

## RESEARCH COMMUNICATION

**Differential distribution of ryanodine receptor type 3 (RyR3) gene product in mammalian skeletal muscles**

Antonio CONTI\*, L. GORZA† and Vincenzo SORRENTINO\*‡§

\*DIBIT, San Raffaele Scientific Institute, via Olgettina 58, 20132 Milano, †Department of Biomedical Sciences, University of Padova, Padova, and ‡Institute of Histology, School of Medicine, University of Siena, Siena, Italy

Activation of intracellular  $\text{Ca}^{2+}$ -release channels/ryanodine receptors (RyRs) is a fundamental step in the regulation of muscle contraction. In mammalian skeletal muscle,  $\text{Ca}^{2+}$ -release channels containing the type 1 isoform of RyR (RyR1) open to release  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum (SR) upon stimulation by the voltage-activated dihydropyridine receptor on the T-tubule/plasma membrane. In addition to RyR1, low levels of the mRNA of the RyR3 isoform have been recently detected in mammalian skeletal muscles. Here we report data on the distribution of the RyR3 gene product in mammalian skeletal muscles. Western-blot analysis of SR of individual muscles indicated that, at variance with the even distribution of the RyR1

isoform, the RyR3 content varies among different muscles, with relatively higher amounts being detected in diaphragm and soleus, and lower levels in abdominal muscles and tibialis anterior. In these muscles RyR3 was localized in the terminal cisternae of the SR. No detectable levels of RyR3 were observed in the extensor digitorum longus. Preferential high content of RyR3 in the diaphragm muscle was observed in several mammalian species. *In situ* hybridization analysis demonstrated that RyR3 transcripts are not restricted to a specific subset of skeletal-muscle fibres. Differential utilization of the RyR3 isoform in skeletal muscle may be relevant to the modulation of  $\text{Ca}^{2+}$  release with respect to specific muscle-contraction properties.

## INTRODUCTION

In skeletal muscle, voltage-mediated activation of the dihydropyridine receptor (DHPR) on the T-tubule/plasma membrane stimulates the ryanodine receptor (RyR)/ $\text{Ca}^{2+}$ -release channels located on the sarcoplasmic reticulum (SR), thus resulting in the release of large amounts of  $\text{Ca}^{2+}$  from the SR. The process of activation of the intracellular RyRs by the DHPRs, also referred to as 'excitation-contraction coupling' [1], seems to depend, in skeletal muscle, on a mechanical link between these two molecules [2–6]. Accordingly, in the regions of skeletal-muscle fibres that are specialized for excitation-contraction coupling, the terminal cisternae of the SR and the T-tubules/plasma membrane are only approx. 15 nm apart, thus bringing the individual RyR1 in close apposition to the DHPR-containing tetrads [7].

Studies made in the electron microscope have shown that, in skeletal muscles, there is a precise pattern in the relative position of RyRs and DHPRs, where only one of every two RyRs is faced by a DHPR-containing tetrad [8]. Recent results further suggest a general occurrence of this structural organization [9]. The presence of RyRs not directly operated by the DHPRs is also supported by studies on the physiology of  $\text{Ca}^{2+}$  release in skeletal-muscle fibres suggesting the existence of a  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release component in triggering bulk  $\text{Ca}^{2+}$  release from the SR [5,10–12]. Whether the  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release mechanism opens (i) the same channels activated by the DHPR or/and (ii) RyRs not directly coupled to DHPR is, however, still not clear.

Of the above two models, the second appears to fit well for most muscles of non-mammalian vertebrates that express equal levels of two isoforms of RyR, referred to as  $\alpha$  and  $\beta$  [13]. These

two isoforms, at least in the frog and the chicken, correspond to the mammalian RyR1 and RyR3 respectively [14,15]. The two channels have been shown to differ in biochemical properties and gating behaviour [16–19]. Given these differences, it has been proposed that the  $\alpha$ -RyR may work coupled to the DHPRs, while the  $\beta$ -RyR could be the non-coupled isoform [18,19]. While the existence of two RyR isoforms in skeletal muscles has been recognized for years in non-mammalian vertebrates, only recently low levels of RyR3 mRNA have been detected also in mammalian skeletal muscles [20–23]. Here we report results of studies on the distribution of the RyR3 isoform in mammalian skeletal muscles.

