THE ACTIVATION AND CONTROL COMPONENTS

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EXCITATION-CONTRACTION COUPLING: THE LINK BETWEEN THE SURFACE AND THE INTERIOR OF A MUSCLE CELL

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SUMMARY

The control of the contractile state of a muscle cell, a process called excitation-contraction coupling, involves a sequence of steps and is passed along through a series of cellular structures. The excitation of the fibre, initiated at the motor end-plate, spreads rapidly over the surface of the fibre and into the fibre along the T-system networks as an action potential. This excitation is coupled to the sarcoplasmic reticulum (SR) by a mechanism not yet identified with certainty. The SR then responds by releasing stored calcium from its interior compartment into the myoplasm. The diffusion and binding of this calcium to regulatory proteins initiates the mechanical events of contraction, which subsequently are turned off when calcium is removed from these regulatory binding sites. Some of this calcium may temporarily bind to proteins in the myoplasm, but eventually it gets pumped back into the internal compartment of the SR by a calcium-ATPase in the SR membrane. The system then is poised for a repeat performance.

The full contractile tension of a skeletal muscle fibre typically is developed a few tens to a few hundreds of milliseconds after the upstroke of the surface action potential. This generally is an insufficient time for an activator, such as calcium ion, to diffuse from the surface to the centre of the fibre (Hill, 1948, 1949). Yet we know that the centre of the fibre is activated, even in a single twitch (Gonzalez-Serratos, 1971). Measurements of the quantity of calcium entry through the surface membrane per contraction (Bianchi & Shanes, 1959) give values that are at least an order of magnitude smaller than the amount of calcium thought to be needed for full activation of contraction. Additionally, more recent evidence indicates that this calcium entry through the surface occurs too late to be directly involved in contraction activation (Sanchez & Stefani, 1978). All these results argue against a mechanism for coupling electrical activity at the surface of the fibre to contraction of the interior (E-C coupling) that depends on entry and diffusion of calcium ions from the surface to the contractile fibrils.

It now is known that E-C coupling in skeletal muscle fibres of vertebrates and many invertebrates involves the release of calcium from storage sites inside the fibre,

Key words: Muscle, excitation-contraction coupling, contraction.

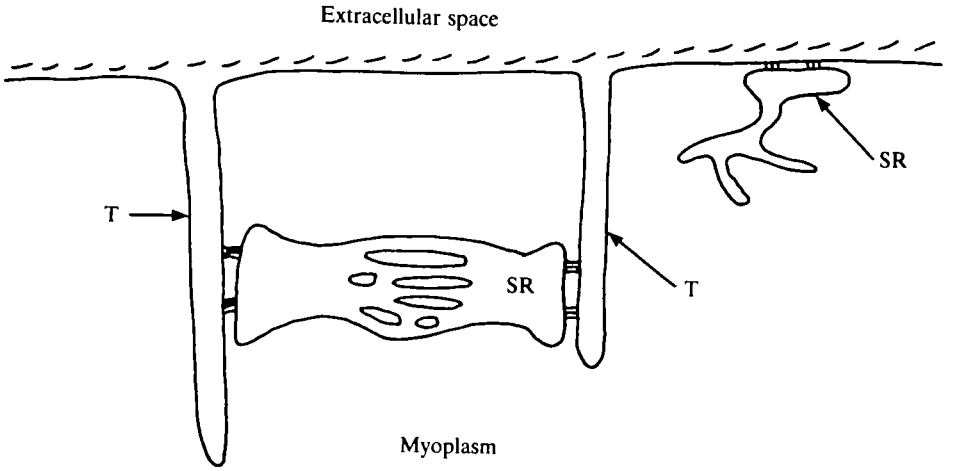

Fig. 1. Drawing showing two forms of coupling of SR to the surface of a muscle cell. The simpler form (right) consists of a direct apposition of a portion of SR to the inner surface of the plasma membrane of the muscle cell. The more complex form (left) has couplings at specific locations between the SR and infolded T-tubules extending into the fibre from its surface. In both cases, the couplings show a characteristic fine structure, and they are thought to be the sites where excitation of the surface of the fibre triggers the SR to release calcium.

specifically within the sarcoplasmic reticulum (SR). The surface action potential is coupled to the SR, either by direct connection at surface foci or by means of the transverse tubules (T-system) (Fig. 1). A sequence of steps, spread out over space and time, carries out the overall function of E-C coupling.

