

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# What Does Maurocalcine Tell Us About the Process of Excitation-Contraction Coupling?

Michel Ronjat and Michel De Waard

*Joseph Fourier University & INSERM U836, Grenoble Institute of Neuroscience  
France*

## 1. Introduction

Cardiac and skeletal muscle contraction is triggered by the arrival of an action potential that locally and transiently depolarizes the plasma membrane. The entire chain of molecular events that link the arrival of the action potential and muscle contraction is called excitation-contraction coupling. This chapter will focus on the way membrane depolarization is sensed and how it triggers a massive increase in the cytosolic  $\text{Ca}^{2+}$  concentration. Plasma membrane depolarization is detected by L-type voltage-gated calcium channels whose activation allows  $\text{Ca}^{2+}$  entry into muscle cells. Cardiac and skeletal muscle voltage-gated calcium channels differ by their  $\text{Ca}^{2+}$  permeability. While cardiac channels lead to an important and rapid  $\text{Ca}^{2+}$  influx, skeletal muscle channel activation allows a moderate and rather slow  $\text{Ca}^{2+}$  entry (Bean, 1989). In fact, the molecular identity of the pore-forming channel subunit differs in both tissues, cardiac fibers expressing the  $\text{Ca}_v1.2$  isoform while skeletal muscles express the  $\text{Ca}_v1.1$  isoform. Nevertheless, the overall subunit composition of both channels, illustrated in Figure 1, bears interesting similarities.

The opening of these ion channels in response to plasma membrane depolarization results from a complex series of conformational changes occurring mainly in the pore-forming subunits. In these subunits, positively charged S4 segments act as voltage-sensing mechanical transducers by altering their position within the plasma membrane as a function of voltage value (Nakai, Adams, Imoto, & Beam, 1994). How movements of other structural elements are related to these S4 repositioning is still an open but particularly important question with regard to the excitation-contraction coupling process as we will see later. In both tissues, the activation of L-type voltage-gated calcium channels produces the opening of another calcium channel located in the membrane of the sarcoplasmic reticulum, the main intracellular  $\text{Ca}^{2+}$  store in muscles. This process leads to a transient and important increase in cytoplasmic  $\text{Ca}^{2+}$  concentration that is at the basis of muscle contraction. This particular ion channel presents several interesting features: i) it is sensitive to the plant alkaloid ryanodine (and is therefore named “ryanodine receptor”), ii) it is positioned in such a way that it faces the L-type channel in order to maximize activation efficiency, and iii) in both tissues, its activation and inactivation are controlled by cytoplasmic  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations (Figure 2).

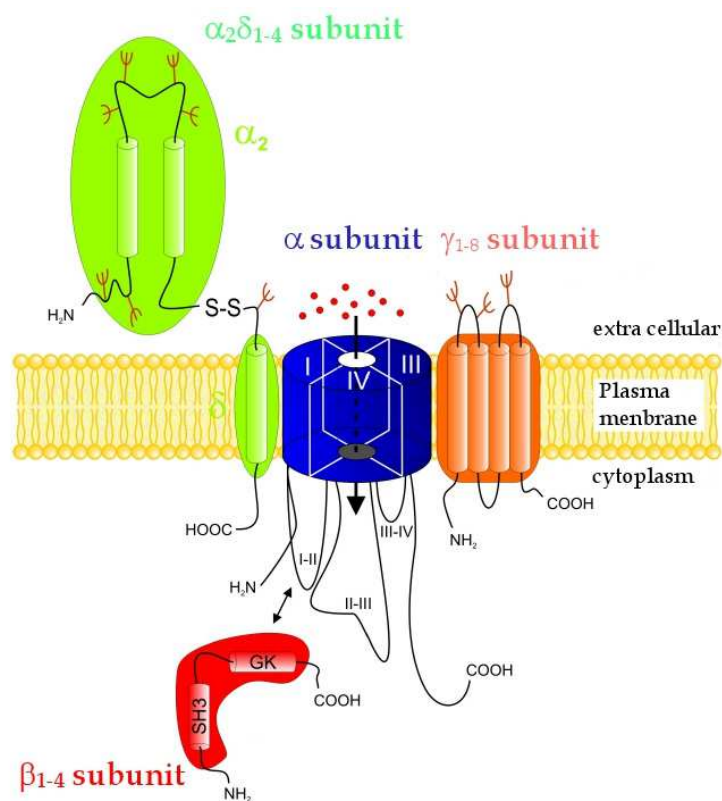


Fig. 1. Schematic representation of an L-type voltage-dependent calcium channel.  $Ca_v\alpha$  is the voltage-sensing and pore-forming subunit, whereas  $\alpha_2\delta$ ,  $\beta$  and  $\gamma$  are auxiliary subunits.  $Ca_v\alpha$  is constituted of four homologous hydrophobic domains linked by cytoplasmic loops. The II-III loop, that links domains II and III, is the focus of this review. The  $\beta$  subunits is the only protein that is fully cytoplasmic.

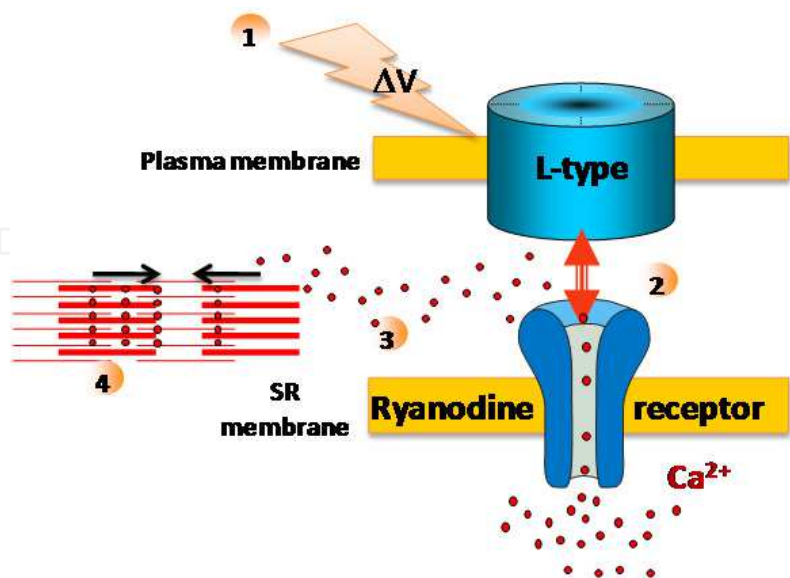


Fig. 2. Cartoon describing the general process of excitation-contraction coupling, starting by membrane depolarization (1), L-type channel activation and transfer of conformational change to the ryanodine receptor (2), ryanodine receptor opening and sarcoplasmic reticulum  $Ca^{2+}$  release (3), and muscle contraction (4).

As for the L-type calcium channels, two different types of ryanodine receptors are respectively expressed in skeletal (type 1 ryanodine receptor) and cardiac (type 2 ryanodine receptors) muscles. These two types of ryanodine receptors differ in their sensitivity to cytoplasmic  $\text{Ca}^{2+}$  concentration. Type 1 ryanodine receptor appears to be more sensitive to inhibition by high cytosolic  $\text{Ca}^{2+}$  concentration than type 2 ryanodine receptors. In contrast, there does not seem to be major differences with regard to activation by cytosolic  $\text{Ca}^{2+}$  (Chu, Fill, Stefani, & Entman, 1993; Fruen, Bardy, Byrem, Strasburg, & Louis, 2000; Michalak, Dupraz, & Shoshan-Barmatz, 1988). *In vivo*, cardiac type 2 ryanodine receptor appears to be less repressed by L-type calcium channel than type 1 ryanodine receptors as witnessed by the far greater likelihood to observe spontaneous channel openings (spark events) (H. Cheng, Lederer, & Cannell, 1993; Wier & Balke, 1999). The differences in biophysical properties between skeletal and cardiac L-type calcium channels on one hand, and skeletal and cardiac ryanodine receptors on the other hand, led to differences in excitation-contraction coupling mechanisms between these two tissue. In cardiac tissues,  $\text{Ca}^{2+}$  entering through activated L-type channels is sufficient to directly activate type 2 ryanodine receptors. This chain of events is called  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release. In this case, opening of the type 2 ryanodine receptor strictly depends on extracellular  $\text{Ca}^{2+}$  (Nabauer, Callewaert, Cleemann, & Morad, 1989). Indeed, the  $\text{Ca}^{2+}$  release process through type 2 ryanodine receptors is a U-shape function of voltage and follows the U-shape voltage-dependence of external  $\text{Ca}^{2+}$  entry. Moreover, blocking external  $\text{Ca}^{2+}$  entry precludes voltage-activated  $\text{Ca}^{2+}$  release through type 2 receptors. Type 1 ryanodine receptors in skeletal muscles differ from type 2 ryanodine receptors in their mode of activation. In spite of the capability of type 1 ryanodine receptors to perform  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release, the amplitude of  $\text{Ca}^{2+}$  entry through activated skeletal L-type channels is too low and its kinetic too slow to trigger a cardiac type  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release mechanism. Indeed, in these cells, the  $\text{Ca}^{2+}$  release process is no longer U-shaped in response to voltage increase and strictly follows the sigmoidal voltage-dependence of activation of L-type channels.  $\text{Ca}^{2+}$  permeability through skeletal L-type channels plays therefore a negligible role in the skeletal type excitation-contraction coupling. In skeletal muscles, the voltage-dependent trigger of type 1 ryanodine receptor activation is the changes in L-type channel conformation. This process has been termed voltage-induced  $\text{Ca}^{2+}$  release (Schneider & Chandler, 1973; Tanabe, Beam, Powell, & Numa, 1988). While it may seem comfortable for intellectual construction to oppose  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release to voltage-induced  $\text{Ca}^{2+}$  release, one should keep in mind that such a drastic distinction between these two mechanisms also presents intrinsic restrictions. In skeletal muscles, the conformation transmission between L-type channel and type 1 ryanodine receptor requires a physical interaction between these channel types. While conceptually, the process of  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release in cardiac tissues does not require a physical interaction between the two channels, it would be a mistake to eliminate the hypothesis that conformational changes in cardiac L-type channels can also be transmitted directly to type 2 ryanodine receptor. Conversely, while skeletal L-type conformational changes are essential to trigger  $\text{Ca}^{2+}$  release through type 1 ryanodine receptors in skeletal muscles, ruling out a contribution of  $\text{Ca}^{2+}$  (originating from internal sources) in the release of  $\text{Ca}^{2+}$  itself may also represent a shortcut attitude. In this review we will therefore not oppose the two modes of  $\text{Ca}^{2+}$ -release but simply take into account that in cardiac tissues external  $\text{Ca}^{2+}$  is an important factor, while in skeletal muscle conformational changes in L-type channels is a more important factor. We will assume that cardiac L-type channels might also be in interaction with the ryanodine type 2 receptor. The next paragraph will

describe what is known in terms of molecular interactions between L-type channels and ryanodine receptors, focusing mainly on the most important determinants for excitation-contraction coupling.

