Chapter4

Study of Structure and Principles of Ion Channeling: Analysis of Ip_3R and RyR Sequences towards Ca^{2+} Channeling

4.1 Summary

Inositol 1,4,5-triphosphate receptors (Ip_3Rs) and ryanodine receptors (RyR) act as cationic channels transporting calcium ions from the endoplasmic reticulum to cytosol (Berridge and Irvine, 1989) 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. Putative calcium binding motifs have been investigated in these sequences. Prediction methods indicate the presence of six transmembrane helices in the C-terminal domain, one of the three domains conserved between Ip_3R and RyR receptors. Recently, the crystal structure of tetrameric K^+ channel (Doyle *et al*., 1998) revealed that two transmembrane helices, an additional pore helix and a selectivity filter are responsible for selective ion K^+ channeling. The last three TM helices of Ip_3R and RyR are particularly well-conserved and analogous pore helix and selectivity filter motif is found in these sequences. Three-dimensional structural model for permeation pathway of the channel tetramer is generated by extrapolating the distant structural similarity to the K^+ channels.

4.2 Signal Transduction Pathways

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 (Ip_3R) and the ryanodine receptor (RyR), act as channels for the release of intracellular Ca^{2+}. Ip_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), as well as 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*., 1999). Ip_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 (Caterrall, 1991). The other two RyR isoforms are often referred to as the 'heart' and 'brain' forms, but the actual cell and tissue distribution of the isoforms is more complex than is suggested by this nomenclature (For reviews see 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 Ip3R 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. Ip_3R and RyR are thought to occur as homotetramers. Their monomers are of length ~3000 and ~5000 amino acids respectively (Mignery *et al.*, 1989; Serysheva *et al.*, 1995; Galvan *et al*., 1999).

4.3 Materials and Methods

The sequences of ip3r_rat, rynr_human and ip3r_drome have been extracted from the SWISSPROT protein sequence database (Appel *et al*., 1994). Blast searches were made in PRODOM database (Altschul *et al*., 1999). Sequences have been aligned using ClustalW multiple alignment program (Thompson *et al*., 1994). Several methods, both for secondary

structure prediction and membrane spanning region prediction, were used: PHD (Rost *et al*., 1995), PREDATOR (Frishman and Argos, 1997), JPRED (Cuff *et al*., 1998) were used to obtain secondary structure prediction. For the prediction of membrane spanning regions, PERSCAN (is a general purpose method; Donnelly *et al*., 1994), PHD (Rost *et al*., 1995), HMMTOP (Tusnády *et al*., 1998), TMHMM (Sonnhammer et al., 1998), TMPRED (Hofmann and Stoffel, 1993), SOSUI (Hirokawa *et al*., 1998) and TOPPRED II (Claros *et al*., 1994) were used. 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 Ip_3R and RyRs. The tetramer co-ordinates were obtained by means of rigid-body superposition from the K^+ channel tetramer co-ordinates using the program SUPER (B.S. Neela, personal communication). The protomers were moved systematically away from the pore axis using SCHELAX (Chou *et al*., *1984*; Sowdhamini *et al*., 1992) by 1.5 Å to suit the reported dimensions of Ca^{2+} channels.

4.4 Results and Discussions

4.4.1 Calcium-binding Sites on Primary Sequence

Ip_3R and RyR are poorly selective and high conductance Ca^{2+} channel with estimated permeability ratio (divalent/monovalent) of both the receptors is nearly six (Bezprozvanny and Ehrlich, 1994; Tinker and Williams, 1992). Calcium is known to be a regulator of both the receptor channels. Both the properties demand existence of calcium binding motifs on the channel structure. However no classical calcium binding motifs are reported for both the receptor channels. Analysis of individual domains suggested by PRODOM (Corpet *et al*., 1999) has been carried out for this purpose. The domain arrangement of Ip_3R and RyR, with putative helix transmembrane helix positions (see later), as suggested by PRODOM is as shown in Figure 4.1 a, b.

