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MINI-REVIEW

The Unraveling Architecture of the Junctional Sarcoplasmic Reticulum

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Abstract

The sarcoplasmic reticulum (SR) of skeletal muscle controls the contraction–relaxation cycle by raising and lowering the myoplasmic free-Ca^{2+} concentration. The coupling between excitation, i.e., depolarization of sarcolemma and transverse tubule (TT) and Ca^{2+} release from the terminal cisternae (TC) of SR takes place at the triad. The triad junction is formed by a specialized region of the TC, the junctional SR, and the TT. The molecular architecture and protein composition of the junctional SR are under active investigation. Since the junctional SR plays a central role in excitation–contraction coupling and Ca^{2+} release, some of its protein constituents are directly involved in these processes. The biochemical evidence supporting this contention is reviewed in this article.

Key Words: Skeletal muscle; sarcoplasmic reticulum; membrane proteins; calsequestrin; Ca^{2+} release; Ca^{2+} channel.

Introduction

The skeletal muscle sarcoplasmic reticulum (SR), a specialized endomembrane network, controls the contraction–relaxation cycle by raising and lowering the myoplasmic free-Ca^{2+} concentration. The coupling between excitation, i.e., depolarization of sarcolemma and transverse tubule (TT), and contraction, i.e., Ca^{2+}-dependent interaction of myosin and actin, proceeds through a chain of events that is only partially understood. Following a propagated action potential, a "signal" is generated and/or released at

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the triad where a specialized region of the terminal cisternae (TC), the junctional SR, and the TT are junctionally associated via bridging structures, or "feet" (Franzini-Armstrong, 1970). The "signal" or messenger, whose nature is still unknown or, at best, controversial (Somlyo, 1985), is able to evoke Ca^{2+} release from the TC of SR. The mechanism of TT–TC coupling, the mechanism underlying Ca^{2+} release from SR, and the molecular composition and localization of Ca^{2+} channels involved in excitation–contraction coupling are very important, yet unsettled, questions of muscle physiology.

In the early 1970s, isolated SR fractions were described as membrane systems with a relatively simple protein and lipid composition (see Meissner *et al.*, 1973). After the successful isolation of light and heavy SR fractions, referable to longitudinal SR and TC, respectively (Meissner, 1975), it became clear that SR was subspecialized, and the heterogeneity and complexity of both function and protein composition of different SR areas began to be reported.

It is now established that each region of the SR network plays a specific role and has its own morphological, functional, and structural characteristics: Ca^{2+} is actively accumulated by a Ca^{2+} pump uniformly distributed in the "free" or nonjunctional SR (Jorgensen *et al.*, 1982); between uptake and release cycles, Ca^{2+} is stored, bound to calsequestrin (CS) that is localized in the lumen of TC (Jorgensen *et al.*, 1979); Ca^{2+} is released via Ca^{2+} release channels localized in the TC membrane (Smith *et al.*, 1985, 1986*a*); and coupling of TT depolarization to Ca^{2+} release from TC involves the junctional SR.

The distinct morphological features of the junctional SR have been recently highlighted by Ferguson *et al.* (1984), Saito *et al.* (1984), and Franzini-Armstrong *et al.* (1987). The myoplasmic leaflet of junctional SR shows squarelike projections ("feet"), large structures composed of four subunits that are disposed in periodic arrangement over the membrane (Ferguson *et al.*, 1984). Aggregates or paracrystalline arrays of CS (Saito *et al.*, 1984) are "associated" with the internal leaflet of the junctional SR where the feet are located. CS seems to form a three-dimensional internal network and to be "anchored" to the junctional SR by thin or peripheral strands (Franzini-Armstrong *et al.*, 1987). Deep-etched rotatory-replicated freeze fractures of skeletal muscle fibers have indicated that such "strands" penetrate within the junctional SR (Franzini-Armstrong *et al.*, 1987) and should likely provide a structural linkage between CS and the junctional SR.

