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Review Article

Mechanosensitive components of integrin adhesions: Role of vinculin

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ABSTRACT

External forces play a key role in shaping development and normal physiology. Aberrant responses to forces, or changes in the nature of such forces, are implicated in a variety of diseases. Cells contain several types of adhesions, linking them to their external environment. It is through these adhesions that forces are both sensed (from the outside inwards) and applied (from inside to out). Furthermore, several adhesion-based proteins are sensitive to changes in intracellular forces, utilising them for activation and regulation. Here, we outline how vinculin, a key component of integrin-mediated adhesions linking the actin cytoskeleton to the extracellular matrix (ECM), is regulated by force and acts as force transducing protein. We discuss the role of vinculin *in vivo* and its place in health and disease; summarise the proposed mechanisms by which vinculin is recruited to and activated at integrin-ECM adhesions; and discuss recent findings that place vinculin as the major force sensing and transmitting component of cell–matrix adhesion complexes. Finally, we discuss the role of vinculin in regulating the cellular responses to both the physical properties of the external environment and to externally applied physical stimuli.

Contents

1. Introduction

The human body can be thought of as a collection of chemical signalling events, intertwining to regulate physiological processes. However, the large collection of tissues and organs comprising the human anatomy has a second key regulator: exposure to the physical environment of the tissue and to ever-changing mechanical stimuli. Human anatomy consists of a myriad of tissues, ranging from the very soft gyri and sulci of the brain, to the very hard rigid trabeculae of bones. In addition to this heterogeneity, tissues continuously experience environmental forces, generated by blood pressure and movement. Understanding how the cells

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that constitute these tissues sense and respond to this multitude of mechanical stimuli is becoming ever important, and is known to regulate development and tissue homeostasis. It is now apparent that the response to force occurs at a protein level and the underlying molecular mechanisms are under intense investigation.

The 117 kDa focal adhesion protein vinculin (from the Latin *vinculum* meaning "bond", "link" or "tie") was discovered in 1979 as a protein localizing at the distal ends of microfilament bundles at the cell membrane [25]. Since its initial discovery, vinculin has become one of the best-characterised proteins of the focal adhesion (FA) where it has emerged as one of the main components of the mechanosensory machinery. Recent advances in microscopy have allowed us to gain a deeper insight into the precise location of vinculin within a FA. Elegant super-resolution microscopy experiments have placed vinculin within a 'force-transduction layer' where it links actin filaments to the extracellular matrix (ECM), through talin and integrin [10,35]. This imaging work supports functional molecular studies that show separate roles for the head domain of vinculin in regulating integrins (through its association with talin) and of the tail in regulating the link to the actomyosin machinery [30].

In this review we focus on the role of vinculin *in vivo*; its force-dependent recruitment and activation within cells; how vinculin acts to transmit forces from inside the cell to the extracellular matrix; and the regulation of the cellular response to mechanical stimuli by vinculin. In addition to its function at FAs, recent work has highlighted a role for vinculin at cell–cell junctions (reviewed by DeMali et al. [20]).

2. Role of vinculin *in vivo*

Genetic ablation of vinculin in mice is embryonic lethal, with defects seen by day E8 and termination by E10. Specific abnormalities are seen in the development of the nervous system, most likely due to defects in cell migration, and also in the developing heart [55]. Interestingly, vinculin appears to have a key function in the heart (a tissue under considerable forces), which fits with its role as a mechanosensitive protein. Vinculin was observed to localize to the intercalated discs joining cardiomyocytes [40] and knock-down of vinculin in primary cardiac myocytes disrupted cell shape and the alignment and assembly of myofibrils [48]. Furthermore, a missense mutation within the vinculin gene that reduced the levels of the protein within the intercalated discs was identified in a patient with hypertrophic cardiomyopathy [53].

Much of the work to elucidate the role of vinculin in cardiac tissue has been done using animal models (reviewed in detail by Zemljic-Harpf et al. [58]), where authors have shown that cardiac-specific knockout of vinculin leads to either sudden death or the development of dilated cardiomyopathy [59]. Additionally, artificially inducing stress to the hearts of heterozygous vinculin knockout mice led to the development of cardiomyopathy [60]. More recently, vinculin has been identified as a regulator of cardiac function during aging, with overexpression of vinculin being protective against stress and increasing the lifespan of *D. melanogaster* by $>150\%$ [36].