2. Molecular determinants of the interaction between L-type channels and ryanodine receptors

2.1 Contribution of two separate domains of the II-III loop of Ca<sub>v</sub>1.1 in voltage-induced Ca<sup>2+</sup> release

Because voltage-induced Ca<sup>2+</sup> release clearly relies on a direct interaction between skeletal muscle L-type and type 1 ryanodine receptor, most of the researches have focused on the interaction between these two channels. In this context, the use of animal models has been decisive in identifying molecular determinants critical for the excitation-contraction coupling process. Three mice models have been widely used: i) the dysgenic mice *mdg* corresponding to a natural knockout of the pore-forming subunit of skeletal L-type channels (Tanabe et al., 1988), ii) the dyspedic mice, a knock-out of type 1 ryanodine receptor (Takeshima et al., 1995), and iii) the β-null mice, a knock-out of the β auxiliary subunit of the skeletal L-type channel. All these gene knock-outs produce lethality at birth by defective respiratory muscle function implying that studies are done with primary myotubes isolated from late embryos.

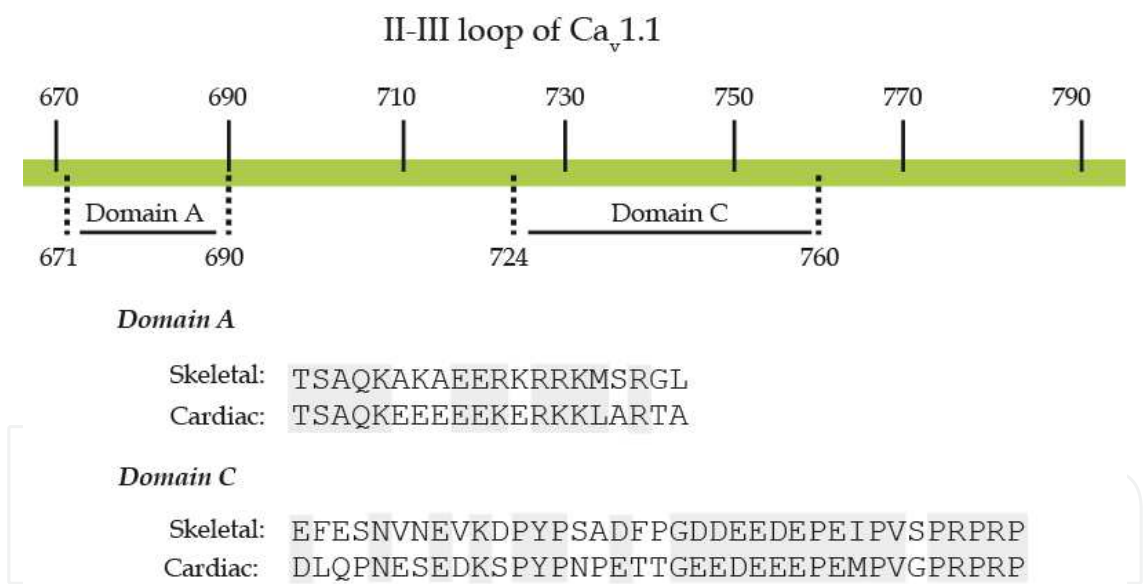


Fig. 3. Amino acid positions of skeletal muscle Ca<sub>v</sub>1.1 Domains A and C. Amino acid sequences of these domains are compared to those of equivalent domains of the cardiac L-type Ca<sub>v</sub>1.2 channel.

In a first series of precept experiments, *mdg* myotubes were used to express chimeras between the pore-forming Ca<sub>v</sub>α subunits of skeletal and cardiac L-type channels in order to identify a skeletal muscle-specific L-type determinant responsible for voltage-induced Ca<sup>2+</sup> release. This approach led to the identification of one particularly important determinant, the large cytoplasmic loop between the second and third hydrophobic domains (called II-III loop) of Ca<sub>v</sub>1.1 (Nakai, Tanabe, Konno, Adams, & Beam, 1998) (see



Figure 3). According to structural predictions, this loop would be of 137 (Ca<sub>v</sub>1.1) to 146 (Ca<sub>v</sub>1.2) amino acids long. Further, restriction of the skeletal L-type domain responsible for voltage-induced Ca<sup>2+</sup> release through type 1 ryanodine receptors locates a sequence of 36 amino acids, 62 amino acids downstream of the start of I-II loop sequence. This domain is called *Domain C*. While this region is undoubtedly important in Ca<sub>v</sub>1.1, the sequence homology with the equivalent *Domain C* of Ca<sub>v</sub>1.2 remains nevertheless quite high. The replacement of Ala<sup>739</sup>, Phe<sup>741</sup>, Pro<sup>742</sup> and Asp<sup>744</sup> of Ca<sub>v</sub>1.1 by, respectively, Pro, Thr, Thr and Glu of Ca<sub>v</sub>1.2 reduces voltage-induced Ca<sup>2+</sup> release (Kugler, Grabner, Platzer, Striessnig, & Flucher, 2004; Kugler, Weiss, Flucher, & Grabner, 2004).

While these studies clearly demonstrated that *Domain C* is critical for allowing voltage-induced Ca<sup>2+</sup>-release by L-type channels, the exact mechanism whereby this domain contributes to the process has still not been resolved. There is no solid evidence for a direct interaction between *Domain C* and the ryanodine receptor, suggesting that its mode of action is indirect or that this interaction relies on a transient conformation of the Ca<sub>v</sub>α subunit controlled by voltage. Following the identification of the II-III loop as an important determinant in voltage-induced Ca<sup>2+</sup> release, synthetic peptides corresponding to fragments of this loop were screened for their ability to interact with purified ryanodine receptors and modify their gating activity. Curiously, this approach led to the identification of a ryanodine receptor-interacting domain of the II-III loop that is different of *Domain C*. This sequence has been termed *Domain A* and corresponds to the amino acid region Thr<sup>671</sup> to Leu<sup>690</sup> of Ca<sub>v</sub>1.1 (Figure 2). One particular feature of this domain is the presence of a stretch of basic residues (8 amino acids out of 20 are either Arg or Lys residues). *Domain A* of Ca<sub>v</sub>1.2 bears sequence homology (10 residues are identical out of 20 and 3 have homology). In spite of this high sequence homology, cardiac *domain A* is considerably enriched in acidic residues suggesting that its function may have evolved differently than its skeletal muscle counterpart. Proof of functional differences between cardiac and skeletal *domain A* comes from the fact that skeletal *domain A* interacts with type 1 ryanodine receptor but not type 2 (O'Reilly et al., 2002). Conversely, cardiac *domain A* does not interact with type 1 ryanodine receptor (el-Hayek, Antoniu, Wang, Hamilton, & Ikemoto, 1995; O'Reilly et al., 2002). Curiously, there are no reports on the potential interaction between cardiac *domain A* and type 2 ryanodine receptor thereby illustrating the little credit the scientific community is giving to the possibility that cardiac L-type and type 2 ryanodine receptors physically interact. While direct interaction of skeletal *domain A* with type 1 ryanodine receptor, and evidence for functional effects (reviewed in paragraph 3), would ensure that this domain plays a critical role in excitation-contraction coupling, this possibility is hotly debated. In 2000, a study reported that the partial scrambling of skeletal *domain A* had little impact on excitation-contraction coupling (Proenza, Wilkens, & Beam, 2000). Careful examination of the scrambled sequence reveals that scrambling occurs on the ten last residues of *domain A* and that many of the residues remain in fact at the same position. Moreover a characteristic feature of *domain A*, a rich content in basic amino acid, is conserved in the scrambled sequence. At the functional level, while excitation-contraction coupling is preserved in principle, no quantification of the extent of this conservation is performed. While these data are interesting, it would have been preferable to perform a more extensive scrambling of the sequence (using the entire domain and avoiding conservation of position for some residues). Other evidences question the role of *domain A* in excitation-contraction coupling. Replacement of the skeletal Ca<sub>v</sub>1.1 II-III loop by the II-III loop of *Musca domestica* Ca<sub>v</sub>α

channel, which has a poor homology with  $\text{Ca}_v1.1$ , largely prevents excitation-contraction coupling, but not completely, while reintegration of skeletal type *domain C* in this construct is enough to restore voltage-induced  $\text{Ca}^{2+}$ -release (Wilkens, Kasielke, Flucher, Beam, & Grabner, 2001). It should be mentioned that the chimera construct in which the entire II-III loop is replaced displays very little  $\text{Ca}^{2+}$  permeability, possibly questioning the correct functioning of the chimera channel. The study lacks also a chimera construct with the integration of skeletal muscle *domain A* rather than *domain C*. Also, expression of two skeletal  $\text{Ca}_v1.1$  hemi-channels, one lacking *domain A*, reconstitutes voltage-induced  $\text{Ca}^{2+}$  release in *mdg* myotubes (Ahern, Arikath et al., 2001). Similarly, complete deletion of *domain A* in  $\text{Ca}_v1.1$  does not affect the amplitude of voltage-induced  $\text{Ca}^{2+}$  release, although it seems to speed the process (Ahern, Bhattacharya, Mortenson, & Coronado, 2001). Very curiously, in the later study, the same authors demonstrate that deletion of both *domains A* and *C* results in the reappearance of some voltage-induced  $\text{Ca}^{2+}$  release. While these studies indicate that the presence of *domain A* is not strictly necessary to observe voltage-induced  $\text{Ca}^{2+}$  release in skeletal muscles, it is hard to conclude that it has no function at all. Also, while *domain C* appears to be important to observe the excitation-contraction coupling process, the data tend to indicate that there might be some interdependence between II-III loop domains to exert their function. Indeed, it was observed *in vitro* that *domain C* exerts an inhibitory effect on the activating function of *domain A* on  $\text{Ca}^{2+}$  release from type 1 ryanodine receptor or on  $[^3\text{H}]$ -ryanodine binding (el-Hayek, Antoniu et al., 1995; Ikemoto & el-Hayek, 1998).

