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MUSCLE STRUCTURE, FIBER TYPES, AND PHYSIOLOGY

Scott Medler and Donald L. Mykles

Abstract

Crustacean muscles are striated muscles exhibiting a wide range of structural characteristics and physiological capabilities. Slow fibers possess relatively wide sarcomeres and produce slow sustained contractions used for diverse biological functions. Fast fibers possess narrow sarcomeres and generate the power needed for quick movements and bursts of locomotion. Differences in contractile rates are determined by sarcomere width and by alternate myosin heavy chain (MHC) isoforms. Crustacean fibers exhibit thin-filament regulation of muscle contraction and possess different isoforms of troponins and tropomyosin that influence the kinetics of muscle activation. Crustacean muscles commonly contain fibers that do not fit neatly into one category, but lie along a continuum of fiber types. The sarcolemma of crustacean fibers is often folded into clefts that penetrate deeply into the fiber interior. These clefts are lined with mitochondria and presumably facilitate diffusional exchange across the membrane in large fibers.

INTRODUCTION

Crustacean skeletal muscles have provided a rich area of research for decades, and a number of compelling reasons exist for studying crustacean muscles. For crustacean biologists, skeletal muscles are integral components of many key processes, making their biology relevant to researchers with a wide range of interests. Growth and molting are essential features of crustacean life history, and these complex processes must be coordinated with skeletal muscle atrophy and subsequent growth. Many crustaceans are highly energetic and mobile animals, and any studies of locomotion or other types of performance must incorporate an understanding of muscle function. Crustaceans, principally decapods, are also important components of the aquaculture and commercial seafood

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industries that represent hundreds of millions of dollars of economic value annually, so an interest in their skeletal muscles is not completely academic (Oesterling 2012).

For those interested in basic skeletal muscle structure and function, crustacean muscles have provided a number of unique models for comparison with the more intensively studied vertebrate skeletal muscles. Beginning in the mid-1960s, Ashley and colleagues performed a series of experiments in which they injected the calcium-sensitive jellyfish fluorescent protein aqueorin into the giant muscle fibers of barnacles (Ashley and Ridgeway 1970). When the muscles were stimulated to contract, the aqueorin emitted light as calcium was released within the muscle to elicit contraction. These were some of the first experiments demonstrating the inextricable connection between Ca^{2+} ions and muscle contraction. Not quite a decade later, Fred Lang and colleagues embarked on a series of studies focused on the developmental changes in muscle fiber type that occur in juvenile lobster claws (Lang et al. 1977a,b, Govind and Lang 1977). These studies showed that fibers could be completely remodeled from fast to slow (and vice versa) and provided one of the first examples of invertebrate muscle plasticity (see Chapter 5 in this volume). More recently, Steven Kinsey's laboratory has used the exceptionally large muscle fibers of crabs to examine the diffusional limitations in muscle fibers more generally (Kinsey et al. 2007, Hardy et al. 2009, Kinsey et al. 2011; see Chapter 12 in this volume). These studies have provided insights not only into the constraints of cell dimensions on basic physiologic function in crustacean muscles, but also help explain how these limitations have affected the evolution of skeletal muscle design more broadly. Collectively, these studies represent just some of the many insights gained from studying these diverse and fascinating skeletal muscles.

In this chapter, we provide a general overview of crustacean muscle structure and physiological function. We begin by providing some examples of the varied and complex roles crustacean muscles play in an animal's basic biology and life history. Next, we focus on crustacean muscle structure, from the whole-muscle level down to fiber ultrastructure. We then focus on the classification of crustacean muscles into discrete fiber types, with a particular focus on the alternate isoforms of myofibrillar proteins that help define these fiber types. Although the current classification system is limited to just a few species, it is clear that a significant amount of overlap exists even among these well-defined fiber types. Finally, we conclude the chapter with a discussion of crustacean muscle physiology. Throughout the chapter, some natural overlap in subject areas is unavoidable. For example, it is impossible to adequately discuss muscle fiber types without covering the structural feature of sarcomere width or physiological parameters like shortening velocity. Although we have attempted to minimize this type of repetition, some degree of redundancy should be anticipated.

MUSCLE STRUCTURE

Overview

Crustacean muscles come in a variety of sizes, structural organizations, and even subtle colors. Each of these characteristics can be related to the functional roles of specific muscles, and crustacean muscles are highly diverse both in terms of their structural organization and function. Like all skeletal muscles, crustacean muscles are made up of a few to hundreds of individual muscle cells or fibers. These fibers are invariably anchored to the exoskeleton at their origin and are attached to a moveable connection point at their insertion. In certain instances, the insertion point is a well-defined tendon, called the *apodeme*, as is the case in the opener and closer muscles of claws. In others, muscles may insert directly onto a different region of the exoskeleton, as occurs with the extensor and flexor muscles of the tail in lobsters and crayfish. Although many crustacean muscles are organized into bipinnate structures with a central apodeme, the precise architecture of diverse muscles varies considerably.

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The wide range in functional organization of crustacean muscles reflects their diverse specialized functional roles. A muscle's functional organization is closely integrated with the exoskeleton to which it is coupled. In some cases, the joints of crustaceans are formed by simple hinges (Warner and Jones 1976, Schenk and Wainwright 2001), whereas others represent highly complex structures with specialized regions of calcification in the exoskeleton that provide essential mechanical properties (Patek et al. 2004, 2007). Several well-studied musculoskeletal systems demonstrate the close coupling between the structure of the exoskeleton and the functional organization of the associated musculature. We discuss three of these systems here, to illustrate the diversity in muscle organization resulting from the functional requirements of the muscles.

The claws of many crustaceans exhibit significant dimorphism in which one claw is more heavily built. The closer muscles of both claws exhibit morphological and cellular adaptations that produce a slow forceful closure of the major claw and a more rapid closure of the minor claw. The major claw, designated the *crusher* in clawed lobsters, has a greater mechanical advantage than its partner, owing to the construction of the dactyl and the insertion point of the closer muscle on the apodeme (Warner and Jones 1976, Costello and Lang 1979, Schenk and Wainwright 2001). In addition to having an architecture that facilitates a forceful claw, all of the fibers that comprise the lobster crusher closer muscle are long-sarcomered slow fibers (Lang et al. 1977a). The slender *cutter* claw in the lobster has a mechanical advantage that favors rapid claw closure, and its closer muscle is built from approximately 65% fast muscle fibers (Lang 1977, see Chapter 5 in this volume).

The first thoracic appendages of mantis shrimp (order Stomatopoda) are specialized into raptorial or hammer-like structures used for predation and defense (Patek et al. 2004, 2007). The velocities generated during the rapid strike from these appendages are among the highest in the animal kingdom and exceed speeds that can be actively generated by contracting skeletal muscles (Burrows 1969, Patek et al. 2004). The function of the appendages has aptly been compared to the operation of a crossbow, in which the energy input to draw back the bow is exceedingly slow in comparison to the rapid release of energy that ensues when the trigger is activated (Patek et al. 2007). In the mantis shrimp, most of the exoskeleton of the merus is very thin and supple, but specialized regions of calcification in the distal portion of the appendage provide structures that function to both store energy and to effectively "cock" the structure (Patek et al. 2007). Although the precise mechanism of energy storage is not well understood, it is clear that a series of calcified ridges in the distal part of the merus are essential to the storage of potential energy generated by the contracture of the extensor muscles (Patek et al. 2007, Zack et al. 2009). The primary muscles used to generate tension for a strike are two large extensors of the carpus that insert onto two calcified sclerites that function as a "click-joint" to lock the carpus in a fully flexed position (Burrows 1969, Burrows and Hoyle 1972, McNeill et al. 1972). Two smaller carpus flexors are used to lock the "cocked" appendage in place for a short period before it is released (Burrows and Hoyle 1972). Each of these muscles is a slow contracting muscle, with ultrastructural and physiological features common to other slow muscles in crustaceans (McNeill et al. 1972). A similar mechanism is used by snapping shrimp (family Alpheidae) to generate their loud snapping sounds (Ritzmann 1974, Versluis et al. 2000; see Chapter 5 in this volume).

In crabs of the family Portunidae, the fifth pereopod has become specialized into a broad paddle that is used as a swimming appendage (Hartnoll 1971, Spirito 1972). These animals are capable of sustained swimming at speeds of up to 1 meter/s and are highly maneuverable (Spirito 1972). The muscles used to generate power for swimming are complex, in terms of both the anatomical arrangement of the muscles and of their fiber composition (White and Spirito 1973, Tse et al. 1983). Swimming is powered by the interaction of four sets of muscles that receive separate innervation and are responsible for distinct physiological activities. White muscles power short-term escape responses, whereas pink-colored muscles drive sustained swimming for prolonged periods. Both types of fibers have structural and physiological features characteristic of fast muscles, but the more

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pigmented fibers are subdivided by membrane clefts lined with high concentrations of mitochondria (Tse et al. 1983, Henry et al. 2001). The specialization of these muscles into aerobic engines that power swimming behavior has evolved in several different species of crabs (Hardy et al. 2010). Clearly, these muscles have evolved a phenotype that is well matched to the paddle-like appendage necessary for swimming.

The common theme illustrated by these examples is that crustacean muscles are highly sophisticated organs, precisely matched to the exoskeletal structures with which they are integrated. Some of these systems have evolved for force production, others for explosive speed, and some for sustained power output. They are highly diverse in terms of their structural, metabolic, and physiological properties. Although the physiological systems of invertebrates are sometimes described as being simple, the diversity and complexity of organization are arguably greater in crustacean muscles than in those of vertebrates.

Ultrastructural Organization

Crustacean muscles exhibit a wide variety of structural organization at the cellular level. One of the major differences between crustacean and vertebrate skeletal muscles fibers is that crustacean fibers display a wide range of sarcomere widths (from 3 to 20 μm), while those of vertebrates are uniformly short (~2.5–3 μm; Hoyle 1967, 1983). Sarcomere width has commonly been used to identify different physiological fiber types in crustaceans because long-sarcomered fibers tend to be innervated by slow motor neurons and contract slowly. Fibers with short sarcomeres are frequently innervated by a fast motor neuron and are fast contracting, whereas intermediate fibers exhibit intermediate sarcomere widths and are often innervated by both fast and slow motor neurons (Atwood 1976, Govind and Atwood 1982). Sarcomere width is not simply a descriptive correlate of contractile phenotype but is a direct determinant of contractile strength and speed (Huxley and Niedergerke 1954, Josephson 1975). Short-sarcomered fibers have a proportionately greater number of these contractile units in series and thereby contract with greater speed than the fibers with longer sarcomeres. Long-sarcomered fibers generally have a greater number of myosin cross-bridges available per sarcomere and therefore produce greater forces than do fibers with short sarcomeres. This pattern is consistent with theoretical expectations, and contractile force is directly correlated with sarcomere width in crustacean muscles (Jahromi and Atwood 1969, Taylor 2000).

