Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 3345–3347, March 1999

Commentary

Luminal loop of the ryanodine receptor: A pore-forming segment?

*David Balshaw, Ling Gao, and Gerhard Meissner**

Departments of Biochemistry and Biophysics, and Physiology, University of North Carolina, Chapel Hill, NC 27599-7260

In this issue of the *Proceedings*, Lynch *et al.* (1) report a new mutation I4898T in the skeletal muscle Ca^{2+} release channel/ryanodine receptor (RyR1). The mutation disrupts Ca^{2+} release channel function, causing severe alterations in muscle structure and function. Ryanodine receptors (RyRs) are Ca^{2+} channels that control intracellular Ca^{2+} levels by releasing Ca^{2+} from the sarco/endoplasmic reticulum (SR), an intracellular Ca^{2+} storage compartment. Mammalian tissues express three closely related RyR isoforms encoded by separate genes. All three isoforms are found in a variety of tissues, with RyR1 and RyR2 being the primary isoform in skeletal and cardiac muscle, respectively, and RyR3 being found in diaphragm, slow twitch skeletal muscles, and other tissues at low levels (2, 3).

Release of Ca^{2+} ions from skeletal muscle SR leads to muscle contraction through complex mechanisms. One of the primary players is RyR1, a large, ligand gated ion channel in specialized junctional areas of the SR (4). RyR1 consists of four large subunits, each with a molecular mass of 565 kDa, ≈5,000 aa, and four small 12-kDa FK506 binding proteins. RyR1 mediates the release of Ca^{2+} from the SR lumen in response to an action potential (5–7). In skeletal muscle, Ca^{2+} release is triggered by direct activation of RyR1 by the dihydropyridine receptor, a voltage-sensing calcium channel in the surface membrane (8).

Many endogenous and exogenous effectors contribute to RyR1 regulation (5–7). RyR1 is partially activated by Ca^{2+} binding to high-affinity, Ca^{2+}-specific sites and by Ca^{2+} and Mg^{2+} binding to low-affinity, less selective sites, giving rise to the characteristic Ca^{2+} dependence of channel activity illustrated in Fig. 1. Other endogenous molecules and pathways that modulate RyR1 function include adenine nucleotides, monovalent cations and anions, redox active molecules, and protein phosphorylation. Exogenous effectors include caffeine, volatile and local anesthetics, and ryanodine. Ryanodine is a plant alkaloid that binds with high affinity and specificity to RyRs in skeletal and cardiac muscle, brain, and other tissues. Ryanodine modifies the conductance and gating of the RyR ion channels by forming a partially open subconductance state at nanomolar concentrations and a fully closed channel at elevated concentrations. [^{3}H]Ryanodine binding is widely used as an indicator of channel activity because of its preferential binding to open channels.

RyR1 Mutations Linked to Malignant Hyperthermia and Central Core Disease

Two congenital myopathies have been linked to mutations in RyR1, malignant hyperthermia (MH) and central core disease (CCD). MH is a pharmacogenetic disorder resulting in the failure of terminating SR Ca^{2+} release after exposure to inhaled anesthetics and depolarizing muscle relaxants. CCD is a similar disorder, which, in addition to the response to anesthetics, is accompanied by muscle weakness and musculoskeletal defects. Seventeen single base mutations have been previously identified in humans that result in MH susceptibility, and of these, five also are associated with CCD (9, 10). In this issue of *The Proceedings,* Lynch *et al*. (1) report a new mutation I4898T from a large Mexican pedigree that is associated with CCD. A striking aspect of the mutation is that it is predicted to lie inside the SR lumen and inactivates the RyR1. Expression of the I4898T mutant in HEK cells yielded greatly reduced [^{3}H]ryanodine binding levels. Furthermore, the response to the two RyR1 channel agonists, halothane and caffeine, was completely abolished.