This paper will discuss the mechanisms underlying each step, to the extent that they are known, and relate these to the overall contractile function of the cell.*

The T-system provides an electrical pathway from the surface of a skeletal muscle fibre to all regions of the fibre (Figs 2, 3). The T-system networks have a complex structure and their three-dimensional arrangement is best illustrated with stereoscopic electron micrographs (see Franzini-Armstrong & Peachey, 1982; Peachey & Franzini-Armstrong, 1983). Functionally, the T-system acts as an inward, electrical extension of the surface membrane. A surface action potential produces an impulse in the T-system that spreads throughout the network in a few milliseconds at room temperature for a frog twitch fibre (Adrian, Costantin & Peachey, 1969; Adrian, Chandler & Hodgkin, 1970; Costantin, 1970). These action potentials are blocked by tetrodotoxin and require external sodium ions. They have been modelled as propagating action potentials of the Hodgkin-Huxley type in three dimensions (Adrian & Peachey, 1973).

* This review will be developed on a somewhat historical basis, with a few representative references. For a more complete set of references, see Chapters 1–3 and 10–15 in *The Handbook of Physiology*, Section 10: *Skeletal Muscle*, (eds L. D. Peachey, R. H. Adrian & S. R. Geiger). American Physiological Society, Bethesda, MD, U.S.A., 1983.

A strong suggestion that the T-tubules carry excitation into the depth of the fibre came from the observation that contractions could be elicited in amphibian twitch muscle fibres by depolarization of a patch of membrane over a Z-line of the myofibrils and not by depolarization over an A-band (Huxley & Taylor, 1958). This localization of sensitivity to depolarization correlated with the location of T-tubule openings in these fibres. A similar correlation also held in three other kinds of fibres with different T-system arrangements (e.g. Huxley & Peachey, 1964). Thus T-tubule depolarization, induced where the T-tubule joins the surface membrane, seems clearly to be an early step in the excitation-contraction coupling mechanism.

It should be noted that the time taken for excitation to spread inward along the T-system is short compared to the time required to get full activation of contraction. It appears that this step has evolved to be fast enough to ensure reasonably good synchrony between fibrils near the surface of the fibre and those at the centre.

It is not so clear how the T-system communicates with the SR. The space between the two membranes is bridged by electron dense components called 'feet' (Fig. 4). Sometimes the feet have a light core, as if they had a hole down the middle, and an increased frequency of such cores has been reported in muscle that had been activated (Eisenberg & Eisenberg, 1982). Thus, there is a possibility that a current channel

