

An updated view of the structural basis for dihydropyridine receptors-ryanodine receptors direct molecular interaction in skeletal muscle

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Abstract

This presentation reviews images of electron micrographs from various skeletal muscles identifying a consistent association of dihydropyridine receptors (DHPR) tetrads with alternate ryanodine receptors. Imaging of the junctional gap in triads from various sources provide direct evidence for the association of four dihydropyridine receptors (DHPRs), clustered into tetrads, with alternate ryanodine receptors (RyRs). It is not clear whether firing of all four components of a tetrad is necessary to fully activate the opening of the RyR channel.

Key Words: skeletal muscle; excitation-contraction coupling; T tubules; sarcoplasmic reticulum; dihydropyridine receptors; ryanodine receptors.

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In skeletal muscle, direct functional coupling between the calcium channels of transverse T tubules (dihydropyridine receptors, DHPRs)¹ and the calcium release channels of the sarcoplasmic reticulum (named ryanodine receptors, RyRs, and seen as “feet in electron micrographs of thin sections”²) is thought to be facilitated by the highly specific positioning of the two channels within the T tubule-SR triads. DHPRs are natively grouped into tetrads or groups of four DHPRs around a common center.³ It is proposed that the precise alignment and spacing of the T tubule DHPR tetrads along the T tubule axis is due to the precise alternate positioning of tetrads relative to the RyRs feet, in the facing sarcoplasmic reticulum (SR) membrane, thus providing sites of direct molecular interactions.³ However, the relative location of the two channels have been deduced from indirect structural information in thin sectioned and freeze fractured images from related but not identical structures. Figure 1 illustrates the type of images from which the commonly accepted structural hypothesis of the 2:1 relationship between tetrads and feet has been derived. The top image is from a freeze-fracture and the bottom from a thin section. Alignment of the two images confirms that while both DHPRs tetrads (top image) and RyR feet (bottom) are distributed into rows parallel to the T tubule longitudinal axis, tetrads are spaced at a distance equal twice that between RyRs. The alternate positioning of tetrads relative to RyR is fully consistent with the disposition of tetrads within clusters on the surface of

cultured cells where DHPR and RyRs are expressed in the presence of RyRs,^{4,5} but again the evidence is indirect. A direct confirmation of the 2:1 relationship requires visibility of DHPR tetrads and of RyR in single images. No such image was ever published, but examples are shown in this communication. The images of Figures 2 A-C illustrates rare micrographs from thin cross sections of skeletal muscles culled from a large selection of

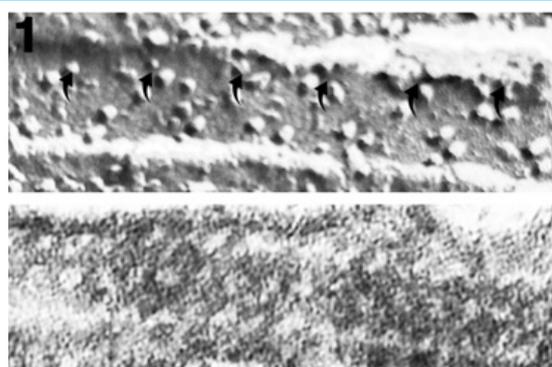
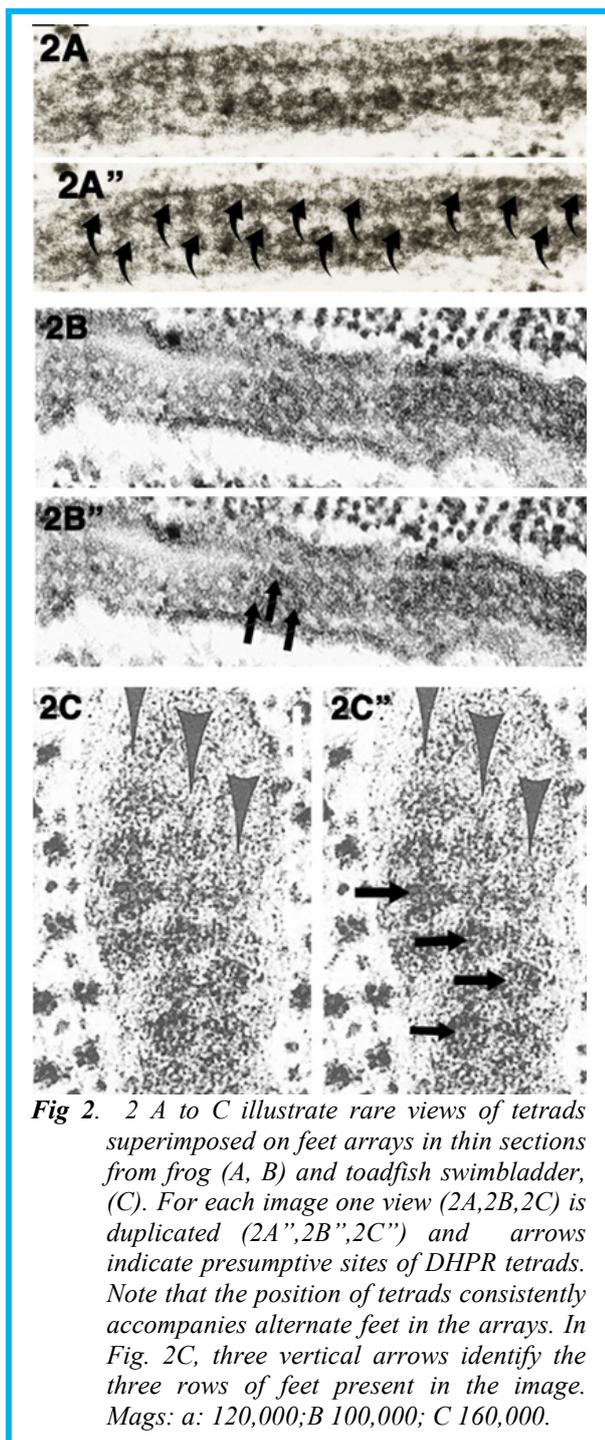
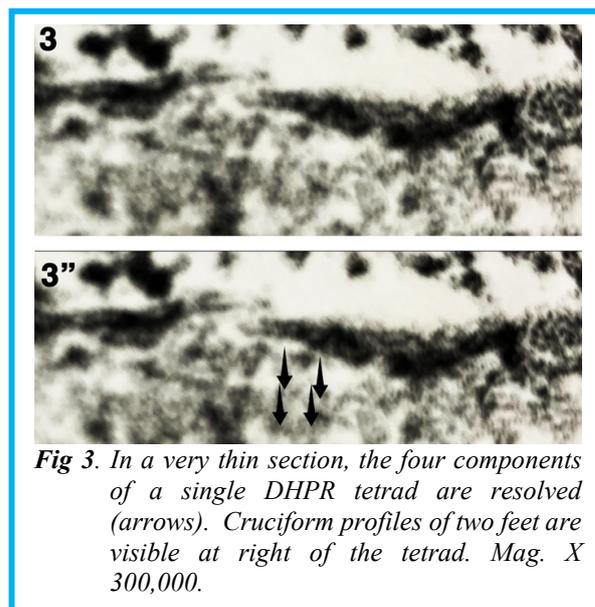