## 2.2 The skeletal muscle L-type channel contains other important determinants for excitation-contraction coupling

While the II-III loop of  $\text{Ca}_v1.1$  has focused much of the attention in the comprehension of the excitation-contraction coupling process, it is important to mention that other relevant L-type channel determinants have been proposed to play a role in the process. Two of these determinants are present on the  $\text{Ca}_v1.1$  pore-forming subunit (the III-IV loop and the C-terminal domain), and the second is constituted by the auxiliary  $\beta_{1a}$  subunit. Their implication is summarized hereunder.

Suspicion of the involvement of the III-IV loop of  $\text{Ca}_v1.1$  arose as a consequence of the identification of a point mutation ( $\text{Arg}^{1086}\text{His}$ ) associated to malignant hyperthermia, a skeletal muscle pathology in which inhalation of volatile anesthetics provokes enhanced  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum, excessive ATP hydrolysis, heat production and therefore muscle damage (Monnier, Procaccio, Stieglitz, & Lunardi, 1997). A functional analysis of this mutation highlights a putative role of this loop in controlling the voltage-dependence of the  $\text{Ca}^{2+}$  release thru type 1 ryanodine receptor and its caffeine sensitivity (Weiss et al., 2004). Caffeine is a well characterized exogenous activator of all types of ryanodine receptors favors  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release by sensitizing the ryanodine receptors to activating cytosolic  $\text{Ca}^{2+}$ . A potential interaction site of this loop on type 1 ryanodine receptor has been identified that encompasses amino acids 922 to 1112 (Figure 4) (Leong & MacLennan, 1998b).

It is noteworthy that this III-IV loop is extremely conserved among  $\text{Ca}_v\alpha$  subunits (with 46 amino acids identical on 53 between  $\text{Ca}_v1.1$  and  $\text{Ca}_v1.2$ ). This suggests that the cardiac III-IV loop may also interact with type 2 ryanodine receptor.

The C-terminus of  $\text{Ca}_v1.1$  has also been shown to be an interesting determinant. The amino acid region 1543 to 1647 of  $\text{Ca}_v1.1$  plays a role in the transport and targeting of  $\text{Ca}_v1.1$  to the triad (Flucher, Kasielke, & Grabner, 2000; Proenza, Wilkens, Lorenzon, & Beam, 2000). The 1393 to 1527 amino acid region of  $\text{Ca}_v1.1$  binds  $\text{Ca}^{2+}$  and calmodulin. In the absence of calmodulin, this domain has been shown to bind type 1 ryanodine receptor at amino acid position 3609 to 3643. A synthetic peptide corresponding to the 1487 to 1506 region of  $\text{Ca}_v1.1$  inhibits both ryanodine binding and ryanodine receptor channel gating in bilayers (Sencer et al., 2001; Slavik et al., 1997). The authors of these findings suggest that these domains could participate to  $\text{Ca}^{2+}$  and/or calmodulin regulation of voltage-induced  $\text{Ca}^{2+}$  release, but more studies are warranted to confirm their importance.

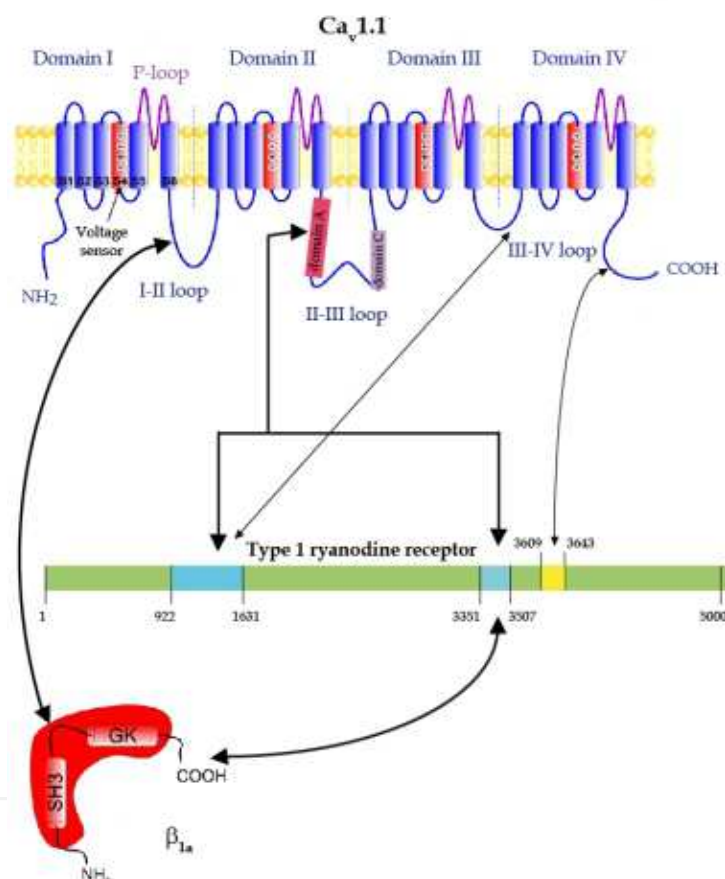


Fig. 4. Summary of known protein interactions between skeletal muscle L-type  $\text{Ca}_v1.1$  and  $\beta$  subunits with type 1 ryanodine receptor. The amino acid positions of Domains A and C are defined in the text.

As mentioned above, absence of the  $\beta_{1a}$  subunit of the L-type calcium channel led to a complete loss of excitation-contraction coupling in skeletal muscle. Recently, this subunit has been shown to directly interact with the type 1 ryanodine receptor (W. Cheng, Altafaj, Ronjat, & Coronado, 2005). Binding experiments identified a cluster of positively charged residues of type 1 ryanodine receptor that control this interaction. Neutralization or deletion of these amino acids severely depress the amplitude of the depolarization induced  $\text{Ca}^{2+}$  release through type 1 ryanodine receptor, indicating that this interaction is important in the regulation of the excitation-contraction coupling in skeletal muscles.



### 3. Is *domain A* of relevance to excitation-contraction coupling?

Although the functional relevance of *domain A* is questioned by studies that have used chimera  $\text{Ca}_v\alpha$  channels, we still believe that it is of interest to investigate the precise role of *domain A* in excitation-contraction coupling. Indeed, it is important to note the intrinsic limitations of several reports that rely on the use of chimera channels and *mdg* myotubes. For a chimera channel to integrate into the triad (the plasma membrane loci where skeletal muscle excitation-contraction takes place), it needs to preserve sufficient molecular determinants that guarantee normal cell trafficking and correct localization with regard to type 1 ryanodine receptor. The role of these determinants on excitation-contraction coupling, and specifically on voltage-induced  $\text{Ca}^{2+}$ -release, cannot be investigated. Also, these studies and conclusions are all based on the assumption (that is not yet proven) that  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release by cardiac L-type channels and type 2 ryanodine receptors can occur in the absence of conformational changes in  $\text{Ca}_v1.2$  (invariably triggered by voltage changes). Testing this hypothesis would require to block voltage-dependent  $\text{Ca}_v1.2$  charge movements without blocking  $\text{Ca}^{2+}$  permeability. While this is a provocative opinion, one could conclude that the chimera studies have demonstrated that skeletal muscle *domain C* governs the requirement on external  $\text{Ca}^{2+}$  to produce  $\text{Ca}^{2+}$  release: when skeletal *domain C* is present, external  $\text{Ca}^{2+}$  would not be required, while when absent, external  $\text{Ca}^{2+}$  is mandatory. *Domain C* is undoubtedly important in participating to voltage-induced  $\text{Ca}^{2+}$  release. However, how is *domain C* voltage-dependent and how does it transmit voltage-dependent conformational changes to type 1 ryanodine receptor without directly interacting with this channel?

In voltage-induced  $\text{Ca}^{2+}$ -release, which occurs in skeletal muscles, the initial trigger for  $\text{Ca}^{2+}$  release through type 1 ryanodine receptor is brought by the voltage change. However, voltage might be considered as the initial trigger for initiating the  $\text{Ca}^{2+}$  release process, and the released  $\text{Ca}^{2+}$ , once it has reached a critical concentration, may contribute to the process by promoting  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release. This can be expected to occur as type 1 ryanodine receptor is perfectly capable to support  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release instead of voltage-induced  $\text{Ca}^{2+}$  release such as when  $\text{Ca}_v1.2$  replaces  $\text{Ca}_v1.1$ . While there is no doubt that type 1 ryanodine receptor can support both voltage- and  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release, very little is known on the contribution of the different  $\text{Ca}_v1.1$  domains (in particular *A* and *C*) to the balance between these two triggering processes. Among other arguments in favor of a contribution of *domain A* to excitation-contraction coupling is the fact that *domain A* is heavily charged. Obviously, voltage-induced  $\text{Ca}^{2+}$  release requires both the presence of  $\text{Ca}_v1.1$  *domain C* in the context of an operational channel and a change in voltage, but it is expected that one type 1 ryanodine receptor-interacting  $\text{Ca}_v1.1$  determinant is altering its conformation upon depolarization to influence excitation-contraction coupling. The basic nature of *domain A* is of interest in that respect since (i) it binds type 1 ryanodine receptor, (ii) influences the  $\text{Ca}^{2+}$  sensitivity of type 1 ryanodine receptor, and (iii) is susceptible to sense voltage changes owing to its close proximity with the plasma membrane. This latter point has never been investigated of course, but needless to say, *domain C* is not the most convincing candidate as voltage-sensor owing to its location within the channel and the lower net charge of the sequence. Earlier evidences that domains *A* and *C* may interact are also interesting since it could be envisioned that voltage-changes alter the interaction between these two domains thereby dynamically regulating excitation-contraction coupling. Finally, most of the studies on excitation-contraction coupling and on voltage-induced  $\text{Ca}^{2+}$  release in particular have focused their attention on the release process itself but not on the termination of the signal upon membrane

repolarisation. In that respect, it would be interesting to have a careful examination of the role of *domain A* on the ending of voltage-induced Ca<sup>2+</sup> release.