## MATERIALS AND METHODS

**Preparation of microsomal membrane proteins**

Bovine and rabbit skeletal-muscle samples were from a local slaughterhouse. Rat skeletal muscles were dissected from 3–4-month-old Sprague-Dawley rats and adult DBA mice (Charles River). Microsomal membranes were prepared as described [14,21]. Longitudinal and terminal cisternae fractions of skeletal-muscle SR were prepared as described in [24].

**SDS/PAGE and Western-blot analysis**

Protein separation, Western-blotting and antigen detection were performed as previously described [14,21]. Rabbit antibodies specific for RyR1, RyR2 and RyR3 were described previously [21]. A rabbit antiserum against calsequestrin was kindly provided by Dr. P. Volpe. A monoclonal antibody (Y1F4) against

the Ca<sup>2+</sup>-ATPase SERCA1 isoform was kindly provided by Dr. J. M. East [25].

### *In situ* hybridization

Mouse skeletal-muscle cryosections used for *in situ* hybridisation experiments were prepared as described in [26]. Murine probes [27,28] and hybridization conditions were described previously [21,26].

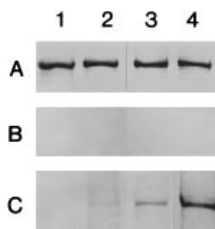
## RESULTS

### Detection of RyR3 protein in microsomal and SR fractions of bovine skeletal muscles

The presence of the three known RyR isoforms was initially analysed in total microsomal membranes and in fractions enriched in terminal cisternae prepared from bovine skeletal-muscle tissue. Figure 1 shows a Western-blot analysis with two different batches of bovine skeletal muscles prepared from abdominal (lanes 1 and 2) and hind-limb (lanes 3 and 4) muscles. The RyR1 gene product was detected in the total microsomal fraction (Figure 1A, lanes 1 and 3) and in the terminal cisternae (Figure 1A, lanes 2 and 4) of these muscles. The RyR2 (the cardiac isoform) was not found (Figure 1B, lanes 1–4). At variance with the equivalent levels of RyR1 in the two batches of muscle analysed, the RyR3 protein was detected in the microsomal fraction of the bovine hind-limb muscle preparation (Figure 1C, lane 3), but not in that from bovine abdominal muscles (Figure 1C, lane 1). It was, however, present in the fraction enriched in terminal cisternae prepared from both muscles (Figure 1C, lanes 2 and 4), suggesting that RyR3 could be enriched in the terminal cisternae of the SR.

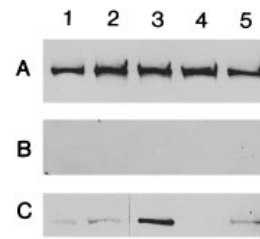
### Differential distribution of the RyR3 isoform in selected rat skeletal muscles

The distribution of the RyR3 protein was further investigated in anatomically distinct skeletal muscles of the rat (Figure 2). In the microsomes from these muscles the RyR1 levels were approximately the same (Figure 2A), while the RyR2 was not detected (Figure 2B), in agreement with previous observations [29–32]. On the other hand, analysis of the RyR3 protein revealed a muscle-type-dependent pattern of RyR3 content, with higher amounts in the microsomal fractions prepared from diaphragm



**Figure 1** Detection of RyR3 in bovine skeletal muscles

Total microsomal vesicles (lanes 1 and 3) and terminal-cisternae membranes (lanes 2 and 4) were prepared from bovine abdominal muscles (lanes 1 and 2) and hind limb (lanes 3 and 4). (A) Immunoblot of 10  $\mu$ g of microsomal proteins (lanes 1 and 3) and 1  $\mu$ g of proteins from terminal cisternae (lanes 2 and 4) with antibodies against RyR1. (B) Immunoblot of 100  $\mu$ g of microsomal proteins (lanes 1 and 3) and 40  $\mu$ g of proteins from terminal cisternae (lanes 2 and 4) with antibodies against RyR2. (C) Immunoblot of 100  $\mu$ g of microsomal proteins (lanes 1 and 3) and 40  $\mu$ g of proteins from terminal cisternae (lanes 2 and 4) with antibodies against RyR3.