The 17 previous MH/CCD mutations in RyR1 map to hot spots between amino acid residues 35–614 and 2163–2458 (10). Five mutations leading to both MH and CCD susceptibility are R163C, I403M, Y522S, R2163H, and R2436H. Tong *et al.* (11) reported that expression of these five CCD-associated mutations in HEK cells results in an increased basal cytosolic Ca^{2+} concentration. Common to the MH and CCD RyR1 mutations was a decreased peak of Ca^{2+} release and a decreased SR Ca^{2+} load. Coexpression of wild-type RyR1 and the I4898T RyR1 mutant in HEK cells decreased the threshold of Ca^{2+} required to initiate opening of wild-type RyR1 and resulted in a reduced release of Ca^{2+} from internal stores, implying that the central defect for MH and CCD mutations in RyR1 is a leaky channel.

Molecular Predictions Based on the I4898T RyR1 Mutation

The N-terminal ≈4,000-aa residues of RyR1 form a large, loosely packed cytosolic foot domain (12). The remaining ≈1,000 C-terminal amino acid residues form the transmembrane domain. Two models for the membrane arrangement of the C-terminal domain suggest 10- (13) and four- (14) transmembrane domains. The four-transmembrane domain model is favored by data using site-directed antibodies (15), single-channel recordings with tryptic fragments (16), and deletion mutants (17). In both models the mutation reported by Lynch *et al.* (1) is positioned in the luminal domain at the end of a transmembrane helix in the 10-transmembrane model or in a luminal loop linking transmembrane domains 3 and 4 in the four-transmembrane model.

Disruption of Protein Interactions. Lynch *et al*. (1) speculate that their results support disruption of the interaction of the RyR1 with associated luminal proteins. RyR1 is known to interact with at least two SR luminal proteins, calsequestrin (18) and triadin (19). Calsequestrin is concentrated at the SR Ca^{2+} release sites (20), binds approximately 40 mol of Ca^{2+} per mol of protein (18), and may have an active role in regulating RyR1 (21, 22), in addition to increasing the Ca^{2+} storing capacity of the SR. Triadin is a membrane-spanning protein with a large luminal component that may form a bridge between RyR1 and calsequestrin (Fig. 2*A*) (23, 24). Triadin recently was reported to regulate RyR channel activity, and this action was modulated by calsequestrin (25). There is also evidence that in addition to the ternary complex between calsequestrin, triadin, and RyR1, there is a quaternary complex involving junctin, another SR-associated protein (26). Al-

The companion to this Commentary is published on page 4164.

*To whom reprint requests should be addressed. e-mail: meissner@med.unc.edu.