Table 1: Putative calcium binding sites in inositol triphosphate $Insp_3R$ and RyR

Amino acid From @	Amino acid to@	Linear Sequence	Comment
97	107	**DLEKKQNETEN**	
228*	255	**D**NK**D**DILKGG**D**VVRLFHAEQ**E**KFLTC *D*E	$Found conserved in domain 1922
317*#	381	**E**V**D**PDF*EEE*CL**E**FQPSVDP*D*QDASRSR LRNAQ**E**KMVYSLVSVP**E**GN*D*ISSIF*E*L **D**PTTLRGG*D*S L	$Found conserved in domain 1922
378*#	450	*D*SLVPRNSYVRLRHLCTNTWVHSTNI PI*D*K**EE**EK PVMLKIGTSPLK**ED**KEAFAIVPVSPAE VR*D*L*D*FAN**D**AS	$Found conserved in domain 1922
528*	544	*D*CG**D**GPMLRL**EE**LG**D**Q	$Found conserved in domain 1922
660*#	733	TNA**D**ILIETKLVLSRFEFEGVSTGENAL EAGED**EEE**VWLFWRDSNK**E**IRSKSV RELAQ**D**AKEGQKE**D**R DILSYY	Found at boundary of domain 1922
741#	849	ARMCL**D**RQYLAINEISGQLDV**D**LILRC MS**DE**NLPY**D/D**R**D**PQ**E**QVTPVKYARL WS**E**IPSEIAID**D**Y**D**SSGTSKDEIKERFA QTMEFV**EE**YLRDVVC	
994#	1059	LCIFKR**E**FD**E**SNSQSS**E**TSSGNSSQEGPS NVPGAL**D**F**E**HIEEQA**E**GIFGGSEENTP LDL**D**DHGGRT	
1107	1121	Q**D**V**D**NYKQIKQ**D**L**D**Q	
1140	1157	DEPMDGASGENEHKKTEE	Unstructured charged loop
1347#	1426	**D**RASFQTLIQMMRSER**D**RM**DE**NSPL	

		MYHIHLV**E** LLAVCT**E**GKNVYTEIKCNSLLPL**DD**IV RVVTH**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*#	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#	2528	K**DD**FILEV**D**RLPNETAVPETG**E**SLAND FLYSDVCRVETG**E**NCTSPAPK E**E**LLPAEETEQDK**E**HTC**E**	Part of luminal loop, domain 1555, Replaced by a charged region in RyR
2589*	2604	**D**TFA**D**LRS**E**KQKK*E***E**	Found conserved in domain 1555

@ corresponds to ip3r_mouse residue numbering

* stretch of residues are found conserved in both Insp3R and RyR.

stretch of residues are reported to bind calcium (Sienaert *et al* ., 1996,1997).

$ - Domain 1922 is N-terminal, which is reported to be ligand binding domain in Insp3R (Miyawaki *et al*., 1991).

/ indicates 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).

PRODOM 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. Interestingly enough, the N-terminal domain in case of Ip3R is 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. 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).

12 Ip_3R sequences and 13 RyR sequences were chosen and aligned at the membrane-traversing transmembrane (TM) domain. The multiple alignment of Ip_3R and RyR sequences show the presence of several conserved negatively charged residues (Table 1) which could act as Ca^{2+} binding sites. While studying Ca^{2+}regulation of Ip_3R receptor at the molecular level and the structural determinants of Ca^{2+} binding, Sienaert and co-workers (Sienaert *et al*, 1996; 1997) had identified 8 linear sites which are shown to bind both calcium and ruthenium red (see Table1). Out of 8 sites, 3 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) at positions (numbering according to rynr_human) 4070-4130, which are at the boundary of the middle domain which is conserved between Ip_3R and RyR receptors. Ip_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 is shown to bind Ca^{2+} (Sienaert *et al*., 1997). Conversely, a region from ip3r_mouse (amino acids 2463-2528) which is the part of C-terminal domain is shown to bind Ca^{2+} but corresponding region in rynr_human is replaced by highly aspartate and glutamate rich region. Thus, the elements that are involved in binding calcium ions on primary structure are conserved and indicate the similar mode of regulation by Ca^{2+}.

4.4.2 Lessons from K+ channel structure

Recently structure of tetrameric K^+ channel (Doyle *et al*., 1998) from *S. Lividens* was reported, revealing many mysteries about the channel structures that had kept physiologists wondering for many decades. Apart from two membrane spanning helices, the loop region connecting the two helices (P-loop) forms the selectivity filter. The amino terminal region of

the P loop is also α-helical (which is termed as pore helix), slanting towards the pore axis from outside. The pore 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 outward thus stabilising the right ions of desired pore size. Sequence alignments from various K^+ channels, both inward and outward rectifiers, shows that most of the residues of pore helix and signature sequence are conserved (Doyle *et al*, 1998; MacKinnon *et al*., 1998; Armstrong, 1998), suggesting that the architecture of the channels is similar irrespective of the direction of ion transfer. Moreover, two membrane spanning helices, pore helix and selectivity filter per monomer would be the minimal requirement and sufficient for forming the functional channel tetramer.

