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ILK: a pseudokinase in the center stage of cell-matrix adhesion and signaling

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Abstract

Integrin-linked kinase (ILK) is a widely expressed and evolutionally conserved component of cell-extracellular matrix (ECM) adhesions. Although initially named as a kinase, ILK contains an unusual pseudoactive site that is incapable of catalyzing phosphorylation. Instead, ILK acts as a central component of a heterotrimer (the PINCH-ILK-parvin complex) at ECM adhesions mediating interactions with a large number of proteins *via* multiple sites including its pseudoactive site. Through higher level protein-protein interactions, this scaffold links integrins to the actin cytoskeleton and catalytic proteins and thereby regulates focal adhesion assembly, cytoskeleton organization and signaling. This review summarizes recent advances in our understanding of the structure and functions of the PINCH-ILK-parvin complex, and discuss emerging new features of the molecular mechanisms by which it regulates diverse cellular, physiological and pathological processes.

Introduction

The interplay between ECM and the actin cytoskeleton is crucial for regulation of diverse cellular processes including cell-ECM adhesion, shape change, migration, proliferation and survival. This interplay depends critically on heterodimeric (α/β) transmembrane receptors, integrins, which bind to ECM proteins *via* their extracellular domains while connecting to actin *via* their cytoplasmic tails (CTs). Understanding how integrin CTs connect to actin has been a major theme of research over the past several decades [1]. Integrin-linked kinase (ILK) was identified in a search for proteins interacting with integrin β CTs [2]. ILK contains an N-terminal ankyrin repeat domain (ARD) and a C-terminal integrin β CT binding kinase-like domain (KLD). Initial studies showed that bacterially expressed ILK not only binds but also phosphorylates integrin β1 CT, suggesting an ILK-dependent phosphorylation mechanism on integrin signaling [2]. Soon after, it was discovered that ILK binds PINCH, which comprises of five LIM domains, via its ARD [3] and α- (also known as CH-ILKBP or actopaxin) or β-parvin (also known as affixin), which comprise two calponin-

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homology (CH) domains, via its C-terminal KLD [4,5]. PINCH, ILK and parvin form a stable ILK-centered heterotrimer (termed IPP thereafter) in cells [4], which is a prerequisite for their localization to mammalian cell-ECM adhesion sites [6,7]. Subsequently, ILK has been shown to interact with many additional proteins either directly or indirectly through PINCH or parvin (Fig 1) to mediate diverse arrays of biological events.

In parallel to the findings that ILK functions as a key scaffold at cell-ECM adhesions, the role of ILK as a kinase has also been extensively examined. ILK was found to regulate phosphorylation of several key signaling intermediates including AKT and GSK-3 [8]. How ILK regulates protein phosphorylation, however, was elusive. It was suggested that ILK directly phosphorylates AKT, GSK-3β, and many other substrates [8]. However, ILK lacks key catalytic residues and thus its ability to function as a "true" kinase was questioned [9]. A series of genetic studies in multiple species also casted doubt on the notion that ILK functions as a kinase *in vivo* as putative kinase dead mutations were able to rescue ILK deficiency-induced developmental and cellular defects [10]. However, despite this, many studies have reported that ILK exerts kinase activity on numerous substrates including AKT and GSK-3 [11]. Thus, although there is a consensus that ILK is biologically important, it remained controversial as to how ILK could confer its functions. Below, we will discuss recent progress on ILK research, focusing on structure-derived pseudokinase features of ILK and how IPP functions as a scaffold in diverse cellular processes.

Structural basis of ILK as a pseudokinase

~10% of kinome lack one or more catalytic residues based on sequence alignment [12]. ILK belongs to this category, which was suggested as a pseudokinase [9]. However, one cannot exclude the possibility that ILK may have an unusual active site where the key missing catalytic residues are spatially compensated by some alternative residues. The compensation was indeed observed in WNK kinase, which lacks the conserved catalytic lysine residue in the subdomain II, uses K233 in its β2 strand as an alternative [13]. To address this issue, a high resolution 3D structure of ILK is necessary. This was achieved in recent crystallization of ILK KLD in complex with α-parvin C-terminal CH2 [14••,15•]. ILK KLD was found to contain conserved kinase domain fold with an ATP bound at the same location as conventional kinases. However, detailed examination revealed multiple pseudoactive site features (Figure 2): (i) an unusual ATP orientation with its γ-phosphate being far away from the putative substrate site; (ii) a nonhydrolyzed ATP in the crystal. ATP is usually hydrolyzed in either active or inactive kinases [16•]; (iii) a severely degraded catalytic loop including the absence of the key catalytic base Asp in the HRD motif and absence of other catalytic residues Lys and Asn. Unlike WNK [13], ILK contains no spatially adjacent residues to compensate these missing catalytic residues; (iv) altered magnesium coordination topology due to DVK that replaces typical metal coordinating DFG motif; (v) unusually short and rigid activation segment that lacks conserved phosphorylation site. The activation segment in a conventional kinase is typically long and flexible required for regulating the kinase activity. These structural features strongly indicated that ILK is a pseudokinase. This was confirmed by the lack of kinase activity of recombinant ILK expressed in either *E. coli* or mammalian cells or endogenous ILK purified from chick tissue [14••,15•]. It is also consistent with the extensive genetic data demonstrating that the kinase activity is not required for embryonic development [17,18,19,20••]. Mn^{2+}, which was recently proposed to promote the kinase function of ILK [11,21], caused no structural difference of ILK as compared to Mg^{2+} [15•]. Furthermore, proteomic analysis revealed no kinase activity against any potential substrates in the presence of either Mg^{2+} or Mn^{2+}, providing definitive biochemical evidence that ILK is a pseudokinase [15•]. The purity of ILK is crucial for definitively examining the kinase activity since a tiny amount of impurity kinase may lead to false-positive result [15•]. The impurity or some unknown kinase

