Oligomerization of the cardiac ryanodine receptor C-terminal tail

Richard STEWART, Spyros ZISSIMOPOULOS and F. Anthony LAI¹

Wales Heart Research Institute, Department of Cardiology, University of Wales College of Medicine, Heath Park, Cardiff CF14 4XN, U.K.

The C-terminal 100 amino acids of the RyR (ryanodine receptor), referred to as the C-terminal tail, is a highly conserved sequence that is present in all known RyR isoforms and which has been implicated in channel function. Deleting the final 15 amino acids from the full-length skeletal muscle RyR resulted in an inactive channel, attributed to impaired assembly of a tetrameric RyR complex [Gao, Tripathy, Lu and Meissner (1997) FEBS Lett. 412, 223–226]. To account for these observations, the C-terminal tail itself may be an important molecular determinant of oligomerization. Alternatively, the large N-terminal cytoplasmic domain may fold back upon itself to interact with the C-terminal tail to provide a correctly folded tetrameric structure.

We explored these possibilities for RyR2 (cardiac RyR) using the yeast two-hybrid interaction assay and *in vitro* translation followed by immunoprecipitation and chemical cross-linking. The data indicate that the C-terminal tail of RyR2 is capable of self-tetramerization. Moreover, a truncated C-terminal tail, lacking the final 15 amino acids, failed to self-associate. These observations suggest that the intrinsic ability of the RyR C-terminal tail to self-tetramerize may be vitally important for the oligomeric assembly of the native RyR channel.

Key words: calcium signalling, cardiac muscle, excitation–contraction coupling, ion channel, oligomerization domain.

INTRODUCTION

The RyR (ryanodine receptor) and the *InsP₃*R (inositol 1,4,5-trisphosphate receptor) constitute distinct families of intracellular CRCs (calcium release channels) that mediate the release of calcium from intracellular stores. Structurally, both CRCs exist as tetrameric complexes [1–5], possessing a large N-terminal cytoplasmic domain and a smaller C-terminal membrane-spanning domain containing the putative pore region [6,7]. The two families of CRCs share limited sequence identity, with the extreme C-terminus (referred to here as the C-terminal tail) being the most highly conserved region. The C-terminal tail is localized on the cytoplasmic face of the sarcoplasmic reticulum/endoplasmic reticulum membrane [8–10] and is implicated in channel subunit oligomerization. For the *InsP₃*R, the emerging picture is that channel assembly is mediated primarily by sequences within the transmembrane-spanning domain [5,11,12], although deletion of the C-terminal tail of rat *InsP₃*R1 was shown to result in a mutant channel with decreased oligomerization efficiency [11]. The C-terminal tail of rat *InsP₃*R2 has been shown to self-interact, and also to interact with the C-terminal tails of rat *InsP₃*R1 and *InsP₃*R3, when assayed in the yeast two-hybrid interaction analysis system [11,13]. In contrast with the *InsP₃*R, the oligomeric assembly properties of the RyR have received little attention to date.

Molecular determinants of RyR oligomerization are thought to reside within both the N-terminal and C-terminal domains [14]. A deletion mutant of the rabbit skeletal muscle RyR, lacking the final 15 amino acids, was completely inactive [15]. This particular mutant did not bind [³H]ryanodine, exhibited no activity in single-channel recordings and sedimented at the top of a sucrose gradient. These observations were consistent with a mutant channel that was unable to assemble into a functional tetramer. Thus the C-terminal tails of both the RyR and the *InsP₃*R are clearly implicated in channel assembly.

We speculated that the role of the RyR C-terminal tail might be to facilitate a stable, correctly folded tetrameric structure through an interaction with the N-terminal domain. Alternatively,

self-association of the RyR C-terminal tail, as shown for the *InsP₃*R C-terminal tail, might constitute an important molecular determinant of oligomerization. Using human cardiac RyR cDNA [16], we generated a comprehensive series of constructs to address these questions. We used yeast two-hybrid analysis and biochemical approaches to show that self-tetramerization of the RyR C-terminal tail is likely to be the key means by which this region may facilitate channel oligomerization.

MATERIALS AND METHODS

Materials

Restriction endonucleases and DNA-modifying enzymes were obtained from Gibco–Invitrogen and Promega Corp. CHAPS was purchased from Roche Molecular Biochemicals, and zwittergent 3–14 was from Calbiochem. All other chemicals were obtained from Sigma.

