

Evolution of skeletal type e-c coupling

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Muscle cells are regulated by large movements of calcium ions and these in turn are controlled by complex systems of membranes. All muscles, from the most primitive to the most evolved organisms share a common set of organelles and proteins that are involved in calcium homeostasis. A calcium pump protein of the sarcoplasmic reticulum and an homologous pump in the plasmalemma are the major contributor to the maintenance of the very low resting concentrations of cytoplasmic calcium. A major SR calcium release channel, the ryanodine receptor (RyR) has been detected from worms up. In worms as well as in all other animals RyRs are located at specific sites (calcium release units) mostly associated with either the plasmalemma or its invaginations the T tubules. A unique interaction between the L type calcium channels of plasmalemma/T tubules and the RyR, allowing a novel mechanism of e-c coupling involving direct intermolecular signaling, first appears in low vertebrates.

In honor of Prof. Ernest Gutmann, whose deep understanding of muscle guided my early experience with this most fascinating tissue. CFA

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A major step in the evolution of vertebrates seems to be the duplication of genes that allowed the differentiation of muscle specific myosin II into separate cardiac and skeletal isoforms and thus the appearance of a separate cardiac system [13]. Skeletal and cardiac muscles of higher vertebrates differ in the mechanism of muscle activation, or excitation-contraction (e-c) coupling, and in the proteins involved. The evolution from a single mechanism of e-c coupling for all muscle fibers, to one that differentiates between cardiac and skeletal muscles seems to have occurred at the transition between chordates and vertebrates in parallel to the myosin II dichotomy [10]. The transition involves the appearance of separate calcium release channels for the two types of striated muscles [2] and the acquisition by skeletal muscle of a novel mechanism for controlling the initiation of contraction.

The two main molecules essential to e-c coupling are dihydropyridine receptors (DHPRs) and ryanodine receptors (RyRs), both located in Calcium Release Units (CRUs). DHPRs are L-type channels that sense the membrane voltage and are located in junctional domains of plasmalemma and its invaginations (T tubules). RyRs are calcium release channels of the endoplasmic reticulum or sarcoplasmic reticulum (SR). In muscle cells, both RyRs and DHPRs are strategically located within Calcium Release Units (CRUs), so that RyRs can

receive a signal from the DHPRs, but this is achieved by different mechanisms. In skeletal muscle, a direct RyR-DHPR interaction is mediated by a specific link between the two molecules, but in cardiac muscle of vertebrates and in body muscles of invertebrates RyR and DHPRs interact indirectly and are only loosely associated. The structural expression of the specific RyR-DHPR association is the disposition of DHPRs in arrays of tetrads, dictated by their link with the tetrameric RyR channels. DHPR tetrads are present in skeletal muscle, but not in vertebrate cardiac muscle and in the body muscles of invertebrates.

We have focused on the transition from random to tetradic positioning of DHPRs and its correlation to e-c coupling modes in the course of evolution, thus testing the hypothesis that one of the evolutionary steps leading to vertebrates is the development of a novel means of activating muscle based on a specific coupling of DHPRs to RyRs [3]. Amphioxus, a protochordate, is appropriately placed in a transitional position between invertebrates and vertebrates. Hagfish, one of the most primitive craniates, is considered to be either a transition between low chordates and vertebrates or the most primitive living vertebrate [5, 11, 12]. Lamprey is an early vertebrate and garfish is an ancient bony fish. Data from more advanced fish are already available in the literature [1, 6]. Our results show that a significant

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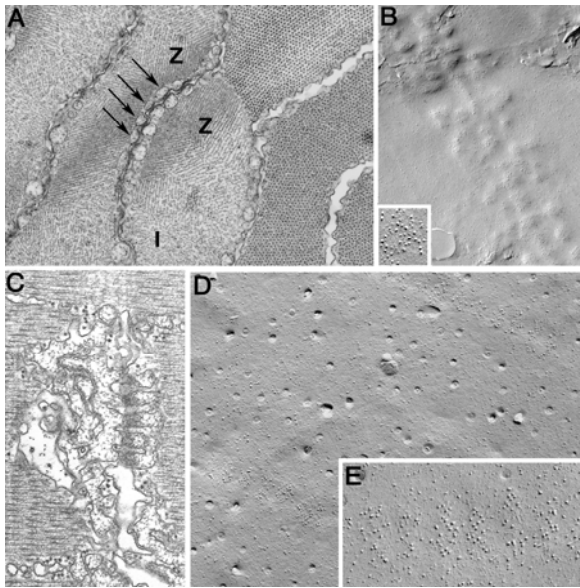


