

Regulation of Skeletal Muscle Contraction

The main feature of muscle contraction is the interaction of actin, myosin and ATP. This fundamental process of contraction is regulated by the tropomyosin-troponin- Ca^{2+} system. It is accepted, that in the resting muscle tropomyosin (TM) is positioned in the groove of the actin double helix in a way that it sterically blocks the combination of myosin with actin. This is illustrated in Fig. RE1a, which shows a thin filament composed of actin, tropomyosin, and the components of troponin (TN-C, TN-I, TN-T). In the absence of Ca^{2+} (Relaxed state), TM blocks the crossbridge binding sites on actin. Binding of Ca^{2+} to TN-C (Activated state) initiates the TM movement, through TN-T, from the center of the actin strand to its side, thereby releasing the steric blocking. In addition, the TN-C- Ca^{2+} complex removes TN-I from its inhibitory position on actin; thus the combination of the myosin head with actin can take place. Since in the thin filament there is only one TN and one TM molecule per seven G-actin molecules, one has to assume that cooperative interactions play a major role in the regulation of contraction.

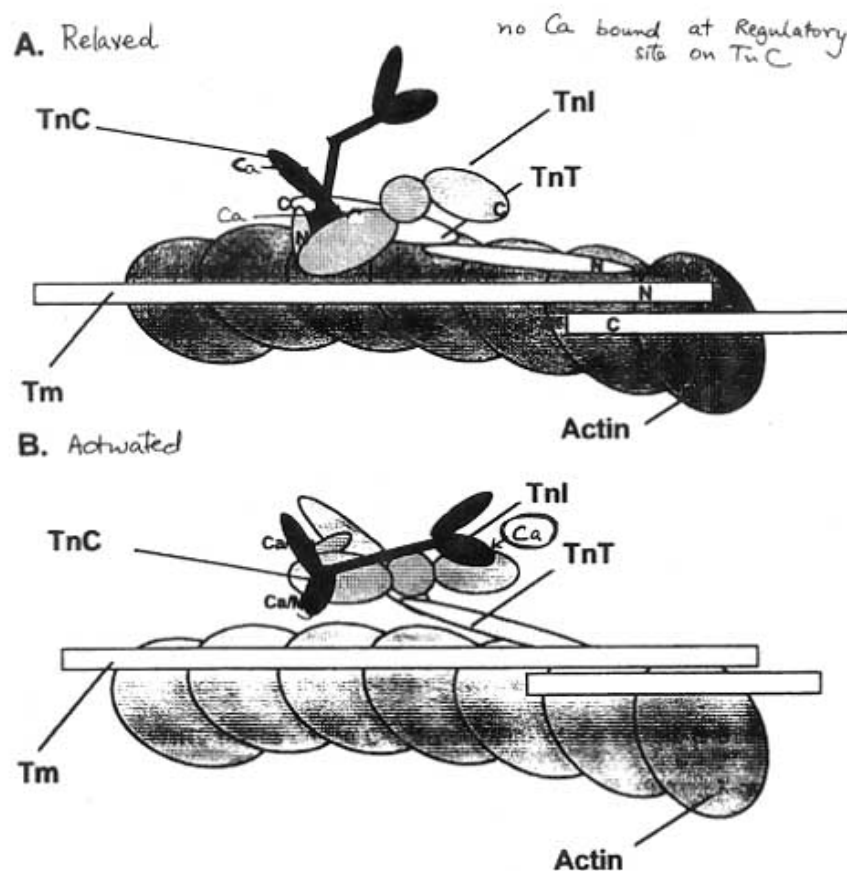


Fig. RE1a. The functional unit of the thin filament in relaxed (A) and Ca^{2+} -activated states (B). (Courtesy of Dr. Helen Rarick).

Lehman and collaborators (1994) were the first to provide for a TM based steric mechanism in *Limulus* thin filaments. Further experiments with frog skeletal muscle confirmed the steric-model for activation of muscle thin filaments (Vibert et al., 1997). Next, by using cryoelectron microscopy and helical image reconstruction the location of tropomyosin in troponin regulated thin filaments has been resolved under both relaxing and activating conditions (Xu et al., 1999).

