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SKELETAL MUSCLE DIFFERENTIATION, GROWTH, AND PLASTICITY

Donald L. Mykles and Scott Medler

Abstract

During embryogenesis, muscle progenitor cells fuse and form multinucleate myotubes that differentiate into myofibers. Fibers increase in length and diameter as animals move through larval, juvenile, and adult stages. In large fibers, infolding of the cell membrane and the concentration of mitochondria adjacent to the cell membrane reduce diffusional distances for energy metabolism. As fibers grow, nuclei are added and occupy more central locations to maintain a relatively constant myonuclear domain. Contractile properties and size are altered by physiological conditions. Transformation requires coordinated expression of fiber type-specific isoforms of myosin, actin, paramyosin, tropomyosin, and troponin-I and -T. Muscle load and contractile frequency change significantly as crustaceans grow; these size-related differences require cellular remodeling of muscles for locomotion. Proteasome/ubiquitin-dependent and calpain-dependent proteolytic systems degrade myofibrillar proteins and are upregulated in atrophic muscle. Myostatin and mechanistic Target of Rapamycin signaling pathways control the protein turnover required for remodeling fiber myofibril structure.

INTRODUCTION

This chapter reviews the current state of knowledge of skeletal muscle myogenesis, growth, and plasticity. There are few modern studies of muscle specification and differentiation during embryogenesis, and little is known about the genes that control myogenesis. Once established in the larval stages, the muscle fibers grow incrementally in length and width after each molt. Because the number of muscle fibers remains relatively constant, the fibers can achieve large

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dimensions in adults. In the American lobster (*Homarus americanus*), for example, fibers in the crusher claw closer muscle can be a centimeter in length and 1–2 mm in diameter. As in other taxa, crustacean skeletal muscles display a remarkable plasticity, and this chapter focuses on the best-known examples. Transformation of fibers from one phenotype to another occurs in the American lobster, snapping shrimp, and Christmas Island red crab. A molt-induced atrophy occurs in the closer muscles of large-clawed decapods, such as American lobster, fiddler crab, crayfish, and blackback land crab. Autotomy of an appendage causes an unweighting atrophy in the corresponding thoracic muscles of green shore crab, fiddler crab, crayfish, and blackback land crab.

Skeletal muscle development begins in the embryo and is completed during the larval stages. The early stages of myogenesis are similar to those in other taxa. Muscle progenitor cells arise in the mesoderm and fuse to form multinucleate myotubes, which differentiate into striated muscle fibers. Some crustaceans develop from a free-swimming nauplius larva, whereas in others the nauplius stage takes place within the egg. In the free-swimming larva, propulsive muscles develop to provide locomotion, and the adult muscles develop later (Kreissl et al. 2008, Hertzler and Freas 2009). In taxa that develop without a free-swimming nauplius, the muscles present in the adult are evident from the earliest stages of development (Harzsch and Kreissl 2010). In the lobster, early muscle fibers are recognized by the appearance of cells containing developing myofilaments and many myonuclei with diffuse chromatin (Kirk and Govind 1992, Govind 1995). Next, thick and thin myofilaments begin to form a regular latticework, but a characteristic sarcomeric banding pattern is not yet recognizable. At this time, signs of innervation, including neuromuscular terminals with clear synaptic vesicles, become evident. Shortly after innervation, distinct myofibrils with recognizable sarcomeric structure become visible (Govind 1995, Lang 1977). Myogenesis in developing crustacean muscles is asynchronous because both long- and short-sarcomered fibers are already present in the larva, whereas undifferentiated myoblasts are present in the same individual (Lang 1977, Jirikowski et al. 2010). The close association between relatively undifferentiated myoblasts and the growing ends of motoneurons suggests that myogenesis and motor neuron growth take place together in newly forming muscles (Harzsch and Kreissl 2010).

Many adult decapods exhibit continual growth throughout their lives. Molting creates additional space for tissue growth (Mykles 1980, Taylor and Kier 2006). Because muscle fibers remain anchored to the new exoskeleton, stretching due to exoskeleton expansion stimulates muscle growth during the postmolt period. This growth can be rapid. For example, in juvenile lobsters, fibers grow to fill most of the available space in the claws by 3 days postecdysis (Medler et al. 2007). In many crustacean species, an individual will increase in mass by several orders of magnitude over its lifetime. This kind of indeterminate growth presents a number of physiological challenges as the animal grows. In terms of skeletal muscles, several different aspects of integrative muscle function are affected by organismal size. One of the most obvious of these is the classical problem of strength-to-weight ratios. As an animal grows in size, its mass increases in proportion to its linear dimensions^3, whereas muscle strength increases in proportion to the cross-sectional area of the muscles (approximately linear dimensions^2; see Schmidt-Nielsen 1984). This reduction in relative muscle strength has varying levels of significance for a species depending on whether it is aquatic or lives primarily a terrestrial existence. Lobsters and king crabs can grow to sizes of several kilograms, but their relatively sedentary existence, where much of their mass is supported by water, largely mitigates the impact of body mass. For a variety of semiterrestrial crabs, increases in body size are expected to have a more significant impact. The largest terrestrial arthropods are members of the infraorder Anomura, and these hermit crabs can attain sizes of up to 3 kg (Greenaway 2003). However, the notion that increases in effective

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load constrain the operation of the musculoskeletal systems is not well supported. For example, even large hermit crabs are sufficiently strong relative to their weight that they exhibit effective locomotor abilities, can climb trees, and can even open coconuts (Herreid and Full 1986a, 1986b, Greenaway 2003). Small hermit crabs are able to carry shells equaling their own body mass or more without affecting running velocity (Herreid and Full 1986b). Exoskeletal strains and the safety factor against limb buckling in running ghost crabs are comparable to values for vertebrate bones (Blickhan et al. 1993). Moreover, performance parameters for these crabs (stride frequency, speed at trot-to-gallop transition, muscle shortening velocity) are also similar to comparably sized mammals (Blickhan and Full 1987, Full 1987, Full and Weinstein 1992, Blickhan et al. 1993). A more likely explanation for limitations on crustacean body size stems from limitations in gas exchange rather than the perceived burden of carrying a heavy exoskeleton (Kaiser et al. 2007).

Skeletal muscle can alter its size and fiber type composition in response to a variety of physiological conditions. Heterochely is common in decapod crustaceans (Mellon 1981, Govind 1992, Mariappan et al. 2000), and species with dimorphic claws are well suited for the study of fiber transformation. Some species, such as *Callinectes sapidus, Carcinus maenas*, and *Menippe mercenaria*, exhibit a distinct "handedness," in which the major claw is usually located on one side (Mariappan et al. 2000). In the hermit crab *Pagurus pollicaris*, the crusher claw is always on the right side and the cutter claw is always on the left side (Stephens et al. 1984). Other species, such as *H. americanus, Alpheus heterochaelis*, and *Nephrops norvegicus*, exhibit an equal distribution of left- and right-handed individuals (Mariappan et al. 2000). The claws of larvae are identical in morphology and fiber type composition and then differentiate into major and minor claws during the juvenile stage. Existing fibers of one type transform to a different type, which involves coordinated expression of fiber type-specific genes, as well as remodeling of the contractile apparatus. This is exemplified by *H. americanus*, in which fast fibers transform to slow fibers in the presumptive major (crusher) claw and slow fibers transform to fast in the presumptive minor (cutter) claw (Mykles 1997b). A similar process probably occurs in other species, in which the major and minor claws differ in fiber type composition, such as the fiddler crab *Uca pugilator* and the hermit crab *P. pollicaris* (Stephens et al. 1984, Govind et al. 1986, Ismail and Mykles 1992). Fiber transformation can also occur in adults. Shifts in fiber type occur during claw reversal in snapping shrimp (*Alpheus* sp.), in which the minor claw (pincer) transforms to the major claw (snapper) when the snapper claw is lost. Fast fibers die, and the remaining slow fibers transform to the snapper slow fiber phenotype over several molts. Claw reversal in the blue crab *C. sapidus* does not result in changes in muscle fiber properties (Govind and Blundon 1985). Claw reversal occurs in the stone crab *M. mercenaria*, but fiber transformation was not examined (Simonson 1985). In preparation for long-distance migration, the fibers in the walking legs of adult red crabs (*Gecarcoidea natalis*) transform from a slow-twitch (S_1) phenotype to a more fatigue-resistant slow-tonic (S_2) phenotype.

Species with large claws, such as *H. americanus*, freshwater yabby (*Cherax destructor*), and males of *Gecarcinus lateralis* and *U. pugilator*, have proved to be excellent models for the study of muscle growth and atrophy (Mykles 1997b). Fiber size is determined by the balance between the protein synthetic and degradative rates. When synthesis exceeds degradation, fibers increase in diameter (hypertrophy). Conversely, when degradation exceeds synthesis, fibers decrease in diameter (atrophy). Two types of atrophy occur in crustaceans. Unweighting from claw or leg autotomy causes a "disuse" atrophy of the corresponding thoracic musculature that operates the appendage. A molt-induced atrophy of the claw closer muscle facilitates withdrawal of the claws from the old exoskeleton at ecdysis. The net loss of protein results in a reduction of fiber diameter, whereas an increased protein turnover is associated with remodeling of the contractile apparatus that results from a preferential loss of thin filaments.

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MYOGENESIS AND EARLY MUSCLE DEVELOPMENT

Several recent studies of muscle development in crustaceans have shed some light onto these processes, although much remains unknown (Kreissl et al. 2008, Hertzler and Freas 2009, Harzsch and Kreissl 2010, Jirikowski et al. 2010). A common theme among these studies of early myogenesis is that the process appears to follow the establishment of a founding muscle cell that serves as a point of muscle development. These cells, termed *pioneer cells* after the myogenic cells in grasshopper embryos (Ho et al. 1983), apparently migrate to specific anatomical locations to effectively "seed" specific muscles throughout the body (Fig. 5.1). Following the initial muscle establishment by the pioneer cells, some differences in the initial steps of myogenesis have been reported. In isopod muscles, single pioneer cells appear to establish each primordial muscle cell (Kreissl et al. 2008), whereas in lobsters and dendrobranchiate shrimp, muscles are established as a common syncytial muscle precursor that then divides to form several muscles (Harzsch and Kreissl 2010). It is currently unclear whether the founding cells divide on their own to become polynucleate or whether they act to recruit other cells that fuse with the founding cell.

