

The brains behind the brawn

Why does weight-training have such a profound effect on the human physique?
The answer lies in the flexibility of the genes that program muscle fibres

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SOME things never change, but muscles are not one of them. As the glistening torsos on the covers of body-building magazines so amply testify, the fibres that power the human skeleton are mutable.

Extremely mutable. Even without resorting to anabolic steroids, by lifting weights the proverbial seven-stone weakling can put on 50 per cent more muscle within a year or so. The human physique, you could be forgiven for believing, is anything but a prisoner of its genes.

Or is it? The mutability of muscles cuts both ways. Muscle atrophy is a serious problem not only for people who are bedridden or confined to wheelchairs, but also for astronauts living in the microgravity environment of space. When denied the opportunity to stretch or contract, a human postural muscle may lose up to 40 per cent of its mass within a few weeks. The message is simple. With admirable economy, muscles adjust their size and behaviour in response to mechanical stimulation—or, as in the case of life in space, the lack of it. Far from being free of genetic control, muscle fibres have a sophisticated yet flexible genetic program which instructs them to tailor themselves to the body's needs.

Deciphering this genetic program has consumed much of my working life. When I began studying skeletal muscles, some 25 years ago, biologists were well versed in the architecture of muscle fibres, but knew almost nothing about their molecular genetics. We could explain roughly how a muscle fibre contracts, but couldn't say what effect stimulating the fibre—or failing to stimulate it—would have on the activities of the genes stowed in its nuclei.

As a result, answers to some of the most fundamental questions seemed impossibly remote. To what extent was genetic make-up influencing the way muscles respond to exercise? When weightlifters, sprinters and marathon runners engaged in their different training routines, what was happening to their muscle fibres at a genetic level? Would we ever be able to use molecular genetics to "diagnose" athletic potential in young people, or to "reprogram" the muscle cells of bedridden people and astronauts to prevent atrophy?

Elusive answers

Today these questions continue to intrigue me, and while firm answers are still proving elusive, the past few years have witnessed some dramatic breakthroughs. In the mid-1980s, molecular biologists in several laboratories around the world began to isolate and clone the genes that encode the rich variety of proteins from which muscle fibres are constructed.

Many of these genes have since been identified and at least partially sequenced, allowing us to probe their responses to exercise. My colleagues and I, for instance, have begun to investigate what happens to certain muscle genes—in particular, a family of genes that encode a protein called myosin heavy chain—when muscle fibres are stimulated and stretched.

As the genetic secrets of muscle growth unfold, so the prospects for genetically manipulating muscle fibres improve. While this is certainly a good thing for people suffering from muscle wasting, it holds a host of potential problems for the world of sport—problems that could make steroid abuse passé. How many athletes would be able to resist injecting themselves with DNA that promised to "reprogram" their muscles to grow without the need for strenuous training and which was afterwards untraceable? Or a recombinant protein that promoted fatigue resistance in muscles?

Such concerns may be hypothetical now but the pace of present research suggests they are unlikely to remain so for much longer. Two years ago Jon Wolff and his colleagues at the University of Wisconsin surprised everyone when they discovered it was possible to transfer genes into the muscle cells of mice simply by injecting pure DNA into the muscle tissue. Previously, the assumption was that gene transfer could only be accomplished using a special vehicle, such as a retrovirus, to ferry the DNA into cells. Building on the American discovery, my team is now collaborating with Kay Davies and her colleagues at the University of Oxford and a team at Guy's Hospital in London to test gene transfer on people with Duchenne muscular dystrophy, the severest form of the inherited muscle-wasting disease.

The genetic defects that cause muscular dystrophy reside in a gene encoding the protein dystrophin. One snag is that, comprising some two million base pairs of DNA, the dystrophin gene is simply too large to transfer without modification. So in our tests we will be injecting a truncated version of the gene that has the potential to slow, but not halt, muscle degeneration. Experiments on mice suggest that this "minigene" is likely to be taken up by a few per cent of muscle fibres close to the site of injection—enough to "salvage" a few fibres. Muscles in the thigh and around the backbone are probably the most important ones to treat, at least initially.