Yeast two-hybrid analysis

Using human cardiac RyR2 16 kb cDNA as a template [16], overlapping fragments of the predicted N-terminal domain, the C-terminal tail and the predicted M6–M7 cytoplasmic loop [16,17] were generated by PCR using *Pfu* polymerase and oligonucleotide primers incorporating engineered restriction sites (Table 1). The RyR2 cDNA constructs generated, and their co-ordinates relative to full-length human RyR2 (4967 amino acid residues), are illustrated schematically in Figure 1(A). PCR products were digested with appropriate restriction enzymes and cloned into the bait vector, pGBKT7 (Clontech), as fusions to the Gal4 DNA-binding domain, to generate the series of bait (BT) constructs. Constructs were each confirmed by nucleotide sequencing. The coding sequences of the C-terminal tail (BT8) and the M6–M7 cytoplasmic region (BT1B) were also cloned into the prey vector, pACT2, as fusions with the activation domain in order to generate constructs AD8 and AD1B respectively. Protein interaction

Abbreviations used: CRC, calcium release channel; *InsP₃*R, inositol 1,4,5-trisphosphate receptor; RyR, ryanodine receptor.

¹ To whom correspondence should be addressed (e-mail LaiT@cardiff.ac.uk).

Table 1 Primer sequences

Construct	Primer	Primer sequence	Engineered restriction site
BT4	Forward	5' CGCGGAACCATGGCCGAT	<i>NcoI</i>
	Reverse	5' CTTCTCCACCTGGGTAT	Internal <i>BglII</i>
BT3	Forward	5' AGTTCATGGTGGACCACACAGAG	<i>NcoI</i>
	Reverse	5' CAGCTCCTCTATGCCATT	Internal <i>BamHI</i>
BT5	Forward	5' AGAACATATGCCTCTCTCGGCGGGA	<i>NdeI</i>
	Reverse	5' AAGTCTCGACTCAAAGCTGCCGTATCT	<i>Sall</i>
BT6	Forward	5' GTCGCATATGGAAGAAGCCATCAAAATC	<i>NdeI</i>
	Reverse	5' GCTAGTCAGAAATCTATAACAAG	<i>EcoRI</i>
BT7	Forward	5' CTGGAGAAGACCATGGAA	<i>NcoI</i>
	Reverse	5' ATAAGTCGACAGCCTGCTGTGTGCCAAA	<i>Sall</i>
BT1B	Forward	5' CAGCCCATGGTTAT TTTAAGCGAGAA	<i>NcoI</i>
	Reverse	5' CTCCTCGACAAAAGTTGCGAGCAAAATAGT	<i>Sall</i>
BT8	Forward	5' CCAGCCATGGATGCTTTTGGAGAACTA	<i>NcoI</i>
	Reverse	5' GTTACTCGAGACATCGCTGGCAATTCAC	<i>XhoI</i>
Δ15BT8	Forward	5' CCAGCCATGGATGCTTTTGGAGAACTA	<i>NcoI</i>
	Reverse	5' GCAATCCCCTGCTTAGAAAAATCCC	Vector-derived <i>XhoI</i>

Figure 1 (A) Schematic depiction of RyR constructs, and (B) results of yeast two-hybrid screening assay

(A) The RyR2 linear structure is shown schematically (top row), with the shaded region representing the cytoplasmic domain, the open region depicting the transmembrane domain and the striped region corresponding to the C-terminal tail. The various AD and BT constructs depicted underneath represent RyR2-derived fragments (numbers represent amino acid residues of RyR2) fused to the activation domain of vector pACT2 or the DNA-binding domain of vector pGBKT7. **(B)** Colony-lift β -galactosidase assays were performed with cells grown on selective medium. Development of a blue phenotype (weak, +; moderate, ++; strong, +++) constitutes a positive interaction. Only the C-terminal tail constructs (AD8 and BT8) showed any evidence of an interaction (+++, $n = 3$).

assays were performed using strain Y190 (Clontech) transformed according to the MATCHMAKER II protocol (Clontech).

