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Structural understanding of the transmembrane domains of inositol triphosphate receptors and ryanodine receptors towards calcium channeling

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Inositol 1,4,5-triphosphate receptors ($Insp_3Rs$) and ryanodine receptors (ryRs) act as cationic channels transporting calcium ions from the endoplasmic reticulum to cytosol by forming tetramers and are proteins localized to the endoplasmic reticulum (ER). Despite the absence of classical calcium-binding motifs, calcium channeling occurs at the transmembrane domain. We have investigated putative calcium binding motifs in these sequences. Prediction methods indicate the presence of six transmembrane helices in the C-terminal domain, one of the three domains conserved between $Insp_3R$ and ryR receptors. The recently identified crystal structure of the K^+ channel, which also forms tetramers, revealed that two transmembrane helices, an additional pore helix and a selectivity filter are responsible for selective K^+ ion channeling. The last three TM helices of $Insp_3R$ and ryR are particularly well conserved and we found analogous pore helix and selectivity filter motif in these sequences. We obtained a three-dimensional structural model for the transmembrane tetramer by extrapolating the distant structural similarity to the K^+ channels.

Keywords: cation channels/distant structural similarity/superfamily/three-dimensional modelling/transmembrane domain

Introduction

The release of intracellular Ca^{2+} is an intermediate step in many cellular signaling processes (Berridge and Irvine, 1989; Tsein and Tsein, 1990). In vertebrates, two classes of proteins, the inositol 1,4,5-triphosphate receptor ($Insp_3R$) and the ryanodine receptor (ryR), act as channels for the release of intracellular Ca^{2+}. $Insp_3R$ causes release of intracellular Ca^{2+} in response to Ip3 which is generated during signaling mechanisms that involves the activation of phospholipase C (Majerus *et al.*, 1985). This signal transduction pathway is used in processes as diverse as the response to hormones, growth factors and neurotransmitters (Berridge and Irvine, 1984) and in various sensory systems such as olfaction (Reed, 1992), gustation (Hwang *et al.*, 1990) and vision (Payne *et al.*, 1988) [for a recent review, see Patel *et al.* (Patel *et al.*, 1999)]. The $Insp_3R$ pathway must also function in the central brain, the tissue from which it was initially purified and cloned (Furuichi *et al.*, 1989; Mignery *et al.*, 1990).

Ryanodine receptor function is best understood in vertebrate skeletal muscle. It is required for the intracellular Ca^{2+} release that occurs prior to muscle contraction, in response to nerve impulses delivered to the muscle plasma membrane (Caterall, 1991). The other two ryR isoforms are often referred to as the 'heart' and 'brain' forms, but the cellular and tissue distribution of the isoforms is more complex than is suggested by this nomenclature [reviews are available (Coronado *et al.*, 1994; Meissner, 1994; Striggow and Ehrlich, 1996)]. Functional studies have shown that the channel may be regulated by various endogenous effector molecules including Ca^{2+}, ATP, cADP ribose and calmodulin, depending upon the isoforms. In addition, both $Insp_3R$ and ryR have been postulated to function during Ca^{2+}-induced Ca^{2+} release in neuronal and non-neuronal tissues requiring Ca^{2+} oscillations (Tsein and Tsein, 1990). The presence of these intracellular Ca^{2+} channels in such diverse tissues indicates that they are likely to be involved in many different cellular functions. Calcium is known to be a regulator of both the receptor channels, although no specific binding motifs are known. Both $Insp_3R$ and ryR are poorly selective and high-conductance Ca^{2+} channels. The estimated permeability ratio (divalent/monovalent) of both the receptors is nearly six (Tinker and Williams, 1992; Bezprozvanny and Ehrlich, 1994). The skeletal muscle ryR has been visualized using cryomicroscopy and angular reconstitution at 30 Å, which showed the structure of the entire system to be a mushroom-shaped tetramer with the transmembrane domain being a part of the stem (Serysheva *et al.*, 1995). Obtaining the detailed molecular structure of these assemblies by X-ray crystallographic or NMR techniques is challenging owing to their membrane-spanning regions and large dimensions (molecular weight 4×450 kDa).