Myogenesis in crustaceans shares a number of similarities with muscle development and differentiation in *Drosophila*, a model organism that has provided one of the most detailed views of muscle development. In *Drosophila*, muscle *founder cells* differentiate from cells within the mesoderm and then migrate to specific locations within the developing embryo. These founder cells then attract and fuse with fusion-competent myoblasts to form the earliest multinucleate

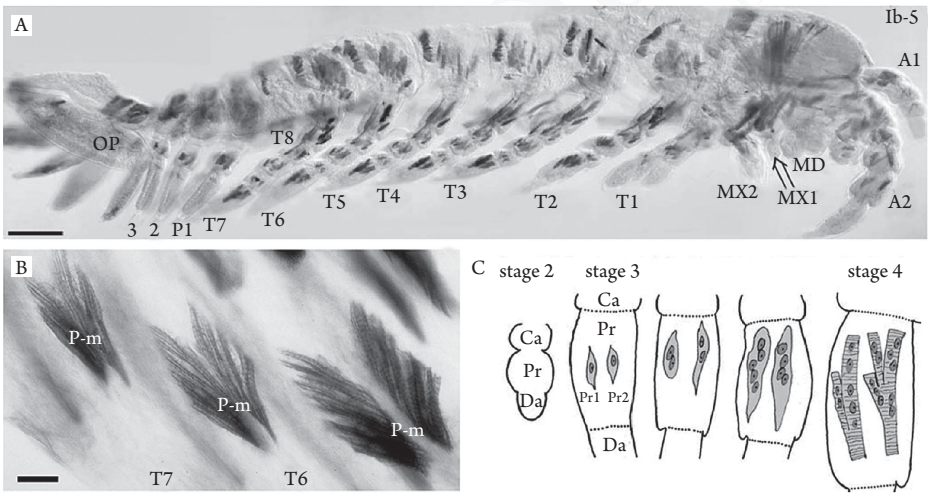

Fig. 5.1.
Myogenesis in crustaceans. (A) Muscle precursor cells in whole mount of a prehatching isopod embryo (*Idotea balthica*). Myogenic cells are labeled (dark) with a monoclonal antibody directed against myosin heavy chain (MHC). Abbreviations: A1, 2, antenna 1 and 2; MD, mandible; MX1, 2, maxilla 1 and 2; OP, operculum; P1-3, pleopods 1 to 3; T1–7, thoracic limbs 1–7 (T1 is a maxilliped); and T8, thoracomere 8. Scale bar = 100 μm. Reprinted from Kreissl et al. (2008), with permission from Springer. (B) Primordial muscles (P-m) in the endopodites of the thoracic limbs of embryonic lobsters (*H. americanus*; embryonic stage E45%). The developing muscles were labeled with a monoclonal antibody against MHC, and these muscle precursors will develop into the closer muscle of these leg segments. Scale bar = 25 μm. Reprinted from Harzsch and Kreissl (2010), with permission from Elsevier. (C) Schematic representation of myogenesis in the propodus of the thoracic limbs of the isopod, *I. balthica*. Muscle founder cells (Pr1 and Pr2) are observed in stage 3 embryos. In subsequent stages, the founder cells enlarge, become multinucleate, and subdivide into distinct subunits. Abbreviations: Ca, carpus; Da, dactylus; and Pr, propodus. From Kreissl et al. (2008), with permission from Springer.

muscle precursor cell. The growing myofiber then elongates toward tendon cells with which they subsequently fuse to form a muscle attachment site (Baylies et al. 1998, Schejter and Baylies 2010). The tendon cells secrete spatial cues that the growing myofibers use to seek out as they elongate toward their future attachment sites (Schejter and Baylies 2010). The founder cell then directs the ongoing differentiation of the developing fiber, with different fiber types being determined by the original founder cell's phenotype. Although our understanding of these events in crustaceans is far from complete, the available data are consistent with a similar developmental process. The common processes between the crustacean muscle development and fly development suggests that arthropod muscles may generally follow a pattern in which founder cells (*pioneer cells* in crustaceans) migrate from undifferentiated mesoderm into a specific anatomical position and then initiate fusion of undifferentiated cells and eventually form a specific muscle fiber type. Further work in this area is clearly needed to understand crustacean muscle differentiation more fully.

Myogenesis in all animals is directed, in part, by groups of transcription factors that orchestrate the processes of muscle differentiation (Baylies et al. 1998, Wigmore and Evans 2002). In vertebrate skeletal muscles, these include the basic helix-loop-helix proteins Myf 5, MyoD, Myf 4, and myogenin, which play complementary roles in directing muscle development (Wigmore and Evans 2002). In *Drosophila*, they include comparable proteins like Twist and Nautilus (Baylies et al. 1998). Although similar myogenic regulatory factors are presumably involved in crustacean myogenesis, the specific proteins have not yet been identified in crustacean muscles.

MUSCLE GROWTH

Once specific muscles have been established during the early stages of development, muscles continue to grow in both length and diameter. The available evidence suggests that increases in overall muscle size in crustaceans are primarily accomplished by increases in the size of individual fibers rather than through increased number of fibers. The hypertrophic growth that characterizes most crustacean muscles means that fiber size may continue to increase over an animal's lifetime. This pattern is fundamentally different from that seen in mammals of vastly different size, in which the size of individual fibers are essentially constant, being on the order of 25–75 μm in diameter (Hoppeler and Fluck 2002, Liu et al. 2009). The mechanism of increasing fiber length is either through the addition of new sarcomeres at the ends of existing muscle fibers, by increasing the length of sarcomeres throughout the fibers, or some combination of both mechanisms (Govind et al. 1974, 1977, Bittner and Traut 1978, El Haj et al. 1984). In fully differentiated lobster muscles, new sarcomeres are added to the ends of existing fibers, but these are the same width as existing sarcomeres (El Haj et al. 1984). The addition of new sarcomeres to the ends of existing fibers is closely linked to the process of molting, when the linear dimensions of the newly formed exoskeleton may increase by approximately 15% (El Haj et al. 1984). Once the old exoskeleton has been shed and the new one has expanded, fiber length increases with the addition of new sarcomeres. It may be that the mechanical stretch provided as the newly formed exoskeleton expands is the physiological cue that initiates fiber elongation (El Haj et al. 1984). In muscles of crayfish, increase in fiber length is accomplished by lengthening of existing sarcomeres. In several different crayfish fibers, sarcomere length increases by more than double in fast, short-sarcomered fibers and up to about fivefold in the fibers of slow muscles (Bittner and Traut 1978). Increasing the length of sarcomeres throughout a fiber would entail an ongoing process of fiber remodeling, whereas the addition of new sarcomeres at the fiber ends would require more restricted remodeling. Altering sarcomere length will also directly impact muscle shortening velocity, with increased sarcomere widths resulting in a slower contracting fiber.

One of the consequences of hypertrophic muscle growth is that diffusion-dependent processes may become limiting (Kinsey et al. 2007, 2011). Large crustacean fibers are often many times larger than mammalian skeletal muscle fibers, and the large size may restrict the diffusion of oxygen, intracellular phosphagens, Ca^{2+}, and other molecules. Kinsey and colleagues have considered this problem in detail and found that, in most cases, diffusion is not limiting to the metabolic processes but may often be on the verge of being diffusion-limited (Kinsey et al. 2011). In the largest anaerobic crustacean muscle fibers, the rate of arginine phosphate resynthesis following exercise is slower than the rate that could become limited by diffusion (Kinsey et al. 2005). However, there are significant structural and physiological adaptations evident in aerobic crustacean fibers that prevent significant diffusion limitation. Aerobic muscle fibers in several crustacean muscles rely on a common mechanism to deal with diffusional limitations associated with hypertrophic growth. In these fibers, the surface membrane of larger fibers becomes invaginated to form clefts that penetrate the fiber center (see Chapter 4 in this volume). In some cases, the fibers become so highly subdivided that it is difficult to determine whether the original fiber is still a single cell or whether it has completely separated into multiple fibers. The mitochondria in these fibers are highly concentrated near the membrane invaginations, so that almost 90% of the mitochondria are found in this location. These aerobic fibers are particularly evident in the muscles used for swimming in portunid crabs (Hardy et al. 2010), as well in the proximal and distal regions of muscle used for running in ghost crabs (Perry et al. 2009).

A related issue is the number and placement of the myonuclei within a muscle fiber. All skeletal muscle fibers are multinucleate, frequently with hundreds of distinct nuclei that originate from multiple myoblasts during development (Baylies and Michelson 2001, Biressi et al. 2007). Each nucleus within a mature muscle fiber is thought to direct the expression of proteins within a limited cytoplasmic space termed the *myonuclear domain* (Allen et al. 1999). In vertebrate muscles, myonuclear domain size is relatively fixed. As vertebrate skeletal muscles grow hypertrophically, nuclei are added to existing fibers to maintain a relatively constant myonuclear domain size (Allen et al. 1999). In mammals ranging over a 100,000-fold difference in body size, the myonuclear domain increases with size but only on the order of three- to fivefold (Liu et al. 2009). In crustaceans, hypertrophic growth of existing muscle fibers also leads to the addition of new nuclei to the fibers to maintain myonuclear domain size (Hardy et al. 2009, Jimenez et al. 2010, Kinsey et al. 2011). In smaller fibers, the nuclei are restricted to the periphery of fibers as they are in mammalian fibers, but, with additional growth, the new nuclei are distributed throughout the fiber, including the fiber interior (Hardy et al. 2009, Kinsey et al. 2011). Overall, the myonuclear domain size in crustacean muscles is comparable to sizes observed in mammalian muscles (~10,000–100,000 μm^3 per nucleus; Liu et al. 2009, Jimenez et al. 2010). In vertebrate muscles, the source of the new nuclei is the population of undifferentiated myoblasts (satellite cells) that lie between the basement membrane and the sarcolemma (Allen et al. 1999, Zammit et al. 2006). In crustacean muscles, putative satellite cells have been identified morphologically, but their role in muscle growth has not been established (Novotová and Uhrík 1992). In snapping shrimp claw muscles undergoing a cycle of degeneration followed by regeneration of a new fiber type, the remnants of the degenerated fibers serve as scaffolding for myoblasts that differentiate into new fibers. The source of these myoblasts was speculated to be either existing satellite cells or from undifferentiated blood cells that transformed into myoblasts (Govind and Pearce 1994).

A different way in which skeletal muscles are impacted by body size is in the frequency of muscle contraction during locomotion. A universal pattern observed among diverse animal taxa is that smaller animals move with higher frequencies in running, flying, and swimming (Hill 1950, Heglund et al. 1974, Full 1997, Medler 2002). As a consequence, the skeletal muscles of smaller animals must have faster intrinsic shortening velocities than in larger animals (Hill 1950, McMahon 1975, Medler and Hulme 2009). The most well-defined example of this pattern is seen in mammalian skeletal

muscles, where homologous isoforms of the myosin heavy chain (MHC) proteins have evolved subtle differences that produce faster muscles in smaller species (Seow and Ford 1991, Reggiani et al. 2000, Pellegrino et al. 2003, Marx et al. 2006). The relative proportion of fast fiber types is greater in the muscles of smaller mammals than in the same muscles of larger animals (Goldspink 1977). In addition to faster muscle shortening, muscles operating at higher contractile frequencies must be able to become activated and inactivated more quickly than in slower contracting muscles (Rome and Lindstedt 1997, Rome 2006). It follows that one mechanism that has evolved to ensure rapid activation and deactivation is a high density of sarcoplasmic reticulum (SR) within muscles used at high frequencies. This ensures that the calcium ions that trigger muscle contraction can be released and then sequestered quickly. The rates of muscle activation, deactivation, and operational frequency are positively correlated with SR density in arthropod muscles, including those of crustaceans (Fahrenbach 1963, Josephson and Young 1987, Stokes and Josephson 1992, Lagersson 2002). In vertebrate muscles, contractile frequency is correlated with concentrations of Ca^{2+}-binding proteins, such as parvalbumin, which buffer cytosolic Ca^{2+} and enhance muscle relaxation (Thys et al. 2001, Rome 2006, Coughlin et al. 2007). Currently, there have not been any parvalbumins or similar proteins identified in crustacean muscles, but it seems likely that they are present. Another mechanism that influences the rate of muscle activation and relaxation is the expression of alternate isoforms of the thin filament proteins that regulate muscle contraction. These could include tropomyosin; troponin (Tn)-I, -T, and -C; or even actin itself. In two fast fiber types in the claws of the freshwater yabby *Cherax destructor*, expression of alternate TnI isoforms is correlated with Ca^{2+}-sensitivity and muscle activation rate (Koenders et al. 2004). In fish muscles, shifts in contractile frequency are correlated with changes in the expression of alternate TnT and TnI isoforms (James et al. 1998, Thys et al. 1998, 2001). In dragonfly flight muscles, the expression of two different TnT splice variants is correlated with contractile frequency and power output (Fitzhugh and Marden 1997, Marden et al. 1999, 2001).

