Molecular determinants of the structural and functional organization of the sarcoplasmic reticulum

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Abstract

The endo-sarcoplasmic reticulum of striated muscle cells consists of distinct functional domains that are extremely well organized both in terms of functional specialization and of spatial organisation. Here we shall review recent evidence on the potential involvement of recently identified novel proteins and of cytoskeletal components in the structural and functional organization of the sarcoplasmic reticulum with respect to the surface membrane/T-tubule system and the contractile apparatus.

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1. Endoplasmic reticulum and sarcoplasmic reticulum in striated muscle cells

The endoplasmic reticulum in animal cells is a morphologically continuous network of membrane tubules and flattened cisternae [1]. It consists of structurally distinct domains that include the nuclear envelope (NE), the rough and smooth endoplasmic reticulum. The endoplasmic reticulum is a multifunctional organelle that sustains a variety of cellular functions including protein synthesis, translocation of proteins across the endoplasmic reticulum membrane, integration of proteins into the membrane, and folding and modification of proteins in the endoplasmic reticulum lumen [2]. Specialized sub domains can be identified in the endoplasmic reticulum based on their high level of organization. These may include endoplasmic reticulum exit sites, where proteins to be exported are actively sorted and partitioned for delivery to selected sites. Additional functions include the synthesis of phospholipids and steroids, which occur on the cytosolic side of the endoplasmic reticulum membrane, and the storage of Ca^{2+} ions, which are sequestered in the endoplasmic reticulum lumen, from where they are released into the cytosol, following cell stimulation [3].

Differentiation of mature muscle cells results in a major reorganization of the endoplasmic reticulum that results in the development of a novel organelle, the sarcoplasmic reticulum [4]. The sarcoplasmic reticulum is a highly specialised form of the smooth endoplasmic reticulum that is dedicated to the regulation of intracellular Ca^{2+} homeostasis, thus participating to regulation of the relaxation–contraction cycle of striated muscles. Within the sarcoplasmic reticulum we can morphologically distinguish at least two well-characterised regions: the terminal cisternae, containing the ryanodine receptor Ca^{2+} release channels (RyR), and the longitudinal tubules, which contain the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps. The allocation of these proteins in two specific domains of the sarcoplasmic reticulum results in functionally distinct sarcoplasmic reticulum subdomains, where terminal cisternae represent the site of Ca^{2+} release while the longitudinal tubules are specialised in Ca^{2+} uptake.
Specific subdomains enriched with respect to components of the Ca$^{2+}$ release machinery (including Ca$^{2+}$ channels, Ca$^{2+}$ pumps, and additional accessory proteins) are observed in the endoplasmic reticulum of most cells. However, it is in the sarcoplasmic reticulum of striated muscle cells that this specialization of endoplasmic reticulum function has been better studied in the molecular details of its organization.

Regulated release of Ca$^{2+}$ from the sarcoplasmic reticulum is dependent on the close apposition of the juxtaional side of the terminal cisternae with the surface membrane/T-tubule system in order to receive the depolarization-mediated signal that triggers the opening of ryanodine receptors Ca$^{2+}$ channels. To this aim, in parallel to the development of the sarcoplasmic reticulum, a system of branching tubules originating as special invaginations of the plasma membrane, named T-tubule, starts to develop. Both the T-tubule system and the sarcoplasmic reticulum make contact with each other, forming specific contacts, called junctions. This highly organized system of membranes complex develops in the interior of the muscle cells where it surrounds each single myofibril [5,6]. In this way, Ca$^{2+}$ release and Ca$^{2+}$ storage sites are precisely positioned with respect to the specific regions of the each sarcomere within the myofibrillar apparatus, where variations in Ca$^{2+}$ levels are translated in cycles of contraction/relaxation.

