Integrin-linked kinase: integrin's mysterious partner

Carsten Grashoff, Ingo Thievessen, Katrin Lorenz, Siegfried Ussar and Reinhard Fässler[1]

Integrin-mediated cell adhesion regulates a vast number of biological processes including migration, survival and proliferation of cells. It is therefore not surprising that defects in integrin function are often rate-limiting for development and profoundly affect the progression of several diseases. The functions of integrins are mediated through the recruitment of cytoplasmic plaque proteins. One of these is integrin-linked kinase, which connects integrins to the actin cytoskeleton and transduces signals through integrins to the extracellular matrix and from integrins to various subcellular compartments.

Addresses
Department of Molecular Medicine, Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany
[1]e-mail: Faessler@biochem.mpg.de

Current Opinion in Cell Biology 2004, **16**:565–571

This review comes from a themed issue on
Cell-to-cell contact and extracellular matrix
Edited by Kathleen Green and Fiona Watt

Available online 21st July 2004

0955-0674/$ – see front matter

DOI 10.1016/j.ceb.2004.07.004

Abbreviations

αPIX	PAK-interactive exchange factor-α
BM	basement membrane
CH	calponin homology
CPI-17	protein-kinase-C-dependent phosphatase inhibitor of 17 kDa
Dock180	180-kDa protein downstream of CRK
EB	embryoid body
ECM	extracellular matrix
EMT	epithelial-to-mesenchymal transition
FA	focal adhesions
GSK-3	glycogen synthase kinase 3
ILK	integrin-linked kinase
ILKAP	ILK-associated phosphatase
Mig-2	mitogen inducible gene-2
MLC	myosin light chain
PAK	p21-activated serine/threonine kinase
PDK	3-phosphoinositide-dependent kinase
PH	pleckstrin homology
PHI-1	phosphatase holoenzyme inhibitor 1
PI3K	phosphatidylinositol-3-kinase
PINCH	particularly interesting new cysteine-histidine-rich protein
PIP3	phosphoinositol trisphosphate
PTEN	protein tyrosine phosphatase and tensin homolog

Introduction

Cell adhesion is mediated by multiprotein complexes composed of adhesion receptors, extracellular matrix (ECM) proteins and cytoplasmic plaque proteins. The cell adhesion receptors determine the specificity of the cell–cell or the cell–ECM interaction and recruit cytoplasmic plaque proteins to the cell adhesion site. The cytoplasmic plaque proteins transduce signals initiated by the adhesion receptor, link the adhesion receptors to the cytoskeleton and regulate the functional properties of the adhesion receptors themselves.

Integrins are a large family of adhesion receptors comprising >20 members that mediate highly dynamic cell–cell and cell–ECM interactions. The association and the release of integrin–ligand interactions are achieved by the ability of integrins to adopt different conformations. The active conformation is triggered by intracellular signals and cytoskeleton assembly and results in ligand binding, integrin clustering and recruitment of cytoplasmic plaque proteins into integrin attachment sites called focal adhesions (FAs) [1,2]. One protein that plays a central role in integrin activation and signaling is integrin-linked kinase (ILK) [3]. ILK is composed of three structurally distinct domains: three ankyrin repeats near the N terminus (a fourth ankyrin repeat was identified in human ILK but lacks well-conserved residues), a short linker sequence, and a kinase domain at the C terminus. The linker domain, together with sequences from the N terminus of the kinase domain, shares some similarities with pleckstrin homology (PH) domains (Figure 1).

In the present review we will discuss the functional properties of ILK, which are governed by ILK's interaction partners and kinase activity. The first part of this review summarizes biochemical and cell biological studies of ILK and the second part deals with *in vivo* experiments from invertebrates and mice.

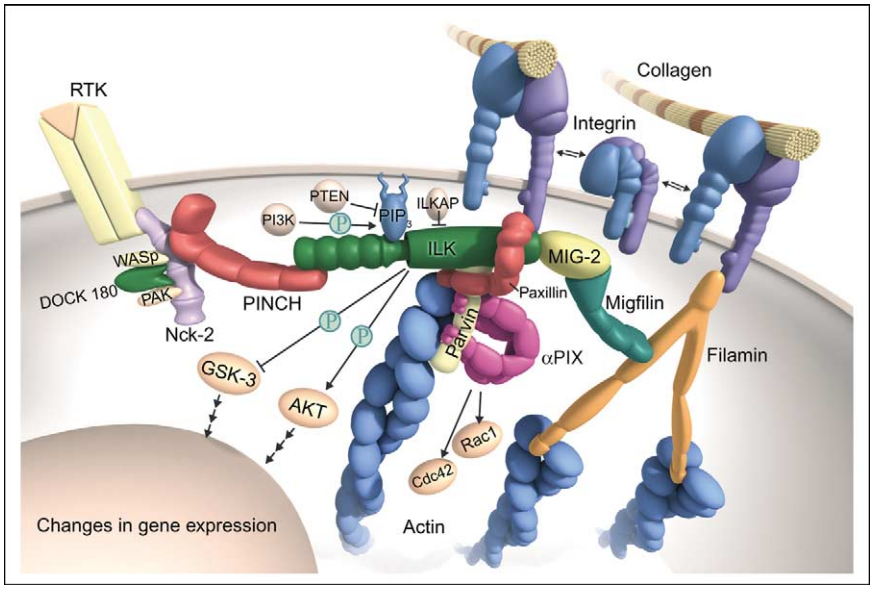
Cell biology and biochemistry of ILK

Overexpression of ILK as well as loss or reduction of ILK expression in cells profoundly affects their morphology and function. The most striking changes are impaired cell spreading, abnormal cell adhesion to and assembly of ECM proteins, delayed formation of FAs and altered cell proliferation [3–6,7••]. How can these defects be explained? Important hints have come from the identification of ILK binding partners (Table 1), from their mode of interaction with ILK and from the identification of substrates for the ILK kinase domain (Table 2).

