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Visions & Reflections

Triadin: a multi-protein family for which purpose?

I. Marty

INSERM 607, CIS, DRDC/CEA Grenoble, 17 rue des Martyrs, 38054 Grenoble cedex 9 (France),
Fax: +33 (0)4 38 78 50 41, e-mail: imarty@cea.fr

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Abstract. Triadin is a protein first identified as a member of the muscle calcium release complex, involved in calcium release for muscle contraction. However, its precise function in this complex is still undefined. Recently, triadin has been shown to be a multi-protein family, with different distribution of the various splice variants within the sarcoplasmic reticulum, raising the possibility of multiple functions for this family of polypeptides. Such functions may include involvement in excitation-contraction coupling, in triad targeting, in structural function or in muscle differentiation. The putative role(s) of triadin(s) will be discussed here.

Key words: Triadin; skeletal muscle; calcium release complex; ryanodine receptor; triad.

Introduction

In skeletal muscle, contraction is induced by a massive calcium release from the sarcoplasmic reticulum into the cytoplasm. The release of calcium results from the depolarization of the plasma membrane which in turn leads to the activation of a macromolecular complex, the calcium release complex. The calcium release complex is localized in a particular area of muscle cell : specifically, the triad in skeletal muscle (diad in cardiac muscle). A triad is formed by the apposition of one T-tubule (an extension of plasma membrane inside the skeletal muscle cell) with two terminal cisternae of sarcoplasmic reticulum [1]. The calcium release complex constitutes a physical and functional link between the two membrane systems, the T-tubules of sarcolemma and sarcoplasmic reticulum. Its exact composition has yet to be fully defined. The two major protein components are the dihydropyridine receptor (DHPR), which is the calcium channel of the plasma membrane (T-tubule), and the ryanodine receptor (RyR), which is the sarcoplasmic reticulum calcium channel. Other proteins are associated with these channels, such as calsequestrin, FKBP12, junctin, calmodulin, S100 and triadin (fig. 1). Although some of these accessory proteins are well known (i.e. calsequestrin, which traps calcium inside the reticulum), others are not fully characterized, and their role in the calcium release complex remains elusive. Such is the case for triadin. Initially identified in skeletal muscle in 1990 [2], then cloned in 1993 from rabbit skeletal muscle [3, 4], triadin took its name from its specific localization at the triads of skeletal muscle. Expression, cloning and characterization of cardiac triadin at the diad came only later [5, 6]. The major focus now is to determine the precise function of the triadins. This review will discuss their putative functions in the context of recent findings.

A multi-protein family

Difficulty in undestanding triadin function is exacerbated by the presence of multiple isoforms, which are expressed from the same gene by alternative splicing [7]. The human triadin gene is 420 kb long, and the coding sequence of Trisk 95, the longest and best-known skeletal isoform, is only 2.2 kb. It is composed of 41 exons, and is thus extremely fragmented [7]. In addition to the 95-kDa triadin isoform expressed in skeletal muscle (Trisk 95),

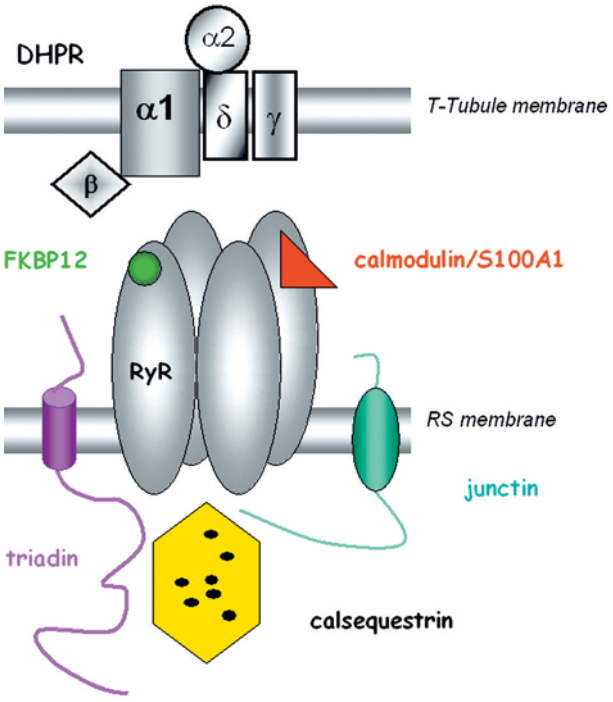

Figure 1. Putative organization of the skeletal muscle calcium release complex.

