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Review

The network of calcium regulation in muscle✪

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In this review the molecular characteristics and reaction mechanisms of different Ca^{2+} transport systems associated with various membranes in muscle cells will be summarized. The following topics will be discussed in detail: a brief history of early observations concerning maintenance and regulation of cellular Ca^{2+} homeostasis, characterization of the Ca^{2+} pumps residing in plasma membranes and sarco(endo)plasmic reticulum, mitochondrial Ca^{2+} transport, Ca^{2+}-binding proteins, coordinated expression of Ca^{2+} transport systems, a general background of muscle excitation-contraction coupling with emphasis to the calcium release channels of plasma membrane and sarcoplasmic reticulum, the structure and function of dihydropyridine and ryanodine receptors of skeletal and cardiac muscles, and finally their disposition in various types of muscles.

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Abbreviations: $[Ca^{2+}]_M$, free Ca^{2+} concentration in the mitochondrial matrix; CHO cells, Chinese hamster ovary cells; DHPR, dihydropyridine receptor; E–C, excitation–contraction coupling; ER, endoplasmic reticulum; F_{SR} leak, the leak of Ca^{2+} from the sarcoplasmic reticulum into the cytoplasm; NCX, Na^+:Ca^{2+} exchanger; P_i, inorganic phosphate; PDH_A, pyruvate dehydrogenase; PMCA, plasma membrane Ca^{2+}-ATPase; PTP, permeability transition pore; RAM, rapid uptake mode; RR, ruthenium red; RyR, ryanodine receptor; SERCA, sarco(endo)plasmic reticulum Ca^{2+}-ATPase; SR, sarcoplasmic reticulum; T tubules, transverse tubules.

CELLULAR CALCIUM HOMEOSTASIS

Brief history of early observations

The recognition of the role of Ca^{2+} as the regulator of a broad range of cellular processes is one of the great accomplishments of the 20th century biology (Cambell, 1983; Carafoli & Klee, 1999; Putney, 2000). It evolved from Ringer's observations that Ca^{2+} is required for cardiac muscle contractility (Ringer, 1883), continued with observations of a similar role of Ca^{2+} in skeletal muscle (Kamada & Kinoshita, 1943; Heilbrunn & Wiercinsky, 1947), and culminated in the discoveries of the Ca^{2+}-transport ATPase of SR (Ebashi, 1961; Hasselbach & Makinose, 1961) and the regulation of actomyosin activity by Ca^{2+} (Weber, 1959) through its interaction with troponin (Ebashi, 1963; Ebashi *et al.*, 1999). In the intervening years the messenger role of Ca^{2+} has been demonstrated in virtually all living cells and all cellular compartments, in processes as diverse as cell division, cellular motility, hormone secretion, intermediary metabolism, neural activity, protein trafficking, gene expression, developmental regulation and apoptosis (Berridge *et al.*, 1998; Brini & Carafoli, 2000; Martonosi, 2000; Berridge, 2001; Mellstrom & Naranjo, 2001; Bootman *et al.*, 2002).

The cytoplasmic free Ca^{2+} concentration of muscle and other cells at rest is around 20–50 nM; this is 10^3–10^4 times lower than the free Ca^{2+} concentration in the extracellular space (usually millimolar) or in the lumen of sarco(endo)plasmic reticulum (SR/ER) (0.1–2.0 mM). The large Ca^{2+} gradients across cellular boundaries are established and maintained by powerful Ca^{2+} pumps located in the cell surface membranes, and in the endoplasmic reticulum (MacLennan *et al.*, 1997; Guerini & Carafoli, 1999; Philipson & Nicoll, 2000), with contributions by the mitochondria (Rizzuto *et al.*, 2000; Duchen, 2000).

During activation by electric stimuli or various agonists, Ca^{2+} enters into the cytoplasm through Ca^{2+} channels located in the plasma membrane (Catterall, 2000) and the SR/ER (Meissner, 1994; Mar & Marks, 2000; Ogawa *et al.*, 2002), causing an increase in cytoplasmic, mitochondrial, and nucleoplasmic Ca^{2+} concentration (Carafoli *et al.*, 2001) that triggers a broad range of coordinated physiological responses (Bootman *et al.*, 2001) from contractile activity (Rios & Stern, 1997; Niggli, 1999) to energy production (Kunz, 2001) and nuclear processes (Santella, 1996; Mazzanti *et al.*, 2001). The Ca^{2+} signals are terminated by the Ca^{2+} pumps that return the cytoplasmic and nucleoplasmic Ca^{2+} concentrations to resting levels.

The role of Ca^{2+} in the biosynthesis of SR (Martonosi, 2000), contractile proteins (Allen & Leinwand, 2002) and mitochondria (Ojuka *et al.*, 2002) is beginning to be recognized.

The Ca^{2+} pumps of the plasma membranes

The plasma membrane Ca^{2+}-ATPase (PMCA). A (Mg^{2+} + Ca^{2+})-activated ATPase of about 130 kDa is present in the plasma membranes of most eukaryotic cells, including plants (Guerini & Carafoli, 1999; Strehler & Zacharias, 2001). It catalyzes the ATP-dependent transport of Ca^{2+} from the cytoplasm into the extracellular space. The Ca^{2+}-dependent cleavage of ATP occurs with the transient formation of an acylphosphate enzyme intermediate, that is linked to Ca^{2+} translocation. The enzyme has high affinity for Ca^{2+} when complexed with calmodulin ($K_m < 0.5\ \mu M$), that serves as a physiological activator; other activators are acidic phospholipids and phosphorylation by several protein kinases. The predicted structure of PMCA contains 10 transmembrane domains; about 80% of its mass protrudes into the cytoplasm, and only short loops are exposed to the outside.

Mammalian PMCAs are encoded by four distinct genes, yielding the PMCA1-4 isoforms (Table 1), (Strehler & Zacharias, 2001). Additional isoform variants are generated by alter-

native splicing of the primary gene transcripts at two major conserved locations, that correspond to the regulatory domains of the pump. PMCA1 and 4 are present in most adult tissues, while PMCA2 and PMCA3 are primarily expressed in the nervous system and in muscles. The distinct functional characteristics of the isoforms, their tissue-specific localization, and the dynamic regulation of their expression during development and in adult animals suggest specific roles for each isoform in Ca^{2+} homeostasis.

Table 1. Classification of Ca^{2+}-ATPase present in mammalian tissues

Protein	Gene	Membrane	Tissue/Cell
SERCA1a/b	ATP2A1	SR	adult and neonatal fast-twitch skeletal muscle
SERCA2a	ATP2A2	SR	slow-twitch skeletal, cardiac and smooth muscle
SERCA2b	ATP2A2	SR/ER	non-muscle, smooth muscle
SERCA3	ATP2A3	SR/ER	muscle and non-muscle tissues including nervous system, various epithelial cells, lymphocytes, thrombocytes
PMCA1	ATP2B1	plasma membrane	most of non-muscle tissues and muscle
PMCA2	ATP2B2	plasma membrane	nervous system, cardiac muscle
PMCA3	ATP2B3	plasma membrane	nervous system
PMCA4a/b	ATP2B4	plasma membrane	most of tissues, erythrocytes
SPCA	ATP2C1	Golgi/ER	ubiquitous

The surface membrane Ca^{2+} pumps are of primary importance in the regulation of total cell Ca^{2+} content. Their cellular concentration and activity are adjusted to compensate for the influx of extracellular Ca^{2+}, but very little is known about the mechanism of this regulation. In healthy red blood cells the ATP-dependent Ca^{2+} pump is sufficient to balance the relatively slow influx of extracellular Ca^{2+}. In excitable cells (heart, skeletal muscle, nervous system), where a large influx of Ca^{2+} occurs during activity, the Na^+:Ca^{2+} exchange system is also required to restore the cell Ca^{2+} content to normal levels.

The Na^+:Ca^{2+} exchanger (NCX). The Na^+:Ca^{2+} exchangers catalyze the electrogenic exchange of 3 Na^+ for 1 Ca^{2+} across the surface membrane (Blaustein & Lederer, 1999; Philipson & Nicoll, 2000). The exchange is driven by the transmembrane gradient of Na^+, that in turn is maintained by the Na^+, K^+-transport ATPase (Skou, 1985; Sweadner & Donnet, 2001; Kaplan, 2002). The net direction of Na^+:Ca^{2+} exchange is determined by the Na^+ and Ca^{2+} concentrations in the medium and the cytoplasm, and by the membrane potential. The primary role of the exchanger is the export of Ca^{2+} from the cell when the cytoplasmic Ca^{2+} concentration is elevated upon activation. Due to its relatively low Ca^{2+} affinity (K_m >1 μM), it is likely to operate most efficiently in its Ca^{2+} efflux mode in areas of high local Ca^{2+} concentration at the peaks of Ca^{2+} transients. Under some special conditions the exchanger may reverse and catalyze net Ca^{2+} import, but the physiological significance of this is not clearly established.

The exchanger is present in particularly high concentration in the surface membranes of excitable cells (heart and nerve cells). The Na^+:Ca^{2+} exchange is not coupled to the hydrolysis of ATP, but ATP serves as a physiological activator by phosphorylation and by promoting the synthesis of phosphatidylinositol 4,5-bisphosphate. In addition to serving as substrates for transport, Na^+ and Ca^{2+} exert distinct regulatory effects on the exchange activity. Na^+ inactivates the exchanger by binding to the internal Na^+ transport sites

at low (about 1 μM) Ca^{2+}. The Na^{+}-dependent inactivation is counteracted by Ca^{2+} binding to internal high affinity Ca^{2+} regulatory sites at elevated Ca^{2+} concentration (15 μM).

The three known mammalian Na^{+}:Ca^{2+} exchangers (NCX1-3) are encoded by distinct genes (Philipson & Nicoll, 1999). NCX1 and 2 are ubiquitously expressed in various tissues, while NCX3 is confined to the brain. Several splice variants of NCX1 have been detected that are functionally distinct and show tissue specific distribution.

The full-length mature cardiac sarcolemmal NCX1 contains 938 amino-acid rsidues. In contrast to earlier predictions of 11 transmembrane helices, its revised topology now contains 9 transmembrane domains with a large cytoplasmic loop of about 550 amino-acid residues between transmembrane helices 5 and 6 (Philipson & Nicoll, 1999). The N-terminus is glycosylated at position 9 and presumed to be extracellular. The large cytoplasmic loop contains the regulatory Ca^{2+}-binding sites and the regions of alternative splicing. The human NCX1 gene is organized into 12 exons and is located on chromosome 2. The mouse NCX1, NCX2 and NCX3 genes were mapped to chromosomes 17, 7, and 12, respectively.

The Ca^{2+} pump of sarco(endo)plasmic reticulum (SERCA). The ATP-dependent Ca^{2+} pumps of sarco(endo)plasmic reticulum (SERCA) constitute a large family of proteins of 100–110 kDa (de Meis, 1995; Moller *et al.*, 1996; Toyoshima *et al.*, 2000; Toyoshima & Nomura, 2002; Lee, 2002; MacLennan *et al.*, 2002). They are structurally distinct from the Ca^{2+} pumps of the plasma membrane, but share similarities in the mechanism of Ca^{2+} translocation. The intracellular location of SERCA ATPases exclusively in SR/ER membranes is maintained by the presence of specific retention/retrieval motifs in their primary sequences (Newton *et al.*, 2003).

The SR Ca^{2+} pump catalyzes the electrogenic transport of 2 Ca^{2+} ions per one ATP molecule hydrolyzed from the cytoplasm into the lumen of SR. The key steps of the process are outlined in Fig. 1. The Ca^{2+} transport is reversible and under favorable conditions results in the formation of 1 ATP molecule for 2 Ca^{2+} ions released from the lumen of SR (Sumbilla *et al.*, 2002). The transfer of 2 Ca^{2+} ions represents four positive charges. Countertransport of H^{+} and fluxes of ions through the anion and cation channels of SR prevent large changes in membrane potential during Ca^{2+} transport (Yu *et al.*, 1993).

The SERCA pumps have high affinity for Ca^{2+} (K_m about 0.1 μM), and are capable to maintain a resting cytoplasmic [Ca^{2+}] of 10–20 nM. Like in other P-type ATPases, transient phosphorylation of the enzyme by ATP on an active site aspartyl group is a key step in Ca^{2+} translocation. The activity of the Ca^{2+} pump is dependent on membrane phospholipids, but in distinction from the plasma membrane Ca^{2+}-ATPase, it is not influenced by calmodulin. The recently obtained high resolution structure of SERCA1 Ca^{2+}-ATPase in two conformations (Toyoshima *et al.*, 2000; Toyoshima & Nomura, 2002; Lee, 2002) will reveal the structural basis of Ca^{2+} translocation and the mechanism of its regulation.

The SERCA family of Ca^{2+}-ATPases includes 3 major isoforms (SERCA1–3, Table 1) coded by distinct genes. The ATP2A1 gene encoding the SERCA1 protein is located on human chromosome 16p12.1-p12.2 (Callen *et al.*, 1995). The ATP2A2 gene for the SERCA2 protein is on chromosome 12q23-q24.1 (Otsu *et al.*, 1993). The ATP2A3 gene of SERCA3 is on chromosome 17q13.3 (Dode *et al.*, 1996). The SERCA isoforms and their splice variants are functionally distinct (MacLennan *et al.*, 1992), their expression is tissue-specific and subject to differential control by innervation, contractile activity and hormones both during development and in adult animals (Martonosi, 2000; Pette, 2001).

