

# An Overview of the Muscle Cell Cytoskeleton

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## Introduction

The structure of muscle has been the subject of intense study for many years. The function and location of the proteins involved in contraction (namely myosin, actin, tropomyosin and troponin) is now known with considerable detail. However, our knowledge of the nature of the interconnections between the myofilaments, the myofibrils, and the cell membrane is incomplete in spite of recent progress. The purpose of this review is to summarize current work regarding the muscle cell cytoskeleton. This review will deal only briefly with the role of titin, nebulin and desmin; these proteins will be described in the paper immediately following by Richard Robson.

## Basic Muscle Structure

A diagram showing the levels of organization of skeletal muscle is shown in Figure 1. Muscle, which is a tissue, is composed of long cylindrical cells that are termed myofibers or fibers. They are surrounded by collagen fibers in the extracellular space. Muscle cells are packed with smaller cylindrical organelles called myofibrils that occupy over 80% of the cell volume. There may be as many as 1000 of these 1-2 $\mu$ m diameter myofibrils in a cross section of a muscle fiber. Observation of these organelles (which remain intact when muscle tissue is homogenized) in a phase contrast microscope reveals alternating light and dark bands. Electron microscopy shows that the bands arise because of the presence of two major filaments: thick (15nm diameter) in the A band and thin (6-8nm diameter) in the I bands (as well as a variable distance into the A band). A dense line bisects the I band perpendicular to the myofibril's long axis and is termed the Z line. An M line is located in the middle of the A band. The filaments are composed of proteins, with myosin being the major constituent of the thick filaments while actin, tropomyosin and troponin make up most of the thin filaments.

## Muscle Cytoskeleton- A Definition

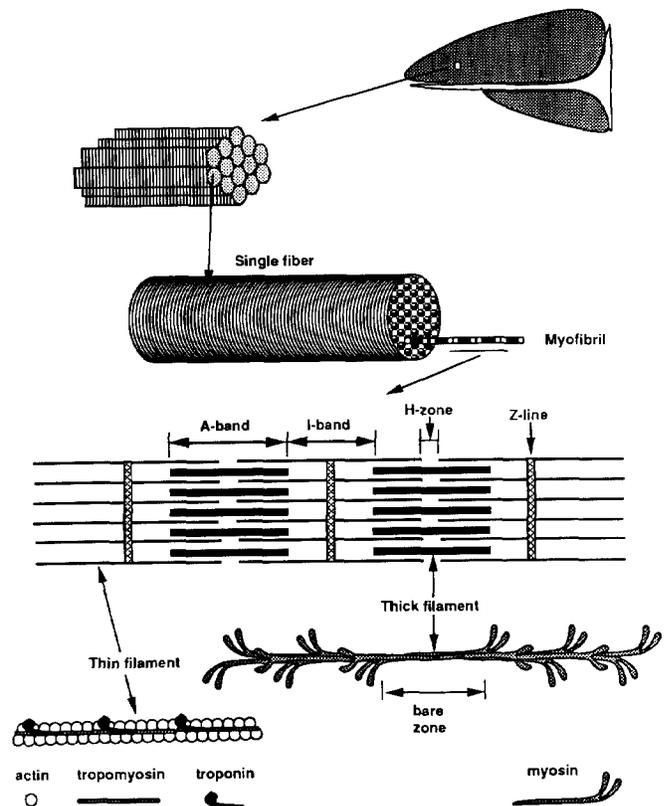
The term "cytoskeleton" was coined to describe a system of intracellular structures that maintain cell shape, interconnect organelles to each other, and often attach to the cell membrane. There are classically three major groups of filaments that constitute the cytoskeleton: microfilaments (6-8nm diameter) containing actin; intermediate filaments

(10nm diameter) containing desmin, vimentin, keratins, etc., depending on the cell type; and microtubules (25nm diameter) containing primarily tubulin. In muscle, the microfilaments are found in ordered arrays in the myofibrils, but in most other cells they occur as bundles or networks that are involved in movement or in maintaining cell shape (Matsudaira, 1991). Intermediate filaments are also found in many cells and tissues, including muscle (Carmo-Fonseca and David-Ferreira, 1990; Steinert and Liem, 1990; Stromer, 1990). Microtubules are fairly sparse in skeletal muscle, averaging less than one per  $\mu$ m<sup>2</sup>, a size corresponding to the approximate cross-sectional area of a myofibril (Goldstein and Cartwright, 1982).