Detailed three-dimensional structures are not available for either of these two classes of receptors. We have carried out an analysis of several $Insp_3R$ and ryR sequences with a view to identifying residues important for calcium binding. We have particularly focused on the transmembrane domain, which is involved in Ca^{2+} channeling. The ryR and $Insp_3R$ share sequence homology in parts (see below) and have the same quaternary structure (Wagenknecht and Radermacher, 1997). Both $Insp_3R$ and ryR occur as homotetramers where the protomers contain ~3000 and ~5000 amino acids, respectively (Mignery *et al.*, 1989; Serysheva *et al.*, 1995; Galvan *et al.*, 1999). It is well known that both the receptors share high sequence similarity at the C-terminal TM domain (Mignery and Sudhof, 1993; Galvan *et al.*, 1999; Ramos-Franco *et al.*, 1999). PRODOM (Corpet *et al.*, 1999) records the N-terminal domain (domain id PD001922) of around 550 amino acids with ip3r_mouse-numbering 143–671 and rynr_human-numbering 180–650 to be similar. The N-terminal domain in the case of $Insp_3R$ was shown to be the ligand-binding domain (Mignery and Sudhof, 1990; Miyawaki *et al.*, 1991). Furthermore, a middle domain of 168 amino acids (domain id PD002036; ip3r_mouse-numbering 1963–2131 and rynr_human-numbering 3751–4123) shares high sequence similarity among $Insp_3Rs$ and ryRs. The C-terminal transmembrane domain is divided into more than one domain according to PRODOM and a region of around 300 amino acids (domain id PD001555; ip3r_mouse-numbering 2382–2674 and rynr_human-numbering 4612–5032) shares relatively high sequence

similarity (36% sequence identity). Immuno-gold electron microscopy data (Mignery *et al.*, 1989) and glycosylation data (Michikawa *et al.*, 1994) demonstrate the positions of the N- and C-termini and the location of particular loops with respect to the cytoplasm, respectively; however, knowledge of the number, location and boundaries of the TM helices is valuable.

The main objective of this paper is to discuss the similarity between the two receptors, its implications on receptor regulation by calcium and the overall structure of the channel-forming domain of the receptors. Structure prediction studies on the transmembrane region of $Insp_3R$ and ryR have been pursued which emphasize the fact that the C-terminal parts of the TM domains of the two classes of receptors, constituting the last three TM helices, share the highest similarity. This paper also reports a novel attempt at the recognition of the minimum channel requirements in $Insp_3R$ and ryRs: two transmembrane helices, the channel pore-helix and selectivity filter as observed in the potassium channels. The strong similarity between these receptors and the K^+ channels has allowed the construction of a three-dimensional structural model of the C-terminal, structurally conserved helices of the transmembrane region despite differences in pore diameter and direction of ion transfer. These findings are supported by experiments using sequence analysis (Galvan *et al.*, 1999) reported subsequent to our observations.

Materials and methods

The sequences of ip3r_rat, rynr_human and ip3r_drome were extracted from the SWISSPROT protein sequence database (Appel *et al.*, 1994). BLAST searches (Altschul *et al.*, 1997) were performed in the PRODOM database. Sequences were aligned using the multiple alignment program CLUSTALW (Thompson *et al.*, 1994). Multiple methods were employed for structure prediction: PHD (Rost *et al.*, 1995), PREDATOR (Frishman and Argos, 1997), JPRED (Cuff *et al.*, 1998) were used to perform secondary structure prediction; for the prediction of membrane-spanning regions, PERSCAN (Donnelly *et al.*, 1994), PHD (Rost *et al.*, 1995), HMMTOP (Tusnády and Simon, 1998), TMHMM (Sonnhammer *et al.*, 1998), TMPRED (Hofmann and Stoffel, 1993), SOSUI (Hirokawa *et al.*, 1998) and TOPPRED II (Claros and von Heijne,

Table I. Putative calcium binding sites in inositol triphosphate receptors ($Insp_3R$) and ryanodine receptors (ryR)

Amino acid from[a]	Amino acid to[a]	Linear sequence[d]	Comment
97	107	**DLEKKQNETEN**	
228[b]	255	**D**NK**D**DILKGG**D**VVRLFHAEQ**E**KFLTC*D*E	Found conserved in domain 1922[e]
317[bc]	381	EV**D**PDF*EEE*CL**E**FQPSVDP*D*QDASRSRLRNA QEKMVYSLVSVP**E**GN*D*ISSIF*E*L**D**PTTLRGG *D*SL	Found conserved in domain 1922[e]
378[bc]	450	*D*SLVPRNSYVRLRHLCTNTWVHSTNIPI*D*K**E** **E**EKPVMLKIGTSPLK**ED**KEAFAIVPVSPAEV R*DLD*FAN**D**AS	Found conserved in domain 1922[e]
528[b]	544	*D*CG**D**GPMLRL**EE**LG**D**Q	Found conserved in domain 1922[e]
660[bc]	733	TNA**D**ILIETKLVLSRFEFEGVSTGENALEAG ED**EEE**VWLFWRDSNK**E**IRSKSVRELAQ**D**AK EGQKE**D**RDILSYY	Found at boundary of domain 1922
741[c]	849	ARMCL**D**RQYLAINEISGQLDV**D**LILRCMS**D** **E**NLPY**D/D**R**D**PQ**E**QVTPVKYARLWS**E**IPSEI AID**D**Y**D**SSGTSKDEIKERFAQTMEFV**EE**YLR DVVC	
994[c]	1059	LCIFKR**E**FD**E**SNSQSS**E**TSSGNSSQEGPSNVP GAL**DF**EHIEEQAEGIFGGSEENTPLDL**D**DHG GRT	
1107	1121	Q**D**V**D**NYKQIKQ**D**L**D**Q	
1140	1157	**D**EPMDGASGENEHKKTEE	Unstructured charged loop
1347[c]	1426	DRASFQTLIQMMRSER**D**RM**DE**NSPLMYH IHLV**E**LLAVCT**E**GKNVYTEIKCNSLLPL**DD** IVRVVTH**ED**CIP**E**VKIAYINFL	
1685	1719	**D**RGYG**E**KQISI**D**ES**E**NA**E**LPQAP**E**A**E**NST**E** Q**E**L**E**P	
2124[bc]	2146	IKKAYMQGEVEFEDGENGEDGAA	Found at boundary of domain 2036 and replaced by two EF-hands in RyR, unstructured loop
2178	2186	QVDGDEALE	Unstructured charged loop
2463[c]	2528	K**DD**FILEV**D**RLPNETAVPETG**E**SLANDFLY SDVCRVETG**E**NCTSPAPKE**E**LLPAEETEQD KEHTC**E**	Part of luminal loop, domain 1555. Replaced by charged region in RyR
2589[b]	2604	DTFADLRSEKQKK*E*E	Found conserved in domain 1555