The SERCA1a and SERCA1b isoforms are preferentially expressed in adult and neonatal fast-twitch skeletal muscles, respectively. SERCA2a is the principal isoform in

slow-twitch skeletal, cardiac and neonatal skeletal muscles, but is also expressed in non-muscle cells. The SERCA2b isoform is primarily located in smooth muscles and in non-muscle cells. The SERCA3 gene product is broadly distributed in various muscle and non-muscle cells. The differential but overlapping expression pattern and regulation of the various SERCA isoforms is a key feature of the functional plasticity of muscles displayed in response to altered physiological requirements.

most types of muscles, and the high density of Ca^{2+} transport sites within the membrane, 1–2 cycles of Ca^{2+} transport are usually sufficient to restore resting cytoplasmic [Ca^{2+}] during muscle relaxation. The Ca^{2+} transport activity of fast-twitch muscle fibers is greater than that of slow-twitch muscle fibers, nearly in proportion to their rate of relaxation. Experimentally induced changes in fiber type composition are reflected in corresponding changes in Ca^{2+} transport activities. Transcriptional control of the expression of

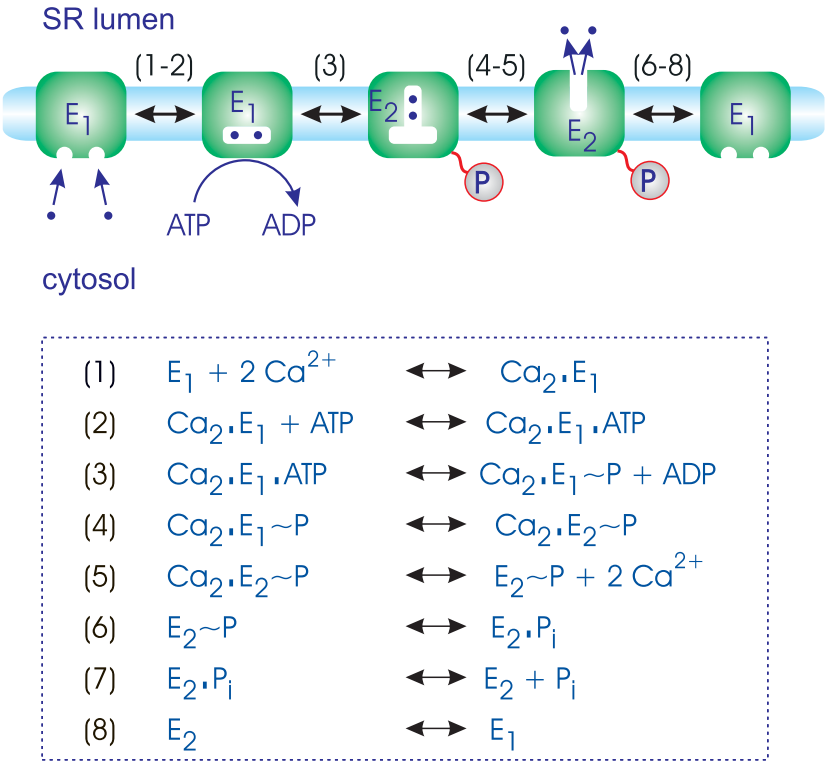

Figure 1. Ca^{2+}-ATPase reaction scheme.

Ca^{2+} transport is initiated by interaction of two Ca^{2+} ions and one ATP molecule with the E_1 conformation of Ca^{2+}-ATPase on the cytoplasmic side (steps 1–2), leading to the phosphorylation of the enzyme by ATP and the occlusion of Ca^{2+} ions within the Ca^{+}-binding pocket of $Ca_2 \cdot E_1{\sim}P$ (step 3). The conversion of the ADP-sensitive $Ca_2 \cdot E_1{\sim}P$ into the ADP-insensitive $Ca_2 \cdot E_2{\sim}P$ form (step 4) is accompanied by a decrease in the Ca^{2+} affinity of Ca^{2+}-ATPase and Ca^{2+} is released on the lumenal side of the membrane (step 5). After Mg^{2+}-catalyzed hydrolysis of $E_2{\sim}P$ (step 6) and the release of P_i on the cytoplasmic side (step 7), the cycle is completed by the isomerization of the E_2 form of Ca^{2+}-ATPase, that has low Ca^{2+} affinity, back into the E_1 form of high Ca^{2+} affinity (step 8).

The density of Ca^{2+} transport sites in the SR membrane is very high (about 30 000/μm^2 surface area) and in fast-twitch fibers the Ca^{2+} transport ATPase may account for 80% of the protein content of the SR membrane (Fig. 2). Due to the extensive development of SR in

Ca^{2+}-ATPase and contractile protein isoforms (Martonosi, 2000; Pette, 2001), together with fiber type specific proteolysis (Sultan *et al.*, 2001), are involved in this adaptation, but the detailed mechanism of the process is unknown.

Mitochondrial Ca^{2+} transport

Ca^{2+} influx pathways of mitochondria. Isolated mitochondria are capable to accumulate large amounts of Ca^{2+} in the presence of inorganic phosphate through a Ca^{2+} uniport of low apparent Ca^{2+} affinity (app. K_m >10 μM) located in the inner mitochondrial membrane (Bernardi, 1999; Duchen, 2000; Buntinas *et al.*, 2001). The Ca^{2+} uptake is driven by the negative membrane potential of −80 to −200 mV across the inner mitochondrial membrane generated either by the respiratory chain or by the hydrolysis of ATP. In the presence of phosphate the transported Ca^{2+} is precipitated in the mitochondrial matrix as Ca^{2+}-phosphate and the Ca^{2+} loading capacity of mitochondria may exceed the total Ca^{2+} content of the cell. Although the mitochondrial Ca^{2+} uniport was discovered nearly a half century ago, it has never been isolated in pure form, and its molecular structure is unknown. Its kinetic properties were characterized largely through flux studies.

The Ca^{2+} uptake rates of mitochondria are steeply dependent on cytoplasmic [Ca^{2+}], suggesting that the Ca^{2+} uniport channel opens only at elevated cytoplasmic [Ca^{2+}]. ATP, inorganic phosphate (P_i) and Mg^{2+} modulate its opening. The Ca^{2+} flux through the open uniport may be greater than 10 000 ions per second, i.e., faster than most ion pumps (Babcock & Hille, 1998). At cytosolic free Ca^{2+} concentrations of 0.1–1.0 μM with a mitochondrial membrane potential of −180 mV, the equilibrium free Ca^{2+} concentration in the mitochondrial matrix ([Ca^{2+}]$_M$) should rapidly reach 0.1–1.0 μM (Rizzuto *et al.*, 2000); the actually measured free [Ca^{2+}]$_M$ in resting cells using mitochondrially targeted aequorin or fluorescent Ca^{2+} indicators is only 100–200 nM, i.e., a tiny fraction of its expected equilibrium value. These data are consistent with observations by electron X-ray microanalysis and by electron energy loss imaging, that

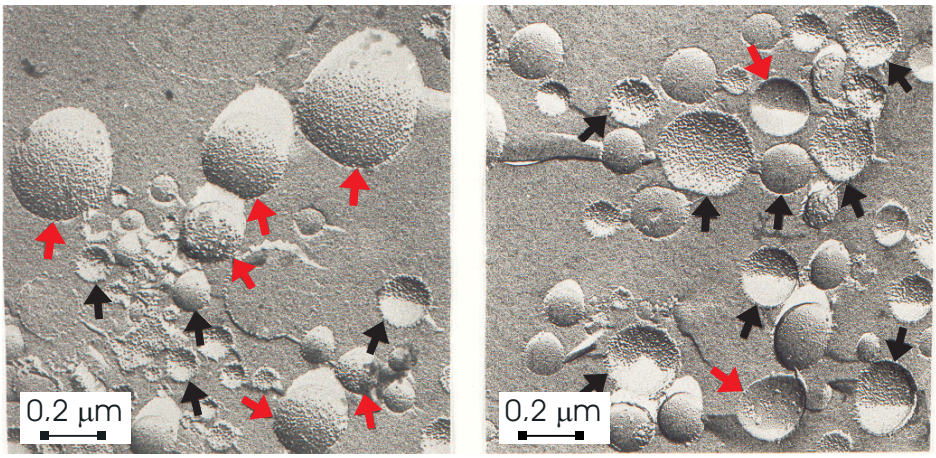

Figure 2. Freeze-etch replicas of SR vesicles isolated from rabbit fast-twitch skeletal muscles.

Fragmentation of SR membranes, a necessary step in isolation of membranous fractions from skeletal muscles, leads to formation of membrane vesicles of various orientation. Most of the vesicles is right-side-out oriented, i.e. with cytoplasmic leaflet of the membrane facing outside (the fraction enriched with right-side oriented vesicles is shown on right panel; this fraction is characterized by the presence of 7.5-nm particles on the concave or outer fracture faces of the vesicle replicas). Hovewer, 10–15% of total number of vesicles tends to form inside-out oriented structures (left panel). Different orientation of obtained vesicles explains why 7.5-nm intramembranous particles, representing the Ca^{2+}-ATPase molecules, are visible on the concave faces of right-side-out oriented vesicles (right panel, black arrows), and in opposite, on the convex or inner fracture faces of inside-out oriented vesicles (left panel, red arrows) (photographs obtained by curtessy of Dr. M. Gabriela Sarzala-Drabikowska).

show surprisingly low total Ca^{2+} content in the mitochondria of various cells. For example, the total Ca^{2+} content of frog skeletal muscle mitochondria measured by electron X-ray microprobe was only 1.7 mmol/kg dry mass, while the corresponding value in the SR was 117 mmol/kg (Somlyo *et al.*, 1981). The mitochondrial Ca^{2+} content of muscle did not change significantly during tetanus or fatigue. Similar low values were obtained for the mitochondria of liver and other cells (Somlyo *et al.*, 1986). These mitochondrial Ca^{2+} contents represent only about 5% of the total cell Ca^{2+}.

The low mitochondrial Ca^{2+} content of resting cells *in vivo* is consistent with the low apparent Ca^{2+} affinity of mitochondrial Ca^{2+} transporter measured *in vitro* (K_m >10 μM) that would render it essentially inactive at the resting cytosolic [Ca^{2+}] of 20–50 nM. These observations led to the suggestion that in differentiated skeletal muscle and perhaps in most other cells, mitochondria play only a secondary role in the regulation of cytoplasmic [Ca^{2+}].

The main physiological role of mitochondrial Ca^{2+} transport is the control of Ca^{2+} concentration in the mitochondrial matrix ([Ca^{2+}]$_M$), upon which the activity of key enzymes of mitochondrial energy metabolism depends (Kunz, 2001; Jouaville *et al.*, 1999). These include three dehydrogenases (McCormack & Denton, 1999) and the F_OF_1-ATPase (Territo *et al.*, 2000), all of which are activated by submicromolar [Ca^{2+}]$_M$. The activation of NAD-isocitrate dehydrogenase and α-ketoglutarate dehydrogenase by Ca^{2+} is allosteric and increases their affinity for the oxidizable substrate. In the case of pyruvate dehydrogenase Ca^{2+} activates a protein phosphatase that converts the inactive phosphorylated form of pyruvate dehydrogenase into an active dephosphorylated form (PDH_A) (Reed *et al.*, 1985).

Increases in cytoplasmic [Ca^{2+}] caused by a variety of interventions (membrane depolarization, agonists, hormones, etc.) causes rapid increase in the [Ca^{2+}]$_M$ (Robert *et al.*, 2001), that is followed by increase in the concentration of NADH due to activation of dehydrogenases (McCormack & Denton, 1999), increase in PDH_A (Reed *et al.*, 1985), increased oxygen consumption, and elevation of ATP concentration (Jouaville *et al.*, 1999; Territo *et al.*, 2001). The Ca^{2+} activation of mitochondrial ATP synthesis coincides with the Ca^{2+} activation of muscle contraction and other ATP-dependent cellular processes.

The electrophoretic Ca^{2+} uniport is inhibited by ruthenium red (RR) that also blocks the activation of Ca^{2+}-sensitive dehydrogenases and the Ca^{2+} agonist-induced stimulation of ATP synthesis (Rizzuto *et al.*, 2000; Territo *et al.*, 2001). Liver and heart mitochondria contain a RR-insensitive Ca^{2+} uptake mechanism, designated as rapid uptake mode (RAM) (Bernardi, 1999; Rizzuto *et al.*, 2000; Buntinas *et al.*, 2001). The RAM has much higher conductivity at the beginning of a pulse than the uniporter, but this conductivity rapidly declines as the [Ca^{2+}] rises, due to Ca^{2+} binding to an external regulatory site (Buntinas *et al.*, 2001). Little is known about the physiological role of RAM.

In view of the low apparent Ca^{2+} affinity of the mitochondrial Ca^{2+} uniport, it was suggested that the changes in [Ca^{2+}]$_M$ are more closely related to the large amplitude fluctuations of [Ca^{2+}] in microdomains of the cytoplasm adjacent to mitochondria (such as the space between the mitochondria and the SR or the plasma membrane) than to the average cytoplasmic [Ca^{2+}] derived from measurement with Ca^{2+} indicators (Rizzuto *et al.*, 2000). This would imply that mitochondrial Ca^{2+} transport involves mainly the portion of mitochondrial surface that faces the Ca^{2+} release channels of the SR or the Ca^{2+} influx pathways of the plasma membrane (Hajnoczky *et al.*, 2000; Seppet *et al.*, 2001).

The mitochondrial [Ca^{2+}]$_M$ under physiological conditions probably do not exceed a few μmol per litre; at much higher [Ca^{2+}] would uncouple the mitochondrial oxidative phosphorylation. Such uncoupling was found to be

negligible up to 600 nM $[Ca^{2+}]_M$ (Territo *et al.*, 2000; 2001). The $[Ca^{2+}]_M$ reflects a steady state between Ca^{2+} influx and Ca^{2+} efflux. The kinetic properties of these pathways effectively balance the $[Ca^{2+}]_M$ within a physiological concentration range.

Pathways of Ca^{2+} efflux from mitochondria. The equilibration of mitochondrial $[Ca^{2+}]_M$ under the influence of the mitochondrial membrane potential is opposed by Na^+-dependent and Na^+-independent Ca^{2+} exporters (Bernardi, 1999; Rizzuto *et al.*, 2000), and by a non-selective high conductance permeability transition pore (PTP) (Rizzuto *et al.*, 2000; Di Lisa *et al.*, 2001) that facilitate the export of Ca^{2+} from the mitochondrial matrix space back into the cytoplasm.

The Na^+-dependent Ca^{2+} efflux is catalyzed by the mitochondrial Na^+:Ca^{2+} exchanger, that is distinct from the Na^+:Ca^{2+} exchanger of the plasma membrane. The mitochondrial Na^+:Ca^{2+} exchanger operates with a probable stoichiometry of 3 Na^+:1 Ca^{2+}; its K_m for Na^+ is 8–10 mM and its V_{max} is 18 nmol Ca^{2+}/mg protein per min in the heart. The Ca^{2+} efflux is inhibited by Sr^{2+}, Ba^{2+}, Mg^{2+}, Mn^{2+}, ruthenium red, verapamil, diltiazem and by the specific inhibitor CGP37157 (Co & Matlib, 1993). The Na^+-dependent Ca^{2+} efflux is primarily responsible for balancing the electrophoretic Ca^{2+} uniport, and maintaining mitochondrial Ca^{2+} loads within the physiological range of 1–4 μmoles Ca^{2+}/mg protein.

The Na^+-independent Ca^{2+} efflux is insensitive to RR and may occur *via* an nH^+:Ca^{2+} exchange mechanism (Kunz, 2001). It saturates at a Ca^{2+} load of 25 nmoles/mg protein and its V_{max} is only 1.2 nmoles Ca^{2+}/mg protein per min (Rizzuto *et al.*, 2000).

The PTP opening is regulated by membrane potential, Ca^{2+}, pH, and inhibited by cyclosporin A (Rizzuto *et al.*, 2000; Di Lisa *et al.*, 2001). PTP opening causes a Ca^{2+}-dependent increase in the permeability of mitochondria to ions and molecules up to 1500 Da, including NAD^+ (Di Lisa *et al.*, 2001). The physiological role of PTP is not clear. It contributes to cell death associated with post-ischemic reperfusion injury of the heart (Di Lisa *et al.*, 2001), and perhaps to other forms of apoptosis (Duchen, 2000; Ferri & Kroemer, 2001).

