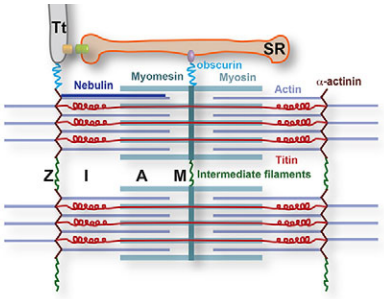
INSIDE JEB

Muscle revisited

Muscle is one of the most ubiquitous tissues on the planet. From the tiniest insects to colossal blue whales, the vast majority of most organisms' movements are directly powered by the relentless motion of ATP-consuming myosin heads on thick myosin filaments shuffling along thin actin filaments. This so-called sliding filament explanation for muscle contraction, where actin and myosin filaments slide past each other, was first proposed independently in two papers published in *Nature* in 1954, by Hugh Huxley and Andrew Huxley in collaboration with co-workers from their respective laboratories. JEB Editor-in-Chief Hans Hoppeler says, 'At that time, muscle was viewed as tissue that can contract, but beyond that there wasn't much interest in muscle and many physiologists considered it to be a rather dull tissue'. However, all of that was set to change in the early 1990s. With the advent of the molecular revolution, Hoppeler recalls that it became possible to explore the mechanisms that underlie the tissue's malleability, allowing scientists to begin dissecting out at last the fine details of the underlying molecular architecture and signalling pathways that support muscular contraction and plasticity.

In the intervening decades, our knowledge has flourished into an understanding of the hundreds of associated regulatory and cytoskeletal proteins that are responsible for the unique structure and plasticity of muscle, which can be fine-tuned to a wide range of movements and remodelled in response to exercise and diet. With over 40 years of experience in the field of muscle plasticity, Hoppeler observes, 'Muscle doesn't only consist of actin and myosin, and I felt an update in the various areas of research was overdue'. Inviting colleagues from many different branches of muscle research to contribute to this specially commissioned issue of *Journal of Experimental Biology*, Hoppeler has assembled reviews discussing the molecular architecture and assembly of the structures that are fundamental to muscle function through to our current understanding of fuel use and how muscle powers locomotion.

Muscle cytoskeleton



A schematic view of the sarcomeric cytoskeleton.

While the basic mechanics of muscle contraction have been understood for more than 60 years, the fine details of the arrangement of the cytoskeletal infrastructure that correctly positions the actin and myosin contractile elements in the sarcomere (the contractile unit of striated muscle) have only recently begun to divulge their secrets. In their review, Mathias Gautel from Kings College London, UK and Kristina Djinović-Carugo from the University of Vienna, Austria, discuss the assembly and relative architecture of the proteins that construct the sarcomere's Z disk – which define the ends of the sarcomere – and M band – the middle of the sarcomere (pp. 135-145). They explain that myofibril assembly begins at the Z disk and that titin – the giant protein that is linked to the myosin filaments – is also anchored to the Z disk, adding that the Z disk also caps the extension of actin filaments, preventing them from extending into the neighbouring sarcomere.

Regulation of actin length has been a major mystery that has puzzled the muscle physiology community for decades, as efficient contraction is dependent on the precise regulation of the length of actin myosin filaments. One candidate for the role of molecular ruler was the muscle protein nebulin, which ranges in size from 34 kDa up to a colossal 900 kDa. However, Miensheng Chu, Carol Gregorio and Christopher Pappas, from The University of Arizona, USA, explain that it is becoming clear that instead of functioning as a molecular ruler that specifies the length of contractile actin filaments, nebulin stabilises the filaments, allowing them to assemble to the correct length (pp. 146-152). In addition, the trio lists other cellular functions in which nebulin has been implicated, including sarcoplasmic reticulum calcium handling, Z-disk integrity, force generation and directing actomyosin interactions. Chu, Gregario and Pappas say, 'Nebulin is not merely a structural protein, but contributes to proper muscle function', and they add, 'nebulin is clearly a multifunctional protein with additional functions likely to be discovered'.

