The PINCH–ILK–parvin complexes: assembly, functions and regulation

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Abstract

Cell–extracellular matrix (ECM) adhesion is mediated by transmembrane cell adhesion receptors (e.g., integrins) and receptor proximal cytoplasmic proteins. Over the past several years, studies using biochemical, structural, cell biological and genetic approaches have provided important evidence suggesting crucial roles of integrin-linked kinase (ILK), PINCH and CH-ILKBP/actopaxin/affixin/parvin (abbreviated as parvin herein) in ECM control of cell behavior. One general theme emerging from these studies is that the formation of ternary protein complexes consisting of ILK, PINCH and parvin is pivotal to the functions of PINCH, ILK and parvin proteins. In addition, recent studies have begun to uncover the molecular mechanisms underlying the assembly, functions and regulation of the PINCH–ILK–parvin (PIP) complexes. The PIP complexes provide crucial physical linkages between integrins and the actin cytoskeleton and transduce diverse signals from ECM to intracellular effectors. Among the challenges of future studies are to define the functions of different PIP complexes in various cellular processes, identify additional partners of the PIP complexes that regulate and/or mediate the functions of the PIP complexes, and determine the roles of the PIP complexes in the pathogenesis of human diseases involving abnormal cell–ECM adhesion and signaling.

Keywords: PINCH; ILK; Parvin; Cell–extracellular matrix adhesion; Cytoskeleton; Signaling

1. Introduction

Virtually all cells in multi-cellular organisms are, at least during certain stages of their life span, in contact with extracellular matrix (ECM). Cell–ECM contact regulates a variety of cellular processes including shape change, motility, proliferation, differentiation and survival. At the molecular level, cell–ECM contact is mediated by transmembrane cell adhesion receptors (e.g., integrins) and a number of highly selective, receptor proximal cytoplasmic proteins. These proteins are assembled into multiple complexes, through which the ECM is physically linked to the actin cytoskeleton and signals are transduced bi-directionally between the ECM and the intracellular compartment [1–11]. Identification of cell–ECM adhesion complexes, determination of their functions in various cellular processes and elucidation of the mechanisms by which cell–ECM adhesion complexes are assembled and regulated, therefore, are of central importance to our understanding of the molecular mechanisms underlying ECM control of cell behavior. One important cytoplasmic component of the cell–ECM adhesions, integrin-linked kinase (ILK), was identified approximately 8 years ago in a yeast two-hybrid screen based on its interaction with the β1 integrin cytoplasmic domain [12]. Subsequently, it was found that PINCH, a protein consisting of primarily five LIM domains [13], forms a stable complex with ILK in cells [14,15]. More recently, it was demonstrated that PINCH, ILK and CH-ILKBP, a member of the CH-ILKBP/actopaxin/affixin/parvin (abbreviated as parvin herein) family [16–19], form a ternary complex in mammalian cells [17,20]. Over the past several years, studies from a number of laboratories have provided important evidence suggesting crucial roles of ILK, PINCH and parvin proteins in ECM control of cell behavior. One important theme emerging from these studies is that the formation of protein complexes consisting of ILK, PINCH and parvin is pivotal to the functions of PINCH, ILK and parvin in many, if not all, of the cellular processes. In this review, I will discuss our current understanding of three important aspects of the PINCH–ILK–parvin (PIP) complexes, namely their assembly, functions and regulation, and identify issues that are pertinent to future studies in this exciting area. For other
aspects of ILK, PINCH and parvin, including more detailed information on the history, structures and their roles in specific biological and pathological processes, readers are referred to several other reviews [15,21–26].

2. Mammalian cells assemble multiple PIP complexes

PINCH [13,27], ILK [12] and parvin [16–19] are multi-domain proteins that are widely expressed in human tissues and well conserved in evolution [28–34]. PINCH and parvin contain domains (five LIM domains for the former and two CH domains for the latter) that mediate exclusively molecular interactions. ILK, on the other hand, exhibits both protein-binding activity and protein serine/threonine kinase activity (reviewed in Ref. [24]). Through two specific interactions, one mediated by the second zinc finger within the PINCH N-terminal-most LIM1 domain and the ILK N-terminal ankyrin (ANK) repeat domain [14,35,36], and the other mediated by the ILK C-terminal kinase domain and the parvin C-terminal CH2 domain [17], PINCH, ILK and parvin form a ternary complex in cells [20] (Fig. 1).