**Figure 2** Detection of the RyR3 protein in rat skeletal muscles

Microsomes were isolated from tibialis anterior (lane 1), abdominal muscles (lane 2), diaphragm (lane 3), EDL (lane 4), and soleus (lane 5) and proteins separated on SDS/PAGE. (A) Immunoblot of 5  $\mu$ g of microsomal proteins probed with antibodies against RyR1. (B) Immunoblot of 70  $\mu$ g of microsomal proteins probed with antibodies against RyR2. (C) Immunoblot of 70  $\mu$ g of microsomal proteins probed with antibodies against RyR3.

and soleus (Figure 2C, lanes 3 and 5) and lower levels in those from tibialis anterior and abdominal muscles (Figure 2C, lanes 1 and 2). No RyR3 protein was detected in similar fractions from extensor digitorum longus (EDL) (Figure 2C, lane 4). A similar pattern was observed also in microsomal fractions from rabbit skeletal muscles (results not shown).

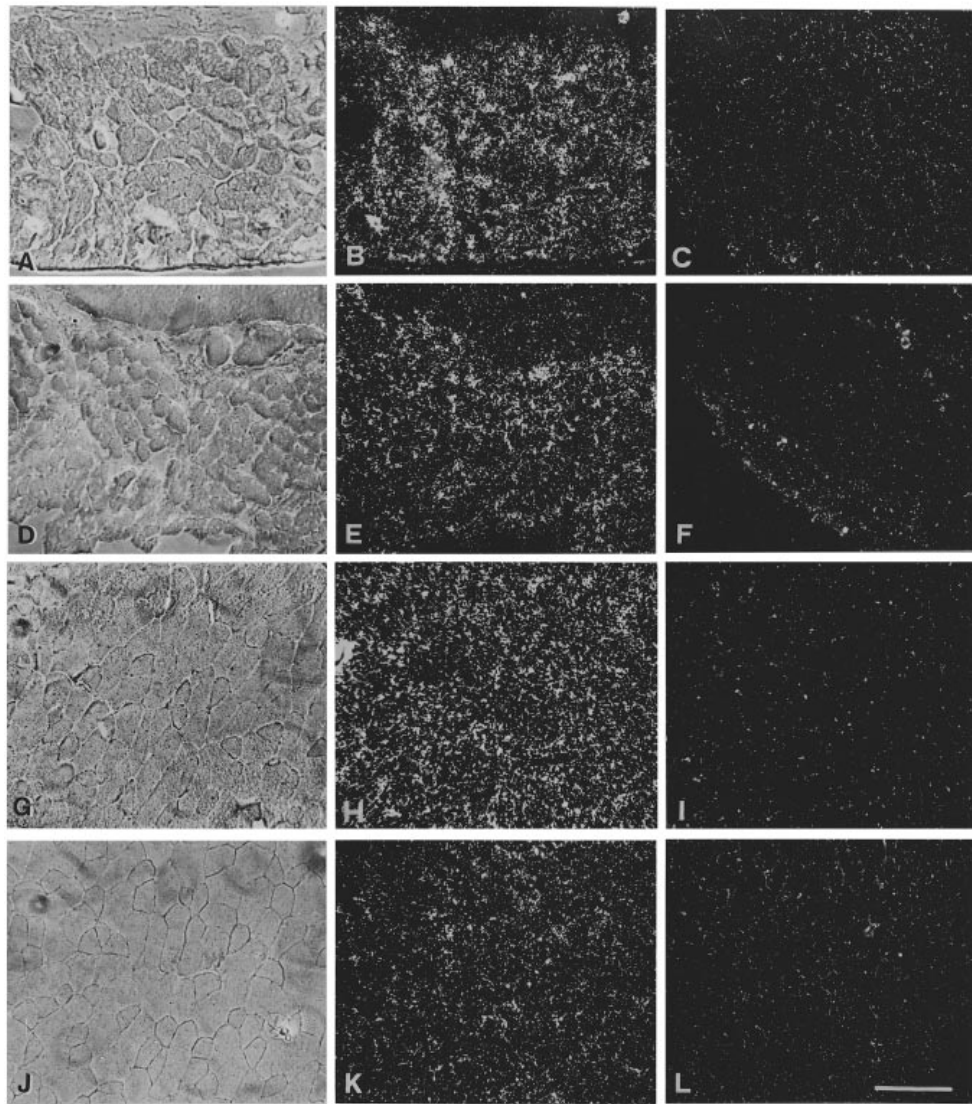
Densitometric analysis of RyR3-specific bands from diaphragm, soleus and tibialis anterior muscles indicated that the levels of RyR3 in these muscles are in the ratio of approx. 10:1.5:0.2 (results not shown). By comparing the relative levels of RyR3 with the respective fibre-type composition of the same rat muscles (diaphragm: 7% type 2B, 35% type 2A, 33% type 2X, 25% type 1; soleus: 10% type 2A, 90% type 1; tibialis anterior: 48% type 2B, 20% type 2A, 18% type 2X, 2% type 1 and 12% of fibres with mixed properties of the different type 2 fibres) a relationship between fibre-type composition [33–35] and levels of RyR3 in these muscles appears unlikely.