The molecular architecture and protein composition of the junctional SR are underactive investigation and are beginning to unravel. Since the junctional SR plays a central role in TT–TC coupling and Ca^{2+} release, some of its protein constituents ought to be directly involved in these processes. The biochemical evidence supporting this contention is reviewed here.

Protein Composition of the Junctional SR of Skeletal Muscle

Costello *et al.* (1986) have described a procedure for the isolation of junctional SR membrane fragments from TC that relies on selective detergent solubilization of the nonjunctional SR. The junctional SR displays a unique protein pattern. Table I lists several proteins that have been attributed to the junctional SR by different laboratories; however, little is known about the function of many of these proteins.

CS is the major component of the junctional SR (Costello *et al.*, 1986). It functions as a Ca^{2+} storage site in close proximity to the Ca^{2+} release channel, which seems to be localized in the junctional SR as well (see below). CS is not an integral membrane protein (Volpe *et al.*, 1987) and might associate with the junctional SR via a CS-binding protein; one such protein (Table I) has been recently identified by Mitchell *et al.* (1988) as a 26-kDa protein that binds CS with high affinity and in a Ca^{2+}-regulated fashion. CS proteins are expressed not only in striated muscle fibers (MacLennan *et al.*, 1983), but also in nonmuscle cells (Volpe *et al.*, 1988), and seem to be localized within the lumen of membrane-bound organelles ("calciosomes") equivalent to the SR and responsible for rapid-scale control of Ca^{2+} transients.

The feet protein, or, at least, one of its components, is the 350-kDa protein. It is the "spanning" protein that closes the gap between TC and TT (Kawamoto *et al.*, 1986); it is an integral membrane protein (Volpe *et al.*, 1987) and is a Ca^{2+}-binding protein (Zorzato and Volpe, 1988*a*); it has binding site(s) for several Ca^{2+} release modulators, i.e., doxurubicin (Zorzato *et al.*, 1986), ryanodine (Campbell *et al.*, 1987; Lai *et al.*, 1987; Inui *et al.*, 1987), calmodulin (Seiler *et al.*, 1984), and ATP (Lai *et al.*, 1988); and it displays Ca^{2+} channel activity after reincorporation into a lipid bilayer (Imagawa *et al.*, 1987; Lai *et al.*, 1988; Hymel *et al.*, 1988). The internal topology of the 350-kDa protein is not yet known in any detail. On the basis of stepwise trypsin digestion studies, Chu *et al.* (1988) have proposed the occurrence of a large crevice, likely the opening of the Ca^{2+} channel, leading to a central polar environment. The architecture of the channel pore and, more importantly, the position of the mouth of the channel relative to the triadic space, and the number and location of ligand-binding site(s) remain to be determined. The relevance of this Ca^{2+} release channel to the physiological Ca^{2+} release channel is discussed later.

Other junctional SR proteins might have a role in receiving the transduction signal from the TT and/or in modulating Ca^{2+} release. One such protein is that having an apparent M_r of 170,000 (Table I): it is a Ca^{2+}-binding protein (Zorzato and Volpe, 1988*a*), is labeled by doxorubicin (Zorzato *et al.*, 1986), and might thus be either a subunit of the Ca^{2+} release channel or the Ca^{2+}-sensing device of the Ca^{2+} channel given the Ca^{2+}

Table I. Protein Composition of Junctional SR

Apparent M_r $\times 10^3$	Integral protein[a]	Ca^{2+}-binding protein[b]	Notes
350[c]	Yes	Yes	Ryanodine receptor[d] Doxorubicin-binding protein[e] Ca^{2+} release channel[f] Calmodulin-binding protein[g] Substrate of cAMP and calmodulin PK[g]
325	Yes	Yes	Calmodulin-binding protein[g] Substrate of cAMP and calmodulin PK[g] Breakdown product of the 350-kDa protein[h]
200	No	Yes	?
170[i]	No	Yes	Blue-staining band[j] Doxorubicin-binding protein[e] Breakdown product of the 350-kDa protein[h]
140	No	Yes	?
118[i]	Yes	Yes	?
80	Yes	No	Anchoring protein[k]?
79[i]			
63	No	Yes	Calsequestrin
52–49	No	Yes	?
38			Aldolase[l]
37	Yes	No	?
34			GDP-DH[l]
32	Yes	No	?
30	Yes	No	?
28[i]			?
26			Calsequestrin-binding protein[m]
17[n]			Calmodulin?
6	Yes	No	?

