Research Articles

Preliminary report: Homology modeling of Human Ryanodine Receptor-1

Waraphan Toniti[1*] Pranom Puchadapirom[2] Aekkapot Chamkasem[3]

[1]Department of Pre-clinic and Applied Animal Sciences, Faculty of Veterinary Science, Mahidol University, Phuttamonthon 4 Rd., Salaya, Phuttamonthon, Nakorn-pathom 73170, Thailand

[2]Department of Pathobiology, Faculty of Science, Mahidol University, Rama VI Rd., Ratchathewi, Bangkok 10400, Thailand

[3]Faculty of Veterinary Science, Mahidol University, Phuttamonthon 4 Rd., Salaya, Phuttamonthon, Nakorn-pathom 73170, Thailand

[*]Corresponding author, E-mail address: waraphan.ton@mahidol.ac.th

Abstract

Excitation-contraction (E-C) coupling is the series of events in which an electrical stimulus is converted into a mechanical contraction. Ryanodine receptors (RyRs), the Ca^{2+} release channels, located at the sarcoplasmic reticulum membrane and played role in E-C coupling. In this study, human RyR1sequence was studied by sequence of P21817. The *in silico* RyR1 models were generated using homology modeling. RyR1 is the largest known ion channels and composes of 15 important subdomains; cytoplasmic assembly and transmembrane assembly. This study focused on the larger cytoplasmic assembly that is composed of 10 subdomains. The results show that the shapes and the pocket sites of each domain of RyR1 are different. Each domain has its own pocket sites which facilitateinteraction between RyR1 and modulators. Future studies will certainly resolve additional structural differences among species of interest and may apply as model of calcium release channel-modulator interaction.

Keywords: Human RyR1, Calcium channel, Skeletal muscle, Homology modeling, *in silico*

การทดลองเบื้องต้น: การสร้างแบบจำลองของตัวรับไรยาโนดีน-1 ในมนุษย์โดยใช้เทคนิค Homology Modeling

วราพันธ์ โตนิติ[1] ปรานอม ภูชฎาภิรมย์[2] เอกพจน์ แช่มเกษม[3]

[1]ภาควิชาปรีคลินิกและสัตวศาสตร์ประยุกต์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล ถนนพุทธมณฑลสายสี่ ตำบลศาลายา อำเภอพุทธมณฑล จังหวัดนครปฐม 73170
[2]ภาควิชาพยาธิชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล ถนนพระรามหก เขตราชเทวี กรุงเทพมหานคร 10400
[3]คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล ถนนพุทธมณฑลสายสี่ ตำบลศาลายา อำเภอพุทธมณฑล จังหวัดนครปฐม 73170
*ผู้รับผิดชอบบทความ E-mail address: warapha.ton@mahidol.ac.th

บทคัดย่อ

กลไกการเกิดการกระตุ้นควบคู่กับการหดตัวของกล้ามเนื้อ (E-C coupling) เป็นชุดของเหตุการณ์ที่เกิดขึ้นอย่างต่อเนื่องและเป็นลำดับ เมื่อได้รับการกระตุ้นเซลล์กล้ามเนื้อจะเปลี่ยนการกระตุ้นทางไฟฟ้าเป็นการหดตัวเชิงกล ตัวรับไรยาโนดีน (RyRs) ทำหน้าที่เป็นแคลเซียมแชนแนลโดยตัวรับชนิดนี้พบที่เซลล์เมมเบรนของเซลล์กล้ามเนื้อและตัวรับนี้จะทำหน้าที่เกี่ยวข้องโดยตรงกับกลไกการเกิดการกระตุ้นควบคู่กับการหดตัวของกล้ามเนื้อ การศึกษาครั้งนี้ใช้ลำดับกรดอะมิโน P21817 สำหรับศึกษาตัวรับไรยาโนดีน-1 ในมนุษย์โดยใช้ *in silico* models ซึ่งทำการจำลองโครงสร้างตัวรับไรยาโนดีน-1โดยตัวรับชนิดนี้จัดเป็นไอออนแชนแนลที่มีขนาดใหญ่ที่สุดและประกอบด้วยหน่วยย่อยๆ ถึง 15 หน่วย แบ่งเป็นหน่วยย่อยที่อยู่ด้านไซโตพลาสมและอยู่ที่ทรานสเมมเบรน การศึกษาวิจัยครั้งนี้ได้ศึกษาเฉพาะหน่วยย่อยที่อยู่ทางด้านไซโตพลาสม จำนวน 10 หน่วยย่อยจากผลการจำลองโครงสร้างพบว่ารูปร่างและตำแหน่งที่คาดว่าโมดูเลเตอร์จะเข้าทำปฏิกิริยามีความแตกต่างกันในแต่ละหน่วยย่อย โดยพบว่าแต่ละหน่วยย่อยมีตำแหน่งที่คาดว่าโมดูเลเตอร์จะเข้าทำปฏิกิริยาของตนเอง ซึ่งคาดว่าอาจจะเป็นตำแหน่งในการเข้าทำปฏิกิริยาระหว่างตัวรับไรยาโนดีนกับโมดูเลเตอร์ การศึกษาตัวรับไรยาโนดีนอย่างละเอียดจะทำให้เข้าใจความแตกต่างของโครงสร้างตัวรับไรยาโนดีนระหว่างสปีชีส์ และอาจจะปรับใช้เป็นโมเดลในการศึกษาความสัมพันธ์ระหว่างแคลเซียมแชนแนลกับโมดูเลเตอร์ได้ในอนาคต

คำสำคัญ : ตัวรับไรยาโนดีน-1 ในมนุษย์กล้ามเนื้อลาย Homology modeling, *in silico*

Introduction

Calcium is a common second messenger (Campbell, 1983; Carafoli and Klee, 1999; Huxley, 1961 in Fill and Copello, 2002). The release of Calcium ion (Ca^{2+}) from its storage tank into the cytoplasm regulates many processes in cells. Cells have many different types of Ca^{2+} transporters including Ca^{2+} release channel that modulate cytosolic Ca^{2+} levels. Thus Ca^{2+} entry across the surface membrane can substantially elevate cytosolic Ca^{2+} levels providing the Ca^{2+} trigger signals for a large number of physiological processes (Fill and Copello, 2002).

cAMP is one of a common second messenger and plays important roles in varieties of signaling pathways including beta 2-adrenoceptors. cAMP may catalyze the activation of protein kinase A and involve in muscle tone regulatory system. On the other hand, cAMP also plays role in reduction of intracellular calcium ion by inhibiting Ca^{2+} release from intracellular stores. The reduction of intracellular Ca^{2+} is leading to muscular relaxation (Johnson, 1998).

In mammal, the three RyR isoforms; RyR1, RyR2 and RyR3 are encoded by three different genes on different chromosomes (Marks et al, 1989; Mikami et al, 1989; Takeshima et al, 1989; Takeshima, 1993; Zorzato et al, 1990 in Fill and Copello, 2002). Mammalian skeletal muscle is expressed predominantly RyR1 isoform. RyR2 is the major isoform that expressed in cardiac muscle. RyR3 is found in diaphragm, epithelial cells, brain and smooth muscle (Lanner et al, 2010; Capes et al, 2011).