C-terminal tail constructs

Construct BT8 encodes 100 residues from the extreme C-terminus of RyR2 (residues 4867–4967) and has a c-Myc epitope tag at the N-terminal residue (4867). A native, untagged C-terminal tail was generated by digesting construct AD8 (*NcoI/XhoI*) and cloning the coding sequence into vector pET-23d (*NcoI/XhoI*) to generate construct nCTT. Additionally, a truncated C-terminal tail, lacking the final 15 amino acids, was generated by PCR using mutant primers (Δ15BT8 Forward and Reverse; Table 1) and subcloned into vector pCR-Blunt II-TOPO (Invitrogen). The coding sequence was removed by restriction digestion (*NcoI/XhoI*) and

cloned into pGBKT7 in-frame with the c-Myc tag to generate construct Δ15BT8.

Cell-free synthesis

Cell-free protein synthesis of RyR fragments was performed by *in vitro* translation using the TNT Quick-T7 coupled rabbit reticulocyte lysate system (Promega) according to the manufacturer's protocols. For co-immunoprecipitation studies, 40 μ l of lysate was incubated with 1 μ g of each plasmid vector and 28 μ Ci of [³⁵S]methionine/[³⁵S]cysteine (Promix; Amersham) for 90 min at 30 °C. For cross-linking studies, 10 μ l of lysate was mixed with 1 μ g of plasmid vector and 14 μ Ci of Promix for 90 min at 30 °C.

Immunoprecipitation

Typically, 40 μ l of the *in vitro* translation reaction was divided into 4 $\times$ 10 μ l aliquots to be processed individually. Each aliquot was solubilized in 90 μ l of solubilization buffer (150 mM NaCl, 50 mM Tris/HCl, pH 8.0, 1 mM EDTA and 1 % CHAPS) and placed on ice for 30 min. Insoluble material was removed by centrifugation at 100 000 *g* for 30 min. Antibody was added to the supernatant, which was then incubated for 30 min on ice, mixed with 10 μ l of Protein G-agarose (Santa Cruz) and incubated for 2 h at 4 °C. Immunoprecipitates were collected by centrifugation and washed three times with solubilization buffer before being resuspended in 20 μ l of SDS/PAGE sample buffer (1 % SDS, 1 % β -mercaptoethanol, 0.1 M Tris/HCl, pH 7.0, and 5 % glycerol). All immunoprecipitation experiments were performed a minimum of three times.

Antibodies

Anti-c-Myc (Santa Cruz) recognizes the c-Myc epitope tag present in vector pGBKT7. Anti-RyR antibodies Ab2160, Ab2149 and Ab130 are rabbit polyclonals generated in our laboratory using previously published methods [16]. Ab2160 recognizes a consensus sequence at the extreme C-terminus of the RyR (amino acids 4951–4967 of RyR2). The epitope for Ab2149 spans amino acids 4934–4948 of RyR2, and Ab130 recognizes a cytoplasmic sequence (amino acids 4674–4697) within the predicted transmembrane-spanning domain [16].

Chemical cross-linking

Typically, 10 μ l of *in vitro* translation reaction was diluted in 340 μ l of cross-linking buffer (150 mM NaCl, 50 mM Tris/HCl pH 8.0, 5 mM imidazole). Haemoglobin was removed from the translation mixture prior to addition of cross-linking agent by incubating the translation reaction with charged Ni²⁺ affinity resin (Novagen). The cleared sample was then supplemented with CHAPS or zwittergent 3–14 to a final concentration of 1 % (final volume 400 μ l) and solubilized on ice for 30 min. Insoluble material was removed by centrifugation at 100 000 *g* for 30 min. The reaction mixture was mixed with 50 μ l of 0.1 M glutaraldehyde and incubated at room temperature. Aliquots of 40 μ l were removed at regular intervals following addition of glutaraldehyde. Unreacted glutaraldehyde was quenched with 10 μ l of 10 % hydrazine for 1 min. Samples were mixed with 25 μ l of SDS sample buffer (3 % SDS, 3 % β -mercaptoethanol, 0.15 M Tris/HCl, pH 7.0, and 15 % glycerol) and heated at 90 °C for 1 min, and aliquots of 25 μ l were removed for SDS/PAGE analysis. All cross-linking experiments were performed a minimum of three times.

SDS/PAGE and autoradiography

Polyacrylamide gels were prepared using the mini-Protein II system from Bio-Rad. Following electrophoresis, gels were fixed [40% (v/v) methanol and 10% (v/v) acetic acid] for 30 min, followed by a 15 min incubation with Amplify (Amersham). Gels were dried prior to fluorography and exposed to X-ray film (X-OMAT; Kodak) at -70°C . Exposure times for immunoprecipitation studies were typically 16–20 h. Cross-linking studies required longer exposures of between 2 and 4 days.