Much of my team's work, however, focuses on a different muscle protein, the myosin heavy chain, a gargantuan molecule comprising 2000 or so amino acids (most large proteins contain no more than a few hundred amino acids).

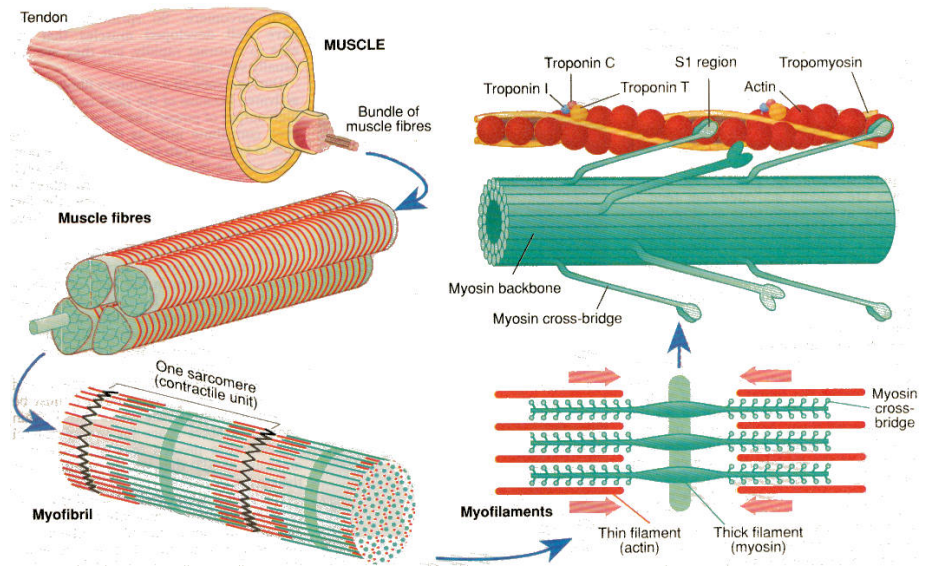
Like virtually all muscle research today, the conceptual roots of the studies lie in a series of electron microscope studies done in the 1950s which revealed the basic structure of muscle fibres and how they contract.

Muscle fibres consist of bundles of rod-like structures, about 1 micrometre in diameter, called myofibrils. Myofibrils are in turn made up of protein filaments arranged in units called sarcomeres (see "Protein Power" above). Each sarcomere consists of sheaths of thin filaments, made out of the protein actin, which slide, telescopically, over a thicker fibre made of the protein myosin. It is this sliding of a muscle's thin filaments over its thick filaments which causes it to contract. The process is triggered by calcium ions—which are released inside muscle cells in response to nerve impulses—and fuelled by ATP. If supplies of ATP run low, the muscle fibre tires.

Over the past two decades many more of the details have become clear. In particular, we now know that the central molecular player in the sliding process is the myosin heavy chain. Each myosin molecule is made of two entwined myosin heavy chains; at the end of each chain is a segment of protein known as the "head group". The myosin molecules coalesce to form the body of the thick filament, leaving short stretches of the protein attached to the head group jutting out like tiny side arms.

These arms, or cross-bridges, play a vital part in contraction. When a muscle contracts they act like banks of tiny oars pulling the sheaths of thin filaments over the thick filament. Exactly how the process works is not completely understood, but as each fibre contracts, the head groups at the ends of its cross-bridges appear to "walk" along the surfaces of actin filaments. The energy for the manoeuvre is supplied by ATP: at the end of a cycle, the cross-bridge detaches from the thin filament and has to be reprimed by ATP before it can go through another cycle of molecular walking.

All muscle fibres, whether they be in the powerful biceps of a weightlifter or the fatigue-resistant thighs of a marathon runner, contract by this same molecular cycle. What distinguishes different muscle fibres is the rate at which their cross-bridges cycle, and their capacity to sustain the cycle. By the mid-1970s, biochemical studies had revealed three main types of muscle fibres: so-called "fast" fibres programmed for fast contractions and powered by ATP produced by anaerobic metabolism; "slow" fibres pro-



Protein power

Muscle myofibrils are not only capable of contracting, but can switch their contractions on and off. How? The answer lies in a complex of regulatory proteins within myofibrils.