To date, two structurally closely related PINCH proteins, PINCH-1 [13] and PINCH-2 [27], have been identified and cloned. PINCH-1 and PINCH-2 are encoded by two different genes but share significant sequence similarity (human PINCH-1 and PINCH-2 are 82% identical at the amino acid level) [27,37]. Both PINCH-1 and PINCH-2 are widely expressed in mammalian cells [13,27,37]. Moreover, they are often co-expressed in the same cells [27]. PINCH-2 [27], like PINCH-1 [14,35,36], binds to the ANK domain of ILK (Fig. 1). The binding of PINCH-1 and PINCH-2 to ILK, however, is mutually exclusive [27]. Thus, in cells expressing both PINCH-1 and PINCH-2, there exist at least two distinctive PIP ternary complexes, one containing PINCH-1 and the other containing PINCH-2.

Mammalian parvin protein family includes three members (α-, β- and γ-parvin) that are encoded by different genes [16–19]. The three parvin proteins share significant sequence similarity, particularly in the C-terminal region encompassing the CH2 domain. Both α- and β-parvin, which were also independently cloned in yeast two-hybrid screens based on interactions with ILK (and hence were also named as CH domain-containing ILK Binding Protein or CH-ILKBP [17] and affixin [19], respectively), bind to ILK through their CH2 domains. Although it remains to be tested experimentally, given the significant sequence similarity between γ-parvin CH2 domain and the ILK-binding CH2 domains of α- and β-parvin [18], it is possible that γ-parvin might also interact with ILK. Based on tissue distribution of human and mouse parvin mRNAs, it appears that both α- and β-parvin are expressed in a wide variety of tissues, whereas the tissue expression of γ-parvin is more restricted, albeit it does overlap with α- and β-parvin in at least certain human tissues such as heart and liver [38]. Thus, it is likely that at least in some human cells, α-, β- and possibly γ-parvin proteins are co-expressed. The presence of multiple ILK-binding parvin proteins further expands the repertoire of different PIP complexes (Fig. 1).

3. Cellular functions of the PIP complexes

A series of recent biochemical, structural, cell biological and genetic studies have shed new light on the cellular functions of the PIP complexes. Based on biochemical and structural analyses of the domains that mediate the interactions between PINCH, ILK and parvin proteins, several dominant-negative inhibitors of the PIP complexes have been developed (Fig. 2). Expression of the dominant-negative inhibitors (e.g., the ILK-binding LIM1 containing PINCH fragments, the PINCH-binding ANK domain-containing ILK fragments, etc.) in mammalian cells effectively disrupted the assembly of the PIP complexes [39,40]. Disruption of the PIP complexes significantly impaired cell shape modulation, motility and ECM deposition [39,40].
to these questions, I will attempt to summarize here recent findings that are pertinent to answering these questions and discuss our current understanding of these important issues.

4. How do the PIP complexes function in ECM control of cell behavior?

Two general mechanisms by which the PIP complexes function in ECM control of cell behavior have emerged from recent studies. First, the PIP complexes provide a crucial physical linkage between integrins and the actin cytoskeleton. In this regard, it is worth noting that components of the PIP complexes are capable of interacting with both the membrane components (e.g., β1 integrins [12]) and the cytoskeletal components (e.g., actin [16,18], Nck-2 [46,47], paxillin [30,48] and Mig-2/UNC-112 [32,49]) of the ECM adhesion structures. Consequently, loss of individual components of the PIP complexes due to gene inactivation or disruption of the assembly of the PIP complexes with the dominant-negative inhibitors compromises the integrin–actin linkage, resulting in defects in cellular processes such as cell–ECM adhesion, spreading, migration and fibronectin matrix assembly. Second, the PIP complexes serve as signaling mediators that transduce signals (e.g., serine/threonine phosphorylation, interaction-induced conformational changes, etc.) to downstream effectors and thereby control cytoskeleton organization, spreading, motility, proliferation and survival. In this regard, it is worth noting that ILK possesses serine/threonine kinase activity [12,50–55] and can phosphorylate several important proteins including protein kinase B (PKB)/Akt [50–52], GSK-3 [50], affixin [19], myosin light chain [51], myosin phosphatase target subunit [54,55] and inhibitors CPI-17 and PHI-1 [53]. A protein serine/threonine phosphatase of the PP2C family, ILKAP, can also associate with ILK [56]. Additionally, PINCH and parvins also directly or indirectly associate with components of other signaling pathways (e.g., small GTPase signaling pathways) [46,57–60] and thereby modulate intracellular signaling. One of the signaling proteins that can directly interact with PINCH is Nck-2 [46,47], a member of the Nck adapter protein family [61,62]. Inactivation of the mouse Nck genes results in embryonic developmental defects that resemble those induced by the loss of components of the integrin/fibronectin signaling pathway [63]. Thus, while Nck proteins can interact with components of multiple signaling pathways and are involved in a variety of cellular processes [61,62], the interactions of Nck proteins with components of the integrin/fibronectin signaling pathway including PINCH [46,47] and FAK [59,64–66] likely play an important role, particularly in the coupling of ECM adhesion to the small GTPase signaling pathways and therefore ECM regulation of actin cytoskeleton remodeling and motility.

