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Titin isn't a sleeping giant

Photobleaching reveals that the backbone of muscle myofibrils is surprisingly mobile.

At 3.7 MDa, titin is the largest protein known to exist (1). This giant polypeptide, also known as connectin, forms the backbone of striated muscle myofibrils by assembling the thin (actin) and thick (myosin) filaments into periodic structural units called sarcomeres. One might expect such an enormous scaffold protein to be immobilized within myofibrils, but da Silva Lopes et al. reveal that sarcomeric titin is actually quite dynamic and able to exchange with soluble pools of the protein (2).

Titin's C terminus is integrated into the central "M-band" of sarcomeres, whereas its N terminus stretches out into the "Z-disc" at sarcomere edges (3). Studies of titin's function have been hampered by the protein's large size and complex splicing pattern. To circumvent these problems, Michael Gotthardt and colleagues at the Max Delbrück Center for Molecular Medicine in Berlin, Germany, generated knockin mice that express titin tagged at its C terminus with green fluorescent protein (GFP). "It's the largest known protein… and we made it larger!" Gotthardt laughs.

The knockin mice were viable, and fluorescently labeled titin was incorporated correctly into the sarcomeres of both cardiac and skeletal muscle (2). Gotthardt and colleagues, led by Katharina da Silva Lopes, isolated embryonic cardiomyocytes from these mice and analyzed titin's mobility by photobleaching its GFP tag. Surprisingly, the bleached titin was replaced by unbleached protein within 14 hours—longer than the few minutes it takes for other, smaller sarcomere components to exchange, but still much faster than the biochemical turnover of titin, which has a half-life of about 3 days (4). Titin fluorescence recovered at the same rate in the presence of the protein synthesis inhibitor cycloheximide, indicating that bleached titin is replaced by the movement of existing titin molecules rather than by newly synthesized protein.

"So even the backbone of the sarcomere is mobile, and the whole structure is in flux," says Gotthardt. Indeed, myofibrils need to be dynamic, he says, because "proteins go bad, especially ones that are under a lot of mechanical tension. If you want to maintain a healthy, working sarcomere, you need to replace the proteins that make it."

To learn more about titin's mobility, Gotthardt and colleagues compared the protein's ability to move in different directions. Based on titin's organization along myofilaments, the team reasoned that, if titin always retains some connection to other structural proteins, it would take longer to move longitudinally than it would take to shift laterally. However, by bleaching differently sized and shaped sections of cardiac myofibrils, da Silva Lopes et al. found that titin moves at the same speed in both directions, suggesting that the protein completely disconnects from the sarcomere and is then free to diffuse either way before reintegrating at a new location.

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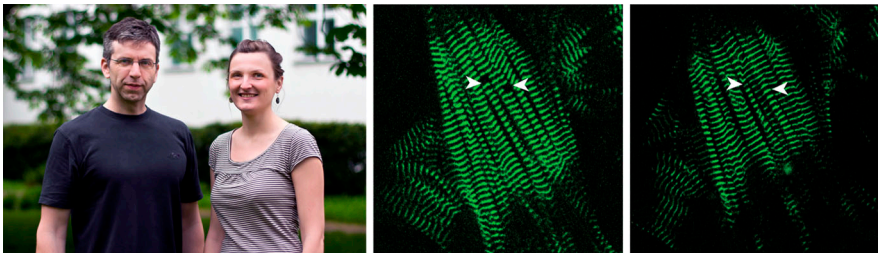
Titin mobility was affected by calcium, however. Bleached areas of cardiac myofibrils recovered their fluorescence more quickly when calcium levels were low, whereas titin's incorporation into sarcomeres was more stable in the presence of high calcium. Gotthardt says that this makes sense because high calcium activates myofibril contraction. "To produce force, the sarcomere proteins need to stay in place and do their work," he explains.

Gotthardt admits that many questions remain about how titin detaches from the sarcomere, how it reintegrates, and how its turnover is regulated. But he plans to use the fluorescent-titin knockin mice to address several other aspects of muscle biology as well. "This mouse also allows us to study how sarcomeres are assembled and how muscle cells fuse to form a multinucleate syncytium. There are a lot of really important questions that we can ask using this model system."

Focal Point



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Michael Gotthardt (left), Katharina da Silva Lopes (right), and colleagues (not shown) create knockin mice expressing a fluorescently tagged version of the giant muscle scaffold protein titin. The researchers analyze titin's dynamics in muscle cells and find that the protein is surprisingly mobile—after photobleaching segments of cardiac myofibrils (arrowheads, center image), titin fluorescence recovers within 14 hours (right). The authors suggest that titin can detach from sarcomeres, move freely by diffusion, and reattach elsewhere in the myofibril.

1. Tskhovrebova, L., and J. Trinick. 2003. *Nat. Rev. Mol. Cell Biol.* 4:679–689.
2. da Silva Lopes, K., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201010099
3. Labeit, S., et al. 1992. *EMBO J.* 11:1711–1716.
4. Isaacs, W.B., et al. 1989. *J. Cell Biol.* 109:2189–2195.