The myofibril's thin filaments are like double pearl necklaces that have been twisted into a spiral. Decorating this spiral are regulatory complexes made up of four proteins: tropomyosin and troponins I, T and C. These complexes adjust the way in which myofibrils respond to ATP and calcium.

The signal to contract is given by calcium ions, which flood into muscle fibres every time the fibres receive a nerve impulse. Some of these ions bind to the regulatory complexes, triggering their shape to change and tropomyosin to move. This shift exposes patches on the surface of the thin filaments with which cross-bridges can interact—pulling the thin filaments over the thick filaments and causing the sarcomere to shorten. The cross-bridges go through repeated cycles of activity until the local energy supply (ATP) runs down. The velocity with which muscle fibres contract correlates with the activity of an enzyme called myosin ATPase, part of the myosin heavy chain protein. The actin attachment site and the ATPase site are located in a fragment at the head of the myosin cross-bridge called "S1". Associated with the S1 fragment are smaller polypeptides or light chains which are believed to influence the cross-bridge cycling rate.

grammed for repetitive, longer-lasting contractions and powered by aerobic metabolism; and an intermediate type of muscle fibre programmed for contractions that are both relatively fast and long-lasting and which are powered by both anaerobic and aerobic metabolism.

Each muscle comprises a mixture of these fibres, the relative proportions depending broadly on what the muscle is used for and the type of exercise we take. For example, the "slow", fatigue-resistant fibres are particularly numerous in postural muscles such as the soleus, which is almost continuously active when we stand, walk and run. They are also found in abundance in the leg muscles of long-distance runners. A weightlifter, on the other hand, for whom strength, not stamina, is important, will develop biceps consisting mainly of fast-contracting fibres.

All of which takes us to the problems that have inspired our research on myosin genes. What makes some muscle fibres contract slowly and others rapidly? Can fibres

metamorphose from one type to another, as the dramatic changes in physique that accompany strenuous training suggest? And if so, how are the interconversions accomplished at the genetic level?

Many of the cellular differences between the main types of muscle fibres simply affect the availability of ATP. Slow muscle fibres, for instance, are invariably packed with mitochondria, the cellular organelles that "burn" oxygen to produce ATP; but not so fast-contracting fibres, which consume ATP produced anaerobically. Intermediate muscle fibres contain a higher density of mitochondria than fast muscle fibres, and so are capable of sustaining an output of high power over a longer period.

Fuelling filaments

Energy production, though, is not the only factor. Equally important is the efficiency with which a fibre uses ATP to fuel the sliding of its thin filaments over its thick filaments. We have recently found that the rate at which cross-bridges consume ATP and hence shunt thin filaments towards the centre of a sarcomere, varies considerably depending on the type of muscle fibre. The cross-bridges of fast muscle fibres cycle more rapidly, and hence consume more ATP than the cross-bridges of slow, postural muscle fibres.

The reason for these different responses lies in protein diversity. Many of the proteins from which muscle fibres are made exist in a variety of slightly different forms, or isoforms. Over the past decade or so researchers have accumulated much evidence showing that a muscle's designation as "fast" or "slow" depends critically on which protein isoforms it is synthesising—and in particular which isoforms of the myosin heavy chain are being produced. The myosin heavy chain is not only large, it is multitasked. In addition to furnishing muscles with cross-bridges, it also reacts with ATP harnessing the energy so released for contraction.

Mammals possess at least seven different versions of the gene that encodes the myosin heavy chain of skeletal muscles. In recent years molecular geneticists have cloned and at least partially sequenced these genes, an accomplishment which has enabled them to synthesise DNA probes that "light up" fibres in which the genes are active. A key finding has been that different members of the myosin gene family are "switched on", or expressed, at different stages in human development. The muscle cells of embryos, for instance, express an embryonic myosin heavy chain which differs slightly from that made in the muscles of newborn babies. This version of the protein in turn differs from those expressed in the muscle fibres of adults. Because the sequences of all these genes are still incomplete, we cannot yet pinpoint the whereabouts of all the structural variations in the myosin protein. However, we expect that most of them lie in the segment of the protein's head group that reacts with ATP as this is where structural variation is likely to have the greatest impact on myosin's contractile properties.