4. Animal toxins presenting an intriguing sequence homology with *domain A*

In the course of ongoing studies on ryanodine receptor channel pharmacology, the group of Coronado identified imperatoxin A as an effector of type 1 ryanodine receptor (Valdivia, Kirby, Lederer, & Coronado, 1992). The toxin has high affinity for the channel (close to 10 nM), produces an increase in channel opening probability by reducing the closure times, induces Ca<sup>2+</sup> release from purified sarcoplasmic reticulum and increases [<sup>3</sup>H]-ryanodine binding by conversion of the ryanodine binding site from a low to a high affinity state (el-Hayek, Lokuta, Arevalo, & Valdivia, 1995). Imperatoxin A was isolated from the venom of the African scorpion *Pandinus imperator*. It is a 33 amino acid peptide containing six cysteine residues and therefore three internal disulfide bridges. The pattern of connectivity results in a fold of the “inhibitor cysteine knot” type with three β-strands (Green et al., 2003). The peptide was first synthesized in 2007 (Zamudio et al., 1997). Since this initial discovery, other toxins have been identified, coming from the venom of other scorpions, that all share high sequence homology with imperatoxin A (Figure 5). These include maurocalcine in 2000 from *Scorpio maurus palmatus* (Mosbah et al., 2000), immediately synthesized in 2000 (Fajloun et al., 2000), two opicalcine variants in 2003 (S. Zhu, Darbon, Dyason, Verdonck, & Tytgat, 2003), hemicalcin in 2007 (Shahbazzadeh et al., 2007), and hadrucalcin in 2009 (Schwartz et al., 2009). All of these peptides have been tested on type 1 ryanodine receptor and shown to possess similar pharmacological properties as imperatoxin A. These toxins therefore define a family of functional homologous peptides.

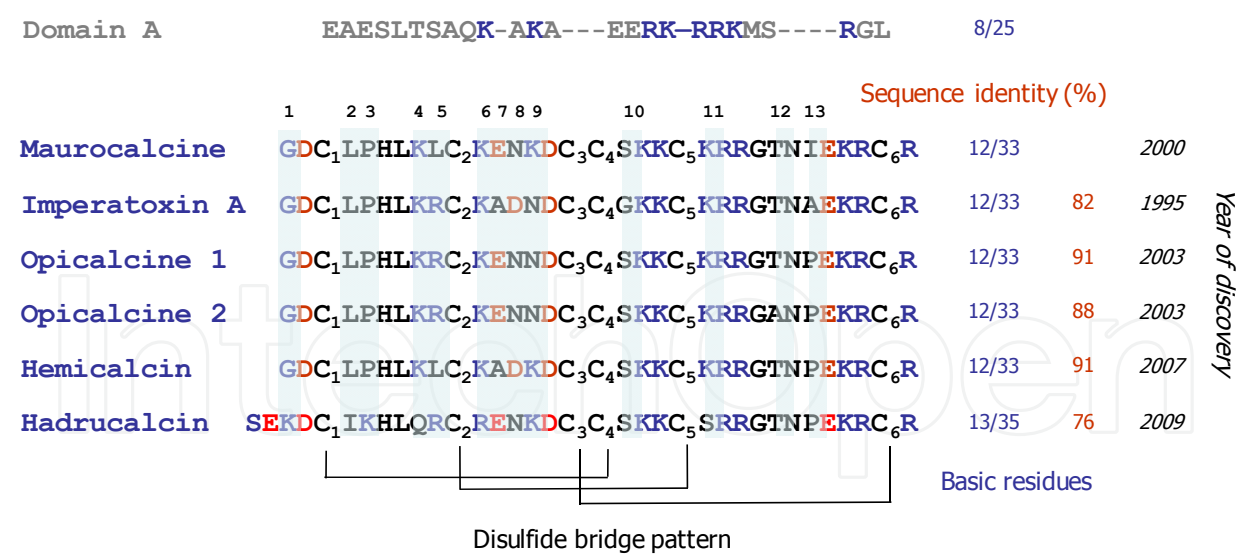


Fig. 5. Amino acid sequences and alignment of maurocalcine-like toxins acting on ryanodine receptors. Sequence homology with skeletal muscle *domain A* is also shown. Positively charged residues are shown in blue, sequence identities are given in red and the year of discovery of each toxin is also provided.

A close comparison of the peptide sequences reveals an important conservation. All peptides are heavily charged, containing mostly Lys residues and some Arg residues, and

the net charge of these peptides is high (+7 if one excludes the potential basic charge of the His residue that is present in all toxins). Most of these basic residues are located on one face of the molecule, creating an important dipole moment in these toxins (Boisseau et al., 2006). A closer examination of these sequences also reveals that a limited stretch of the primary structure of the toxins is highly homologous to the primary structure of skeletal muscle *domain A* (Figure 4). Moreover, modification of *domain A*, meant to further improve the structural similarity between *domain A* and imperatoxin A and maurocalcine, led to a mutant *domain A* with increased functional effects on type 1 ryanodine receptors (Green et al., 2003). These effects include stimulation of  $\text{Ca}^{2+}$  release from sarcoplasmic reticulum vesicles and increased channel opening probability of type 1 ryanodine receptor incorporated in lipid bilayers. Of note, following the action of this optimized *domain A* peptide, these toxins have no cumulative effect on channel activation, strongly suggesting that *domain A* and the toxins share the same binding site on the ryanodine receptors. These experiments highlight in any case the functional relevance of this limited structural homology between *domain A* and the toxins. Further evidence for the importance of this cluster of residues comes from an elegant study of the group of Valdivia. The cardiac *domain A*, which is poorly activator of type 1 ryanodine receptor, can be modified to become a strong type 1 ryanodine receptor activator by selected mutations within this cluster of residues (X. Zhu, Gurrola, Jiang, Walker, & Valdivia, 1999). The mutations were chosen in such a way that cardiac *domain A* integrates the cluster of basic residues of *domain A* that is homologous to the toxin sequences.

### 5. *In vitro* functional similarities between maurocalcine and *domain A*

Among the various toxin activators of ryanodine receptors, maurocalcine is one of the best characterized. Besides sequence similarities with *domain A*, and the emerging evidence that both skeletal muscle *domain A* and maurocalcine share the binding sites on type 1 ryanodine receptor, there is also cumulative indication for functional similarities. We rapidly summarize these similarities hereunder. First, both *domain A* and maurocalcine enhance channel activity by promoting an increase in channel opening probability and the appearance of a subconductance state (Chen et al., 2003; Fajloun et al., 2000; Lukacs et al., 2008; O'Reilly et al., 2002). Both peptides differ however with regard to the level of subconductance state and the duration of channel opening in this subconductance state. Toxins promote long-lasting openings in the subconductance state that are barely observed with *domain A*. Interestingly, the various toxins also differ among each other with regard to the level of subconductance states (Chen et al., 2003; el-Hayek, Lokuta et al., 1995; Tripathy, Resch, Xu, Valdivia, & Meissner, 1998), suggesting that sequence divergences may control this subconductance level. Indeed, we observed that single point mutations in maurocalcine modify this subconductance level (Lukacs et al., 2008). The absence of long-lasting events with *domain A* might also reflect its significantly lower affinity for type 1 ryanodine receptor compared to the toxin. Second, in agreement with their potentiating effects on channel activity, both maurocalcine and *domain A* induce  $\text{Ca}^{2+}$  release from purified sarcoplasmic reticulum vesicles (Dulhunty et al., 1999; el-Hayek, Antoniu et al., 1995; Esteve et al., 2003). These stimulating effects of *domain A* on  $\text{Ca}^{2+}$  release are observed at high concentrations (above 10  $\mu\text{M}$  in general) and are not consistently reported. In one study, application of peptide A was reported to decrease the caffeine- and  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (Chen et al., 2003), indicating in any case a relationship between the binding site of *domain A* and the

$\text{Ca}^{2+}$ -sensitivity of the type 1 ryanodine receptors. In the same study, high concentrations of peptide A actually block the effect of maurocalcine, again suggesting that both peptides bind onto the same site on the type 1 ryanodine receptor. The variable response of type 1 ryanodine receptor to *domain A*, as can be concluded from the various reports, also suggest that peptide conformation or cofactors largely influence the response of type 1 ryanodine receptor to *domain A*. Third, both *domain A* and maurocalcine increase [ $^3\text{H}$ ]-ryanodine binding (Chen et al., 2003; el-Hayek, Antoniu et al., 1995; Gurrola et al., 1999; Lu, Xu, & Meissner, 1994; X. Zhu et al., 1999). This stimulation is expected since conditions that trigger ryanodine channel opening are reported to produce a conversion in the binding site for ryanodine from a low affinity state to a higher one. The amplitude of the increase in [ $^3\text{H}$ ]-ryanodine binding, triggered by *domain A*, maurocalcine or other toxins, appears quite variable. This stimulation depends on other factor, such as the concentrations of cytosolic  $\text{Ca}^{2+}$  (Esteve et al., 2003; Tripathy et al., 1998) and  $\text{Mg}^{2+}$  (unpublished observations). This dependence on cytosolic  $\text{Ca}^{2+}$  concentration deserves some important comments that may be of interest for the understanding of excitation-contraction coupling. Cytosolic  $\text{Ca}^{2+}$  concentration has a biphasic effect of [ $^3\text{H}$ ]-ryanodine binding and channel gating, being an activator at low concentrations (above 100 nM) and inhibitor at higher concentrations (between 10  $\mu\text{M}$  and 1 mM). Interestingly, maurocalcine and imperatoxin A were reported to alter this  $\text{Ca}^{2+}$ -dependence by sensitizing type 1 ryanodine receptors to both the stimulation and inhibitory effects of  $\text{Ca}^{2+}$  (Esteve et al., 2003; Tripathy et al., 1998). For instance, while 100 nM  $\text{Ca}^{2+}$  does not trigger  $\text{Ca}^{2+}$  release from type 1 ryanodine receptor, this same concentration will produce  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release in the presence of maurocalcine. This is an important observation because it means that binding of maurocalcine on type 1 ryanodine receptor makes it prone to perform  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release. The analogy between maurocalcine and peptide A questions the possibility that *domain A* controls the ability of type 1 ryanodine receptors to perform  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release. The possibility that this control is revealed during membrane depolarization opens interesting questions.