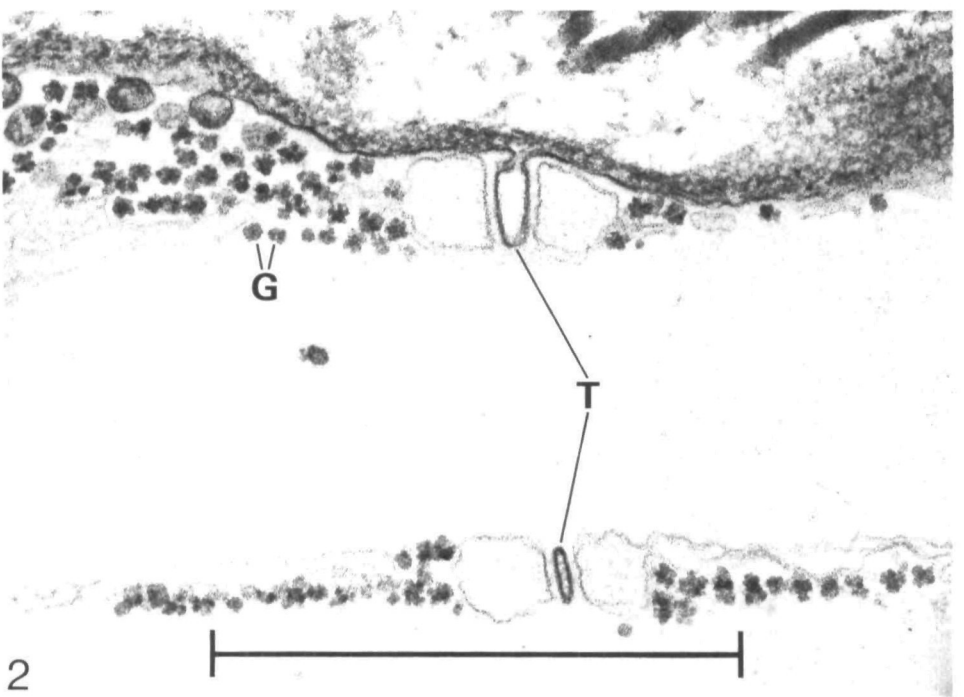

Fig. 2. Electron micrograph of a longitudinal section of a frog sartorius muscle fibre stained with tannic acid and bismuth subnitrate. The stain intensifies the density of the surface membrane, revealing a T-tubule connected to the surface. Deeper in the fibre, another portion of the T-tubule network is seen. Glycogen particles (G) also are stained. The bar indicates 1 μm.

opens during activity, and that ionic current flowing from the T to the SR somehow induces calcium release from the SR (Mathias, Levis & Eisenberg, 1980). I think, however, that at the present time this has to be considered as only one of at least three possible mechanisms. A second possibility is some sort of charge movement in the T-system membrane when it depolarizes, followed by a molecular movement or conformation change in a membrane-associated molecule that carries the signal across to the SR, perhaps through the feet (Schneider & Chandler, 1973; Adrian, 1978). The third possibility is a chemical transmitter, perhaps calcium itself, released from the T-tubule during its depolarization, and diffusion to the SR, where interaction with an appropriate receptor initiates calcium release (Ford & Podolsky, 1972; Endo, 1977).

In any case, we do know that calcium is released from the SR. Electron probe microanalysis has shown that during a tetanus, there is a decrease in calcium concentration within the SR and an associated appearance of calcium in the myoplasm (Somlyo *et al.* 1981). These measurements were made on freeze-dried thin cryosections of rapidly frozen frog muscles, to minimize loss and movement of ions during specimen preparation. Comparison of resting and tetanized muscles showed that about one-half of the calcium contained in the terminal cisternae of the SR in a resting muscle is released into the cytoplasm during a 1·2-s tetanus, and that this is enough to raise the total calcium concentration in the myoplasm by almost 1 mmol l^{-1}.

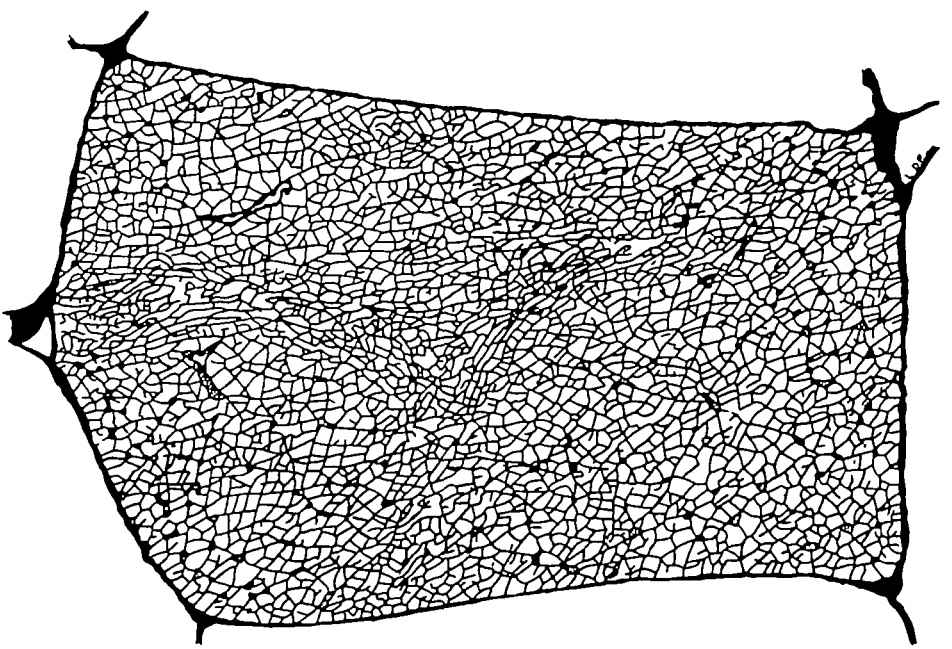

Fig. 3. Reconstruction of the T-system network over the entire cross-section of a frog sartorius muscle fibre, made by reconstruction from serial, high voltage electron micrographs. From Peachey & Eisenberg (1978). Magnification, ×1400.

