

Role of Calsequestrin and Related Luminal Ca^{2+} -Binding Proteins as Mediators of Excitation-Contraction Coupling

Daniela Schreiber, Pamela Donoghue, Clare O'Reilly and Kay Ohlendieck

Department of Biology, National University of Ireland, Maynooth, Co. Kildare, Ireland

Abstract

Changes in cytoplasmic Ca^{2+} -levels regulate the contractile status of skeletal muscle fibres, whereby the finely tuned interplay between voltage sensors, Ca^{2+} -release channels, Ca^{2+} -binding proteins and Ca^{2+} -pumps mediates Ca^{2+} -cycling through the sarcoplasmic reticulum. Although the physical coupling between the α_{1S} -dihydropyridine receptor of the transverse tubules and the ryanodine receptor Ca^{2+} -release channel of the junctional sarcoplasmic reticulum represents the central signal transduction step during excitation-contraction coupling, many other ion-regulatory elements are involved in the formation and maintenance of the supramolecular triad complex. Proper Ca^{2+} -handling could not occur without the existence of luminal Ca^{2+} -binding proteins that perform a dual function both as Ca^{2+} -reservoir components and as endogenous regulators of Ca^{2+} -fluxes. In skeletal muscle, the luminal sarcoplasmic reticulum accommodates the high-capacity Ca^{2+} -binding protein calsequestrin of the terminal cisternae region, its high-molecular-mass isoforms termed calsequestrin-like proteins, the Ca^{2+} -binding/shuttle protein sarcalumenin and calreticulin. This short review discusses their proposed functions in skeletal muscle Ca^{2+} -homeostasis and their potential involvement in muscle disorders such as x-linked muscular dystrophy, malignant hyperthermia, denervation-induced muscular atrophy, diabetes and sarcopenia.

Key words: calcium homeostasis, calreticulin, calsequestrin, calsequestrin-like proteins, excitation-contraction coupling, sarcalumenin.

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In skeletal muscle fibers, the contact zones between the transverse tubular membrane system and the junctional sarcoplasmic reticulum are of central importance for excitation-contraction coupling. The precise temporal and spatial control of Ca^{2+} -fluxes and Ca^{2+} -sequestration underlies the signal transduction process which links surface membrane depolarization to fibre contractions [60]. Ca^{2+} homeostasis is maintained by the physiological interplay between Ca^{2+} -channels, Ca^{2+} -binding proteins and Ca^{2+} -ATPases [63]. Since Ca^{2+} -ions are involved in the regulation of a variety of cellular processes, Ca^{2+} -cycling through intracellular compartments such as mitochondria or the endoplasmic reticulum has to be tightly controlled [6]. In skeletal muscle fibres, the cytosolic Ca^{2+} -level determines the status of the excitation-contraction-relaxation cycle making investigations into ion-handling a key issue in muscle physiology. Over the last decades enormous progress has been made in the elucidation of the molecular mechanisms of the signal transduction processes involved in excitation-contraction coupling, as reviewed in several recent articles [6, 60, 63, 74].

Clearly, differences exist between triadic receptor interactions and Ca^{2+} -handling in the heart and skeletal muscle [74]. While cardiac excitation-contraction coupling is mediated by a Ca^{2+} -induced Ca^{2+} -release mechanism [7], direct protein coupling between the α_{1S} -dihydropyridine receptor and the RyR1 isoform of the junctional ryanodine receptor Ca^{2+} -release channel is responsible for triadic signalling in skeletal muscles [50, 64]. Although the major protein factors involved in excitation-contraction coupling appear to have been identified and well characterised, it remains to be determined how the overall Ca^{2+} -cycling process is integrated physiologically and which proteins are essential for the formation and maintenance of the triad contact zones. Ca^{2+} -buffering in the lumen of the sarcoplasmic reticulum certainly plays a key role in the interactions between energy-dependent Ca^{2+} -uptake and junctional Ca^{2+} -release [11, 55]. This article summarizes the current concepts of luminal Ca^{2+} -binding elements in normal skeletal muscles and discusses the potential involvement of abnormal Ca^{2+} -sequestration in muscle diseases such as x-linked muscular dystrophy.