An important finding has been the identification of peptide A and toxin binding site on type 1 ryanodine receptors. A first report describes the location of biotinylated imperatoxin A binding sites on tetramers of type 1 ryanodine receptors (Samso, Trujillo, Gurrola, Valdivia, & Wagenknecht, 1999). This study illustrates that four imperatoxin A can bind onto a cytoplasmic domain of the tetramer of type 1 ryanodine receptors between the clam and handle domains, 11 nm away from the transmembrane pore. The far distance of binding of imperatoxin A indicates that its effect on channel gating occurs through an allosteric mechanism. In a 3D reconstruction of type 1 ryanodine receptor, the imperatoxin A binding site is in close physical proximity to the  $\text{Ca}^{2+}$ -calmodulin binding site (Wagenknecht & Samso, 2002). More precise location of the binding sites of the toxins has been investigated using recombinant fusion protein of fragments of the type 1 ryanodine receptor. Using biotinylated maurocalcine and peptide A, Altafaj and coll. identified two type 1 ryanodine receptor sequences that bind either *domain A* or maurocalcine (Altafaj et al., 2005). Weak interaction was observed with a fragment encompassing amino acid region 1021 to 1631 that contains the 37 amino acid sequence that was previously shown to bind to the skeletal muscle  $\text{Ca}_v1.1$  II-III loop (Leong & MacLennan, 1998a). Stronger interaction was shown to occur with amino acid region 3351 to 3507. These findings are in agreement with the predicted localization of imperatoxin A



binding site on the 3D structure of type 1 ryanodine receptor. They are also in agreement with the suggestion that these two regions are in close proximity in space, thereby suggesting that they constitute a single binding site. In any case, these data demonstrate for the first time that maurocalcine and *domain A* share the same binding site. Of importance, it has been observed that deletion of the 1272-1455 amino acid region within type 1 ryanodine receptor provokes the loss of depolarization induced  $\text{Ca}^{2+}$  release (Perez, Mukherjee, & Allen, 2003). Combined these observations reinforce the idea that *domain A* and its binding site are somehow involved in the control of voltage-induced  $\text{Ca}^{2+}$  release. While maurocalcine has limited functional effects on type 2 ryanodine receptors, it is interesting to note that it binds to this receptor through an homologous binding site (regions 1033 to 1622 and 3558 to 3609 of type 2 ryanodine receptors) (Altafaj et al., 2007). These results suggest that binding of the toxin can be dissociated from its functional effect. This possibility has lately been reinforced by unpublished observations showing that the effect of maurocalcine depends on the redox state of type 1 ryanodine receptor. Differences in redox states between type 1 and 2 ryanodine receptors may thus explain differences in maurocalcine effects on these two channels. Of note, the redox state of type 2 ryanodine receptor has been shown to control its activity under cardiomyocytes stretch conditions (Prosser, Ward, & Lederer, 2011).

## 6. Use of maurocalcine to understand excitation-contraction coupling in skeletal muscle cells

During the course of maurocalcine characterization, we have shown that external application of maurocalcine on cultured skeletal muscle myotubes produces a small, but detectable, transient cytoplasmic  $\text{Ca}^{2+}$  elevation (Esteve et al., 2003). This effect is also observed in the absence of external  $\text{Ca}^{2+}$  demonstrating the mobilization of intracellular stores. Finally, maurocalcine interferes with the action of 4-chloro-*m*-cresol indicating the involvement of type 1 ryanodine receptors. This was the first demonstration of a pharmacological effect of a maurocalcine-type toxin on intact muscle cells. Since then, similar observations have been made with imperatoxin A and hadrucalcin on cardiomyocytes (Gurrola, Capes, Zamudio, Possani, & Valdivia, 2010; Schwartz et al., 2009). These observations have prompted us to investigate the cell penetration properties of maurocalcine. A series of studies have demonstrated since 2005 that maurocalcine belongs to the cell penetrating peptide family with vector properties that can be derived for various diagnostic, imaging and therapeutic applications (Aroui et al., 2009; Boisseau et al., 2006; Esteve et al., 2005; Jayagopal et al., 2009; Mabrouk et al., 2007; Poillot et al., 2010). A more detailed investigation of the mode of action of maurocalcine in developing skeletal muscle cells and adult fibers reveals that the peptide acts preferentially on type 1 ryanodine receptors that are uncoupled from the L-type calcium channels (Szappanos et al., 2005). Indeed, maurocalcine increases the frequency of spontaneous  $\text{Ca}^{2+}$  release events followed by the appearance of ember-like long lasting  $\text{Ca}^{2+}$  release events in permeabilized adult muscle fibers. Muscle permeabilization is expected to favor the uncoupling between type 1 ryanodine receptors and L-type calcium channels. These observations tend to indicate that maurocalcine has difficulties to act on type 1 ryanodine receptors when *domain A* of L-type channels already occupies the binding site on type 1 ryanodine receptor. Besides this effect of maurocalcine on uncoupled type 1 ryanodine receptors, the effect of the peptide was also investigated on L-type coupled type 1 ryanodine receptors by using high concentrations of



the peptide to gain accessibility to its binding site on the ryanodine receptor. Maurocalcine was injected into adult skeletal muscle fibers and  $\text{Ca}^{2+}$  transients induced by membrane depolarization. In these experiments, incubation with maurocalcine does not induce *per se* a change in resting cytoplasmic  $\text{Ca}^{2+}$  concentrations or amplitude of the depolarization-induced  $\text{Ca}^{2+}$  release. Only minor effects of maurocalcine were observed on the onset kinetics and voltage-dependence of voltage-induced  $\text{Ca}^{2+}$  release. Although these effects are small, they tend to indicate that maurocalcine is able to occupy its binding site during depolarization. Interestingly, much stronger effects of maurocalcine were observed on the kinetics of the termination of  $\text{Ca}^{2+}$  release upon ending membrane depolarization (Pouvreau et al., 2006). These effects correlate with the duration and amplitude of membrane depolarization and confirm that depolarization progressively permits maurocalcine binding onto L-type coupled ryanodine receptors. Taken together, these experiments would suggest that *domain A* acts as a negative clamp on type 1 ryanodine receptor for  $\text{Ca}^{2+}$  release at resting membrane potential. In this configuration, its affinity for *domain A* is too high or the accessibility too restricted to allow maurocalcine binding to type 1 ryanodine receptor. Depolarization produces a change in conformation of *domain A* that reduces its affinity for the ryanodine receptor, thereby relieving the negative clamp for  $\text{Ca}^{2+}$  release. In this situation, maurocalcine gains access to the ryanodine receptor by competing with *domain A* for its binding site. Since maurocalcine does not trigger a gain of function with regard to  $\text{Ca}^{2+}$  release under membrane depolarization, this observation suggests that i) maurocalcine and *domain A* are functionally equivalent under depolarization, and ii) since maurocalcine is an activator of  $\text{Ca}^{2+}$  release, it is likely that *domain A* acquires activating functions under membrane depolarization. Upon membrane repolarization, *domain A* regains access to its binding site on the ryanodine receptor by increased affinity and competitive displacement of maurocalcine. The slow kinetics of maurocalcine displacement by *domain A* explains the slower termination of  $\text{Ca}^{2+}$  release. This sequence of events is depicted in Figure 6.

In the scheme of events presented in Figure 6, *domain A* acts as a functionally versatile ligand: a negative clamp under resting membrane potential and an activator under membrane depolarization. By analogy with maurocalcine action, we hypothesize that the activating function of *domain A* stems from an increase in sensitivity of type 1 ryanodine receptor to cytoplasmic  $\text{Ca}^{2+}$ . Two types of changes in  $\text{Ca}^{2+}$  sensitivity have been observed with maurocalcine: i) a shift in  $\text{Ca}^{2+}$  concentration required to activate  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release towards lower concentrations (from a threshold of 100 to 10 nM  $\text{Ca}^{2+}$ ), and ii) an enhanced amplitude of  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release. With such a mechanism in hand, the initial trigger of  $\text{Ca}^{2+}$  elevation that sparks  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release under these activating functions of *domain A* can be *domain C*. The  $\text{Ca}^{2+}$  sensitizing function of *domain A* under membrane depolarization highlights a new concept for excitation-contraction in skeletal muscles. Rather than opposing voltage-induced  $\text{Ca}^{2+}$  release and  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release, the two phenomena might be closely linked. Voltage-induced  $\text{Ca}^{2+}$  release could be termed voltage-induced  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release. The  $\text{Ca}^{2+}$  responsible for the induction of  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release would originate from the initial opening of type 1 ryanodine receptor. In skeletal muscles, the presence of a negative clamp might be required to avoid spontaneous  $\text{Ca}^{2+}$  release events that otherwise would spark on  $\text{Ca}^{2+}$  release and contraction. In this scheme, the absence of *domain A* mainly alters the  $\text{Ca}^{2+}$  sensitivity of type 1 ryanodine receptors and does not prevent any activating function of *domain C*. When *domain A* is modified, change in  $\text{Ca}^{2+}$  dependence should be observed. However, this is

extremely difficult to address in classical experimental conditions where cytosolic  $\text{Ca}^{2+}$  concentration is not under control.