The precise relationship between endoplasmic reticulum and sarcoplasmic reticulum in striated muscle cells is not obvious and how sarcoplasmic reticulum compartments are sorted from the endoplasmic reticulum is not known [2]. Nor it is clear whether these two compartments are structurally or functionally distinct or represent contiguous domains. In differentiated muscle cells, protein synthesis occurs in the perinuclear region of myofibers, but additional evidence suggests that proteins can be translocated into the interfibrillar membranes [7]. Golgi elements have also been shown to localize around the nuclei and into the interfibrillar spaces [8]. Some classic endoplasmic reticulum markers, like the proteins BiP and PDI, seem to colocalize with specific molecular components of Ca$^{2+}$ stores, e.g., calsequestrin, Ca$^{2+}$ pump and ryanodine receptor [9]. Immuno-localization studies have shown that these markers can be detected in the perinuclear region of the terminal cisternae of myofibers [7,8]. Additional data indicate that endoplasmic reticulum exit sites may be detected in correspondence of Z lines but not of terminal cisternae [10]. These results suggest that the terminal cisternae may contain endoplasmic reticulum elements associated with translocation, glycosylation, and folding functions, but not export function. On the other hand, endoplasmic reticulum domains found around the nucleus and at the Z line may contain also exit sites. In this view, sarcoplasmic reticulum proteins that are synthesized on endoplasmic reticulum compartments not contiguous with sarcoplasmic reticulum need to be transported, through yet unknown vesicular routes, to final destination. Alternatively, sarcoplasmic reticulum-specific proteins may be synthesized within endoplasmic reticulum compartments associated with terminal cisternae, from where they can diffuse to the sarcoplasmic reticulum.

Understanding how the sarcoplasmic reticulum develops and how it forms specific interactions with the surface membrane/T-tubule system and with the myofibrillar apparatus is an open question in muscle cell biology. Here we shall review current information on this topic.

2. Junctional sarcoplasmic reticulum and surface membrane/T-tubule system: the making of junctional complexes

The key proteins for Ca$^{2+}$ release from the terminal cisternae are the ryanodine receptors, the specialised Ca$^{2+}$ release channels present in striated muscle [11]. Both RyR1 and RyR2, which represent the skeletal and cardiac isoforms of ryanodine receptors, respectively, are located in the terminal cisternae and more precisely in the junctional region of the terminal cisternae. The junctional region of the terminal cisternae is the region that faces the surface membrane/T-tubule system and that participates to form structures called triads and diads, found in skeletal and cardiac muscles, respectively [4]. These structures represent junctional complexes between the sarcoplasmic reticulum and the T tubule system that guarantee the functional interaction between dihydropyridine receptors and ryanodine receptors essential for activation of the E–C coupling mechanism [12]. RyR3 channels, the third isoform of ryanodine receptors, which show a developmentally regulated expression in mammalian skeletal muscles, have been recently shown to be localized in the parajunctional membranes immediately adjacent to the junctional region [13]. Although RyRs and DHPRs are functionally coupled, these proteins are not involved in the assembly of membrane junctions between the sarcoplasmic reticulum and the surface membrane/T-tubule system.

In addition to ryanodine receptors, other proteins present in the terminal cisternae have been identified [14]. Triadin is a major integral membrane glycoprotein of the junctional sarcoplasmic reticulum domain. Triadin is a membrane protein containing a single membrane-spanning domain that leaves a short N-terminus protruding in the cytosol, with most of the sequence in the luminal space of the sarcoplasmic reticulum. In the lumen of the sarcoplasmic reticulum, the C-terminus of triadin interacts both with ryanodine receptor and calsequestrin. The junctional sarcoplasmic reticulum contains a second protein with structural homology to triadin, named junctin. Junctin also contains a short cytoplasmic N-terminus followed by a single transmembrane segment that leaves the remaining part of junction in the lumen of the sarcoplasmic reticulum. Junctin is able to interact with triadin, calsequestrin and ryanodine receptor. The interaction of
triadin and junctin with ryanodine receptor is Ca\(^{2+}\)-independent, while that with calsequestrin is inhibited by high Ca\(^{2+}\) concentration. Altogether, it would appear that triadin and junctin are not involved in forming structural contacts with the DHPR or the surface membrane/T-tubule system, while it is widely accepted that the ryanodine receptor, triadin, junctin and calsequestrin may form a quaternary complex, whereby calsequestrin helps to localize high concentrations of Ca\(^{2+}\) near the luminal side of the ryanodine receptor channels through a protein bridge formed by junctin and triadin.