Crustaceans offer compelling examples of size-related changes in muscle function and organization, but it is currently unclear how a systematic shift in operational frequency affects their skeletal muscles. The most complete information comes from studies of semiterrestrial ghost crabs, which are probably the most capable runners of all the crustaceans (Hafemann and Hubbard 1969, Burrows and Hoyle 1973, Full and Weinstein 1992, Perry et al. 2009). These crabs are able to run at top speeds in the range of 1–2 m/sec for short bursts, and the estimated contractile properties are similar to a comparably sized mammal (Hafemann and Hubbard 1969, Burrows and Hoyle 1973, Full and Weinstein 1992, Perry et al. 2009). Stride frequencies during maximal running exhibit a significant correlation with body mass. The smallest ghost crabs reach stride frequencies of approximately 20 Hz, but the larger animals top out at about 4 Hz (Burrows and Hoyle 1973, Blickhan et al. 1993, Perry et al. 2009). Overall, this means that running crabs experience the same scale-dependent shifts in operational frequency as a function of body size known for other kinds of animals. Because these crabs increase in mass by several orders of magnitude over their lifetime, a relevant question is whether the intrinsic properties of the muscles change as the animals grow. A recent study indicates that gradual shifts in the expression of alternate isoforms of MHC, TnT, and TnI is correlated with changes in crab size. Single fibers from the leg extensor and flexor carpopodite muscles express more MHC_2, TnT_1, and TnI_1 in larger crabs than in smaller crabs (Perry et al. 2009). Further work is needed to determine how these differences in myofibrillar protein expression are related to functional differences in the muscles. Over the past two decades, it has become clearer that a significant degree of complexity exists with respect to the number and expression patterns of different myofibrillar isoforms in crustacean muscles (see Chapter 4 in this volume). It is possible that the specific expression of unique combinations of these isoforms provides for a degree of "fine-tuning" in response to the demands placed on different crustacean

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skeletal muscles. It remains to be determined what kinds of changes in muscle fiber type accompany continued growth throughout the lifetime of crustaceans.

SKELETAL MUSCLE PLASTICITY

Fiber Transformation in Juvenile Lobsters

The dimorphic claws of the American lobster *H. americanus* differentiate from isomorphic claws during the early juvenile stages (Govind 1984, 1992, Govind et al. 1987, Mykles 1997b). *H. americanus* has three planktonic larval (zoeal) stages separated by two molts. At the third molt, third-stage larvae metamorphose into fourth-stage juveniles. By the fourth molt, fifth-stage juveniles assume a benthic habitat (Herrick 1895). The claws of fourth-stage animals have identical fiber type compositions: there is a central population of fast fibers and dorsal and ventral populations of slow fibers (Lang et al. 1977, Govind and Lang 1978, Ogonowski et al. 1980). At each subsequent juvenile molt, there is an incremental change in the muscle fiber composition and external morphology as the claws differentiate into the cutter and crusher types (Fig. 5.2; Emmel 1908, Govind and Lang 1978). Fibers in the presumptive cutter claw closer muscle transform from the slow to the fast phenotype, whereas fibers in the presumptive crusher claw closer muscle transform from the fast to the slow phenotype (Govind and Lang 1978, Costello and Lang 1979, Ogonowski et al. 1980). There is no evidence of fiber degeneration and replacement during claw differentiation (Lang et al. 1977, Costello and Lang 1979). Fiber transformation is completed in the cutter claw by the ninth stage, whereas transformation in the crusher claw lags behind the cutter claw and it is not completed until the 13th stage or later (Govind and Lang 1978, Lang et al. 1978, Ogonowski et al. 1980, Medler et al. 2007). Differentiation of the claws begins at the fifth stage, but it requires at least 15 molts over several years before the final cutter and crusher claw morphologies of the adult are attained (Costello and Lang 1979, Emmel 1908).

Changes in the innervation pattern and synaptic properties of fast and slow excitatory motor neurons also occur during claw differentiation. In early juveniles (fourth, fifth, and sixth), most fibers in both claws are innervated by both excitatory neurons (Costello et al. 1981). In adults, this pattern is retained in the crusher claw closer muscle but not in the cutter. In the cutter, most of the fast fibers receive only fast excitatory input, and the slow fibers receive either slow excitatory or both slow and fast excitatory input (Lang et al. 1980, Costello et al. 1981, Costello and Govind 1983). In adults, the cutter fast closer excitatory (FCE) motoneuron fires at higher frequencies for shorter bursts than the crusher FCE motoneuron (Lnenicka et al. 1988). Moreover, there are differences in the response of the excitatory motoneuron cell bodies to sensory stimulation; for both the FCE and slow closer excitatory (SCE) motoneurons, the cell bodies on the crusher side in the thoracic ganglion have greater spike frequencies and longer burst periods than those on the cutter side with the same stimulation (Govind and Lang 1981). Changes in the synaptic properties of the FCE motoneurons coincide with the changes in innervation pattern during claw differentiation. Synaptic facilitation is similar in the symmetrical claws of fourth-stage lobsters (Lnenicka et al. 1988). As the animals transition through the fifth, sixth, and seventh stages, facilitation increases in the presumptive cutter claw but remains unchanged in the presumptive crusher claw (Lnenicka et al. 1988). The changes in the pattern and synaptic properties of the FCE motoneurons in the cutter claw contribute to the faster contraction times needed to capture prey.

The determination of claw laterality is random and is restricted to fourth- and fifth-stage lobsters. In wild populations, the numbers of individuals with left-handed and right-handed crusher

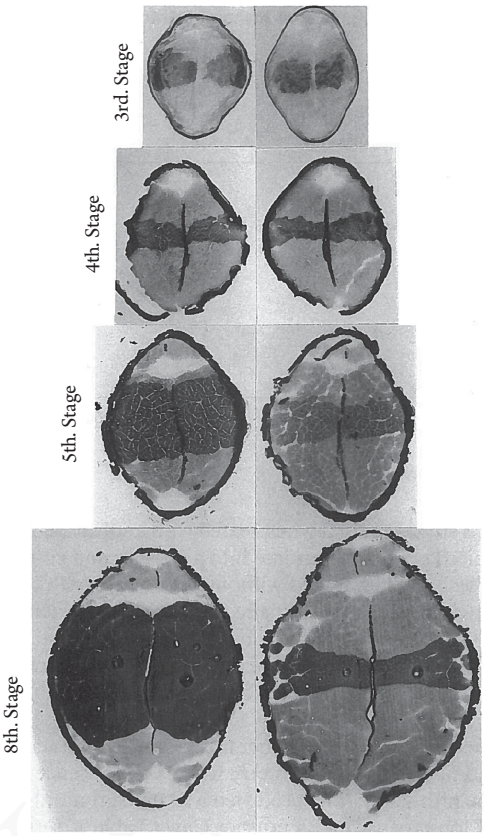

Fig. 5.2.
Fiber transformation during claw differentiation in juvenile American lobster. Transverse sections stained for myofibrillar ATPase activity show developmental changes in the closer muscle as the isomorphic claws of larvae (third and fourth stages) differentiate into the cutter and crusher claws of adults. The central band of dark-staining fast fibers expands in the presumptive cutter claw (left) by transformation of slow fibers to fast fibers. Conversely, the dorsal and ventral bands of slow fibers expand in the presumptive crusher claw (right) by transformation of fast fibers to slow fibers. The fast fibers in the crusher claw closer are completely replaced by slow fibers by the 13th stage. Fiber transformation is restricted to the boundary between fast and slow fiber populations. From Ogonowski et al. (1980), with permission from John Wiley and Sons.

claws are approximately equal (Herrick 1895, Emmel 1908), indicating that claw laterality is not genetically fixed at hatching. Emmel (1908) first demonstrated that autotomy of one of the claws during the fourth or fifth stage induces the remaining claw to differentiate into a crusher. This "forces" the animal to use the intact claw until the next molt, when the contralateral claw regenerate becomes functional (Lang et al. 1978). Once claw laterality is established in an individual, it remains fixed in that individual for the rest of its life (Emmel 1908). Autotomy at larval stages or at sixth and later stages has no effect on claw laterality (Emmel 1908, Lang et al. 1978). Autotomy of both claws during the fourth and fifth stages can delay the critical period for determining claw laterality to the sixth stage (Govind and Pearce 1989).

In an elegant series of experiments, Lang, Govind, and colleagues showed that the establishment of claw laterality requires a minimal reflex activity involving sensory input and neuromuscular output (Govind et al. 1987, Govind 1992). The claw that receives the greater stimulation above a minimum level of activity becomes the crusher claw (Lang et al. 1978, Govind and Kent 1982,

Govind and Pearce 1986, 1992). When juveniles are raised in smooth-bottomed containers without natural substratum or when juveniles receive equal mechanical stimulation through the fourth and fifth stages, most of the animals develop two cutter claws with similar fiber compositions (Lang et al. 1978, Govind and Kent 1982, Govind and Pearce 1986, 1992, Govind et al. 1991). Immobilization has no effect on claw laterality, indicating that a complete reflex arc is needed to determine which claw becomes the crusher (Lang et al. 1978, Govind and Kent 1982). Therefore, the cutter claw is the "default" outcome; a crusher claw only differentiates when one of the claws is used more than the other. The presence of a crusher claw prevents the contralateral claw from differentiating into a crusher. However, "double-crusher" individuals occur rarely in wild populations (Emmel 1908). The fiber compositions differ, even though the claws have the crusher morphology: one of the claws is a "false" crusher because it contains a mixture of fast and slow fibers resembling the composition of the cutter claw; the other claw is a "true" crusher that contains only slow fibers (Govind and Lang 1979). This indicates that fiber transformation can be uncoupled from structural differentiation, presumably from a genetic mutation that prevents repression of the crusher morphology and produces a false crusher.

Changes in muscle protein gene expression occur during claw differentiation. The fast and slow-twitch (S_1) fibers in the differentiated claw of adult *H. americanus* express distinct assemblages of myofibrillar protein isoforms: fast fibers in the cutter claw closer express fast MHC; actinSK3, SK4, and SK5; paramyosin$_1$ (P_1); a 75 kDa protein (P75); troponin-T$_2$ (TnT_2) and troponin-T$_1$ (TnI_1), whereas S_1 fibers in the crusher claw closer express S_1 MHC, actinSK1 and SK2, P_2, TnT_3, and TnI_4 and no P75 (Costello and Govind 1984, Mykles 1985a, 1985b, 1988, Medler and Mykles 2003, Medler et al. 2007, Kim et al. 2009a; see Chapter 4 in this volume). The myofibrillar protein composition of juvenile claw muscle differs from that of differentiated fast and slow fibers in the adult (Fig. 5.3). Claw muscle from fourth-stage animals express P_2, TnT_3, and TnI_4, but not P_1, P75, TnT_2, or TnI_1 (Costello and Govind 1984), which suggests that the fibers are not yet fully differentiated. By the 10th stage, the myofibrillar protein isoform compositions of the cutter and crusher claws resemble those of adults (Costello and Govind 1984), but it may take as long as 2 years for the fast fibers to attain the TnI isoform composition of adults (Fig. 5.3; see Medler et al. 2007). This is supported by analysis of MHC isoform and P75 expression by in situ hybridization and immunocytochemistry, respectively, in juvenile lobsters (Fig. 5.4). In seventh-stage lobsters, P75 and fast and slow MHCs are expressed in all fibers, although P75 and fast MHC are expressed at higher levels in fast fibers and slow MHC is expressed at higher levels in slow fibers (Medler et al. 2007). By the 10th stage, the expression of MHC isoforms and P75 is more discrete between the fast and S_1 fibers (Medler et al. 2007). These data indicate that muscle fibers in juvenile claws are distinct from the fibers in adults and that it may take months or years before the adult fiber phenotypes are achieved.