4.4.3 Secondary Structure Prediction Studies on C-terminal Region

Prediction studies were carried out using methods that use both single sequence and multiple alignment on the sequences of one Ip_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 4.2 for ip3r_mouse sequence. It is interesting to note that the helix marked as "pore helix" is predicted as a membrane-spanning region by three transmembrane region prediction methods while others miss it. However, it is predicted as a helix by all secondary structure prediction methods. Thus, confirming its existence as a helix. The existence of pore helix was confirmed also by applying these methods to the KcsA sequence, where all membrane region prediction methods miss the pore helix. The helix-wheel diagram is shown in Figure 4.3 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 that Ip_3R contains a topology of six membrane spanning helices. Prediction analysis was also performed for the C-terminal domain of ryanodine receptors. PHD TMpred, a method that employs multiple sequence alignments, suggests six membrane spanning helices and a pore helix for RyR, a topology analogous to that suggested for Ip_3R. All the other membrane region prediction methods predict different number of membrane spanning regions, but for the last two membrane spanning helices and

pore helix the results are identical to that for Inp_3R.

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 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 4.4 shows the multiple sequence alignment of the region containing 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% I.D.) The predicted helix positions and certain conserved amino acid positions are indicated. This observed similarity is also in agreement with deletion studies on Ip_3R which demonstrates that the deletion of the first four TM helices of recombinant Ip_3R forms functional calcium channels and mutants lacking the last two helices do not form detectable channels (Ramos-Franco *et al*., 1999). The results of secondary structure prediction, inspection of sequence alignment and the deletion studies (Ramos-Franco *et al*., 1999) strongly suggests that the pore forming regions for both Ip_3R and RyR are similar and conserved.

4.4.4 Structural Paramemters for Calcium channel

From the above discussion and sequence alignment shown in Figure 4.4, 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 Ip_3Rs) is found to be highly conserved, in all known Ip_3Rs and RyR. Mutations of glycine to alanine in this signature sequence in RyR, at first, fourth and sixth positions disrupt the calcium release from the channel (Zhao *et al*., 1999). Also isoleucine to threonine mutation of RyR1 (see Figure 4.4) decreases the threshold of Ca^{2+} requiring to initiate opening of wild type channel and resulted in a reduced release of Ca^{2+} from internal stores (Balshaw *et al*., 1999; Lynch *et al*., 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 in earlier studies that Ca^{2+} channels have pores that are related architecturally to K^+ channels (Roux and MacKinnon 1999; Doyle *et al*., 1998).

Owing to the difference in mechanism of cation conduction, it is obvious that the structural parameters are different for the RyR Ca^{2+} channels than K^+ channels. Ryanodine receptors are reported to have a pore of diameter of ~6-7 Å (McCleskey and Almers, 1985; Tinker and Williams, 1993; Serysheva *et al*., 1999). The length of 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 K^+ channel was determined the experimental value of selectivity filter of K^+ selective channel was reported as 10 Å (Miller, 1982). Blocking studies with the impermeant charged derivative of triethyl amine reveal that this narrowing occurs over 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.

4.4.5 Building the Structure of Permeation Pathway

The three-dimensional structure of RyR human TM domain was derived using 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 length of KcsA sequence and that of RyR C-terminal regions that contains the pore forming region are similar, but both the sequences shares very low sequence similarity (8% ID).

The transmembrane helices, pore helix and selectivity filter region are taken as SCR (structurally conserved regions; Figure 4.5) 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 (Neela, B.S., personal communication). The pore diameter of RyR is 6Å, wider than that of K^+ channel by 3Å as mentioned above. Therefore, in the tetramer of the TM domain, each

monomer was moved 1.5Å away from the pore axis symmetrically, to suit the reported structural parameters. Interprotomer interactions before and after the change in pore dimensions were measured and no major destabilization was found due to the slight enlargement in pore diameter.

The tetramer model has a pore diameter of roughly 6 Å and selectivity filter length around 11 Å, (Figure 4.6) in correspondence with functioning calcium channels. This model satisfies most of the properties of calcium channels both used by binding model and continuum model (Hille B, 1992; Nooner and Eisenberg, 1998; Doyle *et al*., 1998). Figure 4.7 shows the 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 the protomer interface might account for the stability of the tetramer. The model is in agreement with present theory of calcium permeation through large pores, which have large diameters than their preferred ions. The calcium is concentrated by the negatively charged residues, which are concentrated at the mouth of the pore (Figure 4.8a) passing through the selectivity filter region composed of the conserved motif, GGGIGD, which occurs at the luminal mouth of the channel. It can be stabilized by dipole moments of the pore helices and also water molecules present in the middle of the pore (as shown by structure of K^+ channel) and then it passes through the remainder of the pore. This narrow region is relatively short which is consistent with the large conductance of the channel (Latorre and Miller, 1983). The hydrophobic membrane spanning helices form the hydrophobic walls (Figure 4.8b). The pore helices, which are pointing towards the central axis of the pore provide the stabilization to the ions inside the pore by its dipole moments and also holds the amino acids of the selectivity filter region firmly at their position. (Figure 4.9)