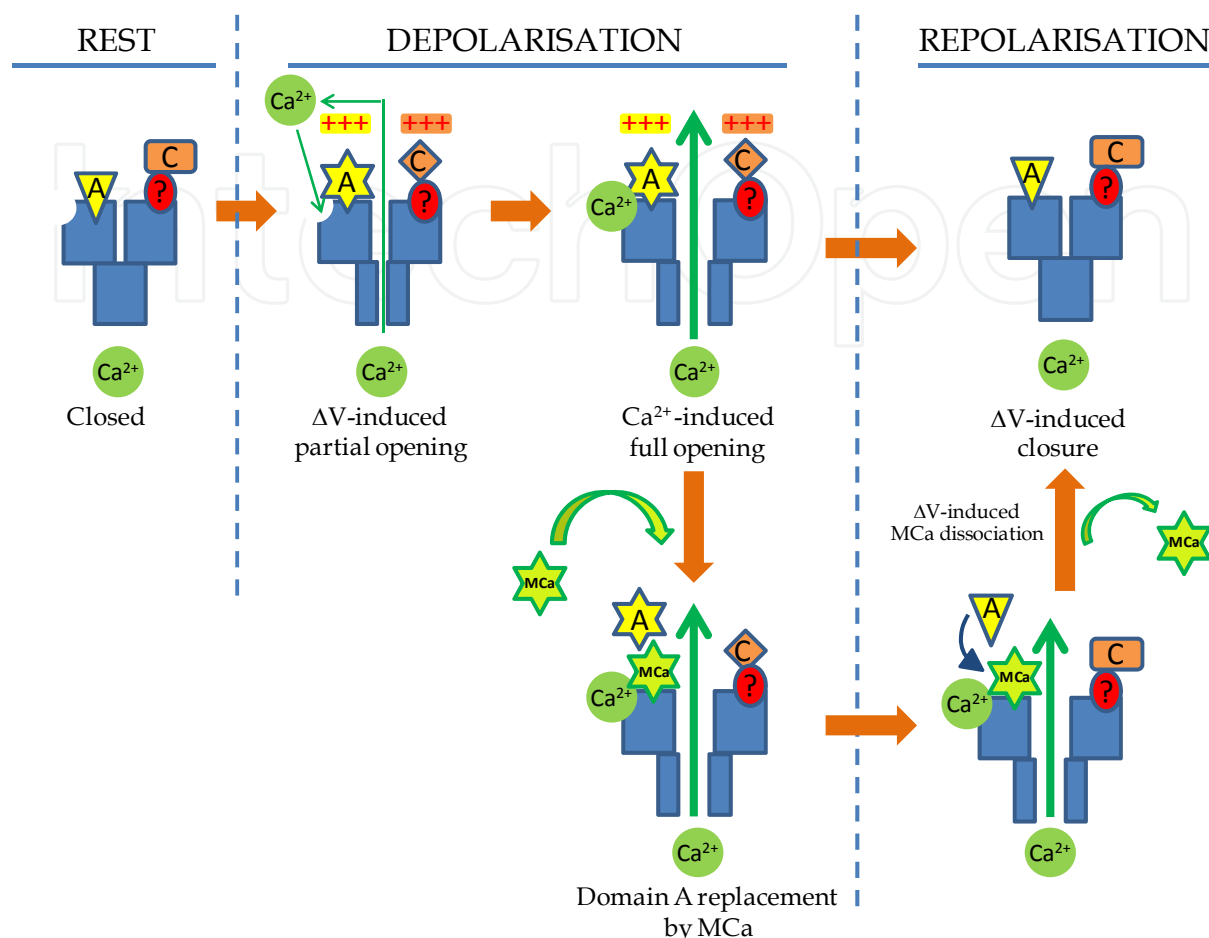


Fig. 6. Putative model of type 1 ryanodine receptor activation in skeletal muscles and role of domain A. A: domain A; C: domain C; ?: unknown putative domain C protein partner or receptor binding site. Membrane depolarization triggers domain C-mediated ryanodine receptor opening, while activating the  $\text{Ca}^{2+}$ -sensitizing function of domain A.  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release becomes the major sources of cytosolic  $\text{Ca}^{2+}$  elevation. Domain A interaction with the ryanodine receptor is weakened and maurocalcine can replace domain A with equivalent function. Upon membrane repolarization, the activating function of domain C is lost, domain A regains its affinity and the  $\text{Ca}^{2+}$  sensitivity is lost. If maurocalcine was occupying domain A binding site on the receptor, it is slowly displaced by the inhibitory conformation of domain A explaining the continuing  $\text{Ca}^{2+}$  release process in the interval, assuming that the activating function of maurocalcine is voltage-independent or at least less voltage-sensitive.

## 7. Conclusion

One common feature between domain A and maurocalcine is that both are positively charged. Interestingly, maurocalcine has been shown to possess a strong affinity for negatively charged membrane lipids (Boisseau et al., 2006; Ram et al., 2008), namely phosphatidylserine and phosphoinositides, but also gangliosides. Also, cell penetration of maurocalcine presents a voltage-sensitive component (Boisseau et al., 2006). These properties

introduce three interesting questions. First, does *domain A* interact with the plasma membrane, and in particular with negatively charged lipids? Second, owing to its close proximity with the plasma membrane and its charged nature, does *domain A* sense changes in membrane potential? Third, if yes, does *domain A* possess some of the plasma membrane translocation properties of maurocalcine by physically invading the plasma membrane during depolarization? While these questions remain unanswered, they may be of interest because these considerations could impact the possible voltage-dependent relationship existing between *domain A* and type 1 ryanodine receptor. Owing to its privileged interaction with type 1 ryanodine receptor through a discrete binding site, *domain A* may also confer voltage-sensitivity to the ryanodine receptor. Maurocalcine, as a tool, clearly demonstrated that the domain of type 1 ryanodine receptor on which maurocalcine or skeletal *domain A* binds plays an important role in the control of channel opening through pore-distant allosteric modifications. Therefore, it is conceivable that depolarization-induced movement of *domain A* within this site could impact the allosteric change that control type 1 ryanodine channel opening. Of note, while skeletal muscle *domain A* has a net positive charge of +6, cardiac *domain A* has a net charge of 0 making it far less suitable to sense changes in membrane potential. By extension, these considerations of voltage-dependence could apply to other domains of the II-III loop of voltage-gated calcium channels. One striking feature of *domain C* is that, contrary to *domain A*, it is negatively charged (12 residues out of 36 are negatively charged in skeletal and cardiac *domain C* – net negative charge -9). On the basis of these observations, one can therefore question the existence of a voltage-sensitivity in the conformation of *domain C*. Also, taking over an idea that has been postulated in the past, it wouldn't be unlikely, considering the electrostatic complementarities of *domains A* and *C*, that both domains interact with each other and that this interaction is under the control of voltage changes. This questions the specific relationship that might exist between *domain A* and *domain C* for the control of voltage-induced  $\text{Ca}^{2+}$  release.

Most studies that have been performed up to now in order to understand the roles of channel cytoplasmic domains in excitation-contraction coupling have suffered from a lack of structural knowledge. The identification of a restricted binding site of maurocalcine and *domain A* on ryanodine receptors opens the door to a better definition of the critical interacting amino acid residues through RMN studies. These structural studies are currently underway and indicate that some specific type 1 ryanodine receptor amino acids are involved in the interaction with both *domain A* and maurocalcine (unpublished results). At last, the identification of the essential amino acids will allow refined functional studies based on more precise  $\text{Ca}_v1.1$  or ryanodine receptor channel mutagenesis. In the process of comparing the functional homologies between *domain A* and maurocalcine, it would be of interest to substitute *domain A* by maurocalcine sequence in  $\text{Ca}_v1.1$ .

The importance of maurocalcine as a tool to investigate excitation-contraction coupling is demonstrated by the fact that, for the first time, this peptide allows a perturbation of the excitation-contraction coupling process in native cells without requiring the modification of any of the proteins involved, by mutagenesis or chimeras. Swapping domains between various ion channel isoforms is indeed a particularly tricky approach considering that there must be a delicate voltage-dependent conformational relationship between numerous important structural determinants. The use of maurocalcine has highlighted two important matters in the study of excitation-contraction coupling. First, it illustrates that the nature of the relationship between *domain A* and type 1 ryanodine receptor changes during membrane

depolarization. Second, it uncovers the unexpected importance of *domain A* in the termination of  $\text{Ca}^{2+}$  release during membrane repolarization. The precise description of this voltage-induced type 1 ryanodine closure is lacking so far. Nevertheless, this result is the first evidence for the role of  $\text{Ca}_v1.1$  *domain A* in this silencing process. The importance of this step in the control of  $\text{Ca}^{2+}$  homeostasis and contraction would justify that more studies be devoted to this issue.

The properties of maurocalcine to cross the plasma membrane suggest that it would be an interesting tool to modify excitation-contraction coupling in skeletal muscle *in vivo*. The fact that maurocalcine shares the same binding site than *domain A* on type 1 ryanodine receptor restricts its accessibility to L-type coupled ryanodine receptors. Obviously, it would thus be very interesting to investigate its effect on ryanodine receptors under conditions where its accessibility to the channel is not restricted, i.e. in the absence of  $\text{Ca}_v1.1$ . Effects of maurocalcine on *mdg* myotubes, lacking  $\text{Ca}_v1.1$ , are currently under investigation in our research team. These studies will provide important information on the effects of maurocalcine, and by inference on *domain A*, in a cellular context independent of the voltage-dependent channel environment and therefore also of the contribution of *domain C*.

The evidence for a voltage-modulated contribution of *domain A* to excitation-contraction coupling prompted us to emphasize the limits existing in the opposition between voltage-induced and  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release mechanisms. Investigations focused on  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release tend to take into account only the cell entry of  $\text{Ca}^{2+}$  from the extracellular space as trigger for  $\text{Ca}^{2+}$  release. The possible contribution of cytoplasmic or sarcoplasmic  $\text{Ca}^{2+}$  calcium in this process is not taken into account because of technical limitations. Indeed, it is quite difficult to maintain cytoplasmic  $\text{Ca}^{2+}$  at a certain concentration while at the same time measuring  $\text{Ca}^{2+}$  release from sarcoplasmic reticulum. Conversely, the possibility that type 2 ryanodine receptor  $\text{Ca}^{2+}$  release undergoes voltage-dependence might be masked by the voltage-dependence of external  $\text{Ca}^{2+}$  entry. If one takes into account these limitations, it is possible to draw a number of working hypotheses that do not necessarily oppose cardiac and skeletal muscle excitation-contraction coupling. Indeed, in light of the maurocalcine effects, we propose that the voltage-dependent modification of type 1 ryanodine receptor by *domain A* induces an increase of its sensitivity to cytoplasmic  $\text{Ca}^{2+}$ . Therefore, we propose to introduce the concept of voltage-induced- $\text{Ca}^{2+}$  induced- $\text{Ca}^{2+}$  release by which depolarization concomitantly promotes a change in  $\text{Ca}^{2+}$  sensitivity, through *domain A* of  $\text{Ca}_v1.1$ , and the opening, through *domain C*, of type 1 ryanodine receptor. In this model, the difference between cardiac and skeletal muscle excitation contraction coupling mechanism only resides on the absence of this change of  $\text{Ca}^{2+}$  sensitivity of the type 2 ryanodine receptor due to the absence of interaction of cardiac *domain A* with type 2 ryanodine receptor.

