Components of cell-matrix adhesions

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Focal adhesions (FAs), also known as ‘focal contacts’, are specialized, extracellular matrix (ECM) attachment and signaling organelles, usually measuring a few square microns, located along the ventral plasma membrane of adherent cultured cells. Interference reflection microscopy, electron microscopy and micro-mechanical measurements indicate that the cell membrane in these sites is tightly connected with the substrate surface and that the gap remaining is only ~10-15 nm. At their cytoplasmic aspects, FAs are associated with bundles of actin microfilaments and apparently play an important role in cell spreading and migration.

FAs are transmembrane anchorage sites, in which the ECM is indirectly linked to the actin cytoskeleton via a web of submembrane ‘anchor proteins’. Thus, characterization of the molecular components of these adhesions and their precise organization appears to be important if we are to understand the function of FAs in both the mechanical interaction with the matrix and the generation and transduction of adhesion-mediated signals. Immunofluorescence and immunoelectron microscopy studies have revealed that FAs contain a surprisingly large number of proteins. The major transmembrane ECM receptors in these sites belong to the integrin family, but additional transmembrane molecules, such as heparan sulfate proteoglycans, the hyaluronan binding molecule layilin and urokinase receptor, appear also to be present. Integrins are heterodimers of α and β subunits, each of which contains a large extracellular domain responsible for ligand binding, a single transmembrane domain and a cytoplasmic domain that can interact with the cytoskeleton. There are several α- and β-subunits, the combination of which defines the binding specificity to the ECM.

Across the membrane, FAs contain a meshwork of ‘anchor proteins’ that link the membrane to the actin cytoskeleton. As can be seen in the poster, the molecular complexity of this ‘submembrane plaque’ is enormous, and >50 different proteins have been reported to localize to it. Given the rather sporadic manner at which FA components were discovered over more than two decades, it appears likely that the actual number of molecular residents of FA (constitutive and transient) is considerably larger. Given modern tools that allow cell-based functional genomic screening, it is likely that the full repertoire of FA molecules will be soon unraveled.
The molecular complexity of the FA is probably considerably greater than that shown, not just because many of the components are still unknown but because many of these molecules can appear in more than one form - owing to post-translational modification, proteolytic processing, alternative splicing and conformational reorganization. For example, tyrosine-specific protein phosphorylation, one of the hallmarks of FAs, can affect binding to SH2 domains in different proteins, and PtdIns(4,5)P2-induced conformational changes in vinculin can alter its interactions with some of its partners (see below).

Let us take a closer look at the known components of FA. Sorting these proteins according to their biochemical activity reveals among them cytoskeletal proteins (defined by their direct or indirect association with actin and the absence of enzymatic activity), such as tensin, vinculin, paxillin, α-actinin, parvin/actopaxin and talin, tyrosine kinases, such as members of the Src family, FAK, PYK2, Csk and Abl, serine/threonine kinases, such as ILK, PKC and PAK, modulators of small GTPases, such as ASAP1, Graf and PSGAP, phosphatases, such as SHP-2 and LAR PTP, and other enzymes, such as PI 3-kinase and the protease calpain II. Are all these components constitutively located in all ECM adhesions in all cells? Probably not, because many of the components may be expressed in a cell-type-specific manner, and others may be differentially enriched in specific subtypes of FA or FA-related adhesions, as fibrillar adhesions, podosomes or focal complexes. Thus, the scheme shown should not be regarded as a representative snapshot of FA architecture.

What are the molecular interactions that take place between FA components? This question can be slightly rephrased according to the experimental approach selected to tackle it. One can study biochemically the repertoire of interactions that the various molecules can participate in, study those that actually occur in living cells or specifically address the order in which they take place during the adhesive process. In vitro binding studies have revealed a multitude of protein-protein interactions between the various components, as well as interactions between these components and molecules not stably localized in FA. As the poster shows, some of the interactions within FAs are mediated by known binding motifs, such as SH2 and SH3 domains, but the majority of binding motifs are still poorly characterized. Note that some of the interactions (e.g. those between SH2 domains and tyrosine phosphorylated proteins) can be regulated by specific enzymatic activities (tyrosine phosphorylation or dephosphorylation, in this case) and thus may be switched on and off.