RyRs are the largest known ion channels (Takeshima et al, 1989; Nakai et al, 1990; Otsu et al, 1990; Zorzato et al, 1990; Hakamata et al, 1992 in Lanner et al, 2010). The massive size, multiple modulators, and the dynamic nature of RyRs make their structural analysis a challenge (Lanner et al, 2010). RyRs form homotetramers of square prism shape (Liu et al, 2000; Fill and Copello, 2002; Samso, Shen and Allen, 2006; Lanner et al, 2010).

RyR1 consists of two major components: a larger cytoplasmic assembly and a smaller transmembrane assembly (TA). RyR1 has been divided into 15 important subdomains. The cytoplasmic area is a large area with cavities and micro-structures: foot and clamps. The corners of the cytoplasmic area are connected through the "handle" domain. The central rim domain of the cytoplasmic area is connected to the membrane region through the "column" (Lanner et al, 2010).

Excitation-contraction (E-C) coupling is the series of events in which an electrical stimulus is converted into a mechanical contraction. Skeletal-type E-C coupling is thought to require a direct interaction between RyR1 and the Dihydropyridine receptors (DHPRs) in the absence of extracellular Ca^{2+} (Perez et al, 2003).

This study focused on the cytoplasmic assembly of human RyR1. We aimed to simulate three dimensional structures of RyR1 by homology modeling technique.

Materials and Methods

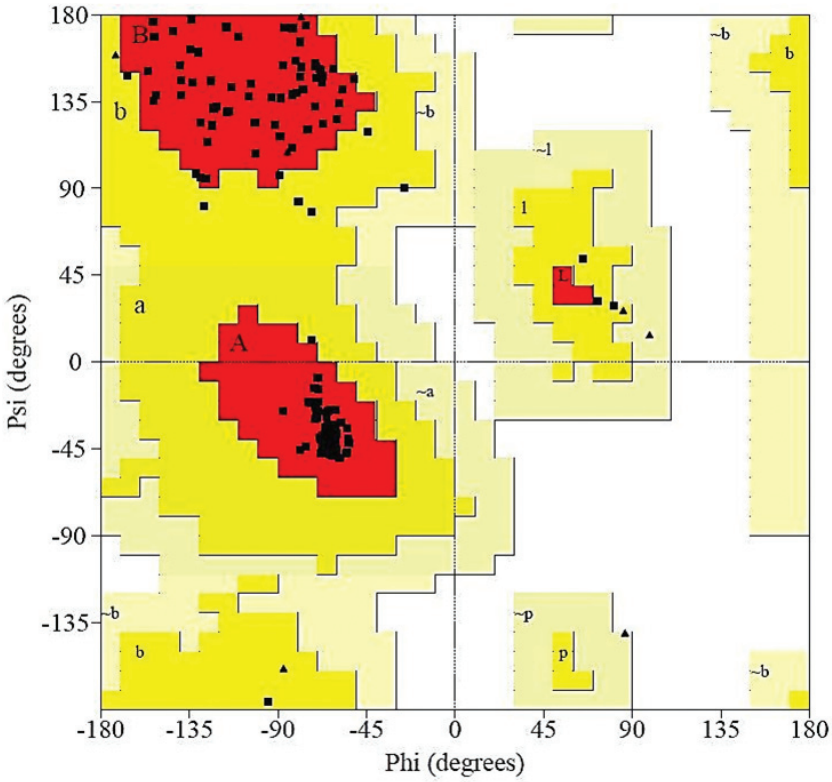
Sequences and structures

Amino acid sequence of human RyR1 was collected from Protein Data Bank (PDB). Human RyR1 sequence was downloaded from UniProt (http://www.uniprot.org) by sequence of P21817. Structural data for RyR1 was obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB-PDB).

Homology model construction

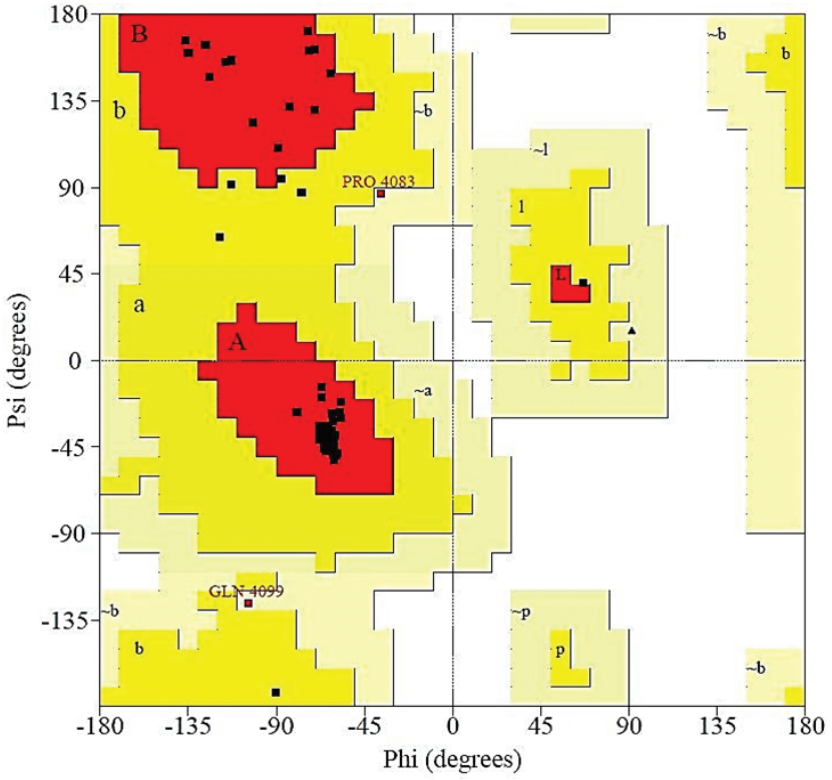
Coordinate files were assigned to the cytoplasmic domain of human RyR1. The next step was close comparison with available structures and constructed the three dimensional structures of particular subdomains (http://salilab.org/modweb).Then Q-site finder (http://www.modelling.leeds.ac.uk/qsitefinder) simulated the pocket sites of RyR1 by hydrophobic probing. The tenth most favorable binding energy of each subdomain was marked.

