

Clathrin's muscle-building regimen

The clathrin heavy chain forms a membrane scaffold that organizes skeletal muscle sarcomeres.

Clathrin is best known for its role in endocytosis and other membrane trafficking events, but the protein's heavy chain has several additional functions, including organization of mitotic spindles and coordination of actin filaments at intercellular adhesions. Vassilopoulos et al. now reveal that the clathrin heavy chain (CHC) also helps to organize skeletal muscle sarcomeres by promoting their attachment to muscle cell membranes (1).

Stéphane Vassilopoulos, a member of Marc Bitoun's team at the Institut de Myologie in Paris, was interested in CHC's function in skeletal muscle because the protein is expressed in a characteristic, striated pattern in muscle fibers, overlapping with the actin-rich I-band of the sarcomeres. Clathrin has been proposed to promote myofibril assembly (2), and mutations in the GTPase dynamin 2, clathrin's partner in both endocytosis and actin filament organization, cause an autosomal-dominant form of centronuclear myopathy (3, 4). "We wanted to revisit the role of clathrin in skeletal muscle," Vassilopoulos says.

Vassilopoulos et al. examined CHC's distribution in adult muscle fibers by confocal and electron microscopy (1) and discovered that the protein colocalized with the actin-binding protein α -actinin in costameres, peripheral structures where sarcomeres attach to the plasma membrane and surrounding extracellular matrix. Costameres are said to be the "Achilles' heel" of skeletal muscle because the contractile apparatus falls apart when its connection to the plasma membrane is disrupted (5).

Vassilopoulos and colleagues then knocked down CHC in cultured muscle cells. α -Actinin usually concentrates at the plasma membrane of differentiating muscle cells before adopting a striated pattern of localization to the costameres and sarcomeric Z-discs. In the absence of CHC, however, α -actinin aggregated in the cytoplasm. "And if you let these cells differentiate for longer, they never become striated," Vassilopoulos explains.

FOCAL POINT

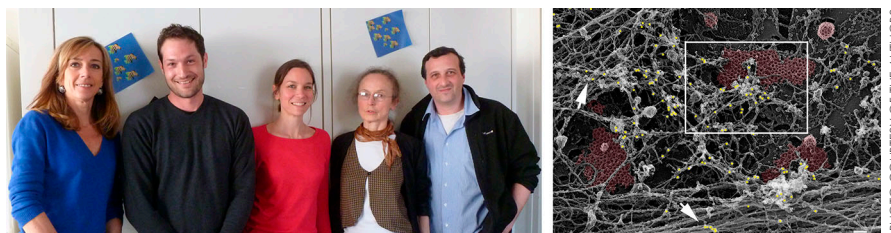


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(Left to right) France Piétri-Rouxel, Stéphane Vassilopoulos, Christel Gentil, Jeanne Lainé, Marc Bitoun, and colleagues (not pictured) reveal that the clathrin heavy chain acts as a membrane scaffold that helps to organize the sarcomeres of skeletal muscle. Clathrin localizes to costameres, the sites where sarcomeres attach to the plasma membrane of muscle cells. Quick-freeze, deep-etch electron microscopy shows how large clathrin lattices (pseudocolored red) form at the membrane of differentiated myotubes. These clathrin "plaques" organize a subcortical network of branched actin filaments associated with the actin-binding protein α -actinin (immunogold labeling shown in yellow). This actin network connects to and organizes the muscle's sarcomeres. In the absence of clathrin, the sarcomeres detach from the plasma membrane and become disorganized.

Depleting CHC from the muscle fibers of adult mice also disrupted the striated pattern of α -actinin and F-actin, leading to a loss of contractile properties and muscle degeneration. "There's a strong disorganization of the contractile apparatus and large regions of detachment from the plasma membrane," Vassilopoulos says.

To investigate how CHC might promote the attachment and assembly of muscle sarcomeres, Vassilopoulos worked with John Heuser and Robyn Roth at Washington University in St. Louis to observe clathrin's organization in cultured muscle cells by quick-freeze, deep-etch electron microscopy. This approach revealed that clathrin forms numerous "plaques" on the plasma membrane of muscle cells, flat lattices that are

much larger than the coated pits formed during clathrin-mediated endocytosis. "And we saw that branched actin filaments form a web underneath these clathrin plaques," Vassilopoulos says. These structures were also enriched in α -actinin and the adhesion protein $\beta 5$ integrin and appear to be part of the costamere linking sarcomeres to the extracellular matrix. Other components of the costamere, such as the dystrophin complex, were unaffected by CHC knockdown,

but α -actinin and costameric actin became disorganized in clathrin's absence, disrupting the connection to muscle sarcomeres. "We think that you need to have this membrane scaffold and subcortical actin filaments to attach to the sarcomeres," Vassilopoulos says. "If you disrupt this scaffold, the sarcomeres won't be organized correctly."

Clathrin plaques were recruited to the plasma membrane by the adaptor protein AP2. Depleting AP2 in cell culture phenocopied the loss of CHC, redistributing α -actinin to the cytoplasm. Knocking down dynamin 2 had the same effect, suggesting that dynamin 2 might also organize the actin cytoskeleton at clathrin plaques and that this function might be compromised in centronuclear myopathy patients.

One outstanding question, says Vassilopoulos, is how muscle cells regulate the formation of clathrin plaques. "Phosphorylation by the kinase Src might be a good candidate to be involved in this process. This could happen during muscle differentiation, leading to more plaques and fewer clathrin-coated pits undergoing endocytosis."

1. Vassilopoulos, S., et al. 2014. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201309096>.
2. Kaufman, S.J., et al. 1990. *Exp. Cell Res.* 191:227–238.
3. Bitoun, M., et al. 2005. *Nat. Genet.* 37:1207–1209.
4. Durieux, A.C., et al. 2010. *Hum. Mol. Genet.* 19:4820–4836.
5. Ervasti, J.M. 2003. *J. Biol. Chem.* 278:13591–13594.

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