Sarcomere width in crustacean muscles is also correlated with other common features of myofibrillar organization. Short-sarcomered fibers tend to have straight and well-aligned Z lines, I bands, and A bands. Long-sarcomered fibers often possess Z lines that appear jagged or wavy in longitudinal sections, and the alignment between adjacent myofibrils is often staggered (Jahromi and Atwood 1969, Mykles and Skinner 1981, Mellon and Stephens 1992, West et al. 1992; Fig. 4.1). In many cases, the H zone in the middle of the A band is well-defined in short-sarcomered fibers but is less visible or absent in the fibers with long sarcomeres. In cross-section, short-sarcomered fibers possess thick filaments surrounded by a highly regular array of six thin filaments (Jahromi and Atwood 1969, West et al. 1992). The thick filaments in the long-sarcomered fibers are surrounded by a higher number of thin filaments (~12 or more) that are scattered around the filament without any obvious order (Jahromi and Atwood 1969, 1971, Mykles and Skinner 1981, West et al. 1992). The filaments themselves are narrower in fast muscles with short sarcomeres than in slow muscles. In both muscle types, an organized array of myosin subfilaments is thought to surround a core of paramyosin. Those of slow muscles are composed of a greater number of these myosin subfilaments and have a thicker core of paramyosin than those of fast muscles (Jahromi and Atwood 1969, Chapple 1982). It is generally thought that the combination of wider thick filaments and a higher number of thin filaments associated with slow fibers results in a greater level of generated force than in fast fibers (Mellon and Stephens 1992, Royuela et al. 2000).

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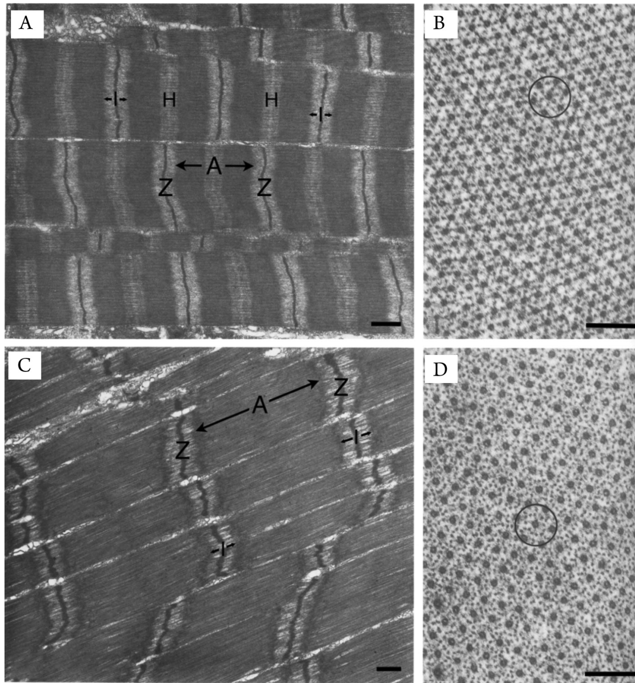

Fig. 4.1.
Structural differences between short- (A, B) and long-sarcomered (C, D) fibers from the claw closer muscles of the Australian yabby, *Cherax destructor*. Longitudinal section of short-sarcomered fiber (A) shows distinct A and I bands, and these regions of adjacent myofibrils line up in register. The H zones and M lines (middle of H zones) in the middle of each A band are distinct. In cross-section (B), each thick filament is surrounded by six thin filaments (enclosed in circle). Longitudinal section of long-sarcomered fiber (C) show that Z lines are less straight, do not line up in register between adjacent myofibrils, and are not always perpendicular to the fiber axis. The H zones and M lines are not readily apparent. In cross-section (D), the long-sarcomered fibers exhibit thick filaments surrounded by an average of 12 thin filaments (enclosed in circle). All figures are transmission electron micrographs (TEM), scale bars = 0.2 μm. From West et al. (1992), with permission from Springer.

Each myofibril within a muscle fiber is surrounded by a collar of membranes formed by the sarcoplasmic reticulum (SR) that is contacted by the network of tubular membranes invaginating from the sarcolemma (Rosenbluth 1969, Franzini-Armstrong et al. 1986, Ushio and Watabe 1993; Fig. 4.2). The extent of the SR varies greatly, depending on the speed and frequency of contractions produced by the muscle. In slow muscles, the SR and corresponding tubular system tend to be less developed than in fast fibers (Ushio and Watabe 1993, Lagersson 2002), although in some cases these differences are minor (Jahromi and Atwood 1969, Jahromi and Atwood 1971). In certain specialized high-frequency muscles, such as the antennal remoter muscle in lobsters, the relative proportion of SR makes up the majority of the fiber volume, and myofibrils only account for about a quarter of the total volume (Rosenbluth 1969).

Crustacean fibers possess a well-developed internal membrane system that functions to carry depolarizations of the sarcolemma into the muscle fiber during excitation-contraction coupling (Peachey 1967, Selverston 1967, Franzini-Armstrong et al. 1986, Ushio and Watabe 1993). In addition to the well-known T tubules that are present in vertebrate skeletal muscles, crustacean fibers variably also possess surface membrane clefts and Z tubules (Peachey 1967, Franzini-Armstrong et al. 1986). The clefts of crustacean fibers are often highly developed and greatly increase the surface area of the sarcolemma. Various tubular systems, running not only transversely, but also in

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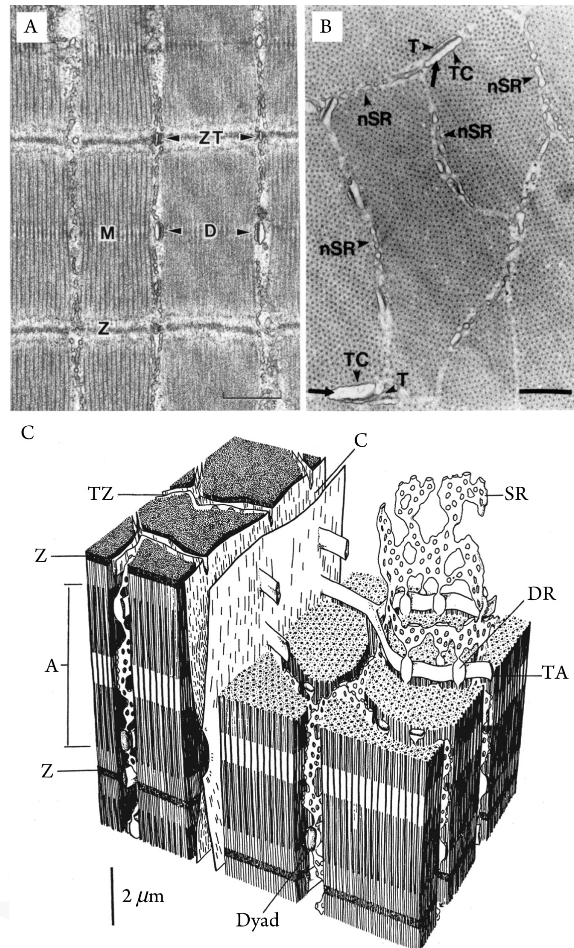

Fig. 4.2.
Membrane systems in crustacean fibers. Longitudinal- (A) and cross-sections (B) of muscle fibers demonstrate the membrane invaginations that form networks around myofibrils (TEM). In both figures, diads are visible where T tubules come into contact with the terminal cisternae of the SR (D in (A); T and arrows in (B)). Other abbreviations: (A) Z tubule, ZT; M line, M; Z line, Z; (B) nonjunctional sarcoplasmic reticulum, nSR; terminal cisternae, TC. Three-dimensional reconstruction (C) of the membrane systems in crustacean fibers demonstrates the relationships among membrane clefts, C, T tubules ("A tubules," TA), SR, and the terminal cisternae ("dilated cisternae," DR). T tubules arise as invaginations of the membrane clefts (C). Other abbreviations in (C): Z tubules, TZ; A band, A; Z line, Z. (A) From Stokes and Josephson (1992), with permission from Springer; (B) from Ushio and Watabe (1993), with permission from Wiley and Sons, Inc.; (C) from Peachey (1967), with permission from Oxford University Press. Scale bars in A and B = 0.5 μm; scale bar in C = 2 μm.

longitudinal and oblique directions, arise from these clefts (Hoyle 1983). Of these, Z tubules penetrate into fibers at the level of the Z line. The T tubules are defined by the fact that they form junctions with the SR and are integral to the process of excitation-contraction coupling, although some junctions with the SR are also formed directly with the clefts themselves. The T tubules form flattened cisternae that are directly opposed to the SR at these positions, where they form dyads and triads (Peachey 1967, Franzini-Armstrong et al. 1986). Fast muscles tend to have a greater density of these connections than slow muscles. In some slow fibers, T tubules regularly penetrate into the fiber at the level of the outer borders of the A bands, but, overall, there is no regular placement of

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these tubules. Particularly in fast muscles, the T tubules may penetrate the fiber in different locations (Jahromi and Atwood 1969, Rosenbluth 1969, Franzini-Armstrong et al. 1986, Stokes and Josephson 1992). Franzini-Armstrong et al. (1986) reported that the Z tubules are distinct from the T-tubule system, although continuities between the systems do exist. The Z tubules do not apparently form junctions with the SR, and their function is poorly understood (Jahromi and Atwood 1971, Franzini-Armstrong et al. 1986).

Ultrastructural studies have provided evidence of foot processes that connect the T tubule to the SR at dyadic and triadic junctions that are similar to those observed in vertebrate muscles (Fig. 4.2A,B; see Mellon and Stephens 1992). We now know that these feet are ryanodine receptors (RyR) responsible for coupling depolarization of the T tubule with intracellular Ca^{2+} release by the SR (Franzini-Armstrong and Protasi 1997, Di Biase and Franzini-Armstrong 2005). In vertebrate skeletal muscles, the ryanodine receptors are physically coupled to the dihydropyridine receptors (DHPR) embedded in the T-tubule membranes and function together as Ca^{2+} release units. In the muscles of crustaceans and other invertebrates, the ryanodine receptors serve a similar role in excitation-contraction coupling, but there is not a direct association with the DHPRs (Di Biase and Franzini-Armstrong 2005; and see the section "Excitation-Contraction Coupling").

