Cell

PTRF Triggers a Cave In

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Caveolae are small membrane invaginations important for cell signaling that are characterized by the presence of caveolin proteins. Hill et al. (2008) have now identified PTRF as a new constituent of the caveolar coat. In the absence of PTRF, caveolae flatten and caveolin-1 is released into the cell membrane, where it is rapidly internalized and degraded.

Caveolae are small invaginations in the plasma membrane that mediate multiple functions, including signal transduction and endocytosis. The major protein constituent of caveolae is caveolin-1 (Cav-1) (Rothberg et al., 1992). One of the most fundamental questions in the formation of caveolae is how Cav-1 gives rise to 50–60 nm wide flask-shaped invaginations. Does this process involve several intermediates and many membrane compartments? In the current work, Parton and colleagues (Hill et al., 2008) show that PTRF (polymerase I and transcript release factor)/Cavin—originally identified as an RNA Pol I transcription termination factor (Jansa et al., 1998)—has an important functional role in caveolae biogenesis.

The assembly of caveolae begins in the endoplasmic reticulum (ER). Immediately after synthesis in the membrane of the ER, Cav-1 assembles as 12–18 mers in a stoichiometric complex with cholesterol (Monier et al., 1995). Cholesterol is a prerequisite for association of recombinant caveolin with liposomes in vitro (Murata et al., 1995), and the initial binding to cholesterol is expected to trigger oligomerization of the protein. Cav-1 oligomerization is tightly coupled with the passage of protein through ER/Golgi membrane systems and its exit to the plasma membrane (Machleidt et al., 2000); mutant Cav-1 proteins that fail to oligomerize are retained and degraded. Caveolin-coated domains exiting from the Golgi complex fuse with the plasma membrane (Tagawa et al., 2005) and must then deform the membrane to form flask-shaped mature caveolae. It is in this final step that PTRF is predicted to contribute to caveolae biogenesis (Figure 1).

The authors of the current study identified PTRF in a screen for cytosolic proteins that are specifically enriched in detergent-resistant membranes from wild-type mouse embryonic fibroblasts compared to mutant cells devoid of caveolin (Hill et al., 2008). PTRF is known to be a peripheral membrane protein that binds to phosphatidylserine and is localized in caveolae (Aboulaich et al., 2004). How might PTRF be recruited to caveolae? Recruitment of PTRF to the cell surface is dependent on the presence of the mature form of caveolae (and not just caveolin); mutant Cav-3 (such as P104L) or cross species isoforms (such as *C. elegans* Cav-1), which either fail to exit the Golgi or fail to form morphological caveolae at the plasma membrane, are defective in PTRF recruitment. PTRF and Cav-1 are present in stoichiometric amounts (~1:1) at caveolae on the cell surface, suggesting that PTRF could be a component of the caveolar coat, although this association may only take place at the plasma membrane. Consistent with this notion, it is only at the cell surface that caveolin and PTRF molecules are in close proximity, as assessed by fluorescence resonance energy transfer (FRET). This nanometer proximity between Cav-1 and PTRF is sensitive to cholesterol depletion, a treatment that also destroys caveolar architecture. Despite mutation of different domains in Cav-1 and PTRF, the authors do not detect a domain in either Cav-1 or PTRF that is involved in the direct binding of these two proteins. Caveolin peptides generate regions in lipid vesicles that are enriched for cholesterol and phosphatidylserine (Wanaski et al., 2003). Consistent with caveolae being a site of enrichment

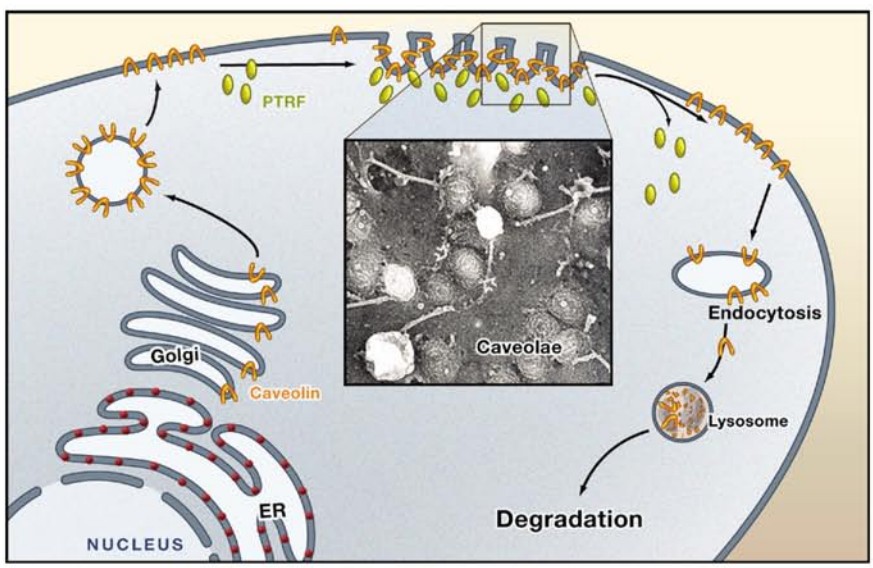

Figure 1. PTRF in the Life Cycle of Caveolin
Caveolin proteins (orange) are delivered to the cell surface, where they can associate with PTRF (green) and generate flask-shaped caveolae. Downregulation of PTRF renders caveolin mobile in the plasma membrane, at which point it can be endocytosed and rapidly degraded. Inset image of caveolae is reproduced from Rothberg et al. (1992).

for cholesterol and phosphatidylserine, a homolog of PRTF called SDPR (serum deprivation response factor; a phosphatidylserine-binding protein) was also identified in the current screen.

What are the implications of the presence of PTRF in caveolae? Knockdown of PTRF by RNA interference leads to a reduction in caveolae density in cultured cells and in zebrafish tissues. Loss of morphologically identifiable caveolae is accompanied by greater mobility of caveolin and its rapid clearance from the cell surface by internalization and degradation in lysosomes. Reduced PTRF in zebrafish leads to defective tissue architecture and a shortening and curving of the notochord and tail. A similar phenotype is also observed when caveolin levels are reduced (Nixon et al., 2007). During notochord development in the fish, caveolin is expressed earlier than PTRF although morphologically distinguishable caveolae appear only after PTRF expression. This observation suggests a role for caveolin outside of caveolae. One possibility is that PTRF could modulate the relative amounts of caveolae-bound caveolin to free caveolin in the plasma membrane. This ratio is likely to influence signaling outcomes from caveolin-associated proteins and to modulate caveolin turnover.

PTRF is a phosphorylated protein (Aboulaich et al., 2004), and its association with caveolae could be regulated by reversible phosphorylation. Pelkmans and Zerial (2005) implicated a serine/threonine kinase *ARAF1* in the regulation of caveolae assembly; knockdown of *ARAF1* by RNA interference dissembles caveolae and results in highly mobile caveolin. It is tempting to speculate that kinases such as *ARAF1* could regulate the phosphorylation of PTRF and thus control caveolae biogenesis. This could have consequences for the regulation of insulin signaling in adipocytes, as caveolae are known to concentrate insulin receptors along their margins (Foti et al., 2007). Moreover, PTRF is a substrate for insulin-stimulated phosphorylation, and it moves to the cytosol and nucleus after insulin treatment.

Characterization of PTRF as a functional component of caveolae has broad implications ranging from caveolae assembly to regulation of signaling via caveolin. Although the precise function of caveolae and a detailed understanding of their biogenesis still elude researchers, piecing together a molecular jigsaw puzzle regarding the assembly and control of caveolae biogenesis is likely to shed new light on this fascinating field.

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