Whilst these studies clearly demonstrate that vinculin is involved in the adaptation of tissues to forces, the ability of vinculin to regulate the actin cytoskeleton also appears to be important for normal homeostasis of bone tissue. Bone resorption is driven by osteoclasts at actin-rich structures known as the sealing zone. Osteoclast-specific knockout of vinculin in mice led to smaller sealing zones and increased bone mass, with the cellular phenotype rescued by expression of wild-type vinculin, but not by expression of actin binding deficient mutants [24].

Taken together, the *in vivo* data shows a clear function of vinculin in both regulating adaptations to forces and in regulating the actin cytoskeleton. These roles are reflected at the molecular level, where vinculin is regulated by intracellular forces and is also involved in force transduction, and at the cellular level, where vinculin regulates cellular responses to mechanical stimuli.

3. Mechanisms of recruitment and activation of vinculin

In cells plated on stiff 2D substrates, integrin-dependent cell–matrix interactions form at the leading edge as focal complexes (FX) and mature into FAs under actomyosin-mediated tension. Both tension independent FX, as well as tension dependent FAs, contain vinculin [57] and several models of how vinculin becomes recruited to these sites have been proposed, including force-dependent and force-independent mechanisms. Most of these models are based on the initial biochemical characterisation of vinculin by Johnson and Craig [34] which revealed that vinculin is formed of three functional groups: the head, neck and tail domains. Bakolitsa et al. [5] determined that the full-length, 1066 amino acid structure is formed of 5 domains. *In vitro*, domains 1–3 of the head form a 'pincer', holding domain 5 (the tail) tightly bound. This conformation is not able to bind to talin suggesting it is an autoinhibitory, inactive conformation [17]. Point mutations to disrupt this auto-inhibition lead to increased binding of talin (*via* the head domain) and actin at the tail domain. These biochemistry results suggest that when vinculin is activated *in vivo*, the autoinhibitory bond is broken leading to the unmasking of a number of binding sites for other FA proteins [9]. The precise mechanism (s) underpinning how vinculin in cells is recruited to adhesion sites and how the autoinhibitory bond is broken remain open to discussion and will be outlined below.

3.1. Recruitment by talin alone

A prerequisite for FA formation and for vinculin recruitment to FAs is the presence of talin. Talin has 11 vinculin binding sites (VBS), the majority of which are thought to be cryptic, requiring specific unmasking events [19]. Talin is clearly the major binding partner for vinculin in FAs; while talin forms the essential structural link between integrin and the intracellular actomyosin machinery, vinculin reinforces this link [1,30]. Integrin-talin engagement at nascent adhesions induces an enrichment of PIP_2 in the inner membrane leaflet [41], and PIP_2 binding to talin is one proposed mechanism for talin's activation; indeed, sequestering PIP_2 disrupts FAs [43]. Actomyosin-mediated forces across talin are proposed to unmask VBSs [8] and early studies suggested that, once activated, talin alone is sufficient to activate vinculin [31]. However, this model has been questioned, as the autoinhibitory interaction between the vinculin head and tail is extremely strong ($Kd < 1$ nM) and a single ligand is considered insufficient to break this bond for full activation of vinculin. Furthermore, *in vitro*, the talin rod only binds to vinculin constructs lacking the auto-inhibitory bond [17]. These results suggest there is a requirement for another vinculin binding partner for the vinculin–talin interaction to occur, and the currently preferred model includes a combination of signals for vinculin activation.

3.2. Recruitment by talin and PIP_2

The vinculin tail domain contains a binding site for PIP_2 (phosphatidylinositol 4,5-bisphosphate) [6] and early studies suggested that this interaction, together with talin binding at the vinculin head, could activate vinculin [26]. This model was reinforced by the finding that PIP_2 binding at the vinculin tail could induce a conformational change in vinculin, possibly forming the

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