MOLECULAR BIOLOGY

Titanic Protein Gives Muscles Structure and Bounce

An athlete with a biological bent may pause a moment while lifting weights or taking a jog to appreciate the molecular choreography that takes place when muscles contract: the ratchetlike sliding actions of filaments made of actin and myosin molecules. But few athletes are likely to know the identity of another key muscle protein, the molecule responsible for their muscles' ability to spring back into shape after being stretched. While this protein does not have the name recognition of actin and myosin, it does have its own claim to fame. It is the largest protein known, a single chain of nearly 27,000 amino acids, with a molecular weight of about 3 million. Few other proteins have molecular weights greater than 200,000.

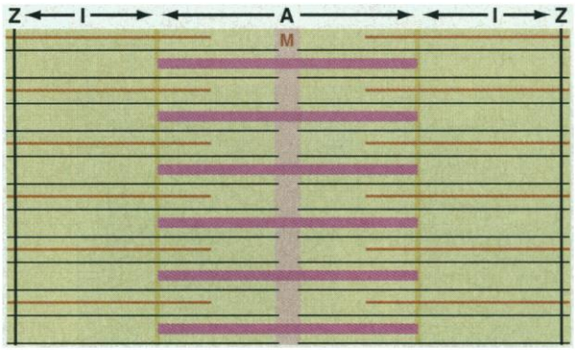
Fittingly, the muscle protein is called titin, and its size has made determining its entire sequence difficult. But on page 293 of this issue, molecular biologist Siegfried Labeit and his student Bernhard Kolmerer, of the European Molecular Biology Laboratory in Heidelberg, Germany, report that they have accomplished the feat.* And their achievement is drawing high praise. "This is really a fascinating work, not only because they were able to complete the sequence ... but also because of the information that was revealed," says Jeffrey Chamberlain, who studies muscle proteins at the University of Michigan Medical School in Ann Arbor.

Indeed, the sequence reveals a brand-new type of structural motif that may account for the protein's springiness. What's more, the length of this motif can vary, and that may explain why some muscles are more elastic than others. Other parts of the protein's sequence suggest how it may perform another key function, acting as a ruler to aid the precise placement of proteins within muscle fibers.

Labeit and Kolmerer determined titin's structure in the usual way, making DNA copies of the messenger RNA that directs titin's synthesis, sequencing that DNA, and converting that sequence into the amino acid sequence of the protein. Because of the huge size of the messenger RNA, they couldn't make a single copy, but had to piece the sequence together from roughly 50 overlapping copies.

Their efforts reveal that 90% of the titin protein consists of 244 copies of two well-known protein motifs, known as the fibronectin type III (FN3) and immunoglobulin (Ig) domains for the proteins in which they were first discovered. Researchers already knew from partial sequences of titin that these motifs were there, and even suspected that they might comprise virtually all of the protein. But Labeit and Kolmerer found, in the center of titin, a new protein motif that has never been seen before. They call it PEVK, from the symbols for the amino acids proline, glutamate, valine, and lysine, which make up 70% of the motif.

With titin's complete sequence in hand, the researchers could begin to develop a better picture of the roles titin plays in the structure and function of sarcomeres, the repeating units of which muscles are made. Microscopic studies in the 1980s had revealed that



Ratchets and springs. Each titin molecule *(black)* spans half a sarcomere, from Z line to M line. The sliding filaments of actin *(tan)* and myosin *(red)*, on the other hand, meet midway.

individual titin molecules span half the length of the sarcomere, from the Z line that marks the border between sarcomeres to the M line, which runs down the center of the sarcomere. Between these two endpoints, the stringlike titin molecules pass through two distinct zones of the sarcomere, the I band, dominated by filaments made of actin, and the A band, where the actin filaments overlap with others made of myosin.

Because of its size, a single titin molecule can span that entire 1-micron distance; the actin and myosin filaments by contrast are made of hundreds of individual molecules. Indeed, because titin spans the distance from Z to M, it seemed a good candidate for controlling the layout of sarcomere proteins, especially in the highly ordered A band. That idea has been supported by the current work. "Now that we have the whole sequence, we can see that the primary structure of the molecule very nicely matches the complicated structure of the [A band]," says John Trinick, who studies titin at the University of Bristol, United Kingdom.

For example, a set of accessory proteins is arranged in 11 stripes that cross the myosin-containing A band in each half-sarcomere. And titin's structure correlates with that arrangement. In the part of the protein that spans the 11-stripe region, the Ig and FN3 motifs, which have the ability to bind other proteins, are ordered in a "super-repeat," a regular pattern of Ig and FN3 motifs, repeated 11 times. The super-repeats had already been discovered in titin, but the new work shows that they line up with the protein stripes. It also reveals other structural patterns in titin that correspond to the placement of other A-band proteins. That buttresses the idea that, for laying down A-band proteins, titin "probably is like a great big template," says Trinick.

The structure may also shed light on how titin contributes to the ability of muscles to spring back after they are stretched. As muscles expand and contract, the sarcomere changes length, mostly in the I band. That, says Michigan's Chamberlain, means that the part of titin that crosses the I band "has to be extremely flexible, because it is going to be moving in and out like an accordion." Earlier experiments suggested, however, that titin behaves more like a spring, pulling the muscle back into shape after being stretched, than like the passive bellows of an accordion. Labeit and Kolmerer now propose that the PEVK sequence may in fact be the spring. For one thing, it is located in the middle of the expandable I band, where the spring would be expected to be.

And, more suggestive yet, when the researchers compared titins from cardiac and skeletal muscle, they found that the PEVK region was only 163 amino acids long in the stiff cardiac muscle, whereas in the much more elastic skeletal muscle it was more than 2000 amino acids long. "That correlates nicely with elasticity," says Trinick, and may "start to explain how different muscles have different stiffnesses."

That tantalizing correlation suggests experiments to test the springiness of PEVK directly. For example, says Labeit, researchers can dot the titin protein with antibodies specific for different parts of the protein, then by watching the spacing of the antibodies when the muscle is stretched, they can pinpoint the stretchiest part of the protein and see if it indeed contains PEVK. And while researchers pursue such questions, athletes can play with a new imagery, visualizing within their muscles not just tiny molecular ratchets, but titanic molecular springs as well.

–Marcia Barinaga

* Sequence information is available at **http://www.aaas.org/science/science.html** (see "Beyond the Printed Page").