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Excitation-contraction coupling

Current muscle proteome projects attempt to identify the entire protein complement of skeletal muscle fibres [36] including the identification of all sarcoplasmic reticulum and triad proteins involved in Ca^{2+} -cycling and signal transduction. Until comprehensive data on the muscle proteome is available, it is difficult to judge the complexity of the Ca^{2+} -handling apparatus and difficult to evaluate how many auxiliary proteins are involved in the regulation of the excitation-contraction coupling process. However, some progress has been made by traditional biochemical approaches in identifying novel triadic regulators. In addition to the junctional triad markers represented by the dihydropyridine receptor, the ryanodine receptor and calsequestrin [50, 55], several protein species have been identified over the past few years that may be involved in Ca^{2+} -homeostasis, receptor coupling and/or the maintenance of triad structure [63]. This includes triadin, junctin, JP-45, JP-90 and FKBP-12 [3, 13, 28, 33, 37, 38, 42, 59, 86]. It remains to be determined how many other triad proteins are directly linked to voltage sensing, receptor coupling, Ca^{2+} -release and Ca^{2+} -reuptake and the stabilisation of the supramolecular triad-associated membrane assembly.

Despite our incomplete knowledge about the complexity of the excitation-contraction coupling apparatus, the overall membrane structure containing the main signal transduction components is well established. Electron microscopical studies have revealed the position of triad couplings at the A-I junction in mammalian skeletal muscle [24, 25]. The close association between a central transverse tubule and the two surrounding terminal cisternae forms a tight contact zone for receptor coupling. In mature skeletal muscles, the excitation-contraction-relaxation cycle is regulated by the physiological interplay between the voltage-sensing α_{1S} -subunit of the dihydropyridine receptor, the ryanodine receptor RyR1 isoform of the junctional Ca^{2+} -release channel and the Ca^{2+} -ATPases. The flow chart in Fig. 1 summarizes the main steps involved in excitation-contraction coupling.

Once a critical amount of acetylcholine is released from the innervating motor neuron into the sub-synaptic cleft, a sufficient amount of neurotransmitter will bind to the nicotinic acetylcholine receptor complex to trigger sarcolemmal depolarization. The propagation of an action potential is then mediated by the activation of voltage-dependent Na^+ -channels in neighboring membrane patches and will eventually reach invaginations of the surface membrane. In the junctional transverse tubular region, charge movement in response to depolarization activates the α_{1S} -subunit of the dihydropyridine receptor [14]. Physical coupling between the II-III loop domain of the voltage sensor and the cytosolic foot region of the RyR1 tetramer initiates the fast release of luminal Ca^{2+} -ions [9, 50]. Thus,