Results



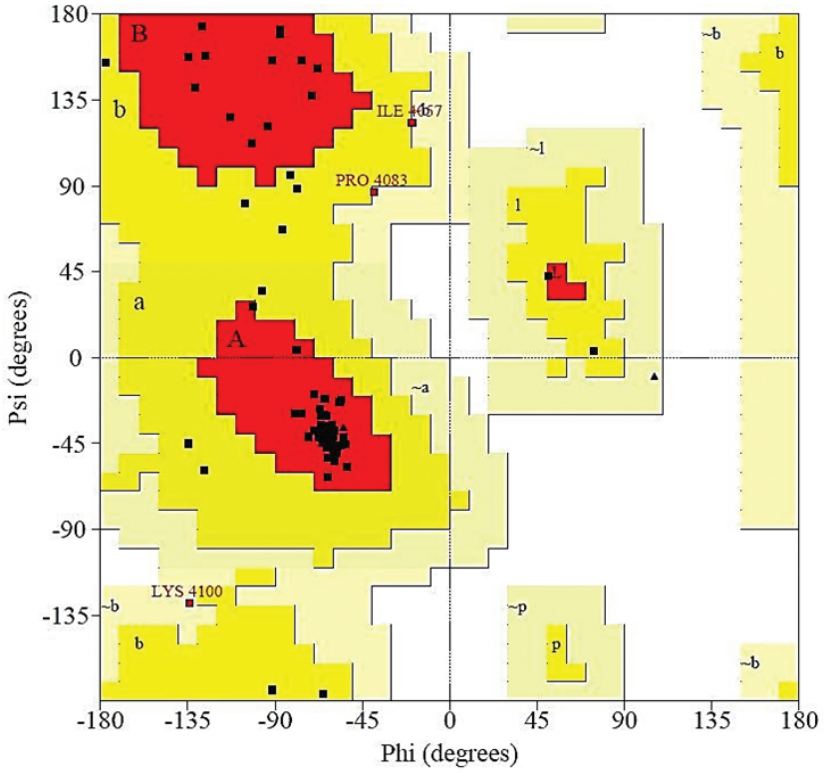
A

NFDPRPVETLNVIIPEKLDSFINKFAEYTHEKWAFDKIQNNWSYGENIDEELKTHPMLRPYKTFSEKDKEIYRWPIKESLKAMIAWEWT IEKAREGEEEKTEKKKTRKISQSAQTYDPREGYNPQPPDLSAVTLSRELQAMAEQLAENYHNTWGRKKKQELEAKGGGTHPLLVPYDTL TAKEKARDREKAQELLKFLQMNGYAVTRG