MUSCLE FIBER TYPES

Overview

The use of specialized muscle cells to generate contraction and movement is a defining characteristic of the Animal Kingdom. All animals, from creeping worms to the fastest vertebrates, rely on the same fundamental processes of muscle contraction to power their movements. Myosin motor proteins are organized into thick filaments that interdigitate with thin filaments of actin and produce force when the myosin motor pulls on the actin filaments. Within this general scheme exists a diverse array of specific levels of muscle organization (Hoyle 1967, Hoyle 1983, Paniagua et al. 1996). In smooth muscles, thick and thin filaments interdigitate with one another, but the spatial organization of the thick and thin filaments within the muscle cell is not well defined. In cross-striated muscles, thick and thin filaments are organized into alternating A bands and I bands along the fiber length, which produces the characteristic repeated banding pattern of these muscles (Fig. 4.1). Obliquely striated muscles are similar to striated muscles in having thick and thin filaments organized into well-defined regions, but the angle of these regions is much less than the right angles observed in striated muscles. Broadly, different animals possess a continuum of muscle fiber types ranging from smooth muscles, to obliquely striated, to striated fibers (Hoyle 1983, Paniagua et al. 1996). In most animal taxa, including the vertebrates and mollusks, multiple types of muscles are present within an organism and even within the same muscle (Paniagua et al. 1996, Royuela et al. 2000). Among the arthropods, all of the muscles are cross-striated, including the muscles of the heart and other visceral organs (Mellon 1992, Paniagua et al. 1996).

Diverse muscles within the Animal Kingdom also differ with respect to their mechanisms of muscle activation (Lehman and Svent-Gyorgyi 1975, Svent-Gyorgyi 1975, Royuela et al. 2000, Hooper et al. 2008). At rest, all muscles possess mechanisms that prohibit the myosin heads of the thick filaments from interacting with the thin filament actins. In the muscles of all animals, an increase in intracellular Ca^{2+} is required to initiate the force-producing interaction between actin and myosin (Szent-Gyorgyi 1975, Hooper et al. 2008). In some muscles, an inhibitory state of the myosin motor must be removed to initiate muscle contraction (*thick filament regulation*). In

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molluskan muscles and vertebrate smooth muscles, a light chain of myosin serves as the regulator of muscle contraction (Szent-Gyorgyi 1975, Hooper et al. 2008, Himmel et al. 2009). In others, troponin and tropomyosin proteins associated with the actin filaments effectively inhibit muscle contraction by blocking the myosin binding site on the actin filaments (*thin filament regulation*; Szent-Gyorgyi 1975, Royuela et al. 2000, Hooper et al. 2008). As with the structural organization of muscles, animals broadly possess a whole range of mechanisms used for muscle activation. Many animals possess both thick filament-regulated and thin filament-regulated muscle fiber types (Lehman and Svent-Gyorgyi 1975, Szent-Gyorgyi 1975, Royuela et al. 2000, Hooper et al. 2008). Arthropods, including crustaceans, rely primarily or exclusively on thin filament regulation during muscle activation. However, there is evidence that some crustacean slow muscles possess dually regulated systems (Lehman and Svent-Gyorgyi 1975, Szent-Gyorgyi 1975, Royuela et al. 2000, Hooper et al. 2008). Crustacean muscles are therefore very similar to vertebrate skeletal muscles with respect to their cross-striated organization and in predominantly possessing thin filament regulation of actomyosin activation.

General Classification of Muscle Fiber Types

Striated skeletal muscles are composed of populations of individual cells, or muscle fibers, which represent the cellular basis of muscle contraction. In all animals, distinct populations of muscle fibers are present, providing specialization of contractile function for differing mechanical requirements (Rome et al. 1988, Rome and Lindstedt 1997). Fast-contracting muscles are needed for bursts of power, whereas slower muscles are used for activities that require more prolonged periods of sustained force generation. Metabolic properties of different fiber types are often matched with shortening speed, with faster muscles tending to be less aerobic and mainly relying on glycolysis and intracellular phosphagens to fuel muscle contraction (Rome and Lindstedt 1997). Within these general parameters, specific muscle fiber types from diverse species exhibit a wide range of contractile and metabolic properties (Rome and Lindstedt 1997). Crustacean muscle fibers are as diverse as those of any animal group. Slow fibers often control appendages and body regions where forces need to be maintained over a period of time, but rapid contraction is not a requirement. These include fibers of claw opener and closer muscles and many of the superficial muscles of the abdomen (Jahromi and Atwood 1969, Ogonowski and Lang 1979, Mykles 1988, Fowler and Neil 1992, Neil et al. 1993, Sohn et al. 2000, Medler et al. 2004). Fast fibers are important in muscles that power rapid locomotion, such as the deep abdominal muscles in lobsters and crayfish, as well as in the leg muscles of running crabs (Jahromi and Atwood 1969, Ogonowski and Lang 1979, Mykles 1985a, Li and Mykles 1990, Cotton and Mykles 1993, Medler and Mykles 2003, Perry et al. 2009). Fast fibers are also found in the cutter claws of lobsters and the pincer claws of snapping shrimp (Mellon and Stephens 1978, O'Connor et al. 1982, Govind 1987). Although slow fibers tend to be more aerobic than fast fibers, this is not a strict correlation, and there are many examples of fast aerobic fibers characterized by high mitochondrial densities (see the section "Aerobic Capacity"). Some muscles are specialized to generate not only fast contractions, but have also evolved to produce muscle twitches at high contractile frequencies (Fahrenbach 1963, Rosenbluth 1969, Stokes and Josephson 1992, Josephson and Stokes 1994). These muscles exhibit specializations that include high densities of T-tubule systems and SR necessary to produce rapid increases and decreases in Ca^{2+} concentrations that trigger muscle activation and relaxation, respectively.

Over the years, skeletal muscle biologists have been aware of differences in muscle fiber types and have attempted to classify these fiber types into some logical framework. Initially, muscles were grouped simply into "red" versus "white" fiber types based on their superficial

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appearance. In other cases, physiological measurements allowed different fibers to be identified based on their contractile properties as either "fast" or "slow." The development of histochemical assays using frozen muscle sections, particularly myofibrillar ATPase histochemistry, allowed for the identification of several different fiber types (Brooke 1970, Barnard et al. 1971). These techniques led to the classification of mammalian fiber into three fundamental groups: slow (I), fast glycolytic (IIB), and fast oxidative (IIA). Subsequently, the myosin heavy chain (MHC) motors responsible for generating contraction in mammalian muscles were identified (Schiaffino and Reggiani 1996, 2011). These different isoforms are encoded by several distinct genes, and the specific MHC isoform(s) expressed in single fibers has become the standard for classifying muscle fiber types (Schiaffino and Reggiani 1996, 2011, Pette and Staron 2000, 2001). Different MHC isoforms within mammalian fibers are now identified either through labeling muscle sections with monoclonal antibodies directed against specific MHC isoforms or through single-fiber sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) analysis (Booth et al. 2010, Pandorf et al. 2010).

Each of these approaches has been used to distinguish crustacean muscle fiber types. In crustaceans, fast-contracting glycolytic fibers are often large in diameter and appear either translucent or pearly white in coloration. Fast and slow fibers with varying degrees of aerobic capacity range from light brown or tan in coloration, to varying shades of pink or red. Histochemical procedures adapted from those used to identify mammalian muscle fiber types have been used to successfully distinguish crustacean fibers (Ogonowski and Lang 1979, Ogonowski et al. 1980, Silverman and Charlton 1980, Tse et al. 1983, Maier et al. 1984, Rathmayer and Maier 1987, Mykles 1988, Gunzel et al. 1993). These techniques stain crustacean fast muscles dark brown to black but leave slow fibers relatively unstained. Preincubating tissue sections with buffers of different pH reverses the staining reaction and reveals a range of intermediate fiber types. This range of fiber types identified through histochemical methods can be directly correlated with the physiological properties and innervation patterns of single fibers (Rathmayer and Maier 1987). It is reasonable to assume that these staining differences are directly correlated with the expression of distinct MHC isoforms present within different fibers, as is the case for mammalian fiber types (Staron and Pette 1986, Staron and Hikida 1992). This pattern has been confirmed in at least one study of crustacean muscles (Neil et al. 1993). Several different MHC isoforms in a limited number of species have been identified using single-fiber SDS-PAGE analysis (LaFramboise 2000, Medler and Mykles 2003, Medler et al. 2004, Perry et al. 2009). A better understanding of the number and types of crustacean MHC isoforms is needed to objectively classify these fiber types with a classification scheme similar to that used for mammalian fibers. Another common method used to classify crustacean fiber types is sarcomere width, which roughly varies from 2.5 to 20 μm. This range of sarcomere dimensions is starkly different from mammalian fibers, which have evolved to a constant width of ~2.5 μm in fast and slow fiber types alike (Hoyle 1983). Crustacean fast fibers are constructed from narrow sarcomeres (2.5–4 μm) and slow fibers from long sarcomeres (12–20 μm), whereas many fibers possess sarcomeres of intermediate width (Atwood 1976, Govind and Atwood 1982).

A pattern commonly observed in different crustacean muscles is that specialized fiber types are anatomically segregated within the same muscle. As a general rule, the more aerobic and slower fiber types tend to be localized closer to the joint at the most proximal and distal regions of the muscle (Mykles et al. 2002, Medler and Mykles 2003, Perry et al. 2009). In the closer muscle of the cutter claw of lobsters and in the pincer of snapping shrimp, the muscle has a central band of fast muscle fibers surrounded by slow fibers (Ogonowski et al. 1980, Govind 1987). These different fiber types provide for a range of muscle contraction rates for distinct types of claw movements.

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Identified Fiber Types in Lobster Muscles

Distinct muscle fiber types identified by specific MHC and other myofibrillar isoforms have revealed a level of diversity and complexity that could not be detected using histochemical techniques alone. The most comprehensive understanding of myofibrillar isoforms in crustacean muscle is from the American lobster, *Homarus americanus*, where alternate isoforms have been identified for MHC, myosin light chains (MLCs), paramyosin, tropomyosin, troponin T, troponin I, troponin C, and actin (Fig. 4.3, Tables 4.1 and 4.2). Here, we discuss the current understanding of these myofibrillar isoforms and what is known from other crustacean species for comparison.