[a] Volpe *et al.* (1987). Two radioactive phosphatidylcholine analogs carrying the photoactivatable aryl-azido group at different levels of one of the fatty-acid chains were used to identify integral membrane proteins.

[b] Zorzato and Volpe (1988*a*). Specific Ca^{2+}-binding proteins were identified by ^{45}Ca ligand overlay of nitrocellulose blots.

[c] The M_r of this protein has not been determined yet and it has been reported to be either 450,000 (Imagawa *et al.* 1987), ~400,000 (Lai *et al.*, 1988), 360,000 (Inui *et al.*, 1987), 350,000–290,000 (Seiler *et al.*, 1984), or 325,000 (Kawamoto *et al.*, 1986). Throughout the text, we refer to this protein as the 350-kDa protein.

[d] Campbell *et al.* (1987), Lai *et al.* (1987), Inui *et al.* (1987).

[e] Zorzato *et al.* (1986).

[f] Imagawa *et al.* (1987), Lai *et al.* (1988), Hymel *et al.* (1988).

[g] Seiler *et al.* (1984); PK, protein kinase.

[h] Campbell *et al.* (1987), Lai *et al.* (1988). Proteolytic digestion of the 350-kDa protein yielded a 170-kDa protein (Chu *et al.*, 1988); thus, the 170-kDa band might be heterogeneous.

[i] Costello *et al.* (1986).

[j] Campbell *et al.* (1983).

[k] Caswell and Brunschwig (1984).

[l] Kawamoto *et al.* (1986); GDP-DH, glyceraldeydephosphate dehydrogenase.

[m] Mitchell *et al.* (1988). The proteins having M_r of 30,000, 28,000, and 26,000 might be the same.

[n] Campbell (1986).

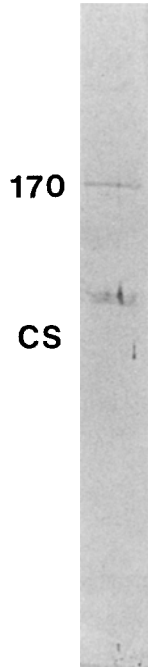

Fig. 1. Immunoblot of rabbit TC fraction with anti-(170-kDa protein) serum. SDS–polyacrylamide gel electrophoresis of rabbit TC (20 μg protein) was carried out as described (Zorzato *et al.*, 1986). Electrophoretic transfer to nitrocellulose paper was carried out overnight at 0.1 A. Immunoblot was revealed as described (Salvatori *et al.*, 1988) by incubating with anti-(170-kDa protein) serum (1 : 100 dilution) and then with alkaline phosphatase-conjugated anti-(chicken) IgG. The anti-(170-kDa protein) serum was raised in chickens by weekly injections of a suspension containing the 170-kDa-, blue staining-band identified by Stains-All staining of SDS–polyacrylamide slab gels (Zorzato *et al.*, 1986). Abbreviations: 170, 170-kDa protein; and CS, calsequestrin.

sensitivity of the Ca^{2+} release *in vitro* (Lai *et al.*, 1988). The 170-kDa protein identified as a blue-staining band, after stains-All staining of SDS–polyacrylamide slab gels, and doxorubicin-binding protein (Zorzato *et al.*, 1986) does not appear to be a breakdown product of the 350-kDa protein, as suggested by Lai *et al.* (1988). In fact, Fig. 1 shows that polyclonal antibodies raised against the 170-kDa, blue-staining band recognize the 170-kDa protein and cross-react with a band of $M_r < 100{,}000$, but do not label the 350-kDa protein (P. Volpe and F. Zorzato, unpublished results).