### *In situ* hybridization analysis of the expression of the RyR3 gene in different murine skeletal-muscle fibres

*In situ* hybridization analysis of mouse skeletal muscles confirmed that skeletal-muscle fibres accumulate RyR3 transcripts (Figure 3, panels E and K, and Figure 4), in addition to RyR1 mRNA (Figure 3, panels B and H). RyR3 mRNA appears to be more concentrated in diaphragm with respect to tibialis anterior (Figure 3; cf. panels E and K) and tensor fasciae latae muscles (results not shown), in agreement with previous results [21]. Note that the RyR3 mRNA hybridization signal appears to be homogeneously distributed across the different skeletal-muscle sections (Figure 3, panels E and K), similarly to what is observed for RyR1 mRNA (Figure 3, panels B and H). Furthermore, as illustrated by Figure 4, no significant differences in RyR3-specific silver-grain accumulation can be detected among skeletal-muscle fibres either in the diaphragm (Figure 4A) or in the tibialis anterior (Figure 4B). In a similar manner, homogeneous hybridization signals were observed in skeletal-muscle fibres of the soleus muscle (Figure 4C). No hybridization signal above background was detected when RyR1 and RyR3 sense probes (Figure 3, panels C, F, I and L) were used.

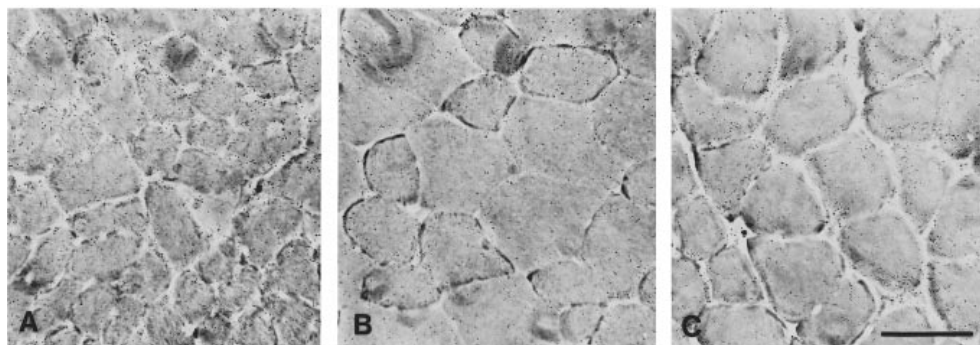
### High levels of RyR3 in diaphragm muscles of different mammalian species

Interestingly, the fibre composition of the diaphragm muscle changes considerably among species: fast-twitch fibres are predominant in the mouse, slow-twitch fibres in the cow, while an



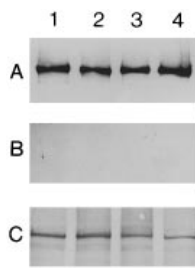
**Figure 3** *In situ* hybridization analysis of RyR1 and RyR3 mRNAs in mouse diaphragm and tibialis anterior muscles

Dark-field micrographs of cryosections of diaphragm (A–F) and a superficial portion of tibialis anterior (G–L) hybridized with RyR1 antisense (B and H) and sense (C and I) probes and RyR3 antisense (E and K) and sense (F and L) probes. Autoradiographies of sections hybridized with RyR1 sense and antisense cRNAs and RyR3 sense and antisense cRNAs were exposed for 3 days and 8 days respectively. Bright fields are illustrated by panels (A), (D), (G) and (J). Note that the superficial portion of the tibialis muscle is composed exclusively of type 2B fibres (large fibres) and type 2X fibres (small fibres) [34]. The bar represents 100  $\mu\text{m}$ .