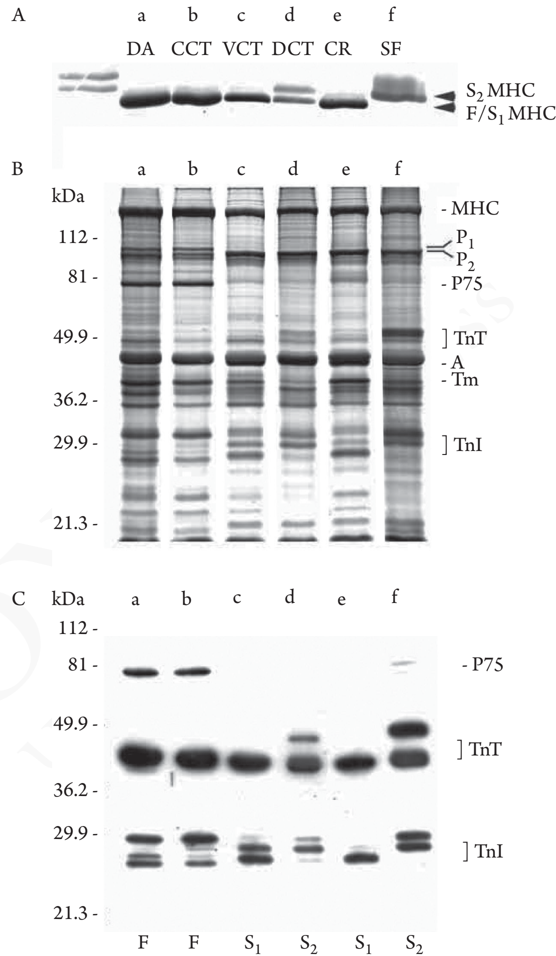

Fig. 4.3.
Myofibrillar protein assemblages in several muscles of the adult lobster *Homarus americanus*. Myosin heavy chain isoforms are shown in (A). Silver-stained gel of multiple myofibrillar proteins are shown in (B). Composite Western blot of P75, TnT isoforms, and TnI isoforms are shown in (C). Multiple isoforms are expressed for many of the myofibrillar proteins, and specific fiber types are characterized by unique combinations of these isoforms. Muscle fibers: deep abdominal (DA), central cutter closer (CCT), ventral cutter closer (VCT), distal cutter closer (DCT), crusher (CR), superficial flexor (SF). These fibers can be classified as fast (F), slow twitch (S_1), or slow tonic (S_2). Abbreviations: myosin heavy chain, MHC; paramyosin, P; 75 kDa protein, P75; troponin T, TnT; actin, A; tropomyosin, Tm; troponin I, TnI. From Medler and Mykles (2003), with permission from The Company of Biologists, Inc.

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Table 4.1. Myofibrillar protein isoforms in fiber types of the American lobster *Homarus americanus*. Actin isoforms have been identified from nucleotide sequences alone. Isoforms of MHC, Tm, and TnC have been identified at both the protein and nucleotide levels. Isoforms of paramyosin, P75, TnT, TnI, and MLCs have been identified at the protein level using SDS-PAGE.

Protein	**Fiber Type**		
	Fast	**Slow Twitch (S_1)**	**Slow Tonic (S_2)**
MHC	Fast	S_1	S_2
Paramyosin	P_1>>P_2	P_2	P_2
P75	+	-	-
TnT	T_2	T_3>>T_2	T_1, T_3
Actin	SK4>5>3 (CT) SK8>5>>7 (DA)	SK1>2	
Tm	Fast	S_1	S_2
TnI	I_1>I_2>I_4>I_5>I_3 (CT) I_1,I_5>I_3>I_2 (DA)	I_4>I_2 (CR) I_3>I_2>I_4 (SA)	I_2>I_4 (CR) I_2>I_3 (SA)
TnC	C_3 (CT) C_2 (DA)	C_1, C_3	
MLC (alpha)	LC_2>>LC_1	LC_2>>LC_1>LC_3	LC_2>>LC_1
MLC (beta)	LC_1 (CT) LC_2 (DA)	LC_1	LC_1

Abbreviations: CT, cutter claw closer; CR, crusher claw closer, DA, deep abdominal muscle; SA, superficial abdominal muscle. Table compiled from Mykles 1985a, Mykles 1985b, Mykles 1988, Li and Mykles 1990, Cotton and Mykles 1993, Mykles et al. 1998, Medler and Mykles 2003, Koenders et al. 2004, Kim et al. 2009, Chao et al. 2010. Abbreviations for proteins: myosin heavy chain, MHC; 75 kDa protein, P75; troponin T, TnT; tropomyosin, Tm; troponin I, TnI; troponin C, TnC; myosin light chain, MLC.

MHC exists as at least three isoforms designated fast, slow twitch (S_1), and slow tonic (S_2) in lobster muscles (Li and Mykles 1990, Cotton and Mykles 1993, Medler and Mykles 2003, Medler et al. 2004, 2007). These isoforms have been identified at the protein level using SDS-PAGE analysis (Medler and Mykles 2003, Medler et al. 2004; Fig. 4.3, Table 4.1) and the 3′ terminal sequences that encode the carboxy-terminal rod region of each isoform has been cloned (Cotton and Mykles 1993, Medler and Mykles 2003, Medler et al. 2004; Table 4.2). The overall sequence similarity among the identified sequences is approximately 80% within the open reading frame, and each isoform has a distinct 3′ untranslated region (UTR), which suggests that the alternate isoforms may be encoded by distinct genes. The fast MHC is found within several different muscles, including the closer muscles of the cutter claw and within the deep extensor and flexor muscles of the abdomen. The S_1 MHC is expressed in various slow muscles, including those of the claw openers and within the closer muscles of the crusher claw. This isoform is also expressed to varying levels within the more superficial postural muscles of the abdomen. The S_2 MHC isoform is expressed in muscles that appear to correspond to the physiologically identified slow tonic fibers. These fibers are frequently located within the proximal and distal regions of muscles near joints, and they are likely used to maintain muscle contractions over a period of time. Within the claw closers, S_2 fibers are found in a distal bundle of fibers, and the S_1 isoform is frequently co-expressed to varying degrees within single fibers (Medler and Mykles 2003). A similar pattern of co-expression at both

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Table 4.2. Myofibrillar nucleotide sequences of the American lobster *Homarus americanus*. References: [a](Cotton and Mykles 1993), [b](Chao et al. 2010), [c](Garone et al. 1991), [d](Kim et al. 2009), [e](Koenders et al. 2002), [f](Medler and Mykles 2003), [g](Medler et al. 2004), [h](Mykles et al. 1998).

Protein	Isoform	Alternative ID	Sequence Information	GenBank Accession #	Ref
MHC	Fast		Partial cds (1529 bp) C-term	U03091.1	a
	S_1		Partial cds (1795) bp C-term	AY232598.1	f
	S_2		Partial cds (813) bp C-term	AY521626	g
Paramyosin			EST (317 bp)	GO271460.1	
P75			Partial cds (766 bp)	AY302591.1	f
TnT	–	–	–	–	
Actin	SK1		Complete cds (1386 bp)	FJ217207	d
		α actin		AF399872	e
	SK2		Complete cds (1395 bp)	FJ217208	d
	SK3		Complete cds (1224 bp)	FJ217209	d
	SK4		Complete cds (1248 bp)	FJ217210	d
	SK5		Complete cds (1295 bp)	FJ217211	d
	SK6		Complete cds (1243 bp)	FJ217212	d
	SK7		Complete cds (1276 bp)	FJ217213	d
	SK8		Complete cds (1245 bp)		d
Tm	Fast		Complete cds (896 bp)	AF034954.1	h
	S_1		Complete cds (2,223 bp)	AF034953.1	h
	S_2		Complete cds (1526 bp)	AY521627	g
TnI			EST (657 bp)	FD699253.1	
			EST (660 bp)	FD467672.1	
TnC	TnC_1		Complete cds (814 bp)	FJ790218	b
		aa sequence		P29289	c
	TnC_{2a}		Complete cds (639 bp)	FJ790219	b
		aa sequence		P29290	c
	TnC_{2b}		Complete cds (2094 bp)	FJ790220	b
		aa sequence		P29291	c
	TnC_{2b}''		Complete cds (2136 bp)	FJ790221	b
	TnC_3		Complete cds (1046 bp)	HM448422	b
	TnC_4'		Complete cds (1667 bp)	FJ790223	b
	TnC_4''		Complete cds (842 bp)	FJ790222	b
	TnC_4'''		Partial cds (563 bp)	FJ790225	b
	TnC_6		Complete cds (2439 bp)	GQ259153	b
	TnC_{6x}		Complete cds (2171 bp)	GQ259154	b
MLC			EST (695 bp)	FE044128.1	
			EST (194 bp)	GO271581.1	

Abbreviations: aa, amino acid; cds, coding sequence; C-term, C-terminal and 3′ untranslated region; EST, expressed sequence tag; EST identified sequences are only included where published sequences are lacking. Other ESTs encoding *H. americanus* MHC, actin, Tm, TnI, and TnC exist in the GenBank database.

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the protein and mRNA levels is observed within the superficial extensor and flexor muscles of the abdomen (Medler et al. 2004). In these single fibers, the S_1 and S_2 isoforms are expressed in varying levels, forming a continuum from the "pure" S_1 and "pure" S_2 fibers. Co-expression of multiple MHCs within single fibers is common in many lobster muscles, even within fibers traditionally classified as either fast or slow.

Six complete MHC sequences have recently been identified from the abdominal muscles of three shrimp species, and these represent the first full-length MHC sequences from crustaceans (Koyama et al. 2012a,b, 2013). Based on sequence comparisons, these represent two different fast MHC isoforms (MHC_1 and MHC_2) expressed in deep abdominal muscles of the shrimp (Koyama et al. 2012a,b, 2013). In addition, several partial sequences from adult pleopod muscles and from developing shrimp muscles have also been identified (Koyama et al. 2013). One of the adult pleopod MHCs exhibits sequence similarities suggesting homology with lobster S_2 MHC (Koyama et al. 2013). In crayfish muscles there are 3–4 different myosin isoforms expressed within different muscles, but their correspondence to the lobster isoforms has not been determined (Sakurai et al. 1996, LaFramboise 2000). Perry et al. (2009) identified three MHC isoforms distributed among distinct fiber types within the carpus extensor and flexor muscle of the ghost crab *Ocypode quadrata*. The 3′ terminal coding sequences and UTRs were also cloned from three distinct MHC isoforms. The similarity among these sequences, and in comparison to those from the lobster, was approximately 80%, but there was no clear correspondence between the crab and lobster MHCs, thus indicating that these may not represent homologous genes. In the large anaerobic fibers from the ghost crab muscles, two MHC isoforms (MHC_1 and MHC_3) were always expressed in approximately 50:50 proportions. More aerobic fibers near the proximal and distal ends of the muscle expressed a distinct isoform (MHC_2), usually as a single isoform but sometimes with one of the isoforms from the fast fibers.

MLCs exist in lobster muscles as 21–23 kDa (α) and 18–18.5 kDa (β) proteins. Three α and two β MLC isoforms are expressed to varying degrees in different lobster fiber types (Table 4.1). A similar pattern occurs in crayfish muscles, where each protein (α and β) is expressed as a fast and slow isoform. In addition, a third 31 kDa MLC is expressed in slow muscles (Sakurai et al. 1996).