ILK — a platform for actin regulatory proteins

Almost all adaptor proteins that bind either directly or indirectly to ILK regulate the actin cytoskeleton and

Figure 1



ILK binds Pinch and parvin and this ternary complex subsequently locates to the plasma membrane through the interaction with the cytoplasmic domain of activated β1 and β3 integrin subunits as well as unknown FAs component(s). Binding to phospholipids results in the activation of the kinase function of ILK, which in turn leads to the phosphorylation of GSK3β and PKB/Akt. Finally, ILK can recruit several adaptor proteins, which are able to regulate actin dynamics or actin attachment to FAs. The molecules presented in Figure 1 are not drawn to scale. AKT, protein kinase B/Akt; RTK, receptor tyrosine kinase; WASP, Wiskott-Aldrich syndrome protein.

hence could be responsible for the shape change and FA dysfunction associated with altered ILK expression (Figure 1). Pinch ('particularly interesting new cysteine-histidine-rich protein') was the first interactor to be identified [8]. Pinch-2, a Pinch homologue, was subsequently identified in mice and humans [9,10]. They are both composed of five LIM domains and a nuclear localization signal (NLS) at the C terminus. The first LIM domain binds the first ankyrin repeat of ILK. The interaction has been well-characterized using structural [11], biochemical and cell biological approaches [8,9]. The fourth LIM domain of Pinch-1 was shown to bind with very low affinity to the SH2/SH3 adaptor protein Nck2, which in turn interacts with growth factor receptors and recruits a large number of proteins, including actin modulators such as Dock180 (180-kDa protein downstream of CRK) and the p21-activated serine/threonine kinase (PAK) [8,12,13]. Whether Pinch-1 interacts with Nck2 *in vivo* is not clear. Since mice and cells lacking Nck2 are normal [14] but mice lacking Pinch-1 die during implantation (F Stanchi and R Fässler, unpublished) this interaction does not seem to be crucial for Pinch-1 function. It has been shown that Pinch-2 can translocate into the nucleus [9]. Its role there, however, is unclear.

Table 1

ILK interacting proteins, the location of their binding site on ILK and the method(s) used to confirm their interaction.

Interactor	Domain	Detection	Reference
β1 integrin	C terminus	Y2H/IP	[3]
β3 integrin	C terminus	IP	[3,61]
ILKAP	N terminus	Y2H/IP	[62]
Mig-2/Kindlin-2	C terminus	Y2H	[21••]
α-parvin	C terminus	Y2H/IP	[18]
β-parvin	C terminus	Y2H/IP	[19]
paxillin	C terminus	IP	[15]
Pinch-1	N terminus	Y2H/IP/CC	[8,11]
Pinch-2	N terminus	IP	[9]
PIP3	PH	—	[26]

CC, co-crystallization; IP, co-immunopreciptiation; Y2H, yeast-two-hybrid assay

Table 2

Putative targets of the ILK kinase activity and the amino acid residue(s) phosphorylated by ILK.

Target	Phosphorylation site	Reference
ILK	(Ser343)	[35,40]
β1 integrin	(Ser785)	[3]
β3 integrin	—	[61]
β-parvin	—	[19]
GSK-3β	(Ser9)	[26,62]
PKB/Akt	(Ser473)	[26]
MLC-20	(Thr18/Ser19)	[42]
MYPT-1	(Thr695, Thr495/Thr709)	[43,44]
CPI-17	(Thr38)	[45]
PHI-1	(Thr57)	[45]

MYPT1, myosin phosphatase target subunit isoform 1.

A search for paxillin binding proteins showed that the kinase domain of ILK contains sequences resembling a paxillin binding subdomain (PBS) motif, which firmly binds paxillin [15]. The ILK–paxillin interaction is necessary but not sufficient to recruit ILK into FAs, where the complex may modulate the function of other paxillin binding proteins such as vinculin, α-actinin, talin and FAK.

Several laboratories have simultaneously shown that parvins, a new family of F-actin binding proteins, bind the kinase domain of ILK [16–19]. The parvins comprise three members (α-parvin or actopaxin or CH-ILK binding protein; β-parvin or affixin; and γ-parvin) and are composed of two calponin homology (CH) domains that bind ILK, paxillin and F-actin. β-parvin was shown to interact with the guanine nucleotide exchange factor α-PIX (PAK-interactive exchange factor-α), which may activate Rac1 and Cdc42 [20]. Parvins are found in FAs and apparently do not colocalize to stress fibers [16,17]. An important future task will be to map the binding sites of ILK, paxillin and F-actin on the CH domains and to test whether their binding occurs simultaneously or is mutually exclusive.