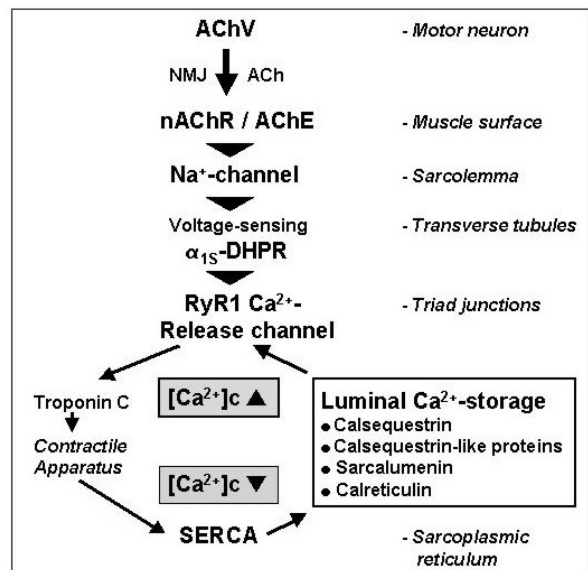


Figure 1: Flow chart summarising the main steps involved in the regulation of excitation-contraction coupling and muscle relaxation. Release of neurotransmitter from acetylcholine vesicles (AChV) from the innervating motor neuron leads to the diffusion of the neurotransmitter across the sub-synaptic cleft, and binding to the nicotinic acetylcholine receptor (nAChR) complex. Surplus neurotransmitter is quickly degraded by the acetylcholinesterase (AChE) located on the basal lamina within the neuromuscular junction (NMJ). The surface action potential is propagated via the activation of voltage-dependent Na^+ -channels and is transferred into the muscle interior by invaginations of the surface membrane, the so-called T-system of the transverse tubules. The voltage-sensing α_{1S} -subunit of the dihydropyridine receptor (α_{1S} -DHPR) directly interacts with the cytosolic domain of the RyR1 isoform of the junctional Ca^{2+} -release channel complex. Increased cytosolic Ca^{2+} -levels cause occupation of ion-binding sites on Troponin C thereby triggering actin-myosin interactions. Muscle relaxation is introduced by the energy-dependent re-uptake of Ca^{2+} -ions by SERCA type Ca^{2+} -ATPases of the sarcoplasmic reticulum. The physiological linkage between the Ca^{2+} -release process and the Ca^{2+} -uptake mechanism is provided by the luminal Ca^{2+} -storage complexes consisting of calsequestrin, calsequestrin-like proteins, sarcalumenin and calreticulin.

surface membrane depolarization becomes coupled to the Ca^{2+} -release channel causing excitation-contraction coupling [47]. Probably, Ca^{2+} -induced Ca^{2+} -release is triggered in uncoupled RyR1 Ca^{2+} -channels in a second step greatly amplifying the Ca^{2+} -flux signalling cascade

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Table 1: List of the established main luminal Ca^{2+} -binding proteins of the sarcoplasmic reticulum from skeletal muscle fibres

Luminal protein	Molecular mass	References
Calsequestrin	63 kDa	[10, 56, 85]
CLP-220	220 kDa	[11, 15, 57]
CLP-170	170 kDa	[11, 15, 57]
CLP-150	150 kDa	[11, 15, 57]
Sarcalumenin	160 kDa	[20, 46]
Calreticulin	55 kDa	[26, 61]

[60]. In contrast, muscle relaxation depends on the swift reversal of the cytosolic Ca^{2+} -signal. Energy-dependent uptake of Ca^{2+} into luminal regions is accomplished by the SERCA type Ca^{2+} -pumps located in the longitudinal tubules and terminal cisternae [63]. Ca^{2+} -removal by the calmodulin-dependent PMCA Ca^{2+} -pump of the sarcolemma or the $\text{Na}^+/\text{Ca}^{2+}$ -exchanger does not appear to be involved in skeletal muscle relaxation to a large extent. Besides Ca^{2+} -release and Ca^{2+} -uptake, proper luminal Ca^{2+} -storage is a prerequisite for triadic signal transduction.

Luminal Ca^{2+} -handling proteins

A very steep concentration gradient exists for Ca^{2+} ions between the cytosol and the lumen of the sarcoplasmic reticulum on the one hand and the extracellular space on the other hand [55, 60]. A large proportion of luminal Ca^{2+} is bound to high-capacity Ca^{2+} -binding proteins within the terminal cisternae and the longitudinal tubules [11, 39]. Table 1 lists the major species of luminal Ca^{2+} -binding elements found in skeletal muscle fibres, i.e. calsequestrin, the calsequestrin-like proteins CLP-150, CLP-170 and CLP-220, sarcalumenin and calreticulin. Ca^{2+} -buffering by protein binding sites in the sarcoplasmic reticulum establishes two important physiological parameters. Firstly, due to the lowering of the free luminal Ca^{2+} -concentration, the Ca^{2+} -ATPase units have to transport ions against a less steep gradient, which significantly decreases the rate of ATP hydrolysis and thereby saves energy equivalents. Secondly, several-fold more Ca^{2+} -ions can be stored in the sarcoplasmic reticulum via Ca^{2+} -binding proteins, as compared to a protein-free luminal area, thereby greatly enhancing the overall Ca^{2+} -reservoir capacity [55]. This is of central importance for the functional integrity of the excitation-contraction machinery. Since the presence of high cytosolic Ca^{2+} -levels is crucial for triggering muscle contraction, and the absence of Ca^{2+} -ions is absolutely critical for initiating muscle relaxation, a fast Ca^{2+} -cycling mechanism and an efficient Ca^{2+} -storage process must exist in skeletal muscles. It is not well understood how the different Ca^{2+} -binding elements interact with each other, which exact luminal domains