Many of the other nonmyosin proteins are also present as multiple isoforms, and their expression largely mirrors that of the MHC isoforms. Many of these isoforms were identified decades ago using SDS-PAGE gels (Costello and Govind 1984, Mykles 1985a,b, 1988). More recently, a significant degree of progress has been made in identifying the gene sequences and tissue-specific expression patterns of these different isoforms. Paramyosin is a large (~105–110 kDa) protein that forms the core of thick filaments in many different invertebrate muscles, and two isoforms of this protein have been identified at the protein level (Table 4.1; Mykles 1985a). In the lobster, fast cutter and deep abdominal muscles preferentially express the larger P_1 isoform, whereas slow crusher claw and superficial abdominal muscles exclusively express the smaller P_2 isoform (Mykles 1985a). Little information is currently available about the gene sequences or mRNA distribution of these paramyosin isoforms, although a putative sequence has been identified as an expressed sequence tag (Table 4.2).

Tropomyosin is formed as a coiled-coil dimeric protein and is coupled to the troponin proteins (TnI, TnT, and TnC) to form the Ca^{2+}-sensitive "switch" in thin-filament regulation of muscle contraction (Hooper and Thuma 2005). In the relaxed state, tropomyosin (Tm) lies in the groove of the actin filament and physically prevents MHC from binding to the actin and generating muscle contraction. When activated, intracellular Ca^{2+} concentrations rise, the Ca^{2+} ions bind to troponin C, and a conformational change takes place in the troponin/tropomyosin complex that moves the tropomyosin away from the myosin binding sites on the actin filament. Therefore, it is anticipated that alternate isoforms of any of these proteins might affect the steepness of the force–Ca^{2+} relationship and thereby influence the sensitivity of muscle activation. Each of the proteins that operate as

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a component of this switch (tropomyosin, troponin T, troponin I, and troponin C) exists as multiple isoforms in lobster muscles (Tables 4.1 and 4.2). Tropomyosin exists as three known isoforms, designated as fast, S_1, and S_2, following the MHC nomenclature (Mykles et al. 1998, Medler et al. 2004; Tables 4.1 and 4.2). Each isoform is encoded by a single gene, and specific isoforms are generated through alternative splicing (Mykles et al. 1998, Medler et al. 2004). Three skeletal muscle Tm isoforms are also present in different muscles of the spiny lobster *Panulirus japonicus* and appear to correspond to the isoforms in the American lobster (Ishimoda-Takagi et al. 1997). In addition, an isoform specific to the heart muscle is also present (Ishimoda-Takagi et al. 1997).

Troponin T also exists as three different isoforms, designated simply as TnT_1, TnT_2, and TnT_3 in the order of migration on SDS-PAGE gels ($T_1 < T_2 < T_3$). TnT_2 is preferentially expressed within the fast muscles, TnT_3 in slow twitch (S_1) muscles, and TnT_1 is found specifically within the slow tonic (S_2) fibers (Table 4.1). We do not currently have information about the gene sequences encoding the TnT isoforms in lobster muscles.

Troponin I exists as five different isoforms, with multiple isoforms frequently being expressed within single fibers. During the juvenile stages of muscle differentiation in lobsters, single fibers express several isoforms, but the patterns of expression become more limited as the lobsters reach adulthood (Medler et al. 2007). In fully differentiated fast fibers of the cutter claw, the predominant isoform is TnI_1, whereas in the S_1 fibers of the crusher TnI_4 is the major isoform (Mykles 1985a, Medler et al. 2007). Adult S_1 fibers in the abdominal muscles predominantly express TnI_3 with some levels of TnI_4; S_2 fibers primarily express TnI_2, whereas fast fibers express TnI_1 in combination with other isoforms (Mykles 1985a, Medler et al. 2004). Many fibers exhibiting phenotypes intermediate to the S_1 and S_2 fiber types express varying levels of the TnI isoforms (Medler et al. 2004). Information about the genes encoding the different TnI isoforms is currently lacking, being limited to partial sequences identified as expressed sequence tags (Table 4.2).

Troponin C is the Ca^{2+} binding protein that functions as the Ca^{2+}-sensitive switch in thin filament-regulated muscles. In lobster muscles, three isoforms of TnC have been identified at the protein level from lobster claw and abdominal muscles using SDS-PAGE analysis (Mykles 1985a), and three have been identified through protein purification and amino acid sequencing of isoforms expressed in the abdominal muscles (Garone et al. 1991; Tables 4.1 and 4.2). However, a recent study has revealed a much greater level of complexity than has previously been anticipated. cDNA sequences for 11 different TnC isoforms have now been identified from lobster tissues, with 6–8 of these being predominantly or exclusively expressed within the skeletal muscles (Chao et al. 2010; Table 4.2). The 11 different isoforms are encoded by seven different genes, with several isoforms being generated by alternative splicing of the same gene (Chao et al. 2010). Three of the isoforms identified by their nucleotide sequences identified by Chao et al. (2010) corresponded to those previously identified by Garone et al. (1991).

Until recently, the protein actin that forms the backbone of the thin filament was known to exist as a single isoform in lobster skeletal muscles. However, new data have shown that at least 12 actin isoforms are expressed within various lobster tissues, eight of which are primarily or exclusively expressed within the skeletal muscles (Kim et al. 2009; Table 4.2). These different isoforms are the products of distinct genes, and the expression of specific isoforms is muscle-specific (Kim et al. 2009). In the land crab *Gecarcinus lateralis*, several actin isoforms are present (9–15, encoded by 7–11 genes), but their tissue-specific expression patterns are not known (Varadaraj et al. 1996). In *Artemia*, 8–10 actin genes are present, and four have been cloned (Macias and Sastre 1990). Similar patterns of actin expression are observed in crustaceans from other taxonomic groups, but the functional significance of this diversity is poorly understood (Hooper and Thuma 2005). In *Drosophila* spp., six different actin isoforms are expressed, four being specific to skeletal muscles (Fyrberg et al. 1998, Lovato et al. 2001, Hooper and Thuma 2005). These isoforms are selectively expressed in different muscles (Lovato et al. 2001) and have been shown to possess nonequivalent physiological

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functions (Fyrberg et al. 1998). These patterns suggest that different actin isoforms confer subtly different physiological properties to muscles with different functions (Fyrberg et al. 1998, Lovato et al. 2001, Hooper and Thuma 2005).

The multiplicity of myofibrillar isoforms present in crustacean skeletal muscles suggests that the precise contractile properties of the muscle are determined by the specific combination of myofibrillar isoforms within a fiber. In vertebrate muscle fibers, it is well established that muscle shortening velocity is determined directly by the MHC isoform(s) expressed. Alternate isoforms of MHC generally provide for a range of shortening velocities, with the fastest isoforms being roughly 5–10 times greater in their velocities than the slowest (Schiaffino and Reggiani 1996, Reggiani et al. 2000). In lobster muscles, histochemical staining clearly shows that muscles possessing the fast MHC hydrolyze adenosine triphosphate (ATP) at higher rates than the slow S_1 MHC (Ogonowski and Lang 1979, Ogonowski et al. 1980). ATPase activity measured from isolated myofibrillar proteins indicates that the lobster fast MHC hydrolyzes ATP at approximately 2–5 times the rate of the slow S_1 MHC (Mykles 1985a), and ATP hydrolysis rate is directly correlated with the speed of muscle shortening (Schiaffino and Reggiani 1996). Histochemical analysis indicates that the slow S_2 MHC is even slower than the S_1 isoform (Mykles 1988, Fowler and Neil 1992, Neil et al. 1993), and mechanical measurements from the Norway lobster *Nephrops norvegicus* are consistent with this interpretation (Holmes et al. 1999). In many single fibers in lobster muscles, multiple MHC isoforms are expressed (Medler and Mykles 2003, Medler et al. 2004). These fibers are known as "hybrid" fibers and are often interpreted to be transitional fibers, caught in the process of switching from one phenotype to another (Pette and Staron 2000). More recently, it has become clear that hybrid fibers are common components of many normal muscles, in which MHC coexpression is often the rule rather than the exception (Stephenson 2001, Caiozzo et al. 2003). In lobster muscles, a significant level of coexpression of different MHC isoforms is present at both the mRNA and protein levels (Medler and Mykles 2003, Medler et al. 2004, 2007). In the slow superficial muscles of the abdomen, a continuum exists between pure S_1 and S_2 fibers in terms of MHC expression and other myofibrillar isoforms as well (Medler et al. 2004). In the leg muscles of the ghost crab *O. quadrata*, three different MHC isoforms are present, and single anaerobic fast fibers typically express MHC_1 and MHC_3 in approximately 50:50 proportions (Perry et al. 2009). The physiological significance of MHC co-expression is not completely understood, but in mammalian muscles, hybrid fibers possess contractile properties intermediate to the pure fiber types (Reiser et al. 1985, Larsson and Moss 1993, Bottinelli et al. 1996). This suggests that blending of two or more MHCs within single fibers may provide for a continuum of contractile properties.

Alternate isoforms of myofibrillar proteins other than MHC also likely contribute to functional differences among fibers, but their role is even less well understood than that of the MHC isoforms. In principle, alternate isoforms of the thin filament regulatory proteins (tropomyosin, TnI, TnT, and TnC) should affect the sensitivity of muscle activation to Ca^{2+} concentration. Consistent with this expectation, alternate isoforms of TnI in two populations of fast fiber in the yabby, *Cherax destructor*, affect the steepness of the Ca^{2+}–force curve (Koenders et al. 2004). The population of fibers with the greater Ca^{2+}-sensitivity also has slightly shorter sarcomeres, which is also consistent with faster muscle contraction. In running ghost crabs, size-dependent differences exist in the relative proportions of TnI and TnT isoforms that may be related to operational frequency during running (Perry et al. 2009). In dragonfly flight muscles, alternatively spliced variants of TnT significantly influence muscle power output and flight performance (Fitzhugh and Marden 1997, Marden et al. 1999, Marden et al. 2001, Marden and Allen 2002). Collectively, these trends suggest that the relative proportions of the thin filament regulatory proteins may influence the kinetics of muscle activation and deactivation. However, it is possible that some of the diversity in myofibrillar isoforms may simply represent functional redundancies or vestiges of past functional specialization. It is surprising, for example, that so many distinct isoforms of actin are expressed within lobster tissues

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(Kim et al. 2009). Actin is generally viewed as being a passive participant in muscle contraction, and a functional role for different actin isoforms would be an unexpected finding. Further studies linking the myofibrillar isoform assemblage with the physiological properties of muscles are needed to reveal how specific isoforms affect muscle function.