Moving on from the regulation of actin filament length, Angela Bagni, Barbara Colombini and Marta Nocella from the University of Florence, Italy, discuss muscle fibre stiffness and the role of the giant muscle sarcomere protein, titin, in muscle fibre static stiffness (pp. 153-160). After describing how the phenomenon was first identified in intact frog muscle, Bagni and colleagues then list the characteristics of the static stiffness, pointing out that it is a property of the intrinsic structure of muscle and is unrelated to myosin–actin cross-bridge formation. They also describe how the time course of static stiffness is similar to the time course of calcium release from the sarcoplasmic reticulum in the muscle. Knowing that the stiffness of spring regions in one isoform of titin (N2A) increases in the presence of calcium, Bagni's laboratory measured static stiffness in muscles with different calcium release profiles and contractile properties, confirming that static stiffness is an intrinsic property of the tissue, unrelated to the stiffness of the myosin cross-bridge. Proposing two possible calcium-mediated mechanisms by which titin could be stiffened to produce static stiffness, the team also suggests that stiffening could be essential to stabilising the organisation of myofibrils in the sarcomere during the

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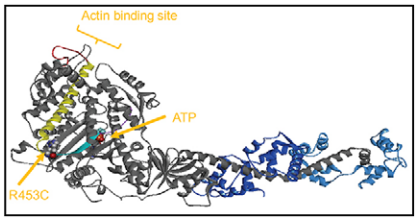
early stages of contraction before activation is synchronised across sarcomeres.

Mechanisms of muscle contraction and excitation–contraction coupling



Beta-cardiac myosin motor domain.

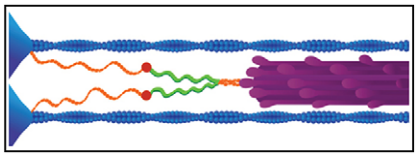
Myosin motors are ubiquitous throughout eukaryotic cells, performing myriad different roles from transport to cell division, and the group of myosin molecules that are produced in muscle tissues are no less diverse. 'It is interesting to know what details of the structure and the function of these motors has been specifically modified by nature to accommodate certain contractile functional qualities', says Hoppeler.

One myosin protein that shows a high degree of variation in the head domain of the protein is human β-cardiac myosin, which is implicated in 35% of cases of the inherited cardiovascular disease hypertrophic cardiomyopathy, which causes thickening of the heart muscle and hypercontractility. James Spudich and colleagues, from Stanford University School of Medicine, USA, discuss how the myosin lever arm amplifies allosteric movements in the myosin head motor domain to drive contraction, and also report that many of the mutations associated with the disease can change the time taken for a myosin head to undergo a complete cycle of ATP hydrolysis (pp. 161-167). The mutations can also produce small changes in intrinsic force or the time that the myosin is in a strongly bound state to actin. The team suggests that the mutations in one arginine-rich region on the surface of the myosin motor domain could account for the hypercontractility associated with this heart disease. These mutations could reduce the affinity of the patch of surface amino acids for proteins that usually detain the myosin head from interacting with actin during a contraction, and Spudich suggests that a loss of binding could result in an increase in the availability of myosin molecules that could actively contribute to contraction, resulting in hypercontractility.

Following on from Spudich's review of the role of myosin in heart disease, Michael Geeves, Jonathan Walklate and Zoltan Ujfalusi, from the University of Kent, UK, discuss how each of the 13 myosin II molecules that can be expressed in different mammalian muscle types differ from each other (pp. 168-174). The sarcomeric isoforms are all remarkably similar (>80% sequence identity) while the non-muscle and smooth muscle forms are more dissimilar (40% identical to sarcomeric myosin). Geeves and colleagues then review how the kinetics and velocity of the ATPase–actin binding cycle differ across mammalian muscle myosins. They say, 'Slow myosin has… been adapted for efficient tension holding and slow contraction velocities, while the fast muscle myosins are adapted for high speed and large power generation'. Considering the myosins that are expressed in human muscle tissue, Geeves and co-authors explain that although the sequence differences between the myosins are known, it is hard to predict how these differences will alter the protein's function. They then focus on one myosin mutation that has been implicated in the heart disease hypertrophic cardiomyopathy, and explain how the simple replacement of an arginine amino acid by a cysteine slows the hydrolysis of ATP sufficiently to significantly affect the heart's function during exercise.