[a]Corresponds to ip3r_mouse residue numbering.
[b]Stretch of residues is found conserved in both Insp3R and ryR.
[c]Stretch of residues is reported to bind calcium (Sienaert *et al.*, 1996, 1997).
[e]Domain 1922 is N-terminal, which is reported to be the ligand binding domain in Insp3R (Miyawaki *et al.*, 1991).
[d]The symbol / indicates a gap in the sequence. Amino acids in bold letters indicate the conserved charged residues, when both families are compared. Conservation only in $Insp_3R$ is shown in italics. The domain numbering is as follows: domain 1922 corresponds to N-terminal residues 180–650, domain 2036 corresponds to middle region of residues 1963–2131 and domain 1555 corresponds to C-terminal region of residues 2382–2674 (numbering according to ip3r_mouse).

1994) were employed. The comparative modeling program COMPOSER (Sutcliffe *et al.*, 1987; Blundell *et al.*, 1988; Srinivasan and Blundell, 1993) was used to derive the three-dimensional structure of the last two TM helices of $Insp_3R$ and ryRs. The tetramer coordinates were obtained by means of rigid-body superposition from the K^+ channel tetramer coordinates using the program SUPER (B.S.Neela, personal communication). The protomers were moved systematically away from the pore axis [calculated by SCHELAX (Chou *et al.*, 1984; Sowdhamini *et al.*, 1992)] by 1.5 Å to suit the reported dimensions of Ca^{2+} channels.

Results and discussion

Sequence alignment between two receptors and its implications for calcium binding and channel domain

Twelve $Insp_3R$ sequences and 13 ryR sequences were chosen and aligned at the membrane-traversing transmembrane (TM)