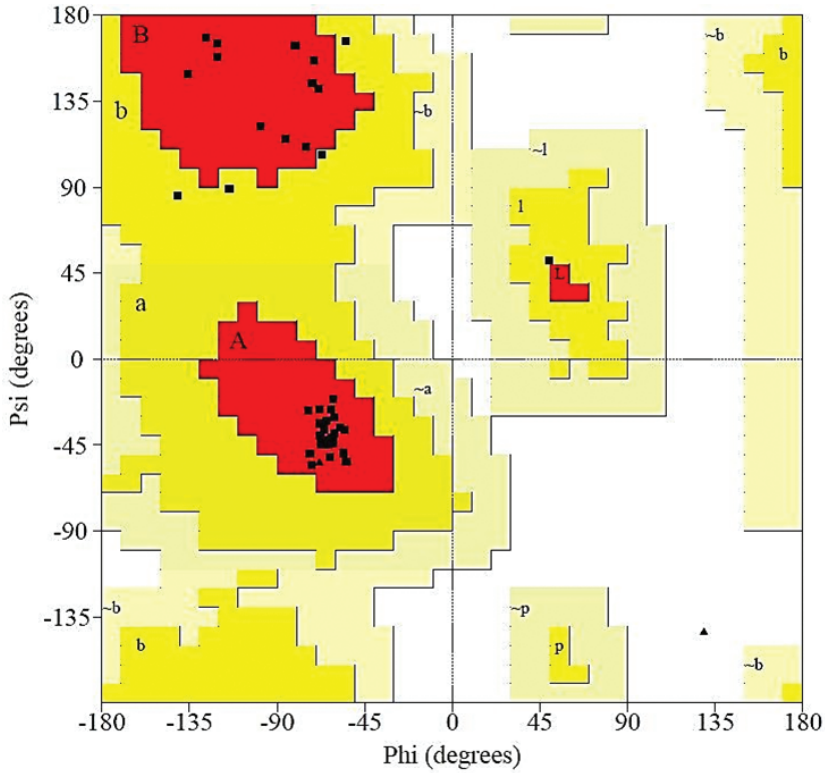
B

SSNVEMILKFFDMFLKLKDIVGSEAFQDYVTDPRGLISKKDFQKAMDSQKQFSGPEIQFLLSCSEADENEMINCEEFANRF



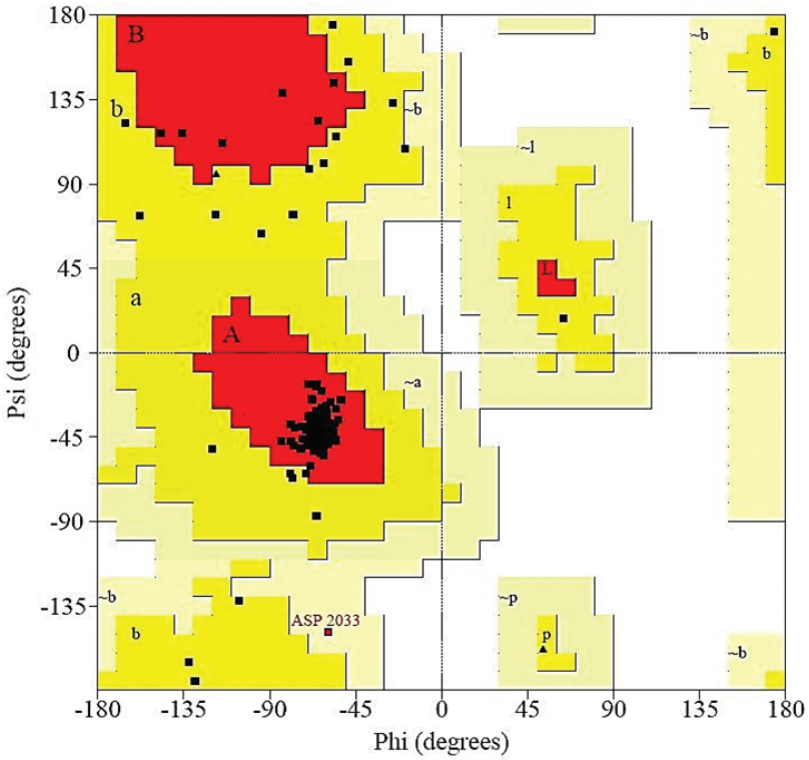
C

GMIARQMVDMLVESSSNVEMILKFFDMFLKLKDIVGSEAFQDYVTDPRGLISKKDFQKAMDSQKQFSGPEIQFLLSCSEADENEMINCE
EFANRFQEP



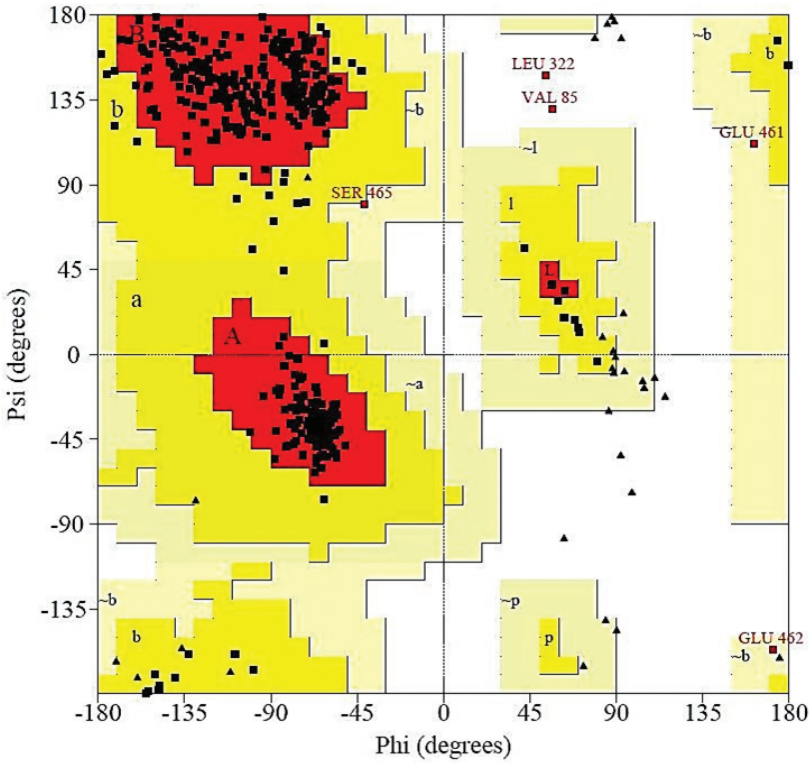
D

TEEEEEEDEEEEGEEEDEEEKEEDEEETAQEKEDEEKEEEEAAEGEKEE



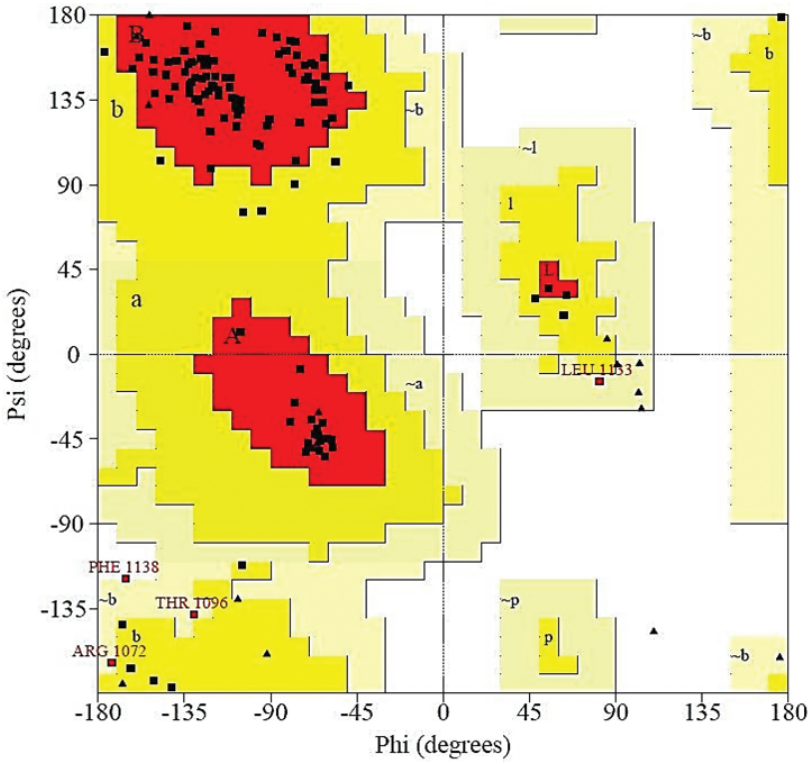
E

IFGDEDVKQILKMIEPEVFTEEEEEEDEEEEGEEEDEEEKEEDEEETAQEKEDEEKEEEEAAEGEKEEGLEEGLLQMKLPESVKLQMCH
LLEYFCDQELQHRVESLAAFAERYVDKLQANQRSRYGLLIKAFSMTAAETARRTREFRSPPQEQINMLLQFKDGTDEEDCPLPEEIRQD
LLDFHQDLLAHCGIQLDGEEEEPEEETTLGSRLMSLLEKVRLVKKKEEKPEEERSAEESKPRSLQELVS



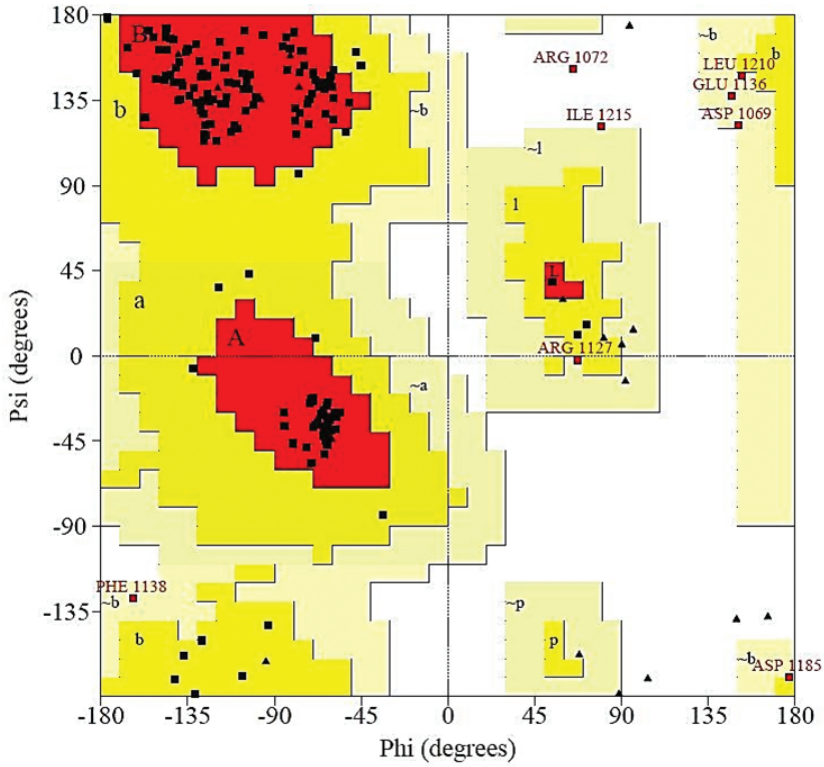
F

QFLRTDDEVVLQCSATVLKEQLKLCLAAEGFGNRLCFLEPTSNAQNVPPDLAICCFVLEQSLSVRALQEMLANTVEAGVESSQGGGHRT
LLYGHAILLRHAHSRMYLSCLTTSRSMTDKLAFDVGLQEDATGEACWWTMHPASKQRSEGEKVRVGDDIILVSVSSERYLHLSTASGEL
QVDASFMQTLWNMNPICSRCEEGFVTGGHVLRLFHGHMDECLTISPADSDDQRRLVYYEGGAVCTHARSLWRLEPLRISWSGSHLRWGQ
PLRVRHVTTGQYLALTEDQGLVVVDASKAHTKATSFCFRISKEKLDVAPKRDVEGMGPPEIKYGESLCFVQHVASGLWLTYAAPDPKAL
RLGVLKKKAMLHQEGHMDDALSLTRCQQEESQAARMIHSTNGLYNQFIKSLDSFSGKPRGSGPPAGTALPIEGVILSLQDLIIYFEPPS
EDLQHEEKQSKLRSLRNRQSLFQEEGMLSMVLNCIDRLNVYTTAAHFAEFAGEEAAESWKEIVNLLYELLASLIRG