As researchers get to grips with the complex genetics of myosin, additional members of the gene family are coming to light. One team in Sydney, Australia, has discovered a special version of the myosin gene which is expressed only in the jaw muscles of cats and similar carnivores. The gene, dubbed "superfast", enables jaw muscles to generate force even faster than conventional fast fibres: a bonus for any animal that uses its jaws as an offensive weapon.

Exactly why human muscles synthesise different versions of the myosin heavy chain at different stages in development is not completely understood, but one can hazard some guesses. Floating in a sac of amniotic fluid, an embryo does very little work. So the puzzle is how its muscles manage to grow. One possibility is that the embryonic version of the myosin heavy chain helps to free muscle fibres from their dependency on stimulation for growth. Circumstantial evidence for this comes from a simple observation: when muscle fibres become damaged, their cells tend to revert to synthesising the embryonic version of the myosin protein, as though this will help them to repair the damage. Would switching on the "embryonic" member of the myosin gene family offer a way of protecting normal muscle fibres from atrophy? Nobody yet knows, but the idea is intriguing.

But we can be certain of one thing: the myosin gene family holds the key to muscle plasticity. With so many different versions of the myosin heavy chain to "choose" from, muscle fibres are inherently flexible. In theory, they can alter their contractile properties by rebuilding their myofibrils using a different type of myosin heavy chain. A fast muscle fibre, for instance, could turn into a slow fibre simply by switching off the gene for the fast myosin heavy chain and switching on the gene for the slow version of the protein.

Striking proof that this does indeed happen has come from a series of experiments done in my own laboratory. Most genes are switched on or off by the indirect actions of signalling molecules such as hormones or growth factors. These may bind to receptor molecules on the surfaces of cells, for instance, which when stimulated set in motion a chain of biochemical events that leads to the activation of a specific gene or family of genes. Muscle genes, by contrast, are regulated largely by mechanical stimuli. It is the stretching or contracting of a muscle fibre which turns specific genes on or off.

We have developed a way of monitoring the activities of myosin genes in slow and fast muscle fibres of rabbits. Our approach is to immobilise the muscle of interest—either a fast leg muscle (the *tibialis anterior*), or slow soleus muscle of the calf of the leg—in a plaster cast, then electrically stimulate it with a specially built microcircuit and then measure the activities of different myosin genes using DNA probes. By varying the frequency of the stimulation we can control the amount of force the muscle generates, while by altering the cast we can change the degree of stretch.

With the fast leg muscle, we found that stretch alone and electrical stimulation alone only mildly affected the activities

of myosin genes. Together, though, these stimuli produced a dramatic result. The fibres virtually shut down synthesis of the fast myosin heavy chain, switching almost exclusively to the slow version of the protein. We had succeeded in "reprogramming" the muscle fibres from the fast type to the slow type. Our experiments on the soleus muscle revealed something no less interesting. The fast myosin heavy chain is the "default" option. An immobilised soleus muscle reverts to expressing the fast myosin gene: it needs to be repeatedly stretched to sustain its synthesis of the slow myosin heavy chain. Both observations are consistent with what we know

about the effects of different training regimes on human muscles (see "Growing the best fibres" below).

Our work on animal muscles has also shed light on how fibres grow. One of the most contentious issues in muscle research has been whether strenuous exercise increases the number as well as the sizes of our muscle fibres. Frustratingly for body builders, it seems that the answer is no. Studies on rodents indicate that the total number of fibres is genetically determined. Athletes must learn to make the most of the muscle fibres they are born with.

2: Growing the best fibres

TRAINING can alter the contractile properties of a muscle in two ways. It can promote the interconversion of fibres—for example, fast fibres into slow fibres—or it can encourage the selective growth of a given fibre type.