In the concluding article in the section focusing on the contraction mechanism, Roger Bannister from the University of Colorado, USA, reviews our current understanding of the process of excitation–contraction coupling, where an electrical impulse from a nerve is transmitted by the L-type Ca^{2+} channel ($Ca_v1.1$), located in invaginations of the muscle membrane within the core of the muscle fibre, to the ryanodine receptor (RyR1), 10 nm away in the membrane of the sarcoplasmic reticulum, to release calcium into the muscle fibre and trigger a muscular contraction (pp. 175-182). Having discussed the functional roles of various domains of the $Ca_v1.1$ protein in excitation–contraction coupling and the correct location of the channel in the plasma membrane–sarcoplasmic reticulum junction, Bannister then explains how recent improvements in our understanding of the structure of the channel have helped us to better understand its function. Bannister then describes how the β_{1a} subunit of $Ca_v1.1$ is essential for communication with the ryanodine receptor, before discussing the Stac3 protein, which is also a component of the channel and was recently identified in the muscle of mutant zebrafish larvae. However, he points out that it is currently unclear whether Stac3 is the elusive intermediary protein that directly links $Ca_v1.1$ to the ryanodine receptor or is essential for the correct location of $Ca_v1.1$ in the muscle membrane.

Eccentric contractions



Schematic diagram of skeletal muscle sarcomeres.

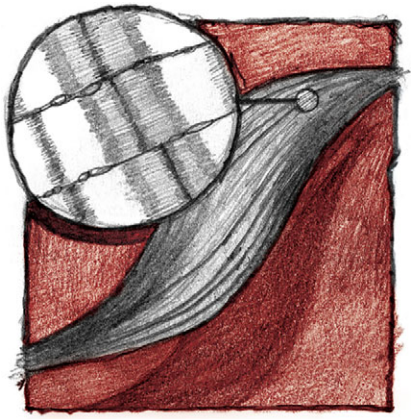
Muscles are remarkably adaptable, adjusting their contraction properties to suit the task in hand, from shortening (concentric) contractions, which drive the movement of limbs, to eccentric contractions – where muscles lengthen while the muscle fibres are activated – which perform as shock absorbers and result in smooth motion. Stan Lindstedt, from Northern Arizona University, USA, reviews the history of research into muscle physiology, from the ancient Greek concept of the 'pneuma' fluid flooding muscle to produce shortening, to A. V. Hill's work, showing that the maximum force is generated when there is no muscle shortening, whereas muscles contract at their maximum velocity when shortening occurs at zero force (pp. 183-188). He then goes on to discuss how Wallace Fenn characterised the lengthening contractions that later became known as eccentric contractions, which require less energy to perform than isometric contractions (where the length remains the same) when doing the same amount of work. Moving on, Lindstedt discusses the stabilising role of eccentric contraction

in normal movement before considering recent work showing how eccentric exercise can increase muscle strength in elderly patients that are unable to perform other forms of exercise. Lindstedt explains that during eccentric contractions, muscle fibres behave as if there is an elastic element combined with the sliding filaments, and it was only after the discovery of the giant elastic muscle protein titin that it was suggested that the novel molecule could function like a bungee cord to store and recover elastic recoil energy.

Continuing on the theme of the role of titin in eccentric contraction, Kiisa Nishikawa, from Northern Arizona University, USA, adds that muscle force continues increasing after the muscle has ceased stretching during these lengthening contractions. Nishikawa outlines several possible mechanisms that could explain how titin functions as a structural element to enhance force production during muscle activation, and proposes a role for the elastic protein where it winds around thin actin filaments as the myosin cross-bridges translate and rotate the actin filament (pp. 189-196). She then explains how this mechanism could account for the low-cost residual force production that is characteristic of eccentric contractions, as stretched elastic titin stores energy and produces force with no additional energy consumption.