This is considerably greater than the rise in free cytoplasmic calcium ion concentration measured with indicator dyes, and more than enough to saturate calcium-specific binding sites on troponin.

However, dynamic studies of calcium movements using calcium-sensitive dyes and theoretical considerations suggest that the rate constants of the various calcium release and binding steps have to be considered before one can say what concentrations are bound to what sites at any given time (see Robertson, Johnson & Potter, 1981; Baylor, Chandler & Marshall, 1983) (Fig. 5). This is especially true in frog muscle, which contains relatively high concentrations of the calcium-binding protein parvalbumin, which can bind a significant fraction of the total myoplasmic calcium ion (Gillis, Thomason, Lefevre & Kretsinger, 1982). These measurements and simulations suggest that initially upon release there is a rapid binding of calcium to troponin and to parvalbumin. A greater proportion of the early binding is to troponin than to

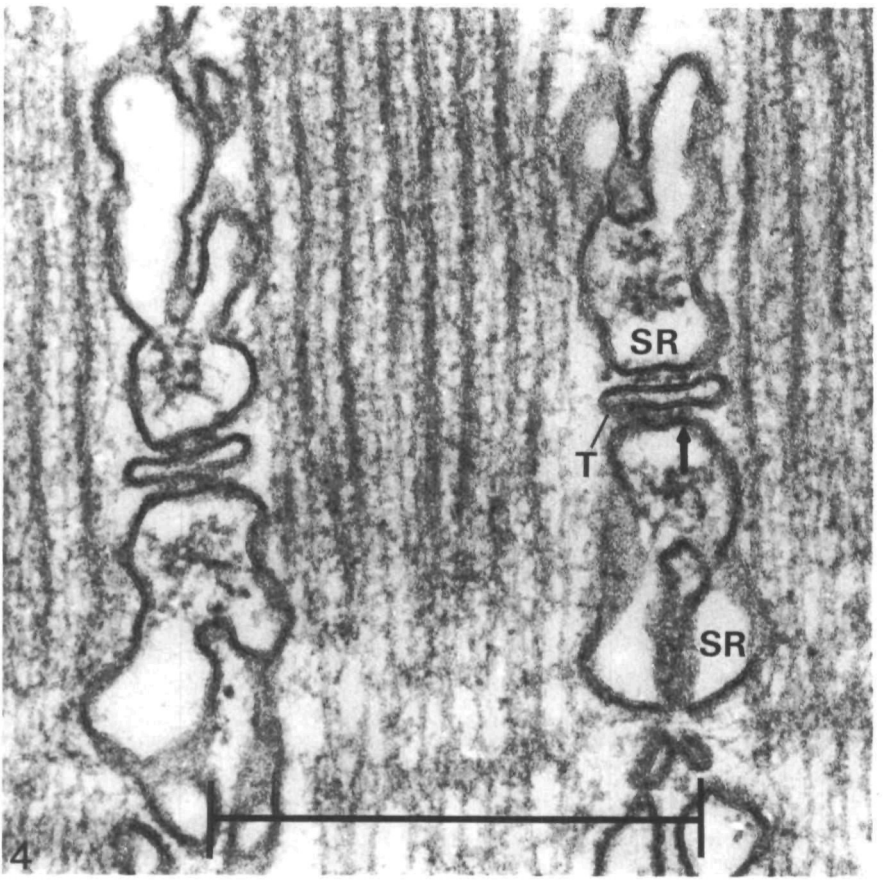

Fig. 4. View of the junction between the SR and T-system in a toadfish swimbladder muscle fibre. Arrow points to a 'foot' connecting between the two structures. From Peachey & Franzini-Armstrong (1983). Bar indicates 0·5 μm.