RESULTS AND DISCUSSION

Yeast two-hybrid assay

A series of overlapping RyR cDNA fragments that spanned the predicted N-terminal domain were constructed in the bait vector (pGBKT7) for use in a yeast two-hybrid screen to detect any protein–protein interactions with the RyR C-terminal tail (Figure 1A). A control construct AD1B, encoding a known cytoplasmic region located between putative transmembrane-spanning segments M6 and M7 [17], was also included. Correct expression of RyR2 protein fragments corresponding to each of the constructs in yeast cells was confirmed by immunoblot analysis (results not shown). The yeast two-hybrid screen results derived from colony-lift filter assays indicated that no protein–protein interaction occurred between the C-terminal tail of RyR2 (AD8) and any of the cytoplasmic domain constructs (BT3 to BT7; Figure 1B). In contrast, the 100-residue C-terminal tail construct was observed to be uniquely capable of a very robust self-interaction (AD8 with BT8). The construct encoding a predicted inter-transmembrane segment region, AD1B, showed no evidence of interaction with any of the constructs, and was employed subsequently as a control.

In vitro immunoprecipitation analysis

The positive result indicating RyR2 C-terminal self-interaction observed in the yeast two-hybrid assay was tested further by binding interaction experiments utilizing *in vitro* translation of RyR2 fragments. The construct BT8 used in yeast two-hybrid experiments, encoding the c-Myc-tagged RyR2 C-terminal tail, also contains a T7 promoter that can drive expression in a rabbit reticulocyte lysate system (Figure 1A). To enable use in co-immunoprecipitation studies, the c-Myc tag was removed by subcloning only the coding sequence of the C-terminal tail into vector pET-23d to generate nCTT, an untagged C-terminal tail construct. The presence of 1% CHAPS was found to greatly reduce background during immunoprecipitations. Functional, full-length RyR has been successfully solubilized using CHAPS concentrations greater than 1% [2,15]; thus inclusion of this detergent should not interfere with any potential interactions.

To determine presence of any *in vitro* interaction, BT8 and nCTT were co-translated, solubilized in 1% CHAPS and immunoprecipitated with an anti-c-Myc antibody. Figure 2 (upper panel) shows that the anti-c-Myc antibody was able to precipitate both the tagged construct BT8 and the untagged construct nCTT, implying that a specific protein–protein interaction occurred between these C-terminal fragments. Immunoprecipitations performed with Ab2160 (to the C-terminus) and Ab130 (to a transmembrane region) were used as positive and negative controls respectively.

To assess the possibility of an interaction between the C-terminal tail and the c-Myc tag, construct BT1B containing a c-Myc epitope tag was used as a negative control. No interaction of the C-terminal tail (AD8) with BT1B had been found in

Figure 2 Co-precipitation studies

Upper panel: co-precipitation of c-Myc-tagged C-terminal tail (BT8) and untagged C-terminal tail (nCTT). RyR2 constructs BT8 and nCTT were co-translated *in vitro* and reciprocal protein interaction was examined by immunoprecipitation with various antibodies and SDS/PAGE analysis (20% polyacrylamide gels). The positions of ^{35}S -labelled proteins were determined by fluorography. The immunoprecipitating antibodies are indicated above the gel. Ab2160 is specific for the extreme RyR C-terminus of both constructs and is a positive control for the presence of both proteins. Anti-c-Myc recognizes only BT8; thus co-precipitation of nCTT would indicate a specific BT8–nCTT interaction. Ab130 recognizes an epitope present only within construct BT1B, and represents a negative control. The right-hand lane (– for all antibodies) represents sample incubated with Protein G–agarose only. Co-precipitation of the untagged C-terminal tail by the anti-c-Myc antibody provides evidence for an association between BT8 and nCTT. Lower panel: lack of co-precipitation of c-Myc-tagged C-terminal tail (BT8) and control construct BT1B. RyR2 constructs BT8 and BT1B were co-translated *in vitro* and reciprocal protein interaction was examined as described above. The immunoprecipitating antibody is indicated above the gel. Anti-c-Myc recognizes the c-Myc epitope tag present in both constructs and is a positive control for the presence of both proteins. The C-terminus antibody Ab2160 is specific for BT8, while Ab130 recognizes only BT1B. The right-hand lane (– for all antibodies) represents sample incubated with Protein G–agarose only. There is no evidence of co-precipitation using either Ab2160 or Ab130, indicating that no interaction occurs between BT8 and BT1B.

the yeast two-hybrid assay (Figure 1B). When BT1B was co-translated with BT8, neither of the antibodies showed evidence of co-precipitation, indicating there was no association between BT8 and BT1B (Figure 2, lower panel). As BT1B was fused in-frame with the c-Myc tag, the data strongly suggest that the self-interaction of the C-terminal tail was specific, and not attributable to an association with the c-Myc tag.