Fig 1. Images of junctional T tubule domains from two triad junctions of frog muscle illustrating the arrays of DHPR tetrads in a freeze fracture image (above) and of feet in a thin section (below). Arrows identify the position of tetrads, emphasizing the double spacings of tetrads relative to feet. Mag: X 120,000.



archived images. In these micrographs the plane of the section is parallel to the orientation of the junctional gap between T tubules and SR, and provides a view of feet arrays associated with limited grazing view of tetrads where the section includes a view of the plane above/below that of the feet. In these limited views, indicated by arrows, profiles of tetrads are superimposed on profiles of feet and are recognized by the additional density of the structure where feet and tetrads are included within the same section thickness. The images



are limited in extent because tetrads occupy a narrow strip over the edges of RyRs and partial views of the tetrads are not easily recognized. Where no tetrads are visible, the density profiles of feet occupy two, occasionally three, rows in the junctional gap and are dominated by the top views of the cytoplasmic RyR domains (Figure 1). Individual feet have a slightly distorted square profile that is connected to adjacent feet along and across the rows insuring the precise alignment of feet. In the rare micrographs where tetrads are clearly distinguished three identifying characteristics are immediately visible (Figures 2A-C). One is the fact that the density profiles of tetrads are located at some distance from each other and, differently from feet, show no signs of connecting to each other. Secondly, tetrad profiles are located in position corresponding to that of alternate feet from the feet arrays (Figures 2 A''-C'' arrows). Thirdly tetrad profiles differ significantly from that of feet in the sense that the center of each profile appears empty. At higher magnification (Figure 3) a single tetrad profile shows four subcomponents derived from the four DHPR subunits that constitute a tetrad. Images of tetrads are mostly limited to very short portion of the feet array, mostly due to the fact that imaging of tetrads is limited to critical section thickness and very precise alignment of the section plane. The extra density marking the position of tetrads is strictly and consistently associated with alternate feet profiles confirming that the "alternate" association of tetrads with feet is a general rule. Ultimately that means that four DHPR molecules are available for interacting with two RyRs (e.g., see Figure 4 in Paolini C, Protasi F, Franzini-Armstrong C. 2004).³ Even when not guided by the elongated T-SR junction structures, feet and DHPR naturally assemble into ordered arrays in which the DHPR/tetrad relationship in maintained.^{4,5}

In conclusion, imaging of the junctional gap in triads from various sources provide direct evidence for the

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association of four DHPRs, clustered into tetrads, with alternate RyRs. It is not clear whether firing of all four components of a tetrad is necessary to fully activate the opening of the RyR channel.

List of acronyms

DHPR - dihydropyridine receptor

RyR - ryanodine receptor

SR - Sarcoplasmic reticulum

T tubules - transverse tubules

Contributions of Author

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