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Parvin-ILK

An intimate relationship

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Integrin-linked kinase (ILK), PINCH and Parvin proteins form the IPP-complex that has been established as a core component of the integrin-actin link. Our recent genetic studies on Drosophila parvin, reveal that loss of function mutant defects phenocopy those observed upon loss of ILK or PINCH in the muscle and the wing, strengthening the notion that these proteins function together in the organism. Our work identified that ILK is necessary and sufficient for parvin subcellular localization, corroborating previous data indicating a direct association between these two proteins. Further genetic epistasis analysis of the IPP-complex assembly at integrin adhesion sites reveals that depending on the cell context each component is required differently. At the muscle attachment sites of the embryo, ILK is placed upstream in the hierarchy of genetic interactions required for the IPP-complex assembly. By contrast, in the wing epithelium the three proteins are mutually interdependent. Finally, we uncovered a novel property for the CH1-domain of parvin: its recruitment at the integrin-containing junctions in an ILK-dependent manner. Apparently, this ability of the CH1-domain is controlled by the inter-CH linker region. Thus, an intramolecular interaction within parvin could serve as a putative regulatory mechanism controlling the ILK-Parvin interaction.

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Cell-matrix adhesion in multicellular organisms is mediated primarily by the integrin family of heterodimeric transmembrane receptors.[1] Through the extracellular domain of their α and β subunits integrins are able to connect to ligands of the extracellular matrix (ECM). Inside the cell the cytoplasmic tails of both integrin subunits orchestrate the assembly of a multi-protein network—the integrin adhesome—which provides docking sites for the actin cytoskeleton.[2] Thus, a structural continuity between the extracellular microenvironment and the cytoskeleton is achieved facilitating the cell-matrix adhesion. One of the essential and evolutionary conserved modules participating in the integrin-adhesome is the IPP-protein complex, comprised of integrin-linked kinase (ILK), PINCH and Parvins.[3,4]

In vertebrates, the parvin family is represented by three members (α-, β- and γ-parvin), whereas in invertebrates there is only one ortholog gene.[4] Parvins consist of an N-terminal region with no obvious homology to other known functional domains, followed by two calponin homology (CH) domains tandemly arranged and separated by a rather long linker sequence in the C-terminal region, similar to fimbrin.[5] Although considered as "atypical," both parvin CH domains -designated CH1 and CH2- constitute the most distinct feature of this protein family, that enables them to bind F-actin.[6-9] Among the three parvin genes, only deletion of α-parvin results in embryonic lethality in mice, although β-parvin may provide functional compensation at earlier developmental stages.[10,11] Interestingly, γ-parvin whose expression is restricted to hematopoietic cells is dispensable.[12]

Representing relatively simple systems, with the advantage of having sufficient anatomical complexity, but less redundancy by closely related proteins,

Drosophila provides an ideal model to study integrin-mediated processes.[13] The characterization of IPP-complex function in Drosophila has been recently completed by our genetic studies of the single fly parvin gene.[14] Phenotypic analysis of parvin null mutant embryos reveals that actin filaments are not able to maintain their connection with the muscle ends. This muscle phenotype is remarkably similar to that of previously described integrin hypomorphic mutations[15] and identical to the ILK and PINCH loss of function mutants.[16,17] Thus, it demonstrates that parvin is essential for the completion of embryogenesis being required in the molecular machinery that mediates the integrin-actin link at muscle attachment sites. Both the muscle defects and the lethality associated with parvin mutants can be completely rescued to adult viability, upon substitution of moderate protein levels in muscle and tendon cells. However, the rescued mutant flies carry wing blisters—in agreement with similar observations regarding compromised integrin function in the wing epithelium[16,18]—implying that parvin is also involved in the maintenance of adhesion between the two wing surfaces.

The pattern of endogenous parvin distribution in the early embryo reveals minimal levels of parvin expression in the amnioserosa and the leading edge of epidermal cells during dorsal closure. After completion of the germband retraction parvin increasingly accumulates in mesoderm-derived tissues while in later developmental stages it is found specifically localized at the tips of muscle myosin filaments at muscle attachment sites. Being heavily accumulated at muscle attachment sites, parvin does not appear to co-distribute with actin filaments in the living fly embryo therefore, its role as an actin-binding protein in vivo remains highly questionable. Given that mammalian parvins exhibit a differential ability for actin binding, Drosophila CH-domain interaction with F-actin appears to be either less favored or only occur in a highly regulated manner at sites of integrin adhesion.[19] To clarify this, specific point mutations within the putative actin binding domain of parvin should be tested in vivo.

At muscle attachment sites, parvin co-localizes both with βPS integrin and integrin adhesome components including, Talin, ILK, PINCH, Tensin and Paxillin. Interaction with ILK appears to be essential and sufficient for both parvin protein stability and subcellular localization. In agreement with previous reports,[20] endogenous protein levels are drastically reduced in the absence of ILK.

In our in vivo studies, we exploit the recruitment of individual CH-domains at integrin adhesion sites as an assay to identify the domain requirements of the parvin-ILK interaction. Structural data have previously illustrated the direct association between the kinase domain of ILK and the parvin CH2-domain.[21] Indeed, in Drosophila, the CH2 domain is also sufficient for parvin localization both at muscle attachment sites and around the cortex in epithelial cells. Our detailed functional analysis further reveals that CH1-domain stability and subcellular localization are also closely correlated with ILK levels suggesting an interaction that confers to fly parvin a second novel localization signal. Mutation analysis within the ILK kinase domain enables us to predict that, although with different affinity, both parvin CH domains share the same ILK binding site. Interestingly, our data suggests that the localization signal within CH1 can be masked by the linker sequence separating the two parvin CH domains. Given that parvin recruitment at muscle attachment sites is tightly regulated by ILK, this linker sequence might then function as a regulatory module ensuring that only high affinity interactions can occur between these two proteins. These data are in support of the hypothesis put forward by Gimona and colleagues[5] which suggests that although parvin contains a tandem CH-domain actin-binding site, its CH-domains and inter-CH domain linker sequence are so unique that this is a strong likelihood of alternative functions. The alternative hypothesis that instead of a direct interaction with ILK, recruitment of CH1 at muscle attachment sites is indirectly mediated by ILK could be a possibility that we favor less. To elucidate further the mode of ILK-parvin interaction in vivo, specific CH1-domain mutation analysis that could disrupt its ability to localize at muscle attachments sites is necessary.