The mechanism by which the asymmetry in the nervous system directs the orderly transformation of fibers over a period of many molts is poorly understood. Transformation is restricted to the boundary between the fast and slow fiber populations as fibers with intermediate histochemical properties are localized to the boundary zone (Govind et al. 1987). Excitatory motoneuron activity probably plays a role. Chronic electrical stimulation induces changes in fiber properties to a more slow-tonic-like phenotype in crayfish abdominal muscles (Cooper et al. 1998, Gruhn and Rathmayer 2002). In the limb opener muscle, synaptic properties of the excitatory motoneurons may affect the contractile properties of the fibers they innervate; the slow-tonic (S_2) phenotype is correlated with larger excitatory postsynaptic potentials and greater short-term synaptic facilitation (Mykles et al. 2002). However, the innervation pattern and synaptic properties do not strictly correspond with fiber type (Costello et al. 1981, Lnenicka et al. 1988). This suggests that highly localized interactions between fast and slow fibers are needed to restrict transformation to the boundary zone. Further research is needed to determine whether fiber transformation is controlled through direct contacts and/or by paracrine factors.

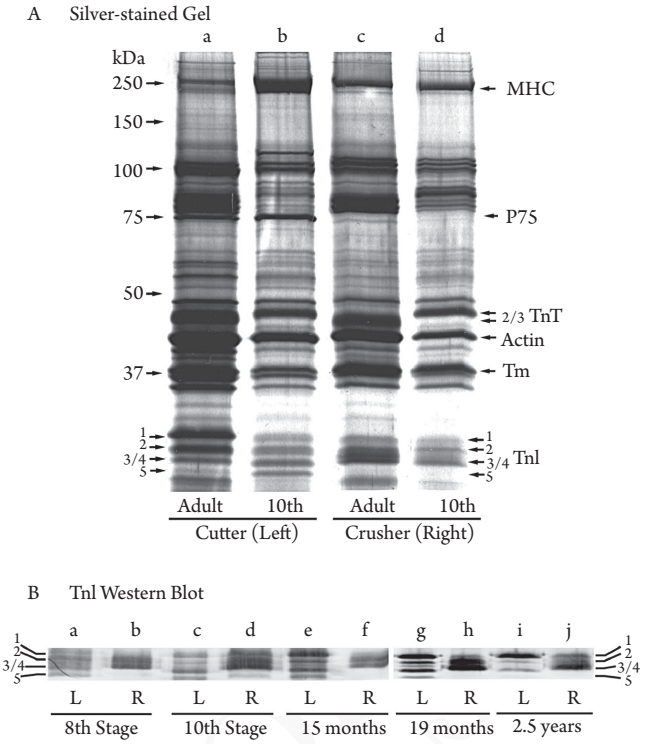

Fig. 5.3.
Differences in myofibrillar proteins between juvenile and adult lobster claw muscles. (A) Myofibrillar protein composition of fibers from cutter and crusher claws from adult and 10th-stage juvenile lobsters (*H. americanus*). Proteins were separated by sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) and stained with silver. Fast fibers from adult cutter claw express TnI_1 as the predominant isoform. In contrast, fast fibers from the central region of the presumptive cutter claw of 10th-stage juveniles express all five TnI isoforms at comparable levels. Fast fibers in cutter claw from both developmental stages express P75. The slow fibers from the adult crusher claw primarily express the $TnI_{3/4}$ isoforms, whereas the juvenile fibers express less of the $TnI_{3/4}$ isoforms. Both claws in juveniles express a TnT isoform with slower electrophoretic mobility than the TnT_2 and TnT_3 isoforms in adult cutter and crusher claws, respectively. Abbreviations: MHC, myosin heavy chain; Tm, tropomyosin; TnI, troponin-I; and TnT, troponin-T. Positions of molecular mass markers (kDa) indicated at left. (B) Western blot of troponin-I isoforms in dimorphic claws during lobster development. The left claw of fourth-stage larvae was autotomized, which induces the right claw to differentiate into the crusher claw; the left claw regenerates and differentiates into the cutter claw (Govind and Pearce, 1989). Fibers from the central region of the left claw (L; presumptive cutter) and the peripheral region of the right claw (R; presumptive crusher) of juvenile lobsters at eighth stage, 10th stage, 15 months, and 19 months and adult lobster at 2.5 years were analyzed. Proteins were separated by SDS-PAGE, transferred to nitrocellulose membrane, and probed with a lobster TnI antibody. Positions of the TnI isoforms indicated at the sides. It may take 2 years for the presumptive cutter claw to achieve the TnI composition of the mature adult. Reprinted with permission from Medler et al. (2007).

Fig. 5.4. (*Continued*)
the seventh molt stage, the developing crusher muscles have a distinct central region of fast fibers and distinct dorsal and ventral regions containing slow fibers (claw from 3 days postmolt animal). (B) Developing seventh-stage cutter claws are primarily composed of fast fibers by this stage (claw from 7 days postmolt animal). The opener muscle and the ventral region of the claw closer are entirely slow. (C, D) 10th-stage claw muscles possess similar staining patterns, but the fast and slow fiber regions are more distinct than the claws from seventh-stage claw muscles (molt stage unknown in 10th-stage claws). This is especially evident in the cutter claw, which completes fiber transformation before the crusher claw. Reprinted with permission from Medler et al. (2007).

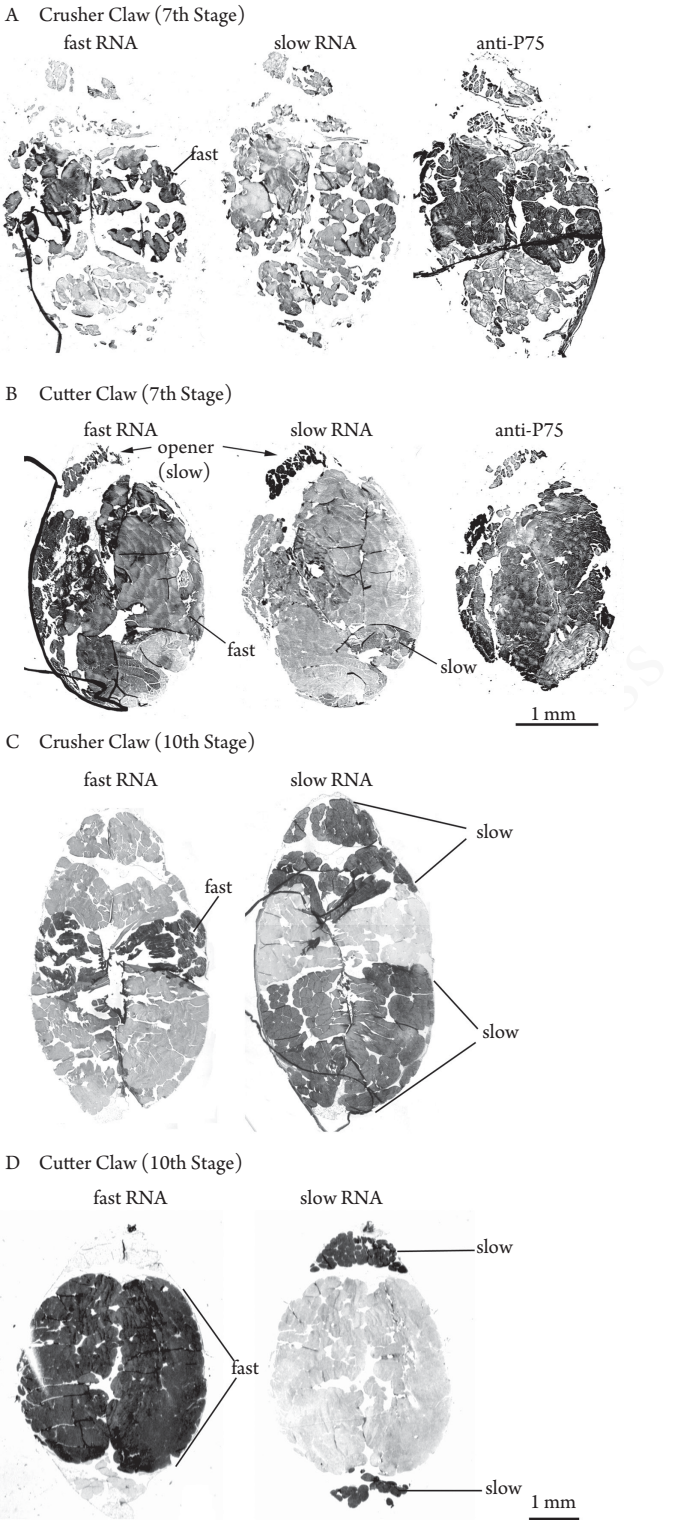

Fig. 5.4.
Differences in myosin heavy chain (MHC) isoform and P75 expression between developing crusher claw muscles and cutter claw muscles in *H. americanus*. Serial cross-sections of muscles were labeled with fast MHC RNA probe (left), S_1 MHC RNA probe (middle), and anti-P75 antibody (seventh stage only) (right). (A) By

Fiber Death and Transformation in Snapping Shrimp

Adult snapping, or pistol shrimps (*Alpheus* and *Synalpheus* spp.) possess dimorphic claws that differ greatly in morphology and function (Mellon 1981, 1999, Govind et al. 1987). The major claw, designated the snapper, has an enlarged dactyl that fits into a corresponding socket in the propodus (Fig. 5.5; Wilson 1903, Darby 1934, Knowlton and Moulton 1963). It is used for prey capture and agonistic behaviors (Darby 1934, Conover and Miller 1978, Knowlton and Keller 1982, Herberholz and Schmitz 1998, Mellon 1999). A rapid release of the dactyl results in a loud popping sound (Ritzmann 1973, Versluis et al. 2000), producing a continuous crackling noise in areas where large populations occur (Johnson et al. 1947, Everest et al. 1948, Knowlton and Moulton 1963, Potter and Chitre 1999). The noise produced by snapping shrimps can serve as a cue for orientation and settlement by invertebrate larvae on coral reefs (Stanley et al. 2010, Vermeij et al. 2010). The minor claw, designated the pincer, is much smaller and is used for feeding and burrowing (Mellon 1999). Right- and left-handed individuals occur in about equal numbers in wild populations (Wilson 1903, Darby 1934). Autotomy of one of the claws in third- or fourth-stage juveniles results in a snapper developing on the contralateral side (Young et al. 1994). These data indicate that claw laterality is established randomly in early juveniles probably by a mechanism similar to that in lobsters.

Unlike lobsters, the laterality of the major and minor claws is not fixed in adults. If the snapper claw is lost, the remaining pincer claw differentiates into a snapper claw over several subsequent

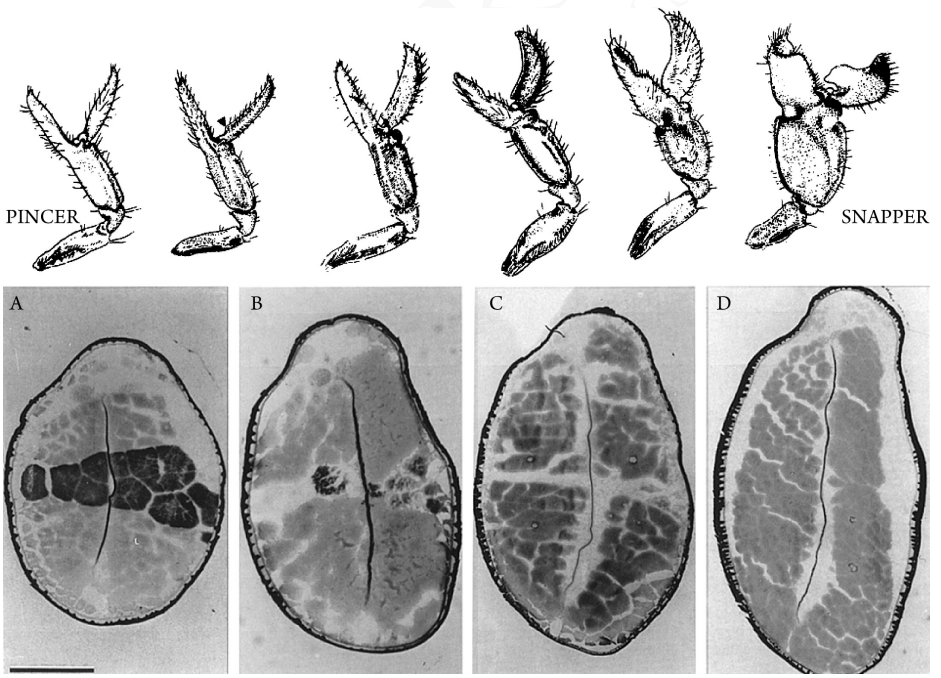

Fig. 5.5.
Claw transformation in snapping shrimp. Autotomy of the snapper claw causes the contralateral pincer claw to differentiate into a snapper over several molts (upper panel), and the pincer regenerates at the location of the original snapper. Myofibrillar ATPase histochemistry shows the dark-staining central band of fast fibers in the pincer (A) degenerating after the first (B) and second (C) molts to the slow fiber composition after the third molt (D). Over the next several molts, the slow fibers transform from the pincer phenotype with intermediate sarcomere lengths (6–8 μm) and a 5–6:1 filament ratio to the snapper phenotype with longer sarcomere lengths (10–15 μm) and a 7:1 filament ratio. Scale bar = 1 mm. Upper panel from Govind et al. (1987), with permission from Oxford University Press; lower panel from Govind and Pearce (1994), with permission from Springer.