A second group of sarcoplasmic reticulum proteins that have been proposed to have a more direct role in the organization of junctional complexes between surface membrane/T-tubule system and junctional sarcoplasmic reticulum has been recently identified. Mitsugumin 29 is a transmembrane protein found at the triadic junction in skeletal muscle that shares significant structural features with members of the synaptophysin family of proteins [15]. A possible functional role of Mitsugumin 29 in maturation and development of the triads in skeletal muscle has been postulated on the basis of results obtained from studies in muscles of mice KO for mg29. In these mutant mice, swollen T-tubules, vacuolated sarcoplasmic reticulum networks and misalignment of triad junctions were observed [16]. Such defects in membrane ultrastructure may have an impact on the Ca\(^{2+}\) signalling process in skeletal muscle. In fact, in addition to alterations in the junctional membrane structure, mice KO for mg29 present an alteration in store operated Ca\(^{2+}\) channels (SOC) located in the plasma membrane and in sarcoplasmic reticulum Ca\(^{2+}\) homeostasis, resulting, on the physiological level, in an increased susceptibility of muscles to fatigue [17].

A major advancement on the molecular mechanisms underlying the formation of junctional membrane structures has been the identification of a family of new proteins, named junctophilins, that appear to take part in the formation of the junctional membrane structures in excitable cells [18]. Junctophilins contain two major domains, a carboxyl-terminal hydrophobic segment spanning the sarcoplasmic reticulum membrane and a cytoplasmic region interacting with the plasma membrane. Four junctophilin subtypes, namely, junctophilin-1, -2, -3 and -4, have been identified in mammalian tissues. Junctophilin-1 is specifically expressed in skeletal muscle. Junctophilin-1-knockout mice show neonatal lethality probably due to defective excitation-contraction coupling as skeletal muscles from these mice show altered development of triad junctions [19,20]. Junctophilin-2 is expressed in both skeletal and cardiac muscle. Junctophilin-2-knockout mice have early lethal phenotype showing embryonic cardiac arrest [18]. Cardiac myocytes of these mice have altered peripheral coupling and the abnormal Ca\(^{2+}\) release. Interestingly, expression of junctophilins in non-muscle cells resulted in the formation of junctional membrane complexes between the plasma membrane and endoplasmic reticulum [18,20]. Junctophilin-3 is predominantly found in the brain. Accordingly, junctophilin-3-knockout mice have impaired performance in motorcoordinated tasks, likely due to abnormal neural signalling [21]. The last junctophilin subtype, junctophilin-4, encodes a protein that shares characteristic structural features with other junctophilin subtypes, and is also expressed in brain [22].

While it is accepted that T-tubules are necessary to allow for the coordinated contraction of muscle fibers, how the T-tubule system develops from the plasma membrane is not known [4–6]. A protein that may participate in the formation of T-tubules is a muscle-specific isoform of Amphiphysin [23]. Amphiphysin is a protein originally identified at the presynaptic nerve terminal where it recruits dynamin to sites of clathrin-mediated endocytosis. A muscle-specific Amphiphysin has been found in the T-tubule system of striated muscles and has been proposed to participate to formation of T-tubules [23]. In vitro studies have shown that Amphiphysin is able to induce tubulation of lipid vesicles. These results are supported by similar evidence in C2C12 cells in which Amphiphysin induces the formation of T-tubule-like structures from the plasma membrane [24]. These data are complemented by studies in mutant animal models. Drosophila flies lacking the Amphiphysin gene are viable but flightless. Interestingly, the skeletal muscles of the mutant fly presented a reduced number and a disorganized architecture of the T-tubule system [25]. In mice, Amphiphysin knockout resulted in perinatal death. Amphiphysin knockout mice have no detectable alteration in skeletal muscles, but morphological alterations were found in cardiac muscle cells [26].