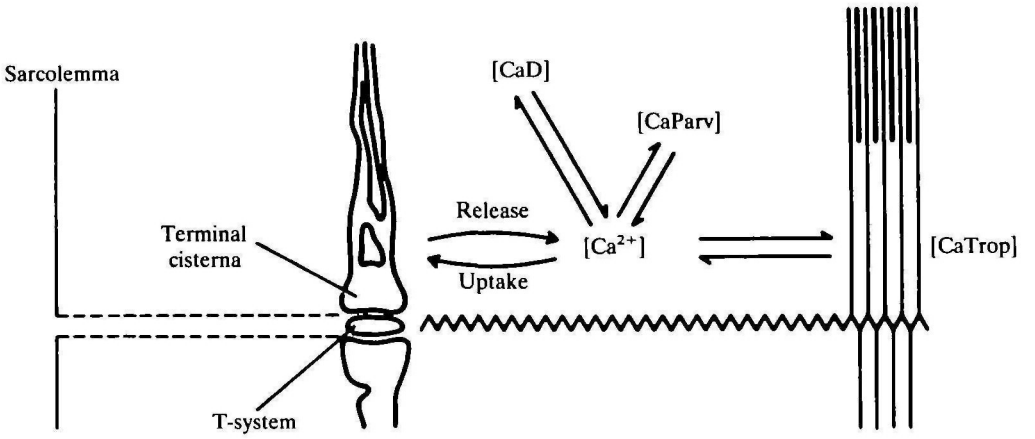

Fig. 5. Kinetic pathways for calcium movement in muscle during excitation-contraction coupling and relaxation. CaParv, calcium-parvalbumin; CaTrop, calcium-troponin; CaD, calcium-dye complex (used to monitor free calcium concentration). From Baylor, Chandler & Marshall (1983).

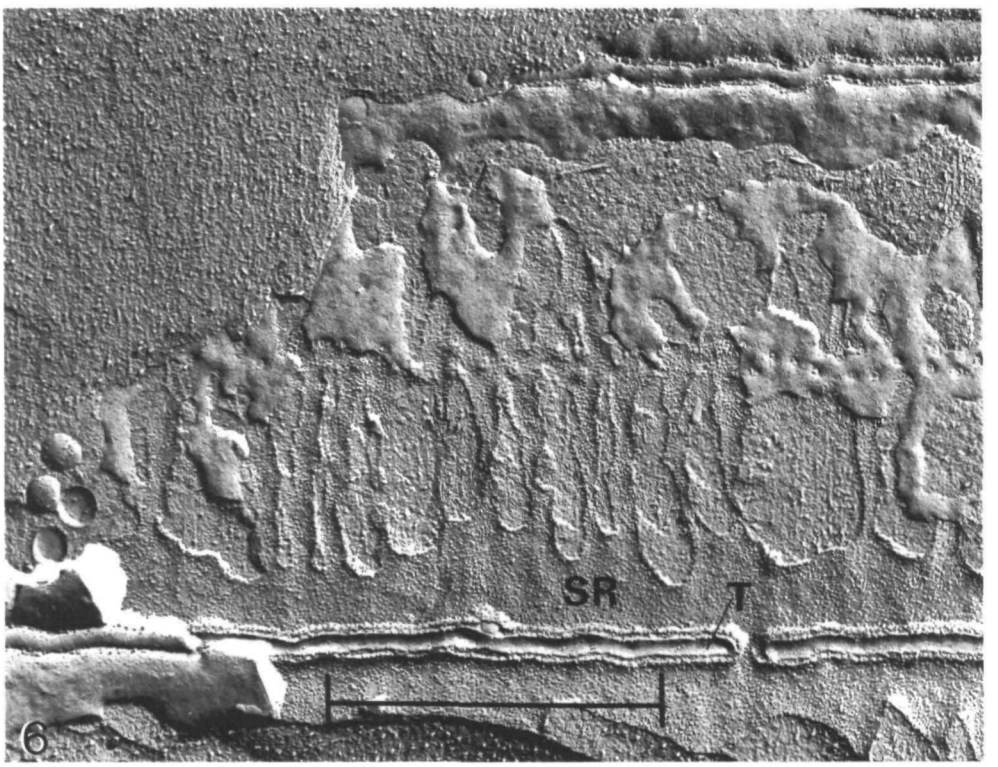

Fig. 6. Freeze-fracture electron micrograph of SR and T-system in a fish muscle. The smooth membranes of the T-tubules can be distinguished from the particle-studded membranes of the longitudinal SR. The particles represent the calcium pump protein in these membranes. From Peachey & Franzini-Armstrong (1983).