A recent paper identified an additional ILK binding partner in *Caenorhabditis elegans*, termed UNC-112 [21••]. UNC-112 contains a FERM domain [22] and is important for the recruitment of the ILK orthologue, Pat-4, to muscle attachment sites. The mammalian orthologue of UNC-112, Mig-2/Kindlin-2, was shown to bind the LIM-domain-containing adaptor protein migfilin, which in turn binds filamin [23•]. It will be interesting to see whether Mig-2/Kindlin-2 also binds ILK in mammalian cells and whether this interaction modulates the function of filamin, which is mutated in a variety of human diseases.

ILK, Pinch and parvin — a ternary complex required for stability and focal adhesion localization

The association of ILK, Pinch and parvin into a ternary protein complex happens before their recruitment into FAs [24•] and serves at least two purposes: it stabilizes the individual proteins and targets the individual components into FAs [24•,25•]. Loss of ILK expression in cells leads to the degradation of Pinch and parvin and, conversely, loss of Pinch expression diminishes ILK and parvin levels [25•]. The degradation can be prevented either by inhibiting the proteasome [25•] or by expressing short N-terminal fragments of ILK (the ankyrin repeats) in ILK-deficient cells (C Grashoff, R Fässler, unpublished data) or Pinch (the first LIM domain) in Pinch-deficient cells (F Stanchi, R Fässler, unpublished data). Their recruitment into FAs, however, cannot be rescued with these fragments. These results support the notion that ILK and Pinch must have binding partner(s) that facilitate FA targeting. Possible candidates for ILK targeting partners are integrins, paxillin and Mig-2/Kindlin-2. It has been shown that nematodes lacking β integrin fail to localize ILK to cell attachment sites [21••]. Mammalian cells may have a similar requirement for β integrin to localize ILK, but this has not been shown yet with cell lines lacking either β1 or β3 or both integrin subunits. Paxillin binds ILK via its N-terminal leucine-rich motifs and targets to FAs via the C-terminal LIM domains. Mutation in the paxillin binding site of ILK prevents ILK/Pinch/parvin recruitment to FAs [15]. Mig-2/Kindlin-2 could also play a role since the worm orthologue UNC-112 is essential for localization of Pat-4/ILK to integrin-containing attachment sites [21••]. No candidate binding partners are currently known that could promote recruitment of Pinch into FAs.

The dependence of ILK, Pinch and parvin stability on the formation of a ternary complex has implications for the interpretation of overexpression experiments. Accumulation of ILK in the cytoplasm of ILK-overexpressing cells may cause a partial depletion of Pinch and parvin from FAs, resulting in an impaired FA function. This could explain why cells either lacking [7••] or overexpressing ILK [3] have similar phenotypes: they both show a rounded morphology and have decreased adhesive properties.

The kinase activity of ILK

Despite the sequence differences between the ILK kinase domain and other protein kinases (important residues in the activation loop of the kinase are not conserved) the similarity was immediately recognized and investigated [3]. Initial studies showed that GST-tagged ILK purified from bacteria or mammalian cells could phosphorylate serine and threonine residues in peptides representing the β1 integrin tail, and model substrates such as myelin basic protein [3].

ILK kinase activity took center stage when it was suggested to be directly associated with cell proliferation, tumor growth and metastasis [4,26–29]. On the one hand, overexpression of ILK in cells results in anchorage-independent cell cycle progression [5] and epithelial-to-mesenchymal transition (EMT) of non-tumorigenic as well as tumorigenic epithelial cells [4,29]. Inhibition of ILK kinase activity, on the other hand, suppresses cell growth in culture as well as growth of human colon carcinoma cells in SCID mice [30]. Several lines of experimental evidence suggest that these phenotypes are largely attributed to enhanced ILK kinase activity and phosphorylation of GSK3β and PKB/Akt [26], two key enzymes involved in a diverse array of cell functions including cell proliferation, survival and insulin responses [31,32]. ILK-dependent phosphorylation of GSK3β in epithelial cells downregulates GSK3β kinase activity [26]. This in turn is associated with reduced E-cadherin expression, enhanced AP1 activity and increased β-catenin–Lef/Tcf activity [4,33], which induces the expression

of cell-cycle-promoting genes such as cyclins and c-myc [5,34]. The reduced E-cadherin expression could be due to a direct effect of the β-catenin-Lef/Tcf complex on E-cadherin gene expression [4]. Alternatively, ILK can reduce E-cadherin levels indirectly by triggering snail expression, which in turn represses E-cadherin gene expression [30].