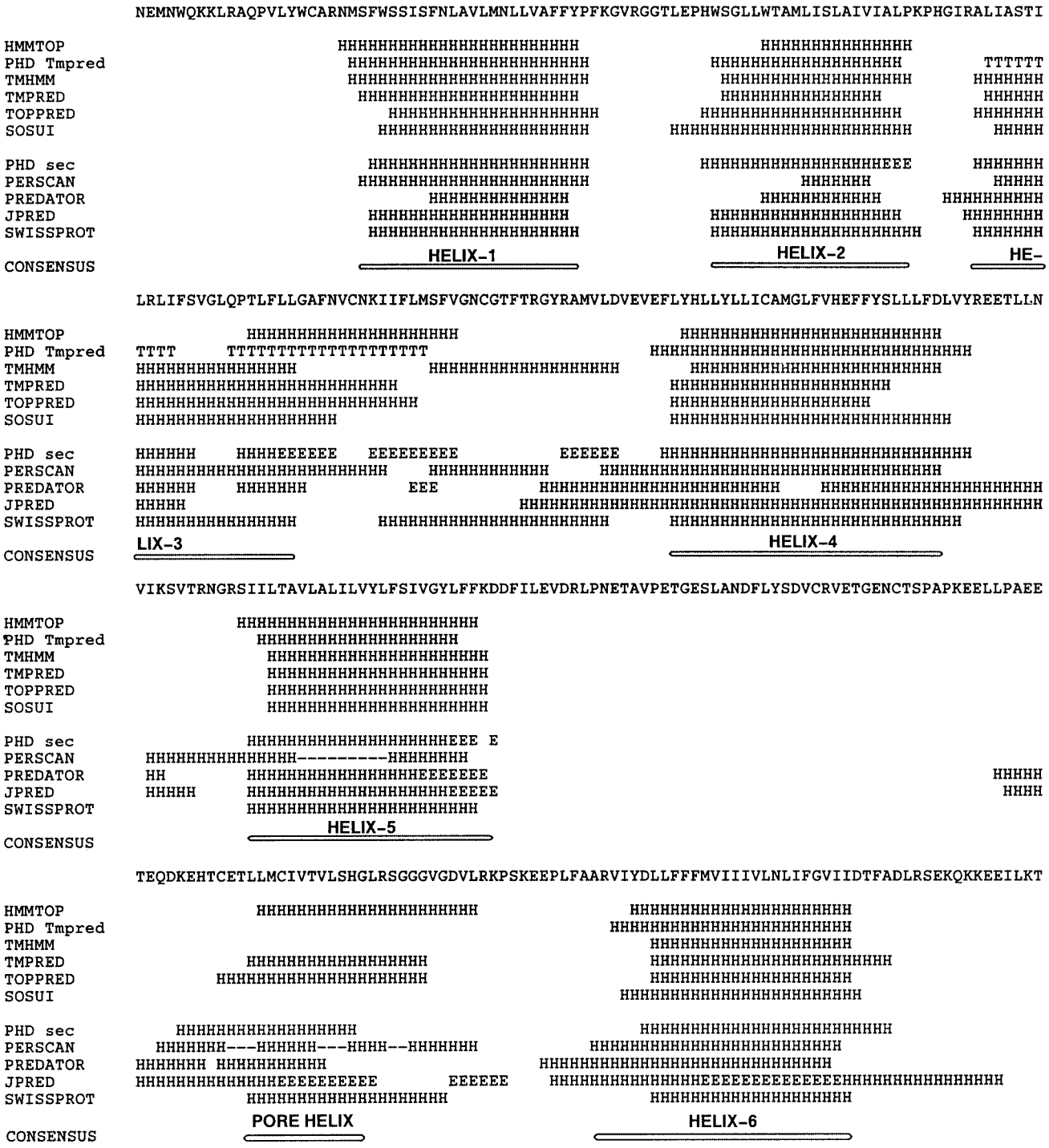

Fig. 1. Consensus secondary structure prediction of transmembrane (TM) helices in the C-terminal domain of inositol triphosphate receptor ($Insp_3R$) sequence. Various methods used for predicting TM helix positions are mentioned (see Materials and methods for details). Most methods identify six TM helices but a few of them predict an additional shorter helix, termed the pore helix, before the last TM helix.

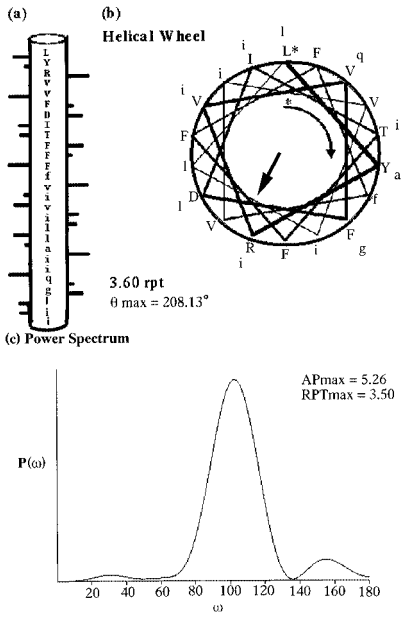

Fig. 2. Helix-wheel projection of the sixth TM helix using the PERSCAN method (Donnelly *et al.*, 1994). The helix positions are predicted by the periodicity and pattern in the occurrence of hydrophobic residues. (**a**) The occurrence of amino acids along the predicted TM helix is shown schematically, where the helix is shown as a cylinder. (**b**) The distribution of conserved residues on the predicted TM helix is projected down the helix axis. Several hydrophobic residues are distributed around the putative TM helix. (**c**) Fourier transform of this periodicity corresponds to an angle of 100°, consistent with the prediction of an α-helix.

domain. The multiple alignment of $Insp_3R$ and ryR sequences show the presence of several conserved negatively charged residues (Table I) which could act as Ca^{2+} binding sites. While studying Ca^{2+} regulation of $Insp_3R$ receptor at the molecular level and the structural determinants of Ca^{2+} binding, Sienaert and co-workers (Sienaert *et al.*, 1996, 1997) identified eight linear sites which were shown to bind both calcium and ruthenium red (see Table I). Of the eight sites, three are in regions where the two classes of receptors share high sequence identity. The regulatory calcium binding sites are therefore novel conserved motifs. Two EF-hand Ca^{2+} binding domains have been identified in lobster skeletal muscle ryR (Xiong *et al.*, 1998), corresponding to 4070–4130 of rynr_human, which are at the boundary of the middle domain conserved between $Insp_3R$ and ryR receptors. $Insp_3R$, however, does not contain an equivalent EF-hand motif, but is replaced by an aspartate–glutamate-rich region (2124–2146 of ip3r_mouse) which was shown to bind Ca^{2+} (Sienaert *et al.*, 1997). Conversely, a region from ip3r_mouse (amino acids 2463–2528), which is a segment of the C-terminal domain shown to bind Ca^{2+}, is replaced by a highly aspartate- and glutamate-rich region in rynr_human.