molts, while the contralateral claw regenerates into a pincer (Wilson 1903, Mellon and Stephens 1978, Stephens and Mellon 1979). The transforming claw becomes a functional snapper after two molts, but the final snapper morphology is not attained until eight molts after autotomy of the contralateral snapper (Mellon and Stephens 1978, Stephens and Mellon 1979, Mellon and Greer 1987). Claw reversal occurs when only the snapper claw is autotomized, and laterality is maintained when only the pincer claw is autotomized or when both claws are autotomized (Wilson 1903, Darby 1934, Mellon and Stephens 1978). This indicates that there is some "memory" of claw asymmetry but that it can be overcome when the snapper claw is lost or is not functional. Transection of the nerve to the pincer claw prevents or delays reversal (Wilson 1903, Young et al. 1996). Although not as effective as pincer claw autotomy, either transection of the nerve, transection of the apodeme to the closer muscle (tenotomy), or removal of the dactyl (dactylotomy) to the snapper triggers transformation of the contralateral pincer, resulting in animals with two snappers; immobilization of the dactyl has no effect (Mellon and Stephens 1978, Govind et al. 1988, Young et al. 1994, Read and Govind 1997a, 1997b). Maintaining animals in communal tanks increases the incidence of double-snapper individuals (Pearce and Govind 1987). These data indicate that the nervous system transmits the loss of the snapper claw to the contralateral pincer and induces differentiation of the pincer to the snapper.

Differentiation of the pincer to the snapper involves a loss of fast fibers and transformation of slow fibers to a longer sarcomere phenotype. The pincer main closer muscle has a central band of fast fibers flanked dorsally and ventrally by slow fibers (Fig. 5.5), whereas the snapper contains only slow fibers (O'Connor et al. 1982, Mearow and Govind 1986, Quigley and Mellon 1986, Pearce and Govind 1987, Govind et al. 1988, Mellon and Quigley 1988). However, the sarcomere lengths and thin-to-thick filament ratios of the slow fibers in the main closer muscles differ between the two claws: the pincer slow fibers have intermediate sarcomere lengths (6–8 μm) and lower filament ratios (5–6:1) than snapper slow fibers (10–15 μm and 7:1, respectively; Stephens and Mellon 1979, Mellon and Stephens 1980, Stephens et al. 1983, Govind and Pearce 1994). This suggests that the intermediate and long-sarcomere fibers in the main closer muscles of the pincer and snapper claws, respectively, represent the two slow phenotypes. The snapper fibers express a larger TnT isoform that is consistent with the S_2 phenotype (Quigley and Mellon 1984, Govind et al. 1986, Mykles 1988, Ismail and Mykles 1992), but this must be confirmed by a thorough analysis of myofibrillar protein isoforms. The fast fibers are completely gone by the second molt after snapper claw autotomy (Fig. 5.5; Mearow and Govind 1986, Mellon and Quigley 1988, Quigley and Mellon 1986, Govind and Pearce 1994, Young et al. 1996). Following fast fiber degeneration, there is a transformation of the slow fibers in the pincer main closer muscle to the longer sarcomere phenotype in the snapper, which is completed within eight molts after autotomy of the snapper (Stephens and Mellon 1979, Mellon and Stephens 1980, Govind and Pearce 1994). The remodeling of the fibers coincides with the downregulation of pincer myofibrillar protein isoforms (e.g., pincer TnT and fast myosin light chain-2) and the upregulation of snapper myofibrillar proteins (e.g., snapper TnT and slow myosin light chain-2; Quigley and Mellon 1984, Mellon and Quigley 1988). Transection of the nerve to the transforming claw within 2 days after autotomy of the contralateral snapper prevents fast fiber degeneration even though claw morphological changes proceed normally (Mellon and Quigley 1988).

Little is known about the mechanisms controlling the changes in fiber type composition during transformation of the pincer to the snapper. The rapid degeneration of the fast fibers after the first molt is reminiscent of the programmed cell death in the abdominal intersegmental muscles (ISMs) of the hawkmoth *Manduca sexta*. At the end of pupation, contraction of the ISMs splits the pupal case, which allows the adult to escape. After the adult emerges, the ISMs are no longer needed, and the fibers completely degenerate by 30 h after emergence (Schwartz 2008). ISM cell death is triggered by a decline in hemolymph ecdysteroid (molting hormone) titer (Schwartz 2008). Fast fiber degeneration in the pincer claw occurs when ecdysteroid titers are low, which

suggests that a decrease in hemolymph ecdysteroid also triggers programmed cell death in snapping shrimp. Whatever the mechanism, it must explain why fast fibers die while slow fibers do not. The transformation of slow fibers from the pincer phenotype to the snapper phenotype has not been investigated in any detail. Gross motoneuron distribution does not appear to determine the fiber phenotype because the innervation patterns of fast and slow excitatory motoneurons are similar between the pincer and snapper claw closer muscles, and the patterns do not change during transformation in *A. californiensis* (Mellon and Stephens 1979, Stephens et al. 1983). Moreover, synaptic properties are correlated with claw type rather than with fiber type; synapses at fast and intermediate fibers in the pincer are characterized by low quantal neurotransmitter output and minimal facilitation, whereas synapses at long-sarcomere fibers in the snapper are characterized by large quantal output and greater facilitation (Mellon and Stephens 1979, Stephens and Mellon 1979, Stephens et al. 1983). This does not exclude trophic factors or other nerve properties, such as firing patterns and peripheral branching patterns, by which the nervous system may control fiber transformation. The cell bodies of the motoneurons in the thoracic ganglion differ in size, with the cell bodies on the snapper side larger than the cell bodies on the pincer side (Mellon 1981, Mellon et al. 1981). Claw reversal results in the reversal in cell body size after one molt, which is consistent with the primacy of the nervous system in driving fiber transformation (Mellon et al. 1981).

Fiber Transformation Associated with Seasonal Migration of Red Crabs

The red crabs (*G. natalis*) of Christmas Island in the Indian Ocean undergo a seasonal long-distance breeding migration. This species, as well as other members of this family, are obligate air breathers (Adamczewska and Morris 2000). The lining of the branchial chamber is elaborated into a highly vascularized lung-like tissue that allows high aerobic capacity. During the months of the dry season (April–October), animals remain inactive in burrows in the interior of the island to avoid desiccation. At the first monsoon rains, adult males and females begin a migration to the shore that may require walking 1 km/day for 5–6 consecutive days (Adamczewska and Morris 2001a). Upon arrival at the coast, males dig burrows where mating occurs. Males return inland and females remain in the burrows while the eggs develop. After about 2 weeks, females enter the ocean, where contact with seawater stimulates hatching and release of the zoea larvae. Over the course of 3–4 weeks, larvae undergo several molts and metamorphose into juvenile crabs, which come ashore and migrate to interior locations. Thus, there is a single "wave" of coastal migration, followed by three consecutive waves of inland migration: first adult males, then adult females about 2 weeks later, and then juveniles about 4 weeks after that.

The transition of sedentary to migratory phases requires physiological mechanisms for sustained locomotory activity. *G. natalis* has a unique shunt from lungs to the gills, which allows greater oxygenation of the hemolymph during exercise, that is lacking in other land crab species (Adamczewska and Morris 2000, Morris 2002). Even so, dry-season *G. natalis* rely on anaerobic metabolism for sustained moderate exercise. By contrast, wet-season *G. natalis* are completely aerobic and show no metabolic acidosis during migration (Adamczewska and Morris 2001a,b).

Migration causes a change in the fiber-type composition of the muscles in the walking legs to a more fatigue-resistant phenotype. Postel et al. (2010) analyzed 2,118 expressed sequence tags (ESTs) from muscles in the merus of walking legs from wet- and dry-season *G. natalis*. Differences in the expression of several myofibrillar protein isoforms are consistent with a shift from slow-twitch (S_1) to slow-tonic (S_2) fibers in preparation for migration. Histochemical studies show that S_2 fibers have a lower myofibrillar ATPase activity and higher aerobic capacity than S_1 fibers (Mykles 1988). Muscles from dry-season animals express higher levels of an ortholog of lobster actinSK1, which is the dominant actin isoform in S_1 fibers in the lobster crusher claw (Kim et al. 2009a, Medler et al. 2005, Medler and Mykles 2003). Orthologs of lobster slow tropomyosin-1 (Ha-sTm$_1$) and

tropomyosin-2 (Ha-sTm$_2$) are also expressed differentially between dry- and wet-season *G. natalis*. In lobsters, Ha-sTm$_1$ is preferentially expressed in S_1 fibers, and Ha-sTm$_2$ is preferentially expressed in S_2 fibers (Medler et al. 2004). In *G. natalis*, the S_2 tropomyosin ortholog (Gn-TmS2) is expressed at higher levels in migratory animals, whereas there is little or no change in the expression of the S_1 ortholog (Gn-TmS1; Postel et al. 2010). There are also changes in 3 TnI and 2 other actin transcripts, but assignment to specific fiber types could not be made (Postel et al. 2010). These data indicate that a shift to slow-tonic fibers in the walking legs contribute to the ability of *G. natalis* to migrate long distances without fatigue.

Transformation of S_1 and S_2 fibers requires remodeling the myofibrillar structure. In fiddler crabs, S_1 fibers have longer sarcomere lengths and higher thin filament-to-thick filament ratios than do S_2 fibers (Ismail and Mykles 1992). Consistent with this is the upregulation of two muscle LIM proteins, Mlp (Contig 131) and paxillin (Contig 140), in leg muscles of migrating *G. natalis* (Postel et al. 2010). Orthologs of these genes are implicated in structural remodeling of insect and mammalian skeletal muscles (Postel et al. 2010). This suggests that LIM proteins facilitate the incorporation of newly synthesized myofibrillar protein isoforms into the contractile apparatus so that animals can initiate migration as soon as the monsoon begins.