In essence, the steric-blocking mechanism is based on the interaction between actin and TM, the fine details of which are shown on Figure RE2. In the Ca^{2+} “off” state, TM (red) contacts actin’s outer domain (which contains subdomain 1 and 2). In the presence of Ca^{2+} TM (yellow) moves azimuthally away from most of actin outer domain. TM (green) is positioned over actin’s inner domain (consisting of subdomains 3 and 4). It follows that there are three distinct positions of TM on the actin filament: 1) In the *blocked* position TM is located on the myosin binding site of actin and, thereby blocks the combination of actin with myosin. 2) In the presence of Ca^{2+} TM is in the *closed* position which only partially blocks the myosin binding site of actin. 3) In the *open* position TM has completely moved away from the myosin binding site and, thus, actin and myosin can combine, (Brown and Cohen, 2005; Brown et al., 2005).

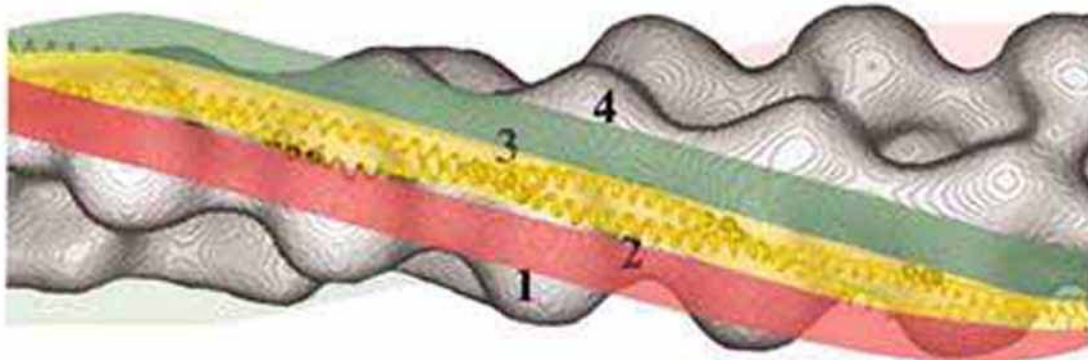


Fig. RE2. Details of the interaction between actin and TM. The azimuthal positions of TM, in the off (red), Ca^{2+} -activated (yellow) and fully activated (green) states, on F-actin (gray) is shown. The four subdomains of actin, 1,2,3, and 4 are indicated. (Reprinted with permission from Brown, J.H., Zhou, Z., Reshetnikova, L., Robinson, H., Yammani, R.D., Tobacman, L.S., and Cohen, C., Part (a) of Figure 4 in the paper entitled Structure of the mid region of tropomyosin: Bending and binding sites for actin. Proc. Natl. Acad. Sci. USA, 102, 18,878 -18,883, 2005. Copyright, 2005, National Academy of Sciences, USA).

The Role of Ca^{2+} in Regulation of Skeletal Muscle Contraction

Historical experiments: In 1883, Ringer observed that contraction of an isolated frog heart ceases when CaCl_2 was omitted from the bathing solution. This was reversible.

Fifty years later, Heilbrunn and Wiercinsky showed that injection of CaCl_2 directly into skeletal muscle fibers causes contraction and no other cation duplicated this effect. They concluded that "Calcium might be an activator of muscle."

A.V. Hill, the founder of muscle physiology, predicted that the activator of muscle must come from an internal source, since membrane depolarization is quickly followed by mechanical response, e.g., a skeletal muscle can be fully activated within a few milliseconds. Diffusion of an activator from the surface to the interior of a fiber would take a much longer time.

The experiments of Huxley and Taylor (1958): These authors raised the question, which part of the sarcomere is involved in activation of contraction? They mounted a single frog fiber under the microscope, immersed in physiological salt solution. Electrical pulse was applied through a very narrow pipette to the fiber, so that the tip of pipette was placed either to the center of the A band or I band. The I band shortened when the pipette was applied to the I band, whereas no response was obtained when the pipette was applied to the A band (Fig. CA1). It was shown later that in frog muscle the junction of transverse tubules (T tubules) and the sarcoplasmic reticulum (SR) are located in the I band, thus these experiments supported the concept that electrical stimulation of the tubules in muscle releases Ca^{2+} from SR.

