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Review

Vinculin and metavinculin: Oligomerization and interactions with F-actin

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ABSTRACT

Vinculin, and its splice variant metavinculin, are scaffolding proteins that localize to cellular adhesions. Vinculin is a key player in mediating cell adhesion, motility, and cellular response to force. In the past decade, a number of new studies have evaluated the importance of vinculin oligomers, especially in their role of bundling F-actin. Emerging evidence also suggests that vinculin oligomerization is important for vinculin's scaffolding function. Here we describe the latest findings on vinculin's interaction with F-actin and we clarify the different known vinculin oligomers. Differences in these functions between vinculin and metavinculin provide key insights to the structure and function of these oligomers, and should guide further studies.

1. Introduction

Since its identification in 1979 [1], vinculin has remained one of the most-studied scaffolding proteins in cellular adhesions. These sites of adhesion contain hundreds of proteins and mediate cell attachment to the extracellular matrix and other cells. Vinculin is a key protein in the regulation of these adhesions and, subsequently, adhesive forces, and cell movement. Though much is known about vinculin structure, localization, and binding partners (as reviewed in [2–4]), there is still much we do not understand, especially its roles in controlling cell morphology, motility, and force transduction. Recently, many studies have highlighted the differences in the structure and function of vinculin and its splice variant, metavinculin, as metavinculin mutations have been identified in heart disease (i.e., dilated cardiomyopathy). This review will highlight differences in ligand binding, oligomerization, and actin bundling properties of vinculin and metavinculin. Furthermore, we explore what these differences reveal about the structure and function of vinculin and the challenges introduced when using truncated proteins of different lengths to study vinculin function.

2. Vinculin and metavinculin

Vinculin is a highly conserved scaffolding protein localized to focal adhesions (FAs) and adherens junctions (AJs) [5]. At these sites of adhesion, vinculin mediates the recruitment of a number of binding partners and is a crucial regulator of cellular responses to tensional forces by mediating the link between transmembrane receptors and the actin cytoskeleton. Vinculin controls FA formation, strength, and migration [3]. In AJs, recent evidence suggests that vinculin regulates the structural integrity of cell–cell adhesions by mediating the mechano-response of E-cadherin [2,6,7], E-cadherin dependent border cell migration by acting downstream of myosin IV [8], and the expression of E-cadherin to the surface cells through its interaction with β-catenin [9].The importance of vinculin is demonstrated through knock-out studies in mice where the embryos die by day E10.5 [10]. Furthermore, fibroblasts isolated from these knock-out mice have a number of defects including difficulties adhering and spreading to substrates, increased migration, elevated FAK and paxillin signaling, reduction in cell stiffness, and resistance to apoptosis and anoikis [11–17].

Vinculin is an α-helical protein that contains three domains: a head (Vh, 91 kDa), a proline-rich linker (4.6 kDa), and a tail (Vt, 21 kDa) [18,19] (Fig. 1A, C). As a scaffolding protein, vinculin binds to many different ligands. Vh binds the cytoskeletal proteins talin [20], α-catenin [21], and α-actinin [22], while the proline-rich

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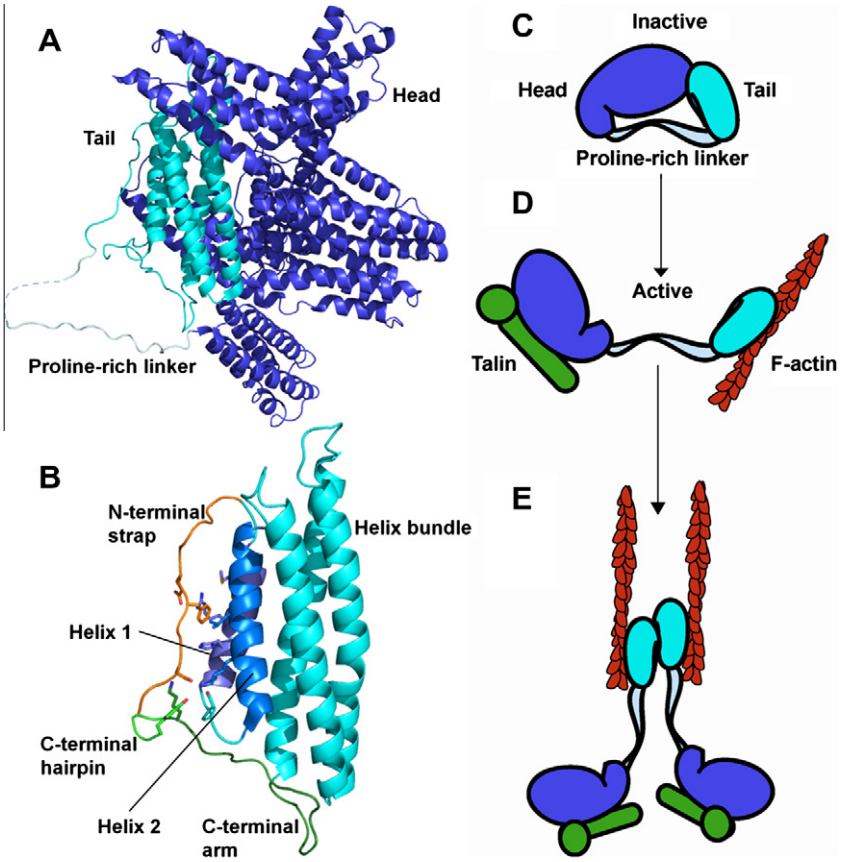