The ILK-dependent parvin stability and recruitment at muscle attachment sites becomes also apparent when high levels of the protein are ectopically expressed. Distinct to either ILK or PINCH behavior, elevated amounts of parvin—upon overexpression in the muscle using the UAS/GAL4 system—result in cytoplasmic accumulation of the protein and induces dominant lethality (**Fig. 1A**). Actually, dominant effects including lethality are also observed upon parvin overexpression in a variety of tissues and developmental stages examined (our unpublished results). Parvin accumulation in the muscle cytoplasm could be possibly explained as a consequence of limited binding sites available for parvin at muscle attachment sites. Interestingly, such a limitation can be overcome once elevated ILK protein levels are simultaneously available (**Fig. 1B**). Complete reversion of the dominant negative phenotype suggests the requirement of tight regulation of parvin levels along with a crucial stoichiometric protein dependence on ILK.

Although parvin recruitment and subcellular localization depends on ILK, parvin absence appears not to affect the localization of any of its interacting proteins namely ILK, paxillin, α-actinin. Nor does it have an influence on integrin associated proteins like PINCH, tensin and zyxin or FAK activation. These data show that although parvin is a central player in the function of integrin adhesion machinery, it does not participate in the assembly of the integrin-actin linker complex. It is rather involved in strengthening the integrin-actin link at muscle attachment sites. Interestingly, in a different tissue, the wing epithelium, our genetic studies show that interactions among the IPP-complex components differ and, like in mammalian cells, the three proteins are mutually dependent for their stability and subcellular localization at sites of integrin adhesion.[22] Indeed, integrin-actin link components are shown to be variably used in different tissues and developmental processes and can be differentially affected upon genetically induced perturbations.[23]

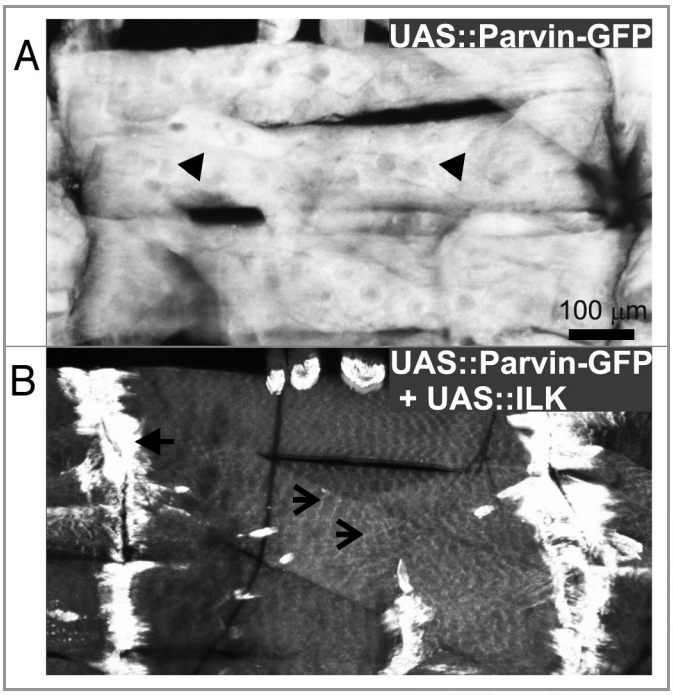

Figure 1. Muscle attachment site recruitment of overexpressed Parvin-GFP upon coexpression with ILK in third instar larvae. Projections of confocal sections of lateral longidutinal muscles from 3rd instar larvae expressing under the *mef2Gal4* driver, UAS::Parvin-GFP alone (A) or with wild type UAS::ILK (B). Parvin-GFP was observed at muscle attachment sites (arrow), in the cytoplasm (arrowhead) and at the Z-lines (open arrows).

Our findings also highlight that the two well established models of integrin-mediated adhesion in Drosophila, namely the muscle attachment sites and the basal side of the wing epithelium, do exhibit diverse molecular strategies capable to mediate distinct adhesion properties in the developing organism.

Could these findings imply differential involvement of each of the IPP components in the establishment of the integrin-actin link? Phenotypic analysis of null mutants for all the three proteins comprising the IPP-complex in the embryo shows muscle defects indistinguishable from those characterizing the loss of either ILK, PINCH or Parvin. Hence, it clearly indicates that at the muscle attachment sites of the Drosophila embryo, IPP-complex proteins work together to facilitate stable linkage of actin to the integrin-containing junctions. This IPP-complex function is however compromised once a component is missing. ILK functions as a key player in the stability and recruitment of both PINCH and parvin.[14,24] While for parvin our findings suggest a direct interaction with ILK, a recent report shows that a direct association of ILK with PINCH is in fact not essential.[25] Thus, the IPP-complex as a physical entity has to be revisited and include an unidentified factor that mediates PINCH recruitment at integrin adhesion sites in an ILK-dependent manner.

Perspective

Our on-going research is focused on elucidating the molecular mechanism by which parvin functions in the developing organism. The "IPP-complex" research community has experienced several surprises over the last decade, and certainly we anticipate more to follow from the parvus (latin word for small) point of view.

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