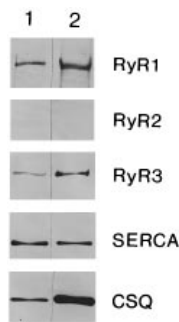
**Figure 4** RyR3 mRNA in mouse skeletal-muscle fibres

Bright-field micrographs of cryosections from diaphragm (A), tibialis anterior (B) and soleus (C) muscles hybridized with RyR3 antisense probe. The bar represents 50  $\mu\text{m}$ .



**Figure 5 High content of RyR3 in diaphragm muscles of different species**

Diaphragm muscle strips were excised from mice (lane 1), rat (lane 2), rabbit (lane 3) and cow (lane 4), and the microsomal fractions separated. Immunoblots containing 5 and 50  $\mu\text{g}$  of microsomal proteins were decorated with antibodies against RyR1 (A) and RyR2 (B) respectively. Immunoblots containing 100  $\mu\text{g}$  of microsomal protein were decorated with antibodies against RyR3 (C).



**Figure 6 Preferential localization of the RyR3 isoform in the terminal-cisternae fraction of skeletal-muscle SR**

Total cellular membranes from bovine diaphragm muscle were fractionated on a discontinuous sucrose gradient and the two fractions corresponding to longitudinal cisternae (lane 1) and to terminal cisternae (lane 2), were analysed. Immunoblots containing 5  $\mu\text{g}$  of protein for each fraction were incubated with antibodies against RyR isoforms. Immunoblots containing 3  $\mu\text{g}$  of protein from each fraction were incubated with a rabbit antiserum against calsequestrin (CSQ), kindly provided by Dr. P. Volpe, and with a monoclonal antibody (Y1F4) against the  $\text{Ca}^{2+}$ -ATPase SERCA1 isoform (SERCA).

intermediate situation is observed in rat and rabbit [30,36,37]. In spite of these wide changes in fibre composition, microsomes prepared from diaphragm muscles of all these species were consistently found to contain relatively high levels of RyR3 (Figure 5, lanes 1–4).

#### Localization of the RyR3 isoform in the terminal-cisternae fraction of the SR of skeletal muscles

To verify whether the RyR3 are distributed in the SR differentially from RyR1, total microsomal vesicles prepared from bovine diaphragm muscle were separated on a discontinuous sucrose gradient. The first fraction is enriched in longitudinal cisternae, although it also contains about 10% of contaminating terminal cisternae [24]. The second fraction consists mainly of terminal cisternae. In agreement with previous data, RyR1 and calsequestrin were detected mostly in the fraction containing terminal cisternae (Figure 6). The presence of low levels of RyR1 and calsequestrin in the fraction containing longitudinal cisternae is due to the contamination of this fraction by some terminal cisternae [24]. Similarly to RyR1 and calsequestrin, most of the RyR3 immunoreactivity was also detected in the terminal-

cisternae fraction (Figure 6). The equivalent loading of the gel lanes, was confirmed by the equal distribution of the  $\text{Ca}^{2+}$ -ATPase pump.

#### DISCUSSION

The data reported demonstrate that the RYR3 gene product is present in mammalian skeletal muscles. In contrast with the skeletal-muscle isoform RyR1, which is expressed at equivalent high levels in all muscles analysed, the RyR3 content appeared to vary among rat skeletal muscles, being more abundant in diaphragm and soleus and below detectable levels in the EDL. The differential distribution of RyR3 isoform in these muscles did not appear to reflect their fibre-type composition. Indeed, in certain muscles, the fibre-type content varies among species, while overall functional properties are maintained [38]. Eventually the relatively higher amount of RyR3 observed in diaphragm and soleus muscles of different mammalian species could be tentatively explained in the light of the involvement of these muscles in continuous pattern of activity, in relation with postural control or respiratory function [39,40]. Furthermore, RyR3 content, rather than velocity of contraction, appears to be directly related to the presence of oxidative fibres, while it is inversely related to the proportion of fast 2B fibres. We cannot exclude the possibility that post-transcriptional regulation acting at a fibre-type level may generate further heterogeneity of RyR3 distribution as for the inositol 1,4,5-trisphosphate receptor in rat skeletal muscle [41]. Unfortunately, the so-far-available antibodies cannot offer any information on RyR3 protein expression at the single-muscle-fibre level.