Temperature Plasticity

Temperature is a significant abiotic factor that affects muscle contraction. Cyprinid fishes exhibit a range of skeletal muscle plasticity in response to temperature changes to maintain muscle performance. In carp, three temperature-specific isoforms of MHC genes are expressed (Watabe 2002). In crustaceans, studies have examined MHC isoform diversity in the context of evolutionary adaptation to environmental temperature and of acclimation to temperature changes. An Antarctic isopod (*Glyptonotus antarcticus*) expresses an MHC isoform that is not present in a temperate isopod species (*Idotea resecata*) or a cold water amphipod species (*Eulimnogammarus verrucosus*; Holmes et al. 2002). This isoform may have evolved to function in the cold Antarctic waters, but further work is needed to demonstrate its functional role. In a subsequent study of seven amphipod species, the expression of four MHC isoforms is correlated with latitudinal distribution (Rock et al. 2009). Although it was concluded that the number of expressed isoforms increased in the more northern populations, one cannot rule out the possibility that these isoforms simply represent differences in common fiber types (i.e., similar to fast, S_1, and S_2 MHC isoforms). Indeed, two MHC isoforms were later identified as being differentially expressed in fast versus slow muscles (Whiteley et al. 2010). Temperature-specific MHC isoforms have not been identified within any crustacean muscles. Temperature has no effect on the expression of MHC isoforms in the abdominal muscle of lobsters (*H. gammarus*) reared at 10°C, 14°C, and 19°C (Whiteley and El Haj 1997, Magnay et al. 2003). These limited data suggest that crustacean muscles do not possess the level of temperature-dependent plasticity observed in some fish species.

Muscle Atrophy

Disuse Atrophy: Effects of Unweighting, Tenotomy, Denervation, and Immobilization

Crustacean muscles undergo a "disuse" atrophy in response to treatments that reduce the load on the fibers, such as tenotomy and limb autotomy. Tenotomy shortens the fiber resting length 15–35% in the crayfish (*Procambarus clarkii*) claw opener muscle, resulting in a 20% decrease in mean fiber diameter by 15 days (Boone and Bittner 1974). Fiber diameter is reduced about 50% one to two months after tenotomy (Boone and Bittner 1974). Autotomy of a walking leg in *C. maenas* and *U. pugilator* and a claw in crayfish (*Procambarus* sp.) causes an approximately 50% reduction in mass

of the thoracic muscles that operate the appendage, relative to the contralateral muscle (Moffett 1987). Interestingly, the diameter of the fibers in the weighted contralateral anterior levator (AL) muscle is reduced, but not as much as the fibers in the unweighted muscle (Schmiege et al. 1992). Thus, there appears to be an autotomy-induced atrophy in weighted muscles. Innervation remains intact and the muscles continue to function (Boone and Bittner 1974, Velez et al. 1981, Moffett 1987). Atrophy occurs in intermolt animals, indicating that it does not require ecdysteroids. The atrophic changes are reversed when the leg regenerates and becomes functional after the animal molts (Schmiege et al. 1992). Unlike mammalian skeletal muscle, there is no change in fiber phenotype in unweighted *P. clarkii* thoracic muscle (Griffis et al. 2001). Moreover, denervation and immobilization have little to no effect on crayfish claw opener muscle (Boone and Bittner 1974, Velez et al. 1981), which indicates that isometric tension per se is not essential for maintaining muscle mass.

Ultrastructural changes associated with unweighting were examined in *C. maenas* and crayfish (*P. clarkii* and *P. simulans*; Velez et al. 1981, Schmiege et al. 1992). Both autotomy and tenotomy cause disorganization of the sarcomere in *C. maenas* and crayfish, respectively, including fragmentation and loss of the Z line (Velez et al. 1981). Striations are indistinct in tenotomized muscle, probably due to hypercontraction (Velez et al. 1981). The myofibrils have extensive areas of myofilament erosion, and the intermyofibrillar space is increased (Velez et al. 1981, Schmiege et al. 1992). These features are indicative of increased degradation of myofibrillar proteins. There are reductions in mitochondria and nuclei, which are correlated with the presence of multivesicular bodies and lysosomes (Velez et al. 1981, Schmiege et al. 1992).

Molt-induced Atrophy of Claw Closer Muscle

In many decapod crustaceans, the first pair of pereopods develops into powerful claws that have offensive and defensive functions. The penultimate segment (propodus) is enlarged and contains a massive closer muscle that enables the dactyl to close with great force. Although large claws confer a competitive advantage, it creates a mechanical challenge at ecdysis when the claws must be withdrawn through the small basi-ischial joint that connects the claw to the body (Fig. 5.6).

Consequently, the mass of the closer muscle is reduced during the premolt period. The physical problem of withdrawing the claw was recognized by biologists in the 19th century (Salter 1860, Herrick 1895), but it was Couch (1837, 1843) who reported a molt-induced atrophy in *Cancer pagurus* and proposed it functioned in extricating the claws at molt. Skinner (1966) rediscovered the phenomenon in the blackback land crab, *G. lateralis*, in which the claw muscle mass decreases about 40% during the premolt period. Various aspects of this molt-induced muscle atrophy have been reviewed (Mykles and Skinner 1982a, 1985a, 1990a, 1990b, Mykles 1992, 1997b, 1999a, West 1997). The major conclusions are:

1. Atrophy is specific to the claw closer muscle, with fiber types in the claw responding differently to the atrophic signal. Muscles in the walking leg and cephalothorax do not atrophy during the premolt period (Mykles and Skinner 1982a, Schmiege et al. 1992, Griffis et al. 2001). In *G. lateralis*, the claw closer muscle is composed mostly of large-diameter S_1 fibers, with small-diameter S_2 fibers located centrally (Mykles 1988). The major claw of the fiddler crab (*U. pugnax*) contains mostly S_1 fibers with small numbers of S_2 fibers, and the minor claw contains only S_2 fibers (Mykles 1988, Ismail and Mykles 1992). In both species, the S_1 fibers undergo a greater atrophy than the smaller S_2 fibers (Mykles and Skinner 1981, Ismail and Mykles 1992). The claw closer muscle in the yabby *C. destructor* has equal numbers of slow (long sarcomere) and fast (short sarcomere) fibers (West 1997, Koenders et al. 2004). The slow fibers in late premolt animals show morphological changes associated with atrophy, whereas fast fibers do not (West et al. 1995, West 1997). Interestingly, the major claw muscle of

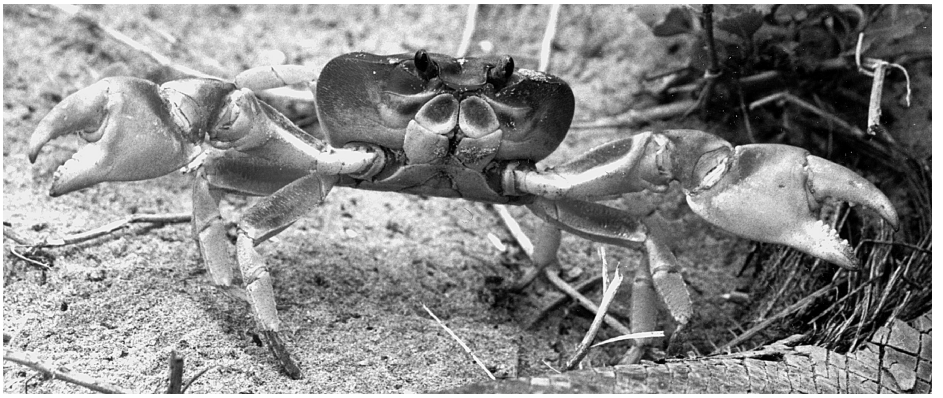

Fig. 5.6.
Dominant male blackback land crab, *G. lateralis*, in threat display. During premolt, the claw closer muscle atrophies. The reduction in muscle mass enables the animal to pull the large claws through the basi-ischial joints at ecdysis. Photograph by D. L. Mykles.

snapping shrimp does not atrophy (Mellon 1999). These animals use a different mechanism to extricate the claw. The exoskeleton splits open at ecdysis, providing a larger opening to remove the appendage (Mellon 1999).

2. The magnitude of claw muscle atrophy is determined by the regeneration load. Growth of limb regenerates occurs during the premolt period, so that a functional claw or leg is restored at ecdysis (Hopkins 2001, Mykles 2001). A large number of limb regenerates can place a significant demand for amino acids needed for tissue growth. In *G. lateralis* and *U. pugnax*, there is about a 40% decrease in mass in animals regenerating no more than one walking leg and about a 78% decrease in mass in animals regenerating 6–8 walking legs (Fig. 5.7; Skinner 1966, Mykles and Skinner 1981, Ismail and Mykles 1992). Thus, muscle protein degradation is accentuated by regeneration of 6–8 walking legs, which would provide amino acids for regenerate growth at a time when animals stop feeding and must rely on endogenous nutrient stores.
3. There is no fiber degeneration. In *G. lateralis*, the twofold reduction in fiber diameter is proportional to the fourfold reduction in myofibrillar cross-sectional area (Mykles and Skinner 1981), indicating that fibers retain the same number of myofibrils. The decrease in myofibrillar diameter results from the removal and degradation of myofilaments, as indicated by areas of myofibrillar erosion and enlarged intermyofibrillar space in atrophic muscle (Fig. 5.7; Mykles and Skinner 1981, Ismail and Mykles 1992, West et al. 1995, West 1997). In *C. destructor*, maximum Ca^{2+}-activated tension decreases about fourfold in both fast and slow fibers in claws of animals immediately before ecdysis (West 1999). There is also a compensatory decrease in the SR, and organelles, such as mitochondria and nuclei, retain their normal appearance (Mykles and Skinner 1981). Secondary lysosomes containing mitochondria and myelin figures indicate increased breakdown of mitochondria and SR membrane as fiber volume decreases (Mykles and Skinner 1982a).
4. There is an extensive remodeling of the sarcomere structure due to a preferential degradation of thin filaments. In *G. lateralis*, 11 thin filaments are removed for each thick filament, resulting in a decrease in the thin-to-thick filament ratio from about 9:1 to 6:1, a 31% decrease in the actin-to-MHC ratio, and a 72% increase in thick filament packing (Fig. 5.8; Mykles and Skinner 1981, 1982b). Similar changes occur in the major claw of male fiddler crabs *U. pugnax* (Ismail and Mykles 1992). In the S_1 fibers, the thin-to-thick filament ratio decreases from about 9:1

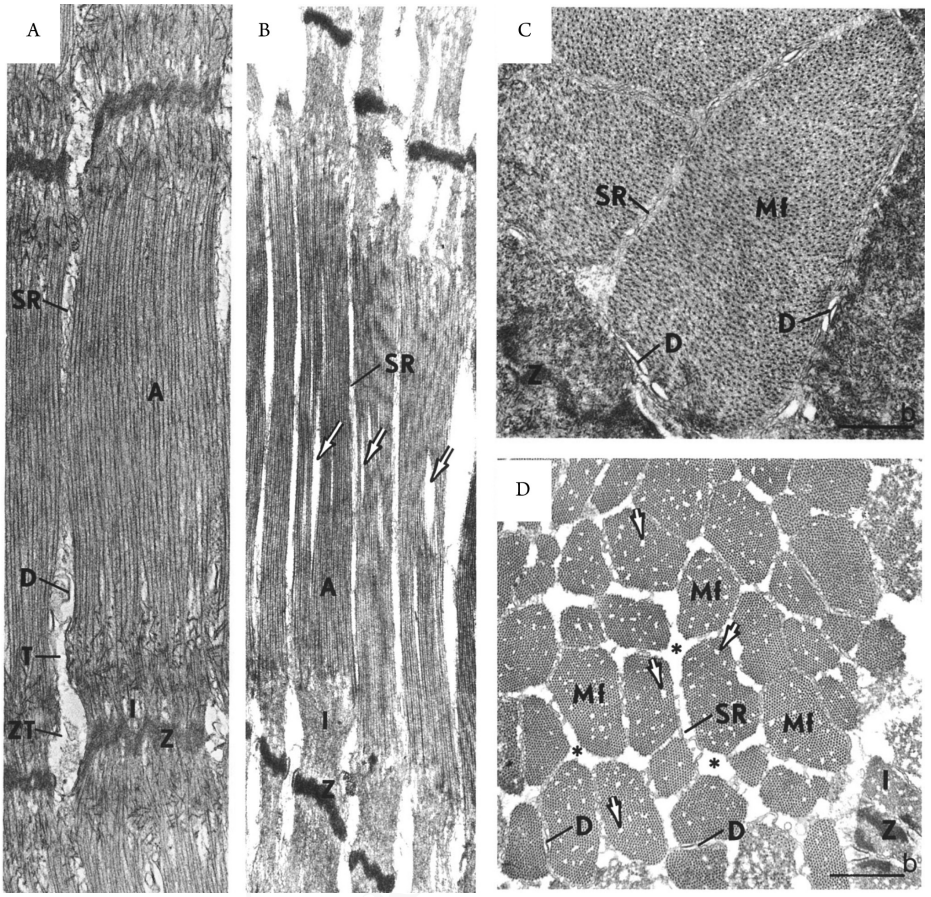

Fig. 5.7.
Molt-induced atrophy in the claw closer muscle of blackback land crab. Electron micrographs show the ultrastructure of the myofibrils in fibers from intermolt (A, C) and late premolt (B, D) animals in longitudinal (A, B) and transverse (C, D) sections. Myofibrillar cross-sectional area is reduced in late premolt fibers while the structure of the sarcomere is retained. Arrows indicate areas of erosion within the myofibrils, and asterisks (*) indicate enlarged interfibrillar space in the premolt fibers. Abbreviations: A, A band; D, dyad; I, I band; Mf, myofibril; SR, sarcoplasmic reticulum; T, transverse tubule; Z, Z-line; and ZT, Z tubule. Scale bar (C, D) = 1 μm. From Mykles and Skinner (1981), with permission from Elsevier.

to 6:1, the actin-to-MHC ratio decreases 74%, and the thick filament packing increases 51% (Ismail and Mykles 1992).