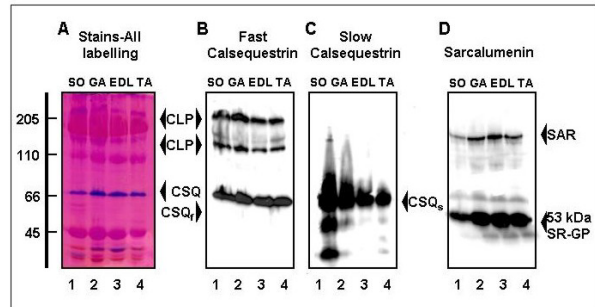


Figure 2. Immunoblot analysis of luminal Ca^{2+} -binding proteins in the sarcoplasmic reticulum from skeletal muscle fibres. Shown is a Stains-All labelled gel (A) and identical immunoblots decorated with antibodies to the fast calsequestrin isoform CSQ_f (B), the slow calsequestrin isoform CSQ_s (C) and the skeletal muscle SAR isoform of sarcalumenin (D). High-molecular mass CSQ isoforms, termed calsequestrin-like proteins (CLP) are indicated in panels (A) and (B). The alternative splice product of the SAR gene, the sarcoplasmic reticulum glycoprotein (SR-GP) of apparent 53 kDa, is marked in panel (D). Lanes 1 to 4 represent microsomal membranes derived from soleus (SO), gastrocnemius (GA), extensor digitorum longus (EDL) and tibialis anterior (TA) muscle homogenates, respectively. Subcellular fractionation, gel electrophoretic separation and immunoblotting was carried out by standard procedures [20, 29]. The position of molecular mass standards (in kDa) are indicated on the left. Stains-All labelled bands and immuno-decorated Ca^{2+} -binding proteins are marked by arrow heads.

they occupy and what changes in their expression patterns and protein-protein interactions occur during prenatal development, postnatal myogenesis, fibre type maturation and muscle ageing. Our current understanding of the biochemical and physiological properties of individual members of the Ca^{2+} -binding family of sarcoplasmic reticulum proteins is discussed below.

In Fig. 2 is illustrated the identification of calsequestrin, calsequestrin-like proteins and sarcalumenin by immunoblotting and dye binding. The cationic carbocyanine dye 'Stains All' labels Ca^{2+} -binding proteins with a characteristic blue colour, while other microsomal components stain in a pink tone [12]. The dye has been proposed to interact with anionic sites within Ca^{2+} -binding proteins producing a dye-protein complex, which absorbs at 600 to 615 nm [12]. All muscle types investigated exhibited a major blue band at approximately 66 kDa and two minor blue bands of higher molecular mass, in addition to a heterogeneous mixture of low-molecular-mass components (Fig. 2A). This agrees with previous work by Fliegel et al. [22]

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and Damiani et al. [17]. Immunoblotting with a monoclonal antibody to fast calsequestrin identified the upper three bands as calsequestrin itself and two CLPs (Fig. 2B). Slow calsequestrin was found at a higher expression level in the predominantly slow soleus muscle, as compared to gastrocnemius, extensor digitorum longus and tibialis anterior preparations (Fig. 2C). In contrast, the relative density of sarcalumenin and the 53 kDa sarcoplasmic reticulum glycoprotein was lower in slow twitching fibres and relatively comparable in microsomes derived from gastrocnemius, extensor digitorum longus and tibialis anterior (Fig. 2D). This shows that different fibre types do not exhibit the same complement of luminal Ca^{2+} -binding proteins, which probably reflects physiological adaptations to different ion binding requirements in fast versus slow muscles.