Interestingly, the RyR3 isoform has been localized to the terminal cisternae of the SR, a site consistent with a potential involvement in the  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release component of excitation-contraction coupling [5,10–12]. In the last few years, subtle differences in the composition of the excitation-contraction coupling machinery have been detected that may reflect a fine tuning of this process to the specific requirement across species, and even among different muscles [1]. These differences include variations in the ratio between DHPR and RyRs [30,42–45], higher numbers of RyRs in fast- versus slow-twitch fibres [30,44–46], and differences in the organization of  $\text{Ca}^{2+}$ -release units [7,47]. Differential expression of RyR isoforms in specialized muscles in non-mammalian vertebrates appears to be a way to regulate  $\text{Ca}^{2+}$  release in relation to particular contracting properties of those muscles. In birds and fish, in fact, expression of the  $\alpha$ -RyR isoform alone has been demonstrated in muscles with very fast contracting properties [48]. In agreement, no expression of RyR3, the mammalian homologue of the  $\beta$ -RyR isoform, could be detected in rat EDL, a typical fast-twitch muscle. In an opposite symmetry, RyR3 is preferentially more abundant in diaphragm, a muscle that, independently from its fibre composition, possesses an extraordinary resistance to fatigue, and in soleus, a postural muscle with slow-twitch properties [40]. The data reported above indicate that differential usage of RyR isoforms also takes place in mammalian skeletal muscle, where it may contribute to the diversification of the excitation-contraction coupling machinery, to meet eventually the specific requirement of specific muscles.

Data in the literature support the hypothesis that the  $\alpha$ -RyR of non-mammalian vertebrates and the RyR1 in mammals are coupled to the DHPR in skeletal muscles [49–51]. In the Crooked Neck Dwarf (*cn/cn*) chicken mutant, absence of the  $\alpha$ -RyR1 results, in spite of normal expression of the  $\beta$ -RyR, in lack of electrical coupling in skeletal myocytes [52]. Similar results have been reported in the *skrr<sup>m1</sup>/skrr<sup>m1</sup>* mice, which carry a null

mutation in the skeletal RyR (RYR1) gene [53]. Interestingly, myocytes from *skrr<sup>m1</sup>/skrr<sup>m1</sup>* mice, similarly to muscle cells from *cn/cn* chickens [52], still release Ca<sup>2+</sup> from the SR in response to caffeine (a known agonist of RyRs) [23,53], which probably reflects the presence of the RyR3 isoform in mammalian skeletal-muscle SR [20,21,23]. It is noteworthy that the levels of Ca<sup>2+</sup> induced by caffeine in skeletal muscles of *skrr<sup>m1</sup>* mice are only about one-tenth of those induced in normal mice [23]. These data suggest that the RyR3-encoded channel may still contribute to elicit significant Ca<sup>2+</sup> transients in mammalian skeletal muscles. Thus, while RyR1, in addition to its role in Ca<sup>2+</sup> release, appears to be essential in conveying to the SR the voltage signal sensed on the plasma membrane by the DHPR, RyR3 could work, at a distal step not directly in association with the DHPR, to modulate Ca<sup>2+</sup> release from the SR in association with RyR1.

For most body muscles of non-mammalian vertebrates, the two-channel model, with the  $\alpha$ -RyR isoform coupled with the DHPR and the  $\beta$ -RyR isoform likely to be operated by Ca<sup>2+</sup>, can be proposed. This model cannot be transposed directly to mammalian skeletal muscles, where, even in the soleus and diaphragm, RyR3 is less represented than RyR1. In the absence of evidence for heterotetrameric channels, three possibilities can be envisaged to explain this discrepancy: (1) 1:1 ratio of Ca<sup>2+</sup>-release channels containing the RyR1 and the RyR3 isoform is present in restricted regions of the triad, while the rest of the triad contains mainly Ca<sup>2+</sup>-release channels containing the RyR1 isoform; (2) a Ca<sup>2+</sup>-release channel containing the RyR3 isoform is scattered every 10–30 Ca<sup>2+</sup>-release channels containing the RyR1 isoform; (3) either one of the two patterns proposed in (1) and (2) could be present only in some of the Ca<sup>2+</sup>-release units present around the T-tubules [7,9]. Whether this variability represents a way of affecting the size of the Ca<sup>2+</sup> transient or to facilitate channel activation requires further studies.

