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RESEARCH NEWS

Nebulin no longer nebulous

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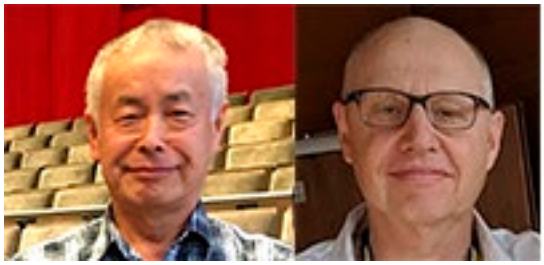
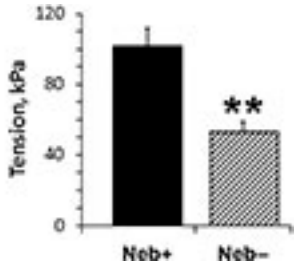
JGP study probes how nebulin affects muscle function.





Research by the laboratories of Masataka Kawai (left) and Henk Granzier (right) explored why nebulin is essential for generating high force levels in skeletal muscle (see graph).

Nemaline myopathy is a muscular disorder characterized by skeletal muscle weakness and difficulty swallowing and breathing, which are brought on by mutations in a protein called nebulin. Nebulin, a component of the contractile apparatus in skeletal muscles, is a giant protein (up to 900 kD in size), whose purpose is poorly understood. In a paper appearing this month in *JGP*, Kawai et al. explain what nebulin does in muscle fibers, and why it is so important in muscle function (1).

In muscle fibers, myosin-based thick filaments are arranged parallel to and partially overlapping with actin-based thin filaments. The heads of myosin molecules form cross-bridges by binding to the thin filament and use ATP hydrolysis to power a conformational change that pulls the thick filament along the thin filament, thereby shortening the muscle fiber to produce contraction. Nebulin, like other thin filament accessory proteins such as tropomyosin and troponin, winds around the strand of filamentous actin that makes up the core of the thin filament.

"The name 'nebulin' comes from the fact that when it was discovered, it was totally nebulous what its functions might be," says Henk Granzier, a professor at the University of Arizona.

"It has been generally assumed that it supports the thin filament. But nobody knew what it was really for," adds Matasaka Kawai, a professor at the University of Iowa. "Our interest was: What is it doing in muscle? Why do we need it?"

Nebulin has been difficult to study because its large size precludes its expression in cell culture. Genetically engineered mice that lack nebulin die soon after birth (2), but Granzier's group recently created a conditional knockout mouse model that lacks nebulin expression only in adult muscle (3). These mice exhibit deficits in skeletal muscle force generation similar to nemaline myopathy patients.

In hopes of gaining insights about the role of nebulin, Granzier sent soleus muscles obtained from wild type and conditional knockout mice to Kawai's laboratory. There, research assistant Tarek Karam dissected them into individual fibers, dissolved their cell membrane, and compared them. Initial studies showed that fibers lacking nebulin generated less tension and were less stiff than wild type, exhibiting a greater degree of extensibility. This makes muscle fibers more brittle, because nebulin-deficient muscle fibers rapidly deterioriate with repeated activations. Interestingly, a recently completed x-ray diffraction study by Granzier's group provides structural evidence for the importance of nebulin in thin filament stiffness (4). But reduced stiffness could only explain some, not all, of the fibers' impaired contraction, so the researchers continued their investigation.

"I have developed a technique called sinusoidal analysis with which we can characterize elementary steps of the cross-bridge cycle," explains Kawai. Using this approach, the researchers could study the steps of actomyosin cross-bridge formation, power stroke, and cross-bridge detachment by manipulating ambient levels of ATP or its hydrolysis products, and probe the rate constants governing these steps (5). Based on previous work, Granzier expected that nebulin might alter the number of active cross-bridges formed between actin and myosin. But that's not what the data showed.

"In contrast, our results suggest that there is an increase in the force per cross-bridge when nebulin is present," note Kawai and Granzier. This is reminiscent of the effect of another thin filament–associated protein, tropomyosin (6). They speculate that, like tropomyosin, nebulin may alter actin's conformation to favor interaction with myosin.

Together, these data highlight many ways in which nebulin affects muscle function. Now, Granzier is curious to see whether function can be restored by reconstituting nebulin to nebulin-deficient thin filaments, while Kawai is interested in nebulette, a counterpart of nebulin found in cardiac muscles.

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2. Bang, M.-L., et al. 2006. *J. Cell Biol.* 173:905–916.
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