The role of some of the junctional SR proteins will be assessed once the protein composition of the junctional TT is clarified. Other junctional SR proteins might be involved in different functions, such as stabilization of the "junctional processes" (Chu *et al.*, 1988) and glucose metabolism, which is turned on by an increased myoplasmic Ca^{2+} concentration. A few proteins

listed in Table I might also belong to TT and mitochondrial membrane fragments that are minor, but unavoidable, contaminants of SR fractions.

Is the Ca^{2+} Release Channel in the Junctional SR?

The TT–TC coupling has been explained on the basis of either a mechanical or chemical hypothesis. The mechanical hypothesis, recently reviewed by Rios and Brum (1987), postulates that charge movements, generated by the dihydropyridine receptor at the TT membrane level, control Ca^{2+} channels in the junctional SR by altering "long-connecting molecules" in the feet. The chemical hypothesis states that a specific chemical transmitter, e.g., Ca^{2+}, inositol 1,4,5-trisphosphate (IP_3), or yet another messenger to be identified, is released within the triad junction in response to an action potential and interacts with Ca^{2+} channels of the junctional SR (Volpe *et al.*, 1986). Simple diffusion across the 120 to 150-Å junctional space requires about 1 μsec, much less than the latency between the upswing of the TT action potential and the rise of myoplasmic free Ca^{2+}, i.e., $\sim$2.5 msec (Vergara and Delay, 1986). Regardless of which hypothesis will turn out to be true, the Ca^{2+} release channel involved in excitation–contraction (E–C) coupling is very likely localized in the junctional SR. Although it has been known for quite some time that Ca^{2+} was stored and released from the TC *in vivo* (for a review, see Somlyo *et al.*, 1981), it was not known whether the Ca^{2+} channels were distributed over the entire TC membrane area or were restricted to the junctional SR area. It has now been determined that one type of Ca^{2+} release channel is in the junctional SR only.

Several probes or modulators of *in vitro* Ca^{2+} release have been used to identify putative components of the Ca^{2+} release channel(s), as summarized in Table II. Using doxorubicin as a natural photoaffinity ligand, Zorzato *et al.* (1986) first reported that three junctional SR proteins (M_r of 350,000, 170,000, and 80,000) were putative components of a Ca^{2+} release channel (Table II). Using antibodies against junctional SR proteins and against the 350-kDa protein, Zorzato and Volpe (1988*b*) confirmed that the doxorubicin-, Ca^{2+}-, caffeine-sensitive Ca^{2+} release channel is in the junctional SR and that the 350-kDa protein is a channel constituent. Using ryanodine, Imagawa *et al.* (1987), Lai *et al.* (1988), and Hymel *et al.* (1988) identified the 350-kDa protein as the Ca^{2+}-, caffeine-, ruthenium-red-, and Mg^{2+}-sensitive, high-conductance Ca^{2+} channel (Table II). Since doxorubicin and ryanodine act, very likely, on the same Ca^{2+} release pathway, the question is whether the 350-kDa protein is the only channel subunit. Single-channel measurements would so indicate (see, e.g., Lai *et al.*, 1988). However, doxorubicin might either recognize additional regulatory components of the Ca^{2+} channel or

Table II. SR Proteins Involved in Ca^{2+} Release Activity

Probe	Labeled proteins $M_r \times 10^3$	Localization	Type of Ca^{2+} release
Ryanodine[a]	350	Junctional	Ca^{2+}-, caffeine-, ATP-, Mg^{2+}-, ruthenium-red-sensitive
Doxorubicin[b]	350, 170, 80	Junctional	Ca^{2+}-, caffeine-, ruthenium-red-sensitive
CaM-depend prot. kinase[c]	60	?[c]	Ca^{2+} sensitive
DACM[d]	32	?	Ca^{2+}-, caffeine-sensitive; depolarization-induced
DCCD[e]	350, 170, 140, 53, 30	?	Alkaline-, TPB-, (ATP + P_i)-sensitive