Prediction studies and analysis of C-terminal region of Insp3R and ryR

Recently, a structure of the tetrameric K^+ channel (Doyle *et al.*, 1998) from *Streptomyces lividens* was reported, revealing many mysteries about the channel structures that had intrigued physiologists for many decades. Apart from two membrane-spanning helices, the loop region connecting the two helices (P-loop) forms the selectivity filter. The N-terminal region of the P loop is also α-helical (which is termed pore helix), slanting towards the pore axis from outside. The helix is followed by a signature sequence: five amino acids in this zone, corresponding to VGYGD, form the lining of the selectivity filter orienting their main chain carbonyls towards the pore axis and their side chains outwards, thus stabilizing the right ions of the desired pore size. Sequence alignments from various K^+ channels, both inward and outward rectifiers, show that most of the residues of the pore helix and signature sequence are conserved (Armstrong, 1998; Doyle *et al*, 1998; MacKinnon *et al.*, 1998), suggesting that the architecture of the channels is similar, irrespective of the direction of ion transfer. Moreover, two membrane-spanning helices per monomer would be the minimum requirement and sufficient for forming the functional channel tetramer.

Prediction studies were carried out on the sequences of one $Insp_3R$ and one ryR, to map the putative transmembrane region on both the receptors. Various transmembrane region prediction methods available on SWISSPROT server (www.expasy.ch) were employed. The results from various methods with the predicted positions of the transmembrane helices are shown in Figure 1 for ip3r_mouse sequence. We confirmed this result by applying these methods to the KcsA sequence, where all methods miss the pore helix. The helix-wheel diagram is shown in Figure 2 for the region predicted to contain the sixth TM helix of ip3r_mouse by PERSCAN (Donnelly *et al.*, 1994). It is clear from the prediction studies reported here that $Insp_3R$ contains a topology of six membrane-spanning helices.

Prediction analysis was also performed for the ryanodine receptors, where PREDICTPROTEIN, which employs multiple sequence alignments, suggests six transmembrane helices and a pore helix. The pore helix is predicted in the loop region between the putative fifth and sixth membrane-spanning helices of the receptors, which is known to be analogous to the P loop of voltage-activated Ca^{2+}, Na^+ and K^+ channels (Mignery and Sudhof, 1993). It is also implicated to be the pore-forming segment (Balshaw *et al.*, 1999).

Figure 3 shows the multiple sequence alignment of the region containing the putative last three helices of both the receptors where the highest sequence similarity extends to a further 100 amino acids towards the C-terminus (36% sequence identity). The predicted helix positions and certain conserved amino acid positions are indicated. This is also in agreement with deletion studies on $Insp_3R$ which demonstrate that the deletion of the first four TM helices of recombinant $Insp_3R$ forms functional calcium channels and mutants lacking the last two helices do not form detectable channels (Ramos-Franco *et al.*, 1999).

Location of calcium permeation pathway and molecular structure of the channel-forming region

From the above discussion and the sequence alignment shown in Figure 3, it is clear that the conserved C-terminal region also contains the predicted pore helix, which has a length of 10 amino acid residues. Following the pore helix, a motif, GXRXGGGXGD (starting from 4820 of ryRs and 2540 of $Insp_3Rs$), is found to be highly conserved in all known $Insp_3Rs$ and ryRs. Mutation of glycine to alanine in this signature sequence in ryR, at the first, fourth and sixth positions, disrupts the calcium release from the channel (Zhao *et al.*, 1999). Also, the isoleucine to threonine mutation of ryR-1 (see Figure 3) decreases the threshold of Ca^{2+} required to initiate opening of the wild-type channel and results in a reduced release of Ca^{2+} from internal stores (Balshaw *et al.*, 1999; Lynch *et al.*,