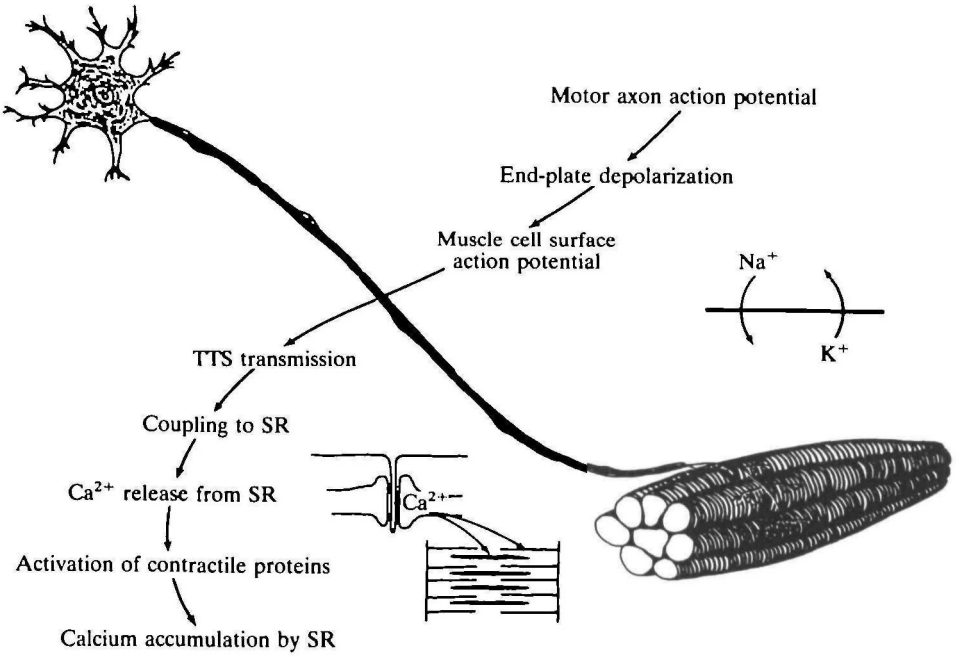

Fig. 7. Summary of events in excitation-contraction coupling. TTS, T-system.

parvalbumin because most of the parvalbumin present is complexed to magnesium, and this complex dissociates slowly compared to the time required for calcium binding to free parvalbumin or to troponin. Later, as parvalbumin exchanges magnesium for calcium, the bulk of the myoplasmic calcium moves from troponin to parvalbumin, with a consequent relaxation of tension.

The release of calcium from the SR, its diffusion over short distances of the order of a micrometre, and binding to troponin would seem to be sufficient to activate the fibre. Of course, the contractile cycle is not complete until the contraction is inactivated and the muscle fibre relaxes. This again is a function of the SR. The longitudinal part of the SR membrane, which is a majority of its surface area, is essentially a pure preparation of calcium-pump ATPase molecules (Fig. 6). The pumping activity of these membranes lowers the myoplasmic calcium concentration to a very low level, inducing release of calcium from both troponin and parvalbumin, until the resting distribution of calcium in the cell is restored. This completes the cycle of contraction and relaxation (Fig. 7).

It is worth noting that the structural and functional parameters that determine the rate of rise of tension are different from those that determine its rate of fall. The overall size of the fibre, the degree of development of T-tubules, and the size and efficiency of the calcium releasing part of the SR all would be expected to influence the rate of rise of tension. The rate of fall of tension can be expected to depend upon the activity of calcium binding proteins, such as parvalbumin, and the pumping efficiency of the SR.

These two sets of parameters should be potentially independent of each other. This underlines the possibility that a cycle of contraction and relaxation has two independently adjustable phases, the rise of tension and the fall of tension.

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