Crustacean Muscle Proteins as Allergens

One discipline that has provided unexpected insights into our knowledge of crustacean myofibrillar proteins is the field of seafood allergen research. Crustaceans and mollusks, collectively referred to as shellfish, represent a major proportion of all seafood consumed worldwide (Lehrer et al. 2003, Lopata and Lehrer 2009). A significant number of individuals within the population exhibit allergic reactions to these foods, and, in some cases, the allergic reactions prove to be fatal. Several proteins from crustacean muscles have been identified as allergens, including arginine kinase, SR Ca^{2+} binding protein, and tropomyosin (Lopata and Lehrer 2009). Of these, tropomyosin has by far been the most consistently identified as a major allergen from multiple species of crustaceans, as well as from mollusks (Reese et al. 1999, Ayuso et al. 2002, Lehrer et al. 2003). Tropomyosin is the only major shrimp allergen, and more than 84% of the total IgE antibodies in shrimp-allergic patients are directed against this protein (Lehrer et al. 2003). In addition to allergic reactions caused from consuming or coming in contact with crustacean muscles, tropomyosins from other arthropods (cockroaches and house mites) can cause allergic responses as well (Lehrer et al. 2003). The tropomyosin amino acid sequence is highly conserved among crustaceans, thus providing several common antigenic sites among different tropomyosin isoforms (Motoyama et al. 2007, Suma et al. 2007).

PHYSIOLOGICAL PROPERTIES OF CRUSTACEAN MUSCLES

Nerve–Muscle Interactions

The initial identification of physiological fiber types in crustacean muscles was made independently from the molecular and biochemical determination of muscle fiber types. Several comprehensive reviews of the principles of neuromuscular organization and physiology of crustacean muscles have been published (Atwood 1976, Govind and Atwood 1982, Hoyle 1983, Govind 1987, Govind 1995, Millar and Atwood 2004). These topics are also covered in greater depth in the chapter by Atwood (see Chapter 4 in volume 3), so they will be covered only briefly here. Crustacean muscle innervation patterns significantly complicate the relationship between the physiological properties of different fiber types because a single fiber may be controlled by anywhere from one to five excitatory motor neurons. In addition, many fibers are also affected by an inhibitory motor neuron that can modulate muscle contraction.

Single-fiber analyses of the claw closer muscle in the crab *Eriphia spinifrons* provide an example of the complexities that exist between muscle phenotype and innervation patterns (Rathmayer and Maier 1987; Fig. 4.4). Four different fiber types are present in this muscle, as identified through histochemical, electrophysiological, and enzymatic properties. The fibers are variably innervated by two excitatory motor neurons, one fast and one slow, as well as by a common inhibitory neuron. Type I fibers are classified as slow oxidative. They exhibit low ATPase activities, slow contractions, and are innervated by all three motor neurons. Type II and type III fibers are both classified as fast oxidative glycolytic and exhibit fast contractions and high ATPase activities. The two fiber types also possess moderate to high levels of glycolytic and oxidative enzymes. However, type II fibers receive innervation from all three motor neurons, whereas type III fibers exclusively receive the fast motor neuron. The largest, type IV fibers are fast glycolytic. They exhibit fast contraction, high

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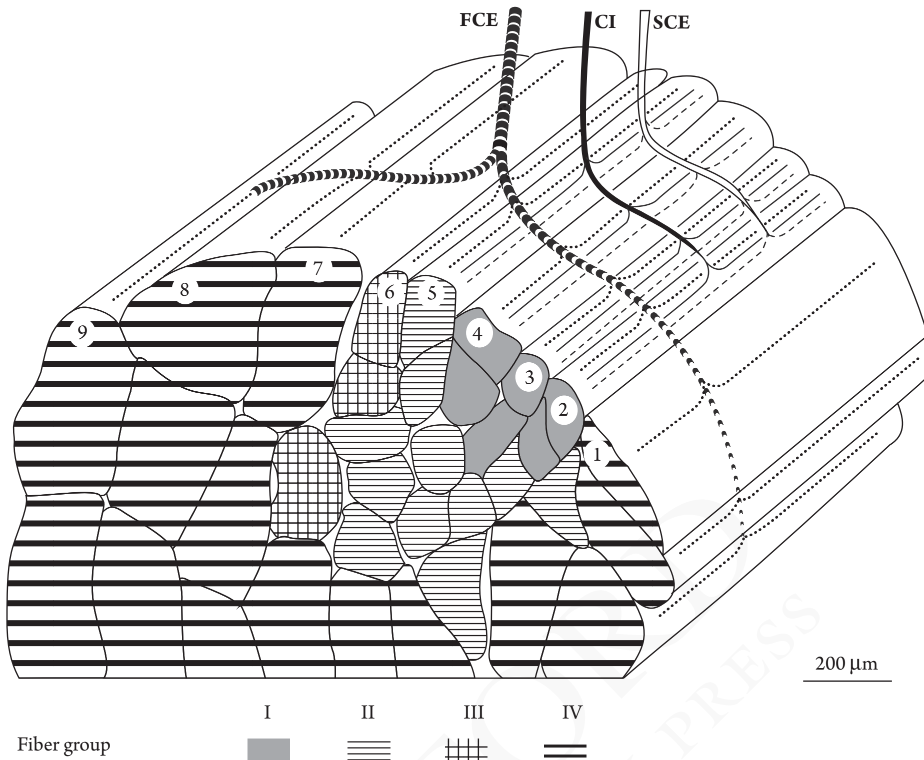

Fig. 4.4.
Schematic diagram of four fiber types present in the leg closer of the crab *Eriphia spinifrons*. Fiber types are identified using combined histochemical, electrophysiological, and biochemical analyses. Three motor neurons variably innervate the different fibers: a fast excitatory motor neuron (FCE), a slow excitatory motor neuron (SCE), and a common inhibitory neuron (CI). Type I fibers are classified as slow oxidative and are controlled by all three neurons. Type II fibers are fast oxidative/glycolytic and are also controlled by all three neurons. Type III fibers are fast oxidative/glycolytic and are controlled only by the FCE. Type IV fibers are fast glycolytic and are also controlled exclusively by the FCE. From Rathmayer and Maier (1987), with permission from Oxford University Press.

ATPase activities, and high levels of glycolytic enzymatic activity, but low oxidative capacity. Like the type III fibers, these fibers are controlled exclusively by the fast motor neuron. These patterns illustrate the principle that crustacean muscle physiology is not determined by fiber type alone, but by potentially complex interactions between the activity of different motor neurons and the cellular and molecular composition of different individual fibers. When contrasted with the organization of mammalian skeletal muscle and motor nerves, some general differences are apparent in crustacean neuromuscular systems. Crustacean muscles tend to have relatively few motor neurons that supply a single muscle, but the number of contacts along a single muscle is greater than in mammalian muscles, which only have a single synapse per fiber (Hoyle 1983, Belanger 2005).

In addition to neurotransmitters released at neuromuscular junctions, crustacean skeletal muscles respond to a number of different neuromodulatory peptides (Kreissl et al. 1999, Mercier et al. 2003, Weiss et al. 2003). These compounds are thought to be released into the circulation from various sources, and they affect different physiological systems including the heart and circulation, digestive system, and skeletal muscles. Their precise role in relation to skeletal muscle function is not completely understood, but we do know that both excitatory and inhibitory peptides exist. Proctolin and FMRFamide-like peptides tend to potentiate muscle contraction, whereas

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allatostatins act in an inhibitory capacity (Kreissl et al. 1999, Mercier et al. 2003, Weiss et al. 2003). These effects are exerted through both presynaptic mechanisms and directly on the muscle itself to modulate muscle contractility. There is some evidence that these compounds influence skeletal muscle contractile characteristics through selective phosphorylation of myofibrillar proteins (Brüstle et al. 2001).

Excitation-Contraction Coupling

Overall, the process of excitation-contraction coupling in crustacean muscles appears to be most similar to that in vertebrate cardiac muscles and other invertebrate muscles (Ashley et al. 1993, Palade and Györke 1993, Lea 1996, Quinn et al. 1998, Weiss et al. 2001, Takekura and Franzini-Armstrong 2002). A depolarization of the sarcolemma is carried along the tubular system into the muscle fiber to the dyadic and triadic junctions between the tubule and the enlarged cisternae of the SR. Although the threshold potential needed to initiate contraction is variable among muscles, for most crustacean fibers, the resting potential is more negative than threshold. In muscles that exhibit all-or-none contractions, the threshold is approximately 20–30 mV more positive than the resting potential. In tonic fibers that produce graded contractions, the threshold is closer to the resting potential, and the amount of tension developed is proportional to the level of depolarization (Chapple 1982). Unlike mammalian muscle fibers, the activating depolarization is primarily carried by the inward current of Ca^{2+} ions, rather than by Na^{+} (Ashley et al. 1993, Ushio et al. 1993, Weiss et al. 2001). L-type Ca^{2+} channels within the tubular membranes open, and the inward flux of Ca^{2+} can then activate TnC to initiate contraction, but Ca^{2+} ions also bind to ryanodine (RyR) receptors present in the SR membrane and initiate the release of stored Ca^{2+} (Weiss et al. 2001). This represents a process of Ca^{2+}-induced Ca^{2+} release (CICR), which is the principal mechanism of Ca^{2+} release occurring in the muscles of invertebrates and lower vertebrates (Palade and Györke 1993, Lea 1996, Quinn et al. 1998, Weiss et al. 2001). The threshold pCa for CICR through the RyR in the SR of lobster muscles is approximately 6.0–6.4 (Lea 1996, Quinn et al. 1998). The degree to which Ca^{2+} influx from the extracellular fluid versus that released from the SR initiates muscle contraction probably varies among different muscles, but, in most fibers, CICR appears to play an essential role (Palade and Györke 1993, Ushio et al. 1993, Lea 1996, Quinn et al. 1998, Weiss et al. 2001).

In most vertebrate skeletal muscles, the depolarization of the T tubule triggers a conformational change in the dihydropyridine sensitive L-type Ca^{2+} channels (DHPRs), and a direct mechanical coupling between this protein and the RyR leads to opening of the RyR on the SR membrane (Franzini-Armstrong and Protasi 1997, Endo 2009). In these muscles, the DHPR functions primarily as a voltage sensor rather than a Ca^{2+} channel, and the process is not CICR, being instead a direct coupling between depolarization of the T-tubule membrane and opening of the RyR of the SR (Franzini-Armstrong and Protasi 1997). This is seen as a more advanced form of excitation-contraction coupling and apparently evolved early in the evolution of vertebrates (Di Biase and Franzini-Armstrong 2005). This direct coupling depends, in part, on a physical coupling between the DHPRs within the T-tubule membrane and the RyRs on the SR (Franzini-Armstrong and Protasi 1997, Takekura and Franzini-Armstrong 2002, Di Biase and Franzini-Armstrong 2005). In vertebrates, each RyR is associated with four DHPRs arranged into a square pattern, where each DHPR is attached to one of the four subunits of the RyR (Di Biase and Franzini-Armstrong 2005). Invertebrate muscles, including those of crustaceans, lack the highly ordered arrays of DHPRs, and there is no evidence of a close association between these Ca^{2+} channels and the RyRs (Loesser et al. 1992, Takekura and Franzini-Armstrong 2002, Di Biase and Franzini-Armstrong 2005). This lack of direct coupling between these two molecules supports the view that CICR, rather than direct coupling, provides the mechanism to link depolarization with muscle contraction.