The preferential usage of one isoform in mammalian skeletal muscles versus the more frequent employment of two isoforms in most non-mammalian vertebrates is suggestive of an overall capability of the RyR1 isoform to fulfil most of the needs of mammalian skeletal muscles. However, the reported differential pattern of expression of the RyR3 isoform among different mammalian skeletal muscles suggests that usage of two isoforms of RyR may still be important to fulfil specific functional demands of certain muscles. This would in fact justify the fact that it has been conserved, even though with some variations, for over hundreds of millions of years.

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## REFERENCES

- Meissner, G. (1994) *Annu. Rev. Physiol.* **56**, 485–508
- Fleischer, S. and Inui, M. (1989) *Annu. Rev. Biophys. Chem.* **18**, 333–364
- Lu, X., Xu, L. and Meissner, G. (1994) *J. Biol. Chem.* **269**, 6511–6516
- Marty, I., Robert, M., Villaz, M., De Jongh, K., Lai, Y., Catterall, W. A. and Ronjat, M. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2270–2274
- Schneider, M. F. (1994) *Annu. Rev. Physiol.* **5**, 463–484
- Schneider, M. F. and Chandler, W. K. (1973) *Nature (London)* **242**, 747–751
- Franzini-Armstrong, C. and Jorgensen, A. O. (1994) *Annu. Rev. Physiol.* **56**, 509–534
- Block, B. A., Imagawa, T., Campbell, K. P. and Franzini-Armstrong, C. (1988) *J. Cell Biol.* **107**, 2587–2600
- Franzini-Armstrong, C. and Kish, J. W. (1995) *J. Muscle Res. Cell Motil.* **16**, 319–324
- Jacquemond, V., Csernoch, L., Klein, M. G. and Schneider, M. F. (1991) *Biophys. J.* **60**, 867–873
- Rios, E. and Pizarro, G. (1991) *Physiol. Rev.* **71**, 849–908
- Rios, E., Pizarro, G. and Stefani, E. (1992) *Annu. Rev. Physiol.* **54**, 109–133
- Olivares, E. B., Tanksley, S. J., Airey, J. A., Beck, C. F., Ouyang, Y., Deerinck, T. J., Ellisman, M. H. and Sutko, J. L. (1991) *Biophys. J.* **59**, 1153–1163
- Ottini, L., Marziali, G., Conti, A., Charlesworth, A. and Sorrentino, V. (1996) *Biochem. J.* **315**, 207–216
- Oyamada, H., Murayama, T., Takagi, T., Iino, M., Iwabe, N., Miyata, T., Ogawa, Y. and Endo, M. (1994) *J. Biol. Chem.* **269**, 17206–17214
- Airey, J. A., Grinsell, M. M., Jones, L. R., Sutko, J. L. and Witcher, D. (1993) *Biochemistry* **32**, 5739–5745
- Bull, R. and Marengo, J. J. (1993) *FEBS Lett.* **331**, 223–227
- O'Brien, J., Valdivia, H. H. and Block, B. A. (1995) *Biophys. J.* **68**, 471–482
- Percival, A. L., Williams, A. J., Kenyon, J. L., Grinsell, M. M., Airey, J. A. and Sutko, J. L. (1994) *Biophys. J.* **67**, 1834–1850
- Giannini, G., Clementi, E., Ceci, R., Marziali, G. and Sorrentino, V. (1992) *Science* **257**, 91–94
- Giannini, G., Conti, A., Mammarella, S., Scrobogna, M. and Sorrentino, V. (1995) *J. Cell Biol.* **128**, 893–904
- Sorrentino, V. (1996) in *Ryanodine Receptors* (Sorrentino, V., ed.), pp. 85–100, CRC Press, Boca Raton, FL