we have now observed in rat skeletal muscle the presence of at least three other isoforms: Trisk 51 [8], Trisk 49 and Trisk 32 [Vassilopoulos S. et al., unpublished]. Several isoforms have also been described in heart, even though only one appears to be predominantly expressed. The main cardiac triadin is a small protein (CT1, 33 kDa) which differs from all the major skeletal muscle triadins (Trisk 95, 95 kDa; Trisk 51, 51 kDa; Trisk 32, 32 kDa). All the isoforms shares a common N-terminal cytoplasmic segment, transmembrane alpha helix and luminal part of variable length. The specificity of each isoform occurs in the C-terminal end (fig. 2). The N-terminal cytoplasmic segment has been shown to interact with the RyR [9], while the luminal part has been shown to interact both with RyR and with calsequestrin [10] via a KEKE repeat [11]. These two domains are common to all the triadins identified to date. Interestingly, the classic isoforms (Trisk 95, Trisk 51 and the cardiac CT1) are localized in the junctional reticulum. However, Trisk 49 and Trisk 32, the two new skeletal isoforms, are localized in longitudinal sarcoplasmic reticulum. Their localization outside of the triad makes them unlikely to interact with RyR or calsequestrin. This indicates that the presence of putative in vitro interaction sites presented in figure 2 does not provide compelling evidence of an interaction in vivo. The different cellular and tissular localization of these isoforms leads to the postulation of a specific function for each isoform.

Tissue localization

Triadin tissue localization drives the hypothesis concerning its function: should we search for muscle-specific function or calcium release specific function? To date, triadin has been found only in striated muscle (skeletal muscle and cardiac muscle). Unsuccessful attempts at detection have also been made in other tissues using Northern blot hybridization from whole tissue homogenate. However, this technique lacks sensitivity, and subtle expression patterns in particular cells of a tissue may have be missed. In particular, this technique does not allow the detection of triadin in whole brain homogenate or in cerebellum ([5] and our group, unpublished). Nevertheless, it is possible to imagine triadin expression in particular neurons, for example in cerebellar granule cells, where RyR and DHPR have been demonstrated to be associated in a calcium release complex [12]. In these cells, triadin could be present and associated with this complex. As triadin has been shown to be associated with RyR1 in skeletal muscle, and RyR2 in cardiac muscle, it could be worthwhile look to for its presence in cells expressing either one of these isoforms.

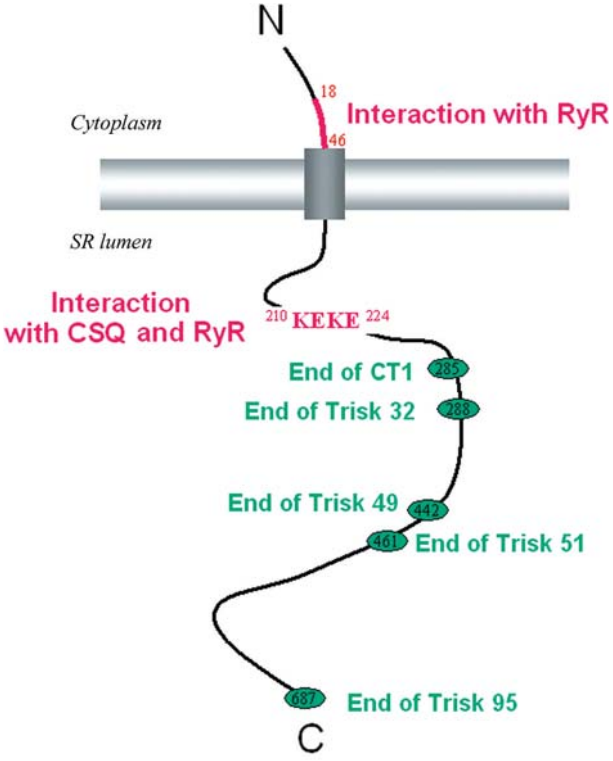

Figure 2. Structure of triadin isoforms. The four skeletal muscle triadins (Trisk 32, Trisk 49, Trisk 51 and Trisk 95) and the cardiac triadin (CT1) are represented on the same schema, with the number of the last C-terminal amino acid for each isoform, which is preceded by a short sequence of specific amino acids (not represented). The in vitro interaction sites with RyR and calsequestrin (CSQ) are represented.

What could be the function of triadin?