associated with partially purified ILK may explain some of the previous studies that showed kinase activity.

In addition to WNK, a few previously proposed pseudokinases have also been shown to have residual kinase activity such as CASK and HER3/ErbB3 [16•]. However, the kinase activities of these "active" pseudokinases are extremely low and hence their physiological relevance as effective enzymes remain to be further investigated. As a comparison, ILK belongs to the pseudokinase Group 2 including STRADα and VRK3, which have severely degraded active site with no structural alternative and no residual kinase activity [16•].

IPP functions as a key interaction hub at ECM adhesions

How does ILK act as a pseudokinase to regulate cell behavior and intracellular signaling? Emerging evidences suggest that pseudokinases utilize their pseudokinase domains to recognize specific targets. In certain cases, this leads to conformational changes of the target proteins including kinases and thus triggering signaling [16•]. ILK may function in similar manners by interacting with different proteins through multiple binding sites. In particular, ILK binds tightly to PINCH via its ARD [3,6,22,23] and to parvin via its KLD [4,5, 14••], leading to formation of a stable ILK-centered IPP complex [6,10]. The formation of IPP is critical for stability of PINCH, ILK and parvin [24,25,26] and for their localization to cell-ECM adhesions in mammalian cells [6], where they engage in interactions with a large number of additional proteins [10,27]. In invertebrates such as *C. elegans* and *Drosophila*, ILK also forms a ternary complex with PINCH and parvin [18,28,29,30,31•,32•]. While the core interactions (e.g., PINCH-ILK and ILK-parvin) are well conserved, certain binding partners are gained or lost (e.g. PINCH binding partner UNC-98 [33]) during evolution. Similar to what was found in mammalian cells [6,24,25,26], PINCH, ILK and parvin are mutually dependent for their stability and localization to ECM adhesions in the wing epithelium in *Drosophila* [32•]. However, *Drosophila* ILK can localize to strong muscle attachment sites in the absence of PINCH [30,31•]. In *C. elegans*, ILK can localize to but fails to properly organize in muscle attachment sites in the absence of PINCH [34].

The structure of ILK KLD/α-parvin CH2 complex revealed that the CH2 binding site involves the conserved helices (αEF and αG) and part of P+1 loop, which is the substrate site for conventional kinases [14••,15•]. Thus, ILK has evolved to utilize a unique pseudoactive site to specifically engage with its target. Based on the high sequence homology of parvin family members, we expect that β- and γ-parvin CH2 bind to ILK KLD in similar manner. Consistently, the interactions of ILK with α- and β-parvin are mutually exclusive [35]. Interestingly, the ILK KLD/parvin CH2 complex can still bind integrin β1/β3 CTs, suggesting that the integrin-binding site on ILK KLD is different from that of parvin CH2 [14••]. The structure of ILK ARD/PINCH complex has also been resolved, which involves a large interface consistent with the tight affinity [22,23]. These distinct binding sites on ILK thus provide a basis for understanding the formation of IPP and its connection to integrin. Because parvin has also been shown to bind to F-actin [36], we can now envision an integrin β CT-IPP-actin pathway (Fig 3) that may mechanically link ECM with cytoskeleton. Such pathway may also communicate with other pathways through interactions of IPP with other signaling/adaptor proteins (Fig 1) thereby regulating diverse arrays of cellular events. The structures of several binding interfaces of the IPP-mediated complexes have been determined including those of PINCH LIM4 bound to Nck2 [37] and parvin CH2 bound to paxillin LD motif [38,39]. The interfaces of integrin β CT [14••], Nck2 [37], and paxillin [38,39] on the IPP complexes do not overlap, which suggest a scaffolding mechanism. More than two dozens of IPP binding proteins have been identified (Fig 1) and it is anticipated that more structures of IPP-mediated complexes will be forthcoming, which

will provide a framework to design definitive mutation experiments to dissect functions of various IPP-mediated pathways.