Figure 1: A) Cross sectioned myotome in amphioxus shows “lamellae”, each representing a single cell containing one myofibril. Opposite Z lines (Z) and the adjacent I bands, several jSR sacs associate with the plasmalemma forming peripheral couplings (arrows). B) Freeze-fracture replica of the cytoplasmic leaflet from the plasmalemma in an Amphioxus lamella. 2-3 parallel rows of dome-shaped plasmalemma domains are located at every Z line. The plasmalemma domes are decorated by clusters of particles that are consistently larger than those decorating the rest of cytoplasmic leaflet (inset). C) The myotome muscle of garfish shows a peculiar finger-like shape of the jSR. D, E) The plasmalemma domains that face the finger like jSR vesicles are decorated by clusters of particles arranged in tetrads.

structural transition marks the protochordate to craniate evolutionary step.

Amphioxus myotomes are composed of extremely thin (1-2 μm) sheet-like cells, called lamellae, containing a single myofibril and no T-tubules system [16]. The sarcoplasmic reticulum is located at the periphery between the myofibril and the plasmalemma [4, 8, 9, 14, 15]. and numerous junctional SR (jSR) vesicles are located at the level of the Z line and adjacent I bands (Fig. 1A, arrows). The vesicles are associated with feet or RyRs [9] and form peripheral couplings by associating with the plasmalemma, thus constituting the CRUs of amphioxus lamellae.

Unlike amphioxus, all muscles from lower vertebrate that we examined have larger muscle fibers and contain T tubules. The jSR in these muscles forms both peripheral couplings at the periphery and dyads/triads (SR-T junctions) internally. For example, in garfish the jSR cisternae are in the form of longitudinally oriented finger-like extensions (Fig. 1C) that wrap around the T

tubules and also face the plasmalemma at the fiber's edge, forming peripheral couplings, positioned at the level of the Z lines. Lamprey and hagfish have different SR geometries, but they also present both internal CRUs (dyads and triads) and peripheral couplings.

In all these muscles it is expected that freeze-fracture of the surface plasmalemma will reveal membrane domains that are associated with sites of peripheral couplings and that the sites can be identified on the basis of their frequency, size, shape and location relative to the bands of the sarcomere. Indeed, all muscles present special domains of the plasmalemma that are distinguished by clusters of large intramembranous particles visible in the fractured cytoplasmic leaflet of the surface membrane and are appropriately located.

The fractured plasmalemma of amphioxus shows 2-3 parallel rows of dome-shaped protrusions located in circumferential belts centered over the Z lines, where peripheral couplings usually reside (Fig. 1B). The plasmalemma of hagfish has few and very small patches of the large particles, in keeping with the fact that peripheral couplings are not frequent and of small size in these fibers. The lamprey's plasmalemma shows frequent clusters of large particles occupying elongated, rectangular patches of surface membrane in proximity of the Z lines, with a long axis oriented along the circumference of the fiber. This fits very well with the observation that peripheral couplings in this fish are located at the Z lines, they are frequent and they have extensive rows of feet when seen in cross sections of the muscle fibers, that is when observed in the direction of the fiber's circumference. Finally in the garfish muscle fibers, membrane domains occupied by large particles, are located at the Z line level, they are narrow, elongated in the direction parallel to the fiber's long axis, and arranged into groups of 5-8, again in keeping with the presence of numerous elongated peripheral couplings that are clustered into small groups (Figs. 1 D and E).

The arrangements of large particles within the clusters defined above fall into two major categories. In amphioxus the large particles are clustered in fairly close proximity to each other, but there is no detectable order in their disposition within the clusters (Figs. 2A-D): the particles are neither aligned with each other along straight lines, nor consistently grouped into a recognizable pattern. Muscle fibers from all other samples (Figs. 2E-H for hagfish; 9I-L for lamprey and Fig. 2M-P for garfish) show grouping of the particles into sets of four located at the corners of small squares (tetrads), as first seen in bony fish [1, 6]. The tetrads however are often incomplete and also are distorted by the fracturing process. So each tetrad may be missing one or more particles and the position of the particles may not define a perfect square. This is attributed to a combination of incomplete fracturing and/or lack of components within the tetrads.