Cross-linking studies

In order to determine the stoichiometry of the self-interaction of the C-terminus of RyR2, CHAPS-solubilized nCTT was translated *in vitro* and cross-linked with glutaraldehyde (Figure 3A). The predicted molecular mass of nCTT is 12 kDa, and in the absence of cross-linking agent nCTT was observed as a band migrating between the 7 and 17 kDa molecular mass markers, corresponding to a monomer. Within 5 min of adding glutaraldehyde, an additional higher-molecular-mass species was visible that migrated between the 17 and 32 kDa molecular mass markers. This was consistent with a dimeric form of nCTT with a

Figure 3 Chemical cross-linking of C-terminal tail (nCTT) and control construct BT1B

RyR2 constructs nCTT and BT1B were translated *in vitro* and cross-linked with glutaraldehyde as described in the Materials and methods section, followed by SDS/10%-PAGE analysis. The position of ^{35}S -labelled proteins was determined by fluorography. (A) In 1% CHAPS, the 12 kDa nCTT cross-links to higher-molecular-mass species that correspond to predicted multimers of the C-terminal tail (24 kDa dimer, 36 kDa trimer, ~48 kDa tetramer-doublet). (B) In the presence of 1% zwittergent 3-14, there is no evidence for cross-linked nCTT multimers. (C) In 1% CHAPS, there is no evidence for multimers of control construct BT1B, in contrast with nCTT. ori, origin.

predicted molecular mass of 24 kDa. Between 10 and 20 min a doublet appeared above the 42 kDa molecular mass marker, consistent with the formation of a tetrameric form of nCTT, which would have a predicted molecular mass of 48 kDa. A possible explanation for the presence of a doublet could be the formation of the nCTT tetramer cross-linked in two distinct forms, a linear and closed-circular arrangement, as has been found in studies of the Shaker potassium channel revealing self-tetramerization of the T1 domain [18]. Interestingly, after 30–40 min, a band migrating between the 32 and 42 kDa molecular mass markers became visible. This could be attributed to a trimeric species with a predicted molecular mass of 36 kDa. Notably, appearance of the trimer after formation of the doublet corresponding to the tetramer suggests that tetramer formation is likely to be the result of a dimer–dimer interaction.

The RyR [2] and the InsP₃R [5] are both known to form functional tetramers in the absence of covalent bonds, as indicated by the loss of tetrameric structure in the presence of the linear zwitterionic detergent, zwittergent. Hence we analysed whether multimerization of the C-terminal tail was also susceptible to disruption by treatment with zwittergent [2]. As shown in Figure 3(B), solubilization of nCTT in zwittergent 3–14 totally abolished the formation of multimers, consistent with the loss of oligomeric structure. Additionally, the cross-linking of control

Figure 4 Co-immunoprecipitation analysis of a truncated C-terminal tail ($\Delta 15\text{BT8}$) and a full-length C-terminal tail (BT8)

RyR2 constructs BT8 and $\Delta 15\text{BT8}$ were co-translated *in vitro* and reciprocal protein interaction was examined by immunoprecipitation with various antibodies and SDS/20%-PAGE analysis. The position of ^{35}S -labelled proteins was determined by fluorography. The epitopes recognized by anti-c-Myc and Ab2149 are present in both constructs and act as a positive control. Ab2160 recognizes only BT8. The right-hand lane (— for all antibodies) represents sample incubated with Protein G-agarose only. There is no evidence for co-precipitation of $\Delta 15\text{BT8}$ by Ab2160, indicating a lack of interaction between BT8 and $\Delta 15\text{BT8}$.