While it is increasingly clear that ILK and its binding partners are crucial in intracellular signaling, how they function in intracellular signaling is incompletely under-
stood. One of the downstream targets of ILK signaling is PKB/Akt. ILK can directly phosphorylate PKB/Akt at Ser473 [50,52], which is essential for full activation of PKB/Akt [67]. Loss of ILK in human 293 cells and mouse macrophages diminished the Ser473 phosphorylation of PKB/Akt [68]. Overexpression of dominant-negative forms of ILK also inhibited PKB/Akt activation [41,50,69]. In addition, ILK can regulate Ser473 phosphorylation indirectly [70]. Loss of α-parvin/CH-ILKBP, another component of the PIP complex, prevented the membrane translocation of PKB/Akt and therefore compromised the activating phosphorylation of PKB/Akt [71]. Thus, ILK and associated proteins could directly or indirectly regulate PKB/Akt activation, which likely explains, at least in part, observations that elevation in the level or the activity of ILK is frequently associated with cancers and other hyper-proliferative and/or anti-apoptotic conditions [52,72–84]. Despite the compelling evidence for an important role of ILK in PKB/Akt activation in at least some cells, there is equally compelling evidence suggesting that ILK is not the only kinase that can phosphorylate PKB/Akt at Ser473. Expression of ILK mutants with reduced kinase activity in ILK-deficient flies and worms fully rescued the phenotypical defects induced by the loss of ILK [31,32,85]. Furthermore, biochemical studies suggest that there exists a Ser473 kinase that is distinct from ILK [86]. Finally, loss of ILK in certain mouse cells failed to prevent the activating phosphorylation of PKB/Akt [43,44]. Thus, while ILK is important in promoting PKB/Akt activation in some cells, ILK is unlikely an obligate kinase that is solely responsible for the Ser473 phosphorylation of PKB/Akt in some other cells. Currently, it is not known why ILK is essential for PKB/Akt activation in some but not other cell types. Because many studies showing that ILK regulates PKB/Akt phosphorylation were done in oncogenically transformed cells [41,50,52,70], it is tempting to suggest that PKB/Akt activation depends more heavily on ILK in cancer cells. This hypothesis, if proven correct, could potentially provide a new strategy to selectively suppress the activation of PKB/Akt in cancer cells.

5. Focal adhesion recruitment and regulation of the PIP ternary complexes

Focal adhesion proteins are recruited to cell–ECM adhesion sites in response to cell contact with ECM, which leads to growth and maturation of the cell–ECM adhesion structures [7,87]. Understanding how focal adhesion proteins are recruited to cell–ECM adhesion sites, therefore, is essential for understanding focal adhesion assembly and regulation. In mammalian cells, the assembly of the PIP ternary complexes occurs prior to the formation of cell–ECM adhesions [88], suggesting that PINCH, ILK and parvin proteins are recruited to ECM adhesion sites as pre-assembled protein complexes. Furthermore, mutations that ablate the formation of the PIP ternary complexes abolish the ability of PINCH, ILK or parvin proteins to localize to cell–ECM adhesions [17,27,35,88], indicating that the formation of the PIP ternary complexes not only precedes but also is obligatory for their localization to cell–ECM adhesions. How does the assembly of the PIP ternary complexes promote their localization to cell–ECM adhesions? One likely possibility is that it enhances the association with cell–ECM adhesions, as components of the PIP ternary complexes are known to interact with several other components of cell–ECM adhesions including integrins, actin, paxillin and Mig-2/UNC-112. In this regard, it is worth noting that although the assembly of PIP ternary complexes is essential for their efficient localization to cell–ECM adhesions, there is evidence suggesting that interactions with other proteins are also involved in this process [48,88]. Additionally, the assembly of the PIP ternary complexes also promotes the stability of the proteins [89] and might facilitate the transport of the complex components to cell–ECM adhesions.