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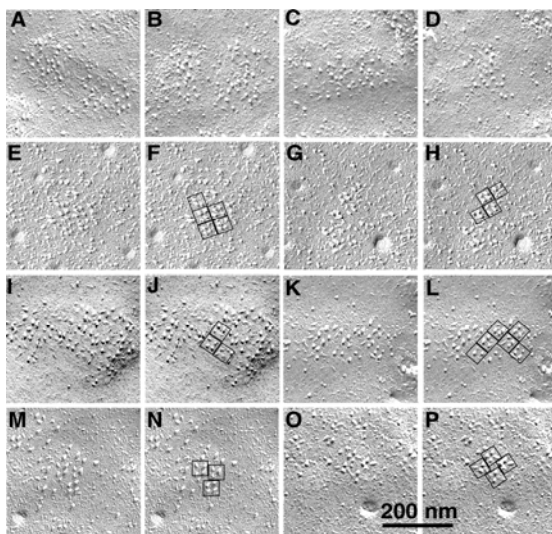


Figure 2: Details of large particle clusters in amphioxus (A-D); hagfish (E-H); lamprey (I-L); garfish (M-P). In amphioxus the particles are clustered but show no special arrangement within the clusters. In hagfish, lamprey and garfish the particles are grouped into small orthogonal arrangements of four (tetrads, within squares). The tetrads are not always complete but they clearly form part of a larger array. Two arrangements are visible. In hagfish (E-H) and lamprey (I-L), the tetrads abut side by side; in garfish (M-P) on the other hand, the tetrads are in proximity near their corners. The latter arrangement is typical of most vertebrates, including higher fish.

One way of determining the parameters of a tetrad array is to outline the tetrads with a square tangent to their outer profile (Figs. 2 F, H, J, L, N, P). When this is done, it is clear that two different arrangements of tetrads are displayed, lamprey and hagfish differing from garfish. In lamprey and hagfish the squares delimiting tetrads lie side by side, so that lines connecting the centers of adjacent tetrads are parallel to lines connecting the subunits of the tetrads along the square edges. In garfish, on the other hand the tetrads are displaced by one half width relative to each other, and the lines connecting the centers of adjacent tetrads are at an angle relative to the lines delimiting the tetrad squares.

In summary all muscles examined have clusters of large intramembrane particles that are located in correspondence of peripheral couplings. In all craniata the particles are arranged in tetrads and this directly identifies them as representing L-type channels or DHPRs, as previously established [20]. In the case of Amphioxus, even though the particles do not have a specific arrangement, we rely on the analogy with muscles of invertebrates and cardiac muscle [17, 19, 23]

to propose that they also represent the Ca channels responsible for the large inward Ca current in these muscles [8, 15]. This structural evidence can be directly translated into information of molecular and functional diversity. It is well established that the special relationship between DHPR and RYR disposition evidenced by the presence of DHPR tetrad arrays is directly linked to the presence of the skeletal specific isoforms of the two proteins and to their ability of mechanically interacting [7, 18, 21]. The appearance of tetrads is thus a direct indication of the new acquisition of the skeletal muscle specific isoforms of RyR (RyR1 or homologous) and α 1DHPR (SkDHPR or homologous), which occurs at the transition between low chordates and vertebrates.

The acquisition of DHPR tetrads by low craniata corresponds quite well with the acquisition of a DHPR-RyR interaction that does not require Ca permeation through the L type channels by the same species [10]. This then represents a transition to a more advanced form of excitation contraction coupling that was made possible by the ability of the two molecules to form a supramolecular complex and to directly influence each other. In addition to allowing a direct interaction with RyR, the skeletal muscle DHPR carries little calcium current [22] thus reducing the metabolic burden on the muscle fiber.

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Abbreviations

e-c, excitation-contraction, jSR junctional SR; DHPR dihydropyridine receptors, L type voltage dependent calcium channels; CRU Calcium Release Unit

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