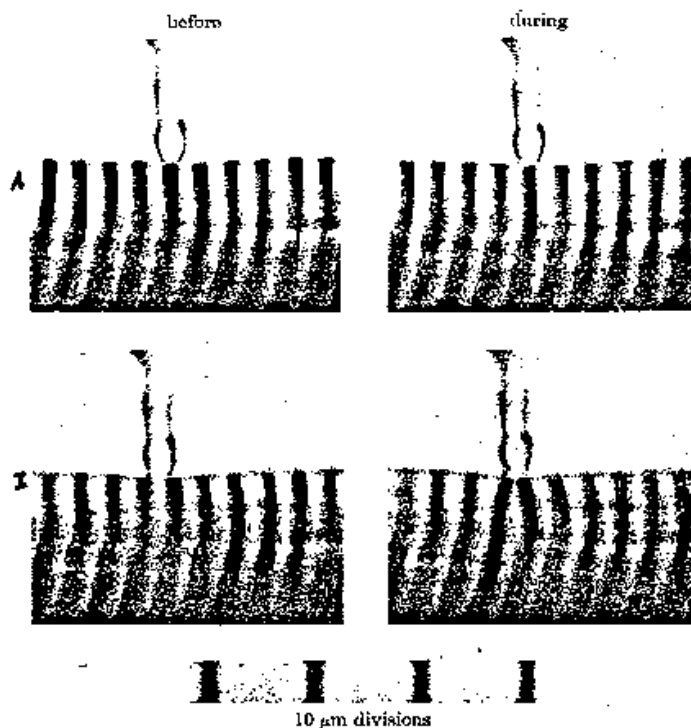


Fig. CA1. Local activation of a twitch fiber from the semitendinosus muscle of *Rana temporaria* (From Huxley and Taylor, 1958). In the upper pictures, the pipette was applied to the A band, in the lower pictures, the pipette was applied to the I band.

Current concepts: Ca^{2+} is a very versatile intracellular signal that can regulate many different intracellular functions. In skeletal and cardiac muscle, where they allow for excitation-contraction (E-C) coupling, these signals reach their largest amplitude and fastest rates (Rios, 2006). In fast twitch muscle the signal aims to saturate TN-C and then

make it Ca^{2+} free, all in milliseconds. This requires up to 1 mM Ca^{2+} to move from the stores to cytosol. Fast change is achieved by juxtaposition of two large fluxes (Ca^{2+} release and removal). Fluxes up to 250 mM s^{-1} for release and 50 mM s^{-1} for removal are made possible by finely interspersed Ca^{2+} sources (release channels, or RyRs) and sinks (pumps and Ca^{2+} binding molecules). Top flux is reached in less than 1 ms and it is turned off entirely within about 4 ms.

A specialized intracellular store, the SR, controls the level of intracellular Ca^{2+} in muscle, in response to depolarization of the plasmalemma. The E-C transduces the depolarization of the sarcolemma to Ca^{2+} release. The two membrane systems, plasmalemma and SR, communicate with each other at specific structure called calcium release units (CRUs) or junctions. The key elements of the structure are: the ryanodine receptors (RyRs), large intracellular channels ($\sim 2260 \text{ kDa}$) that allow Ca^{2+} to exit the SR in response to depolarization of the plasma membrane and the dihydropyridine receptors (DHPRs), L-type voltage gated Ca^{2+} channels that are present in the exterior membranes of the muscle cells and that control the opening of RyRs (reviewed in Protasi et al., (2003).

The process starts from voltage sensors (DHPRs) of the T tubule, and include the interactions of channels with Ca^{2+} (Ca^{2+} -induced Ca^{2+} release and Ca^{2+} -dependent inactivation). In addition there are ancillary proteins (calmodulin, immunophilins triadin, junction, calsequestrin, or sarcalumenin) candidates for modulating further the function of the release channels (Rios, 2006).