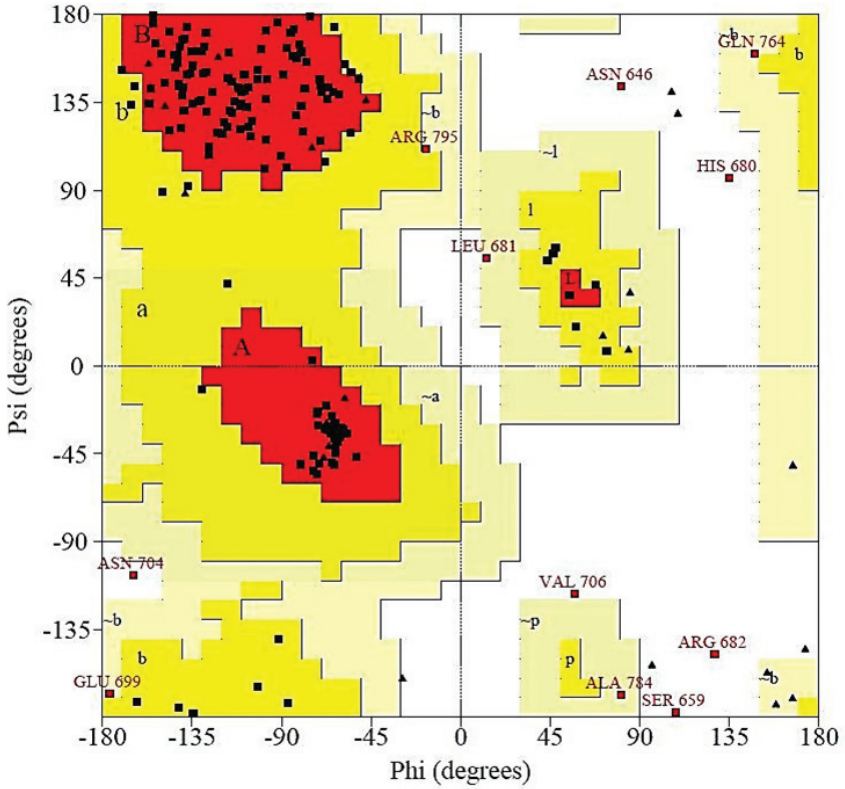
G

SQVENQSRCDRVRIFRAEKSYTVQSGRWYFEFEAVTTGEMRVGWARPELRPDVELGADELAYVFNGHRGQRWHLGSEPFGRPWQPGDVV
GCMIDLTENTIIFTLNGEVLMSDSGSETAFREIEIGDGFLPVCSLGPGQVGHLNLGQD



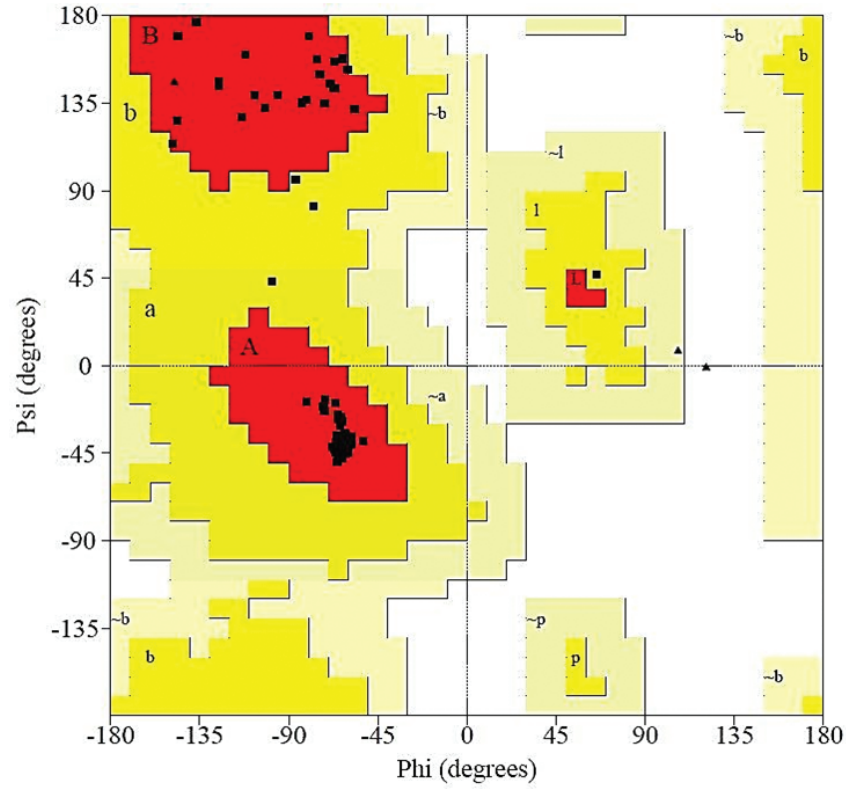
H

GYGYNIEPPDQEPSQVENQSRCDRVRIFRAEKSYTVQSGRWYFEFEAVTTGEMRVGWARPELRPDVELGADELAYVFNGHRGQRWHLGS
EPFGRPWQPGDVVGCMIDLTENTIIFTLNGEVLMSDSGSETAFREIEIGDGFLPVCSLGPGQVGHLNLGQDVSSLRFFAICGLQEGFEP
FAINMQRPVTT



I

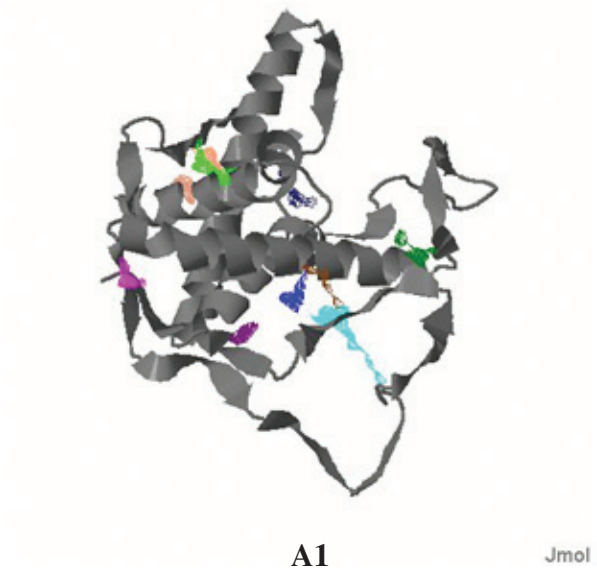
ENLLPGRELLLQTNLINYVTSIRPNIFVGRAEGTTQYSKWYFEVMVDEVTPFLTAQATHLRVGWALTEGYTPYPGAGEGWGGNGVGDDL
YSYGFDGLHLWTGHVARPVTSPGQHLLAPEDVISCCLDLSVPSISFRINGCPVQGVFESFNLDGLFFPVVSFSAGVKVRFLLGGRHGEF
KFLPPPGYAPCHEA



J

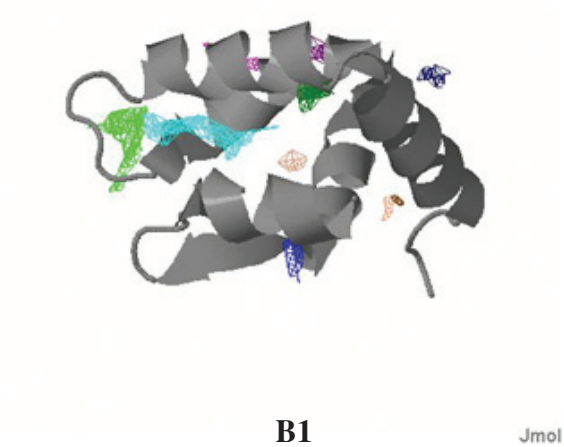
DFVPCPVDTVQIVLPPHLERIREKLAENIHELWALTRIEQGWTYGPVRDDNKRLHPCLVDFHSLPEPERNYNLQMSGETLKTLLALGCHV

Figure 1 Ramachandran plots and amino acid sequences of each subdomain (A-J).