Ca^{2+}-binding proteins and their role in calcium homeostasis and signaling

The regulation of Ca^{2+} homeostasis also involves a large array of Ca^{2+} binding proteins with a broad spectrum of Ca^{2+} affinities, located in the cytoplasm (Kawasaki & Kretsinger, 1994; Niki *et al.*, 1996), in the lumen of SR/ER (Chevet *et al.*, 1999; Brittsan & Kranias, 2000; Corbett & Michalak, 2000; Martonosi, 2000; Tupling *et al.*, 2002), in the nuclei (Santella, 1996; Bootman *et al.*, 2002), and in the mitochondria (McCormack & Denton, 1999; Duchen, 2000; Rizzuto *et al.*, 2000; Territo *et al.*, 2001). Some of these are Ca^{2+}-sensitive enzymes such as the phosphorylase kinase and the mitochondrial dehydrogenases that adjust the energy production of the cell to physiological demands. Others like calmodulin or troponin serve as Ca^{2+}-dependent regulators of the activity of enzymes such as the plasma membrane Ca^{2+}-ATPase (Guerini & Carafoli, 1999), or actomyosin (Farah & Reinach, 1995). Some Ca^{2+}-binding proteins of the nuclei serve as regulators of gene expression (Santella, 1996; Bootman *et al.*, 2002), while those in the SR lumen participate in the direct control of lumenal $[Ca^{2+}]$ and perhaps in other less clearly defined functions (Michalak, 1996). Phospholamban is an important inhibitor of the Ca^{2+}-ATPase of cardiac SR (Brittsan & Kranias, 2000), calsequestrin is the major Ca^{2+} buffer in the terminal cisternae (Milner *et al.*, 1992), and calreticulin and calnexin serve as chaperones in protein folding (Chevet *et al.*, 1999; Corbett & Michalak 2000).

In the resting muscle much of the cell Ca^{2+} content is stored in the SR lumen and a major portion of SR Ca^{2+} is bound to the low affinity

Ca^{2+} of the lumenal Ca^{2+}-binding proteins. Upon activation of muscle, Ca^{2+} is rapidly released from SR through the ryanodine-sensitive Ca^{2+} channels (Meissner, 1994; Ogawa *et al.*, 2002) with increase in the $[Ca^{2+}]$ of the cytoplasm, mitochondria and nuclei. Depending on the amplitude and kinetics of the Ca^{2+} signal, the high affinity Ca^{2+}-binding proteins of these compartments rapidly bind Ca^{2+}. In skeletal and cardiac muscle much of the Ca^{2+} released from the SR during activation is bound to troponin causing activation of actomyosin ATP hydrolysis and muscle contraction (Holmes & Geeves, 2000; Huley, 2000; Gordon *et al.*, 2000). Simultaneous activation of glycolysis and oxidative phosphorylation serve to maintain the ATP concentration of the muscle at nearly constant level, despite the high rate of ATP utilization (Booth & Thomason, 1991). Depending on the frequency and amplitude of Ca^{2+} signals, other components of the Ca^{2+} signalling networks, including those involved in the regulation of gene expression, are also activated permitting an accurate matching of both the activity and the concentration of the various enzymes to the physiological demands.

The Ca^{2+} pumps of the plasma membrane and the SR have relatively slow turnover rates ($<10\ s^{-1}$) compared with the conductance of Ca^{2+} release channels ($10\,000\ s^{-1}$) that generate the Ca^{2+} signals. Therefore, the Ca^{2+} pumps have only modest effect on the rapidly rising phase of the Ca^{2+} transients during muscle activation. By contrast, troponin and parvalbumin bind Ca^{2+} rapidly and most of the activating Ca^{2+} ends up bound to these proteins.

At the beginning of muscle relaxation, the repolarization of the plasma membrane causes the closure of the Ca^{2+} release channels of SR. The cytoplasmic free Ca^{2+} concentration begins a gradual decline toward resting level, due to the operation of Ca^{2+} pumps and the continued binding of calcium to troponin, parvalbumin and other high affinity Ca^{2+}-binding proteins. The Ca^{2+} binding to troponin causes continued increase in contractile tension, while the cytoplasmic free $[Ca^{2+}]$ is lowered to near resting level (Ashley *et al.*, 1993). Muscle relaxation is eventually brought about by the slow release of bound Ca^{2+} from troponin. The relationship between contractile tension and the kinetics of the Ca^{2+} transient is consistent with the kinetics of Ca^{2+} binding to troponin measured *in vitro*, and was realistically modeled in frog skeletal muscle assuming that the concentration of troponin, parvalbumin and the SR Ca^{2+} pump are 0.24, 1.0, and 0.19–0.49 mM, respectively, and the rate constants of Ca^{2+} binding are $0.575 \times l0^8\ M^{-1}s^{-1}$ for troponin, and $1.25 \times l0^8\ M^{-1}\ s^{-1}$ for parvalbumin, and the rate constants of Ca^{2+} release are $115\ s^{-1}$ for troponin and $0.5\ s^{-1}$ for parvalbumin (Baylor *et al.*, 1983). The simulations suggest that following a single action potential, about 300 μM Ca^{2+} was released from the SR into the cytoplasm. The free cytoplasmic $[Ca^{2+}]$ rose only to a peak value of 2.1 μM, because much of the released Ca^{2+} was bound to troponin (about 110 μM) and parvalbumin (about 170 μM). Repetitive stimuli cause the additional release of only about one third as much Ca^{2+}. Parvalbumin is present in resting muscle largely complexed with Mg^{2+}; therefore, the Ca^{2+} binding to parvalbumin occurs with a slow displacement of Mg^{2+} from its binding site. As a result, Ca^{2+} binding to troponin precedes that of parvalbumin during activation and some of the Ca^{2+} released from troponin during relaxation is bound by parvalbumin before it is returned to the SR lumen. The buffering action of parvalbumin requires the release of nearly twice the amount of Ca^{2+} from the SR than in its absence.

There is a precise stoichiometry between actin, tropomyosin, troponin and myosin in the myofibrils, and the amount of Ca^{2+} released from the SR must be sufficient to saturate a large portion of the Ca^{2+}-binding sites on troponin, without reaching harmful concentrations in the cytoplasm. This delicate balance requires that the expression of each

component must be coordinated to yield cellular concentrations that match the Ca^{2+} requirement of the contractile apparatus. The amplitude and time course of the Ca^{2+} transient was similar in frog and fast-twitch mouse skeletal muscles (Hollingworth *et al.*, 1996). The slow release of Ca^{2+} after each Ca^{2+} pulse from the high affinity Ca^{2+}-binding sites of the various Ca^{2+} receptors is a general feature of Ca^{2+} signalling networks that prolongs the physiological effects of Ca^{2+} beyond the observed lifetime of the brief cytoplasmic Ca^{2+} spikes.

Coordinated expression of calcium transport systems

The relative contribution of the various Ca^{2+} transport systems to the intracellular Ca^{2+} homeostasis varies with cell type and species as determined by their specific physiological requirements. Variations in the content and isoform composition of the Ca^{2+} signalling network of muscle can be imposed by exercise, cross-innervation or chronic electric stimulation with major changes in contractility (Pette, 2001). The range of contribution of individual components of the regulatory network can be tested by their overexpression or ablation, that may also trigger compensatory changes in other components aimed at maintaining the Ca^{2+} homeostasis within physiological limits. These data permit an assessment of the robustness of Ca^{2+} regulation, defined as the ability of the system to function in the face of large changes in its composition. Such robustness is an important requirement of molecular networks and may serve as an evolutionary principle in their development.

Comparison of Ca^{2+} regulation in skeletal and cardiac muscles. In skeletal muscles there is only small influx of extracellular Ca^{2+} during activity (Bianchi & Shanes, 1959) and contractility can be maintained for a long time in virtually Ca^{2+}-free medium (Armstrong *et al.*, 1972). As over 99% of the activating Ca^{2+} originates from the SR, the total Ca^{2+} content of skeletal muscle remains essentially unchanged during activity, and the plasma membrane Ca^{2+}-ATPases and Na^+:Ca^{2+} exchangers are of only modest importance in cellular Ca^{2+} homeostasis. By contrast, the activation of cardiac muscle is accompanied by large influx of Ca^{2+} from the extracellular medium (Ringer, 1883) through the voltage-sensitive Ca^{2+} channels of the plasma membrane (Cannell *et al.*, 1995), that may represent 10% of the total activating Ca^{2+}. The Ca^{2+} influx triggers Ca^{2+}-induced Ca^{2+} release from the SR, that is a key element of cardiac excitation-contraction coupling (Fabiato, 1989). The extrusion of this excess Ca^{2+} requires the powerful Ca^{2+} pumps and Na^+:Ca^{2+} exchangers of the cardiac plasma membranes (Bers, 2002). Caffeine induces the release of Ca^{2+} from the SR of rabbit cardiac myocytes, and in the presence of caffeine, Ca^{2+} uptake by the SR is prevented. Most of the Ca^{2+} released from the SR by caffeine is extruded from the cells by the Na^+:Ca^{2+} exchanger (Bers *et al.*, 1993). The Ca^{2+} extrusion was reduced by incubation in Na^+- and Ca^{2+}-free solutions that inhibited the Na^+:Ca^{2+} exchanger, with increase in the peak of caffeine-induced contraction, due to increase in cytoplasmic [Ca^{2+}]. The caffeine-induced contraction relaxed slowly in Na^+- and Ca^{2+}-free solution due to slow Ca^{2+} removal by the mitochondria and the plasma membrane Ca^{2+}-ATPase.

It is estimated that in rabbit cardiac muscle during a relaxation from twitch under physiological conditions, 70% of the cytoplasmic Ca^{2+} is removed by the SR Ca^{2+}-ATPase, 28% by the Na^+:Ca^{2+} exchanger, and only the remaining 2% by the plasma membrane Ca^{2+}-ATPase and the mitochondria (Bers *et al.*, 1993; Bassani *et al.*, 1994). The corresponding values for rat cardiac myocytes are 92%, 7%, and 1%, respectively (Bassani *et al.*, 1994). Eventually the Ca^{2+} accumulated by mitochondria is released through the mitochondrial Na^+:Ca^{2+} exchanger and reaccumulated by the SR. The release of Ca^{2+} from the mitochondria can be inhibited by replac-

ing the cytoplasmic Na^+ with choline, that inhibits the mitochondrial Na^+:Ca^{2+} exchanger (Bers *et al.*, 1993). Similar conclusions were reached by Balke *et al.* (1994) who also found negligible contribution by mitochondria and the plasma membrane Ca^{2+}-ATPase to Ca^{2+} removal during a single Ca^{2+} transient in rat ventricular myocytes following inhibition of Na^+:Ca^{2+} exchangers by the removal of internal and external Na^+. Under these conditions the declining phase of the Ca^{2+} transient could be modeled considering only the kinetics of SR Ca^{2+}-ATPase (K_m, V_{max}), the complexation of Ca^{2+} by the Ca^{2+}-binding proteins and the leak of Ca^{2+} from the SR into the cytoplasm (F_{SR} leak). The values of K_m (0.28 μM), V_{max} (0.21 mM s^{-1}) and F_{SR} leak (0.018 mM s^{-1}) derived from these data are consistent with independent estimates based on *in vitro* data.

Contrasting these observations, a more recent study suggests (Choi & Eisner, 1999) that the contribution of plasma membrane Ca^{2+}-ATPase to the Ca^{2+} removal in rat ventricular myocytes may reach 25% of the contribution of Na^+:Ca^{2+} exchanger. Inhibition of PMCA by carboxyeosin increased the resting Ca^{2+} content of SR by 17%, suggesting that in the resting cell the plasma membrane Ca^{2+}-ATPase decreased the Ca^{2+} content of SR by competition with the SR Ca^{2+}-ATPase.

Overexpression and ablation of components of Ca^{2+} homeostasis. The individual components of the Ca^{2+} regulatory network are expressed in proper concentration and isoform composition to maintain the total Ca^{2+} concentration of the cell and the free Ca^{2+} concentrations of the various organelles within physiological ranges both during rest and activity. The robustness of the design was tested by manipulating the expression of the various isoforms of the SR and plasma membrane Ca^{2+}-transport ATPases and Na^+:Ca^{2+} exchangers, followed by analysis of the resulting changes in the pattern of Ca^{2+} regulation (Shull, 2000; Kiriazis & Kranias, 2000; Periasamy & Huke, 2001). The genetic manipulation of the individual components identified the limits of plasticity in the Ca^{2+} signalling network, but also revealed major gaps in our knowledge about the integration of their function and the mechanisms that coordinate their expression.

Reduction of SERCA Ca^{2+}-ATPase content in Brody's disease. Brody' disease is a rare genetically heterogeneous, autosomal recessive or dominant inherited disorder of skeletal muscles, characterized by painless contractures that develop during exercise (Brody, 1969). The slow relaxation of muscle is caused by decreased Ca^{2+} transport activity of SR due to mutations in the ATP2A1 gene coding for the SERCA1 isoform of the Ca^{2+}-ATPase of fast-twitch (type 2) skeletal muscle fibers (MacLennan, 2000; Odermatt *et al.*, 2000). Some of the mutations cause virtually complete loss of both SERCA1 protein and the Ca^{2+}-ATPase activity, presumably due to its defective translation and/or degradation. In other cases, the rate of Ca^{2+} transport was reduced by about 50% without change in the content of Ca^{2+}-ATPase protein (Taylor *et al.*, 1988; Benders *et al.*, 1996). There may also be cases that are not associated with defects in the ATP2A1 gene (Zhang *et al.*, 1995), and involve other unidentified proteins that may modulate SERCA1 activity. The SERCA1a protein accounts for at least 99% of the SERCA isoforms expressed in adult Type 2 fast-twitch muscle fibers. In view of its major role, it is surprising that its severe deficiency or virtual absence in Brody's disease causes a benign clinical condition that usually does not interfere with normal life and frequently goes undiagnosed. Compensatory mechanisms for SERCA1 deficiency have not been identified so far. Refilling of the Ca^{2+} stores of the SR must be a key element of compensation since the activation of contraction that depends on these Ca^{2+} stores is not affected significantly in Brody's disease. The possible contributions of SERCA2, SERCA3, or the capacitative Ca^{2+} entry mechanism have been considered and need to be further explored. Ca^{2+} transport

systems in the plasma membranes or mitochondria are not likely to play a major role in this compensation.