Sarcoplasmic Reticulum

In 1902, Emilio Veratti, an Italian pathologist described, by light microscopy, a reticular structure of the sarcoplasm. Mazzarello et al., (2003) reviewed the history of SR and show how Veratti's discovery was lost and rediscovered sixty years later, following the introduction of electron microscopy.

In 1960, it was also shown that SR preparations exhibit Ca^{2+} -ATPase activity. That is, the SR membrane contains a Ca^{2+} pump that transports Ca^{2+} from the sarcoplasm into the SR lumen at the expense of ATP. The crystal structure of the Ca^{2+} -ATPase of skeletal muscle SR has been reported (Toyoshima et al., 2000) with two Ca^{2+} bound in the transmembrane domain which comprises 10 alpha helices. The two Ca^{2+} are located side by side and are surrounded by four transmembrane helices. The crystallographic and biochemical data suggested that large domain movements take place during Ca^{2+} transport by the Ca^{2+} -pump ATPase. Indeed this was found when the Ca^{2+} pump was crystallized with a bound-ATP analogue and one Mg^{2+} and two Ca^{2+} ions (Toyoshima and Mizutani, 2004). In this structure, the ATP analogue reorganizes the three cytoplasmic domains (A, N and P), which are widely separated without nucleotide, by directly bridging the N and P domains. The structure of the P-domain itself is altered by the binding of the ATP analogue and Mg^{2+} . As a result, the A-domain is tilted so that one of the transmembrane helices moves to lock the cytoplasmic gate of the transmembrane Ca^{2+} -binding site. This appears to be the mechanism for occluding the bound- Ca^{2+} , before releasing it into the lumen of the SR. The same conclusion was reached by Sørensen et al (2004).

The SR is the Ca^{2+} -storage compartment of muscle. It forms a network in the sarcoplasm so that each fiber is surrounded by the reticulum and thereby has easy access to Ca^{2+} . The SR widens at its two ends forming terminal sacs, called cisternae. The functional unit is the triad, consisting of two cisternae belonging to two adjacent SR and of one T-tubule in between (Fig. SR1). The distance between the terminal cisternae and the T-tubule is 10-15 nm. This gap is spanned by "foot structures", also called the junctional foot protein. Later it was identified as ryanodine receptor, which is the Ca^{2+} release channel. In frog muscle the T-tubules are located at the level of the Z line, and in mammalian muscles they are located at the junction of A and I bands.



Fig. SR1. Electron micrograph of T-tubule and SR junction (From Chu et al., 1987; with permission from Arch. Biochem. Biophys., 258, 13-23, 1987, by Academic Press). The transverse tubule (TT) is flanked by two terminal cisternae of SR. The arrows point to the foot structures. LT, longitudinal tubule of SR.

Fig. SR2 is a scheme of the SR and T-tubule junction. The T-tubule is shown invaginating from the sarcolemma. The foot structure spans the gap between the terminal cisternae and T-tubule. Within the SR, Ca^{2+} is bound to calsequestrin, a protein that is anchored to the inner membrane of the terminal cisternae. Upon muscle stimulation, Ca^{2+} leaves the terminal cisternae and binds to troponin-C in the sarcoplasm to initiate the contraction process (see also Fig. EC2). Until the muscle is stimulated, Ca^{2+} is continuously released from the terminal cisternae. However, a part of the Ca^{2+} in the sarcoplasm is recaptured by the longitudinal part of the SR; the Ca^{2+} pump is responsible for the Ca^{2+} uptake and ATP supplies the energy. Once Ca^{2+} is inside the longitudinal tubules, it diffuses back to the terminal cisternae, where it is bound to calsequestrin, at the storage site. A 30-kDa calsequestrin-binding protein (Yamaguchi and Kasai, 1998) regulates the binding of Ca^{2+} to calsequestrin.