The major Ca^{2+} -reservoir complex of the terminal cisternae is represented by calsequestrin clusters [25, 57, 71]. The peripheral protein assembly is probably anchored to junctional sites via the calsequestrin-binding protein named junctin [24, 38]. Since deletion of the carboxy-terminal domain or phosphorylation sites does not affect the segregation of calsequestrin to the junctional sarcoplasmic reticulum, a specific vesicle budding process from the endoplasmic reticulum appears to be involved in calsequestrin routing to the sarcoplasmic reticulum [66-68]. Recently it has been shown by blot overlay assays, chemical crosslinking and differential co-immuno precipitation that calsequestrin forms links not only to junctin, but also to other excitation-contraction coupling proteins [30, 32, 62]. Calsequestrin seems to form mostly self-aggregates, but a subpopulation of it is tightly linked to the ryanodine receptor [30]. Both, direct coupling between the Ca^{2+} -binding protein and the Ca^{2+} -release channel and indirect interactions via triadin appear to exist in skeletal muscle triads [32].

Calsequestrin was originally described by MacLennan and Wong [56] as a high capacity, low-affinity Ca^{2+} -binding protein of the sarcoplasmic reticulum. A slow- and a fast-twitch isoform of calsequestrin exist in skeletal muscle [8]. Using hydrophobic exchange chromatography, calsequestrin can conveniently be isolated in a homogeneous form [10] for biochemical analyses [30]. The observation that Ca^{2+} -sequestration by calsequestrin results in conformational changes in the ryanodine receptor suggests that calsequestrin is an endogenous regulator of the Ca^{2+} -release channel [35, 69]. The ryanodine receptor and calsequestrin appear to exist in a mutual coupling process, i.e. the conformational change within one protein is transmitted to the other [79]. Protein-protein interactions are postulated to play a key role in regulating luminal Ca^{2+} -concentrations, since large calsequestrin clusters exhibit positive co-operativity with respect to high capacity Ca^{2+} -binding. It is not fully understood whether high-

molecular-mass bands recognized by antibodies to calsequestrin (Fig. 2B) represent novel isoforms or just chemically non-reducible aggregates of the 63 kDa monomer [57]. Since the so-called calsequestrin-like proteins (CLPs) are greatly reduced in dystrophic fibres [15], but stabilisation by chemical crosslinking achieves a restoration of the three main CLP bands, CLP-150 to CLP-220 are most likely not distinct isoforms of the terminal cisternae Ca^{2+} -binding protein. Analysis of the crystal structure of calsequestrin by Wang et al. [85] showed that each monomer makes two extensive dimerization contacts and that calsequestrin contains three negative thioredoxin-like domains that surround a hydrophilic centre. It is proposed that conformational changes within these domains account for the variation in Ca^{2+} -binding. Co-operative kinetics seems to play a major role in the coordinated binding and release of Ca^{2+} -ions from calsequestrin clusters [40].