## 8. References

- Ahern, C. A., Arikath, J., Vallejo, P., Gurnett, C. A., Powers, P. A., Campbell, K. P., et al. (2001). Intramembrane charge movements and excitation- contraction coupling expressed by two-domain fragments of the  $\text{Ca}^{2+}$  channel. *Proc Natl Acad Sci U S A*, 98(12), 6935-6940.
- Ahern, C. A., Bhattacharya, D., Mortenson, L., & Coronado, R. (2001). A component of excitation-contraction coupling triggered in the absence of the T671-L690 and L720-



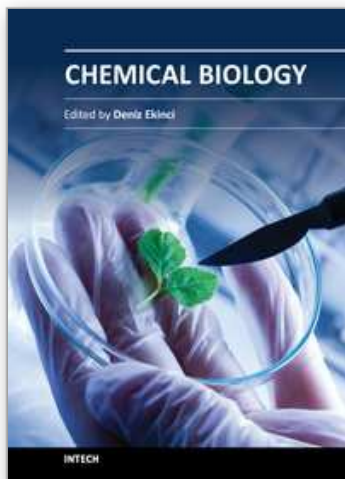
- Q765 regions of the II-III loop of the dihydropyridine receptor  $\alpha(1s)$  pore subunit. *Biophys J*, 81(6), 3294-3307.
- Altafaj, X., Cheng, W., Esteve, E., Urbani, J., Grunwald, D., Sabatier, J. M., et al. (2005). Maurocalcine and domain A of the II-III loop of the dihydropyridine receptor Cav 1.1 subunit share common binding sites on the skeletal ryanodine receptor. *J Biol Chem*, 280(6), 4013-4016.
- Altafaj, X., France, J., Almasy, J., Jona, I., Rossi, D., Sorrentino, V., et al. (2007). Maurocalcine interacts with the cardiac ryanodine receptor without inducing channel modification. *Biochem J*, 406(2), 309-315.
- Aroui, S., Ram, N., Appaix, F., Ronjat, M., Kenani, A., Pirollet, F., et al. (2009). Maurocalcine as a Non Toxic Drug Carrier Overcomes Doxorubicin Resistance in the Cancer Cell Line MDA-MB 231. *Pharm Res*, 26(4), 836-845.
- Bean, B. P. (1989). Classes of calcium channels in vertebrate cells. *Annu Rev Physiol*, 51, 367-384.
- Boisseau, S., Mabrouk, K., Ram, N., Garmy, N., Collin, V., Tadmouri, A., et al. (2006). Cell penetration properties of maurocalcine, a natural venom peptide active on the intracellular ryanodine receptor. *Biochim Biophys Acta*, 1758(3), 308-319.
- Chen, L., Esteve, E., Sabatier, J. M., Ronjat, M., De Waard, M., Allen, P. D., et al. (2003). Maurocalcine and peptide A stabilize distinct subconductance states of ryanodine receptor type 1, revealing a proportional gating mechanism. *J Biol Chem*, 278(18), 16095-16106.
- Cheng, H., Lederer, W. J., & Cannell, M. B. (1993). Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science*, 262(5134), 740-744.
- Cheng, W., Altafaj, X., Ronjat, M., & Coronado, R. (2005). Interaction between the dihydropyridine receptor  $\text{Ca}^{2+}$  channel beta-subunit and ryanodine receptor type 1 strengthens excitation-contraction coupling. *Proc Natl Acad Sci U S A*, 102(52), 19225-19230.
- Chu, A., Fill, M., Stefani, E., & Entman, M. L. (1993). Cytoplasmic  $\text{Ca}^{2+}$  does not inhibit the cardiac muscle sarcoplasmic reticulum ryanodine receptor  $\text{Ca}^{2+}$  channel, although  $\text{Ca}(2+)$ -induced  $\text{Ca}^{2+}$  inactivation of  $\text{Ca}^{2+}$  release is observed in native vesicles. *J Membr Biol*, 135(1), 49-59.
- Dulhunty, A. F., Laver, D. R., Gallant, E. M., Casarotto, M. G., Pace, S. M., & Curtis, S. (1999). Activation and inhibition of skeletal RyR channels by a part of the skeletal DHPR II-III loop: effects of DHPR Ser687 and FKBP12. *Biophys J*, 77(1), 189-203.
- el-Hayek, R., Antoniu, B., Wang, J., Hamilton, S. L., & Ikemoto, N. (1995). Identification of calcium release-triggering and blocking regions of the II-III loop of the skeletal muscle dihydropyridine receptor. *J Biol Chem*, 270(38), 22116-22118.
- el-Hayek, R., Lokuta, A. J., Arevalo, C., & Valdivia, H. H. (1995). Peptide probe of ryanodine receptor function. Imperatoxin A, a peptide from the venom of the scorpion *Pandinus imperator*, selectively activates skeletal-type ryanodine receptor isoforms. *J Biol Chem*, 270(48), 28696-28704.
- Esteve, E., Mabrouk, K., Dupuis, A., Smida-Rezgui, S., Altafaj, X., Grunwald, D., et al. (2005). Transduction of the scorpion toxin maurocalcine into cells. Evidence that the toxin crosses the plasma membrane. *J Biol Chem*, 280(13), 12833-12839.
- Esteve, E., Smida-Rezgui, S., Sarkozi, S., Szegedi, C., Regaya, I., Chen, L., et al. (2003). Critical amino acid residues determine the binding affinity and the  $\text{Ca}^{2+}$  release efficacy of maurocalcine in skeletal muscle cells. *J Biol Chem*, 278(39), 37822-37831.



- Fajloun, Z., Kharrat, R., Chen, L., Lecomte, C., Di Luccio, E., Bichet, D., et al. (2000). Chemical synthesis and characterization of maurocalcine, a scorpion toxin that activates Ca(2+) release channel/ryanodine receptors. *FEBS Lett*, 469(2-3), 179-185.
- Flucher, B. E., Kasielke, N., & Grabner, M. (2000). The triad targeting signal of the skeletal muscle calcium channel is localized in the COOH terminus of the alpha(1S) subunit. *J Cell Biol*, 151(2), 467-478.
- Fruen, B. R., Bardy, J. M., Byrem, T. M., Strasburg, G. M., & Louis, C. F. (2000). Differential Ca(2+) sensitivity of skeletal and cardiac muscle ryanodine receptors in the presence of calmodulin. *Am J Physiol Cell Physiol*, 279(3), C724-733.
- Green, D., Pace, S., Curtis, S. M., Sakowska, M., Lamb, G. D., Dulhunty, A. F., et al. (2003). The three-dimensional structural surface of two beta-sheet scorpion toxins mimics that of an alpha-helical dihydropyridine receptor segment. *Biochem J*, 370(Pt 2), 517-527.
- Gurrola, G. B., Arevalo, C., Sreekumar, R., Lokuta, A. J., Walker, J. W., & Valdivia, H. H. (1999). Activation of ryanodine receptors by imperatoxin A and a peptide segment of the II-III loop of the dihydropyridine receptor. *J Biol Chem*, 274(12), 7879-7886.
- Gurrola, G. B., Capes, E. M., Zamudio, F. Z., Possani, L. D., & Valdivia, H. H. (2010). Imperatoxin A, a Cell-Penetrating Peptide from Scorpion Venom, as a Probe of Ca-Release Channels/Ryanodine Receptors. *Pharmaceuticals (Basel)*, 3(4), 1093-1107.
- Ikemoto, N., & el-Hayek, R. (1998). Signal transmission and transduction in excitation-contraction coupling. *Adv Exp Med Biol*, 453, 199-207.
- Jayagopal, A., Su, Y. R., Blakemore, J. L., Linton, M. F., Fazio, S., & Haselton, F. R. (2009). Quantum dot mediated imaging of atherosclerosis. *Nanotechnology*, 20(16), 165102.
- Kugler, G., Grabner, M., Platzer, J., Striessnig, J., & Flucher, B. E. (2004). The monoclonal antibody mAB 1A binds to the excitation-contraction coupling domain in the II-III loop of the skeletal muscle calcium channel alpha(1S) subunit. *Arch Biochem Biophys*, 427(1), 91-100.
- Kugler, G., Weiss, R. G., Flucher, B. E., & Grabner, M. (2004). Structural requirements of the dihydropyridine receptor alpha1S II-III loop for skeletal-type excitation-contraction coupling. *J Biol Chem*, 279(6), 4721-4728.
- Leong, P., & MacLennan, D. H. (1998a). A 37-amino acid sequence in the skeletal muscle ryanodine receptor interacts with the cytoplasmic loop between domains II and III in the skeletal muscle dihydropyridine receptor. *J Biol Chem*, 273(14), 7791-7794.
- Leong, P., & MacLennan, D. H. (1998b). 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(45), 29958-29964.
- Lu, X., Xu, L., & Meissner, G. (1994). Activation of the skeletal muscle calcium release channel by a cytoplasmic loop of the dihydropyridine receptor. *J Biol Chem*, 269(9), 6511-6516.
- Lukacs, B., Sztrettye, M., Almassy, J., Sarkozi, S., Dienes, B., Mabrouk, K., et al. (2008). Charged surface area of maurocalcine determines its interaction with the skeletal ryanodine receptor. *Biophys J*, 95(7), 3497-3509.
- Mabrouk, K., Ram, N., Boisseau, S., Strappazzon, F., Rehim, A., Sadoul, R., et al. (2007). Critical amino acid residues of maurocalcine involved in pharmacology, lipid interaction and cell penetration. *Biochim Biophys Acta*, 1768(10), 2528-2540.
- Michalak, M., Dupraz, P., & Shoshan-Barmatz, V. (1988). Ryanodine binding to sarcoplasmic reticulum membrane; comparison between cardiac and skeletal muscle. *Biochim Biophys Acta*, 939(3), 587-594.