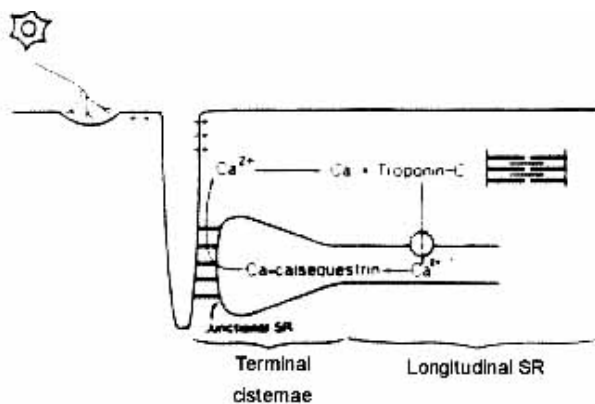


Fig. SR2. Scheme of T-tubule and SR junction (From Paul and Heiny, Copyright 1993, reproduced with permission of Lippincott, Williams & Wilkins).

The terminal cisternae contain the Ca^{2+} release channels of the SR. That channel is a very large protein (MW about 2 million) that spans the entire SR membrane thickness and protrudes up to the T-tubule. The protruding hydrophilic domain of the channel was first visualized in electron micrographs as foot structures (Franzini-Armstrong and Nunzi, 1983), then identified as the ryanodine receptor (RyR). It contains a central axial channel and four radial conduits. Ca^{2+} from the SR enters the sarcoplasm at the junction via the four conduits. As the Ca^{2+} channel opens, Ca^{2+} leaves the SR, calsequestrin releases Ca^{2+} , and more Ca^{2+} moves out into the sarcoplasm.

Signal transduction between T-tubule and SR-junction: The dihydropyridine receptors (DHPRs) of the T-tubule and the Ca^{2+} -release channels of the SR terminal cisternae are participating in signal transduction. The DHPRs act as voltage sensors: as an action potential propagates over the T-tubules (see Fig. EC2) charged regions of the receptor molecule rapidly move and cause a conformational change in the receptor. The DHPRs and the Ca^{2+} -release channels are in direct apposition at the junction. The conformational change in the DHPRs may lead to the opening of the Ca^{2+} -release channels. Several hypotheses have been proposed to connect the measured charge movements in the DHPRs to Ca^{2+} -release from SR.

The structure of DHPR has been studied extensively: Thus, its three dimensional structure, from the skeletal muscle source, has been determined (Wang et al., 2002). Structural interactions between α_{1S} -DHPRs and multiple regions of RyR1 were found in skeletal muscle (Protasi et al., 2002). The β_{1a} subunit was found to be essential for the assembly of DHPR arrays in skeletal muscle (Schredelseker et al., 2005)

Ca^{2+} -sparks: These are brief, localized elevations of myoplasmic $[\text{Ca}^{2+}]$ caused by increased increments of Ca^{2+} via SR Ca^{2+} release channels in muscle. The properties of individual sparks provide information regarding the opening of SR Ca^{2+} channels within functioning cells. Using high speed confocal microscopy Lacampagne et al., (2000) showed that individual Ca^{2+} sparks activated by membrane depolarization in single frog skeletal muscle fibers can be terminated prematurely by repolarization. Thus, either voltage sensor deactivation on repolarization or release channel inactivation during continued depolarization can terminate the Ca^{2+} release channel activity underlying voltage activated Ca^{2+} sparks in skeletal muscle (Figure SR3).

Figure SR3 is the representation of the T-tubule SR junction. Membrane potential-dependent states of the DHPR voltage sensors in T-tubule membrane (left membrane in each panel) and voltage sensor- and ligand-dependent states of the RyR Ca^{2+} release channels in the apposed SR membrane (right membrane in each panel) are shown. Binding sites mediating Ca^{2+} -dependent activation (a) and inactivation (i) are indicated on each RyR (Part A). A 0.3- to 2.0 sec repolarization to -90 mV from a depolarized holding potential of 0 mV was used to reprime a small fraction of the voltage sensor unit (Part A, top DHPR tetrad), so that at most a single voltage sensor unit was likely to be reprimed per junctional region. During a subsequent large depolarization any reprimed voltage sensor units would be activated which, in turn would cause the RyR Ca^{2+} release channel directly coupled to the activated DHPR to open and conduct Ca^{2+} (Part B, top RyR), thereby initiating a Ca^{2+} spark. Neighboring release channels not coupled to

voltage sensors might be activated by Ca^{2+} induced Ca^{2+} release caused by locally elevated $[\text{Ca}^{2+}]$ and mediated by Ca^{2+} binding to the *a* site (Part B, middle RyR). Thus, the ability of repolarization to prematurely turn off Ca^{2+} release during Ca^{2+} sparks, experimented by Lacampagne et al., (2000), provides a unique characteristic of skeletal excitation-contraction coupling that serves as a basis for brief Ca^{2+} release during a skeletal muscle action potential.