Null-mutation of the ATP2A2 gene. The ATP2A2 gene produces two alternatively spliced isoforms of the SERCA2 ATPase. SERCA2a is the principal isoform in cardiac and slow-twitch skeletal muscles, while SERCA2b is present in smooth muscles and non-muscle cells (Wu *et al.*, 1995). Heterozygous transgenic mice were generated by gene targeting that eliminated the promoter and the 5′ end of the ATP2A2 gene (Ji *et al.*, 2000). Homozygous mutants were not observed, indicating that SERCA2 serves functions essential for life and there is no satisfactory compensation to rescue the null-mutant. The heterozygous offspring were viable and appeared healthy. In heart samples of the heterozygous animals, the SERCA2 mRNA level was reduced by 45%, and the SERCA2 protein content and Ca^{2+} transport activity by 35%, without change in the Ca^{2+}-concentration dependence of Ca^{2+} transport. These observations indicate that both copies of the ATP2A2 gene are required for normal activity. The loss of one copy of the ATP2A2 gene and the associated decrease in Ca^{2+}-transport activity have significant physiological consequences. The mean arterial blood pressure and the left ventricular systolic pressure were lower in heterozygous as compared with control animals; this difference was not observed at maximal-adrenergic stimulation (Periasamy *et al.*, 1999) that relieves the inhibition of Ca^{2+}-ATPase by phospholamban (Brittsan & Kranias, 2000; Kiriazis & Kranias, 2000). The reduction in the Ca^{2+} content of SR and in the rate of Ca^{2+} transport was also reflected in slower rise and decline of the left ventricular pressure wave, dP/dt (Periasamy *et al.*, 1999), and a decrease in the rate of shortening and relaxation of heterozygous myocytes (Ji *et al.*, 2000). These effects are similar to those observed after inhibition of the cardiac Ca^{2+} pump by thapsigargin (Kirby *et al.*, 1992). The phospholamban content was reduced by 40% in the transgenic heart, but the basal phosphorylation level of Ser16 and Thr17 of phospholamban was increased approximately 2-fold, relieving the inhibition of Ca^{2+}-ATPase by phospholamban and providing some compensation for the decrease in Ca^{2+}-ATPase content (Ji *et al.*, 2000). The NCX content was 38% higher in the heterozygous than in control heart, without change in the NCX mRNA level, suggesting posttranscriptional regulation of NCX protein expression. The Ni^{2+}-sensitive NCX outward current also increased by about 40% indicating increased NCX activity. The increase in NCX activity in the forward and reverse directions may facilitate both Ca^{2+} extrusion and Ca^{2+} entry, providing inotropic support for the heart (Ji *et al.*, 2000). Loss of function mutation of one copy of the ATP2A2 gene is implicated in the human autosomal dominant skin disorder, the Darier disease (Jacobsen *et al.*, 1999; Zhao *et al.*, 2001). This indicates a role for SERCA2 in the cell adhesion of keratinocytes and an as yet uncertain relationship to mood disorders, epilepsy and mental retardation (Jacobsen *et al.*, 1999). Increased incidence of heart disease has not been observed in Darier patients, and Darier-like skin lesions have not been seen in mice carrying null-mutation of ATP2A2 gene, indicating species differences in the susceptibility to skin or heart disease in SERCA2a deficiency.

Ablation of the SERCA3 isoform of Ca^{2+}-ATPase. The ATP2A3 gene codes for the SERCA3 Ca^{2+}-ATPase that is expressed in endothelial cells, in epithelial cells of the trachea, intestine and salivary glands, in platelets, mast cells, and lymphocytes. Each of these cell types also contain SERCA2b. SERCA3 has lower Ca^{2+} affinity and higher pH optimum than SERCA2b, and it is expected to participate in specialized signalling functions consistent with its limited distribution (Wu *et al.*, 1995). Mice homozygous for null-mutation of the ATP2A3 gene were viable, fertile and did not show overt disease phenotype (Liu *et al.*, 1997). However, after con-

traction with phenylephrine, the acetylcholine-induced relaxation of their aortic rings was impaired. The acetylcholine-induced relaxation is dependent on NO synthesized by the endothelial cells, that diffuses into the smooth muscle and induces relaxation by a cGMP-dependent mechanism. As the aortas of ATP2A3−/− animals relax in response to sodium nitroprusside, an NO donor, their smooth muscle is apparently sensitive to NO (Liu *et al.*, 1997). Therefore, the defective acetylcholine-induced relaxation in these animals may be due to depletion of the acetylcholine-responsive Ca^{2+} stores in the aortic endothelial cells, that would impair the Ca^{2+} signalling required for the activation of endothelial NO synthase. Ablation of SERCA3 also interfered with the epithelium-dependent relaxation of tracheal smooth muscle (Kao *et al.*, 1999). Since SERCA3 is not expressed in tracheal smooth muscle, this effect implies a role for SERCA3 in mediating epithelial Ca^{2+} signal transduction. Based on these studies, SERCA3 does not appear to play a critical role in basic housekeeping functions essential for cell viability either during development or in mature animals. Rather it has some specific role in Ca^{2+} signalling in a restricted set of cells, that requires further investigation.

Overexpression of various isoforms of the Ca^{2+}-ATPase in cardiac muscle of transgenic animals provides an opportunity to analyze the effect of Ca^{2+}-ATPase content and isoform specificity on the contractility of the heart. Besides their basic biological interest, such studies have a potential therapeutic importance. Cardiac failure and chronic pressure overload cardiac hypertrophy are associated with decreased expression levels of the Ca^{2+}-ATPase, altered Ca^{2+} homeostasis, and impaired myocardial function, that may be corrected by increasing the Ca^{2+}-ATPase content of the heart (Martonosi, 2000).

Overexpression of SERCA2a. The rat SERCA2a transgene was overexpressed in various tissues of mice using the chicken β-actin promoter which is not tissue specific (He *et al.*, 1997), or selectively in the heart using the α-myosin heavy chain promoter (Baker *et al.*, 1998). The level of SERCA2a mRNA increased 2.6–7.9-fold in the transgenic hearts, while the increase in Ca^{2+}-ATPase content was only 1.2–1.5-fold. The modest increase in the SERCA2a content may indicate some regulatory mechanisms that control the translation, membrane insertion or degradation of the Ca^{2+}-ATPase in the heart. The increased expression of the Ca^{2+}-ATPase led to increased rates of ATP-dependent Ca^{2+} uptake in cardiac homogenates without change in Ca^{2+} affinity, and to increased rates of contraction and relaxation both in isolated myocytes and in work-performing heart (He *et al.*, 1997; Baker *et al.*, 1998). There was no significant change in the phospholamban and Na^+:Ca^{2+} exchanger content of the transgenic heart (He *et al.*, 1997). The SERCA2a mRNA and protein levels are significantly reduced in hypothyroid as compared with euthyroid mice (Bluhm *et al.*, 1999). Overexpression of SERCA2a in hypothyroid mice compensated for some of the loss of SERCA2a expression due to hypothyroidism and improved the cardiac contractile function. The results obtained with SERCA2a overexpression in transgenic animals confirm the results of earlier studies using adenovirus-mediated transient gene transfer (Miyamoto *et al.*, 2000). Adenoviral-mediated SERCA2a gene transfer also restored contractile function in cardiomyocytes isolated from failing human hearts (Del Monte *et al.*, 1999).

Overexpression of SERCA1a. The SERCA isoform specificity of cardiac function was analyzed by overexpression of the rat SERCA1a isoform in mice (Loukianov *et al.*, 1998). The SERCA1a is the major isoform of fast-twitch skeletal muscle fibers, and it is not expressed normally in heart. SERCA1a overexpression increased the total SERCA content of the transgenic heart 2.5-fold, with 1.7-fold increase in the maximal velocity of ATP-induced Ca^{2+} uptake, indicating functional expression of the SERCA1a isoform in the cardiac SR.

This was reflected in increased rates of contraction and relaxation both in transgenic myocytes and in work-performing heart preparations (Loukianov *et al.*, 1998). The overexpression of SERCA1a caused a 50% decrease in the SERCA2a content of the transgenic hearts, without change in the phospholamban, calsequestrin, actin and tropomyosin content. Similar observations were made using an adenovirus vector for transient expression of SERCA1a in cardiac myocytes (Cavagna *et al.*, 2000). The high level of expression of SERCA1a in transgenic hearts with a 50% reduction in SERCA2a content suggests that SERCA1a competes effectively with SERCA2a for insertion sites in cardiac SR. With the expression levels obtained in these studies the cardiac SR may have reached physical saturation with the Ca^{2+}-ATPase.

Overexpression of SERCA2b. The role of SERCA isoform specificity was further analyzed by overexpression of the rat SERCA2b isoform in transgenic mice (Greene *et al.*, 2000). SERCA2b is found in smooth muscle and in most other cells, but it is only a minor isoform in cardiac muscle. The SERCA2b mRNA increased 20-fold and the SERCA2b protein 8–10-fold in transgenic hearts, without change in SERCA2a mRNA or protein content. The maximal rate of Ca^{2+} uptake in cardiac homogenates remained unchanged, but there was an increase in the Ca^{2+} affinity of the pump consistent with the high Ca^{2+} affinity and low velocity of the Ca^{2+} transport catalyzed by the SERCA2b Ca^{2+}-ATPase. The SERCA2b transgenic hearts also had higher contraction and relaxation rates than control hearts indicating functional insertion of the non-cardiac isoform into cardiac SR. While the SERCA2a isoform is widely distributed in the SR membrane, the overexpressed SERCA2b was preferentially localized near the transverse tubules and the Z line. The distinct localization of the two isoforms implies that they do not compete for the same insertion sites. This may explain that SERCA2b overexpression did not affect the SERCA2a content of the heart. The distinct localization may be related to structural differences between SERCA2b and SERCA2a. SERCA2b has a long carboxyl terminal tail of 50 amino-acid residues (Lytton *et al.*, 1989), that may form an extra transmembrane domain placing the C-terminus of SERCA2b on the lumenal side of the SR membrane. By contrast, SERCA1 and SERCA2a have only 10 putative transmembrane domains with C-termini on the cytoplasmic side.

Overexpression of Na^+:Ca^{2+} exchanger in cardiac muscle. In ventricular myocytes of transgenic mice overexpressing the canine cardiac NCX (Adachi-Akahane *et al.*, 1997; Terracciano *et al.*, 1998), the rates of contraction and relaxation were faster, and the Ca^{2+} content of SR was 69% greater than in ventriculocytes of control litter mates. Even when the Na^+:Ca^{2+} exchanger was inhibited by removing the Na^+ and Ca^{2+} from the medium, the rate of Ca^{2+} removal from the cytoplasm remained faster in the transgenic than in control myocytes, indicating faster Ca^{2+} uptake by the transgenic SR. There was no evidence for changes in the SERCA2, phospholamban, and calsequestrin contents determined by Western blot, suggesting increased activity rather than increased content of the SR Ca^{2+} pump. Reversal of the NCX at low cytoplasmic [Ca^{2+}] may contribute to the increased Ca^{2+} content of SR in transgenic animals. Inhibition of SR Ca^{2+} transport by thapsigargin slows the declining phase of the Ca^{2+} transient. Close to 30% inhibition of SR Ca^{2+} transport by thapsigargin was required to slow the decline of Ca^{2+} transient in transgenic animals to control levels (Terracciano *et al.*, 1999). The increased Ca^{2+}-transport activity of SR may serve to compensate for the increased Ca^{2+} extrusion by the overexpressed Na^+:Ca^{2+} exchanger, but the mechanism of this compensation remains to be established.

A somewhat different relationship between NCX and SERCA expression was observed during development in mouse heart. The ex-

pression of NCX is 2-fold higher in fetal than in adult heart and decreases during development, while the SERCA2 and phospholamban expression increase during the same period nearly 8-fold (Reed *et al.*, 2000). This antithetical relationship between NCX and SERCA expression levels is consistent with the greater contribution of Ca^{2+} influx to the excitation/contraction coupling of fetal heart, while in adult heart Ca^{2+} release from the SR plays a dominant role (Martonosi, 2000). Hypothyroidism tends to reduce the expression of SERCA2a mRNA and protein, while the expression of NCX1 increases. Opposite changes are observed in hyperthyroidism (Reed *et al.*, 2000). More information is clearly needed about the mechanisms that regulate the expression of these two proteins before a functional connection between their expression level could be rationalized.

Overexpression of plasma membrane Ca^{2+}-ATPase. The PMCA4CI isoform (corresponding to human PMCA4b isoform) was overexpressed in rats under the control of ventricle specific myosin light chain-2 promoter (Hammes *et al.*, 1998), resulting in 2.5- and 1.6-fold increase in PMCA protein level in neonatal and adult hearts, respectively. The Ca^{2+}-dependent PMCA ATPase activity in adult hearts increased in proportion to the increase in PMCA content. The PMCA transgenic animals showed no differences in cardiac performance from control animals, and the expression of NCX, SERCA2a Ca^{2+}-ATPase and the endogenous rat PMCA1 mRNA was also unchanged.

These observations suggest that PMCA has no major role in the beat-to-beat regulation of the contraction-relaxation cycle in adult transgenic rat hearts. However, overexpression of PMCA increased about 2-fold the rate of protein synthesis in cardiomyocyte cultures stimulated with fetal calf serum or isoproterenol, suggesting a role in growth regulation during development (Hammes *et al.*, 1998). Several-fold overexpression of PMCA4CI in L6 myogenic cell lines (Hammes *et al.*, 1996) significantly decreased the resting cytosolic free Ca^{2+} levels and accelerated myogenic differentiation as measured by the formation of multinucleated myotubes, and by a 3- to 4-fold increase in the activity of creatine phosphokinase. The relationship of the decrease in cytoplasmic free [Ca^{2+}] to the differentiation promoting effect is unclear since activity-dependent increase in cytoplasmic Ca^{2+} promotes differentiation of muscle (Martonosi, 2000) and nerve cells (Carafoli *et al.*, 1999). PMCA4CI was also stably expressed and correctly targeted to the plasma membrane in Chinese hamster ovary (CHO) cells (Guerini *et al.*, 1995). Based on the amount of phosphorylated intermediate, the level of expressed PMCA4CI in transgenic cells was 10–15 times greater than the PMCA level in control cells, while the concentration of endogenous SERCA pump was reduced. There was no change in Na^+, K^+-ATPase activity. The PMCA transfected cells effectively released Ca^{2+} into the medium, and inside out plasma membrane vesicles isolated from transfected cells accumulated 8–10 times more Ca^{2+} than those of control cells. Cells expressing high levels of PMCA recovered slowly from trypsinization and grew slowly after plating but after this initial growth delay behaved like non-transfected CHO cells.

Stable overexpression of PMCA1a in rat aorta endothelial cells (Liu *et al.*, 1996) yielded 3–4-fold increase in the amount of functional PMCA1a, that was matched by similar increase in the Ca^{2+}-calmodulin stimulated Ca^{2+} pump activity tested in isolated vesicle preparations. The expression and activity of the SERCA pump was down-regulated. There was also an unexplained 2.6-fold increase in Ca^{2+} influx into the PMCA1a expressing cells. Transient overexpression of PMCA4CI in CHO cells (Brini *et al.*, 2000) also reduced the Ca^{2+} content of SR by about 20% without significant effect on cytosolic or mitochondrial free Ca^{2+} levels. The decrease in SERCA expression and/or activity associated with PMCA overexpression is difficult to view as part of a regulatory mech-

anism, since it contributes to a decrease in SR Ca^{2+} content. The possibility must be considered that the transfection technique itself may interfere with the expression of some endogenous proteins.