Full activation of PKB/Akt requires PIP3-dependent phosphorylation of two residues: Thr308 and Ser473 [32]. Whereas PDK-1 (3-phosphoinositide-dependent kinase 1) phosphorylates Thr308, ILK has been identified as 'PDK-2', which phosphorylates Ser473 via a direct interaction at the plasma membrane [26,35]. Besides possessing a kinase activity, ILK fulfils other requirements of a PDK2, including PIP3 binding and regulation of its activity by PI3K (phosphatidylinositol-3-kinase) or PTEN (protein tyrosine phosphatase and tensin homolog) [26,27]. However, some doubts about ILK's kinase activity arose when it was reported that it has no Ser473 phosphorylation activity [36,37•]. These doubts were reinforced by genetic studies in invertebrates and mice that demonstrated normal Ser473 phosphorylation in the absence of ILK [7••,21••,38]. Loss-of-function mutations of ILK in worms and flies show no defects that can be explained by impaired PKB/Akt activity, but develop severe muscle defects that are fully rescued when different kinase-dead versions of ILK are expressed [21••,38]. Similarly, fibroblasts with or without the ILK gene phosphorylate Ser473 to a similar extent following insulin or PDGF stimulation [7••], and neither chondrocytes nor keratinoyctes change their steady-state Ser473 phosphorylation after ILK gene ablation *in vivo* [39] (T Sakai and R Fässler, unpublished). These findings convincingly demonstrate that ILK — even if it has Ser473 phosphorylation activity — is not the only PDK2. These findings, however, do not exclude the possibility that ILK mediates the phosphorylation of PKB/Akt and other target proteins in an indirect manner, for example by recruiting a kinase or inhibiting a phosphatase [37•,40]. Support for such a notion also comes from gene ablation experiments. Monocytes lacking ILK expression show reduced Ser473 phosphorylation [41•]. Similarly, ILK-null fibroblasts, which respond normally to insulin treatment, fail to maintain Ser473 phosphorylation levels to the same extent as normal cells upon PDGF treatment [7••]. Furthermore, they display a slightly reduced steady state level of Ser473 phosphorylation under normal culture conditions (T Sakai and R Fässler, unpublished).

Other targets of the ILK kinase activity (Table 2) are β-parvin [19], the regulatory myosin light chain (MLC) [42], and MLC phosphatase [43,44] and its regulators CPI-17 (protein-kinase-C-dependent phosphatase inhibitor of 17 kDa) and PHI-1 (phosphatase holoenzyme inhibitor 1) [45]. The significance of their phosphorylation, however, is not clear.

Since ILK regulates so many essential cellular functions it is important to settle the debate on ILK's kinase activity. Solving the structure of the ILK kinase domain will be very informative, as will the analysis of mice carrying 'kinase-dead' versions of the ILK gene and the identification of PDK2(s). In addition to these new experimental approaches, new reagents to probe ILK's function will be useful. The E359K mutation in ILK, for example, was originally found to lack kinase activity and was therefore used in many studies as a 'kinase-dead' version of ILK. It turns out, however, that the mutation does not affect kinase activity but rather impairs paxillin binding and FA targeting [46•]. Furthermore, a polyclonal anti-ILK antiserum that recognizes a 59 kDa band of unknown origin instead of the 52 kDa sized ILK has been used in a large number of studies and could potentially have given misleading results [3,6,47].

Studies of ILK/Pinch/parvin in invertebrates and mice

The attachment sites of the body wall muscle to the hypodermis of *C. elegans* are called dense bodies and resemble FA-like structures. They contain β-*pat*-3 integrin (the only β integrin subunit in *C. elegans*), pat-4/ILK, UNC-97/Pinch, pat-6/parvin and UNC-112/Mig-2 and loss-of-function alleles of these proteins lead to severe adhesion defects manifesting as muscle detachment and embryonic lethality [21••,22,48,49•]. The loss-of-function studies also reveal that β-pat-3 integrin is required to recruit ILK to the plasma membrane [21••] and that integrins are partially mislocalized in the absence of pat-4/ILK [21••] or UNC-112/Mig-2/Kindlin-2 [22]. A recent report showed that the Zn^{2+}-finger-containing transcription factor UNC-98 can bind UNC-97/Pinch and is also required for muscle attachment to the body wall [50•]. UNC-98 shuttles between dense bodies and the nucleus where it binds DNA and probably regulates gene transcription. So far an ortholog of the UNC-98 gene has not been identified in flies or mammals.

Drosophila melanogaster has a similar requirement for βPS integrins, ILK and Pinch in muscle cell attachment [38,51••]. Interestingly, loss of βPS integrin function in flies leads to detachment of ECM from the cell membrane, while loss of ILK function leads to detachment of F-actin from the plasma membrane, indicating an important role for ILK in actin stabilization at integrin attachment sites [38]. The severe muscle defect in worms or flies lacking ILK can be fully rescued by the expression of different kinase-dead ILK transgenes, supporting the idea that ILK functions as an important adaptor protein, independent of its kinase activity [21••,38].

The loss of ILK expression in mice leads to peri-implantation lethality similar to what is seen upon loss of β1 integrin expression [7••,52]. The cause of the developmental arrest was studied in embryoid bodies (EBs)

[7••,53,54]; these studies showed that β1-integrin-mutant EBs are unable to deposit a basement membrane (BM), while ILK-null EBs produce a BM but fail to polarize the epiblast (a primitive tissue that will give rise to all three germ layers). Since addition of laminin to β1-integrin-null EBs rescues the BM assembly phenotype and allows epiblast development it is likely that β1 integrin and ILK function independently during the peri-implantation period [54].

Conditional loss of ILK in chondrocytes leads to skeletal growth retardations characterized by abnormal chondrocyte shape and decreased proliferation *in vivo* [39,55], and diminished chondrocyte spreading on ECM and reduced stress fiber formation *in vitro* [39]. Similar, albeit more severe, defects are also observed in mice with a chondrocyte-specific deletion of the β1 integrin gene [56], indicating that β1 integrins and ILK are both required for normal chondrocyte function. The mechanism leading to reduced chondrocyte proliferation in the absence of ILK expression is not understood; altered phosphorylation of PKB/Akt or GSK-3β was excluded [39]. A conditional deletion or reduction of ILK gene expression in macrophages, on the other hand, results in a strong inhibition of the PKB/Akt-Ser473 phosphorylation associated with apoptosis [41•], indicating that ILK kinase activity might differ depending on the cell type.