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RyRs have been isolated from crustacean muscles and studied in isolated vesicles, as well as in intact myofibrillar bundles (Formelova et al. 1990, Seok et al. 1992, Lea 1996, Quinn et al. 1998, Xiong et al. 1998). Each RyR is composed of four approximately 5,000 amino acid subunits, the same as those found in vertebrate skeletal muscles (Franzini-Armstrong and Protasi 1997, Xiong et al. 1998). Like the RyRs in vertebrates, these channels release Ca^{2+} in response to Ca^{2+} concentrations in the micromolar range, but they are inhibited by Ca^{2+} in the millimollar range (Quinn et al. 1998, Xiong et al. 1998). Two EF-hand domains are present on each RyR subunit, but it is currently unclear whether these Ca^{2+}-binding sites function in channel activation or inhibition (Xiong et al. 1998). The evoked Ca^{2+} currents from lobster RyRs are only about half those of mammalian RyRs (Quinn et al. 1998).

In vertebrates, multiple RyR isoforms are expressed in a tissue-specific manner. In mammals, RyR_1 is the principal isoform expressed in skeletal muscles, RyR_2 in cardiac muscle, and RyR_3 in the brain (Franzini-Armstrong and Protasi 1997). In nonmammalian vertebrates, two isoforms, RyRα and RyRβ, are expressed and are homologous to mammalian isoforms RyR_1 and RyR_3, respectively (Franzini-Armstrong and Protasi 1997). Physiological studies of different muscles in the Australian yabby are consistent with the presence of two different RyR isoforms (Launikonis and Stephenson 2000), but there has yet to be an identification made of multiple crustacean isoforms.

Mechanical Properties

Multiple parameters define the functional performance of skeletal muscles. These include the mechanical properties of muscle stress (force/cross-sectional area) and shortening velocity (muscle lengths per second; L/s). Maximal muscle force is proportional to the physiological cross-sectional area of a muscle, whereas muscle stress is largely determined by sarcomere width or, more precisely, A-band width (Huxley and Niedergerke 1954, Josephson 1975, Taylor 2000). When compared with muscles from a wide range of animals representing different phyla, some crustacean muscles are capable of generating the greatest forces known for any animal (Medler 2002). For example, a variety of muscles from mammals, birds, and other vertebrates generate stresses in the range of 150–200 kN/m^2. By comparison, a number of crustacean muscles produce maximal stresses ranging from 400 to 2,000 kN/m^2 or greater (Taylor 2000, Medler 2002). These trends arise from differences in the anatomical arrangement of the sarcomeres because muscle force is proportional to the sarcomere width whereas shortening velocity is proportional to the number of sarcomeres in series (Huxley and Niedergerke 1954, Josephson 1975, Taylor 2000). This relationship represents a tradeoff between muscle strength and muscle speed, which has resulted in a diverse range of fiber types in crustacean muscles adapted for different uses. In vertebrate muscles, by comparison, the stress generated by different muscles is nearly constant, but muscles' shortening velocities vary over orders of magnitude.

Muscle shortening velocities (typically reported as maximum unloaded shortening velocity or V_{max}) for various crustacean muscles have not been studied as extensively as muscle forces, but the available data suggest that these values are similar to if somewhat slower than those of vertebrate muscles. In the broadest comparison among skeletal muscles representing different phyla, maximal shortening velocity ranges from less than 1 to 25 muscle L/s, which appears to represent an upper limit for shortening velocity (Josephson 1993). Keeping in mind that some level of variability exists among measurement parameters and approaches used in different studies, crustacean muscles exhibit shortening velocities comparable to the muscles of other active animals, including vertebrates. The muscles from the barnacle *Balanus nubilus* have very low rates of contraction (V_{max} = 0.15 L/s), but produce stresses of up to 600 kN/m^2. These fibers possess long sarcomeres (~9 μm) but also have low rates of ATP hydrolysis (Griffiths et al. 1990). Slow fibers in the Norway lobster have maximal shortening velocities of about 0.5 L/s

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(Holmes et al. 1999), whereas the flagellum abductor muscle that drives rhythmically active flagella in crabs has a V_{max} of 7.6 L/s (Stokes and Josephson 1994). The extensor and flexor carpus muscles of running ghost crabs were estimated to be capable of maximal shortening velocities of approximately 5–7 L/s (Perry et al. 2009). By comparison, the limb muscles of a comparably sized mouse have shortening velocities that range from 6 L/s (soleus) to 14 L/s (extensor digitorum longus); see Askew and Marsh (1997).

Aerobic Capacity

Crustacean muscles exhibit a range of aerobic capacities, from fibers that possess very few aerobic adaptations to those that are rich in mitochondria and have adaptations that facilitate oxygen exchange (Silverman and Charlton 1980, Tse et al. 1983, Mykles 1988, Stokes and Josephson 1992, Boyle et al. 2003, Johnson et al. 2004, Perry et al. 2009, Hardy et al. 2010). Generally, slow fibers are more aerobic than fast fibers, and slow tonic fibers are more aerobic than slow twitch fibers (Ogonowski and Lang 1979, Lang et al. 1980, Mykles 1988, Fowler and Neil 1992, Neil 1993). However, just as with vertebrate muscles, crustacean fast fibers also exist that possess great aerobic capacities (Silverman and Charlton 1980, Tse et al. 1983, Stokes and Josephson 1992, Hardy et al. 2010). Functionally, aerobic fibers are found in muscles used for slow sustained contractions (Mykles 1988, Fowler and Neil 1992, Neil 1993), those that power swimming and running (Tse et al. 1983, Boyle et al. 2003, Perry et al. 2009, Hardy et al. 2010), and those used for sustained, high-frequency contractions (Silverman and Charlton 1980, Stokes and Josephson 1992). Within single muscles, fibers located near the proximal and distal regions tend to be more aerobic and also tend to be composed of slower fiber types (Lang 1980, Mykles 1988, Mykles et al. 2002, Perry et al. 2009).

A common pattern observed in these crustacean aerobic fibers is one in which the mitochondria are positioned close to the sarcolemma (Fig. 4.5). In many instances, these fibers are highly subdivided by the clefts that penetrate into the fiber from the outer regions, and the subdivisions are lined by high densities of mitochondria. The subsarcolemmal distribution of mitochondria observed in crustacean muscles is distinct from the distribution in mammalian muscle fibers, where the mitochondria are more evenly scattered around the myofibrils (Boyle et al. 2003, Kinsey et al. 2007). The subdivision of individual fibers may be to facilitate exchange of oxygen and nutrients between the hemolymph and the muscle fibers (Hardy et al. 2009). In the aerobic fibers that power swimming in portunid crabs, fibers become more divided by the membrane clefts as they get larger with growth. The result is that the larger fibers in larger crabs are more subdivided, keeping the average width of each subdivision relatively constant (Hardy et al. 2010). We have observed a similar trend in the aerobic fibers of ghost crabs (Medler, unpublished observations). The function of the subsarcolemmal distribution of mitochondria is not completely clear. Although the distribution facilitates the exchange of oxygen between the hemolymph and mitochondria, it requires phosphagens to diffuse across a greater distance from the mitochondria to the fiber interior (Stokes and Josephson 1992, Boyle et al. 2003, Kinsey et al. 2007).

Broadly, crustacean muscle contraction is not only dependent on the contractile properties of the muscle fiber, but also on the motor neuron that controls contraction. Therefore, fatigue resistance in crustacean muscles is not simply a consequence of muscle adaptations, but is also dependent on the motor neuron(s) activating the muscle. Single fibers are often controlled by both a fast motor neuron and a slow motor neuron. The fast motor neurons elicit a greater response from the muscle fiber, but the neurons are quickly fatiguing, whereas the activation from the slow motor neuron generates less force, but the muscles exhibit facilitation over time (Atwood and Cooper 1996, Millar and Atwood 2004).

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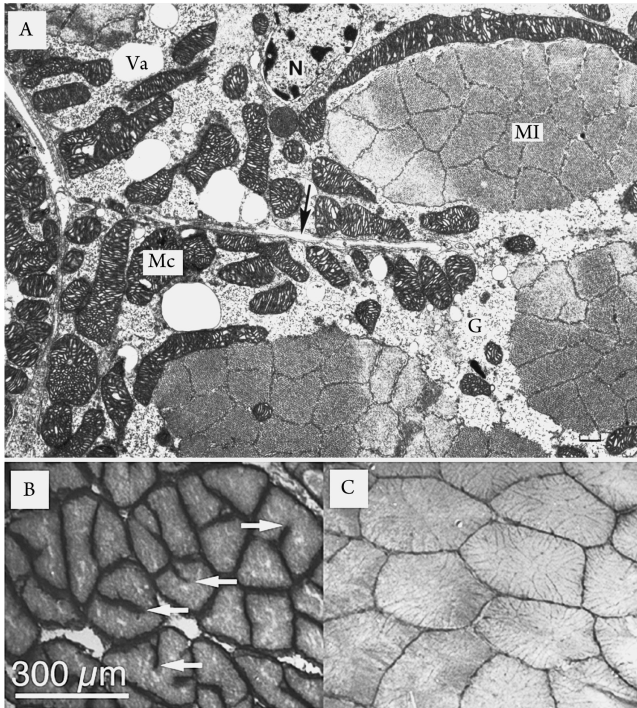

Fig. 4.5.
Structural features associate with aerobic and anaerobic fibers. Aerobic fibers are demonstrated by cross-section of the flagellum abductor (FA) muscle of *Carcinus maenas* (A) and in proximal fibers of the extensor carpus in *Ocypode quadrata* (B). Mid-region fibers of the extensor carpus in *O. quadrata* (C) are not highly aerobic. In *C. maenas*, mitochondria (Mc) are distributed around the fiber periphery in the FA muscle, whereas myofibrils (MI) are located more centrally. A membrane cleft (black arrow) is visible in the figure (TEM). In *O. quadrata*, NADH tetrazolium reductase staining from mitochondrial enzymes reveals a similar distribution in these fibers. In the more proximal fibers (B), high densities of mitochondria are present, and membrane clefts (white arrows) subdivide the fibers. In the mid-region fibers (C), mitochondria are similarly restricted primarily to the subsarcolemmal regions, but with much lower densities. Abbreviations in (A): mitochondria, Mc; glycogen, G; myofibrillar island, MI; nucleus, N; vacuole, Va. (A) is reprinted with permission from Stokes and Josephson (1992), with permission from Springer. (B) and (C) are from Perry et al. (2009), with permission from The Company of Biologists, Inc. The scale bar in A = 0.5 μm.