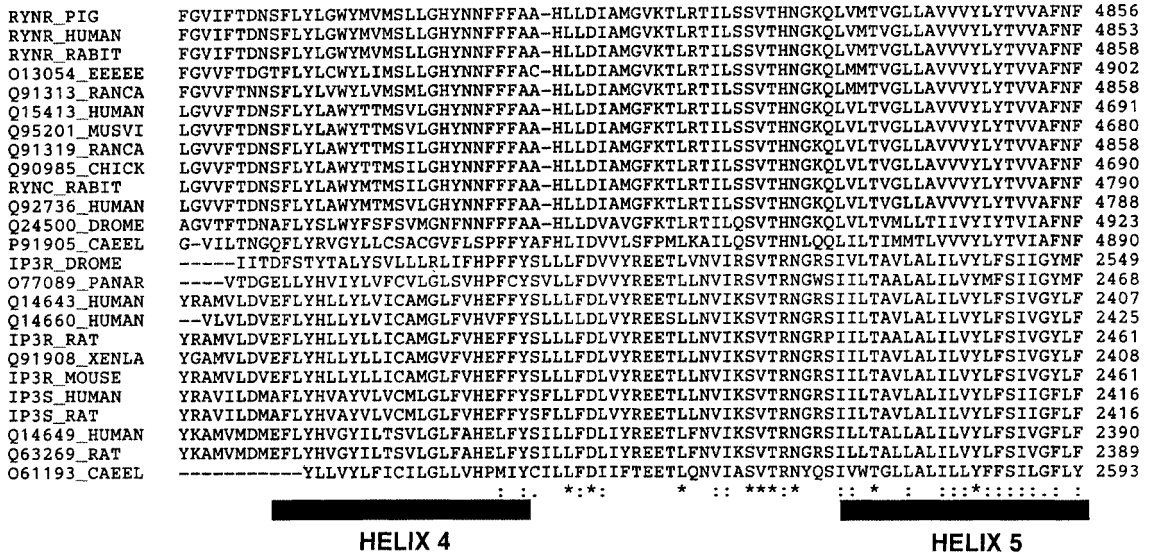

Fig. 3. *Continued overleaf*

1999). These data suggest that this conserved region constitutes channel conduction pathway or the central pore lining of this receptor (Zhao *et al.*, 1999), reaffirming that the same topology is present in the channel-forming region as in the KcsA K^+ channel, viz. fifth helix, pore helix, pore-lining region and sixth helix.

It is anticipated that Ca^{2+} channels have pores that are related architecturally to K^+ channels (Doyle *et al.*, 1998; Roux and MacKinnon 1999). In this paper, we report the three-dimensional structure of ryR human TM domain using the KcsA structure as the template and by employing the COMPOSER homology modeling program (Sutcliffe *et al.*, 1987; Blundell *et al.*, 1988; Srinivasan and Blundell, 1993). The transmembrane helices, pore helix and selectivity filter region are considered as structurally conserved regions (SCRs) and the resulting structure is energy minimized with a fixed backbone conformation. The tetramer positions of the calcium channel are generated from the K^+ channel tetramer by a structure superposition program called SUPER (B.S.Neela, personal communication). Figure 4a shows a ribbon diagram of the tetramer model of ryR human derived by such comparative modeling studies. The presence of leucines and other hydrophobic residues in two adjacent protomers at their interface (see Figure 4a) might account for the stability of the tetramer.

Owing to the difference in size of the cations and the mechanism of cation conduction, it is obvious that the structural parameters are different for the ryR Ca^{2+} channels than the K^+ channels. Ryanodine receptors are reported to have a pore diameter of ~6–7 Å (McCleskey and Almers, 1985; Tinker and Williams, 1993; Serysheva *et al.*, 1999). The length of the selectivity filter region is found to be 10.4 Å (Tinker and Williams, 1993, 1995), which is in good agreement with the KcsA selectivity filter length of 12 Å. Before the structure of the K^+ channel was determined, the experimental value of the selectivity filter of the K^+ selective channel was approximated to 10 Å (Miller, 1982). Blocking studies with the impermeant charged derivative of triethylamine reveal that this narrowing occurs over the first 10–20% of the voltage drop when crossing from the lumen of SR to the cytoplasm, showing that the narrow region (selectivity filter) occurs at the luminal mouth of the channel. Therefore, the tetramer of the TM domain was moved away from the pore axis to suit the reported structural parameters. By monitoring the interprotomer interactions