[a] Lai *et al.* (1988), Imagawa *et al.* (1987), Hymel *et al.* (1988). In previous reports, Lai *et al.* (1987) and Inui *et al.* (1987) described several ryanodine-binding proteins, which later disappeared.
[b] Zorzato *et al.* (1986).
[c] Kim and Ikemoto (1986) found a correlation between the amount of calmodulin (CaM)-dependent P_i incorporation into the 60-kDa protein and the extent of inhibition of Ca^{2+} release. According to Seiler *et al.* (1984), a 60-kDa protein phosphorylated by a calmodulin-dependent protein kinase is present in both junctional and nonjunctional SR.
[d] Morii *et al.* (1986) found that the fluorescence intensity of the membrane-attached DACM [*N*-(7-dimethylamino-4-methyl-3-coumarinyl) maleimide] decreased with the onset of Ca^{2+} release. DACM was reported to label a constituent of the Ca^{2+} release channel or a protein that is in close contact with the channel
[e] Argaman and Shoshan-Barmatz (1988); DCCD, dicyclohexylcarbodimiide; TPB, tetraphenylboron.

label junctional SR proteins other than the 350-kDa protein by mere contiguity (see Zorzato *et al.*, 1986). Two additional proteins having an apparent M_r of 60,000 (Kim and Ikemoto, 1986) and 32,000 (Morii *et al.*, 1986) were described as putative components of the same Ca^{2+} release pathway: the evidence provided, however, is only circumstantial and indirect (see notes to Table II).

Using dicyclohexylcarbodimiide (Argaman and Shoshan-Barmatz, 1988), yet other polypeptides have been involved in the Ca^{2+} release process. Some of these proteins (Table II) might be related to a different Ca^{2+} efflux pathway sensitive to tetraphenylboron and changes of pH. There might be more than one Ca^{2+} efflux pathway in isolated SR (see Palade *et al.*, 1989), each possibly with its own molecular constituents.

A crucial question is whether the 350-kDa protein is the Ca^{2+} release channel involved in E–C coupling. Notwithstanding triumphal and unwarranted claims to this effect (Hymel *et al.*, 1988; Knudson *et al.*, 1988), formal proof is still lacking. The junctional localization, intrinsic conductance, i.e., ~ 100 ps (Smith *et al.*, 1985), and density (Smith *et al.*, 1986*b*) seem to indicate that the ryanodine-sensitive Ca^{2+} channel is the Ca^{2+} release channel. However, this is only a possibility, not yet a fact. This question is

doomed to remain open until the mechanism of E–C coupling is understood (see also Palade *et al.*, 1989).

IP_3 has been also proposed as a messenger for E–C coupling. Although IP_3-induced Ca^{2+} release has been observed in both TC and skinned fibers (Volpe *et al.*, 1985), and all necessary substrates and key enzymes for IP_3 metabolism have been identified in skeletal muscle (for a review, see Volpe *et al.*, 1989), the role of IP_3 in E–C coupling is far from proven. No information is available on the molecular constituents of the IP_3-sensitive Ca^{2+} channel. It is also not known whether IP_3 acts on the same channel modulated by Ca^{2+} and ATP, since Smith *et al.* (1986*a*) and Suarez-Isla *et al.* (1988) have obtained conflicting results. In this context, it is noteworthy that the junctional SR is endowed with a specific IP_3ase activity that appears to be due to an intrinsic, yet to be identified, integral protein (Milani *et al.*, 1988).

Junctional SR during Development and the Control Exerted by Motor Innervation

In mammalian species that are born relatively immature, such as the rat, mouse, and rabbit, TT and SR membranes are sparse at the neonatal stage (Schiaffino and Margreth, 1969). These develop postnatally from, respectively, invaginations of the plasma membrane and from smooth endoplasmic rediculum areas, which are occasionally seen in continuity with rough endoplasmic reticulum (Ezerman and Ishigawa, 1967). The development of the longitudinal SR lags behind that of both TT and junctional SR, especially at the A-band level (Schiaffino and Margreth, 1969; Luff and Atwood, 1971), where an extensive network of SR tubules, characteristic of fast-twitch fibers, is attained only at advanced stages (Margreth *et al.*, 1974). Subsequent to innervation, at times coincident with the onset of motile activity, widespread changes occur in the macromolecular composition of intracellular membranes, e.g., SR and mitochondria (Volpe *et al.*, 1982). At these stages, the ratio CS/Ca^{2+} pump protein in rabbit skeletal muscle microsomes decreases markedly (Volpe *et al.*, 1982), indicating proliferation of nonjunctional SR.