## Myofibril-Myofibril Connections

The existence of transverse connections between myofibrils has been suspected for many years, based on the

Figure 1  
Levels of Muscle Organization



Drawing by Darl Swartz

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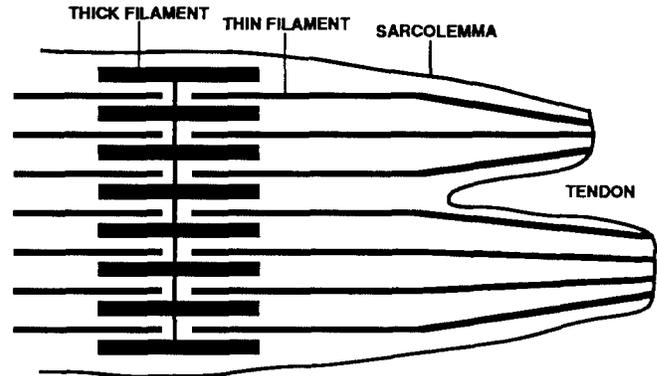
Reciprocal Meat Conference Proceedings, Volume 44, 1991.

observations that the banding patterns of adjacent myofibrils are usually aligned in the muscle cell. Extraction of muscle with high salt solutions removes most of the contractile proteins, but an intricate system of filamentous material remains which can be observed by electron microscopy (Wang and Ramirez-Mitchell, 1983). There appeared to be residual connections at the Z line and M line regions between adjacent myofibril ghosts. The exact composition of these filaments has not yet been determined. Evidence that some form of lateral transmission of force between adjacent myofibrils and to the cell membrane was provided by Street and Ramsey (1965) and Street (1983). They crushed a muscle fiber, breaking all the myofibrils (Figure 2). The injured fiber was then attached to a force transducer and stimulated to contract. Street found that fibers lacking longitudinal continuity in their myofibrils developed almost as much force as fibers that were not injured. Since the only way that the myofibrils could produce tension was by pulling on the sarcolemma, these experiments demonstrated that the myofibrils were somehow laterally connected to the cell membrane and to each other. The fact that almost full force was produced indicated that virtually all the myofibrils were laterally connected since the force would be proportional to the number of contracting elements. Electron microscopy of intact cells has shown transverse filaments at the level of the Z lines and M lines (Nunzi and Franzini-Armstrong, 1980; Pierobon-Bormioli, 1981). These filaments are more easily observed if the muscle has been treated with hypertonic solutions to cause the myofibrils to move farther apart. A pair of proteins termed skelemins is believed to encircle the M line and constitute myofibril to myofibril connections (Price, 1987).

### Myofibril to Cell Membrane Connections

Myofibrils attach to the cell membrane at the ends of the fiber in a structure termed the myotendinous junction (Figure 3). The cell membrane in this region is folded into a series of finger-like processes that are in proximity to collagen bundles in the extracellular space (Ishiwata et al., 1983; Trotter, 1990; Tidball and Law, 1991). Thin filaments come close to the membrane both at their ends and tangentially.

Figure 3  
Myotendinous Junction



The first evidence for specialized proteins being localized in the junction between the myofibrils and the cell membrane was provided by Pardo and coworkers. They found that a protein called vinculin (116 kD) was primarily located near the cell membrane in cross sections of muscle cells viewed after antibody staining (Pardo et al. 1983a,b). Longitudinal sections which just grazed the cell surface revealed riblike stripes of staining which they termed "costameres." These costameres were located adjacent to the I band regions of the subsarcolemmal myofibrils (Figure 4). The positions of these costameres changed with the sarcomere length. Also the cell membrane became bulged in shortened muscle cells with the dense connection points appearing in the I band regions (Shear and Bloch, 1985). The location of vinculin on the cytoplasmic face of the muscle cell membrane is consistent with its postulated role in attaching actin-containing stress fibers to membranes in other types of cells (for review, see Otto, 1990).