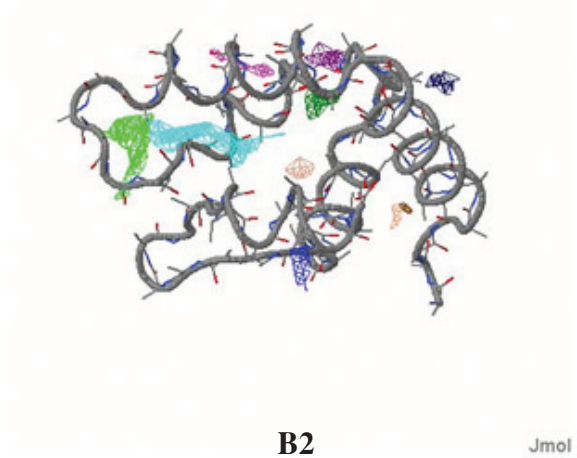


A1

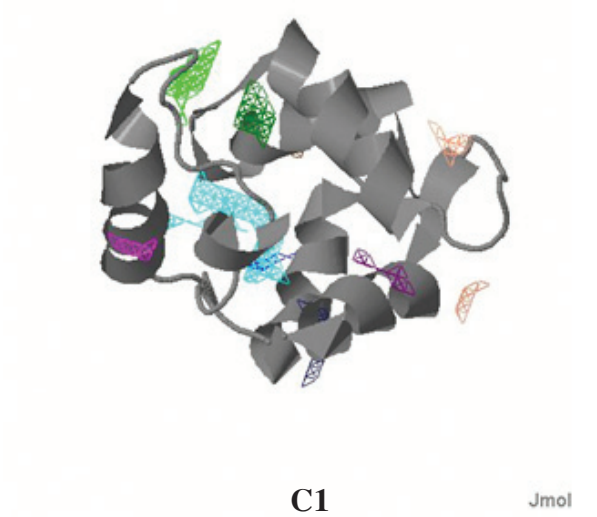
A2



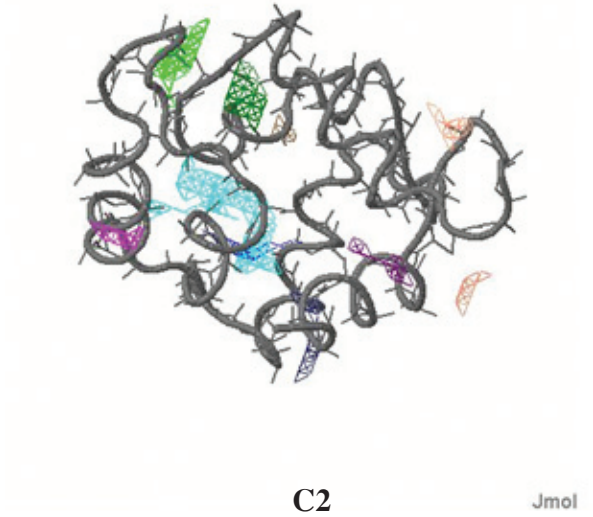
B1



B2



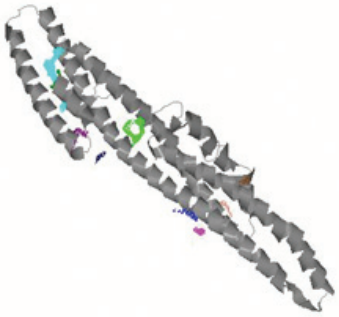
C1



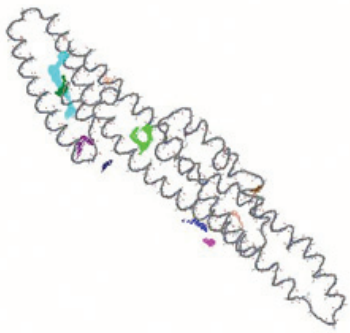
C2

D1

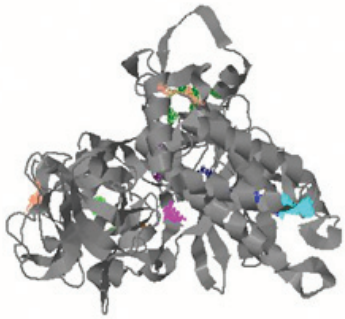
D2



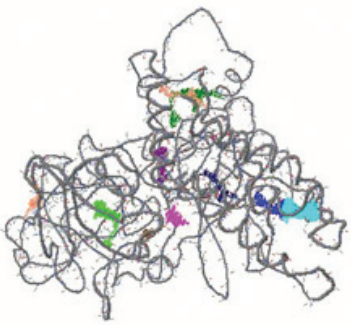
E1



E2



F1



F2

G1

G2

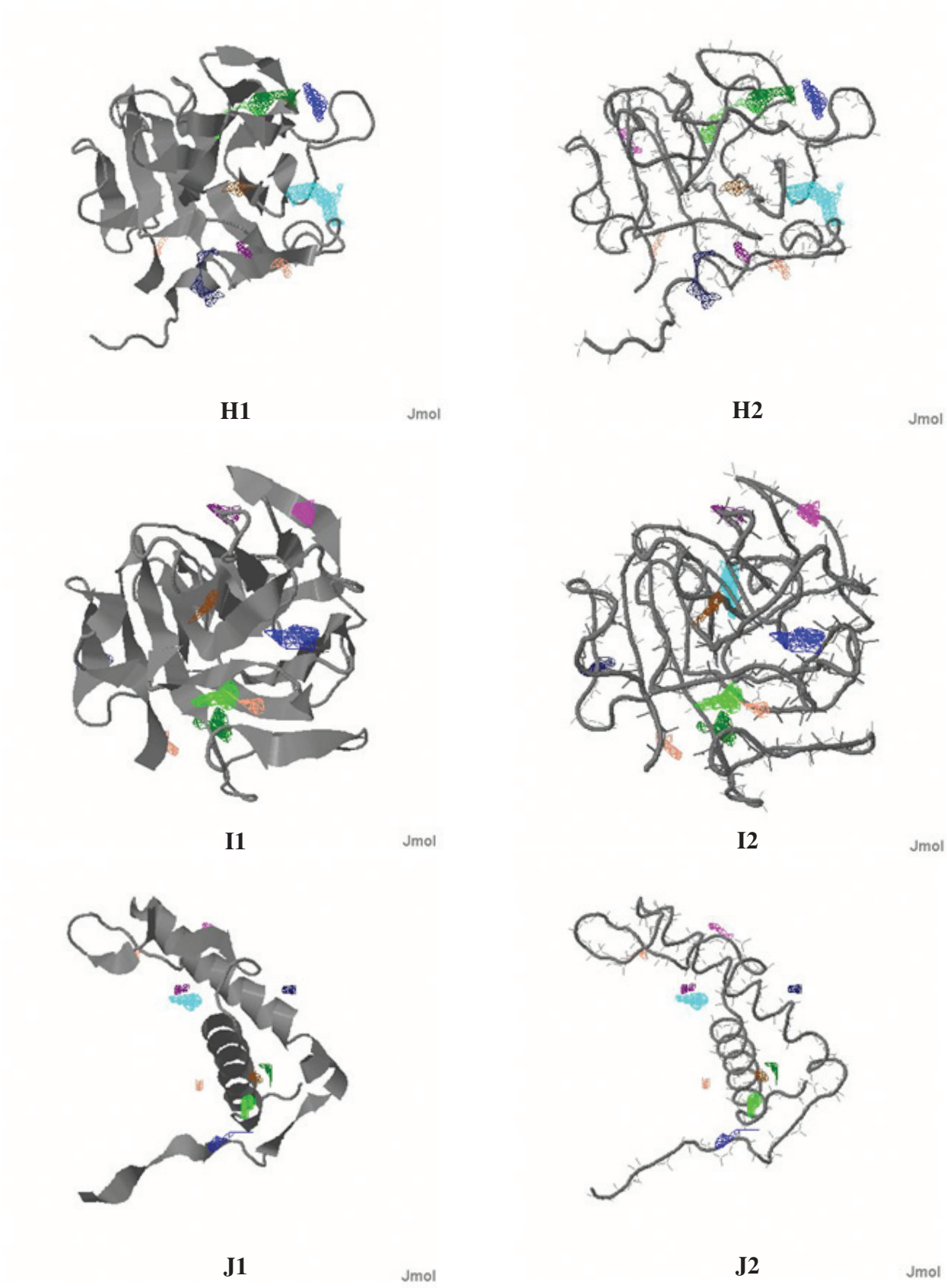


Figure 2 Tertiary structure of each hRyR1 subdomain (A1-J1) and back bone structures of particular subdomain with predicted pocket sites (A2-J2).

Discussion

Skeletal muscle contraction regulation is one of the most popular issues in the application for treating the muscle wasting diseases (Agbenyega et al, 1995; Smith et al, 2002). The varieties of skeletal muscle modulators catalyze directly through the transmembrane receptors such as ADRB2, RyRs. Homology modeling technique may give the valuable information of 3-D structure of the particular receptors including of ADRB2 and RyR1 (Toniti et al, 2012).

As methods for determining three-dimensional structure develop, there are several serious problems of the validity of protein model (Lüthy, Bowie and Eisenberg, 1992; Hooft et al, 1996). The ultimate goal of protein modeling is to predict the correct and compatibility result achieved experimentally (Epstein, Goldberger and Anfinsen, 1963; Krieger, Nauurs and Vriend, 2003; Samso, Shen and Allen, 2006; Amado et al, 2009; Meng et al, 2009; Samso et al, 2009; Tung et al, 2010; Lanner et al, 2010; Capes, Loaiza and Valdivia, 2011). Figure 1 and 2 shows the residues in most favored regions from Ramachandran plots of human cytoplasmic assembly of RyR1 and the predicted pocket sites of each subdomain.Future studies will certainly resolve additional structural differences among species of interest and may apply as model of calcium release channel-modulator interaction.

Conclusion

Thein *silico* human RyR1 model was studied. The cytoplasmic assembly consists of 10 subdomains. The results show that the shapes and the pocket sites of each domain of cytoplasmic assembly are different. Each domain has its own pocket sites which facilitate interaction between the cytoplasmic side of RyR1 and modulators.

Acknowledgement

The authors would like to thank Dr.Paisan Kanthang, Faculty of Science and Technology, Rajamongala University of Technology PhraNakhon, Thailand for valuable advices and suggestions. We are also very grateful to Faculty of Veterinary Sciences, Mahidol University for providing grants to support this research.