Molecular Mechanisms Regulating Protein Turnover in Crustacean Muscle

In mammalian muscle, atrophy results from a net increase in protein degradation, either from increased proteolysis and no change in protein synthesis, decreased synthesis and no change in proteolysis, or increased proteolysis and decreased synthesis (Schakman et al. 2009, McCarthy and Esser 2010, Goodman et al. 2011). Molt-induced claw muscle atrophy is atypical because protein synthesis is increased as much as 13-fold in vivo and in vitro in claw muscle from late premolt animals (Skinner 1965, El Haj et al. 1996, Covi et al. 2010). The large increase in protein synthesis

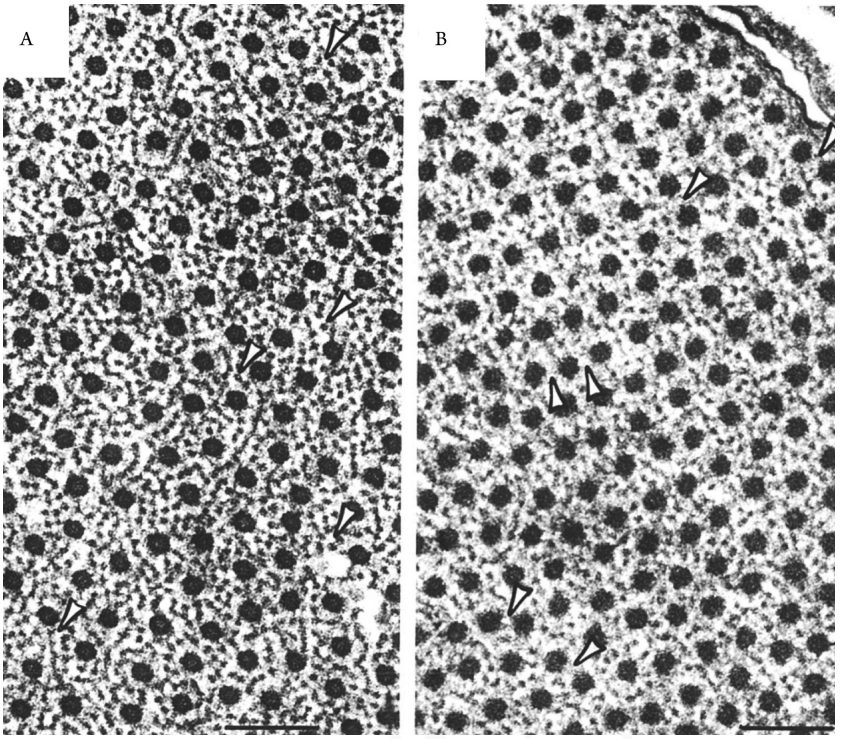

Fig. 5.8.
Molt-induced claw muscle atrophy causes changes in myofilament ratios and packing. (A) Transverse section of myofibril from an intermolt animal showing thick filaments surrounded by 10–15 thin filaments; the thin-to-thick filament ratio is about 9:1. Arrowheads indicate areas where thick filaments are separated by two rows of thin filaments. The average spacing of thick filaments is 51 nm. (B) Transverse section of myofibril from a late premolt animal showing thick filaments surrounded by 7–11 thin filaments; the thin-to-thick filament ratio is about 6:1. Arrowheads indicate areas lacking a row of thin filaments between thick filaments. The average spacing of thick filaments is 45 nm. Scale bars = 0.1 μm. From Mykles and Skinner (1981), with permission from Elsevier.

seems counterproductive because it necessitates an even greater increase in protein degradation to produce a reduction in mass. However, an accelerated protein turnover is necessary for the extensive remodeling of the fibers that occurs during premolt, as described earlier. Increased protein turnover is associated with remodeling in mammalian muscle (Bassel-Duby and Olson 2006). Any modification in the filament lattice requires the exchange of proteins in the myofibril with newly synthesized proteins in the cytoplasm (Russell et al. 2000). For example, the soluble myofilament pool increases under catabolic conditions (Dahlmann et al. 1986). Thus, the restructuring of the sarcomere depends on the flux, or turnover, of protein between the soluble and myofibrillar protein pools.

Protein degradation is mediated by calpain and ubiquitin (Ub)/proteasome proteolytic systems. Their role in molt-induced atrophy is briefly summarized here and emphasizes recent research. The reader is referred to reviews for further details on their biochemical properties (Mykles and Skinner 1985b, 1990a, 1990b, Beyette and Mykles 1999, Mykles 1992, 1993, 1997a, 1997b, 1998, 1999a, 1999b, 2000). Calpains are cytoplasmic Ca^{2+}-dependent proteinases (CDPs) that completely degrade myofibrillar proteins in vitro and in situ (Mykles and Skinner 1982b, 1983, 1986, Mattson and Mykles 1993, Mykles 1990). Four calpain activities, designated CDP I, IIa, IIb, and III, occur in crustacean

muscle, and cDNAs encoding three calpains, designated CalpB, CalpM, and CalpT, have been characterized (Table 5.1; Mykles 2000). Total calpain activity increases twofold in atrophic claw muscle (Mykles and Skinner 1982b).

The Ub/proteasome proteolytic system is stimulated in atrophic claw muscle. Ub is a highly conserved protein that, when conjugated to a protein, targets that protein for degradation by the proteasome (Mykles 1998, Glass 2010). Ubiquitin mRNA, Ub-protein conjugates, and proteasome subunits increase five-, eight-, and twofold, respectively, in atrophic claw muscle of *G. lateralis*, with greater ubiquitin expression in S_1 fibers (Shean and Mykles 1995, Koenders et al. 2002). In *H. americanus*, Ub expression is upregulated in claw muscle during premolt (Koenders et al. 2002). During larval development of the European lobster *H. gammarus*, proteasome activity in the claw muscle is higher during the pre- and postmolt stages (Götze and Saborowski 2011). The proteasome has limited ability to degrade myofibrillar proteins (Mykles 1989, Mykles and Haire 1991, 1995). Its precise role in muscle atrophy has yet to be determined, but its function appears secondary to calpains. As calpains preferentially degrade the Z line (Mykles 1990), calpains initiate protein degradation by releasing filaments from the myofibril; filaments are subsequently degraded by calpain and Ub/proteasome systems (Mykles 1997b).

Ecdysteroids control protein metabolism in the claw closer muscle. Protein synthesis increases during premolt, with maximum rates at late premolt when hemolymph ecdysteroid titers are at their peak (Covi et al. 2010, Mykles, 2011). Coincident with the increase in protein synthesis, there is a decrease in myostatin (Mstn) expression. Mstn, a member of the transforming growth factor-β (TGFβ) family, is an autocrine factor that inhibits protein synthesis and stimulates protein degradation in mammalian muscle (Schakman et al. 2009, McCarthy

Table 5.1. Comparison of masses of deduced sequences from crustacean calpain cDNAs with masses of lobster Ca^{2+}-dependent proteinases (CDPs) characterized biochemically.

Type	Calculated mass (cDNA)	Accession Number	CDP activity (subunit mass)	Reference
B Calpain:				
Gl-CalpB	88.9 kDa	AY639153	CDP IIb (95 kDa[b])	(Kim et al. 2005*a*)
Lv-CalpB	Partial cDNA	GQ179742		unpublished
M Calpain:				
Gl-CalpM	65.2 kDa	AY639152	CDP III (59 kDa[a];	(Kim et al. 2005*a*)
Ha-CalpM	66.3 kDa	AY124009	62 and 68 kDa[b])	(Yu and Mykles 2003)
Nn-CalpM	65.9 kDa	FJ666100		(Gornik et al. 2010)
T Calpain:				
Gl-CalpT	74.6 kDa	AY639154	CDP I or IIa (60 kDa[b])	(Kim et al. 2005*a*)

Abbreviations: Gl, *Gecarcinus lateralis*; Ha, *Homarus americanus*; Lv, *Litopenaeus vannamei*; Nn, *Nephrops norvegicus*.
Native masses of lobster CDPs I, IIa, IIb, and III are 310 kDa, 125 kDa, 195 kDa, and 59 kDa, respectively (Mykles and Skinner 1986). The putative identities of CalpB with CDP IIb and CapT with CDP I or IIa have not been established. The subunit composition of CDP I is not known.
[a] Mass estimated by gel filtration column chromatography (Mykles and Skinner 1986).
[b] Mass estimated by SDS-polyacrylamide gel electrophoresis (Beyette et al. 1993, 1997, Beyette and Mykles 1997, Yu and Mykles 2003). A 62 kDa isoform of Ha-CalpM is expressed in claw muscle, and a 68 kDa isoform is expressed in abdominal muscle (Yu and Mykles 2003).
Modified from Kim et al. (2005*a*).

and Esser 2010). cDNAs encoding the crustacean ortholog have been cloned from *G. lateralis, H. americanus, C. maenas, Eriocheir sinensis, Litopenaeus vannamei, Penaeus monodon*, and *Pandalopsis japonica* (Covi et al. 2008, Cho et al. 2009, Kim et al. 2009b, 2010, MacLea et al. 2010, De Santis et al. 2011, Qian et al. 2013). In *G. lateralis*, Gl-Mstn mRNA is reduced in both the claw closer and thoracic muscles during premolt (Covi et al. 2010). However, the effect of molting is greater in the claw muscle. By late premolt, Gl-Mstn mRNA in the claw muscle decreases by 81% (approximately fivefold) and 94% (approximately 17-fold) in animals induced by eyestalk ablation (ESA) and by multiple leg autotomy (MLA), respectively, and is negatively correlated with ecdysteroids (Covi et al. 2010). Gl-Mstn mRNA in thoracic muscle decreases by 68% (approximately threefold) and 82% (approximately fivefold) in ESA and MLA animals, respectively, and is only weakly correlated with ecdysteroid (Covi et al. 2010). In *L. vannamei*, the effects of 20-hydroxyecdysone (20E) injection on *Lv-Mstn* expression differed between muscles: 20E decreased mRNA levels in abdominal and pleopod muscles, increased mRNA levels in pereopod muscle, and had no effect on mRNA levels in thoracic muscle (Qian et al. 2013). However, because the control injections consisted of an equivalent volume of saline rather than the ethanol vehicle, it is possible that the changes in *Lv-Mstn* mRNA were not strictly in response to 20E (Qian et al. 2013). In *H. americanus*, spontaneous molting results in a larger decrease (82%) in Ha-Mstn expression in crusher claw muscle than in cutter claw (51%) or deep abdominal (69%) muscles (MacLea et al. 2010). However, an acute increase in ecdysteroids caused by ESA has no effect on Ha-Mstn mRNA levels in the three muscles (MacLea et al. 2010). These data suggest that ecdysteroids stimulate protein synthesis by downregulating Mstn. The differential response of the claw and thoracic muscles to ecdysteroid may be due to differences in the expression of the ecdysteroid receptor (Gl-EcR and Gl-RXR isoforms; Kim et al. 2005a,b, Covi et al. 2010).