Fig. 1. Structure and activation of vinculin. (A) Ribbon diagram of the crystal structure of full-length vinculin (PDB 1ST6). Vt is shown in cyan. (B) The crystal structure of Vt (modified from PDB 1ST6). The N-terminal strap, H1 and H2, the C-terminal arm, and C-terminal hairpin are highlighted. Side chains of residues involved in maintaining the interaction between the strap, helix bundle, and C-terminal hairpin are shown as sticks. (C) The inactive conformation of vinculin is maintained by interactions between the head and tail. (D) Synergistic activation of vinculin requires binding of a Vh ligand (talin) and a Vt ligand (F-actin) to switch vinculin from its inactive to active state. (E) Active vinculin is able bind multiple proteins and acts a scaffold. Actin binding to Vt causes a conformational change to promote formation of a dimer that can bundle F-actin filaments.

linker binds vasodilator-stimulated phosphoprotein [23], ponsin/CAP [24], vinexin α/β [25], and the Arp2/3 complex [26]. Vt binds paxillin [27], raver1 [28], phosphatidylinositol-4,5-bisphosphate (PIP_2) [29], and filamentous actin (F-actin) [30].

Structurally, Vt is a five-helix bundle, with an amino (N)-terminal strap (879–893), helices 1–5 (H1–H5; 896–1045), and an extended carboxyl (C)-terminus (1046–1066) (Fig. 1B). The structure of the isolated Vt domain [31] is maintained in the context of the full length protein [18,32]. Both the N-terminal strap and C-terminus lack defined secondary structure, yet both interact with the helix bundle, and each other. As demonstrated in Fig. 1B, the N-terminal strap interacts with H1, H2, and the H1–H2 loop through contributions from D882, F885, and E887. D882 also mediates interactions between the strap and the end of the C-terminus (C-terminal hairpin). The C-terminus interacts with the bottom of the helix bundle (including the H1–H2 and H3–H4 loops) primarily through hydrophobic contacts, and with the N-terminal strap primarily through polar interactions (Fig. 1B).

As a scaffolding protein, vinculin has no enzymatic activity. However, it is often described as existing in "active" or "inactive" states. This corresponds to the ability of vinculin to bind or not bind, respectively, its ligands. Ligand binding is occluded in the inactive state by intramolecular interactions between Vh and Vt, as reviewed by Peng et al. [2]. Release of autoinhibitory contacts and activation of Vinculin is believed to be achieved through synergistic binding of both a head and tail ligand (Fig. 1C–E), as binding of a single ligand is insufficient for full activation [19]. For example, F-actin binding to the tail domain requires an additional interaction with another partner via Vh such as talin or α-catenin [33]. There are several combinations that may activate vinculin, though the biological relevance and consequences of these combinations are unknown. The one exception to the synergistic activation model is the *Shigella* protein, IpaA, which is reported to fully activate vinculin, though this interaction is not native to the host organism [33]. However, later experiments suggest that vinculin activation through IpaA still requires a Vt ligand [34]. Vinculin is also thought to be activated by mechanical forces, as these forces drive vinculin localization to FAs, a common consequence of vinculin activation [3,35–38]. Additionally, phosphorylation of vinculin may weaken autoinhibitory contacts and play a role in vinculin activation [39]. Although we are beginning to understand the process of vinculin activation, the cellular consequences of synergistic and mechanical activation of vinculin are poorly understood, and the topic remains an important area of study.

Metavinculin (150 kDa), a splice variant of vinculin, is co-expressed with vinculin in muscle tissues [40]. Both metavinculin and vinculin are localized to the cell membrane, the I-band in the sarcomere, and to intercalated discs [41]. Metavinculin contains an exon (exon 19) that codes for a 68-residue insert in the tail domain (MVt) between H1 and H2, which alters its function [42] (Fig. 2). Furthermore, while vinculin itself is highly conserved among most species, greater sequence variation is observed in the insert region between human, mouse, and chicken metavinculin than the rest of the molecule [43] (Fig. 2D). The presence of this