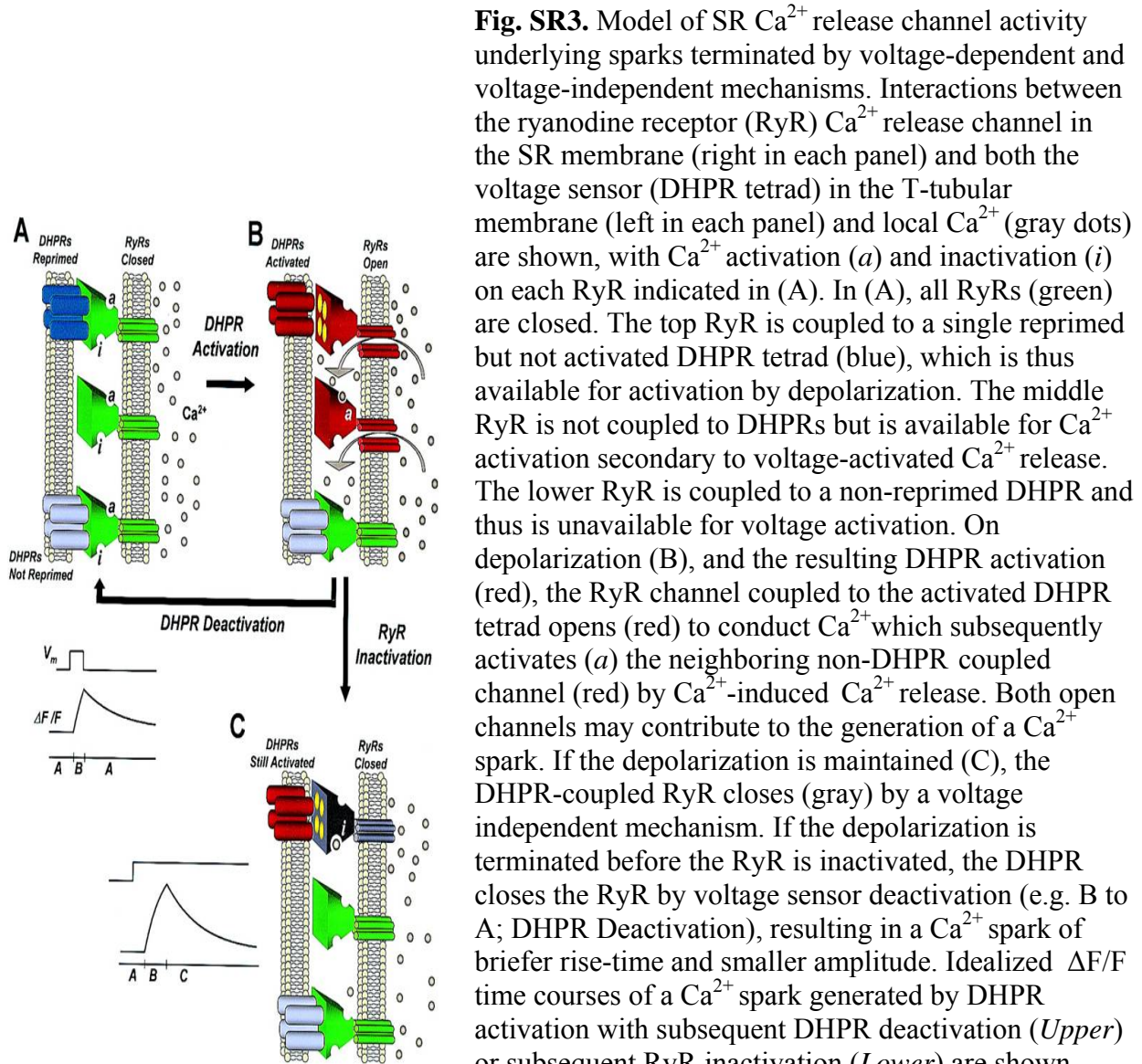


Fig. SR3. Model of SR Ca^{2+} release channel activity underlying sparks terminated by voltage-dependent and voltage-independent mechanisms. Interactions between the ryanodine receptor (RyR) Ca^{2+} release channel in the SR membrane (right in each panel) and both the voltage sensor (DHPR tetrad) in the T-tubular membrane (left in each panel) and local Ca^{2+} (gray dots) are shown, with Ca^{2+} activation (*a*) and inactivation (*i*) on each RyR indicated in (A). In (A), all RyRs (green) are closed. The top RyR is coupled to a single reprimed but not activated DHPR tetrad (blue), which is thus available for activation by depolarization. The middle RyR is not coupled to DHPRs but is available for Ca^{2+} activation secondary to voltage-activated Ca^{2+} release. The lower RyR is coupled to a non-reprimed DHPR and thus is unavailable for voltage activation. On depolarization (B), and the resulting DHPR activation (red), the RyR channel coupled to the activated DHPR tetrad opens (red) to conduct Ca^{2+} which subsequently activates (*a*) the neighboring non-DHPR coupled channel (red) by Ca^{2+} -induced Ca^{2+} release. Both open channels may contribute to the generation of a Ca^{2+} spark. If the depolarization is maintained (C), the DHPR-coupled RyR closes (gray) by a voltage independent mechanism. If the depolarization is terminated before the RyR is inactivated, the DHPR closes the RyR by voltage sensor deactivation (e.g. B to A; DHPR Deactivation), resulting in a Ca^{2+} spark of briefer rise-time and smaller amplitude. Idealized $\Delta F/F$ time courses of a Ca^{2+} spark