- Monnier, N., Procaccio, V., Stieglitz, P., & Lunardi, J. (1997). Malignant-hyperthermia susceptibility is associated with a mutation of the alpha 1-subunit of the human dihydropyridine-sensitive L-type voltage-dependent calcium-channel receptor in skeletal muscle. *Am J Hum Genet*, 60(6), 1316-1325.
- Mosbah, A., Kharrat, R., Fajloun, Z., Renisio, J. G., Blanc, E., Sabatier, J. M., et al. (2000). A new fold in the scorpion toxin family, associated with an activity on a ryanodine-sensitive calcium channel. *Proteins*, 40(3), 436-442.
- Nabauer, M., Callewaert, G., Cleemann, L., & Morad, M. (1989). Regulation of calcium release is gated by calcium current, not gating charge, in cardiac myocytes. *Science*, 244(4906), 800-803.
- Nakai, J., Adams, B. A., Imoto, K., & Beam, K. G. (1994). Critical roles of the S3 segment and S3-S4 linker of repeat I in activation of L-type calcium channels. *Proc Natl Acad Sci U S A*, 91(3), 1014-1018.
- Nakai, J., Tanabe, T., Konno, T., Adams, B., & Beam, K. G. (1998). Localization in the II-III loop of the dihydropyridine receptor of a sequence critical for excitation-contraction coupling. *J Biol Chem*, 273(39), 24983-24986.
- O'Reilly, F. M., Robert, M., Jona, I., Szegedi, C., Albrieux, M., Geib, S., et al. (2002). FKBP12 modulation of the binding of the skeletal ryanodine receptor onto the II-III loop of the dihydropyridine receptor. *Biophys J*, 82(1 Pt 1), 145-155.
- Perez, C. F., Mukherjee, S., & Allen, P. D. (2003). Amino acids 1-1,680 of ryanodine receptor type 1 hold critical determinants of skeletal type for excitation-contraction coupling. Role of divergence domain D2. *J Biol Chem*, 278(41), 39644-39652.
- Poillot, C., Dridi, K., Bichraoui, H., Pecher, J., Alphonse, S., Douzi, B., et al. (2010). D-Maurocalcine, a pharmacologically inert efficient cell-penetrating peptide analogue. *J Biol Chem*, 285(44), 34168-34180.
- Pouvreau, S., Csernoch, L., Allard, B., Sabatier, J. M., De Waard, M., Ronjat, M., et al. (2006). Transient loss of voltage control of  $\text{Ca}^{2+}$  release in the presence of maurocalcine in skeletal muscle. *Biophys J*, 91(6), 2206-2215.
- Proenza, C., Wilkens, C., Lorenzon, N. M., & Beam, K. G. (2000). A carboxyl-terminal region important for the expression and targeting of the skeletal muscle dihydropyridine receptor. *J Biol Chem*, 275(30), 23169-23174.
- Proenza, C., Wilkens, C. M., & Beam, K. G. (2000). Excitation-contraction coupling is not affected by scrambled sequence in residues 681-690 of the dihydropyridine receptor II-III loop. *J Biol Chem*, 275(39), 29935-29937.
- Prosser, B. L., Ward, C. W., & Lederer, W. J. (2011). X-ROS signaling: rapid mechano-chemo transduction in heart. *Science*, 333(6048), 1440-1445.
- Ram, N., Aroui, S., Jaumain, E., Bichraoui, H., Mabrouk, K., Ronjat, M., et al. (2008). Direct Peptide Interaction with Surface Glycosaminoglycans Contributes to the Cell Penetration of Maurocalcine. *J Biol Chem*, 283(35), 24274-24284.
- Samso, M., Trujillo, R., Gurrola, G. B., Valdivia, H. H., & Wagenknecht, T. (1999). Three-dimensional location of the imperatoxin A binding site on the ryanodine receptor. *J Cell Biol*, 146(2), 493-499.
- Schneider, M. F., & Chandler, W. K. (1973). Voltage dependent charge movement of skeletal muscle: a possible step in excitation-contraction coupling. *Nature*, 242(5395), 244-246.
- Schwartz, E. F., Capes, E. M., Diego-Garcia, E., Zamudio, F. Z., Fuentes, O., Possani, L. D., et al. (2009). Characterization of hadrucalcin, a peptide from *Hadrurus gertschi* scorpion venom with pharmacological activity on ryanodine receptors. *Br J Pharmacol*, 157(3), 392-403.

- Sencer, S., Papineni, R. V., Halling, D. B., Pate, P., Krol, J., Zhang, J. Z., et al. (2001). Coupling of RYR1 and L-type calcium channels via calmodulin binding domains. *J Biol Chem*, 276(41), 38237-38241.
- Shahbazzadeh, D., Srairi-Abid, N., Feng, W., Ram, N., Borchani, L., Ronjat, M., et al. (2007). Hemicalcin, a new toxin from the Iranian scorpion *Hemiscorpius lepturus* which is active on ryanodine-sensitive  $\text{Ca}^{2+}$  channels. *Biochem J*, 404(1), 89-96.
- Slavik, K. J., Wang, J. P., Aghdasi, B., Zhang, J. Z., Mandel, F., Malouf, N., et al. (1997). A carboxy-terminal peptide of the  $\alpha$  1-subunit of the dihydropyridine receptor inhibits  $\text{Ca}^{2+}$ -release channels. *Am J Physiol*, 272(5 Pt 1), C1475-1481.
- Szappanos, H., Smida-Rezgui, S., Cseri, J., Simut, C., Sabatier, J. M., De Waard, M., et al. (2005). Differential effects of maurocalcine on  $\text{Ca}^{2+}$  release events and depolarization-induced  $\text{Ca}^{2+}$  release in rat skeletal muscle. *J Physiol*, 565(Pt 3), 843-853.
- Takeshima, H., Yamazawa, T., Ikemoto, T., Takekura, H., Nishi, M., Noda, T., et al. (1995).  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release in myocytes from dyspedic mice lacking the type-1 ryanodine receptor. *Embo J*, 14(13), 2999-3006.
- Tanabe, T., Beam, K. G., Powell, J. A., & Numa, S. (1988). Restoration of excitation-contraction coupling and slow calcium current in dysgenic muscle by dihydropyridine receptor complementary DNA. *Nature*, 336(6195), 134-139.
- Tripathy, A., Resch, W., Xu, L., Valdivia, H. H., & Meissner, G. (1998). Imperatoxin A induces subconductance states in  $\text{Ca}^{2+}$  release channels (ryanodine receptors) of cardiac and skeletal muscle. *J Gen Physiol*, 111(5), 679-690.
- Valdivia, H. H., Kirby, M. S., Lederer, W. J., & Coronado, R. (1992). Scorpion toxins targeted against the sarcoplasmic reticulum  $\text{Ca}^{2+}$ -release channel of skeletal and cardiac muscle. *Proc Natl Acad Sci U S A*, 89(24), 12185-12189.
- Wagenknecht, T., & Samso, M. (2002). Three-dimensional reconstruction of ryanodine receptors. *Front Biosci*, 7, d1464-1474.
- Weiss, R. G., O'Connell, K. M., Flucher, B. E., Allen, P. D., Grabner, M., & Dirksen, R. T. (2004). Functional analysis of the R1086H malignant hyperthermia mutation in the DHPR reveals an unexpected influence of the III-IV loop on skeletal muscle EC coupling. *Am J Physiol Cell Physiol*, 287(4), C1094-1102.
- Wier, W. G., & Balke, C. W. (1999).  $\text{Ca}^{2+}$  release mechanisms,  $\text{Ca}^{2+}$  sparks, and local control of excitation-contraction coupling in normal heart muscle. *Circ Res*, 85(9), 770-776.
- Wilkens, C. M., Kasielke, N., Flucher, B. E., Beam, K. G., & Grabner, M. (2001). Excitation-contraction coupling is unaffected by drastic alteration of the sequence surrounding residues L720-L764 of the  $\alpha$  1S II-III loop. *Proc Natl Acad Sci U S A*, 98(10), 5892-5897.
- Zamudio, F. Z., Gurrola, G. B., Arevalo, C., Sreekumar, R., Walker, J. W., Valdivia, H. H., et al. (1997). Primary structure and synthesis of Imperatoxin A (IpTx(a)), a peptide activator of  $\text{Ca}^{2+}$  release channels/ryanodine receptors. *FEBS Lett*, 405(3), 385-389.
- Zhu, S., Darbon, H., Dyason, K., Verdonck, F., & Tytgat, J. (2003). Evolutionary origin of inhibitor cystine knot peptides. *Faseb J*, 17(12), 1765-1767.
- Zhu, X., Gurrola, G., Jiang, M. T., Walker, J. W., & Valdivia, H. H. (1999). Conversion of an inactive cardiac dihydropyridine receptor II-III loop segment into forms that activate skeletal ryanodine receptors. *FEBS Lett*, 450(3), 221-226.



## **Chemical Biology**

Edited by Prof. Deniz Ekinici

ISBN 978-953-51-0049-2

Hard cover, 444 pages

**Publisher** InTech

**Published online** 17, February, 2012

**Published in print edition** February, 2012

Chemical biology utilizes chemical principles to modulate systems to either investigate the underlying biology or create new function. Over recent years, chemical biology has received particular attention of many scientists in the life sciences from botany to medicine. This book contains an overview focusing on the research area of protein purification, enzymology, vitamins, antioxidants, biotransformation, gene delivery, signaling, regulation and organization. Particular emphasis is devoted to both theoretical and experimental aspects. The textbook is written by international scientists with expertise in synthetic chemistry, protein biochemistry, enzymology, molecular biology, drug discovery and genetics many of which are active chemical, biochemical and biomedical research. The textbook is expected to enhance the knowledge of scientists in the complexities of chemical and biological approaches and stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of chemical biology.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Michel Ronjat and Michel De Waard (2012). What Does Maurocalcine Tell Us About the Process of Excitation-Contraction Coupling?, Chemical Biology, Prof. Deniz Ekinici (Ed.), ISBN: 978-953-51-0049-2, InTech, Available from: <http://www.intechopen.com/books/chemical-biology/what-does-maurocalcine-tell-us-about-the-process-of-excitation-contraction-coupling->

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen