Vinculin-cell membrane interactions

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Focal adhesions (FAs) are macromolecular complexes that connect the actin cytoskeleton to the extracellular matrix (ECM). These cell junctions are highly spatially organized yet dynamic organelles that transduce force from the actin cytoskeleton to transmembrane proteins known as integrins, which are heterodimers that bind to ECM components such as fibronectin, collagen, and laminin. The intracellular tails of integrin connect indirectly to the actin network via interactions with core FA proteins such as talin and α-actinin. Force is transmitted from F-actin to integrins via these core components to promote cell migration by a mechanism that has been termed a “molecular clutch” [1].

Vinculin is an essential protein component of FAs thought to stabilize FAs and to regulate force transmission. Vinculin null mice die during embryogenesis and embryonic fibroblasts derived from these mice display marked defects in cell movement. Vinculin is a 1066 residue protein consisting of a 91 kDa head domain (VH) and a 21 kDa five-helix bundle tail domain (Vt) separated by a proline-rich linker. Cytoplasmic vinculin is kept in a closed, auto-inhibited conformation by hydrophobic interactions between VH and Vt. Activation of vinculin in FAs uncovers binding sites for numerous FA proteins including talin, α-actinin, α- and β-catenin, and E-cadherin in VH, and paxillin, F-actin, and raver1 in Vt. Activated vinculin is also able to bind membrane-bound lipids such as phosphatidylinositol 4,5-bisphosphate (PIP2). Inositol phospholipids are components of numerous signaling pathways but the function of PIP2 binding by vinculin is unclear.

We recently reported the crystal structure of human vinculin bound to PIP2, which revealed that PIP2 binding alters vinculin structure to direct oligomerization and that simultaneous binding of PIP2 and F-actin is structurally possible [2]. The structure also unequivocally identified the vinculin residues involved in PIP2 binding thereby allowing the design of mutant constructs specifically deficient in PIP2 binding. These observations should be interpreted in light of recent reports describing the nanoscale structure of FAs derived from super-resolution microscopy [1]. These studies indicated that FAs are stratified vertically into three layers: a membrane-proximal integrin signaling layer (ISL), an actin regulatory layer (ARL) located ~60 nm higher, and an intermingled force transducing layer (FTL) between the ISL and ARL. Because of the distance between the ISL and the ARL it was suggested that only talin could simultaneously engage integrins and actin. Vinculin is distributed between all three of the axial FA layers but the distance between the ISL and the ARL would preclude a single vinculin molecule from binding simultaneously with PIP2 in the ISL and F-actin in the ARL. Thus, it was suggested that vinculin “climbs” talin like a ladder to reach the ARL and bind F-actin. However, the data from super-resolution microscopy do not provide direct information on the dynamic behavior of proteins at the nanoscale level.

FAs are polarized structures and FRAP studies showed distinct differences in vinculin exchange rates at the distal and proximal tips [5]. The FA residence time of full-length vinculin but not VH correlates with applied force [6]. Finally, it was recently reported that ECM stiffness, such as that found at the invasive border of breast tumors, stabilizes vinculin/talin/actin interactions, which facilitates PIP2 conversion to PIP, and the activation of signaling pathways that might contribute to cancer cell invasion [7].
Clearly, we are a long way from understanding the details of the connection between phosphoinositide signaling and FA-mediated cell migrations but they are likely to have significant functional implications for normal cell function and tumor cell progression and metastasis.

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