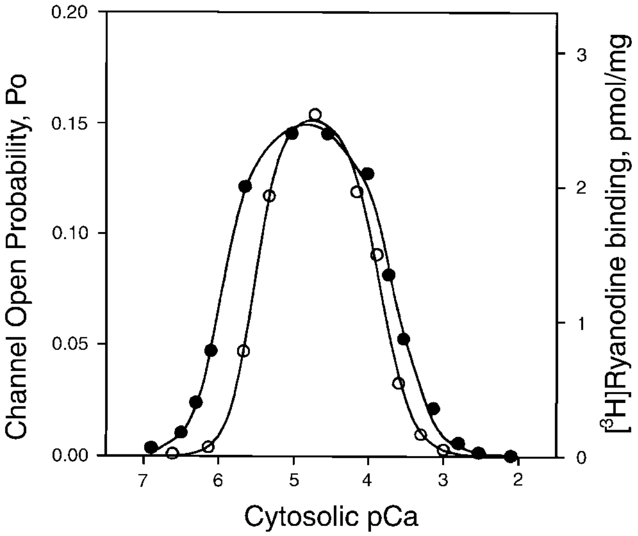


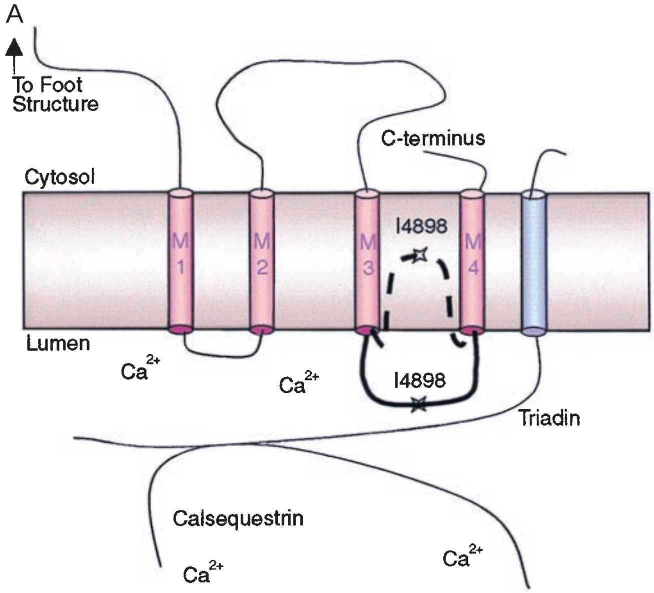
FIG. 1. Calcium dependence of RyR activity. Specific [^{3}H]ryanodine binding to rabbit skeletal muscle SR membranes (●) and purified RyR1 channel open probability (○) are shown as a function of cytosolic free Ca^{2+}. The data are fit to a two-site (one activation, one inhibition) logistic function.

though it is possible that the I4898T mutation may disrupt the regulation of RyR1 by associated luminal proteins in muscle, such an effect would be unlikely in the nonmuscle HEK293 cell expression system used by Lynch *et al.* (1).

Regulation by Luminal Ca^{2+}. Alternatively, the I4898T mutation may alter regulation of the RyR1 channel by luminal Ca^{2+}. As illustrated in Fig. 1, RyR1 is regulated by cytosolic Ca^{2+} concentrations. There is increasing evidence, however, that both skeletal and cardiac muscle RyRs also are regulated by SR luminal Ca^{2+}. Two potential sites mediating this regulation are a Ca^{2+} binding site within the RyR1 luminal domain itself (27, 28) and cytosolic sites involved in activating and inactivating the channel after ion flux to the cytosolic surface (29, 30). A regulatory luminal Ca^{2+} binding site could possibly be disrupted by the I4898T mutation. Resolving the sites of regulation by luminal Ca^{2+} has been difficult, however. The action of luminal Ca^{2+} at cytosolic sites is favored by data that the regulation by luminal Ca^{2+} depends on the channel first being activated by a cytosolic ligand. The dependence of channel activity on the amounts of Ca^{2+} flowing through the channel from the lumen to the cytosol also supports an action of luminal Ca^{2+} at cytosolic channel sites.

Luminal Loop as a Pore-Forming Segment. A third, and favored, explanation for the effects of the I4898T mutation is that this residue constitutes part of the ion conducting pore of RyR1. A recent publication (31) on the structure of the K^+ channel from *Streptomyces lividans* detailed the structure of the pore region of a prototypical ion channel with direct comparisons between K^+ channels and the voltage-gated Na^+ and Ca^{2+} channels. An important finding regarding the K^+ channel

A



B

Human RyR1	RKFYNKSEDE	DEPDMKCDDM	MTCYLFHMYV	GVRAGGGIGD	EIEDPAGDE	YELYRVVFD
RyR CONSENSUS	RKFYNKSED-	D-PDMKCDDM	-TCY-FHMYV	GVRAGGGIGD	EIEDPAGD-	YE-YR--FD
IP3R CONSENSUS	-----	---E--C-TL	LMCIVT--N-	GLR-GGG GD	-LR-PS--E	LF-ARV-YD
COMBINED IDENTITY		C	C	G R GGG GD	P	R D
S. lividans K^+ channel				VGYGD		

FIG. 2. (*A*) Proposed transmembrane arrangements for the RyR showing two possible orientations for the luminal loop between M3 and M4. If this loop is located in the lumen, it may be a site of interaction with triadin, and by extension with calsequestrin. Conversely, if the loop dips back into the membrane, it may form either a part of the conduction pathway or ion selectivity filter. (*B*) Sequence comparison of the proposed pore region. The sequence for the loop between M3 and M4 is given for the human skeletal RyR1 isoform, as well as the residues that are conserved between RyR subtypes, the conserved sequence for the related IP_3Rs, locations for amino acids that are identical between all RyR and IP_3R subtypes, and the analogous residues in the selectivity filter of the *S. lividans* K^+ channel.

structure was the identification of a conserved VGYG motif that comprised the ion selectivity filter. The side chains of the valine and tyrosine residues are oriented away from the pore and are involved in stabilizing the pore structure through hydrogen bonds between the tyrosine and a tryptophan residue on an adjacent helix. This orientation places the valine and tyrosine main chains in the pore where they participate in coordinating K^+ during ion transport.