The first functional data were obtained on skeletal muscle triadin and demonstrated that triadin could regulate RyR function, stabilizing the RyR closed state [9, 13]. But why and how? Most of the experiments performed up to now have aimed at answering these questions directly or indirectly. The triadin KO mouse has not yet been developed, probably because of the expression of the same gene both in skeletal and cardiac muscle. What has been developed is a mouse that overexpresses triadin in cardiac muscle [14]. This mouse developed mild cardiac hypertrophy with overall downregulation of most of the proteins involved in the calcium release/uptake process (RyR, junctin, Ca^{2+}-ATPase) probably to maintain the best possible calcium release. Unfortunately, this does not give precise information about the function of cardiac triadin, except that it is involved in the overall process of calcium release and that its long-term modification results in compensatory downregulation of its partners of the calcium release complex. Beside its implication in the calcium release process of cardiac and skeletal muscle, other functions could be imagined, mainly because of the presence of multiple isoforms.

Triad targeting

One possible function of triadin could be the targeting of triad proteins to the triad. The triad (diad in cardiac muscle) is a very specialized part of the muscle cell. In skeletal muscle, the proteins involved in excitation-contraction coupling are all localized only at the triad: the ryanodine receptor, in the terminal junctional part of reticulum and not in the longitudinal reticulum; the DHP receptor, in the T-tubule membrane, and not in the rest of the plasma membrane; and calsequestrin, at the junctional part of the reticulum. No triad-specific signal has been identified in any of these proteins except for the alpha-1 subunit of DHPR [15], and their specific triad localization could result from interaction with a targeting protein, which helps the protein to reach its correct localization. However, the two recently identified skeletal muscle triadin isoforms, Trisk 32 and Trisk 49, are surprisingly localized in longitudinal sarcoplasmic reticulum and not in the junctional part of sarcoplasmic reticulum [Vassilopoulos S. et al., unpublished]. Should triadin fulfill such a triad-targeting role, it is unlikely that all the isoforms will have the same involvement, unless there exist mechanisms by which triadin brings a cargo to the triad and then reaches another localization.

Structural role of triadin

We have observed that one of the two new triadin isoforms, Trisk 32, colocalized in rat skeletal muscle with the IP_3 receptor (IP_3R), the other intracellular calcium channel. As IP_3R shares homologies with RyR, there could be another calcium release complex, centered on IP_3R, and similar to that of RyR. Up to now, we have not been able to isolate an IP_3R-specific complex, probably because of the low expression level of IP_3R in skeletal muscle. Despite that, as part of a calcium release complex (involving either RyR or IP_3R), triadin may be involved in maintenance of the structure of the complex during skeletal muscle contraction, in association with the cytoskeleton of the cell. Different triadin isoforms may then, via interactions with different cytoskeletal proteins, maintain the different parts of the sarcoplasmic reticulum in correct localization during contraction. The localization of Trisk 32 is also in the part of sarcoplasmic reticulum which is close to mitochondria. The involvement of mitochondria in calcium release is a current focus of study. One of the hypotheses is that sarcoplasmic Ca^{2+} release induces mitochondrial Ca^{2+} increase that in turns activates the ATP production which is necessary for muscle contraction. This process requires close contact between sarcoplasmic reticulum and mitochondria [16], and it is possible to imagine that Trisk 32 could be involved in this process.

Plasticity of the triadin pattern

Could the pattern of triadin isoform expression switch during muscle development and differentiation? This has not been extensively studied to date, but we have some indication that this could be the case. We have observed that Trisk 95 and Trisk 51 appear progressively as myotube are formed, and that the pattern of triadin expression is not the same between embryonic and adult rat muscles [8]. In this context, Trisk 95 or Trisk 51 could be involved in cell fusion during myotube formation. Indeed, cardiac muscle cells, which do not fuse, do not express these isoforms, or only in very minor amounts. Could the triadin pattern switch during muscle pathology? If a triadin isoform switch occurs during differentiation, then in some muscle pathologies where muscle regeneration is more active because of muscle fragility (Duchenne muscular dystrophy, for example), a different triadin pattern should be observed. We are currently exploring this avenue.

Conclusion

Despite intensive studies, the function(s) of triadin(s) remains elusive, and the recent discovery of multiple isoforms opens new fields of research. More experiments must be performed in order to test the different hypotheses developed in this review concerning the function of triadin. As the transgenic mice overexpressing cardiac triadin do not provide much information concerning triadin

function, the key step will be the development of skeletal muscle-specific KO mice now and in the future.

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