ILK-centered IPP mediates diverse functions in embryonic development and diseases

Functional studies in various model organisms have demonstrated that ILK, PINCH and parvin are indispensable for embryonic development [17,18,19,20••,25,32•,40,41]. Furthermore, studies in human patients, tissue- or cell-selective knockout animals and cultured cells have revealed important roles of ILK, PINCH and parvin in organ (e.g., kidney, liver, heart, muscle, etc.) functions and diseases (e.g., cancer). Although detailed discussions on this topic are beyond the scope of this review (readers are referred to several excellent reviews on this topic [10,11,42]), we highlight three general themes emerging from these studies.

First, loss of ILK, PINCH and/or parvin in both invertebrates and vertebrates always inevitably cause defects in cell-ECM adhesion, migration and/or actin cytoskeleton organization, suggesting that IPP functions as an evolutionally conserved essential scaffold in the integrin-actin network. Second, ILK plays additional important roles both in and outside of ECM contacts. For example, ILK is critical for caveolae trafficking in keratinocytes and functions in this process by binding and recruiting IQGAP1 to nascent focal adhesions at the cell cortex and thereby stabilizing microtubules and promoting caveolin trafficking to the plasma membrane [43•]. ILK can also associate with mitotic centrosomes and regulate mitotic spindle organization [44] and centrosome clustering in cancer cells [45]. A fraction of ILK can localize to the nucleus and regulate gene expression [46]. Third, ILK is often considered pro-proliferative. However, there are notable exceptions. For example, removal of ILK from hepatocytes in mice increases hepatocyte proliferation, resulting in hepatomegaly and enhanced liver regeneration [47,48]. IPP may also mediate cross-communications of integrins with other signaling pathways such as EphA signaling [49] and EGF signaling [50]. The different and sometime even opposing effects of ILK on cell proliferation and survival may reflect, at least in part, different signaling proteins with which IPP engages. At pathological level, abnormally high levels of IPP and its network components have been observed in numerous diseases such as failing hearts [51] and various cancers [52]. However, given that ILK is a pseudokinase, the therapeutic strategy that was targeted at the kinase activity should be re-considered.

Conclusions

Since the discovery of IPP more than a decade ago [4], numerous studies have affirmed a central role of this scaffold in connecting integrins to the actin cytoskeleton and signaling proteins. Furthermore, recent structural, biochemical and genetic analyses have definitively shown that ILK, a central piece of IPP, is a *bona fide* pseudokinase. This calls upon a significant revision of some of the previously proposed mechanisms of how ILK may operate to regulate various biological pathways. Considering that ablation or dysfunction of ILK or other components of IPP frequently affects key signaling pathways such as those of AKT and MAP kinases [53,54], the next wave of investigation may focus on the precise mechanisms by which IPP regulates these pathways. It is conceivable that multiple mechanisms might be involved. For example, α-parvin has been shown to facilitate membrane translocation of Akt [55], an obligatory step in Akt activation. Thus, IPP may regulate compartmentalization of signaling complexes and hence influence their activation. Furthermore, some of the IPP interactive proteins may possess kinase or phosphatase activities or function as adaptors linking IPP to kinases or phosphatases. For example, PINCH can associate with phosphatase 1alpha [56•], which leads to inhibition of the

phosphatase activity and thus enhances Akt1 phosphorylation, suggesting another mode of Akt regulation. It is also possible that ILK directly binds to and regulates an unknown kinase. This has been shown in the pseudokinase STRADα, which activates its binding partner LKB kinase [57]. Clearly, the advances in IPP research over the past decade have attested to the power of combined use of structural, biochemical, cellular and genetic approaches for better understanding of cell-ECM adhesion and signaling in diverse biological and pathological responses. With the unraveling of the structures and functions of IPP and its partners, we are entering a new era in which the discoveries may lead to novel therapeutic approaches for pathological processes associated with abnormal ECM adhesion and signaling.

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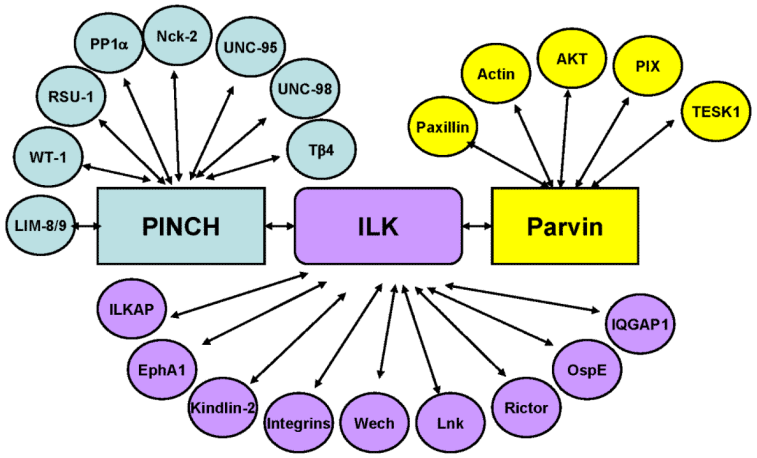

Figure 1.