A sequence related to the K^+ channel VGYG motif is GGIG in RyRs and GGV/IG in the related inositol trisphosphate receptors (IP_3Rs) (Fig. 2*B*). Although the isoleucine (or valine) side chains cannot be involved in stabilization through hydrogen bonds, they may contribute to stabilization through other mechanisms. Conservation of the VGYG motif in the K^+ channels and the related GGI/VG motif in all isoforms of the RyRs and IP_3Rs (Fig. 2*B*) implies that the luminal loop between transmembrane regions 3 and 4 of RyRs may fold back into the membrane in a manner similar to that shown for the K^+ channel (31) and suggested for the related IP_3Rs (32), with I4898 in the membrane as illustrated in Fig. 2*A*. RyR channels are high conductance channels with a wide range of permeabilities not only for divalent cations but also for group 1a cations such as Cs^+, K^+, and Na^+ (5). It therefore is highly likely that the I4898T mutation has wide-ranging consequences for the structure of the pore as well as the ion selectivity of the channel. Further support of the model of Fig. 2*A* is that other mutations (D4900A, D4900R, R4914E, and D4918A in Fig. 2*B*) in the luminal loop between transmembrane regions 3 and 4 result in loss of [^{3}H]ryanodine binding (33) and alter channel K^+ conductance (A. Tripathy and G.M., unpublished studies). The pore-forming or P-segment structure we propose for the RyR (Fig. 2*A*) in analogy to the pore structure of the voltage-dependent K^+ channels also is suggested for the voltage-gated Na^+ and Ca^{2+} channels (34).

The importance of the I4898T RyR1 mutation is borne out by the finding that high-affinity ryanodine binding in isolated vesicles and pharmacologically induced calcium release in a cell-based assay are absent for this mutant (1). Chen *et al.* (35) showed that a mutation lacking channel function can be partially rescued by coexpression with a wild-type channel. By using this approach, Lynch *et al.* (1) demonstrated that the I4898T mutation does alter Ca^{2+} dependence of ryanodine binding, which is believed to be an indicator of channel activation. The stoichiometry of wild type to mutant channels in an intact cellular system is not known, however. Six arrangements of RyR homotetramers based on these coexpression studies are possible. Accordingly, Ca^{2+} release observed in a cellular assay system may result from a wide range of release units, each having distinct properties, thereby complicating the interpretation of the results.

In conclusion, the I4898T mutation in the RyR1 described by Lynch *et al.* (1) raises several intriguing questions regarding the transmembrane location and function of a conserved SR luminal loop region in RyRs. Consequences of the mutation have implications on several aspects of channel structure and function ranging from pore structure and ion selectivity to interactions with sites of regulation by Ca^{2+} and ancillary proteins.

Support by United States Public Service Grants AR18687 and HL27430 is gratefully acknowledged.