- Takekura, H., Yamazawa, T., Ikemoto, T., Takekura, H., Nishi, M., Noda, T. and M. I. (1995) *EMBO J.* **14**, 2999–3006
- Saito, A., Seiler, S., Chu, A. and Fleischer, S. (1984) *J. Cell Biol.* **99**, 875–885
- Colyer, J., Mata, A. M., Lee, A. G. and East, J. M. (1989) *Biochem. J.* **262**, 439–447
- Goza, L., Schiaffino, S. and Volpe, P. (1993) *J. Cell Biol.* **121**, 345–353
- Mattei, M. G., Giannini, G., Moscatelli, F. and Sorrentino, V. (1994) *Genomics* **22**, 202–204
- Sorrentino, V. and Volpe, P. (1993) *Trends Pharmacol. Sci.* **14**, 98–103
- Arai, M., Otsu, K., MacLennan, D. H. and Periasamy, M. (1992) *Am. J. Physiol.* **262**, C614–C620
- Damiani, E. and Margreth, A. (1994) *J. Muscle Res. Cell Motil.* **15**, 86–101
- Inui, M., Saito, A. and Fleischer, S. (1987) *J. Biol. Chem.* **262**, 1740–1747
- Lai, F., Erickson, H., Rousseau, E., Liu, Q.-Y. and Meissner, G. (1988) *Nature (London)* **331**, 315–319
- DeNardi, C., Ausoni, S., Moretti, P., Goza, L., Velleca, M., Buckingham, M. and Schiaffino, S. (1993) *J. Cell Biol.* **123**, 823–835
- Goza, L. (1990) *J. Histochem. Cytochem.* **38**, 257–265
- Kushmerick, M. J., Moerland, T. S. and Wiseman, R. W. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 7521–7525
- Biral, D., Damiani, E., Volpe, P., Salviati, G. and Margreth, A. (1982) *Biochem. J.* **203**, 529–540
- Gauthier, G. F., Lowey, S., Benfield, P. A. and Hobbs, A. W. (1982) *J. Cell Biol.* **92**, 471–484
- Engel, A. G. and C. Franzini-Armstrong (1994) *Myology*, McGraw-Hill, New York
- Pette, D. and Staron, R. S. (1990) *Rev. Physiol. Biochem. Pharmacol.* **116**, 1–76
- Schiaffino, S. and Reggiani, D. (1996) *Physiol. Rev.*, in the press
- Moschella, M. C., Watras, J., Jayaraman, T. and Marks, A. R. (1995) *J. Muscle Res. Cell Motil.* **16**, 390–400
- Anderson, K., Cohn, A. H. and Meissner, G. (1994) *Am. J. Physiol.* **266**, C462–466
- Bers, D. M. and Stiffel, V. M. (1993) *Am. J. Physiol.* **264**, C1587–C1593
- Lamb, G. D. (1992) *J. Muscle Res. Cell Motil.* **13**, 394–405
- Lamb, G. D. and Walsh, T. (1987) *J. Physiol. (London)* **393**, 595–617
- Franzini-Armstrong, C. (1994) in *Myology* (Engel, A. G. and C. Franzini-Armstrong eds.), pp. 176–199, McGraw-Hill, New York
- Takekura, H., Bennett, L., Tanabe, T., Beam, K. and Franzini-Armstrong, C. (1993) *Biophys. J.* **64**, 241a
- O'Brien, J., Meissner, G. and Block, B. A. (1993) *Biophys. J.* **65**, 2418–2427
- Gonzalez, A. and Rios, E. (1993) *J. Gen. Physiol.* **102**, 373–421
- Ma, J., Anderson, K., Shirokov, R., Levis, R., Gonzalez, A., Karhanek, M., Hosey, M. M., Meissner, G. and Rios, E. (1993) *J. Gen. Physiol.* **102**, 423–448
- Rios, E., Karhanek, M., Ma, J. and Gonzalez, A. (1993) *J. Gen. Physiol.* **102**, 449–481
- Ivanenko, A., McKemy, D. D., Kenyon, J. L., Airey, J. A. and Sutko, J. L. (1995) *J. Biol. Chem.* **270**, 4220–4223
- Takekura, H., Iino, M., Takekura, H., Nishi, M., Kuno, J., Minowa, O., Takano, H. and Noda, T. (1994) *Nature (London)* **369**, 556–559