

UNC-45a helps cells manage their stress levels

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The chaperone UNC-45a helps nonmuscle myosin II fold and assemble into contractile stress fibers.

In muscle cells, myosin II assembles together with actin filaments to form the myofibrils that drive muscle contraction. In other cell types, nonmuscle myosin II (NM-II) isoforms also assemble into contractile actomyosin structures, such as the stress fibers that control cell adhesion, migration, and morphology. In this issue, Lehtimäki et al. reveal that nonmuscle cells incorporate myosin into stress fibers via a mechanism similar to the one muscle cells use to assemble myofibrils (1).

In *Caenorhabditis elegans* and other invertebrates, myofibril assembly depends on a chaperone protein called UNC-45, which works together with the heat shock proteins Hsp90 and Hsp70 to ensure that myosin II molecules are folded correctly (2). Vertebrates express two homologues of UNC-45, and the muscle-specific isoform UNC-45b appears to play a comparable role in myofibril assembly. “We thought that nonmuscle cells might use a similar mechanism to assemble stress fibers,” explains Pekka Lappalainen from the University of Helsinki. “However, several papers have used RNAi to knockdown the nonmuscle isoform UNC-45a, which only seems to cause a mild phenotype and has no effect on myosin folding or filament assembly.”

Nevertheless, Lappalainen and colleagues, led by graduate student Jaakko Lehtimäki, examined UNC-45a’s function in U2OS cells and found that, although the protein’s localization and expression level varied from cell to cell, it was often enriched in actomyosin stress fibers. The fact that many cells seem to be perfectly fine expressing low levels of UNC-45a could explain why knocking down the protein by RNAi has little effect. Lehtimäki et al. therefore used CRISPR/Cas9 to generate UNC-45a knockout U2OS cells (1).

“It was really striking. The morphology of these cells was completely different,” Lappalainen says. The cells were very elongated and unable to retract their tails as they migrated. They also formed multiple lamellipodial protrusions extending in different directions, although, despite



Focal Point A team of researchers, including (left to right) Pekka Lappalainen, Jaakko Lehtimäki, Dylan Burnette, and Aidan Fenix, reveal that the myosin chaperone UNC-45a acts in nonmuscle cell types to promote folding of nonmuscle myosin II and facilitates its assembly into bipolar filaments that form contractile structures such as stress fibers. This is similar to the role that UNC-45b plays in the assembly of myofibrils in muscle cells. Compared with control U2OS cells (top), knocking out UNC-45a (bottom) dramatically alters cell morphology and inhibits the formation of stress fibers (stained for F-actin).

PHOTOS COURTESY OF THE AUTHORS.

these defects, they migrated faster than control cells.

“Then we looked at the stress fibers and found that they were more or less absent from UNC-45a knockout cells,” Lappalainen says. NM-II levels were also depleted in these cells. But, when the researchers added the drug MG-132 to inhibit the proteasome, NM-II aggregates accumulated in the cytosol, suggesting that, in the absence of UNC-45a, a large proportion of NM-II fails to fold properly and is targeted to the proteasome for degradation.

“Stress fibers . . . were more or less absent from UNC-45a knockout cells.”

A small fraction of NM-II does manage to fold in UNC-45a knockout cells. However, in collaboration with Aidan Fenix and Dylan Burnette at Vanderbilt University School of Medicine, Lehtimäki et al. found that the absence of UNC-45a also inhibits the assembly of NM-II into functional filaments. Fenix and Burnette used superresolution structured illumination microscopy to show that UNC-45a knockout cells formed fewer bipolar filaments with NM-II motor domains arranged at both ends. “So, UNC-45a has two functions,” Lappalainen explains. “It folds NM-II and then it somehow helps these folded molecules assemble into proper bipolar filaments that are important for contractility.”

Through a series of rescue experiments with different deletion mutants, Lehtimäki et al. found that UNC-45a’s C-terminal region, including the conserved UCS domain that binds to myosin II, mediates NM-II folding. But NM-II’s subsequent assembly into bipolar filaments is enhanced by UNC-45a’s N-terminal TPR domain, which, in the case of *C. elegans* UNC-45, has been proposed to promote myofibril assembly by mediating the chaperone’s oligomerization into linear chains that serve as scaffolds for myosin II filaments (3).

Though knocking out UNC-45a strongly inhibited stress fiber assembly, it had relatively little effect on the formation of another contractile structure, the cytokinetic cleavage furrow that divides cells in two at the end of mitosis. Lappalainen speculates that the small amount of NM-II that folds and forms filaments in UNC-45a knockout cells is sufficient to mediate cytokinesis because there are no other contractile structures in mitotic cells to compete for this limited pool of myosin (4).

Lappalainen and colleagues now want to target UNC-45a as a tool to test the physiological roles of various contractile structures in different cell types.

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