In conclusion, the data on ablation and overexpression of key components of the Ca^{2+} signalling network indicate a surprising degree of robustness of Ca^{2+} regulation. Only complete ablation of the SERCA2 gene in mice caused loss of viability but even in this case, heterozygotes remained essentially healthy. Nearly complete loss of the human SERCA1 protein in some forms of Brody's disease caused only mild delay of muscle relaxation, while ablation of SERCA3 had no effect either on viability or fertility. Massive overexpression of SERCA1, 2a, and 2b in heart improved cardiac performance, and there was no obvious disease phenotype associated with overexpression of Na^+:Ca^{2+} exchanger or the plasma membrane Ca^{2+} transport ATPase. Induced change in the expression level of one component was frequently accompanied by changes in others, but at this stage, it is difficult to assign these changes to cooperative effects that may be expected to improve Ca^{2+} regulation. In fact, some of the associated changes may be artifacts of the genetic manipulation technique. The story of gene regulation by Ca^{2+} is only beginning to unfold.

EXCITATION–CONTRACTION (E–C) COUPLING. THE Ca^{2+} RELEASE CHANNELS OF THE PLASMA MEMBRANES AND THE SARCOPLASMIC RETICULUM

The mechanism of E–C coupling was the subject of intense interest during the last decade that led to a vast expansion of literature. Only key elements of this progress will be briefly reviewed here. For more detailed information the reader is referred to the review articles listed in the text.

The depolarization of the surface membrane of muscle cell initiated by the nerve impulse (Sperelakis & Gonzales-Serratos, 2001; Wahler, 2001) is conducted into the interior of the muscle fiber by extensions of the surface membrane called the transverse (T) tubules (Heiny, 2001). The T tubules establish specialized contacts (T–SR junctions, triads, diads) with the junctional membrane of SR, that contain the two key components of the excitation–contraction coupling apparatus, the dihydropyridine receptor (DHPR) and the ryanodine receptor (RyR) (Franzini-Armstrong, 1999).

The DHPR of the T tubules and surface membrane (Hofmann *et al.*, 1999; Bezanilla, 2000; Catterall, 2000) serves both as voltage sensor and as a calcium channel by responding to changes in membrane potential with intramembrane charge movement and inward calcium current (Hofmann *et al.*, 1999; Bezanilla, 2000; Catterall, 2000). The RyR Ca^{2+} channels of SR briefly open during the action potential permitting the rapid release of stored Ca^{2+} from the SR into the cytoplasm (Meissner, 1994; 2001; Franzini-Armstrong & Protasi, 1997; Niggli, 1999; Mar & Marks, 2000; Ogawa *et al.*, 2002). The activation of RyR is under the tight control of DHPR in both skeletal and cardiac muscles, resulting in efficient coupling of membrane depolarization to calcium release from the SR (Fig. 3). However, the mechanism appears different in skeletal and cardiac muscles due to differences in the structure and disposition of the two receptors.

Skeletal muscles are able to contract in an essentially Ca^{2+}-free medium for several hours (Armstrong *et al.*, 1972); the Ca^{2+} current through DHPR is slow and apparently unrelated to E–C coupling. In skeletal muscles DHPR serves mainly as a voltage sensor that controls the opening of the SR Ca^{2+} channel either by direct interaction with RyR or through contacts with other proteins (Meissner, 2001).

The contraction of cardiac muscles is dependent on extracellular Ca^{2+} (Ringer, 1883).

The voltage-dependent Ca^{2+} current through the activated cardiac DHPR Ca^{2+} channel is fast and sufficiently large to play a fundamental role in the activation of RyR in cardiac SR by a process called Ca^{2+}-induced Ca^{2+} release (Fabiato, 1989; Cannell *et al.*, 1995; Bers, 2002); nevertheless a direct control of RyR by interaction with DHPR may also contribute.

The structure and function of dihydropyridine receptor in skeletal and cardiac muscles

The DHPRs of skeletal and cardiac muscles are structurally distinct; they are coded by different genes, and their expression is differentially regulated (Hofmann *et al.*, 1999; Catterall, 2000). In dysgenic mice the functional DHPR content of skeletal muscle is severely reduced, while in the heart and in the sensory neurons the DHPR content remains normal (Chaudhari, 1995). The skeletal muscle DHPR is a pentamer containing $\alpha 1, \alpha 2\text{-}\delta, \beta$ and γ subunits (Hofmann *et al.*, 1999; Catterall, 2000). The $\alpha 1$ subunit is the central component of the complex and it occurs in two forms with relative molecular masses of 175 kDa and 212 kDa (Tanabe *et al.*, 1987; Catterall, 2000). The 175 kDa form accounts for more than 90% of the $\alpha 1$ subunit content. The $\alpha 1_{175}$ form may arise by post-translational proteolysis from the $\alpha 1_{212}$ form. The $\alpha 1$ subunit of the cardiac DHPR has a molecular mass of 242 771 (Mikami *et al.*, 1989); it is larger than the $\alpha 1_{212}$ subunit of the skeletal muscle DHPR (Tanabe *et al.*, 1987). Analysis of the amino-acid sequence of skeletal and cardiac $\alpha 1$ subunits reveals four internal structural repeats (I–IV), each containing five hydrophobic segments (S1, S2, S3, S5, and S6), and one positively charged segment (S4). The S1 through S6 segments are assumed to represent helical membrane spanning domains arranged around a central Ca^{2+} channel, while the hydrophilic N- and C- terminal regions of the molecule are positioned on the cytoplasmic surface (Hofmann *et al.*, 1999; Catterall, 2000). The S4 segment is assumed to serve as voltage sensor (Bezanilla, 2000).

The structural basis of the differences between E–C coupling in skeletal and cardiac muscles was analyzed by expressing the cDNAs of skeletal and cardiac DHPR in dysgenic myotubes in tissue culture, and studying the characteristics of spontaneous or electrically evoked contractions. The slow Ca^{2+} current together with the voltage-dependent charge movement and E–C coupling can be fully restored to dysgenic muscle by microinjection of the expression plasmid PCAC6 for the $\alpha 1$ subunit of the skeletal muscle DHPR (Adams *et al.*, 1990; Tanabe *et al.*, 1990a). The restored E–C coupling persists in a Ca^{2+}-free medium or in the presence of 0.5 mM Cd^{2+} like the E–C coupling of normal skeletal muscle.

Dysgenic muscles injected with the expression plasmid of cardiac DHPR (pCARD1) expressed a Ca^{2+} current that was similar to the large, fast activating L type Ca^{2+} current of cardiac muscle (Tanabe *et al.*, 1990b). Substitution of Ba^{2+} for Ca^{2+} caused a large increase in peak current, while the skeletal muscle Ca^{2+} channel was only slightly affected by Ba^{2+} (Adams *et al.*, 1990; Tanabe *et al.*, 1990a, 1990b). The pCARD1-injected dysgenic myotubes produced electrically induced contraction in normal Ringer solution, but not in Ca^{2+}-free Ringer solution or in the presence of 0.5 mM Cd^{2+}, consistent with the Ca^{2+} requirement for cardiac E–C coupling (Tanabe *et al.*, 1990b).

Chimeric DHPR cDNAs constructed by replacing various segments of the cardiac DHPR with corresponding segments from skeletal DHPR identified the loop between domains II and III as an important determinant for skeletal or cardiac type E–C coupling (Tanabe *et al.*, 1990a); the highly conserved III–IV loop may also contribute. Peptides corresponding to the II–III loop region activate the skeletal, but not the cardiac RyR Ca^{2+} release channels suggesting that this region is involved in the interaction between DHPR

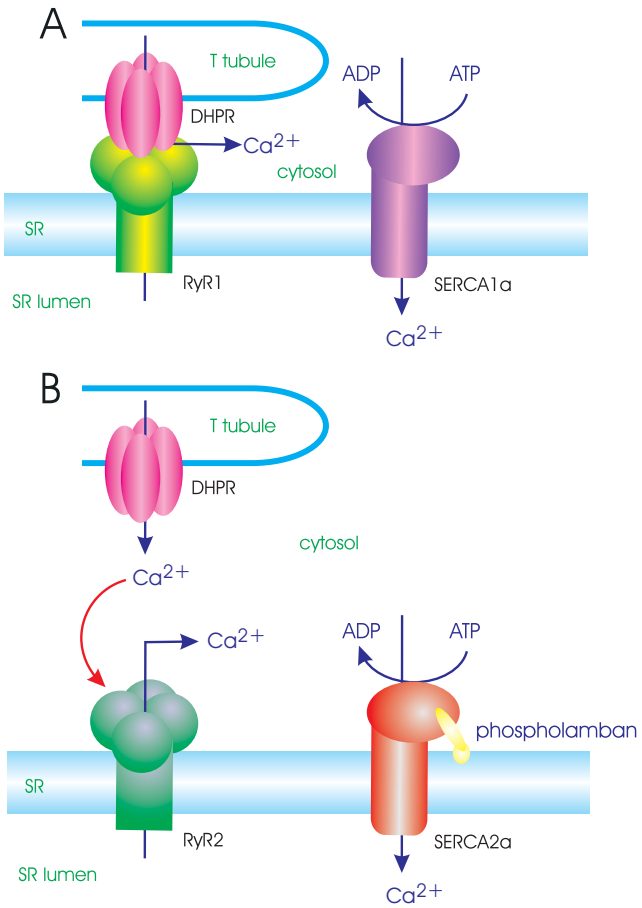

Figure 3. The disposition of Ca^{2+} release channels of plasma membrane and SR in skeletal (A) and cardiac (B) muscles.

Depolarization of the surface membrane of skeletal or cardiac muscle cells initiated by the nerve impulse is conducted into the interior of the muscle fiber by extensions of the plasma membrane, i.e. the T tubules. Therefore, T tubules establish specialized contacts with the SR membrane that contain the two key components of the E–C coupling apparatus, the dihydropyridine receptor (DHPR) and the ryanodine receptor (RyR). Other explanations are within the text.

and RyR in the junctional complex (Leong & MacLennan, 1998; Stange *et al.*, 2001). The effects of this interaction are bi-directional resulting in the activation of RyR1 Ca^{2+} release by depolarization, in the inactivation of RyR1 during repolarization, and in the enhancement of the DHPR Ca^{2+} channel activity. Phosphorylation of Ser687 in the II–III loop by cAMP dependent protein kinase abolished these effects. Repeat I of the 1 subunit determines the kinetics of Ca^{2+} channel activation, that is slow in skeletal and fast in cardiac muscle (Tanabe *et al.*, 1991). The SS1–SS2 segment between helices V and VI is assumed to line the channel pore (Catterall, 2000).

The structure of ryanodine receptor

There are three genetically distinct isoforms of RyR in mammals (Franzini-Armstrong & Protasi, 1997; Mar & Marks, 2000). RyR1 is the dominant isoform in skeletal muscle, RyR2 in cardiac muscle, while RyR3 is ubiquitously distributed in various tissues including muscle (Ogawa *et al.*, 2000). In avian, fish and amphibian muscles, two major isoforms α and β were identified (Franzini-Armstrong & Protasi, 1997). The RyR1 isoform is present both in slow and fast-twitch skeletal muscles. The rabbit and human RyR1 contain 5037 and 5032 amino-acid residues, respectively, with

molecular masses of close to 560 kDa (Zorzato *et al.*, 1990). Much of the mass of the molecule is exposed in the cytoplasm, anchored by four to twelve transmembrane sequences into the junctional SR membrane (Zorzato *et al.*, 1990). The transmembrane helices are assumed to form the Ca^{2+} channels. The gene of human RyR1 was localized to chromosome 19q13.1. Mutation in this gene may be responsible for some forms of malignant hyperthermia (Mickelson & Louis, 1996; MacLennan, 2000).

The RyR2 of rabbit cardiac muscle contains 4969 amino-acid residues with a molecular weight of 564 711 (Nakai *et al.*, 1990; Otsu *et al.*, 1990). The gene for the cardiac ryanodine receptor (RyR2) is on chromosome 1 (Otsu *et al.*, 1990). The RyR1 and RyR2 isoforms have 66% amino acid homology and similar topology, but differ in cellular distribution, channel activation and interaction with DHPRs that contribute to the differences in E–C coupling between skeletal and cardiac muscles. Three-dimensional reconstitution of negatively stained or frozen-hydrated electron microscope images of the skeletal muscle RyR revealed a tetrameric structure with dimensions of 27 × 27 × 14 nm, that is consistent with the dimensions of "junctional feet" seen in triad junctions of skeletal muscle (Sharma *et al.*, 1998; Liu *et al.*, 2001; Samso & Wagenknecht, 2002). In the large cytoplasmic domain there is a central channel which branches into four radial channels that extend to the lateral surface of the feet. These channels probably serve as the pathways for Ca^{2+} release. A square shaped basal platform of 14 × 14 × 4 nm contains the membrane spanning domain. RyR2 and RyR3 have similar structures (Sharma *et al.*, 1998; Liu *et al.*, 2001).

The side of the "feet" facing the T-tubules is presumably attached directly or with the assistance of other junctional proteins to the dihydropyridine receptor tetramers in the T tubules. Residues 922–1112 of RyR1 bind specifically to the II–III and III–IV loops of the skeletal muscle dihydropyridine receptor or to the II–III loop of the Na^{+} channel but there is no interaction with the same region of the cardiac DHPR (Leong & MacLennan, 1998). The corresponding peptide fragment from the cardiac RyR2 did not interact with skeletal DHPR. These observations indicate that the DHPR II–III and III–IV loops interact with a broad region of the RyR1 surface. The mechanical strength of the SR T tubule interaction is critical to prevent disruption during muscle contraction and stretch.

The RyR1 interacts with FKBP12 (Wagenknecht *et al.*, 1996) and calmodulin (Samso & Wagenknecht, 2002) on its cytoplasmic surface, at about 10 nm distance from the transmembrane channel; therefore, the effects of these agents on Ca^{2+} channel activity are mediated by long-range changes in the conformation of RyR. Conformational changes visible by electron microscopy indeed accompany transitions between the closed and the open states of the Ca^{2+} channel (Orlova *et al.*, 1996).

The Ca^{2+} channel is activated by cytoplasmic Ca^{2+} and ATP and inhibited by calmodulin (Meissner, 1994; 2001); phosphorylation on Ser2809 by calmodulin-dependent protein kinase activated the cardiac RyR (Meissner, 1994). The activation by cytoplasmic Ca^{2+} is the biochemical basis of Ca^{2+}-induced Ca^{2+} release. The rate of Ca^{2+} release increases with increase in the Ca^{2+} concentration of SR lumen (Hidalgo & Donoso, 1995). This may be due to Ca^{2+} binding to lumenal Ca^{2+}-binding sites on RyR or to an effect of lumenal Ca^{2+} on junctional proteins that may interact with RyR (Hidalgo & Donoso, 1995).