3. Ankyrins and organization of sarcoplasmic reticulum in striated muscles

How proteins are sorted into specialized domains of the sarcoplasmic reticulum, like the junctional region of the terminal cisternae or the longitudinal tubules, is not clear. A role for ankyrins in the localization of proteins participating in the regulation of intracellular Ca\(^{2+}\) homeostasis in striated muscles was initially proposed by Tuvia et al. [27]. Ankyrins are a family of ubiquitously expressed proteins that participate in the organization of specific membrane domains by linking specific proteins on the plasma membrane with the sub plasma membrane cytoskeleton [28]. Vertebrate genome contains three ankyrin genes: Ank1, Ank2, and Ank3, which encode proteins named originally Ankyrin-R, Ankyrin-B, and Ankyrin-G. Ankyrin gene products are often co-expressed within the same tissue and cells where they seem to have divergent and non-overlapping functions. The primary structure of ankyrins can be divided in at least three common structural domains (membrane-binding domain, spectrin-binding domain, and death domain), where they share a high degree of sequence...
similarity. These proteins are, however, quite divergent in their C-terminal regulatory domains. Transcripts of all three ankyrin genes undergo a regulated splicing processing which results in the generation of several specific ankyrin isoforms from each gene. In addition to their primary localization on the plasma membrane, specific ankyrin isoforms have been found to associate also with the membrane of intracellular organelles such as Golgi, lysosomes and the sarcoplasmic reticulum [29,30]. In striated muscles, ankyrins have been detected at specific sites, including costameres, the postsynaptic membrane and triads [31–34]. More recent studies with ank2 deficient mice have better defined the role of this isoform in the localization of proteins involved in \( \text{Ca}^{2+} \) homeostasis, and especially of the \( \text{InsP}_3 \) receptors at specific domains of the sarcoplasmic reticulum [35–37]. Cardiomycocytes derived from ank1-B+/− and ankyn-B−/− mice display abnormal spontaneous contraction rates and abnormal \( \text{Ca}^{2+} \) dynamics that may be caused by displacement of the ankyrin-B from the T-tubule/sarcoplasmic reticulum sites. Indeed, cardiomycocytes from ank1-B+/− and ankyn-B−/− mice present a reduction, in the T-tubule region, of proteins such as the Na/K ATPase, Na/Ca\(^{2+}\) exchanger, and inositol 1,4,5 trisphosphate (\( \text{InsP}_3 \) receptor) [36]. Since all of these proteins are known to bind ankyn-B, it would appear that a role of ankyn B is to favor the assembly of a complex of proteins responsible of \( \text{Ca}^{2+} \) homeostasis at the T-tubule region [36–38]. Accordingly it has been postulated that reduction of the Na/K ATPase, by causing an increased intracellular Na content, would increase exchange of intracellular Na for extracellular \( \text{Ca}^{2+} \) by the Na/Ca\(^{2+}\) exchanger, thus resulting in the elevated \( \text{Ca}^{2+} \) transients observed in ankyn B−/− cardiomycocytes. Interestingly, these studies in ankyn-B−/− mice have provided a good experimental model to understand the molecular basis of an inherited cardiac arrhythmia (long QT syndrome type 4) reported in a family in which a mutation in the ANKB gene has been found [38]. Patients carrying ankyn-B mutations display varying degrees of cardiac dysfunction including bradycardia, sinus arrhythmia, idiopathic ventricular fibrillation, catecholaminergic polymorphic ventricular tachycardia, and risk of sudden death. Similar symptoms can be observed in ankyn-B+/− mice.

