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Nck by the numbers

Meshing experiments and simulations, study uncovers the stoichiometry of actin-polymerizing proteins.

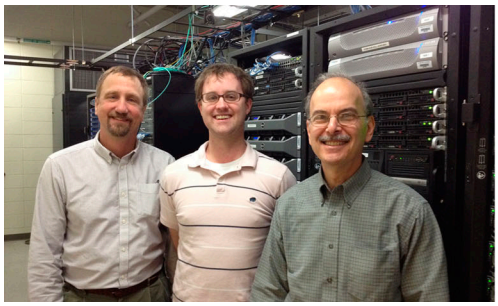
A growing axon, a cancer cell on the move, and a virus spreading from cell to cell have at least one thing in common—they receive a boost from the Nck adaptor proteins that help control actin dynamics. Ditlev et al. reveal that the density and relative amounts of Nck proteins determine their effects on actin polymerization (1).

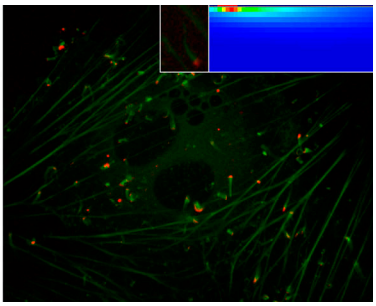
By activating the N-WASp–Arp2/3 pathway, Nck proteins trigger actin polymerization in a variety of situations. For example, they spur kidney cells called podocytes to send out extensions that encircle capillaries and create a blood filter (2). Nck proteins also help orchestrate the actin rearrangements that enable a T cell to form an immunological synapse with an antigen-presenting cell and that propel the membrane projections, or invadopodia, cancer cells use to spread (3, 4). Some pathogens hijack this actin-polymerizing mechanism to move around. For example, the vaccinia virus enlists Nck adaptors to prod its host cell to sprout actin extensions, which chauffeur the virus to neighboring cells it can invade (5).

Nck proteins contain SH3 domains that latch onto N-WASp. In previous work, the researchers used antibodies to force fusion proteins carrying the Nck SH3 domains to cluster at the membrane, spurring cells to produce long actin protrusions called comet tails (6). In the new study, Ditlev et al. quantified the effect of Nck proteins on actin dynamics, combining experiments with simulations that relied on a computational model constructed with the Virtual Cell software.

Again using antibodies to concentrate proteins sporting Nck SH3 domains, the researchers measured comet tail characteristics such as length and peak actin concentration. They found that the number of SH3-carrying fusion proteins in a cluster had little effect on tail dynamics. An alternative possibility is that the density of Nck proteins matters more than the total number of molecules. To test this notion, Ditlev et al. engineered cells to produce SH3-containing proteins as well as nonfunctional fusion proteins that lacked the domains. Density proved crucial, the team found. Membrane clusters that held between 20 and 80% functional SH3 fusion proteins spurred some actin polymerization but yielded no tails. These structures only grew when the density rose above 80%.

Focal Point





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(Left to right) Bruce Mayer, Jonathon Ditlev, Leslie Loew, and colleagues (not pictured) explored the dynamics of the actin-polymerizing Nck proteins. When fusion proteins that contain Nck domains (red, right) cluster at the plasma membrane, cells grow comet tails of actin (green). The inset shows a close-up of a comet tail and a model tail from the researchers' simulations. The team found that Nck proteins are most effective at triggering actin polymerization if they are at high density and at a 4:2:1 ratio with N-WASp and the Arp2/3 complex.

The researchers then used Virtual Cell simulations to determine the stoichiometry of Nck and its partners. It's conceivable that one Nck molecule activates one N-WASp molecule, which in turn flips on one copy of the Arp2/3 complex. However, the Virtual Cell simulations revealed that tail parameters under this 1:1:1 ratio didn't correspond to the researchers' experimental measurements. A 2:2:1 ratio of Nck to N-WASp to Arp2/3 gave a better fit, but a 4:2:1 ratio provided the closest match to the measured values for comet tails. Consistent with this result, a recent paper reported that two N-WASp molecules interact with each Arp2/3 complex (7). "Our work shows that the stoichiometry extends even further," says co-senior author Leslie Loew.

Simulations with the Virtual Cell supported the team's hunch that each N-WASp binds directly to only one Nck molecule. A second Nck connects indirectly through another protein, WIP, that previous research had revealed could link to N-WASp and Nck. The team's experiments on cells lacking WIP confirmed the protein's role as intermediary because these cells did not sprout tails.

The Nck system "is unusual in that actin polymerization is exquisitely sensitive to density," says co-senior author Bruce Mayer. The 4:2:1 ratio of Nck to N-WASp to Arp2/3 could provide an element of safety, the researchers suggest. The requirement for four Nck molecules sets a threshold for stimulation, reducing the likelihood that an extraneous signal could trigger inappropriate actin assembly. One question for future exploration, the scientists note, is how WIP affects N-WASp. Although some work suggests it serves as an inhibitor, "we suspect that WIP is involved in the mechanism of N-WASp activation," says first author Jonathon Ditlev.

> **"Actin polymerization is exquisitely sensitive to [Nck] density."**

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