Although most Ca^{2+} -regulatory proteins undergo distinct isoform changes during a stimulation-induced fast-to-slow transition process [72, 73], the expression of sarcalumenin of 160 kDa is unaltered following skeletal muscle transformation. Sarcalumenin binds approximately 35 mol of Ca^{2+} -ions per mol protein [46]. The monoclonal antibody XIIC4 recognises a 53 kDa glycoprotein that does not bind Ca^{2+} -ions, as well as the sarcalumenin band. Both, sarcalumenin and the alternative splice variant of its carboxy-terminus co-localize with the sarcoplasmic reticulum Ca^{2+} -ATPase [46]. This was confirmed by differential co-immuno precipitation experiments and chemical crosslinking [20]. Both techniques demonstrated a tight linkage between sarcalumenin and the SERCA1 isoform of the sarcoplasmic reticulum Ca^{2+} -ATPase. Sarcalumenin is located to the longitudinal tubules and the terminal cisternae structures and is expressed in relatively comparable levels in fast and slow fibres. It probably functions as a Ca^{2+} -shuttle protein that mediates between the Ca^{2+} -uptake units and the junctional Ca^{2+} -storage/release sites. Cycles of phosphorylation and dephosphorylation of sarcalumenin and the histidine-rich Ca^{2+} -binding protein have been shown to modulate the activity of the junctional ryanodine receptor Ca^{2+} -release channel complex [81]. Since the exact subcellular localization and function of the histidine-rich Ca^{2+} -binding protein is controversial, it is not discussed here. A third Ca^{2+} -binding protein, present especially in developing muscle, is represented by calreticulin [61]. It exhibits a much higher abundance in smooth muscle cells and non-skeletal/cardiac muscles and its expression decreases rapidly during postnatal myogenesis [4, 43]. Calreticulin is only present in a low density in mature skeletal muscle [26].

Muscular disorders involving luminal Ca^{2+} -handling proteins

Abnormal Ca^{2+} -handling is involved in many disease processes. Due to the high Ca^{2+} -concentration in

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extracellular spaces and intracellular organelles, damage to membrane systems often results in the uncontrolled increase of cytosolic calcium in end-stage pathology. Because long-term elevated cytosolic Ca^{2+} -levels are an important pathophysiological mechanism, its fast and efficient removal is essential for the survival of diseased cells and tissues. Besides this general pathophysiological mechanism, numerous muscle disorders have a more specific Ca^{2+} -dependent aspect [27, 54]. Here, we discuss the potential role of abnormal Ca^{2+} -handling using examples of specific disease with a typical neuromuscular involvement. This includes x-linked muscular dystrophy, diabetes, malignant hyperthermia, muscular atrophy, and sarcopenia.

Primary genetic abnormalities in the Duchenne muscular dystrophy gene represent the underlying cause for the most severe forms of x-linked muscular dystrophy [1]. The absence of the membrane cytoskeletal protein dystrophin results in the loss of a sarcolemmal glycoprotein complex, which in turn weakens the linkage between the actin membrane cytoskeleton and the extracellular matrix [70]. Modified surface Ca^{2+} -fluxes and impaired luminal Ca^{2+} -buffering is believed to be the major down-stream effect of sarcolemmal micro-rupturing, eventually leading to muscle weakness in dystrophin-deficient fibres [16]. Hence, abnormal Ca^{2+} -homeostasis in mechanically stressed fibres may lead to the severe degeneration of skeletal muscles. Imbalanced ion cycling through the sarcolemma and the sarcoplasmic reticulum appears to contribute to the enhanced degradation of muscle proteins [2]. An extensive immunoblotting survey of dystrophic muscle fibres with a library of monoclonal antibodies to Ca^{2+} -handling proteins revealed the drastic reduction in all calsequestrin-like proteins and sarcalumenin [15, 20]. While CLP-150 to CLP-220 are presented at 20% to 50% of their normal density [15], the relative expression of sarcalumenin is approximately 70% lower in dystrophic fibres as compared to normal skeletal muscle [20]. Immunofluorescence labelling showed a patchy internal labelling of sarcalumenin in dystrophic fibres that is indicative of the abnormal formation of sarcalumenin domains within the sarcoplasmic reticulum. This might explain the 20% reduction in the overall Ca^{2+} -buffering capacity of the dystrophic sarcoplasmic reticulum. Impaired Ca^{2+} -shuttling between the Ca^{2+} -uptake units and calsequestrin clusters via sarcalumenin may indirectly amplify the Ca^{2+} -leak channel induced elevation of cytosolic Ca^{2+} -levels [16].