Physiological Differences Among Identified Fiber Types

Clear physiological differences exist among the S_1, S_2, and fast fiber types identified in the muscles of lobsters and other decapod crustaceans. The fast fibers of the deep abdominal flexors and extensors produce force at greater rates than the slow fibers, but the maximum force is significantly less (Jahromi and Atwood 1969, Ogonowski and Lang 1979). Muscle fibers found in the legs and claws of the lobster show similar differences (Jahromi and Atwood 1971). There are also more subtle differences between the fast muscles of the abdomen and those that compose the fast closer of the cutter claw, although these have not been well studied. The abdominal muscle fibers appear to be a more "pure" fast fiber type, as seen by significant differences in the expression of slow MHC isoforms. The fast muscles of the claw co-express some level of the S_1 MHC isoform, but the expression of that isoform in the abdominal musculature is nearly zero (Medler and Mykles 2003). Additionally, the fast fibers in cutter closer and deep abdominal muscles differ in expression of TnC

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isoforms (Chao et al. 2010; Table 4.1). The myosin ATP hydrolysis rate, which is directly correlated with muscle shortening velocity, is also higher in the abdominal musculature when compared to the fast claw fibers (Mykles 1985a). As for the slow fiber types, the S_1 fibers have faster rates of contraction and relaxation than those of the S_2 fibers and also exhibit higher ATPase activity than the S_2 fibers in histochemical staining reactions (Mykles 1988, Galler and Neil 1994, Holmes et al. 1999). By comparison, the S_2 fibers are more sensitive to activation by Ca^{2+}, meaning that they become active at lower Ca^{2+} concentrations (Galler and Neil 1994). The S_2 fibers also exhibit a greater degree of neuromuscular facilitation, providing the capability to maintain force production even after the S_1 fibers have fatigued (Mykles et al. 2002; Fig. 4.6). The classification into these discrete fiber types is really an oversimplification because many fibers possess a phenotype intermediate to these extremes (Costello and Govind 1983, Medler et al. 2004).

In addition to the well-defined fiber types just discussed, there are a number of different crustacean fiber types that do not clearly fit into this system. Physiological studies of several of these different fibers provide further insight into the functionality of different fibers. Using myosin ATPase histochemistry, Rathmayer and colleagues (Rathmayer and Maier 1987, Galler and Rathmayer 1992) identified four different fiber types in the closer muscles of the walking legs of the crab *E. spinifrons* (Fig. 4.4). Mechanical measurements from three of these fiber types showed a gradation in shortening velocities, with the fastest fibers being about 2–3 times faster than the slowest. Ca^{2+} sensitivity also differed among fiber types, but there was no direct correlation with shortening velocity. Sarcomere width was correlated with the different fiber types, with the slowest having the longest sarcomere width (14.6 μm), the fastest having the shortest (9.6 μm), and the intermediate fiber type having an intermediate sarcomere width (12.3 μm). Fast muscles of crayfish abdominal extensors and flexors possess ATPase activities that are approximately 5–7 times higher than the slow muscles of the claw opener (Sakurai et al. 1996). West et al. (1992) found that short-sarcomered (3.25 μm) claw closer fibers in the yabby (*C. destructor*) had slightly higher ATPase activities than those of the long-sarcomered fibers (8.57 μm), but these differences were not significant. Their conclusion

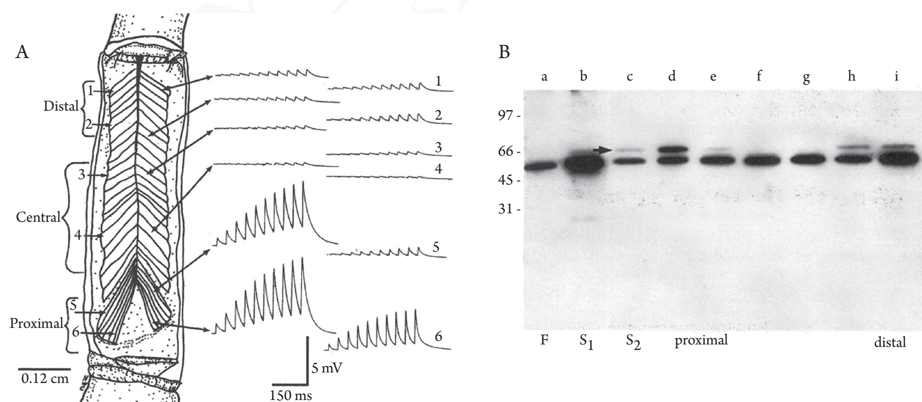

Fig. 4.6.
Myofibrillar protein isoform expression is correlated with synaptic efficacy. Fibers of the crayfish leg opener exhibit regional differences in excitatory postsynaptic potential (EPSP) following stimulation of the excitatory motor neuron (A). Proximal fibers (5 and 6) exhibit the greatest short-term facilitation, the distal fibers (1 and 2) intermediate levels, and fibers of the central region (3 and 4) show the least facilitation. Western blots of TnT isoforms from opener fibers reveal a correlation with these physiological responses (B). The most proximal (d and e) and distal (h and i) fibers express varying levels of TnT_1 (arrow) in combination with TnT_3, identifying them as slow tonic (S_2) fibers. The central fibers (f and g) express only TnT_3 and are slow twitch (S_1). Fibers a–c are controls (F, fast; S_1, slow twitch; S_2, slow tonic). From Mykles et al. (2002), with permission from The Company of Biologists, Inc.

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was that the faster contraction of the short-sarcomered fibers was due primarily to the structural arrangement of the sarcomeres, rather than to differences in the myosin cross-bridge kinetics.

Overall, crustacean muscles comprise highly diverse fiber types, as evident from the range of physiological, ultrastructural, and molecular compositions among fibers within and among species. Currently, no unified system exists that can be used to systematically classify crustacean fiber types in an unambiguous way. In extensively studied mammalian muscles, different fibers are classified according to the MHC isoform(s) that they express, and the genes encoding these isoforms have been fully characterized (Schiaffino and Reggiani 1996, Schiaffino and Reggiani 2011). The most precise classification of muscle fiber types in crustaceans is for the American lobster (*H. americanus*) in which fibers are classified as either S_1, S_2, or fast. This system seems to be relevant for crayfish and at least some crabs, but currently there are not enough data for MHC isoforms in different species to know for certain. In ghost crab leg muscles, three MHC isoforms are expressed in different ratios, but their migration pattern on SDS-PAGE gels is different from that of the lobster (Perry et al. 2009). Similarly, several different isoforms have been identified in crayfish muscles, but their migration pattern is different from the lobster (LaFramboise 2000). Sequence comparisons among orthologous decapod MHCs have failed to identify precise fiber type categories among species, and homologies of these different isoforms remain uncertain (Cotton and Mykles 1993, Medler and Mykles 2003, Medler et al. 2004, Perry et al. 2009). Koyama et al. (2012a,b, 2013) have recently presented phylogenetic relationships among available MHC isoforms from several different species, but these are primarily based on the partial sequences available at this time. Partial sequences are also available for the MHC genes of marine isopods (Holmes et al. 2002, Magnay et al. 2003), but these are for the myosin head near the 5′ end of the molecule and offer no comparison with other known sequences. A more comprehensive dataset consisting of both full MHC sequences and corresponding SDS-PAGE migration patterns would be especially helpful in defining crustacean fiber types more objectively. This type of analysis would also be indispensable for an understanding of the evolution of crustacean fiber type diversity. Crustaceans as a group are highly diverse and represent several distinct evolutionary histories. It could be that several different specific fiber type classification schemes are required to accurately classify the number of distinct fiber types and the interrelationships among these.

FUTURE DIRECTIONS

We are currently very limited in our understanding of the relationships among the diverse fiber types that make up crustacean muscles. These limitations exist for several reasons. First, many of the fiber types that have been identified are still only defined by descriptive parameters like fast versus slow, red versus white, or long- versus short-sarcomered fibers. These definitions are useful for broadly grouping fiber types but are imprecise. A second problem is that fibers have been classified using unique systems for different species, and it is unclear how the identified fiber types correspond to one another. Crab fibers defined as types I–IV by Rathmayer and colleagues (Fig. 4.4) likely correspond in some way to the S_1, S_2, and fast scheme of lobster muscles (Fig. 4.3), but data are not currently available to unravel their relationships. Finally, the identification of myofibrillar protein isoforms has been limited to just a few species, and, in most instances, only partial sequences have been identified if molecular data are available at all.

A better understanding of precisely how many different crustacean fiber types exist and how fiber types among different species are related to one another is clearly needed. An unambiguous fiber type scheme would provide a foundation for other studies, such as those focused on nerve–muscle interactions, dynamic muscle function in locomotion, skeletal muscle plasticity, and other areas related to skeletal muscle biology. We advocate using the MHC isoforms expressed in different

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fibers as the definitive measure of crustacean fiber type. This has been the standard for mammalian skeletal muscle classification for many years and provides an objective measure of fiber type (Schiaffino and Reggiani 2011). Although the full-length MHC sequences from shrimp (Koyama et al. 2012a,b, 2013) are currently the only complete crustacean MHC sequences available, several partial sequences have been published, and we should work to obtain full sequences whenever possible. When a greater number of full-length sequences are available, we will be able to establish how many different fiber types exist and how they are related to one another.

CONCLUSIONS

Crustacean muscles are highly diverse in both their structure and physiological function. These muscles have evolved into specialized tissues that fulfill a variety of processes, including sustained force generation, very rapid contractions, and sustained power output for long periods of time. Although diverse, crustacean muscles all share several unifying features. All crustacean muscles are striated and rely principally on thin filament regulation of muscle contraction. Fast contracting fibers possess short sarcomeres that are roughly the width of those found in vertebrate muscles (~2.5–4 μm), whereas slow fibers have longer sarcomeres of variable width (5–20 μm). A well-developed tubular network carries membrane depolarization into the fiber interior to come in close contact with the SR where Ca^{2+} is released to trigger contraction. Crustacean fibers use DHPR proteins and RyRs to control Ca^{2+} release from the SR. Unlike the process in mammalian muscles, there is not close coupling between the DHPR and ryanodine receptor, and the process in crustacean muscles is more similar to that in other invertebrates, lower vertebrates, and mammalian cardiac muscles.

Crustacean muscles comprise a number of distinct fiber types. These are most clearly defined in the American lobster, where the types include fast, slow twitch (S_1), and slow tonic (S_2) fibers. Each fiber type can be defined by specific assemblages of myofibrillar protein isoforms. These represent alternate forms of MHC, paramyosin, actin, tropomyosin, troponins, and MLCs. Recent studies have found that the number and expression patterns of these isoforms are much more complex than previously appreciated. This diversity of proteins assembled into different fibers is presumably responsible for the range of physiological properties. Physiological responses are also dependent on the number and pattern of innervation from excitatory and inhibitory motor neurons.

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