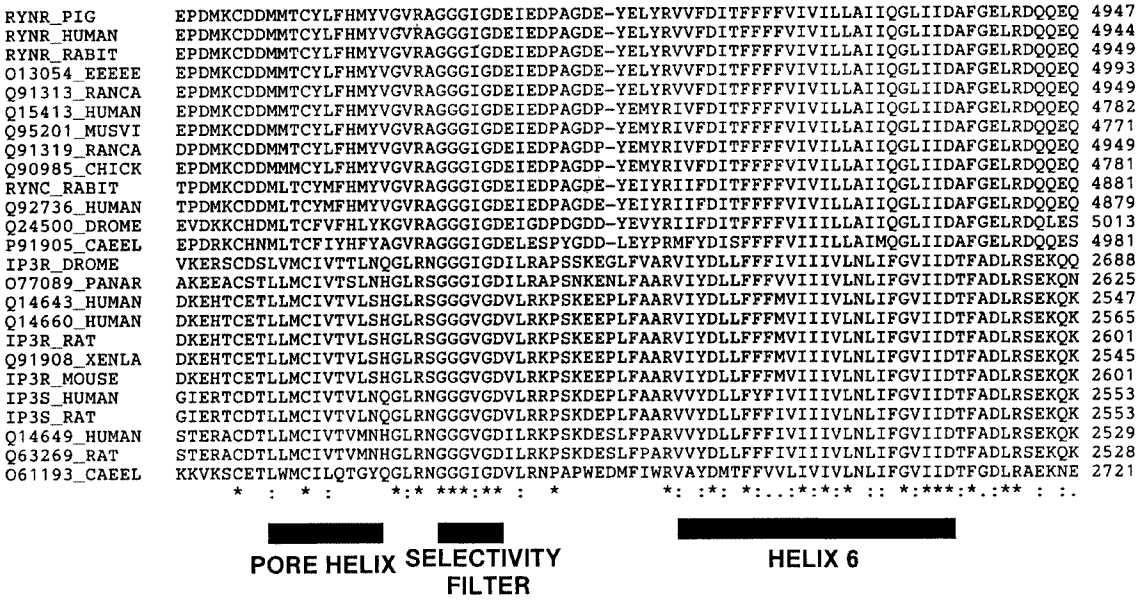

Fig. 3. Multiple sequence alignment of various $Insp_3Rs$ and ryanodine receptors (ryRs) corresponding to the region of the C-terminal, transmembrane (TM) domain that has the highest sequence conservation across the two families (RYNR_PIG, ryanodine receptor, *Sus scrofa* skeletal muscle; RYNR_HUMAN, ryanodine receptor, *Homo sapiens* skeletal muscle; RYNR_RABIT, ryanodine receptor, *Oryctolagus cuniculus* skeletal muscle; O13054_EEEEE, ryanodine receptor ryr1 isoform, *Makaira nigricans*; Q91313_RANCA, α-ryanodine binding protein, *Rana catesbeiana*; Q15413_HUMAN, ryanodine receptor 3, *Homo sapiens* brain; Q95201_MUSVI, ryanodine receptor type 3, *Mustela vison*; Q91319_RANCA, β-ryanodine binding protein, *Rana catesbeiana*; Q90985_CHICK, ryanodine receptor type 3, *Gallus gallus*; RYNC_RABIT, ryanodine receptor, *Oryctolagus cuniculus* cardiac muscle; Q92736_HUMAN, ryanodine receptor 2, *Homo sapiens* cardiac muscle; Q24500_DROME, ryanodine receptor, *Drosophila melanogaster*; P91905_CAEEL, ryanodine receptor, *Caenorhabditis elegans*; IP3R_DROME, inositol 1,4,5-triphosphate binding protein receptor, *Drosophila melanogaster*; O77089_PANAR, inositol 1,4,5-triphosphate receptor, *Panulirus argus*; Q14643_HUMAN, human type 1 inositol 1,4,5-triphosphate receptor, *Homo sapiens*; Q14460_HUMAN, inositol 1,4,5-triphosphate receptor type 1, *Homo sapiens*; IP3R_RAT, inositol 1,4,5-triphosphate binding protein type 1 receptor, *Rattus norvegicus*; Q91908_XENLA, inositol 1,4,5-triphosphate receptor, *Xenopus laevis*; IP3R_MOUSE, inositol 1,4,5-triphosphate binding protein type 1 receptor, *Mus musculus*; IP3S_HUMAN, inositol 1,4,5-triphosphate binding protein type 2 receptor, *Homo sapiens*; IP3S_RAT, inositol 1,4,5-triphosphate binding protein type 2 receptor, *Rattus norvegicus*; Q14649_HUMAN, type 3 inositol 1,4,5-triphosphate receptor, *Homo sapiens*; Q63269_RAT, inositol triphosphate receptor subtype 3, *Rattus norvegicus*; O61193_CAEEL,E f33d4.2a protein, *Caenorhabditis elegans*). The predicted TM helices 4, 5 and 6 are marked. The positions of the predicted functional motifs, the pore helix and selectivity filter are also indicated. Analogous motifs are shown to form the cationic pathway in K^+ channels (Doyle *et al.*, 1998).

before and after the change in pore dimensions, we find that no major destabilization occurs owing to the slight increase in pore diameter. The tetramer model has a pore diameter of ~6 Å and a selectivity filter length of ~11 Å, corresponding with the functioning calcium channels, and satisfies most of the properties of calcium channels (Nonner and Eisenberg, 1998). Our model is in agreement with the current theory of calcium permeation through large pores, which have larger diameters than their preferred ions. The calcium ion is attracted by the negatively charged residues concentrated at the mouth of the pore (Figure 4b), passes through the selectivity filter region, composed of the conserved motif GGGIGD, which occurs at the luminal mouth of the channel, and passes through the remainder of the pore. This narrow selectivity region is relatively short, which is consistent with the large conductance of the channel (Latorre and Miller, 1983). The membrane-spanning helices form the hydrophobic walls and the pore helices which are pointing towards the central axis of the pore