Figure 5 Chemical cross-linking of a truncated C-terminal tail ($\Delta 15\text{BT8}$)

RyR2 construct $\Delta 15\text{BT8}$ was translated *in vitro* and then cross-linked with glutaraldehyde as described in the Materials and methods section, followed by SDS/10%-PAGE analysis. The position of ^{35}S -labelled proteins was determined by fluorography. There is no evidence for multimers of the truncated C-terminal tail, indicating a lack of ability to assemble into high-molecular-mass species.

construct BT1B produced only monomers of the predicted molecular mass (15 kDa), indicating that this region is not capable of assembling into multimers (Figure 3C). Hence, in the presence of CHAPS, evidence for multimerization by chemical cross-linking was therefore specific to the C-terminal tail.

Deletion of C-terminal residues

Given the previous observation that a deletion mutant of full-length RyR1 lacking 15 amino acids of the C-terminal tail region appeared incapable of oligomerization [15], we were interested in assaying an identical deletion introduced into the 100-residue RyR2 C-terminal tail BT8 construct itself. The final 15 amino acids from BT8 were therefore deleted to generate construct $\Delta 15\text{BT8}$. This deletion completely removed the epitope recognized by Ab2160, and so Ab2160 could be used as the co-precipitating antibody when $\Delta 15\text{BT8}$ was co-translated with BT8. Immunoprecipitations using Ab2149 and anti-c-Myc antibody, epitopes for which are present on both constructs, were used as positive controls.

Upon co-translation of BT8 and $\Delta 15\text{BT8}$ followed by solubilization in 1% CHAPS, Ab2160 precipitated BT8, as expected, but failed to co-precipitate $\Delta 15\text{BT8}$ (Figure 4). This indicates that there is no protein–protein interaction detectable between the wild-type and C-terminal tail deletion constructs. Consistent with this result, attempts to chemically cross-link the CHAPS-solubilized $\Delta 15\text{BT8}$ with glutaraldehyde failed to produce any evidence for the existence of multimers (Figure 5).

Taken together, these results show that $\Delta 15\text{BT8}$ is unable to self-associate, resulting in an inability to form the tetramers observed for BT8. Thus the C-terminal deletion study indicates that the extreme 15 residues of the C-terminal tail may play an important role in oligomeric association.

Summary

In conclusion, the C-terminal tail of the cardiac RyR has been shown to self-assemble to form apparent tetramers using yeast two-hybrid analysis, *in vitro* co-immunoprecipitation and chemical cross-linking approaches. Self-tetramerization of the C-terminal tail implies that it may constitute part of the mechanism by which this region contributes to oligomerization of the channel subunits to form a functional tetrameric RyR complex. Furthermore, deletion of the final 15 amino acids from the C-terminal tail of RyR2 resulted in a loss of self-assembly, an observation that could provide an explanation for the functional data described previously for C-terminally truncated skeletal muscle RyR1 [15]. In that study, the finding that a full-length RyR1 mutant lacking the final 15 amino acids was apparently unable to assemble into a functional tetramer may therefore have been attributable to a loss of self-tetramerization of the truncated C-terminal tail.

Our yeast two-hybrid analysis failed to demonstrate an interaction between the RyR2 C-terminal tail and N-terminal domain constructs. However, given the large size and complex structural organization of the N-terminus, there remains the possibility that the C-terminal tail may associate with a region formed by a tertiary intra- or inter-molecular domain. Thus the data do not completely rule out the possibility of additional stabilizing interactions between the N-terminal domain and the C-terminal tail in the native RyR oligomeric complex.

This work was supported by British Heart Foundation grants PG/99087 and PG/03/039/155274 to F.A.L.