Ecdysteroids appear to activate mechanistic target of rapamycin (mTOR)-dependent protein synthesis. mTOR is a highly conserved protein kinase that stimulates translation by phosphorylating ribosomal S6 kinase (S6K) and 4EF-binding protein-1 (Schakman et al. 2009, McCarthy and Esser 2010, Goodman et al. 2011, Frost and Lang 2012). mTOR functions as a sensor that controls cellular growth. Its activity is regulated by nutrients, intracellular energy levels, hypoxia, stress, and growth factors (Laplante and Sabatini 2012). The stimulation of muscle protein synthesis by ecdysteroids is primarily at the translational level, as indicated by increases in ribosomal activity and global protein synthesis during premolt (Skinner 1968, El Haj et al. 1996, Covi et al. 2010). Elevated ecdysteroids have little effect on MHC and actin mRNA levels (Whiteley and El Haj 1997, El Haj 1999, Medler et al. 2005). Expression of Rheb (*R*as *h*omolog *e*nriched in *b*rain), an activator of mTOR, is increased nearly fourfold in claw muscles from premolt *G. lateralis*, and Gl-Rheb mRNA levels are positively correlated with hemolymph ecdysteroid levels (MacLea et al. 2012). The mRNA levels of other mTOR signaling components (mTOR, Akt, and S6K) are not correlated with hemolymph ecdysteroid titers (MacLea et al. 2012). These data indicate that mTOR is activated by Rheb, resulting in increased synthesis of cytosolic and myofibrillar proteins (Covi et al. 2010). Thus, Rheb expression may serve as a molecular marker for tissue growth in crustaceans.

Mstn may have pleiotropic functions in crustaceans. Mstn is expressed in a wide variety of tissues, which suggests its function is not restricted to controlling muscle growth (Covi et al. 2008, Kim et al. 2009b, 2010, MacLea et al. 2010, De Santis et al. 2011, Qian et al. 2013). Studies on shrimp (*P. monodon* and *L. vannamei*) illustrate the complex and potentially multimodal actions of Mstn in crustaceans (De Santis et al. 2011, Qian et al. 2013). The expression of Pm-Mstn in abdominal muscle varies during the molting cycle, although there is not a clear relationship between Pm-Mstn mRNA levels and periods of muscle growth. Pm-Mstn mRNA level is elevated immediately after ecdysis (postmolt stage A), when muscles grow in

response to stretching from the expansion of the new exoskeleton. Pm-Mstn expression is low at intermolt stage when abdominal muscle growth is complete. Expression of *Lv-Mstn* in "mixed muscle" samples exhibits the same pattern (Qian et al. 2013). This suggests that Mstn is a myotropic factor that stimulates muscle protein synthesis. An apparent contradiction is that Pm-Mstn mRNA levels are also elevated at premolt stages, when the abdominal muscle is not growing (De Santis et al. 2011). Moreover, abdominal muscles in decapods with elongated body plans (e.g., shrimp, lobsters, and crayfish) do not atrophy because the abdomen is easily withdrawn through the large opening created at the junction between the cephalothorax and abdomen at ecdysis (Chang and Mykles 2011). However, ribosomal activity and protein synthesis are increased in lobster abdominal muscles during premolt (Whiteley and El Haj 1997). If protein synthesis is similarly elevated in premolt shrimp abdominal muscle, the Pm-Mstn expression pattern over the molting cycle is consistent with its function as a stimulatory growth factor. This is supported by knockdown experiments, in which injections of Pm-Mstn ds-RNA into the abdominal muscle significantly reduced shrimp growth over a 45-day period (De Santis et al. 2011). Interpretation of these results should be tempered by possible systemic effects because the Pm-Mstn knockdown was not restricted to the abdominal muscle. Pleopod muscle harvested at 7 days had a similar decrease (~40%) in Pm-Mstn mRNA as abdominal muscle harvested at 45 days, indicating the effect had spread to other regions of the abdomen. Other tissues with high levels of Pm-Mstn expression, such as heart, gill, eyestalk, and stomach, were not included in the qPCR analysis (De Santis et al. 2011). An alternative explanation is that Pm-Mstn knockdown inhibited molting by decreasing the ecdysteroidogenic activity of the molting glands (Y-organs) because Gl-Mstn is highly expressed in the Y-organ (Mudron and Mykles, data not shown). A reduction in molting frequency would explain the lower growth rate of Pm-Mstn ds-RNA-injected animals. Unfortunately, the molting data were not reported, thus leaving the matter unresolved (De Santis et al. 2011).

Little is known about how ecdysteroids regulate protein degradation in crustacean muscles. In *G. lateralis*, ESA causes a transient (1–3 days) increase in Gl-CalpT and Gl-EcR mRNAs in claw muscle, but not in thoracic muscle; ESA has no effect on Gl-CalpB and Gl-CalpM expression (Kim et al. 2005a). Interestingly, the expression of Gl-CalpT and Gl-EcR is correlated in both claw and thoracic muscles, suggesting the two genes are co-regulated (Kim et al. 2005a). However, the expression of ecdysteroid receptor genes (Gl-EcR and Gl-RXR) and Gl-CalpT is not correlated with ecdysteroid (Covi et al. 2010). In *H. americanus*, Ha-CalpM expression in the crusher claw is not affected by molt stage (Yu and Mykles 2003). These data suggest that sustained activation of calpains in atrophic muscle involves post-transcriptional mechanisms.

Atrophy in response to unweighting has a different effect on the expression of genes involved in protein synthesis and degradation. By contrast to the effects of molt induction in *G. lateralis*, Gl-Mstn mRNA increases threefold and Gl-CalpT mRNA decreases 40% in unweighted muscles with respect to weighted contralateral muscle (Covi et al. 2010). In premolt animals, the decrease in Gl-Mstn mRNA in unweighted muscle parallels the decrease in Gl-Mstn mRNA in weighted muscle, indicating that increased ecdysteroids supersede any effect that unloading has on Gl-Mstn expression. In other words, low ecdysteroids permit the upregulation of Mstn in unweighted thoracic muscle. Gl-Rheb and Gl-S6K are increased 2.2-fold and 1.3-fold, respectively, in unweighted thoracic muscle, indicating that mTOR-dependent protein synthesis is stimulated (MacLea et al. 2012). Unweighting has no effect on Gl-Akt, Gl-mTOR, Gl-EcR, and Gl-RXR mRNA levels (Covi et al. 2010, MacLea et al. 2012). These data, which are consistent with the positive relationship between Gl-Rheb and Gl-Mstn expression in weighted thoracic muscle (MacLea et al. 2012), indicate that Mstn stimulates protein synthesis in thoracic muscle.

FUTURE DIRECTIONS

Research should now turn toward elucidating the molecular control of fiber differentiation, phenotype, and size by neural activity and endocrine and paracrine factors. We know little about the molecular mechanisms regulating muscle differentiation during development, in particular the roles of transcription factors that specify skeletal muscle in other organisms (e.g., Mef2, Myf4, Myf5, MyoD, and myogenin in vertebrates) and how neuronal activity alters myofibrillar gene expression. The control of protein turnover is central to muscle plasticity. Fiber transformation requires the coordinated expression of fiber type-specific myofibrillar protein isoforms, which must be assembled and incorporated into the myofibrils while myofilaments containing protein isoforms of the former fiber type are removed and degraded. The relative rates of protein synthesis and degradation determine whether a muscle grows or atrophies. mTOR probably plays a critical role because increased protein synthesis is associated with skeletal muscle growth and remodeling in diverse organisms. In mammals, Mstn controls muscle growth by suppressing mTOR activity and activating Ub/proteasome-dependent degradation (Schakman et al. 2009, McCarthy and Esser 2010, Goodman et al. 2011, Frost and Lang 2012). The function of Mstn in crustacean muscle is not fully understood. Mstn appears to be a stimulator of protein synthesis in abdominal muscles in *P. monodon* and unweighted thoracic muscle in *G. lateralis* (Covi et al. 2010, De Santis et al. 2011, MacLea et al. 2012). In *G. lateralis* claw muscles, the downregulation of Gl-Mstn and the upregulation of Gl-Rheb indicate that Mstn inhibits mTOR-mediated protein synthesis (Covi et al. 2010, MacLea et al. 2012). Thus, muscles in the same species differ in the relationship between Gl-Mstn and Gl-Rheb expression. In claw muscle, Gl-Rheb and Gl-Mstn mRNAs are negatively correlated in animals induced to molt by ESA but not in animals induced to molt by MLA (MacLea et al. 2012). By contrast, Gl-Rheb and Gl-Mstn mRNAs are positively correlated in weighted thoracic muscle from both ESA and MLA animals (MacLea et al. 2012). The differences in the relationship between Gl-Mstn and Gl-Rheb expression may be due to differences in the responses of the two muscles to ecdysteroids (Covi et al. 2010). Gl-Mstn and Gl-Rheb mRNA levels in claw muscles are significantly correlated with hemolymph ecdysteroid titers, whereas there is no correlation between Gl-Mstn and Gl-Rheb mRNA levels and hemolymph ecdysteroid titers in thoracic muscles (MacLea et al. 2012). The next step is to gain a mechanistic understanding of the complex interactions between endocrine/paracrine factors and signaling pathways controlling protein turnover.

CONCLUSIONS

Relatively little is known about the early stages of crustacean myogenesis, but the available data suggest a process similar to that in insects. Muscle precursor cells migrate to specific locations in the embryo and then differentiate into fibers. Muscle fibers with motor nerves are in place at relatively early stages of development, and subsequent growth over several molt cycles produces adult muscles. The genes that control muscle differentiation remain to be studied. Molting stimulates the growth of muscle fibers, which increase in length by the addition of sarcomeres and/or lengthening of existing sarcomeres and in diameter by sarcomere splitting and/or increases in myofibril cross-sectional area. As in mammals, crustacean skeletal muscle is a dynamic tissue that can undergo dramatic changes in mass and contractile properties. Molecular mechanisms controlling the changes in fiber composition by cell death and fiber transformation are complex and probably involve a combination of neurotrophic, endocrine, and paracrine factors, which have yet to be identified. Changes in fiber composition are coordinated with changes in claw morphology, but they can, on rare occasions, be unlinked. In "double-crusher" lobsters, the closer muscle in the "false" crusher has the cutter claw fiber composition. Also, fiber transformation is completed before the

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adult claw morphology is attained. Because both fiber transformation and molt-induced atrophy involve extensive remodeling of the sarcomeric structure, it is likely that both processes require an increase in protein turnover. The main difference, however, is that there is a net loss of protein in muscle atrophy, whereas there is no net loss or net gain in protein in transforming fibers. LIM proteins may play a role in both processes (Postel et al. 2010). Calpains degrade myofibrillar proteins, but we know little about how their activities are regulated by ecdysteroids. Atrophy of unweighted thoracic muscle is ecdysteroid-independent and is associated with modest increases in the expression of Gl-Mstn, Gl-Rheb, and Gl-S6K and decreases in the expression of Gl-CalT (Covi et al. 2010, MacLea et al. 2012). The stimulation of muscle protein synthesis by ecdysteroids in molt-induced claw muscle atrophy involves both Mstn and mTOR signaling pathways (Covi et al. 2010, MacLea et al. 2012).

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