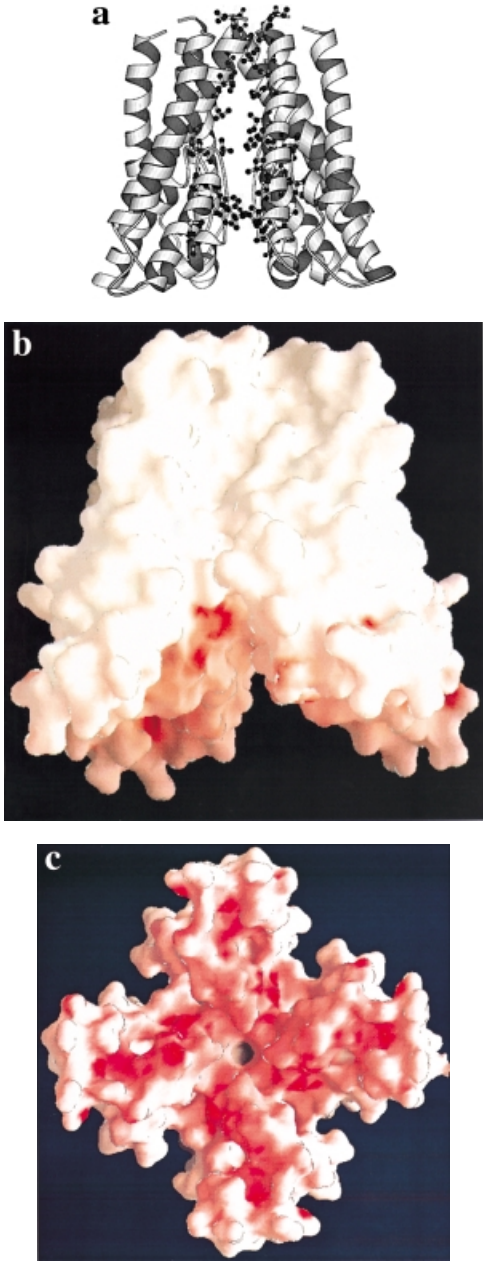

Fig. 4. Three-dimensional model of the last two predicted TM helices of human ryanodine receptor. This corresponds to the highest conserved region amongst most ryanodine receptors (ryRs) and inositol triphosphate receptors ($Insp_3Rs$). Owing to the similarity between Ca^{2+} channels and K^+ channels, the model was built by extrapolating from the K^+ channel structure (Doyle *et al.*, 1998). Ca^{2+} ions pass through the pore helix and the selectivity filter. Two transmembrane helices are shown to be important for channeling activity. (**a**) Ribbon diagram of the Ca^{2+} channel tetramer shown using MOLSCRIPT (Kraulis, 1991). Several hydrophobic residues (shown for two adjacent protomers) line the protomer interface along the TM helices and serve to stabilize the tetramer. (**b**) Electrostatic potential representation of the Ca^{2+} channel tetramer. GRASP (Nicholls *et al.*, 1993) was employed for this representation. Acidic residues are indicated by red patches and blue patches indicate basic residues. (**c**) Same as (b) but down the tetramer pore helix axis. A broad red patch at the mouth of the channel shown in this three-dimensional model of the tetramer might explain how Ca^{2+} ions are attracted towards the channel.

provide stabilization to the ions and also hold the amino acids in the selectivity filter region.

Conclusions

We have identified three regions of $Insp_3R$ and ryR which contain high similarity and are important for Ca^{2+} binding and channel regulation. The high degree of partial sequence similarity between the two receptors suggests that the elements involved in calcium channel formation and selectivity are highly similar and conserved during evolution. It is well known that all of the known Na^+, Ca^{2+} and K^+ channels are made of tetramers of either four internal repeats each containing six membrane spanning helices or four protomers each having six membrane-spanning helices (Hille, 1992). Some channels are tetramers of two transmembrane-spanning α-helices. On the basis of structural principles exemplified by the KcsA K^+ channel structure (Doyle *et al.*, 1998), we have put forth the first atomic level structure of a calcium channel, a single file pore, which is in agreement with existing structural and theoretical studies, which provides clues to the permeation pathway located in the linear sequence and how calcium ions might pass through it. The coordinates of the tetramer model are available from the authors on request. To our knowledge, no previous papers have mentioned a pore helix in $Insp_3R$ or ryRs. The above analysis also confirms that the cationic channel proteins belong to a broad superfamily. It will be interesting to compare the four internal repeats of the Na^+ channels for similarities in secondary structural features.

Note added in proof

A similar work speculating the tertiary structure and mechanism of ion conduction of Insp3R and ryR has been reported recently (Williams, West and Sitsapesan (2001) recognising the structurally similar elements, such as pore helix and selectivity filter, as in K+ channels.

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