In contrast to muscular dystrophy, in diabetic skeletal muscle fibres the expression of calsequestrin and especially that of the calsequestrin-like proteins has been found to be significantly increased [34]. Since no calsequestrin degradation products are detectable in diabetic microsomes, probably no major proteolytic processes occurred. Equilibrium dialysis studies

revealed an overall increase in the Ca^{2+} -binding capacity of the sarcoplasmic reticulum from diabetic skeletal muscle, which agrees with the increased calsequestrin expression [34]. Obviously, a functional correlation exists between the up-regulation of the Ca^{2+} -binding proteins and the expanded buffering of luminal Ca^{2+} -ions. Because no significant changes in luminal Ca^{2+} -binding proteins were shown to exist in the diabetic heart, the up-regulation of the high-capacity Ca^{2+} -binding proteins might represent a specific compensatory mechanism of diabetic skeletal muscle only. Increased calsequestrin levels might sufficiently counter-act elevated cytosolic Ca^{2+} -levels in diabetes. This protective mechanism quickly and efficiently removes excess Ca^{2+} -ions thereby preventing Ca^{2+} -dependent myo-necrosis.

Malignant hyperthermia is a dominantly inherited autosomal predisposition of otherwise healthy people who undergo an uncontrollable skeletal muscle hyper-metabolism when exposed to volatile anesthetics such as halothane [52]. The clinical symptoms of an episode of malignant hyperthermia are extreme skeletal muscle rigidity, hyperkalemia, hypoxia and hyperthermia associated with acidosis. Malignant hyperthermia is primarily a metabolic muscle disease. However, secondary changes may occur in the kidneys, heart and the lungs. Although abnormalities in calsequestrin or other luminal Ca^{2+} -binding proteins might be involved in this pharmacogenetic disease, genetic linkage analysis has so far revealed mutations only in the RyR1 isoform of the Ca^{2+} -release channel and the dihydropyridine receptor [52]. Thus, malignant hyperthermia can be considered a disease of excitation-contraction coupling. The mutated ryanodine receptor exhibits a prolonged channel opening time which causes a transient increase in cytosolic Ca^{2+} -levels during excitation. The drastic rise in cytosolic Ca^{2+} -concentration then leads to glycogenolysis, ATP depletion, mitochondrial oxidation, production of excess lactic acid and CO_2 and ultimately to a disturbance of intra- and extracellular ion homeostasis with consequent muscle cell damage. No major differences were found in the expression of luminal Ca^{2+} -binding proteins in malignant hyperthermia [29], but protein gel shift experiments with halothane-treated sarcoplasmic reticulum showed a clear difference between vesicles from normal and susceptible specimens. Anaesthetic-induced clustering of the RyR1 complex was observed at a significantly lower threshold concentration in the sarcoplasmic reticulum from patients with malignant hyperthermia as compared to normal individuals [29]. Hence, the decreased Ca^{2+} -loading ability of the sarcoplasmic reticulum from susceptible muscle fibres is most likely due to altered quaternary receptor structure and/or modified functional dynamics within the Ca^{2+} -regulatory machinery. The increased receptor complex formation of a hyper-gated Ca^{2+} -channel is

probably at the centre of the development of a metabolic crisis in malignant hyperthermia.

Alterations in mechanical loading conditions, the nutritional supply to muscle fibres, changes in neuromuscular activity or hormonal modifications can have a significant effect on skeletal muscle plasticity [23]. The molecular and cellular adaptation can be observed following endurance training, immobilization or experimental chronic low-frequency stimulation [31, 72, 73]. An extreme type of change in neuromuscular activity is the total disconnection of a skeletal muscle by physical separation from its innervating motor nerve. Denervation-induced muscular atrophy triggers a dramatic loss in tissue mass and a decrease in isometric contractile force within a few weeks. Since fibre type shifting generally has a striking effect on the isoform expression pattern of almost all Ca^{2+} -regulatory proteins, it is not surprising that key Ca^{2+} -pump and Ca^{2+} -binding proteins are affected after nerve crush or complete denervation. While increased levels of sarcocalumenin and calsequestrin have been observed after denervation, the fast Ca^{2+} -ATPase appears to be drastically decreased during muscular atrophy [45, 49, 51, 53, 87]. Thus, altered skeletal muscle activity seems to have a profound effect on the abundance and isoform expression pattern of a subset of Ca^{2+} -handling elements [48, 78]. These molecular changes probably represent physiological adaptations to changed functional demands.