Disposition of dihydropyridine and ryanodine receptors in skeletal and cardiac muscles

The differences in the mechanism of E–C coupling between skeletal and cardiac muscles are in part related to the distinct structural organization of DHPR and RyR in the junctional complexes of the two muscle types.

In both muscles the RyR tetramers form dense structures (feet) visible by electron microscopy, that span the junctional gap of 10 nm between the SR and the T tubules or plasma membranes (Franzini-Armstrong & Protasi, 1997).

In the toadfish swim-bladder muscle and in mammalian skeletal muscles the RyR "feet" are arranged in tetragonal arrays within the junctional SR, facing arrays of tetrads containing four DHPR molecules located in the junctional T tubules and in the plasma membranes (Franzini-Armstrong & Protasi, 1997). This spatial arrangement permits interaction of the four DHPR molecules within a tetrad with the RyR tetramers in alternate feet. The association of alternate RyR feet with the DHPR tetrads provides the structural basis for the direct mechanical activation of half of the RyR Ca^{2+} channels by DHPR (Rios & Pizarro, 1991). RyR feet that are not in contact with the DHPR tetrads may be activated by Ca^{2+} released from adjacent feet. Since mammalian skeletal muscles contain only one major RyR isoform (RyR1), it is not clear why DHPR tetrads interact only with alternate RyR tetramers. In several non-mammalian muscles that contain α and β isoforms of the RyR in similar amounts, only the α RyR tetramers appear to interact with DHPR tetrads, and the β isoform is presumably activated by Ca^{2+}.

In cardiac muscles the DHPR molecules are generally not grouped into tetrads and their position does not correspond to the position of the RyR feet (Sun *et al.*, 1995). Extra-junctional RyRs associated with calsequestrin aggregates are seen in the non-junctional "corbular" SR of cardiac muscles located at some distance from the T tubules or surface membranes (Jorgensen *et al.*, 1993; Franzini-Armstrong *et al.*, 1998). The absence of regular contacts between DHPR and RyR in cardiac muscles supports the primary role of voltage-dependent Ca^{2+} influx and Ca^{2+}-induced Ca^{2+} release in cardiac E–C coupling (Fabiato, 1989).

Purified skeletal muscle DHPR and RyR did not interact *in vitro* (Brandt *et al.*, 1990), and after coexpression of the two proteins in CHO cells there was no junction formation (Takekura *et al.*, 1995). The RyR expressed alone was correctly inserted into the ER membrane of CHO cells and formed functional tetrameric Ca^{2+} channels arranged in arrays similar to those seen in skeletal muscle (Takekura *et al.*, 1995). By contrast, most of the skeletal muscle DHPR expressed in CHO cells was retained in the perinuclear membranes and did not assemble into tetrads, suggesting a requirement for other junctional components for correct targeting and self-assembly of DHPR. Some of these components may be specific to muscle cells since DHPR is correctly targeted in dyspedic mouse embryo muscles lacking RyR1 (Takekura & Franzini-Armstrong, 1999). Even in this case, coexpression of RyR1 was also required for the formation of DHPR tetrads and for their alignment into orthogonal arrays (Protasi *et al.*, 2000). The other skeletal muscle isoform, RyR3, was ineffective in inducing DHPR tetrad formation (Protasi *et al.*, 2000).

Other junctional proteins that interact with DHPR and RyR may play a role in the formation and stabilization of junctions. These include calsequestrin (Milner *et al.*, 1992; Wagenknecht *et al.*, 2002), triadin (Kobayashi *et al.*, 2000; Marty *et al.*, 2000; Kirchhefer *et al.*, 2001), junctin (Zhang *et al.*, 2001), junctate (Treves *et al.*, 2000), junctophilin (Takeshima *et al.*, 2000), mitsugumin (Brandt *et al.*, 2001), 30 kDa protein (Yamaguchi & Kasai, 1998), 45 kDa protein (Zorzato *et al.*, 2000), 90 kDa protein (Froemming *et al.*, 1999), 150–167 kDa proteins (Orr & Shoshan- Barmatz, 1996), annexin VI (Diaz- Munoz *et al.*, 1990, but see also Barrientos & Hidalgo, 2002), aldolase and glyceraldehyde 3-phosphate dehydrogenase (Caswell & Brandt, 1989).

The precise role of these proteins in the structure and function of junctional complexes remains to be defined.

REFERENCES

Adachi-Akahane S, Lu L, Li Z, Frank JS, Philipson KD, Morad M. (1997) Calcium signaling in transgenic mice overexpressing cardiac Na^+–Ca^{2+} exchanger. *J Gen Physiol.*; **109**: 717–29.

Adams BA, Tanabe T, Mikami A, Numa S, Beam KG. (1990) Intramembrane charge movement restored in dysgenic skeletal muscle by injection of dihydropyridine receptor cDNAs. *Nature.*; **346**: 569–72.

Allen DL, Leinwand LA. (2002) Intracellular calcium and myosin isoform transitions. *J Biol Chem.*; **277**: 45323-30.

Armstrong CM, Bezanilla FM, Horowicz P. (1972) Twitches in the presence of ethylene glycol bis (β-aminoethyl ether)-*N,N′*-tetraacetic acid. *Biochim Biophys Acta.*; **267**: 605–8.

Ashley CC, Griffiths PJ, Lea TJ, Mulligan IP, Palmer RE, Simnett SJ. (1993) Barnacle muscle: Ca^{2+}, activation and mechanics. *Rev Physiol Biochem Pharmacol.*; **122**: 149–258.

Babcock DF, Hille B. (1998) Mitochondrial oversight of cellular Ca^{2+} signalling. *Curr Opin Neurobiol.*; **8**: 398–404.

Baker DL, Hashimoto K, Grupp IL, Ji Y, Reed T, Loukianov E, Grupp G, Bhagwhat A, Hoit B, Walsh R, Marban E, Periasamy M. (1998) Targeted overexpression of the sarcoplasmic reticulum Ca^{2+}-ATPase increases cardiac contractility in transgenic mouse heart. *Circ Res.*; **83**: 1205–14.

Balke CW, Egan TM, Wier WG. (1994) Processes that remove calcium from the cytoplasm during excitation–contraction coupling in intact rat heart cells. *J. Physiol.*; **474**: 447–62.

Barrientos G, Hidalgo C. (2002) Annexin VI is attached to transverse-tubule membranes in isolated skeletal muscle triads. *J Membr Biol.*; **188**: 163-73.

Bassani JWM, Bassani RA, Bers DM. (1994) Relaxation in rabbit and rat cardiac cells: species dependent differences in cellular mechanisms. *J Physiol.*; **476**: 279–93.

Baylor SM, Chandler WK, Marshall MW. (1983) Sarcoplasmic reticulum calcium release in frog skeletal muscle fibres estimated from Arsenazo III calcium transients. *J Physiol.*; **344**: 625–66.

Benders AAGM, Wevers RA, Veerkamp JH (1996) Ion transport in human skeletal muscle cell: disturbances in myotonic dystrophy and Brody's diseases. *Acta Physiol Scand.*; **156**: 355–67.

Bernardi P. (1999) Mitochondrial transport of cations: channels, exchangers, and permeability transition. *Physiol Rev.*; **79**: 1127–55.

Berridge MJ, Bootman MD, Lipp P. (1998) Calcium: a life and death signal. *Nature.*; **395**: 645–8.

Berridge MJ. (2001) The versatility and complexity of calcium signaling. In *Complexity in biological information processing*. Novartis Foundation Symposium 239, pp 52–67. John Wiley and Sons.

Bers DM, Bassani JWM, Bassani RA. (1993) Competition and redistribution among calcium transport systems in rabbit cardiac myocytes. *Cardiovascular Res.*; **27**: 1772–7.

Bers DM. (2002) Cardiac excitation–contraction coupling. *Nature.*; **415**: 198–205.

Bezanilla F. (2000) The voltage sensor in voltage-dependent ion channels. *Physiol Rev.*; **80**: 555–92.

Bianchi CP, Shanes AM. (1959) Calcium influx in skeletal muscle at rest, during activity and during potassium contracture. *J Gen Physiol.*; **42**: 803–15.

Blaustein MP, Lederer WJ. (1999) Sodium/calcium exchange: its physiological implications. *Physiol Rev.*; **79**: 763–854.

Bluhm WF, Meyer M, Sayen MR, Swanson EA, Dillmann WH. (1999) Overexpression of sarcoplasmic reticulum Ca^{2+}-ATPase improves cardiac contractile function in hypothyroid mice. *Cardiovasc Res.*; **43**: 382–8.

Booth FW, Thomason DB. (1991) Molecular and cellular adaptation of muscle in response to exercise: perspectives of various models. *Physiol Rev.*; **71**: 541–85.

Bootman MD, Lipp P, Berridge MJ. (2001) The organization and functions of local Ca^{2+} signals. *J Cell Science*.; **114**: 2213–22.

Bootman MD, Berridge MJ, Roderick HL. (2002) Calcium signaling: more messengers, more channels, more complexity. *Curr Biol*.; **121**: R563–5.

Brandt NR, Caswell AH, Wen S-R, Talvenheimo JA. (1990) Molecular interactions of the junctional foot protein and dihydropyridine receptor in skeletal muscle triads. *J Membr Biol*.; **113**: 237–51.

Brandt NR, Franklin G, Brunschwig J-P, Caswell AH. (2001) The role of mitsugumin 29 in transverse tubules of rabbit skeletal muscle. *Arch Biochem Biophys*.; **385**: 406–9.

Brini M, Carafoli E. (2000) Calcium signalling: a historical account, recent developments and future perspectives. *Cell Mol Life Sci*.; **57**: 354–70.

Brini M, Bano D, Manni S, Rizzuto R, Carafoli E. (2000) Effects of PMCA and SERCA pump overexpression on the kinetics of cell Ca^{2+} signalling. *EMBO J*.; **19**: 4926–35.

Brittsan AG, Kranias EG. (2000) Phospholamban and cardiac contractile function. *J Mol Cell Cardiol*.; **32**: 2131–5.

Brody IA. (1969) Muscle contracture induced by exercise. A syndrome attributable to decreased relaxing factor. *N Eng J Med*.; **281**: 187–92.

Buntinas L, Gunter KK, Sparagna GC, Gunter TE. (2001) The rapid mode of calcium uptake into heart mitochondria (RAM): comparison to RAM in liver mitochondria. *Biochim Biophys Acta*.; **1504**: 248–61.

Callen DF, Lane SA, Kozman H, Kremmidiotis G, Whitmore SA, Lowenstein M, Doggett NA, Kenmochi N, Page DC, Maglott DR, Nierman WC, Murakawa K, Berry R, Sikela JM, Houlgatte RA, Uffray C, Sutherland GR. (1995) Integration of transcript and genetic maps of chromosome 16 at near -1-Mb resolution: demonstration of a "hot spot" for recombination at 16p12. *Genomics*.; **29**: 503–11.

Campbell AK. (1983) *Intracellular calcium: its universal role as regulator*. John Wiley & Sons, Chichester.

Cannell MB, Cheng H, Lederer WJ. (1995) The control of calcium release in heart muscle. *Science*.; **268**: 1045–9.

Carafoli E, Klee C. eds. (1999) *Calcium as a cellular regulator*. Oxford University Press, New York.

Carafoli E, Genazzani A, Guerini D. (1999) Calcium controls the transcription of its own transporters and channels in developing neurons. *Biochem Biophys Res Commun*.; **266**: 624–32.

Carafoli E, Santella L, Branca K, Brini M. (2001) Generation, control and processing of cellular calcium signals. *Crit Rev Biochem Mol Biol*.; **36**: 107–260.

Caswell AH, Brandt NR. (1989) Triadic proteins of skeletal muscle. *J Bioenerg Biomembr*.; **21**: 149–62.

Catterall WA. (2000) Structure and regulation of voltage-gated Ca^{2+} channels. *Annu Rev Cell Dev Biol*.; **16**: 521–55.

Cavagna M, O'Donnell JM, Sumbilla C, Inesi G, Klein MG. (2000) Exogenous Ca^{2+}-ATPase isoform effects on Ca^{2+} transients of embryonic chicken and neonatal rat cardiac myocytes. *J Physiol*.; **528**: 53–63.

Chaudhari N. (1995) Muscular dysgenesis. The role of calcium channels in muscle development. In *Ion channels and genetic diseases*. Soc Gen Physiol Series, vol 50, Dawson DC, Frizzel RA. eds, pp 115–24. Rockefeller University Press, New York.

Chevet E, Jakob CA, Thomas DY, Bergeron JJ. (1999) Calnexin family members as modulators of genetic diseases. *Semin Cell Dev Biol*.; **10**: 473–80.

Choi HS, Eisner DA. (1999) The role of sarcolemmal Ca^{2+}-ATPase in the regulation of resting calcium concentration in ventricular myocytes. *J Physiol*.; **515**: 109–18.

Co DA, Matlib MA. (1993) A role for the mitochondrial Na^{+}–Ca^{2+} exchanger in the regulation of oxidative phosphorylation in isolated

heart mitochondria. *J Biol Chem*.; **268**: 938–47.

Corbett EF, Michalak M. (2000) Calcium, a signaling molecule in the endoplasmic reticulum. *Trends Biochem Sci*.; **25**: 307–11.

de Meis L, ed. (1995) Ca transport: pumps and channels. *Biosci Rep*.; **15**: 241–408.

Del Monte F, Harding SE, Schmidt U, Matsui T, Kang ZB, Dec GW, Gwathmey JK, Rosenzweig A, Hajjar RJ. (1999) Restoration of contractile function in isolated cardiomyocytes from failing human hearts by gene transfer of SERCA2a. *Circulation*.; **100**: 2308–11.

Di Lisa F, Menabo R, Canton M, Barile M, Bernardi P. (2001) Opening of the mitochondrial permeability transition pore causes depletion of mitochondrial and cytosolic NAD^+ and is a causative event in the death of myocytes in postischemic reperfusion of the heart. *J Biol Chem*.; **276**: 2571–5.

Diaz-Munoz M, Hamilton SL, Kaetzel MA, Hazarika P, Dedman JR. (1990) Modulation of Ca^{2+} release channel activity from sarcoplasmic reticulum by annexin VI (67-kDa calcimedin). *J Biol Chem*.; **265**: 15894–9.

Dode L, Wuytack F, Kools PF, Baba-Aissa F, Raeymaekers L, Brike F, van de Ven WJ, Casteels R, Brik F. (1996) cDNA cloning, expression and chromosomal localization of the human sarco/endoplasmic reticulum Ca^{2+} ATPase 3 gene. *Biochem J*.; **318**: 689–99.

Duchen MR. (2000) Mitochondria and calcium: from cell signalling to cell death. *J Physiol*.; **529**: 57–68.

Ebashi S. (1961) Calcium binding activity of vesicular relaxing factor. *J Biochem*.; **50**: 236–44.