Antibody staining has shown a number of proteins that appear to be localized both laterally along the plasmalemma as well as at the myotendinous junction (Figure 5 and Table 1). There appear to be two major types of protein complexes that function in myofibril-to-membrane attachment. The first

Figure 2  
Street-Ramsey Experiments

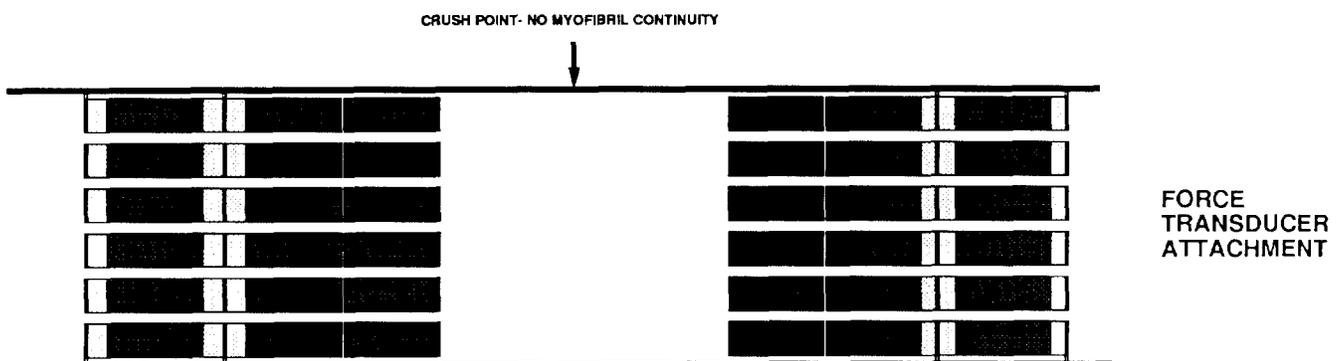
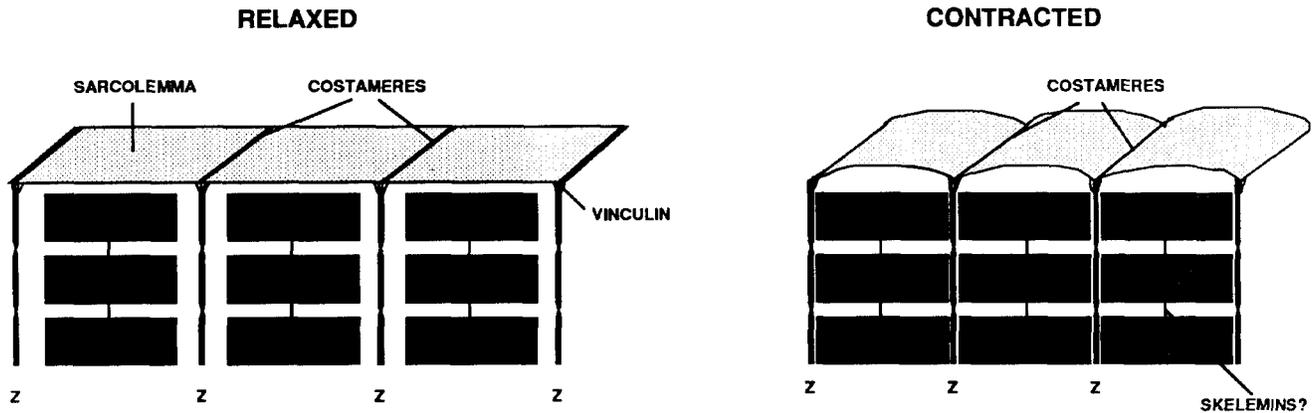


Figure 4  
Costameres



Redrawn from Pardo and coworkers, J. Cell Biol. 97:1081, 1983

includes proteins associated with vinculin. Talin, alpha actinin and integrin have been demonstrated in the same positions of the cell by antibody staining and shown to interact by in-vitro binding experiments (Tidball et al., 1986; Belkin et al., 1986; Burrige et al., 1988; Beckerle and Yeh, 1990; Muguruma et al., 1990; Swasdison and Mayne, 1989; Volk et al., 1990).

The second complex includes a group of glycoproteins (156, 50, 43 and 35 kD) and a protein called dystrophin (Ervasti et al., 1990; Ohlendieck et al., 1991). Dystrophin is a 427 kD protein that has been identified as defective or absent in patients with muscular dystrophy (Hoffman et al., 1987). It is found immediately inside the cell membrane and at the myotendinous junction (Zubrzycka-Gaarn et al., 1991; Miyatake et al., 1991; Tidball and Law, 1991; Wessels et al., 1991). Dystrophin is a rod-shaped protein more than 100 nm long (Murayama et al., 1990; Pons et al., 1990). It also has been shown to bind to actin (Levine et al., 1990). Thus it is believed to function as an anchor for actin filaments. The cellular damage that occurs in muscular dystrophy has been postulated to occur as a result of the lack of attachments between the myofibrils and the cell membrane.