Not only are eccentric contractions more energetically efficient than shortening contractions, but the control strategy employed by the nervous system during eccentric contractions also differs from that used when muscles shorten, explain Jacques Duchateau, from the Université Libre de Bruxelles, Belgium, and Roger Enoka, from the University of Colorado, USA (pp. 197-204). Fewer motor units are recruited during lengthening contractions performed with a similar load, and the researchers explain that 'the discharge rate is systemically less during lengthening actions'. Regarding the mechanisms that control eccentric contractions, Duchateau and Enoka say, 'The neural drive discharged by the spinal cord is less for lengthening than for shortening contractions', and conclude, 'the central nervous system has a specific activation strategy for lengthening contractions'.

Muscle plasticity



Having discussed the architecture and contraction function of muscle in molecular detail, the review collection now moves on to consider muscle plasticity in response to a wide range of factors, from exercise and diet to disuse in hibernation and the factors that permit muscle plasticity.

Hans Hoppeler, from the University of Bern, Switzerland, initially outlines the many influences that contribute to muscle malleability, including strength and endurance training, which can bring about changes in the contractile machinery and the delivery and consumption of oxygen (pp. 205-213). He then provides an overview of the molecular mechanisms that bring about change in muscle. After detailing the physical stressors that are activated during exercise – mechanical loading, neuronal activation, hormonal adjustments and metabolic disturbances – Hoppeler discusses the molecular signalling pathways that are activated to control gene expression and protein degradation during muscle remodelling in response to endurance training. These factors include the PGC1α transcription factor, which regulates mitochondrial and capillary growth; AMP-activated protein kinase, which senses energy consumption; and hypoxia inducible factor, which regulates the equilibrium of oxygen the tissue. In contrast, strength training activates the mTORC1 protein complex, which regulates protein synthesis and, ultimately, the increase in muscle mass. Hoppeler concludes by reviewing the factors – such as inflammation factors, glucocorticoid hormones and hypoxia – that are key when muscle is lost in response to inactivity and disease.

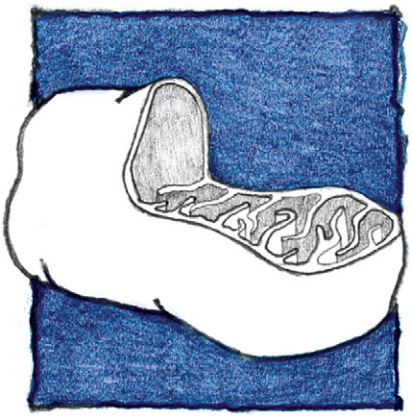
Expanding on the theme of exercise effects on muscle plasticity, John Hawley, based in the UK at Liverpool John Moores University and at the Australian Catholic University, writing with William Smiles and Donny Camera, from the Australian Catholic University, discuss the effects that different energy sources (fat and protein) have on muscle remodelling when undertaking strength and endurance training (pp. 214-225). The team report that a high-protein diet, either in the form of good quality meat or a mixture of dairy and soy, in association with resistance exercise significantly increases muscle mass as a result of increased access to essential amino acids through the activation of the mTOR1 protein complex. However, it is less clear whether fat consumed as part of a training regime has a positive or detrimental effect on muscle accumulation. Considering the impact of protein ingested after endurance training by athletes, Hawley and colleagues suggest that the rise in muscle protein synthesis may counter the increase in protein degradation that accompanies the transition to predominantly oxidative metabolism associated with training. The scientists say, 'We now have a better understanding of the multiplicity and complexity of the pathways involved in these exercise responses', although they add, 'connecting distinct signalling cascades to defined metabolic responses and specific changes in gene and protein expression in skeletal muscle that occur after exercise is complicated'.