Overexpression of ILK in mammary glands of transgenic mice leads to tumor formation [29]. Similarly, pharmacological inhibition of ILK in prostate carcinoma cells causes them to proliferate much less rapidly *in vivo* [57••]. These findings can principally be explained by the oncogenic activities of ILK (activation of PKB/Akt, inhibition of GSK-3β, and stimulation of AP-1, NF-κB and β-catenin–Lef/Tcf transcription factors) and its ability to promote tumor angiogenesis. ILK promotes blood vessel invasion into tumors in two ways: ILK induces HIF1α-dependent VEGF expression in tumor cells, which in turn regulates endothelial cell migration and proliferation in an ILK kinase-dependent manner [57••]. The importance of ILK for tumor pathology is underscored by the fact that a large number of malignant tumors display increased ILK levels and kinase activity [58], and in some tumor types ILK levels correlate with tumor grade [59,60].

Outlook

ILK has many interesting functional facets and work in both invertebrates and mice has revealed an essential role for ILK in development. There is a general consensus that ILK plays a central role in the reorganization of the F-actin cytoskeleton and its attachment to FAs. The role of ILK as a kinase is more controversial. Since a large number of ILK functions rely on kinase activity, including EMT, proliferation and VEGF expression, this controversy should urgently be settled. This can be assisted by solving the structure of the ILK kinase domain, using continued genetic approaches or the well-defined antibodies that have become available over the past few years. As has already been done in flies and worms, it should be tested in mice whether point mutations in the kinase domain of ILK impair the function of the molecule.

An important future task will be to identify the signals that trigger assembly of the ILK/Pinch/parvin complex, to identify the proteins that recruit the core complex into FAs, and to establish how the core complex modulates integrin functions and regulates actin dynamics. The availability of cell lines and mice that lack ILK and the progress in proteomics and live cell imaging should together help to dissect these mechanisms and to clarify ILK's role in integrin-mediated cell adhesion.

Acknowledgements

We thank Drs. Kyle Legate, Cord Brakebusch and Haiyan Chu for discussion, comments and critical reading of the manuscript. The integrin/ILK work is supported by the DFG (SFB413), the Fonds der Chemischen Industrie and the Max Planck Society.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hynes RO: **Integrins: bidirectional, allosteric signaling machines**. *Cell* 2002, **110**:673-687.

2. Brakebusch C, Fässler R: **The integrin–actin connection, an eternal love affair**. *EMBO J* 2003, **22**:2324-2333.

3. Hannigan GE, Leung-Hagesteijn C, Fitz-Gibbon L, Coppolino MG, Radeva G, Filmus J, Bell JC, Dedhar S: **Regulation of cell adhesion and anchorage-dependent growth by a new β1-integrin-linked protein kinase**. *Nature* 1996, **379**:91-96.

4. Novak A, Hsu SC, Leung-Hagesteijn C, Radeva G, Papkoff J, Montesano R, Roskelley C, Grosschedl R, Dedhar S: **Cell adhesion and the integrin-linked kinase regulate the LEF-1 and β-catenin signaling pathways**. *Proc Natl Acad Sci USA* 1998, **95**:4374-4379.

5. Radeva G, Petrocelli T, Behrend E, Leung-Hagesteijn C, Filmus J, Slingerland J, Dedhar S: **Overexpression of the integrin-linked kinase promotes anchorage-independent cell cycle progression**. *J Biol Chem* 1997, **272**:13937-13944.

6. Wu C, Keightley SY, Leung-Hagesteijn C, Radeva G, Coppolino M, Goicoechea S, McDonald JA, Dedhar S: **Integrin-linked protein kinase regulates fibronectin matrix assembly, E-cadherin expression, and tumorigenicity**. *J Biol Chem* 1998, **273**:528-536.

7. •• Sakai T, Li S, Docheva D, Grashoff C, Sakai K, Kostka G, Braun A, Pfeifer A, Yurchenco PD, Fässler R: **Integrin-linked kinase (ILK) is required for polarizing the epiblast, cell adhesion, and controlling actin accumulation**. *Genes Dev* 2003, **17**:926-940.
This paper analyzes the role of ILK during mouse development and in fibroblast cells. It demonstrates that ILK is required for F-actin reorganization, epiblast polarity and cavitation. Fibroblasts lacking ILK expression display reduced adhesion, spreading, F-actin distribution and proliferation but normal GSK3b and PKB/Akt phosphorylation.

8. Tu Y, Li F, Goicoechea S, Wu C: **The LIM-only protein PINCH directly interacts with integrin-linked kinase and is recruited to integrin-rich sites in spreading cells**. *Mol Cell Biol* 1999, **19**:2425-2434.

9. Zhang Y, Chen K, Guo L, Wu C: **Characterization of PINCH-2, a new focal adhesion protein that regulates the PINCH-1-ILK**

interaction, cell spreading, and migration. *J Biol Chem* 2002, **277**:38328-38338.