Summary of IPP interactome. This figure depicts PINCH-, ILK- or parvin-mediated interactions of which functions have been investigated. Many of the IPP binding proteins have been listed in previous reviews [10, 27] except the recently published ones including IQGAP1 [43], PP1α [56], WT-1 [58], Ospe [59], LIM8/9 and UNC-95 [60], and Lnk [61].

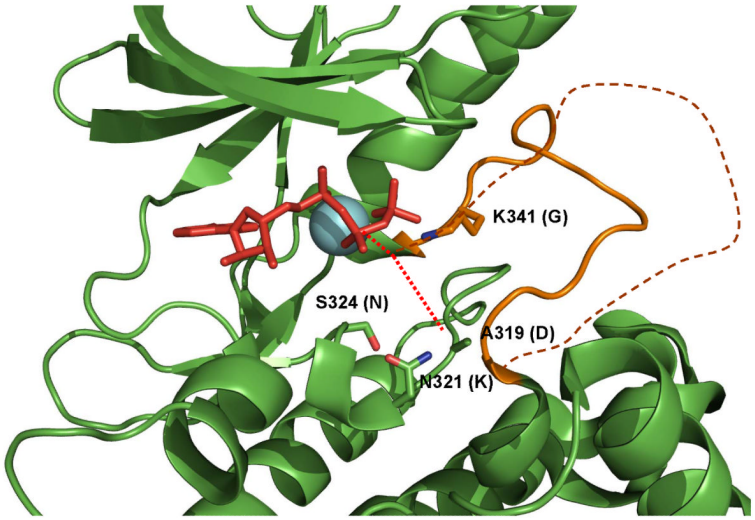

Figure 2.

Pseudoactive site features of ILK: A non-degraded ATP is bound to ILK KLD but its g-phosphate is oriented far away from A319 (see the dotted red line) – a site corresponding to the conventional catalytic base Asp. The typical DFG is replaced by DVK where K341 forms the salt-bridge with the g-phosphate of ATP, facilitating its distinct orientation. In addition to A319 that replaces the conventional catalytic Asp, multiple other catalytically important residues are missing including N321 that replaces Lys and S324 that replaces Asn. These catalytic residues are labeled and their corresponding catalytic residues are provided in the parentheses. Note that there are no surrounding residues that may alternatively substitute these catalytic residues. A single magnesium (cyan) is present in ILK KLD but coordinated differently in contrast to the two magnesium ions present in the conventional kinases. Finally, the activation segment (solid orange line) of ILK KLD is much shorter than the conventional one (dotted orange line).

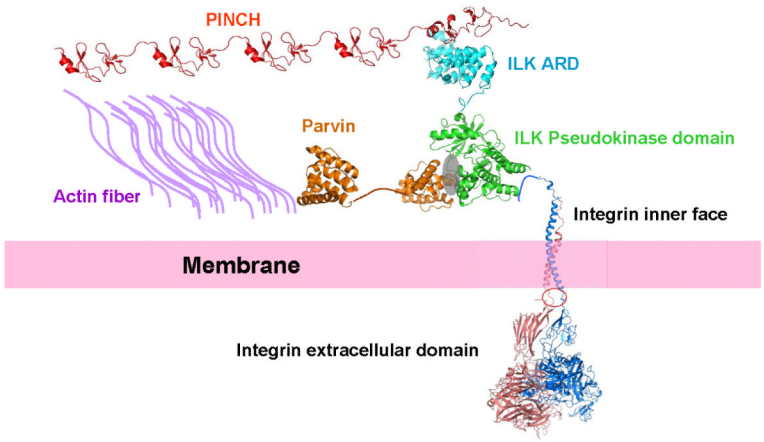

Figure 3.

A proposed structural assembly of IPP and its connection to integrin and actin filaments, allowing the linkage of ECM with actin cytoskeleton. The landscape was created using various PDB coordinates including the integrin extracellular domain (3FCU), integrin transmembrane-cytoplasmic domain (2KNC), ILK KLD/parvin CH2 complex (3KMW), ILK ARD/PINCH LIM1 complex (2KBX), PINCH LIM4 (1NYP), and Parvin CH2 (2K2R).