Athletes also know that retraining a muscle requires less effort than the initial period of training. In his review, Kristian Gundersen, from the University of Oslo, Norway, describes a form of cellular memory that enables humans that have previously performed strength exercise to rebuild muscle mass and strength more quickly after extended periods of inactivity (pp. 235-242). Gundersen explains that additional nuclei are recruited to muscle from satellite cells during exercise training, and suggests that these nuclei are retained when training ceases and muscle fibres atrophy, allowing the muscle to grow more rapidly when exercise is resumed. 'Thus the nuclei represent a functionally important "memory" of previous strength', says Gundersen, adding that the nuclei could be retained for at least 15 years. He also recommends early strength training as a preventative measure against muscle

deterioration in old age, when recruitment of muscle nuclei declines.

While sportsmen and -women are intent on modifying muscle to enhance performance, hibernating animals must simply maintain the muscle status quo under conditions that normally result in extensive wastage. Reviewing the literature, Clark Cotton, from College of St Benedict/St John's University, USA, says, 'Losses of muscle mass, protein and fibre size are typically small during hibernation', adding that the muscle of hibernators also retains strength (pp. 226-234). In addition, the increase in proportion of fast-twitch glycolytic fibres that is normally found in immobile animals does not occur in torpid animals. Cotton then goes on to discuss the mechanisms that could protect hibernators from muscle disuse atrophy, such as the depression of protein synthesis and degradation via various metabolic pathways. He also reviews two possible mechanisms for the maintenance of muscle fibre type ratio, one activated by intracellular calcium that increases when the animal is shivering during arousal from torpor, and the second triggered by circulating free fatty acids that are associated with fasting and exposure to cold temperatures.

Metabolism and tissue cross-talk

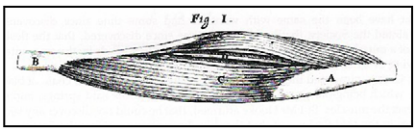


Muscle function is clearly defined by its molecular structure and the ability to adapt in response to use; however, muscle performance is also tightly correlated with the ATP fuel supply, which is generated by mitochondria in the tissue. By oxidising the products of glucose and lipids – pyruvate and NADH – mitochondria generate the energy-rich molecule ATP, which fuels all muscle contractions. However, Kevin Conley, from the University of Washington Medical Center, USA, says, 'Less clear is the direct role that mitochondria play in setting the limits to exercise performance' (pp. 243-249). Conley explains that exercise performance can be improved either by increasing the mitochondrial content of muscle or by increasing the ATP output of the organelles, and then goes on to present the three main processes that can affect mitochondrial ATP production: the electron transport chain, which generates a proton gradient across the inner mitochondrial membrane; ADP phosphorylation, which is driven by the proton gradient to produce ATP; and proton leak, which dissipates the proton gradient to reduce ATP production. Then, Conley lists points in the ATP cycle where production can be augmented, and says, 'the steps in oxidative phosphorylation… are not fixed, but remarkably dynamic'. He also points out that it is possible to specifically target points in the ATP production cycle to augment ATP production and ultimately improve muscle function.

There are also a wide range of fuels available to drive ATP production for muscle contraction, and Jean-Michel Weber, from the University of Ottawa, Canada, explains that muscle performance is highly dependent on fuel supply and the removal of waste products. Focusing on fuel use by fish muscle, Weber explains that anaerobic white muscle is able to function as an almost entirely self-contained system by recycling the lactate that is produced by anaerobic exercise and converting it back into glycogen, which can be reused as fuel later (pp. 250-258). Fish also use lipids that are transported in the form of lipoproteins – in contrast to the albumin-bound fatty acids transported in mammals – from lipid stores to fuel muscle during prolonged exertion. Weber also suggests that adult fish may use protein as a fuel source, citing the example of migrating adult salmon returning to their spawning grounds, but adds, 'the exact contribution is yet to be determined'.

In addition to its role in generating movement, muscle was recently discovered to produce signalling molecules during exercise, know as myokines, which have been shown to improve physiological energy homeostasis (regulation). Mark Febbraio, Lena Cron and Tamara Allen, from the Baker IDI Heart and Diabetes Institute, Australia, explain that the interleukin-6 family of myokines are produced by muscle during exercise. Two members of the family have recently been shown to have beneficial metabolic effects, with IL-6 breaking down fat stores, while CNTF (ciliary neurotrophic factor) increases fat consumption in muscle (pp. 259-265). However, Febbraio and colleagues point out that neither myokine is likely to prove clinically effective in the treatment of obesity and type 2 diabetes because of side effects, although he is optimistic that protein mimics based on both myokines could prove effective in weight loss.

