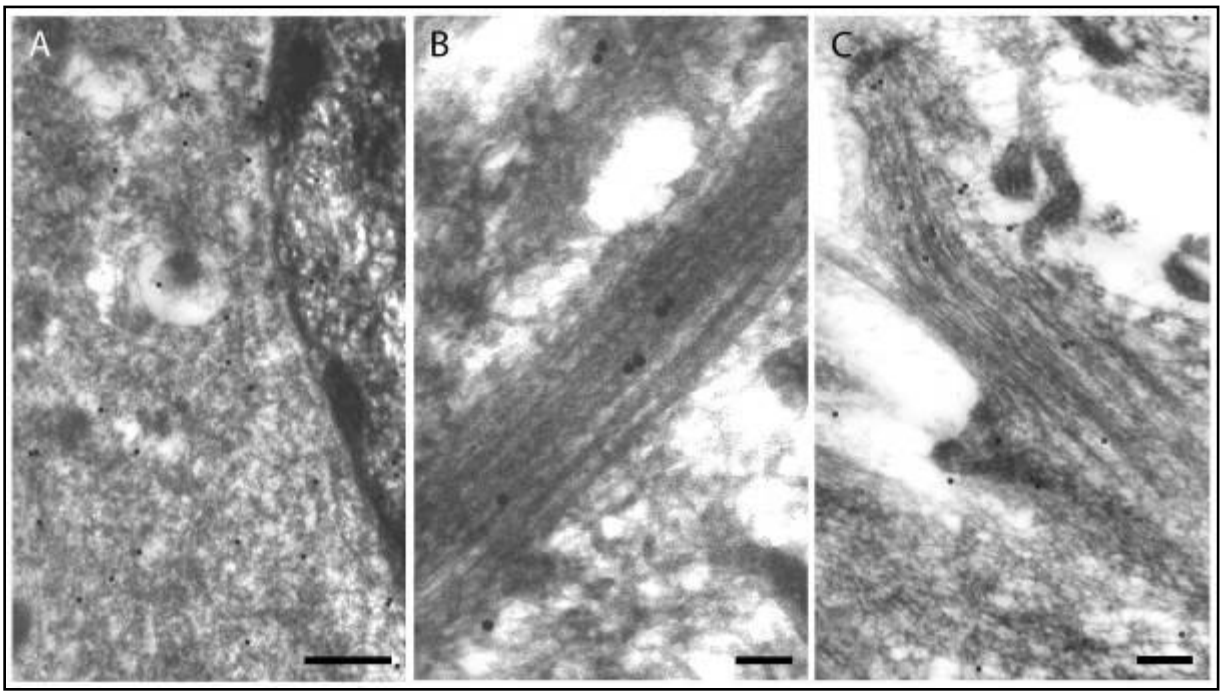

Figure 2. Immonogold labelling of C2C12 cells, at three time points of differentiation, observed at TEM. Myoblasts at T_0 (A); cells at T_1 (B); myotubes at T_2 (C). Bar scale: A = 0.5 µM; B = 0.1 µM; C = 0.25 µM.