The ultrastructure of immature muscle fibers of newborn animals tends to resemble that of denervated muscle fibers. The influence of phasic motor innervation on fast-twitch muscles has been investigated in rabbit skeletal muscles at early periods, i.e., 15 days, after denervation: Salvatori *et al.* (1988) showed that junctional SR is characteristically more developed than nonjunctional SR and that the ratio CS/Ca^{2+} pump is much higher. The content of the 350-kDa protein and the surface area of junctional SR relative to the Ca^{2+} pump membrane are also increased markedly after denervation (F. Zorzato, P. Volpe, E. Damiani, and A. Margreth, unpublished results).

The increase of junctional SR after denervation appears to be due to the increase in number of TC, as well as to an increase of the junctional contact area of TC with TT (Salvatori *et al.*, 1988). The loss of motor innervation, rather than inactivity, as such, appears to be responsible for the hyperplasia of triads in muscle fibers, since the changes appear to be readily reversible upon reinnervation of muscle fibers after nerve crushing (Salvatori *et al.*, 1988).

Denervation not only modifies the ratio of junctional to nonjunctional SR, but also seems to affect the functional properties of the Ca^{2+} release channel. In fact, the Ca^{2+} permeability of TC is decreased after denervation (F. Zorzato, P. Volpe, E. Damiani, and A. Margreth, unpublished results). Denervation-induced changes might affect the channel *per se* or its regulatory mechanisms. Investigation of the nature of these changes is in progress and might shed light on some of the ontogenetic transitions of the Ca^{2+} release channel.

Sequential replacement of discrete cellular and molecular constituents is a general, fundamental ontogenetic rule. By analogy with the occurrence of myosin heavy- and light-chain isoforms (for a review, see Caplan *et al.*, 1983) and SR Ca^{2+} pump isoenzymes (Damiani *et al.*, 1981; Brandl *et al.*, 1987), it may be anticipated that a neonatal form of the Ca^{2+} release channel protein(s) is expressed in newborn animals. This may help to explain, at least in part, the observation that twitch characteristics of mammalian skeletal muscles are relatively uniform at birth and that there is a postnatal speeding of contraction time, mainly of the fast-twitch muscles (Close, 1972). It may also be expected that fiber-type specificity occurs with respect to most of the junctional SR proteins. A pharmacological study on skinned fibers has indirectly indicated that the molecular components of the Ca^{2+} release channel have homologous functions in fast- and slow-twitch muscle, but are not identical (Salviati and Volpe, 1988). I am confident and hopeful that, in the near future (1989?), molecular cloning will enable the isolation of cDNAs that encode distinct isoforms of the Ca^{2+} release channel(s).

Future Directions

The identification of the 350-kDa protein as a Ca^{2+} release channel (Imagawa *et al.*, 1987; Lai *et al.*, 1988; Hymel *et al.*, 1988) has crowned several years of relentless efforts. However, major questions have yet to be addressed—among them, the relevance of this Ca^{2+} release channel to the E–C coupling mechanism, its structure, subunit composition, and regulation. The architecture of the junctional SR still awaits full understaning and the role of its protein constituents is not going to be restricted to

E–C-coupling-related functions. Thus, there is some work for biochemists and much more for physiologists and molecular biologists.

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Note Added at Proof

A. M. Rubstov and A. J. Murphy (*Biochem. Biophys. Res. Commun.* **154**, 462–468, 1988) have recently suggested that the caffeine receptor might be the 170 kDa protein described in Tables I and II.

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