Muscle in motion



One of the first drawings illustrating a uni-pennate muscle, attributed to Christopher Wren. Photo credit: Wellcome Library.

Although muscle powers movement, it does not work in isolation. Muscles apply forces at joints that are amplified by skeletal levers and are also connected in-line to other tissues with springy tendons, which store energy that is released explosively when the tension is relaxed. Tendons can also function as shock absorbers that allow energy to be absorbed rapidly and dissipated or recycled later. Thomas Roberts, from Brown University, USA, says, 'It is difficult, if not impossible, to isolate muscle performance from the influence of elastic structures'.

Outlining the roles of tendons in fast activities, such as jumping, Roberts says that tendons absorb energy when an animal lands, although it is not clear whether tendons increase energy efficiency by recycling energy during running (pp. 266-275). He adds that the giant protein titin has the potential to store significant amounts of energy, although its contribution to elastic energy storage is currently unclear, as there is some intrinsic elasticity in actomyosin cross-bridges, which may contribute to muscle elasticity. Despite these potential sources of muscle elasticity, Roberts shows that tendon is a far more powerful elastic tissue, returning 85–95% of the energy stored, compared with 40% in muscle. He says, 'While the functions provided by

muscle elasticity are not yet established, we can be certain that these springs have yet-undiscovered roles'.

Marco Narici and colleagues, from the University of Nottingham and Liverpool John Moores University, UK, also explain that muscle architecture contributes to the force produced by the tissue during movement (pp. 276-284). The fibres in many muscles are inclined at an angle relative to the tendon to which the muscle attaches – the pennation angle. This arrangement makes it possible to pack more muscle fibres into these so-called pennate muscles than when the fibres run parallel to the direction in which the muscle pulls, and increases the force that can be produced by the muscle. However, the pennation angle declines rapidly when the muscle is not in use for as little as 1 week, and Narici also calculates that a reduction of fascicle (muscle fibre bundle) length of as little as 9% can result in the loss of 2890 muscle sarcomeres along the length of a muscle fibre over the same time. In addition, Narici warns that muscle function deteriorates dramatically with age, with a reduction in fibre size and number causing a decrease in muscle strength and contraction velocity of as much as 50% in the elderly. However, he explains that it is possible to minimise muscle loss through exercise, as eccentric (lengthening) contractions extend the length of muscle fibres, and concentric (shortening) contractions produce thicker fibres, so muscle growth direction can be influenced by the choice of strength training regime.

Concluding this special issue, Andrew Biewener, from Harvard University, USA, reminds us that although muscle architecture is highly conserved at a molecular level, differences in larger-scale architecture, fibre type and activation timing can greatly alter force production as well as the muscle's energetic cost (pp. 285-294). Biewener reviews recent studies of muscle use in flight and terrestrial locomotion, and argues that the large muscles – which have little or no tendon and long parallel muscle fibres – are directly modulated to power movement, while muscles further along the limb tend to have long tendons and pennate architecture to generate forces economically.

Closing remarks

Reflecting on the material covered, Hoppeler says, 'Using molecular biology we have gained much insight into the fine mechanics of the molecular events that lead to contraction, and we've learned that there are a good 1000 additional proteins that are essential for muscle function; in particular titin, which seems to be so important in muscle lengthening'. Hoppeler also says, 'I think the review collection is timely because all of the fields covered by muscle physiology have moved on massively over the last couple of years. It gives anyone who works in this area a series of short, targeted backgrounds that define where the field currently stands'. Looking to the future, he then challenges the next generation of muscle physiologists not to lose track of the physiology that underpins muscle function and plasticity as they probe ever deeper into the structure and function of this remarkable plastic tissue.

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