A striking feature of several of the cytoskeletal proteins is their similarity in amino acid sequence. Dystrophin has 24

Figure 5  
Membrane-Myofibril Attachment Proteins

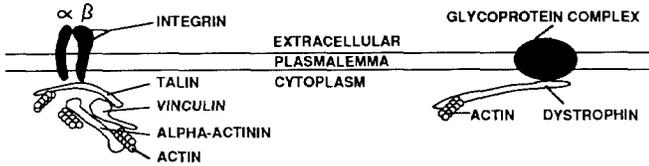


Table 1. Cytoskeletal Proteins in Muscle.

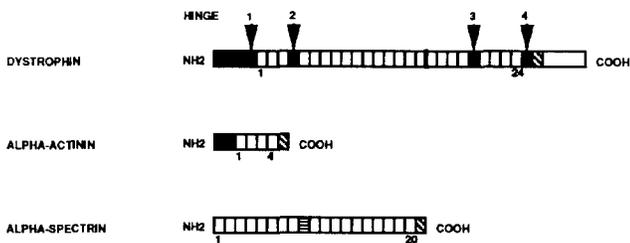
	Location	Binding
<b>Myofibrillar</b>		
Titin	Full Sarcomere	Thick Filaments to Z Lines
Nebulin	Thin Filaments	Actin
Desmin	Z Lines	?
Skelemins	M Lines	?
Alpha-Actinin	Z Lines	Actin
<b>Sarcolemmal</b>		
Integrins	Cell Membrane, MT Junction	Talin
Talin	Cell Membrane, MT Junction	Integrin, Vinculin, Actin
Vinculin	Cell Membrane, MT Junction	Talin, Alpha-Actinin, Actin?
Glycoprotein Complex	Cell Membrane, MT Junction	Dystrophin
Dystrophin	Cell Membrane, MT Junction	Glycoprotein Complex, Actin, Talin
Paxillin	Cell Membrane, MT Junction	?

repeat units (Koenig and Kunkel, 1990) of an approximately 100 amino acid domain that was originally found in spectrin (Speicher and Marchesi, 1984). These triple helical domains are also found in alpha-actinin (Figure 6). A region postulated to be involved in actin binding is found at the amino terminus of both alpha-actinin and dystrophin. In addition, a number of hinge regions have been postulated to occur in dystrophin (arrowheads, Figure 6) which may facilitate actin binding at the cell membrane (Koenig and Kunkel, 1990)

### Postmortem Changes in Cytoskeletal Proteins

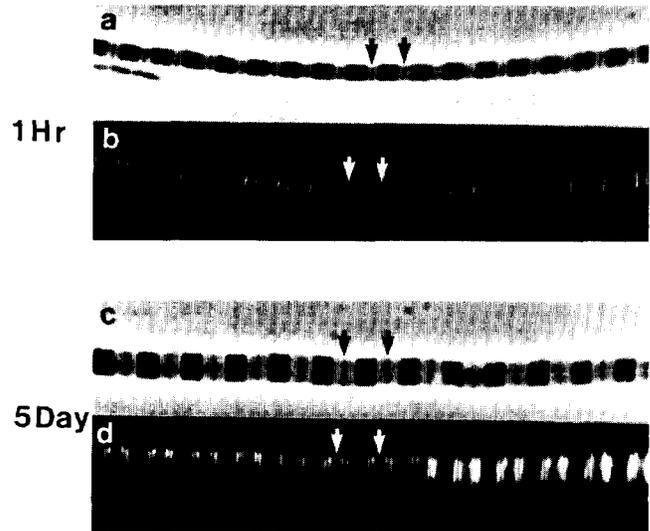
What happens to these cytoskeletal proteins in postmortem muscle? Our answers are still incomplete. Desmin appears to be rapidly degraded after death (see Robson et al., 1984 for review). A monoclonal antibody against titin has been shown to stain two bands per sarcomere in fresh muscle but four bands per sarcomere in most myofibrils from bovine psoas at 48 hours postmortem (Ringkob et al., 1988). An example is shown in Figure 7. Thus titin is either partially degraded or some protein to which it is attached is altered.

Figure 6  
Cytoskeletal Protein Sequence Domains



Redrawn from Koenig and Kunkel, J. Biol. Chem. 265:4550, 1990

Figure 7  
Postmortem Changes in Titin Position



Nebulin is extensively degraded in bovine muscle within the first few days after death (Lusby et al., 1983; Fritz and Greaser, 1991). Details concerning changes in the other proteins described here remain to be explored.

### ACKNOWLEDGEMENTS

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