## Properties of the tubular system network in twitch muscle

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The tubular (t-) system is an invagination of the surface membrane in skeletal muscle that enters the cell in a uniform fashion at the level of the sarcomere. The t-system membrane is closely apposed to the terminal cisternae of the sarcoplasmic reticulum (SR) throughout the cell. This allows the protein-protein contacts essential for excitation-contraction coupling. Also, the t-system acts as conduit for extracellular fluid deep inside the cell. The t-system appears to be a functional longitudinally connected network, as mechanically skinned fibres of fast-twitch rat muscle display spontaneous force responses (Posterino *et al.*, 2000). In the absence of a surface membrane, the t-system appears to remain connected by: (i) the realignment or "crossing-over" of regular t-tubules across misregistered sarcomeres; and (ii) longitudinal connections that run perpendicularly between regular t-tubules. Our aim was to examine the properties of the t-system network in more detail to help define its role in skeletal muscle.

All experiments were approved by the Animal Ethics Committee at The University of Queensland. Cane toads were stunned by a blow to the head and double pithed. The iliofibularis muscles were removed. Rats and mice were killed by asphyxiation and the soleus and extensor digitorum longus (EDL) muscles were removed. Intact fibre bundles were exposed to $Na^+$-based physiological solution containing either Cascade Blue or purified 500- or 2000-kDa fluorescein dextran and imaged on a 2-photon microscope. In other experiments, isolated intact fibres were exposed to either, or a combination of, 500-kDa fluorescein dextran, rhod-2 salt or fluo-5N salt. Single fibres were then mechanically skinned to trap fluorescent dye in the sealed t-system, and then transferred to a custom-built experimental chamber containing a $K^+$-based internal solution. Additionally skinned fibres were stimulated with platinum electrodes in a bath containing a $K^+$-repriming solution with 0.1 mM rhod-2 above an Olympus FV1000 confocal microscope. Cytoplasmic $Ca^{2+}$ release was imaged in linescan mode, with the scanning line parallel to the longitudinal axis of the fibre. This maneouver allowed determination of whether action potentials were propagating radially or longitudinally by the apparent angle of the elicited SR $Ca^{2+}$ release to the scanning line. All experiments were performed at 20 ± 2°C.

Field stimulation of skinned fibres from rat EDL showed an action potential-induced $Ca^{2+}$ release from SR uniformly along the length of the scanning line, indicating stimulation elicited action potentials at each t-tubule, which in turn propagated radially across the fibre. Spontaneously activating $Ca^{2+}$ release was imaged at a consistent angle to the scanning line, indicating action potentials propagated longitudinally along the fibre at a constant rate of 7.4 ± 1.3 mm/s ($n$ = 8). To assess the diameter of the t-system lumen, fluorescein dextrans of 500- and 2000-kDa were employed. These dextrans were calculated to have diameters of 29 and 55 nm, respectively. Imaging of intact fibre bundles exposed to the dyes all showed a strong fluorescence band immediately outside the fibre. Only Cascade Blue and 500-kDa fluorescein dextran showed dye entry into the t-system, consistent with estimates of t-tubule diameter of ~30 nm (Luff & Atwood, 1971). To determine whether the longitudinal connections had a similar diameter as the regular t-tubule, we trapped the 500-kDa fluorescein dextran and rhod-2 (MW = 869) in the t-system of the same skinned fibre preparation. Confocal imaging showed that both the dextran and rhod-2 had access to the entire regular t-system. However, only rhod-2 had access to the perpendicular longitudinal connections between regular t-tubules. This indicated that these longitudinal connections have a smaller diameter than that of the regular t-tubules. Finally it was important to probe the mobility of molecules of different size through the lumen of the t-system network. To do this we measured fluorescence recovery after photobleaching (FRAP) of the 500-kDa dextran and rhod-2 or fluo-5N (MW = 958). The small molecular weight dyes showed full FRAP within 20-30 min in all three twitch fibre types examined. Only partial FRAP of the dextran was observed even after 1 h in the three fibre types examined ($n$ > 3 in all cases).

In conclusion, we have shown that the t-system network is excitable and has a lumen that is fully connected that can evenly distribute small molecules. Furthermore, the regular t-tubules of the network have a significantly larger diameter than the longitudinal connections that run perpendicularly to the regular tubules.

Posterino GS, Lamb GD & Stephenson DG. (2000) *Journal of Physiology*, **527:** 131-7.
Luff AR & Atwood HL. (1971) *Journal of Cell Biology*, **51:** 369-83.