Ebashi S. (1963) Third component participating in the superprecipitation of natural actomyosin. *Nature*.; **200**: 1010.

Ebashi S, Endo M, Ohtsuki I. (1999) Calcium in muscle contraction. In *Calcium as a cellular regulator*. Carafoli E, Klee C. eds, pp 579–95. Oxford University Press, New York.

Fabiato A. (1989) Appraisal of the physiological relevance of two hypotheses for the mechanism of calcium release from the mammalian cardiac sarcoplasmic reticulum: calcium-induced release versus charge coupled release. *Mol Cell Biochem*.; **89**: 135–40.

Farah CS, Reinach FC. (1995) The troponin complex and regulation of muscle contraction. *FASEB J*.; **9**: 755–67.

Ferri KF, Kroemer G. (2001) Mitochondria — the suicide organelles. *BioEssays*.; **23**: 111–5.

Franzini-Armstrong C. (1999) The sarcoplasmic reticulum and the control of muscle contraction. *FASEB J*.; **13** (Suppl): S266–70.

Franzini-Armstrong C, Protasi F. (1997) Ryanodine receptors of striated muscles: a complex channel capable of multiple interactions. *Physiol Rev*.; **77**: 699–729.

Franzini-Armstrong C, Protasi F, Ramesh V. (1998) Comparative ultrastructure of Ca^{2+} release units in skeletal and cardiac muscle. *Ann NY Acad Sci*.; **853**: 20–30.

Froemming GR, Pette D, Ohlendieck K. (1999) The 90-kDa junctional sarcoplasmic reticulum protein forms an integral part of a supramolecular triad complex in skeletal muscle. *Biochem Biophys Res Commun*.; **261**: 603–9.

Gordon AM, Homsher E, Regnier M. (2000) Regulation of contraction in striated muscle. *Physiol Rev*.; **80**: 853–924.

Greene AL, Lalli MJ, Ji Y, Babu GJ, Grupp I, Sussman M, Periasamy M. (2000) Overexpression of SERCA2b in the heart leads to an increase in sarcoplasmic reticulum calcium transport function and increased cardiac contractility. *J Biol Chem*.; **275**: 24722–7.

Guerini D, Schroder S, Foletti D, Carafoli E. (1995) Isolation and characterization of a stable Chinese hamster ovary cell line overexpressing the plasma membrane Ca^{2+} ATPase. *J Biol Chem*.; **270**: 14643–50.

Guerini D, Carafoli E. (1999) The calcium pumps. In *Calcium as a cellular regulator*. Carafoli E, Klee C. eds, pp 249–78. Oxford University Press, New York.

Hajnoczky G, Csordas G, Madesh M, Pacher P. (2000) The machinery of local Ca^{2+} signalling between sarcoendoplasmic reticulum and mitochondria. *J Physiol.*; **529**: 69–81.

Hammes A, Oberdorf-Maass S, Jenatschke S, Pelzer T, Maass A, Gollnick F, Meyer R, Afflerbach J, Neyses L. (1996) Expression of the plasma membrane Ca^{2+}-ATPase in myogenic cells. *J Biol Chem.*; **271**: 30816–22.

Hammes A, Oberdorf-Maass S, Rother T, Nething K, Gollnick F, Linz KW, Meyer R, Hu K, Han H, Gaudron P, Ertl G, Hoffmann S, Ganten U, Vetter R, Schuh K, Benkwitz C, Zimmer HG, Neyses L. (1998) Overexpression of the sarcolemmal calcium pump in the myocardium of transgenic rats. *Circ Res.*; **83**: 877–88.

Hasselbach W, Makinose M. (1961) Die calciumpumpe der "Erschlaffungsgrana" desmuskels und ihre abhangigkeit von der ATP-spaltung. *Biochem Z.*; **333**: 518–28.

He H, Giordano FJ, Hilal-Dandan R, Choi DJ, Rockman HA, McDonough PM, Bluhm WF, Meyer M, Sayen MR, Swanson E, Dillmann WH. (1997) Overexpression of the rat sarcoplasmic reticulum Ca^{2+}-ATPase gene in the heart of transgenic mice accelerates calcium transients and cardiac relaxation. *J Clin Invest.*; **100**: 380-9.

Heilbrunn LV, Wiercinsky FJ. (1947) Action of various cations on muscle protoplasm. *J Cell Comp Physiol.*; **19**: 15–32.

Heiny JA. (2001) Excitation–contraction coupling in skeletal muscle. In *Cell physiology sourcebook. A molecular approach*, 3d edn. Sperelakis N. ed, pp 911–26. Academic Press, San Diego.

Hidalgo C, Donoso P. (1995) Luminal calcium regulation of calcium release from sarcoplasmic reticulum. *Biosci. Rep.*; **15**: 387–97.

Hofmann F, Lacinova L, Klugbauer N. (1999) Voltage-dependent calcium channels: from structure to function. *Rev Physiol Biochem Pharmacol.*; **139**: 33–87.

Hollingworth S, Zhao M, Baylor SM. (1996) The amplitude and time course of the myoplasmic free Ca^{2+} transient in fast-twitch fibers of mouse muscle. *J Gen Physiol.*; **108**: 455–69.

Holmes KC, Geeves MA. (2000) The structural basis of muscle contraction. *Philos Trans R Soc Lond B Biol Sci.*; **355**: 419–31.

Huley HE. (2000) Past, present and future experiments on muscle. *Philos Trans R Soc Lond B Biol Sci.*; **355**: 539–43.

Jacobsen NJ, Lyons I, Hoogendoorn B, Burge S, Kwok PY, O'Donovan MC, Craddock N, Owen MJ. (1999) ATP2A2 mutations in Darier's disease and their relationship to neuropshychiatric phenotypes. *Hum Mol Genet.*; **8**: 1631–6.

Ji Y, Lalli MJ, Babu GJ, Xu Y, Kirkpatrick DL, Liu LH, Chiamvimonvat N, Walsh RA, Shull GE, Periasamy M. (2000) Disruption of a single copy of the SERCA2 gene results in altered Ca^{2+} homeostasis and cardiomyocyte function. *J Biol Chem.*; **275**: 38073–80.

Jorgensen AO, Shen A C-Y, Arnold W, McPherson PS, Campbell KP. (1993) The Ca^{2+}-release channel/ryanodine receptor is localized in junctional and corbular sarcoplasmic reticulum in cardiac muscle. *J Cell Biol.*; **120**: 969–80.

Jouaville LS, Pinton P, Bastianutto C, Rutter GA, Rizzuto R. (1999) Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term priming. *Proc Natl Acad Sci U S A.*; **96**: 13807–12.

Kamada T, Kinoshita H. (1943) Disturbances initiated from the naked surface of muscle protoplasm. *Jap J Zool.*; **10**: 469–93.

Kao J, Fortner CN, Liu LH, Shull GE, Paul RJ. (1999) Ablation of the SERCA3 gene alters epithelium dependent relaxation in mouse tracheal smooth muscle. *Am J Physiol.*; **277**: L264-70.

Kaplan JJ. (2002) Biochemistry of Na^+,K^+-ATPase. *Annu Rev Biochem.*; **71**: 511–35.

Kawasaki H, Kretsinger RH. (1994) Calcium binding proteins 1: EF-hands. *Protein Profile.*; **1**: 343–517.

Kirby MS, Sagara Y, Gaa S, Inesi G, Lederer WJ, Rogers TB. (1992) Thapsigargin inhibits contraction and Ca^{2+}transient in cardiac cells by specific inhibition of the sarcoplasmic reticulum Ca^{2+} pump. *J Biol Chem.*; **267**: 12545–51.

Kirchhefer U, Neumann J, Baba HA, Begrow F, Kobayashi YM, Reinke U, Schmitz W, Jones LR. (2001) Cardiac hypertrophy and impaired relaxation in transgenic mice overexpressing triadin 1. *J Biol Chem.*; **276**: 4142-49.

Kiriazis H, Kranias EG. (2000) Genetically engineered models with alterations in cardiac membrane calcium-handling proteins. *Annu Rev Physiol.*; **62**: 321–51.

Kobayashi YM, Alseikhan BA, Jones LR. (2000) Localization and characterization of the calsequestrin binding domain of triadin. 1. Evidence for a charged beta-strand in mediating protein–protein interaction. *J Biol Chem.*; **275**: 17639–46.

Kunz WS. (2001) Control of oxidative phosphorylation in skeletal muscle. *Biochim Biophys Acta.*; **1504**: 12–9.

Lee AG. (2002) Ca^{2+}-ATPase structure in the E_1 and E_2 conformations: mechanism, helix–helix and helix–lipid interactions. *Biochim Biophys Acta.*; **1565**: 246–66.

Leong P, MacLennan DH. (1998) The cytoplasmic loops between domains II and III and domains III and IV in the skeletal muscle dihydropyridine receptor bind to a contiguous site in the skeletal muscle ryanodine receptor. *J Biol Chem.*; **273**: 29958–64.

Liu B-F, Xu X, Fridman R, Muallem S, Kuo TH. (1996) Consequences of functional expression of the plasma membrane Ca^{2+} pump isoform 1a. *J Biol Chem.*; **271**: 5536–44.

Liu LH, Paul RJ, Sutliff RL, Miller ML, Lorenz JN, Pun RY, Duffy JJ, Doetschman T, Kimura Y, MacLennan DH, Hoying JB, Shull GE. (1997) Defective endothelium-dependent relaxation of vascular smooth muscle and endothelial cell Ca^{2+} signaling in mice lacking sarco(endo)plasmic reticulum Ca^{2+}-ATPase isoform 3. *J Biol Chem.*; **272**: 30538-45.

Liu Z, Zhang I, Sharma MR, Li P, Chen SR, Wagenknecht T. (2001) Three-dimensional reconstruction of the recombinant type 3 ryanodine receptor and localization of its amino terminus. *Proc Natl Acad Sci U S A.*; **98**: 6104–9.

Loukianov E, Ji Y, Grupp IL, Kirkpatrick DL, Baker DL, Loukianova T, Grupp G, Lytton J, Walsh RA, Periasamy M. (1998) Enhanced myocardial contractility and increased Ca^{2+} transport function in transgenic hearts expressing the fast-twitch skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPase. *Circ Res.*; **83**: 889–97.

Lytton J, Zarain-Herzberg A, Periasamy M, MacLennan DH. (1989) Molecular cloning of the mammalian smooth muscle sarco-(endo)plasmic reticulum Ca^{2+}-ATPase. *J Biol Chem.*; **264**: 7059–65.

MacLennan DH. (2000) Ca^{2+} signalling and muscle disease. *Eur J Biochem.*; **267**: 5291–97.

MacLennan DH, Toyofuku T, Lytton J. (1992) Structure-function relationships in sarcoplasmic reticulum type Ca^{2+} pumps. *Ann NY Acad Sci.*; **671**: 1–10.

MacLennan DH, Rice WJ, Green MN. (1997) The mechanism of Ca^{2+} transport by sarco(endo)plasmic reticulum Ca^{2+}-ATPases. *J Biol Chem.*; **272**: 28815–8.

MacLennan DH, Abu-Abed M, Kang C-H. (2002) Structure function relationships in Ca cycling proteins. *J Mol Cell Cardiol.*; **34**: 897–918.

Mar SO, Marks AR. (2000) Ryanodine receptors. In *Calcium signalling.* Putney JW Jr. ed, pp 227–47. Boca Raton, CRC Press.

Martonosi AN. (2000) *The development of sarcoplasmic reticulum*. Harwood Academic Publ., Amsterdam.

Marty I, Thevenon D, Scotto C, Groh S, Sainnier S, Robert M, Grunwald D, Villaz M. (2000) Cloning and characterization of a new isoform of skeletal muscle triadin. *J Biol Chem.*; **275**: 8206–12.

Mazzanti M, Bustamante JO, Oberleithner H. (2001) Electrical dimension of the nuclear envelope. *Physiol Rev.*; **81**: 1–19.

McCormack JG, Denton RM. (1999) Calcium in the regulation of intramitochondrial enzymes. In *Calcium as a cellular regulator*. Carafoli E, Klee C. eds, pp 529–44. Oxford University Press, New York.

Meissner G. (1994) Ryanodine receptor/Ca^{2+} release channels and their regulation by endogenous effectors. *Annu Rev Physiol.*; **56**: 485–508.

Meissner G. (2001) Ca^{2+} release from sarcoplasmic reticulum in muscle. In *Cell physiology sourcebook. A molecular approach,* 3d edn. Sperelakis N. ed, pp 927–40. Academic Press, San Diego.

Mellstrom B, Naranjo JR. (2001) Mechanisms of Ca^{2+}-dependent transcription. *Curr Opin Neurobiol.*; **11**: 312–9.

Michalak M, ed. (1996) *Calreticulin.*, RG Landes and Co., Austin.

Mickelson JR, Louis CF. (1996) Malignant hyperthermia: excitation–contraction coupling, Ca^{2+} release channel, and cell Ca^{2+} regulation defects. *Physiol Rev.*; **76**: 537–92.

Mikami A, Imoto K, Tanabe T, Niidome T, Mori Y, Takeshima H, Narumiya S, Numa S. (1989) Primary structure and functional expression of the cardiac dihydropyridine- sensitive calcium channel. *Nature.*; **340**: 230–3.

Milner RE, Famulski KS, Michalak M. (1992) Calcium binding proteins in the sarcoplasmic /endoplasmic reticulum of muscle and nonmuscle cells. *Mol Cell Biochem.*; **112**: 1–13.

Miyamoto M, del Monte F, Schmidt U, DiSalvo TS, Kang ZB, Matsui T, Guerrero JL, Gwathmey JK, Rosenzweig A, Hajjar RJ. (2000) Adenoviral gene transfer of SERCA2a improves left-ventricular function in aortic-banded rats in transition to heart failure. *Proc Natl Acad Sci U S A.*; **97**: 793–8.

Moller JV, Juul B, le Maire M. (1996) Structural organization, ion transport and energy transduction of P-type ATPases. *Biochim Biophys Acta.*; **1286**: 1–51.

Nakai J, Imagawa T, Hakamata Y, Shigekawa M, Takeshima H, Numa S. (1990) Primary structure and functional expression from cDNA of the cardiac ryanodine receptor/calcium release channel. *FEBS Lett.*; **271**: 169–77.

Newton T, Black JP, Butler J, Lee AG, Chad JE, East JM. (2003) The calcium pump SERCA1 is maintained in the endoplasmic reticulum by a retrieval signal located between residues 1-211. *Biochem J.*; in press.

Niggli E. (1999) Localized intracellular calcium signalling in muscle: calcium sparks and calcium quarks. *Annu Rev Physiol.*; **61**: 311–35.

Niki I, Yokokura H, Sudo T, Kato M, Hidaka H. (1996) Ca^{2+} signaling and intracellular Ca^{2+} binding proteins. *J Biochem.*; **120**: 685–98.