Different types of exercise will encourage different fibres to grow. For sports such as cycling and marathon running, which require stamina not power, fast muscle fibres are seldom activated and tend to atrophy at the same time as slow fibres grow. The end result is muscles that contain a higher proportion of slow cross-bridges compared with fast cross-bridges and which are enriched in mitochondria, certain metabolic enzymes and blood capillaries. In most exercises, slow fibres are activated before fast fibres, because their motor neurons "fire" at a lower threshold.

Weightlifting is simply about generating force—so the bigger the muscles, the better. During the initial stages of weight training, muscle fibres expand to fill the space around them. As they grow into this "extracellular" space, there is no increase in the size of the muscle as a whole. Only later does it appear to expand.

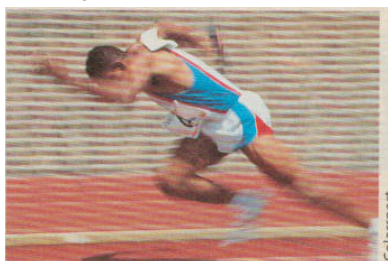
The maximum force that a single muscle can develop during a single contraction is governed by the total number of myosin cross-bridges that interact with the muscle's thin filaments. But providing the fibres are made up of more or less the same mix of myofibrils, one can safely predict the relative strengths of muscle fibres from their thickness.

Even in weightlifting, however, size isn't everything. There is evidence from electron microscopy studies that when untrained people attempt to exert their maximum force not all their muscle fibres are recruited. Trained weightlifters are able to activate about 30 per cent more of their muscle fibres.

What counts for sprinters is the total output of power. Power corresponds to the amount of work done (force x distance) per



Hidden strength: size isn't everything for weightlifters



Sprinters must avoid expressing 'slow' muscle genes

unit time, so the important parameters are the speed with which a muscle can contract and the amount of force it can produce. The higher and the more rapid the force generation, the greater the output of power.

The velocity with which a muscle can contract is, in part, determined by how many sarcomeres it possesses. Long-legged animals and humans have longer muscles and thus more sarcomeres in series. The snag with long limbs is that they suffer from the problem of inertia. The contraction velocity is also influenced by the number and type of cross-bridges within each sarcomere. So sprinters generally

have well developed muscles that are able to contract rapidly, but they are not usually very tall.

During a race all a sprinter's fibres will be activated because of the need to produce a high output of power. The slow fibres, however, contribute little as they will not be able to keep up with the rate of contraction: they will be moved passively by the skeleton. A high proportion of slow fibres in a muscle would tend to hamper the fast fibres, so good sprinters are likely to have a very high proportion of fast muscle fibres.

Slow genes only come into play after long bursts of physical activity. Sprinters should not engage in the kind of training used by weightlifters because overloading a muscle for any length of time causes it to express "slow" genes. Instead, they should rely on short bursts of very intense activity such as sprinting itself.

Long and middle distance runners need sustainable power. Their muscles must be fatigue resistant, so the fibres must be thin enough to leave space for the additional mitochondria needed to supply the vast amounts of ATP that will be needed over the course of a race.

The athlete who excels at stamina events is likely to have an unusually high percentage of slow-contracting fibres. Fibres with the slow myosin cross-bridges are much more efficient and economical for producing the slow repetitive contractions needed for distance running.

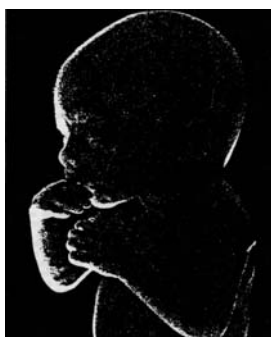
Slow type myosin cross-bridges consume ATP at more or less the same rate as mitochondria can supply it. Therefore, these muscles work in a steady state for most of the race except at the end when the athlete puts on a spurt and the muscles become anoxic.

In endurance events, where it is important to sustain power output, the training has to be directed towards increasing the percentage of "slow" contractile proteins, more mitochondria and improved vascularity. This is best achieved by putting in as many hours on the running track as possible.

Nevertheless, overloading muscles certainly does increase the thickness of their fibres. The thickening appears to occur in two main stages. First, myofibrils split longitudinally into two or more daughter myofibrils—an event triggered by a built-in "mismatch" between thin and thick filaments (see Figure 1). Then, each fibre boosts its synthesis of proteins such as actin, the myosin heavy chain and the proteins that regulate contraction.