generated by DHPR activation with subsequent DHPR deactivation (*Upper*) or subsequent RyR inactivation (*Lower*) are shown. (Reprinted with permission from Lacampagne, A., Klein, M.G., Ward, C.W., and Schneider, M.F. Figure 5 in their paper entitled Two mechanisms for termination of individual Ca^{2+} sparks in skeletal muscle. *Proc. Natl. Acad. Sci. USA*, **97**, 7823-7828, 2000. Copyright 2000, National Academy of Sciences, USA).

Excitation - Contraction Coupling

Excitation-contraction coupling (E-C) describes the events that lead from electrical stimulation of muscle to the initiation of muscle contraction. The time course of Ca^{2+} release from the SR during muscle contraction is of great interest. Ashley and Ridgway (1968) were the first to study this relationship. They monitored changes in Ca^{2+} concentration during muscle contraction by injecting aequorin, a Ca^{2+} -binding bioluminescent protein, into muscle fibers. Upon Ca^{2+} -binding aequorin emits light that can be measured; following the emission of light, aequorin is inactivated and the bound Ca^{2+} is released.

An aequorin-injected muscle fiber was electrically stimulated and first the action potential was registered. This was followed by light emission reflecting changes in intracellular Ca^{2+} concentration. When the Ca^{2+} -mediated light output reached its peak, tension developed, by the time maximal tension was produced the Ca^{2+} -mediated light output died away (Fig. EC1).

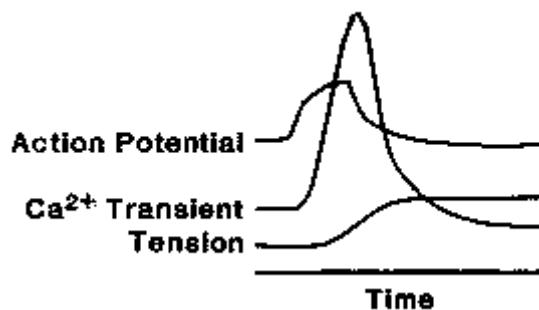


Fig. EC1. Time relationship of action potential, Ca^{2+} transient and tension (From Ashley and Ridgway, 1968).