1. Lynch, P. J., Tong, J., Lehane, M., Mallet, A., Giblin, L., Heffron, J. J. A., Vaughan, P., Zafra, G., MacLennan, D. H. & McCarthy, T. V. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 4164–4169.
2. Giannini, G., Conti, A., Mammarella, S., Scrobogna, M. & Sorrentino, V. (1995) *J. Cell Biol.* **128,** 893–904.
3. Ledbetter, M. W., Preiner, J. K., Louis, C. F. & Mickelson, J. R. (1994) *J. Biol. Chem.* **269,** 31544–31551.
4. Franzini-Armstrong, C. & Protasi, F. (1997) *Physiol. Rev.* **77,** 699–729.
5. Meissner, G. (1994) *Annu. Rev. Physiol.* **56,** 485–508.
6. Sutko, J. L. & Airey, J. A. (1996) *Physiol. Rev.* **76,** 1027–1071.
7. Zucchi, R. & Ronca-Testoni, S. (1997) *Pharmacol. Rev.* **49,** 1–51.
8. Rios, E. & Pizarro, G. (1991) *Physiol. Rev.* **71,** 849–908.
9. Mickelson, J. R. & Louis, C. F. (1996) *Physiol. Rev.* **76,** 537–592.
10. Loke, J. & MacLenann, D. H. (1998) *Am. J. Med.* **104,** 470–486.
11. Tong, J., McCarthy T. V. & MacLennan, D. H. (1999) *J. Biol. Chem.* **274,** 693–702.
12. Wagenknecht, T. & Radermacher, M. (1997) *Curr. Opin. Struct. Biol.* **7,** 258–265.
13. Zorzato, F., Fujii, J., Otsu, K., Phillips, M., Green, N. M., Lai, F. A., Meissner, G. & MacLennan, D. H. (1990) *J. Biol. Chem.* **65,** 2244–2256.
14. Takeshima, H., Nishimura, S., Matsumoto, T., Ishida, H., Kangawa, K., Minamino, N., Matsuo, H., Ueda, M., Hanaoka, M., Hirose, T. & Numa, S. (1989) *Nature (London)* **339,** 439–445.
15. Grunwald, R. & Meissner, G. (1995) *J. Biol. Chem.* **270,** 11338–11347.
16. Wang, J. P., Needleman, D. H., Seryshev, A. B., Aghdasi, B., Slavik, K. J., Liu, S. Q., Pedersen, S. E. & Hamilton, S. L. (1996) *J. Biol. Chem.* **271,** 8387–8393.
17. Bhat, M. B., Zhao, J., Takeshima, H. & Ma, J. (1997) *Biophys. J.* **73,** 1329–1336.
18. MacLennan, D. H. & Wong, P. T. S. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 1231–1235.
19. Caswell, A. H., Brandt, N. R., Brunschwig, J.-P. & Purkerson, S. (1991) *Biochemistry* **30,** 7507–7513.
20. Meissner, G. (1975) *Biochim. Biophys. Acta* **389,** 51–68.
21. Ikemoto, N., Antoniu, B., Kang, J. J., Meszaros, L. G. & Ronjat, M. (1991) *Biochemistry* **30,** 5230–5237.
22. Szegedi, C., Sarkozi, S., Herzog, A., Jona, I. & Varsanyi, M. (1999) *Biochem. J.* **337,** 19–22.
23. Guo, W. & Campbell, K. P. (1995) *J. Biol. Chem.* **270,** 9027–9030.
24. Caswell, A. H., Motoike, H. K., Fan, H. & Brandt, N. R. (1999) *Biochemistry* **38,** 90–97.
25. Ohkura, M., Furukawa, K. I., Fujimori, H., Kuruma, A., Kawano, S., Hiraoka, M., Kuniyasu, A., Nakayama, H. & Ohizumi, Y. (1998) *Biochemistry* **37,** 12987–12993.
26. Zhang, L., Kelley, J., Schmeissner, G., Kobayashi, Y. M. & Jones, L. R. (1997) *J. Biol. Chem.* **272,** 23389–23397.
27. Sitsapesan, R. & Williams, A. J. (1995) *J. Membr. Biol.* **146,** 133–144.
28. Gyorke, I. & Gyorke, S. (1998) *Biophys. J.* **75,** 2801–2810.
29. Tripathy, A. & Meissner, G. (1996) *Biophys. J.* **70,** 2600–2615.
30. Xu, L. & Meissner, G. (1998) *Biophys. J.* **75,** 2302–2312.
31. Doyle, D. A., Cabral, J. M., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T. & MacKinnon, R. (1998) *Science* **280,** 69–76.
32. Michikawa, T., Hamanaka, H., Otsu, H., Yamamoto, A., Miyawaki, A., Furuichi, T., Tashiro, Y. & Mikoshiba, K. (1994) *J. Biol. Chem.* **269,** 9184–9189.
33. Gao, L., Tripathy, A., Xu, L., Pasek, D., Balshaw, D., Xin, C. & Meissner, G. (1999) *Biophys. J.* **76,** A303 (abstr.).
34. Catterall, W. A. (1995) *Annu. Rev. Biochem.* **64,** 493–531.
35. Chen, S. W. R., Ebisawa, K., Li, X. & Zhang, L. (1998) *J. Biol. Chem.* **273,** 14675–14678.