10. Braun A, Bordoy R, Stanchi F, Moser M, Kostka G, Ehler E, Brandau O, Fässler R: **PINCH-2 is a new five LIM domain protein, homologous to PINCH and localized to focal adhesions**. *Exp Cell Res* 2003, **284**:239-250.

11. Velyvis A, Yang Y, Wu C, Qin J: **Solution structure of the focal adhesion adaptor PINCH LIM1 domain and characterization of its interaction with the integrin-linked kinase ankyrin repeat domain**. *J Biol Chem* 2001, **276**:4932-4939.

12. Tu Y, Li F, Wu C: **Nck-2, a novel Src homology2/3-containing adaptor protein that interacts with the LIM-only protein PINCH and components of growth factor receptor kinase-signaling pathways**. *Mol Biol Cell* 1998, **9**:3367-3382.

13. Velyvis A, Vaynberg J, Yang Y, Vinogradova O, Zhang Y, Wu C, Qin J: **Structural and functional insights into PINCH LIM4 domain-mediated integrin signaling**. *Nat Struct Biol* 2003, **10**:558-564.

14. Bladt F, Aippersbach E, Gelkop S, Strasser GA, Nash P, Tafuri A, Gertler FB, Pawson T: **The murine Nck SH2/SH3 adaptors are important for the development of mesoderm-derived embryonic structures and for regulating the cellular actin network**. *Mol Cell Biol* 2003, **23**:4586-4597.

15. Nikolopoulos SN, Turner CE: **Integrin-linked kinase (ILK) binding to paxillin LD1 motif regulates ILK localization to focal adhesions**. *J Biol Chem* 2001, **276**:23499-23505.

16. Nikolopoulos SN, Turner CE: **Actopaxin, a new focal adhesion protein that binds paxillin LD motifs and actin and regulates cell adhesion**. *J Cell Biol* 2000, **151**:1435-1448.

17. Olski TM, Noegel AA, Korenbaum E: **Parvin, a 42 kDa focal adhesion protein, related to the α-actinin superfamily**. *J Cell Sci* 2001, **114**:525-538.

18. Tu Y, Huang Y, Zhang Y, Hua Y, Wu C: **A new focal adhesion protein that interacts with integrin-linked kinase and regulates cell adhesion and spreading**. *J Cell Biol* 2001, **153**:585-598.

19. Yamaji S, Suzuki A, Sugiyama Y, Koide Y, Yoshida M, Kanamori H, Mohri H, Ohno S, Ishigatsubo Y: **A novel integrin-linked kinase-binding protein, affixin, is involved in the early stage of cell–substrate interaction**. *J Cell Biol* 2001, **153**:1251-1264.

20. Rosenberger G, Jantke I, Gal A, Kutsche K: **Interaction of αPIX (ARHGEF6) with β-parvin (PARVB) suggests an involvement of αPIX in integrin-mediated signaling**. *Hum Mol Genet* 2003, **12**:155-167.

21. •• Mackinnon AC, Qadota H, Norman KR, Moerman DG, Williams BD: ***C.elegans* PAT-4/ILK functions as an adaptor protein within integrin adhesion complexes**. *Curr Biol* 2002, **12**:787-797.
Loss of PAT-4/ILK leads to muscle detachment. Genetic studies place PAT-4/ILK downstream of b-PAT-3/intgerin and upstream of UNC-97/Pinch.

22. Rogalski TM, Mullen GP, Gilbert MM, Williams BD, Moerman DG: **The *UNC-112* gene in *Caenorhabditis elegans* encodes a novel component of cell–matrix adhesion structures required for integrin localization in the muscle cell membrane**. *J Cell Biol* 2000, **150**:253-264.

23. • Tu Y, Wu S, Shi X, Chen K, Wu C: **Migfilin and Mig-2 link focal adhesions to filamin and the actin cytoskeleton and function in cell shape modulation**. *Cell* 2003, **113**:37-47.
This paper describes a new ILK adaptor protein termed migfilin, which links Mig-2/ILK to filamin. Knockdown of migfilin leads to altered cell shape, reduced migration and abnormal F-actin distribution. These defects resemble the phenotype of ILK-deficient cells.

24. • Zhang Y, Chen K, Tu Y, Velyvis A, Yang Y, Qin J, Wu C: **Assembly of the PINCH–ILK–CH–ILKBP complex precedes and is essential for localization of each component to cell–matrix adhesion sites**. *J Cell Sci* 2002, **115**:4777-4786.
This paper shows that ILK/Pinch/parvin have formed a ternary complex already prior to recruitment into FAs.

25. • Fukuda T, Chen K, Shi X, Wu C: **PINCH-1 is an obligate partner of integrin-linked kinase (ILK) functioning in cell shape modulation, motility, and survival**. *J Biol Chem* 2003, **278**:51324-51333.
Knockdown experiments show that Pinch and ILK are obligate partners and if not associated will be degraded by the proteasome.

26. Delcommenne M, Tan C, Gray V, Rue L, Woodgett J, Dedhar S: **Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase**. *Proc Natl Acad Sci USA* 1998, **95**:11211-11216.

27. Persad S, Attwell S, Gray V, Delcommenne M, Troussard A, Sanghera J, Dedhar S: **Inhibition of integrin-linked kinase (ILK) suppresses activation of protein kinase B/Akt and induces cell cycle arrest and apoptosis of PTEN-mutant prostate cancer cells**. *Proc Natl Acad Sci USA* 2000, **97**:3207-3212.

