Ryanodine receptors (RyRs) are a family of intracellular channels that mediate Ca\(^{2+}\) release from the endoplasmic and sarcoplasmic reticulum. More than 50 distinct point mutations in one member of this family, RyR1, cause malignant hyperthermia, a potentially lethal pharmacogenetic disorder of skeletal muscle. These mutations are not randomly distributed throughout the primary structure of RyR1, but are grouped in three discrete clusters. In this issue of the *Biochemical Journal*, Kobayashi et al. present evidence that interdomain interactions between two of these mutation-enriched regions play a key role in the gating mechanism of RyR1.

Key words: calcium channel gating, malignant hyperthermia, ryanodine receptor.
the effect of substituting each amino acid of RyR structures on their electrophysiological properties could provide a powerful approach to understanding channel structure–function relationships. However, the time and financial cost of substituting every amino acid of a 5000-amino-acid primary sequence, then analysing the functional consequences, would be prohibitive. As a result, any conceptual approach that can limit the number of residues to be analysed before any experimentation is of considerable value. Nature has provided many clues as to which amino acid residues are key players in RyR gating. Bioinformatic approaches have identified amino acid residues in RyR structures that are highly conserved throughout evolution and so might play crucial roles in channel function. Comparison of RyR primary structures with those of other channel families has also generated insights into the structure–function relationships of these proteins. Using this approach, it is suggested that certain RyR transmembrane helices might form a three-dimensional structure analogous to that of the Streptomyces lividans KcsA potassium channel pore [9]. Mutations in the \textit{RYR1} gene are expressed as susceptibility to MH by virtue of their influence on the function of the channel protein product. The 50 or so RyR1 mutations appear to be grouped into three clusters in the primary structure of this protein: one near the N-terminus, one central and the other near the C-terminus. Provided that it does not arise from biases in polymorphism detection methods and analyses, the distribution of these mutations implies that these three regions are likely to be important determinants of RyR1 channel gating.

In this issue of the \textit{Biochemical Journal}, as well as in a number of recently published observations summarized in their excellent recent review [10], Kobayashi et al. [11] exploit the apparent clustering of MH mutations into discrete domains of RyR1 to explore the roles these regions play in channel gating. They propose that, while in its resting state, the RyR1 channel is blocked by interdomain interactions between regions corresponding to the N-terminal and central MH mutation clusters. Factors that destabilize these interactions, such as endogenous and pharmacological RyR activators or MH mutations, ‘unzip’ the interacting domains, thereby unblocking the channel pore (Figure 1). In order to test this hypothesis further, Kobayashi et al. [11] generated sequence-specific antibodies against the synthetic peptides ‘domain peptide 1’ (DP1, RyR1 residues 590–609) and ‘domain peptide 4’ (DP4, residues 2442–2477). These antibodies specifically recognized their target sequences and did not interact with peptides corresponding to other regions of RyR1. Purified anti-DP1 and anti-DP4 immunoglobulins both enhanced [\textsuperscript{3}H]ryanodine binding to skeletal muscle microsomes not interacting with domains containing residues 799–1172 and residues 3010–3225. However, a fusion protein containing this epitope interacted with this preparation of this Commentary, George et al. [15] published

MH mutation clusters, making the former more accessible to the macromolecular quenching, whereas the small molecular quencher could access the fluorescent label equally well in both ‘zipped’ and ‘unzipped’ conformations. Although available data strongly support the notion that interdomain interactions between the regions defined by DP1 and DP4 are critical in RyR1 gating, intramolecular contacts between other regions are also likely to be important. Studies by Zorzato et al. [12], employing a monoclonal antibody that recognizes an epitope located between RyR1 residues 335–620, also demonstrated that interdomain interactions regulate the gating of the channel. However, a fusion protein containing this epitope interacted with regions containing residues 799–1172 and residues 3010–3225 of RyR1 in overlay assays [12]. These regions are distinct from that encompassing DP4 and one of them (residues 3010–3225) is a calmodulin-binding domain, indicating that accessory proteins might modulate pivotal interdomain interactions within RyR1.

Although the findings presented by Kobayashi et al. [11] represent a significant step forward in resolving the molecular detail of RyR channel gating, there is considerable scope for further investigation. For example, most mutations that cause CCD are located in the C-terminal cluster of RyR1. There is debate over whether such mutations generate channels that are excessively leaky and deplete Ca\textsuperscript{2+} in SR stores, or do not form fully functional channel complexes due to modification of the transmembrane pore [13,14]. Approaches similar to those employed by Kobayashi et al. [11], examining the influence of ‘CCD domain’–specific peptides and antibodies on RyR1 channel gating, might help to clarify this issue. Indeed, during preparation of this Commentary, George et al. [15] published
data using FRET (fluorescence resonance energy transfer) techniques to demonstrate that the transmembrane channel-forming domain of RyR2 interacts functionally with a cytoplasmic region corresponding to amino acid residues 3722–4610. The interacting domains defined by DP1 and DP4 are of suitable size to permit resolution of their solution structures by use of NMR imaging. Such approaches could be employed to define the critical interacting residues between these domains, the conformational changes that result from their binding and the influence of incorporating MH mutations on these molecular contacts. Cryoelectron microscopy and image reconstitution could be used to assign the position of epitopes recognized by domain-specific antibodies to the three-dimensional structure of RyR1, as well as to examine the influence of these probes on channel ultrastructure. We anticipate that interplay between the fields of molecular genetics and channel biochemistry, such as that presented by Kobayashi et al. [11], will continue to increase appreciation of the molecular basis of RyR channel gating and its participation in human disease processes.

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