Limits to growth

Just how dramatic this increase in protein synthesis can be was revealed in our study of the fast muscles of rodents. After four days of regular stimulation the amount of messenger RNA—production of which marks the first step in protein synthesis—in the fibres had increased by 250 per cent, and the muscle had grown by 35 per cent. The disparity between these figures is revealing: it suggests that what limits muscle growth is not the copying of genes into messenger RNA but the translation of messenger RNA molecules into protein.



Easy life: Embryos do very little mechanical work, so how do their muscles grow?

The myosin heavy chain is by no means the only muscle protein which differs in different types of fibre. Tropomyosin and a trio of related proteins called troponins I, C and T, which "sit" on the thin actin filament, regulating its interactions with myosin cross-bridges, also exist in a variety of isoforms—as do the proteins that pump calcium in and out of the membraneous channels, or sarcoplasmic reticulum, that surround myofibrils. Yet so central is the role of the myosin heavy chain that we believe the other protein isoforms serve only to fine-tune a muscle's contractile properties.

The task ahead is to unravel the mechanisms by which mechanical stimuli activate genes in muscle fibres. The rewards of doing so could be immense. If we could trace the biochemical steps leading to the switching on or off of muscle genes, the prospects of developing drugs that could artificially stimulate fibres to grow would improve dramatically. Present knowledge is fragmentary. Calcium and ATP undoubtedly play a crucial part in activating muscle genes, but are unlikely to function without molecular accomplices. Another key goal of muscle research is to determine the three-dimensional structures of the myosin and actin proteins, so that we can work out in detail how their amino-acids interact. A breakthrough came in 1990 when

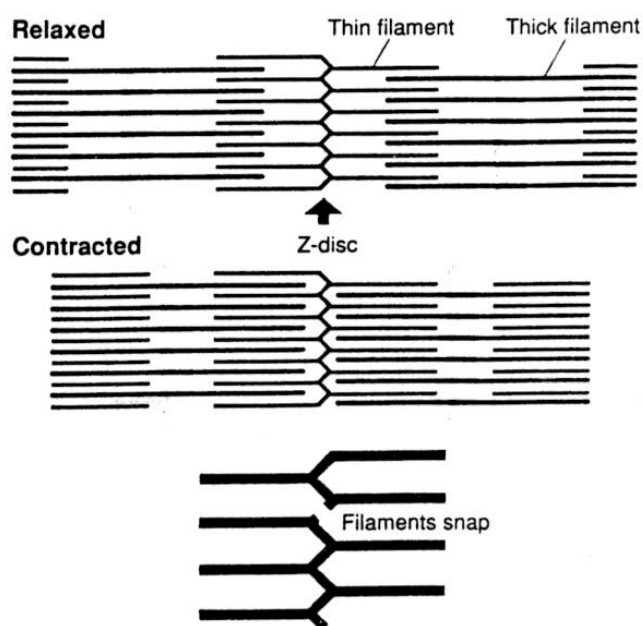


Figure 1
Muscle myofibrils contract by pulling their thin filaments over their thick filaments. As myofibrils grow fatter, their Z discs—where the thin filaments of neighbouring sarcomeres meet—come under increasing stress during contraction, and eventually snap. The snapping of consecutive Z-discs results in the myofibril splitting.

researchers at the Max-Planck Institute in Heidelberg used X-ray crystallography to probe the molecular architecture of actin.

Much work is now being done on sequencing the human genome. Once researchers have catalogued most, if not all, of the genes, studies of genetic differences between individuals will follow. Yet such research is unlikely to provide us with a simple way of diagnosing athletic potential, because athletic performance is the product of many disparate bodily systems. It depends as much on the brain and cardiovascular system as it does on having the right types of muscle fibres in the right place.

The prospects for using molecular biology to optimise training regimes are rather better. In theory, we could extend our work on animal muscles to human athletes, testing different exercise regimes to see which have the greatest impact on gene expression in muscle tissue. The main obstacle is no longer detecting gene activity but how to extract samples of specific types of muscle fibres from athletes.

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