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Talin is cut out for intercellular adhesion

Study reveals that arginylation and proteolysis regulate this focal adhesion protein's function at cell–cell contacts.

The cytoskeletal adaptor protein talin helps cells attach to the extracellular matrix by linking actin filaments to integrin-based focal adhesions. Zhang et al. demonstrate that a proteolytic fragment of talin also promotes the formation of intercellular adhesions and that the generation and function of this fragment is regulated by arginylation (1).

The enzyme arginyltransferase, or Ate1, transfers arginine from tRNAs onto the N-terminal amino group of target proteins. Arginylation was first described in 1963 (2), but the process has remained in the shadows of better-studied protein modifications like phosphorylation and ubiquitination. Its relative obscurity doesn't reflect a lack of importance—Ate1 is essential for the survival of flies and mice (3). Nor does it reflect the modification's prevalence. "Probably at least a quarter of the proteome is arginylated at some point," estimates Anna Kashina, from the University of Pennsylvania in Philadelphia, who found that talin was arginylated in a large-scale analysis of Ate1 targets (4).

"The only target to be somewhat understood mechanistically is β-actin," continues Kashina. "Its arginylation is essential for forming the leading edge of locomoting cells." As well as having motility defects, however, Ate1-deficient cells have problems adhering to the extracellular matrix (5). Kashina and colleagues, led by postdoc Fangliang Zhang (who now runs his own lab at the University of Miami), therefore decided to investigate how arginylation affects the function of talin (1).

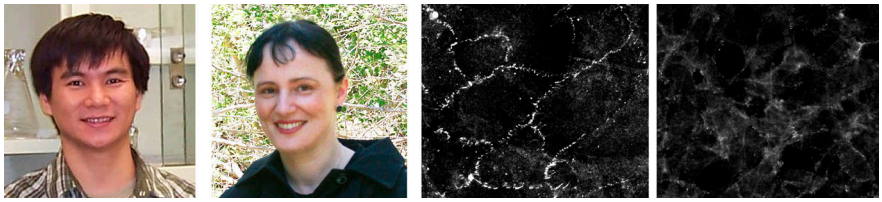
Talin is arginylated on an alanine residue in its C-terminal tail, suggesting that the protein must first be cleaved at this residue to expose its amino group to Ate1. Accordingly, Zhang et al. found that a C-terminal talin fragment of the predicted size—70 kD—was generated in fibroblasts. Depleting or inhibiting calpain 2—a protease known to cleave other sites in talin—blocked production of the fragment, which the researchers named VAD because it contains talin's vinculin-binding, actin-binding, and dimerization sites.

"We expected to find that [the VAD fragment] regulates cells' attachments to the substrate," Kashina admits. "But, to our surprise, we found that it regulates cell–cell adhesion instead." VAD colocalized with the adhesion molecule cadherin at intercellular junctions, and the fragment's production was enhanced in confluent fibroblast monolayers making numerous cell–cell contacts. VAD was absent from cells lacking Ate1, on the other hand, which showed fewer and smaller cell–cell contacts than wild-type fibroblasts. Expressing recombinant VAD in Ate1-null cells rescued the formation of cadherin-based intercellular adhesions but failed to rescue these cells' defects in attachment to the extracellular matrix.

> "It's a novel principle of how proteins can be regulated by arginylation."

VAD appeared to promote intercellular adhesion by stimulating actin polymerization at cadherin-based contact sites. But how does arginylation affect this process? The lack of VAD in Ate1-deficient cells indicated that arginylation promotes the fragment's cleavage from talin, and, indeed, calpain activity was reduced in Ate1-null fibroblasts. In addition, arginylation of VAD itself enhanced its ability to stimulate cadherin-based cell contacts.

Focal Point



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Fangliang Zhang (left), Anna Kashina (right), and Sougata Saha (not pictured) demonstrate that a proteolytic fragment of the focal adhesion protein talin promotes the formation of cadherin-based intercellular adhesions. Both the production and function of the fragment are regulated by arginylation. Compared with wild-type cells (center), fibroblasts lacking the arginyltransferase Ate1 show few cadherin-positive cell–cell contacts (far right).

"Arginylation regulates both the generation and the function of the fragment," says Kashina. "It's a novel principle of how proteins can be regulated by arginylation."

VAD's distinct activity at cell–cell adhesions also represents a novel function for talin, a function that may be particularly important in the heart, where talin is found at the intercalated discs that connect neighboring cardiomyocytes. Intriguingly, Zhang et al. found high levels of VAD in heart tissue, and Ate1-knockout mice show severe defects in cardiac development (6).

Kashina now wants to investigate whether the generation of VAD in confluent fibroblasts helps control their growth and activity in tissues, and whether this talin fragment is linked to contact-independent growth in cancer cells. Kashina also wants to study how and why other cellular proteins are arginylated. In contrast to talin's cleavage and subsequent arginylation, β-actin is arginylated at its N terminus cotranslationally. "Right now, we've discovered two independent mechanisms of arginylation, and I think there will be more," Kashina says.

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