REFERENCES

- Lai, F. A., Erickson, H. P., Rousseau, E., Liu, Q. Y. and Meissner, G. (1988) Purification and reconstitution of the calcium release channel from skeletal muscle. *Nature (London)* **331**, 315–319.
- Lai, F. A., Misra, M., Xu, L., Smith, H. A. and Meissner, G. (1989) The ryanodine receptor calcium release channel complex of skeletal muscle sarcoplasmic reticulum. *J. Biol. Chem.* **264**, 16776–16785.
- Mignery, G. A. and Sudhof, T. C. (1990) The ligand binding site and transduction mechanism in the inositol-1,4,5-trisphosphate receptor. *EMBO J.* **9**, 3893–3898.
- Maeda, N., Kawasaki, T., Nakade, S., Yokota, N., Taguchi, T., Kasai, M. and Mikoshiba, K. (1991) Structural and functional characterization of inositol 1,4,5-trisphosphate receptor channel from mouse cerebellum. *J. Biol. Chem.* **266**, 1109–1116.
- Mignery, G. A., Newton, C. L., Archer, B. T. and Sudhof, T. C. (1990) Structure and expression of the rat inositol 1,4,5-trisphosphate receptor. *J. Biol. Chem.* **265**, 12679–12685.
- Bhat, M. B., Zhao, J. Y., Takeshima, H. and Ma, J. (1997) Functional calcium release channel formed by the carboxy-terminal portion of ryanodine receptor. *Biophys. J.* **73**, 1329–1336.
- Sayers, L. G., Miyawaki, A., Muto, A., Takeshita, H., Yamamoto, A., Michikawa, T., Furuichi, T. and Mikoshiba, K. (1997) Intracellular targeting and homotetramer targeting of a truncated inositol 1,4,5-trisphosphate receptor-green fluorescent protein chimera in *Xenopus laevis* oocytes: evidence for the involvement of the membrane-spanning domain in endoplasmic reticulum targeting and homotetramer complex formation. *Biochem. J.* **323**, 273–280.
- Grunwald, R. and Meissner, G. (1995) Lumenal sites and C-terminus accessibility of the skeletal muscle calcium release channel (ryanodine receptor). *J. Biol. Chem.* **270**, 11338–11347.
- Marty, I., Villaz, M., Arlaud, G., Bally, I. and Ronjat, M. (1994) Transmembrane orientation of the N-terminal and C-terminal ends of the ryanodine receptor in the sarcoplasmic reticulum of rabbit skeletal muscle. *Biochem. J.* **298**, 743–749.
- Mikoshiba, K., Furuichi, T. and Miyawaki, A. (1994) Structure and function of IP3 receptors. *Semin. Cell Biol.* **5**, 273–281.
- Galvan, D. L., Borrego-Diaz, E., Perez, P. J. and Mignery, G. A. (1999) Subunit oligomerization and topology of the inositol 1,4,5-trisphosphate receptor. *J. Biol. Chem.* **274**, 29483–29492.
- Miyawaki, A., Furuichi, T., Ryou, Y., Yoshikawa, S., Nakagawa, T., Saitoh, T. and Mikoshiba, K. (1991). Structure-function relationships of the mouse inositol 1,4,5-trisphosphate receptor. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 15821–15825.
- Magnino, F., Schmidt, K., Mery, L. and Dufour, J.-F. (2001) Rat inositol 1,4,5-trisphosphate receptor isoform 2 interacts with itself in its C-terminal portion and upstream of the first transmembrane domain. *Eur. J. Biochem.* **268**, 5981–5988.
- Chen, S. R. W., Airey, J. A. and MacLennan, D. H. (1993) Positioning of major tryptic fragments in the Ca^{2+} release channel (ryanodine receptor) resulting from partial digestion of rabbit skeletal muscle sarcoplasmic reticulum. *J. Biol. Chem.* **268**, 22642–22649.
- Gao, L., Tripathy, A., Lu, X. and Meissner, G. (1997) Evidence for a role of C-terminal amino acid residues in skeletal muscle Ca^{2+} release channel (ryanodine receptor) function. *FEBS Lett.* **412**, 223–226.
- Tunwell, R. E. A., Wickenden, C., Bertrand, B. M. A., Shevchenko, V. L., Walsh, M. B., Allen, P. D. and Lai, F. A. (1996) The human cardiac muscle ryanodine receptor-calcium release channel: identification, primary structure and topological analysis. *Biochem. J.* **318**, 477–487.
- Zorzato, F., Fujii, J., Otsu, K., Phillips, M., Green, N. M., Lai, F. A., Meissner, G. and MacLennan, D. H. (1990) Molecular cloning of cDNA encoding human and rabbit forms of the Ca^{2+} release channel (ryanodine receptor) of skeletal muscle sarcoplasmic reticulum. *J. Biol. Chem.* **265**, 2244–2256.
- Pfaffinger, P. J. and DeRubeis, D. (1995) Shaker K^{+} channel T1 domain self-tetramerizes to a stable structure. *J. Biol. Chem.* **270**, 28595–28600.

Received 22 April 2003/22 August 2003; accepted 8 September 2003

Published as BJ Immediate Publication 8 September 2003, DOI 10.1042/BJ20030597