In the past, fluorescent Ca^{2+} indicators, such as *fura-2* and *quin-2*, were used to measure changes in intracellular Ca^{2+} concentrations in the nanomolar to micromolar range. The Ca^{2+} -indicators are excited at slightly longer wavelengths when they are free of Ca^{2+} than when in their Ca^{2+} -bound form. By measuring the ratio of fluorescent intensity at two excitation wavelengths the free Ca^{2+} concentration can be calculated.

The release of Ca^{2+} from SR when studied in frog muscle by confocal microscopic imaging of the fluorescent indicator, *fluo-3*, revealed that the release occurs largely in the form of discrete events, termed Ca^{2+} sparks. It is not clear whether the sparks are the results of opening of individual channels or of a concerted opening of channel clusters (Rios et al., 1999). In the recent work from the same laboratory *mag-indo-1* and *rhod-2* indicators were used for imaging Ca^{2+} in the SR and in the cytosol, respectively (Launikonis et al., 2006). An example is shown in Figure EC1a.

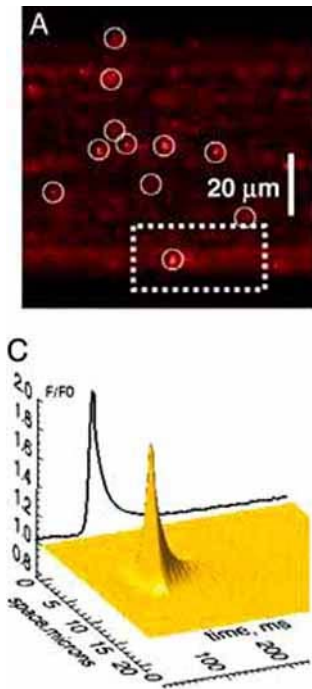


Fig. EC1a. Imaging of cytosolic $[Ca^{2+}]$ in frog skeletal muscle fibers. (A) Line scan of the fluorescence of *rhod-2* in cytosol. Circles mark Ca^{2+} -sparks. (C) Average of subarrays (dashed rectangle in A) of the fluorescence around sparks, space (microns) and time (ms) resolution. (Reprinted with permission from Launikonis, B.S., Zhou, J., Royer, L, Shannon, T. R., Brum, G., and Rios, E. Part A and C of Figure 1 in their paper entitled Depletion “skraps” and dynamic buffering inside the cellular calcium store. *Proc. Natl. Acad. Sci. USA*, **103**, 2982-2987, 2006. Copyright 2006, National Academy of Sciences, USA).

Sequence of events: Upon stimulation of the muscle, an action potential propagates over the sarcolemma, travels through the T-tubules and elicits Ca^{2+} release from the SR into the sarcoplasm. Ca^{2+} binds to TN and the inhibition of actin-myosin combination that prevails at rest is lifted, and contraction ensues. Ca^{2+} is the link between excitation and contraction. Fig. EC2 shows that in the resting muscle (A) the membrane is negative on the inside and positive on the outside (*left part of the Figure*). In the shortened muscle (B), one notes the reversal of polarity upon stimulation and Ca^{2+} release from the terminal cisternae of SR toward the filaments (*right part of the Figure*).

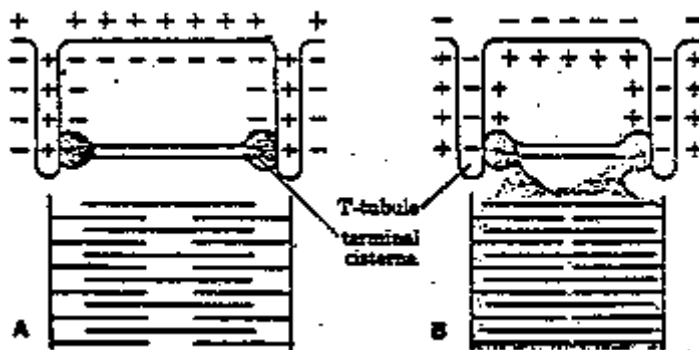


Fig. EC2. Sequence of events during excitation-contraction coupling (From Keeton, 1972).

Suggested readings: Rios (2006); Berridge et al., (2003); Lacampagne et al., (2000); Brown et al., (2005).

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