References

Agbenyega E.T., Morton R.H., Hatton P.A. and Wareham A.C. 1995.Effect of the β_2-adrenergic agonist clenbuterol on the growth of fast- and slow-twitch skeletal muscle of the dystrophic (C57BL6J dy2J/dy2J) mouse.Comparative Biochemistry and Physiology. 111C(3): 397-403.

Amador F.L., Liu S., Ishiyama N., Plevin M.J., Wilson A., MacLennan D.H. and Ikura M. 2009. Crystal structure of type I ryanodine receptor amino-terminal β-trefoil domain reveals a disease-associated mutation "hot spot" loop. Proceedings of the Natural Academic of Sciences. 106 (27): 11040-11044.

Araya R., Liberona J.L., Cárdenas J. C., Riveros N., Estrada M., Powell J.A., Carrasco M. A. and Jaimovich E. 2003.Dihydropyridinereceptors as voltage sensors for a depolarization-evoked, IP3R-mediated, slow calcium signal in skeletal muscle cells. The Journal of General Physiology. 212: 3-16.

Beard, N. A., Wei L. and Dulhunty A. F.2009.Control of muscle ryanodine receptor calcium release channels by proteins in the sarcoplasmic reticulum lumen. Clinical and Experimental Pharmacology & Physiology. 36(3): 340-345.

Berchtold M.W., Brinkmeier H. and Müntener M. 2000.Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. Physiology Review. 80(3): 1215-1265.

Capes, E. M., Loaiza R. and Valdivia H. H. 2011. Ryanodine receptors. Skeletal Muscle. 1(1): 18.

Cheng, W., Altafaj X., Ronjat M. and Coronado R. 2005. Interaction between the dihydropyridine receptor Ca^{2+}channel beta-subunit and ryanodine receptor type 1strengthens excitation-contraction coupling. Proceedings of the National Academy of Sciences of the United States of America. 102(52): 19225-19230.

Chiang W., Allison C.P., Linz J.E. and Strasburg G.M. 2004. Identification of two *αRYR* alleles and characterization of *αRYR* transcript variants in turkey skeletal muscle. Gene. 330: 177-184.

Cordes M.H.J., Burton R.E., Walsh N.P., McKnight C.J. and Sauer R.T. 2000. An evolutionary bridge to a new protein fold. Nature Structural Biology. 7 (12): 1129-1132.

Epstain C.J., Goldberger R.F. and Anfinsen C.B. 1963. The genetic control of tertiary protein structure. Model systems.Cold Spring Harbor Symposia on Quantitative Biology. 28: 439-449.

Evan P.R. 2006. An introduction to stereochemical restrains. ActaCrystallographica. D63: 58-61.

Favero M., Jiang D-J., Chiamuler C., Cangiano A. and Fumagalli G.F. 2008. Expression of small-conductance calcium-activated potassium channels (SK3) in skeletal muscle: regulation by muscle activity. Journal of Physiology. 586.19: 4763-4774.