4. Muscle-specific ank1 isoforms: in between the sarcoplasmic reticulum membrane and the myofibrillar apparatus

In striated muscles, a precise localization of the sarcoplasmic reticulum relative to myofibrils is observed whereby the sarcoplasmic reticulum surrounds the myofibrillar apparatus forming a sleeve-like structure that favours a close apposition between the \( \text{Ca}^{2+} \) stores and the contractile apparatus (Franzini [4]). The highly regulated nature of the arrangement of the sarcoplasmic reticulum around myofibrils is such that specific domains of the sarcoplasmic reticulum involved in the mechanisms of \( \text{Ca}^{2+} \) release and uptake (i.e., terminal cisternae and longitudinal tubules, respectively) are positioned at regular intervals in correspondence of specific regions of the sarcomere [4–6]. In this way, junctional complexes like diads and triads are positioned in correspondence of either the junction between the A-I bands or of the Z disk, as observed in skeletal and cardiac muscles of mammals, respectively [4,39]. However, although the precise localization of the sarcoplasmic reticulum with respect to myofibrils has been known since decades, the molecular mechanism(s) responsible of the interactions between these two cellular structures is not known [3,4,9,39].

A set of muscle-specific transcripts of the ank1 gene has been identified in striated muscles [40,41]. These transcripts encode characteristic muscle-specific ank1 isoforms that lack both membrane and spectrin binding sequences and retain only a short sequence from the COOH-terminus of the large ankyn 1 [42,43]. The NH2-terminal portion of these small muscle-specific Ank1 isoforms contains a transmembrane domain that anchors these proteins to the sarcoplasmic reticulum membrane, while the remaining amino acid sequence extends in the cytosol [41,34]. Immunostaining of adult skeletal muscle has shown that small Ank1 isoforms are targeted to specific domains of the sarcoplasmic reticulum in correspondence of the Z-disks and M lines of the contractile apparatus [34].

Studies aimed at identifying a possible cytosolic interacting protein for the small muscle-specific ankyns have shown that one small muscle-specific ank1 isoform (ank1.5) is capable of interacting with the C-terminus of Obscurin [44,45]. Obscurin is a recently identified muscle protein known to bind titin [46]. Obscurin is an extremely large protein characterized by a modular architecture that contains multiple immunoglobulin-like (Ig-like) domains, two fibronectin-like (FN3-like) domains, and a RhoGEF/PH domain. Additional transcripts of Obscurin have been detected that may also contain one or two serine-threonine kinase domains [47,48]. The modular structure of Obscurin makes this protein a very good candidate for mediating multiple interactions both within the myofibrils and with other cellular structures [49–53].

The interaction between ank1.5 and Obscurin is mediated by an amino acid sequence present in ank1.5, but not in other small ank1 proteins (e.g., ank1.6 and ank1.7). On his own, ank1.5 recognizes a specific sequence present in the non-modal region at the C-terminus of Obscurin [44]. Transfection of plasmids encoding ank1.5 and a fusion protein consisting of the C-terminus of Obscurin cloned in frame with the Green Fluorescent Protein resulted in the association of GFP-Obscurin with the endoplasmic reticulum. In cultured skeletal muscle cells ank1.5 was detected near or at the M line where it colocalizes with Obscurin. Localization of
ank1.5 at the M line required the presence of an intact Obscurin-binding site since a mutation in this sequence prevented the localization of ank1.5 at the M line. These data are compatible with a model where these two proteins may contribute to holding a stable interaction between the sarcoplasmic reticulum and the myofibrils [44].

In conclusion, during the past few years, molecular techniques have helped to identify new genes encoding proteins that may participate in the formation of junctional complexes between the sarcoplasmic reticulum and the plasma membrane T-tubule system. In addition, there is initial evidence that cytoskeletal proteins may interact with proteins on the sarcoplasmic reticulum and help in assembling specific protein complexes required in the formation of stable interactions between the contractile apparatus and the sarcoplasmic reticulum. On this basis it can be expected that in the near future a more detailed understanding of the molecular mechanisms underlying the subcellular organization of the sarcoplasmic reticulum shall be available.

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