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 from cell to cell have at least one other protein, WIP, that previous research had revealed could link to N-WASp and Nck. 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.

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.

Simulations with the Virtual Cell software predicted 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%.

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.

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1. Ditlev, J.A., et al. 2011. J. Cell Biol. http://dx.doi.org/10.1083/jcb.201111113.
2. Jones, N., et al. 2009. J. Am. Soc. Nephrol. 20:1533–1543.
3. Barda-Saad, M., et al. 2005. Nat. Immunol. 6:80-89.
4. Oser, M., et al. 2010. Eur. J. Cell Biol. 90:181–188.
5. Frischknecht, F., et al. 1999. Nature. 401:926–929.
6. Rivera, G.M., et al. 2004. Curr. Biol. 14:11–22.