28. Attwell S, Roskelley C, Dedhar S: **The integrin-linked kinase (ILK) suppresses anoikis**. *Oncogene* 2000, **19**:3811-3815.

29. White DE, Cardiff RD, Dedhar S, Muller WJ: **Mammary epithelial-specific expression of the integrin-linked kinase (ILK) results in the induction of mammary gland hyperplasias and tumors in transgenic mice**. *Oncogene* 2001, **20**:7064-7072.

30. Tan C, Costello P, Sanghera J, Dominguez D, Baulida J, de Herreros AG, Dedhar S: **Inhibition of integrin linked kinase (ILK) suppresses β-catenin-Lef/Tcf-dependent transcription and expression of the E-cadherin repressor, snail, in APC$^{-/-}$ human colon carcinoma cells**. *Oncogene* 2001, **20**:133-140.

31. Cohen P, Frame S: **The renaissance of GSK3**. *Nat Rev Mol Cell Biol* 2001, **2**:769-776.

32. Lawlor MA, Alessi DR: **PKB/Akt: a key mediator of cell proliferation, survival and insulin responses?** *J Cell Sci* 2001, **114**:2903-2910.

33. Troussard AA, Tan C, Yoganathan TN, Dedhar S: **Cell–extracellular matrix interactions stimulate the AP-1 transcription factor in an integrin-linked kinase- and glycogen synthase kinase 3-dependent manner**. *Mol Cell Biol* 1999, **19**:7420-7427.

34. Novak A, Dedhar S: **Signaling through β-catenin and Lef/Tcf**. *Cell Mol Life Sci* 1999, **56**:523-537.

35. Persad S, Attwell S, Gray V, Mawji N, Deng JT, Leung D, Yan J, Sanghera J, Walsh MP, Dedhar S: **Regulation of protein kinase B/Akt-serine 473 phosphorylation by integrin-linked kinase: critical roles for kinase activity and amino acids arginine 211 and serine 343**. *J Biol Chem* 2001, **276**:27462-27469.

36. Balendran A, Casamayor A, Deak M, Paterson A, Gaffney P, Currie R, Downes CP, Alessi DR: **PDK1 acquires PDK2 activity in the presence of a synthetic peptide derived from the carboxyl terminus of PRK2**. *Curr Biol* 1999, **9**:393-404.

37. • Hill MM, Feng J, Hemmings BA: **Identification of a plasma membrane Raft-associated PKB Ser473 kinase activity that is distinct from ILK and PDK1**. *Curr Biol* 2002, **12**:1251-1255.
This paper shows that ILK is part of a protein complex with PKB/Akt Ser473 activity. Interestingly, however, the kinase activity is distinct from ILK.

38. Zervas CG, Gregory SL, Brown NH: ***Drosophila* integrin-linked kinase is required at sites of integrin adhesion to link the cytoskeleton to the plasma membrane**. *J Cell Biol* 2001, **152**:1007-1018.

39. Grashoff C, Aszodi A, Sakai T, Hunziker EB, Fässler R: **Integrin-linked kinase regulates chondrocyte shape and proliferation**. *EMBO Rep* 2003, **4**:432-438.

40. Lynch DK, Ellis CA, Edwards PA, Hiles ID: **Integrin-linked kinase regulates phosphorylation of serine 473 of protein kinase B by an indirect mechanism**. *Oncogene* 1999, **18**:8024-8032.

41. • Troussard AA, Mawji NM, Ong C, Mui A, St -Arnaud R, Dedhar S: **Conditional knock-out of integrin-linked kinase demonstrates an essential role in protein kinase B/Akt activation**. *J Biol Chem* 2003, **278**:22374-22378.
Conditional deletion of ILK in monocytes/macrophages results in reduced phosphorylation of Ser473 of PKB/Akt, indicating that the Ser473 activity of ILK may occur in a cell-type-dependent manner.

42. Deng JT, Van Lierop JE, Sutherland C, Walsh MP: **Ca^{2+}-independent smooth muscle contraction. A novel function for integrin-linked kinase**. *J Biol Chem* 2001, **276**:16365-16373.

43. Kiss E, Muranyi A, Csortos C, Gergely P, Ito M, Hartshorne DJ, Erdodi F: **Integrin-linked kinase phosphorylates the myosin phosphatase target subunit at the inhibitory site in platelet cytoskeleton**. *Biochem J* 2002, **365**:79-87.

44. Muranyi A, MacDonald JA, Deng JT, Wilson DP, Haystead TA, Walsh MP, Erdodi F, Kiss E, Wu Y, Hartshorne DJ: **Phosphorylation of the myosin phosphatase target subunit by integrin-linked kinase**. *Biochem J* 2002, **366**:211-216.

45. Deng JT, Sutherland C, Brautigan DL, Eto M, Walsh MP: **Phosphorylation of the myosin phosphatase inhibitors, CPI-17 and PHI-1, by integrin-linked kinase**. *Biochem J* 2002, **367**:517-524.