Odermatt A, Barton K, Khanna VK, Mathieu J, Escolar D, Kuntzer T, Karpati G, MacLennan DH. (2000) The mutation of Pro789 to Leu reduces the activity of the fast-twitch skeletal muscle sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA1) and is associated with Brody disease. *Hum Genet.*; **106**: 482–91.

Ogawa Y, Kurebayashi N, Murayama T. (2000) Putative roles of type 3 ryanodine receptor isoforms (RyR3). *Trends Cardiovasc Med.*; **10**: 65–70.

Ogawa Y, Murayama T, Kurebayashi N. (2002) Ryanodine receptor isoforms of non-mammalian skeletal muscle. *Front Biosci.*; **7**: d1184–94.

Ojuka EO, Jones TE, Han DH, Chen M, Wamhoff BR, Sturek M, Holloszy JO. (2002) Intermittent increases in cytosolic Ca^{2+} stimulate mitochondrial biogenesis in muscle cells. *Am J Physiol.*; **283**: E1040–5.

Orlova EV, Serysheva II, Van Heel M, Hamilton SL, Chiu W. (1996) Two structural configurations of the skeletal muscle calcium release channel. *Nat Struct Biol.*; **3**: 547–52.

Orr I, Shoshan-Barmatz V. (1996) Modulation of the skeletal muscle ryanodine receptor by endogenous phosphorylation of 150/160 kDa proteins of the sarcoplasmic reticulum. *Biochim Biophys Acta.*; **1283**: 80–8.

Otsu K, Willard HF, Khanna VK, Zorzato F, Green NM, MacLennan DH. (1990) Molecular cloning of cDNA encoding the Ca^{2+} release channel (ryanodine receptor) of rabbit car-

diac muscle sarcoplasmic reticulum. *J Biol Chem*.; **265**: 13472–83.

Otsu K, Fujii J, Periasamy M, Difilippantonio M, Uppender M, Ward DC, MacLennan DH. (1993) Chromosome mapping of five human cardiac and skeletal muscle sarcoplasmic reticulum genes. *Genomics*.; **17**: 507–9.

Periasamy M, Reed TD, Liu LH, Ji Y, Loukianov E, Paul RJ, Nieman ML, Riddle T, Duffy JJ, Doetschman T, Lorenz JN, Shull GE. (1999) Impaired cardiac performance in heterozygous mice with a null mutation in the sarco(endo)plasmic reticulum Ca^{2+}-ATPase isoform 2 (SERCA2) gene. *J Biol Chem*.; **274**: 2556–62.

Periasamy M, Huke S. (2001) SERCA pump level is a critical determinant of Ca^{2+} homeostasis and cardiac contractility. *J Mol Cell Cardiol*.; **33**: 1053–63.

Pette D. (2001) Historical perspectives: plasticity of mammalian skeletal muscle. *J Appl Physiol*.; **90**: 1119–24.

Philipson KD, Nicoll DA. (2000) Sodium–calcium exchange: a molecular perspective. *Annu Rev Physiol*.; **62**: 111–33.

Protasi F, Takekura H, Wang Y, Chen SR, Meissner G, Allen PD, Franzini-Armstrong C. (2000) RyR1 and RyR3 have different roles in the assembly of calcium release units of skeletal muscle. *Biochem J*.; **79**: 2494–508.

Putney JW Jr, ed. (2000) *Calcium signalling*. CRC Press, Boca Raton.

Reed LJ, Damuni Z, Merryfield ML. (1985) Regulation of mammalian pyruvate and branched-chain-keto acid dehydrogenase complexes by phosphorylation dephosphorylation. *Curr Top Cell Regul*.; **27**: 41–9.

Reed TD, Babu G, Ji Y, Zilberman A, Ver Heyen M, Wuytack F, Periasamy M. (2000) The expression of SR calcium transport ATPase and the Na^{+}/Ca^{2+} exchanger are antithetically regulated during mouse cardiac development and in hypo/hyperthyroidism. *J Mol Cell Cardiol*.; **32**: 453–64.

Ringer S. (1883) A further contribution regarding the influence of the blood on the contraction of the heart. *J Physiol*.; **4**: 29–42.

Rios E, Pizarro G. (1991) Voltage sensor of excitation–contraction coupling in skeletal muscle. *Physiol Rev*.; **71**: 849–908.

Rios E, Stern MD. (1997) Calcium in close quarters: microdomain feedback in excitation–contraction coupling and other cell biological phenomena. *Annu Rev Biophys Biomol Struct*.; **26**: 47–82.

Rizzuto R, Bernardi P, Pozzan T. (2000) Mitochondria as all-around players of the calcium-game. *J Physiol*.; **529**: 37–47.

Robert V, Gurlini P, Tosello V, Nagai T, Miyawaki A, Di Lisa F, Pozzan T. (2001) Beat-to-beat oscillations of mitochondrial $[Ca^{2+}]$ in cardiac cells. *EMBO J*.; **20**: 4998–5007.

Samso M, Wagenknecht T. (2002) Apocalmodulin and Ca^{2+}-calmodulin bind to neighboring locations on the ryanodine receptor. *J Biol Chem*.; **277**: 1349–53.

Santella L. (1996) The cell nucleus: an Eldorado to future calcium research? *J Membr Biol*.; **153**: 83–92.

Seppet EK, Kaambre T, Sikk P, Tiivel T, Vija H, Tonkonogi M, Sahlin K, Kay L, Appaix F, Braun U, Eimre M, Saks VA. (2001) Functional complexes of mitochondria with Ca^{2+},Mg^{2+}-ATPases of myofibrils and sarcoplasmic reticulum in muscle cells. *Biochim Biophys Acta*.; **1504**: 379–95.

Sharma MR, Penczek P, Grassucci R, Xin HB, Fleischer S, Wagenknecht T. (1998) Cryoelectron microscopy and image analysis of the cardiac ryanodine receptor. *J Biol Chem*.; **273**: 18429–34.

Shull GE. (2000) Gene knockout studies of Ca^{2+} transporting ATPases. *Eur J Biochem*.; **267**: 5284–90.

Skou JC. (1985) Sodium-potassium pump. In *Membrane transport. People and ideas*. Tosteson DC ed, pp 155–185. American Physiological Society, Bethesda.

Somlyo AV, Gonzalez-Serratos H, Shuman H, McClellan G, Somlyo AP. (1981) Calcium release and ionic changes in the sarcoplasmic reticulum of tetanized muscle: an electron probe study. *J Cell Biol.*; **90**: 577–94.

Somlyo AV, Bond M, Shuman H, Somlyo AP. (1986) Electron-probe-Xray microanalysis of in situ calcium and other ion movements in muscle and liver. *Ann NY Acad Sci.*; **483**: 229–40.

Sperelakis N, Gonzalez-Serratos H. (2001) Skeletal muscle action potential. In *Cell physiology sourcebook. A molecular approach*, 3rd edn. Sperelakis N. ed, pp 865–86. Academic Press, San Diego.

Stange M, Tripathy A, Meissner G. (2001) Two domains in dihydropyridine receptor activate the skeletal muscle Ca^{2+} release channel. *Biophys J.*; **81**: 1419–29.

Strehler EE, Zacharias DA. (2001) Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. *Physiol Rev.*; **81**: 21–50.

Sultan KR, Dittrich BT, Leisner E, Paul N, Pette D. (2001) Fiber type specific expression of major proteolytic systems in fast-to slow-transforming rabbit muscle. *Am J Physiol.*; **280**: C239–47.

Sumbilla C, Lewis D, Hammerschmidt T, Inesi G. (2002) The slippage of the Ca^{2+} pump and its control by anions and curcumin in skeletal and cardiac sarcoplasmic reticulum. *J Biol Chem.*; **277**: 13900–6.

Sun XH, Protasi F, Takahashi M, Takeshima H, Ferguson DG, Franzini-Armstrong C. (1995) Molecular architecture of membranes involved in excitation–contraction coupling of cardiac muscle. *J Cell Biol.*; **129**: 659–71.

Sweadner KJ, Donnet C. (2001) Structural similarities of Na^+,K^+-ATPase and SERCA, the Ca^{2+}-ATPase of the sarcoplasmic reticulum. *Biochem J.*; **356**: 685–704.

Takekura H, Takeshima H, Nishimura S, Takahashi M, Tanabe T, Flockerzi V, Hofmann F, Franzini-Armstrong C. (1995) Coexpression in CHO cells of two muscle proteins involved in excitation–contraction coupling. *J Muscle Res Cell Motil.*; **16**: 465–80.

Takekura H, Franzini-Armstrong C. (1999) Correct targeting of dihydropyridine receptors and triadin in dyspedic mouse skeletal muscle *in vivo*. *Develop Dynam.*; **214**: 372–80.

Takeshima H, Komazaki S, Nishi M, Iino M, Kangawa K. (2000) Junctophilins: a novel family of junctional membrane complex proteins. *Mol Cell.*; **6**: 11–22.

Tanabe T, Takeshima H, Mikami A, Flockerzi V, Takahashi H, Kangawa K, Kojima M, Matsuo H, Hirose T, Numa S. (1987) Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature.*; **328**: 313–8.

Tanabe T, Beam KG, Adams BA. (1990a) Regions of the skeletal muscle dihydropyridine receptor critical for excitation–contraction coupling. *Nature.*; **346**: 567–9.

Tanabe T, Mikami, Numa S, Beam KG. (1990b) Cardiac-type excitation-contraction coupling in dysgenic skeletal muscle injected with cardiac dihydropyridine receptor cDNA. *Nature.*; **344**: 451–53.

Tanabe T, Adams BA, Numa S, Beam KG. (1991) Repeat I of the dihydropyridine receptor is critical in determining calcium channel activation kinetics. *Nature.*; **352**: 800-3.

Taylor DJ, Brosnan MJ, Arnold DL, Bore PJ, Styles P, Walton J, Radda GK. (1988) Ca^{2+}-ATPase deficiency in a patient with an exertional muscle pain syndrome. *J Neurol Neurosurg Psychiatry.*; **51**: 1425–33.

Terracciano CMN, DeSouza AI, Philipson KD. (1998) Na^+–Ca^{2+} exchange and sarcoplasmic reticular Ca^{2+} regulation in ventricular myocytes from transgenic mice overexpressing the Na^+–Ca^{2+} exchanger. *J Physiol.*; **512**: 651–67.

Terracciano CMN, Philipson KD, MacLeod KT. (1999) The relationship between overexpression of the Na^+–Ca^{2+} exchanger and inhibition of the sarcoplasmic reticulum (SR) Ca^{2+}-ATPase in ventricular myocytes from transgenic mice. *J Physiol.*; **518**: 50P–1P.

Territo P, Mootha V, French S, Balaban RS. (2000) Ca^{2+} activation of heart mitochondrial oxidative phosphorylation: role of the F_O/F_1-ATPase. *Am J Physiol.*; **278**: C423-35.

Territo PR, French SA, Dunleavy MC, Evans FJ, Balaban RS. (2001) Calcium activation of heart mitochondrial oxidative phosphorylation. Rapid kinetics of mV_{O2}, NADH and light scattering. *J Biol Chem.*; **276**: 2586–99.

Toyoshima C, Nomura H (2002) Structural changes in the calcium pump accompanying the dissociation of calcium. *Nature.*; **418**: 605–11.

Toyoshima C, Nakasako M, Nomura H, Ogawa H. (2000) Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 Å resolution. *Nature.*; **405**: 647–55.

Treves S, Feriotto G, Moccagatta L, Gambari R, Zorzato F. (2000) Molecular cloning, expression, functional characterization, chromosomal localization and gene structure of junctate, a novel integral calcium binding protein of sarco(endo)plasmic reticulum membrane. *J Biol Chem.*; **275**: 39555–68.

Tupling AR, Asahi M, MacLennan DH. (2002) Sarcolipin overexpression in rat slow twitch muscle inhibits sarcoplasmic reticulum Ca^{2+} uptake and impairs contractile function. *J Biol Chem.*; **277**: 44740–6.

Wagenknecht, Grassucci R, Berkowitz J, Wiederrecht GJ, Xin HB, Fleischer S. (1996) Cryoelectron microscopy resolves FK506 binding protein sites in the skeletal muscle ryanodine receptor. *Biophys J.*; **70**: 1709–15.

Wagenknecht T, Hsieh C-E, Rath BK, Fleischer S, Marko M. (2002) Electron tomography of frozen-hydrated triad junctions. *Biophys J.*, **83**: 2491–501.

Wahler GM. (2001) Cardiac action potentials. In *Cell physiology sourcebook. A molecular approach*, 3rd edn. Sperelakis N. ed, pp 887–98. Academic Press, San Diego.

Weber A. (1959) On the role of calcium in the activity of adenosine-5′-triphosphate hydrolysis by actomyosin. *J Biol Chem.*; **234**: 2764–9.

Wu KD, Lee WF, Wey J, Bungard D, Lytton J. (1995) Localization and quantification of endoplasmic reticulum Ca^{2+}-ATPase isoform transcripts. *Am J Physiol.*; **269**: C775–84.

Yamaguchi N, Kasai M. (1998) Identification of 30 kDa calsequestrin-binding protein which regulates calcium release from sarcoplasmic reticulum of rabbit skeletal muscle. *Biochem J.*; **335**: 541–7.

Yu X, Carroll S, Rigaud JL, Inesi G. (1993) H^+ counter transport and electrogenicity of the sarcoplasmic reticulum Ca^{2+} pump in reconstituted proteoliposomes. *Biophys J.*; **64**: 1232–42.

Zhang Y, Fujii J, Phillips MS, Chen HS, Karpati G, Yee WC, Schrank B, Cornblath DR, Boylan KB, MacLennan DH. (1995) Characterization of cDNA and genomic DNA encoding SERCA1, the Ca^{2+}-ATPase of human fast-twitch skeletal muscle sarcoplasmic reticulum, and its elimination as a candidate gene for Brody disease. *Genomics.*; **30**: 415–24.

Zhang L, Franzini-Armstrong C, Ramesh V, Jones LR. (2001) Structural alterations in cardiac calcium release units resulting from overexpression of junctin. *J Mol Cell Cardiol.*; **33**: 233–47.

Zhao XS, Shin DM, Liu LH, Shull GE, Muallem S. (2001) Plasticity and adaptation of Ca^{2+} signaling and Ca^{2+} dependent exocytosis in SERCA2+/– mice. *EMBO J.*; **20**: 2680–9.

Zorzato F, Fujii J, Otsu K, Phillips M, Green NM, Lai FA, Meissner G, MacLennan DH. (1990) Molecular cloning of cDNA encoding human and rabbit forms of the Ca^{2+} release channel (ryanodine receptor) of skeletal muscle sarcoplasmic reticulum. *J Biol Chem.*; **265**: 2244–56.

Zorzato F, Anderson AA, Ohlendieck K, Froemming G, Guerini R, Treves S. (2000) Identification of a novel 45 kDa protein (JP–45) from rabbit sarcoplasmic reticulum junctional–face membrane. *Biochem J.*; **351**: 537–43.