4.5 Conclusions

To conclude, this chapter reports the regions of Ip_3R and RyR, which share high similarity and its importance for Ca^{2+} binding and channel regulation, are identified. High degree of partial sequence similarity between the two receptors suggests that the elements involved in calcium channel formation, regulation 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 (see for example, Hille, 1992). Else some channels are tetramer of two transmembrane spanning α-helices.

On the basis of structural principles exemplified by the KcsA K^+ channel structure (Doyle *et al*., 1998), the first atomic level structure of a calcium channel has been proposed as a multi ion-single file pore. It 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 novel finding of this work is identification of pore helix in Ip_3R or RyR. The above analysis also confirms that the cationic channel proteins belong to a broad superfamily with highly conserved structures. It will be interesting to compare the four internal repeats of the Na^+ channels for similarities in secondary structural features.

4.6 References

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Figure Legends

Figure 4.1 Doamin structure of Ip_3R (4.1a) and RyR (4.1b) primary sequences as provided by PRODOM domain database (Corpet *et al*., 2000). It shows the domain level similarity shared by both the receptor channels and hence points to tertiary structure similarities.

Figure 4.2 Consensus secondary structure predicton of transmembrane (TM) helices in the C-terminal domain of Inositol triphosphate receptor (Ip_3R) sequence. Various methods used for predicting TM helix positions are mentioned (see text on Materials and Methods for details). Most methods identify six TM helices while a few of them predict an additional

shorter helix before the last TM helix.

Figure 4.3: Helix-wheel projection of the sixth TM helix using 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 helix is shown as a cylinder.

b) shows the distribution of conserved residues on the predicted TM helix 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 amphipathic membrane spanning α-helix.

Figure 4.4 Multiple sequence alignment of various Ip_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: alpha-ryanodine binding protein, *Rana catesbeiana*; Q15413_HUMAN: ryanodine receptor 3, *Homo sapiens* brain; Q95201_MUSVI: ryanodine receptor type 3, *Mustela vison*. Q91319_RANCA: beta-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-trisphosphate-binding protein receptor, *Drosophila melanogaster*; O77089_PANAR: inositol 1,4,5-trisphosphate receptor, *Panulirus argus*; Q14643_HUMAN: human type 1 inositol 1,4,5-trisphosphate receptor, *Homo sapiens*; Q14460_HUMAN: inositol 1,4,5-trisphosphate receptor type 1, *Homo sapiens*; IP3R_RAT: inositol 1,4,5-trisphosphate-binding protein type 1 receptor, *Rattus norvegicus*; Q91908_XENLA: inositol 1,4,5-triphosphate receptor, *Xenopus laevis*; IP3R_MOUSE: inositol 1,4,5-trisphosphate-binding protein type 1 receptor, *Mus musculus*; IP3S_HUMAN: inositol 1,4,5-trisphosphate-binding protein type 2 receptor, *Homo sapiens*; IP3S_RAT: inositol 1,4,5-trisphosphate-binding protein type 2 receptor, *Rattus norvegicus*; Q14649_HUMAN: type 3 inositol 1,4,5-trisphosphate 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).

Figure 4.5 The alignment shows the Structurally Conserved Regions (SCRs) used by COMPOSER (Sutcliffe *et al*., 1987) for generating the monomer model of permeation pathway in RyR on the basis of 1bl8 structure.

Figure 4.6 A portion of three-dimensional model of the permeation pathway in RyR showing the structural parameters. The figure was prepared using SETOR (Evans, 1993)

Figure 4.7 Three-dimensional model of the last two predicted TM helices of human ryanodine receptor. This corresponds to the region that is most conserved between ryanodine receptors (RyRs) and inositol triphosphate receptors (Ip_3Rs). Owing to the similarity between Ca^{2+} channels and K^+ channels, the model has been 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. 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 stabilise the tetramer.

Figure 4.8 Electrostatic potential representation of the Ca^{2+} channel tetramer using GRASP (Nicholls *et al.*, 1993). Acidic residues are indicated by red patches and blue patches indicate basic residues.
a) the pore view (b) view 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**.**

Figure 4.9 Ribbon diagramme showing three-dimensional model of the last two predicted TM helices of human ryanodine receptor. The pore helices and the selectivity filter regions are marked. The figure was preparewd using SETOR (Evans, 1993).