46. • Nikolopoulos SN, Turner CE: **Molecular dissection of actopaxin-integrin-linked kinase–paxillin interactions and their role in subcellular localization**. *J Biol Chem* 2002, **277**:1568-1575.
This report identifies a paxillin binding site in the ILK kinase domain. The interaction of ILK with paxillin is essential for the recruitment of ILK into FAs. Furthermore, the paper provides evidence that the E359K mutation in ILK affects paxillin binding but not the kinase activity.

47. Ahmed N, Oliva K, Rice GE, Quinn MA: **Cell-free 59 kDa immunoreactive integrin-linked kinase: a novel marker for ovarian carcinoma**. *Clin Cancer Res* 2004, **10**:2415-2420.

48. Hobert O, Moerman DG, Clark KA, Beckerle MC, Ruvkun G: **A conserved LIM protein that affects muscular adherens junction integrity and mechanosensory function in *Caenorhabditis elegans***. *J Cell Biol* 1999, **144**:45-57.

49. • Lin X, Qadota H, Moerman DG, Williams BD: ***C. elegans* PAT-6/actopaxin plays a critical role in the assembly of integrin adhesion complexes *in vivo***. *Curr Biol* 2003, **13**:922-932.
A phenotype analysis of PAT-6/parvin-deficient nematodes. Genetic experiments place PAT-6/parvin downstream of PAT-4/ILK and UNC97/Pinch.

50. • Mercer KB, Flaherty DB, Miller RK, Qadota H, Tinley TL, Moerman DG, Benian GM: ***Caenorhabditis elegans* UNC-98, a C2H2 Zn finger protein, is a novel partner of UNC-97/PINCH in muscle adhesion complexes**. *Mol Biol Cell* 2003, **14**:2492-2507.
A Zn^{2+} finger protein called UNC-98 shuttles between FAs and nucleus and interacts with UNC-97/Pinch in dense bodies. The nuclear function of UNC-98 is unknown.

51. •• Clark KA, McGrail M, Beckerle MC: **Analysis of PINCH function in *Drosophila* demonstrates its requirement in integrin-dependent cellular processes**. *Development* 2003, **130**:2611-2621.
Analysis of Pinch-deficient flies demonstrates muscle detachment and wing blisters. Pinch localization to muscle attachment sites is integrin-dependent, whereas ILK localization to integrin attachment sites is not Pinch-dependent. This indicates that in *Drosophila*, ILK and Pinch are not obligate binding partners.

52. Fässler R, Meyer M: **Consequences of lack of β1 integrin gene expression in mice**. *Genes Dev* 1995, **9**:1896-1908.

53. Aumailley M, Pesch M, Tunggal L, Gaill F, Fässler R: **Altered synthesis of laminin 1 and absence of basement membrane component deposition in b1 integrin-deficient embryoid bodies**. *J Cell Sci* 2000, **113**:259-268.

54. Li S, Harrison D, Carbonetto S, Fässler R, Smyth N, Edgar D, Yurchenco PD: **Matrix assembly, regulation, and survival functions of laminin and its receptors in embryonic stem cell differentiation**. *J Cell Biol* 2002, **157**:1279-1290.

55. Terpstra L, Prud'homme J, Arabian A, Takeda S, Karsenty G, Dedhar S, St-Arnaud R: **Reduced chondrocyte proliferation and chondrodysplasia in mice lacking the integrin-linked kinase in chondrocytes**. *J Cell Biol* 2003, **162**:139-148.

56. Aszodi A, Hunziker EB, Brakebusch C, Fässler R: **β1 integrins regulate chondrocyte rotation, G1 progression, and cytokinesis**. *Genes Dev* 2003, **17**:2465-2479.

57. •• Tan C, Cruet-Hennequart S, Troussard A, Fazli L, Costello P, Sutton K, Wheeler J, Gleave M, Sanghera J, Dedhar S: **Regulation of tumor angiogenesis by integrin-linked kinase (ILK)**. *Cancer Cell* 2004, **5**:79-90.
This paper demonstrates a dual role for ILK in tumor growth. ILK in tumor cells promotes VEGF release leading to the recruitment of endothelial cells, and ILK in endothelial cells supports their migration and proliferation in a PKB/Akt-dependent manner.

58. Marotta A, Parhar K, Owen D, Dedhar S, Salh B: **Characterisation of integrin-linked kinase signalling in sporadic human colon cancer**. *Br J Cancer* 2003, **88**:1755-1762.

59. Graff JR, Deddens JA, Konicek BW, Colligan BM, Hurst BM, Carter HW, Carter JH: **Integrin-linked kinase expression increases with prostate tumor grade**. *Clin Cancer Res* 2001, **7**:1987-1991.

60. Dai DL, Makretsov N, Campos EI, Huang C, Zhou Y, Huntsman D, Martinka M, Li G: **Increased expression of integrin-linked kinase is correlated with melanoma progression and poor patient survival**. *Clin Cancer Res* 2003, **9**:4409-4414.

61. Pasquet JM, Noury M, Nurden AT: **Evidence that the platelet integrin αIIbβ3 is regulated by the integrin-linked kinase, ILK, in a PI3-kinase dependent pathway**. *Thromb Haemost* 2002, **88**:115-122.

62. Leung-Hagesteijn C, Mahendra A, Naruszewicz I, Hannigan GE: **Modulation of integrin signal transduction by ILKAP, a protein phosphatase 2C associating with the integrin-linked kinase ILK1**. *EMBO J* 2001, **20**:2160-2170.