

## **IN FOCUS**

## A cell-free screen of caveolae interactions

Ben Short

Researchers reconstitute caveolae assembly in cell-free extracts to investigate how these membrane microdomains interact with signaling proteins.

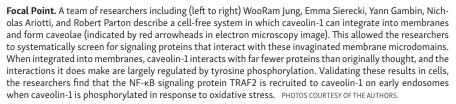
The plasma membranes of most animal cells contain numerous, cholesterol-rich microdomains known as caveolae, which are thought to play important roles in endocytosis and signal transduction. These flaskshaped invaginations are formed by small, integral membrane proteins called caveolins and cytoplasmic, lipid-binding proteins known as cavins. Caveolins were long thought to regulate signal transduction by binding constitutively to a wide variety of cell signaling proteins. In this issue, however, Jung et al. reconstitute caveolae assembly in a cell-free system to show that caveolins are much less promiscuous and that their interactions can be regulated by phosphorylation (1).

The caveolin signaling hypothesis originally proposed that caveolin binds to a wide range of signaling proteins via a conserved region called the caveolin scaffolding domain (CSD) that recognizes a loose consensus sequence called the caveolin binding motif (2–4). But more recent studies have suggested that neither the CSDs of caveolins or the caveolin binding motifs of its putative binding partners are available to mediate protein–protein interactions. The CSD of caveolin-1, for example, is closely associated with the membrane when the protein is assembled into caveolae (5).

"So, soluble binding assays might not represent physiological interactions," explains Robert Parton from The University of Queensland. "We wanted to find a system where we could systematically screen for interactions with caveolin embedded in a membrane."

Parton and colleagues, including first author WooRam Jung and co-senior authors Yann Gambin and Nicholas Ariotti, found that they could express fluorescently tagged caveolin-1 in cell-free extracts of *Leishmania tarentolae* (6) and that the resulting





protein integrated into membranes present in the extracts, forming caveolae-like invaginations that were the same size, and contained similar numbers of caveolin molecules as caveolae in cells (1).

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"We could then coexpress fluorescently tagged versions of putative caveolin binding partners and test for interactions in a multiwell format using fluorescence correlation spectroscopy," Parton says.

Using this approach, Jung et al. found that most proteins proposed to bind to caveolin's CSD fail to interact when caveolin is assembled into caveolae. Endothelial nitric-oxide synthase (eNOS), for example, failed to bind to membrane-integrated caveolin-1 in cellfree extracts. Nor did Jung et al. see an interaction between eNOS and caveolin-1 in cells using a proximity ligation assay. "There's a lot of data showing that caveolin regulates eNOS signaling," says Parton. "We still think that's true, but it's unlikely to be via a direct interaction." Instead, the researchers think caveolins and caveolae may influence eNOS and several other signaling pathways by regulating membrane lipid composition.

Membrane-integrated caveolin-1 did directly interact with a few proteins in cell-free extracts, however, including two Src family kinases and a protein involved in NF-κB signaling called TRAF2 (7). Rather than binding to the CSD, though, the interaction of these proteins depended on the phosphorylation of a tyrosine residue near caveolin-1's N terminus by Abl kinase. This residue—tyrosine 14—is known to be phosphorylated in vivo in response to a variety of cell stresses.

Accordingly, Jung et al. found that treating cells with hydrogen peroxide stimulated caveolin-1 phosphorylation and caused TRAF2 to associate with caveolin-1 on early endosomes. "We're interested in the physiological significance of this interaction," says Parton. "But the idea that phosphorylation stimulates caveolin internalization and the recruitment of proteins to a signaling platform on early endosomes could be important for many signaling processes."

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## bshort@rockefeller.edu.

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