How is the assembly of the PIP ternary complexes regulated? One important regulator of the assembly of the PIP ternary complexes appears to be protein kinase C. Inhibition of protein kinase C down-regulates the assembly of the PIP ternary complexes [88]. Because protein kinase C is known to play important roles in focal adhesion assembly, migration, proliferation and fibronectin matrix assembly [3,90–98], processes in which the PIP complexes are intimately involved, the identification of protein kinase C as an upstream regulator of the PIP complexes suggests that regulation of the assembly of the PIP ternary complexes is likely involved in the control of these protein kinase C-regulated processes. Additionally, the assembly of the PIP ternary complexes is regulated by the phosphatidylinositol-3 (PI-3) kinase signaling pathway, as inhibition of this signaling pathway by PI-3 kinase inhibitors or by overexpression of tumor suppressor PTEN inhibits the interaction between ILK and CH-ILKBP/α-parvin [99]. Given the crucial roles of the PIP complexes in a variety of biological and pathological processes, it is likely that additional regulators of the PIP complexes will be identified in the future. Another important area of future research is to understand the regulation of the PIP complexes at molecular and atomic levels. Recent studies on the three-dimensional structures of PINCH and ILK have provided a promising start [36,47].

6. Why do cells assemble multiple PIP complexes?

As discussed above, multiple PIP ternary complexes are present in at least certain human cells. Are different PIP complexes functionally redundant or are they playing distinct roles? Although we do not yet have a full answer to this question, recent studies suggest that different PIP complexes likely have distinct roles, albeit they may also share certain common functions. For example, there exist two different PIP complexes (PINCH-1–ILK–parvin and
PINCH–ILK–parvin complex in a number of human cells [27]. Although both PINCH-1–ILK–parvin and PINCH-2–ILK–parvin complexes are localized at cell–ECM adhesions [27,39], an elevation of the PINCH-2 expression, which promotes the assembly of the PINCH-2–ILK–parvin complex and concomitantly inhibits the assembly of the PINCH-1–ILK–parvin complex, impaired cell shape modulation and migration [27]. Additionally, we have recently found that depletion of PINCH-1 from human cells destabilized a number of cellular processes including cell spreading and survival [89]. Furthermore, despite its ability to form a complex with ILK and parvin, overexpression of PINCH-2 was unable to rescue the phenotypical defects induced by the loss of PINCH-1 [89], suggesting that the PINCH-2–ILK–parvin complex is incapable of substituting the PINCH-1–ILK–parvin complex in at least some cellular processes. Similarly, although both α- and β-parvin can bind to ILK, there are considerable biochemical and functional differences between different parvin proteins. For example, α-, β-, and γ-parvin proteins likely differ in their ability of being phosphorylated by cyclin B1/cdc2 kinase [100]. Functionally, depletion of CH-ILKBP (α-parvin) in human HeLa cells, which express both α- and β-parvin (Y. Zhang, K. Chen and C. Wu, unpublished observations), induced apoptosis but did not inhibit cell spreading [71]. On the other hand, depletion of either PINCH or ILK in HeLa cells not only induced apoptosis but also impaired cell spreading [89]. These results suggest that while the PINCH–ILK–CH-ILKBP (α-parvin) complex plays a prominent role in cell survival, some other PIP complexes (e.g., PINCH–ILK–β-parvin) likely can substitute the PINCH–ILK–α-parvin complex or even play a more prominent role in cell shape modulation. The presence of multiple PIP ternary complexes, each of which contains one or two structurally and functionally related but distinctive components (Fig. 1), provides a versatile system by which mammalian cells can precisely control cell morphology, motility, survival and other fundamental cellular processes that involve the PIP complexes.

7. Concluding remarks

Studies over the past several years have established the PIP complexes as one of the key regulators of cell–ECM communication. In addition, these studies have begun to uncover the molecular mechanisms underlying the assembly, functions and regulation of the PIP complexes. While these studies have clearly provided an important framework for future research, there are many important questions, including those discussed in this review, awaiting full answers. Among the challenges of future studies are to define the functions of different PIP complexes in various cellular processes, identify additional partners of the PIP complexes that regulate and/or mediate the functions of the PIP complexes, determine the structural basis underlying the assembly, functions and regulation of the PIP complexes and ultimately, fully understand the molecular mechanisms whereby the PIP complexes function in vertebrates as well as invertebrates. A complete understanding of the assembly, functions and regulation of the PIP complexes offers an exciting opportunity of gaining insights into the general mechanism governing cell–ECM adhesion and signaling. Additionally, there is increasing evidence suggesting that ILK and its binding partners are crucially involved in not only cancers (reviewed in Ref. [101]) but also other pathological or physiological processes including renal failure [40,102–105] and differentiation and/or functions of a variety of cell types [69,81,106–111]. Unraveling the mechanisms by which ILK, PINCH and parvin function in various physiological and pathological processes represents another challenge to cell biologists as well as clinical investigators.

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References

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