The age-related decline in skeletal muscle mass and contractile strength is now usually referred to as sarcopenia [65]. The pathobiochemical hierarchy existing within the various molecular and cellular factors leading to senescent muscle weakness is not well understood [41]. The calcium hypothesis of sarcopenia assumes that a drastically reduced level of Ca^{2+} -ion supply for contractile muscle proteins can lead to the muscle weakness associated with aging [21, 44, 58]. The basic process underlying this phenomenon is proposed to be an uncoupling between the excitation-induced signal transduction mechanism and junctional Ca^{2+} -release [18, 19]. If a large number of ryanodine receptor Ca^{2+} -release channel units becomes physiologically disconnected from the α_{1S} -voltage-sensing dihydropyridine receptor, excitation-contraction uncoupling occurs [75]. In addition, many other mechanisms of age-related muscle wasting have been suggested including mitochondrial dysfunction, reduced protein synthesis, inadequate nutritional supply, a drastic reduction in essential hormones and changes within the peripheral nervous system [65, 80, 82, 84]. The analysis of established animal models of sarcopenia agrees with the excitation-contraction uncoupling hypothesis [76]. In both ageing rats and rabbits, a dramatic decrease in the α_{1S} -subunit of the dihydropyridine receptor was observed. An age-related shift to slower fibre type characteristics was indicated

by an increase in the slow/cardiac isoform of calsequestrin in aged rabbit fibres [76]. However, these data were not confirmed by studies into the human ageing process. A comprehensive immunoblot analysis of vastus lateralis specimens from male humans aged 18 to 82 years of age revealed no major changes in the relative abundance of the α_{1S} -dihydropyridine receptor or calsequestrin [77]. This suggests that fundamental physiological differences exist between sarcopenia in animals and humans. Hence, the assumption that abnormal excitation-contraction coupling is responsible for age-related muscle weakness cannot be extrapolated from animal models to senescent human muscle fibres without modifications.

Conclusions

In the past few years there has been great progress in the elucidation of the signal transduction pathway underlying excitation-contraction coupling. The involvement of luminal Ca^{2+} -binding proteins in ion-cycling through the sarcoplasmic reticulum is absolutely critical for this regulatory mechanism. This is especially emphasised by the pathophysiological role of abnormal expression of calsequestrin-like proteins and sarcocalumenin in x-linked muscular dystrophy. Reduced levels of these key Ca^{2+} -binding proteins and endogenous regulators severely impair the integrity of skeletal muscle fibres and lead to necrosis. In this respect, it is encouraging that basic research on Ca^{2+} -handling mechanisms has introduced a new way of treating muscular dystrophy with inhibitors of Ca^{2+} -dependent proteolytic enzymes [5, 83]. The complete cataloguing of the skeletal muscle proteome in the near future [36] should be useful in the identification of novel therapeutic targets within the Ca^{2+} -regulatory apparatus. We still lack an intricate understanding of the fine regulation of excitation-contraction coupling. Although the major proteins responsible for voltage sensing, Ca^{2+} -release, Ca^{2+} -binding and Ca^{2+} -pumping have been identified and extensively characterised, many important physiological steps underlying triadic signal transduction have not yet been revealed. The application of high-throughput intra-proteomics tools such as blot overlay assays [62] may be useful in determining the complex interaction between the various triad proteins involved in the formation, regulation and maintenance of excitation-contraction coupling. Once a comprehensive map of protein-protein interactions between all triad proteins has been established, it will be possible to compose a three-dimensional map of junctional interactions and to fully understand the complexity of excitation-contraction coupling in skeletal muscle.

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Address Correspondence to:

Prof. Kay Ohlendieck, Department of Biology, National University of Ireland, Maynooth, Co. Kildare, Ireland. Tel. 353-1-7083842; Fax. 353-1-7083845.

E-mail. kay.ohlendieck@may.ie

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