Fill, M. and Copello J. A. 2002.Ryanodine receptor calcium release channels. Physiological Reviews. 82(4): 893-922.

Galli L., Orrico A., Cozzolino S., Pietrini V., Tegazzin V. and Sorrentio V. 2002. Mutations in the *RYR1* gene in Italian patients at risk for malignant hyperthermia: evidence for a cluster of novel mutations in the C-terminal region. Cell Calcium. 32 (3): 143-151.

Gommans, I. M., Vlak M. H., de Haan A. and van Engelen B. G. 2002. Calcium regulation and muscle disease. Journal of Muscle Research and Cell Motility. 23(1): 59-63.

Hoffman, E. P. 1995. Voltage-gated ion channelopathies: Inherited disorders caused by abnormal sodium, chloride, and calcium regulation in skeletal muscle. Annual Review of Medicine. 46: 431-441.

Hooft R.W.W., Vriend G., Sander C. and Abola E.E. 1996. Errors in protein structures.Nature. 381 (6580): 272.

Johnson M. 1998. The β-adrenoceptor.American Journal of Respiratory and Critical Care Medicine. 158: S146-S153.

Jorquera G., Juretic N., Jaimovich E. and Riveros N. 2009. Membrane depolarization induces calcium-dependent upregulation of Hsp70 and Hmox-1 in skeletal muscle cells. American Journal of Physiology-Cell Physiology. 297(3): C581ñC590.

Krieger E., Nabuurs S.B. and Vriend G. 2003. "Homology modeling" in structural bioinformatics (Editors, Borne, P.E. & Weissig H.). Wiley-Liss, Inc. 507-521.

Lanner J.T., Georgiou D.K., Joshi A.D. and Hamilton S.L. 2010. Ryanodine receptors: structure, expression, molecular details, and function in calcium release. Cold Spring Harbor Perspectives Biology. 2: a003996.

Liu Z., Zhang J., Sharma M.R., Li P., Chen S.R.W. and Wagenknecht T. 2000. Three-dimensional reconstruction of the recombinant type 3 ryanodine receptor and localization of its amino terminus. Proceedings of the National Academy of Sciences. 98 (11): 6104-6109.

Lüthy R., Bowie J.U. and Eisenberg D. 1992. Assessment of protein models with three-dimensional profiles. Nature. 356: 83-85.

MacLennan, D. H. 2000.Ca^{2+} signalling and muscle disease. European Journal of Biochemistry/ FEBS. 267(17): 5291-5297.

Meng X., Wang G., Viero C., Wang Q., Mi W., Su X-D., Wagenknecht T., Williams A.J., Liu Z. and Yin C-C. 2009. CLIC2-RyR1 Interaction and structural characterization by cryo-electron microscopy. Journal of Molecular Biology. 387: 320-334.

Perez C.F., Voss A., Pessah I.N. and Allen P.D. 2003. RyR1/RyR3 Chimaras reveal that multiple domains of RyR1 are involved in skeletal-type E-C coupling. Biophysical Journal. 84: 2655-2663.

Rebbeck R.T., Karunasekara Y., Gallant E.M., Board P.G., Beard N.A., Casarotto M.G. and Dulhunty A.F. 2011. The β_{1a}subunit of the skeletal DHPR binds to skeletal RyR1 and activates the channel via its 35-residue C-terminal tail. Biophysical Journal. 100: 922-930.

Sambuughin N., Holley H., Muldoon S., Brandom B.W., de Bantel A.M., Tobin J.R., Nelson T.E. and Goldfarb L.G. 2005. Screening of the entire ryanodine receptor type 1 coding region for sequence variants associated with malignant hyperthermia susceptibility in the North American population. Anesthesiology. 102: 515-21.

Samso M., Feng W., Pessah I.N. and Allen P.D. 2009. Coordinated movement of cytoplasmic and transmembrane domains of RyR1 upon gating. PLoS Biology. 7 (4): 0980-0995.

Samso M., Shen X. and Allen P.D. 2006. Structural charcterization of RyR1-FKBP12 interaction. Journal of Molecular Biology. 356: 917-927.

Serysheva I.I. 2004. Structural insights into excitation-contraction coupling by electron cryomicroscopy. Biochemistry (Moscow). 69 (11): 1506-1514.

Sharma M.R., Jeyakumar L.H., Fleischer S. and Wagenknecht T. 2000. Three-dimensional structure of ryanodine receptor isoform three in two conformational states as visualized by cryo-electron microscopy. The Journal of Biological Chemistry. 275 (13): 9485-9491.

Spear B.B., Heath-Chiozzi M. and Huff J. 2001. Clinical application of pharmacogenetics. Trends in Molecular Medicine. 7 (5): 201-204.

Smith W.N., Dirks A., Sugiura T., Muller S., Scarpace P. and Powers S.K. 2002. Alteration of contractile force and mass in the senescent diaphragm with [beta] (2)-agonist treatment. Journal of Applied Physiology. 92(3): 941-948.

Szentesi P., Kovacs L. and Csernoch L. 2000. Deterministic inactivation of calcium release channels in mammalian skeletal muscle. The Journal of Physiology. 528(Pt3): 447-456.

Todd A.E., Orengo C.A. and Thornton J.M. 2001. Evolution of function in protein superfamilies, from a structural perspective.Journal of Molecular Biology. 307: 1113-1143.

Toniti W., Puchadapirom P, Chamkasem A. and Tansatit T. 2012. Preliminary report: homology modeling of human, dog and cat β-adrenergic receptors. Journal of Applied Animal Science. 5(2): 35-42.

Tung C-C., Lobo P.A., Kimlicka L. and Petegem F.V. 2010. The amino-terminal disease hotspot of ryanodine receptors forms a cytoplasmic vestibule. Nature. 468: 585-589.

Vangrevelinghe E., Zimmermann K., Schoepfer J., Portmann R. Fabbro D. and Furet P. 2003. Discovery of a potent and selective protein kinase CK2 inhibitor by high-throughput docking.Journal of Medicinal Chemistry. 46 (13): 2656-2662.

Zhou Q., Wang Q-L., Meng X., Shu Y., Jiang T., Wagenknecht T, Yin C-C., Sui S-F. and Liu Z. 2008. Structural and functional characterization of ryanodine receptor-natrintoxin interaction. Biophysical Journal. 95: 4289-4299.

Zucchi R. and Ronca-Testoni S. 1997. The sarcoplasmic reticulum Ca^{2+} channel/ ryanodine receptors: modulation by endogenous effectors, drugs and disease states. Pharmacological Reviews. 49(1): 1-51.

Zhou H., Jungbluth H., Sewry C.A.,Feng L., Bertini E., Bushby K., Straub V., Roper H., Rose M.R., Brockington M., Kinali M., Manzur A., Robb S., Appleton R., Messina S., D'Amico A., Quinlivan R., Swash M.,Müller C.R.,Brown S.,Treves S. and Muntoni F. 2007. Molecular mechanisms and phenotypic variation in*RYR1*-related congenital myopathies. Brain. 130: 2024-2036.