Evidently, many of the components of FA can interact with more than one (often 5-10 or more) molecular partner; this suggests that they can - at least in theory - assemble in numerous alternative combinations, giving rise to highly diverse supramolecular complexes. However, only a fraction of the interactions identified by biochemical studies can actually take place simultaneously, since many of the partners either compete at the same site or stericly interfere with the binding of other molecules to nearby locations (see, for example, the interactions of many anchor proteins with the short cytoplasmic tail of integrin, the multiple SH2- and SH3-mediated interactions of FAK etc.). This notion does not really 'simplify' the picture but instead suggests that FAs are a molecularly diverse family of structures or consist of a heterogeneous array of supramolecular complexes. Such heterogeneity could, of course, affect the function of the specific adhesion. Indeed, quantitative microscopy indicates that adhesion sites can be molecularly heterogeneous structures that have diverse morphologies, molecular compositions and phosphotyrosine levels (e.g. fibrillar adhesions, focal complexes and podosomes). Given the relatively small number of morphological and molecular variants detected so far, however, some sets of molecular interactions must be particularly robust, leading to the formation of specific networks of components typical to each variant form.

In conclusion, characterization of the molecular complexity of matrix adhesions appears to provide important insights into the structural and functional diversity of these sites in living cells. As indicated here, the known repertoire of FA components is probably incomplete, and the information on their assembly following interaction with the ECM is still very limited. The greatest challenge in future characterization of these sites will be to understand the mechanisms of adhesion-mediated signaling in these sites and how this affects cell behavior and fate.

Abbreviations, synonyms and approximate molecular weights: α-actinin, 103 kDa; Abl, 145 kDa; Actopxin/Parvin, 42 kDa; AKT (PKB), 55 kDa; AND-34, 93 kDa; ASAP1, 130 kDa; C3G, 145-155 kDa; Calpain II, 80 kDa and 30 kDa subunits; AS; Crk-associated substrate, 130 kDa; CASK, calcium/calmodulin-dependent serine protein kinase, 104 kDa; Caveolin-1, 21-24 kDa; Chl, 120 kDa; CH-ILKBP, calpin homology domain-containing ILK-binding protein, 53 kDa; Crk, 33 kDa; Crpl, cystein-rich protein-1, 23 kDa; Csk, C-terminal Src kinase, 50 kDa; Dbl (MCE-2), 115 kDa (proto-Dbl) or 66 kDa (Dbl); DOCK180, 180 kDa protein downstream of Crk, 180 kDa; DRAL, downregulated in rhabdomyosarcoma LIM-protein (FHL2 and SLIM3), 32 kDa; EAST, EGFR-associated protein with SH3 and TAM domains, 68 kDa; ECM proteins, extracellular matrix proteins (a wide variety of proteins such as fibronectin, vitronectin and collagen); ERM family, ezrin radixin moesin, 68-69 kDa; F-Ac tin, filamentous (polymerized) actin; FAK, focal adhesion kinase, 125 kDa; Filamin, 280 kDa; Flimbin, 68-70 kDa; G-Ac tin, globular (monomeric) actin, 42 kDa; Graf, GTPase regulator associated with FAK, 95 kDa; Grb2, growth-factor-receptor-bound protein 2, 25 kDa; Grb7, growth-factor-receptor-bound protein 7, 59 kDa; Heparan-sulfate binding proteins, many ECM molecules, growth factors, protease inhibitors, lipoproteins and others; Hic-5, hydrogen-peroxide-inducible clone 5, 55 kDa; Hyaluronan, hyaluronic acid (−(β1,4-GlcUA−β1,3-GlcNAc)−), 10000-10000 kDa; ILK, integrin-linked kinase, 59 kDa; Integrins, variable αβ heterodimer receptors of ECM proteins; IR, insulin receptor, oligotetramer composed of two heterodimers of 130 kDa and 90 kDa subunits; IRS-1, insulin receptor substate 1, 185 kDa; LAR PTP, leukocyte-common-antigen-related protein tyrosine phosphatase, 200 kDa (protoeolytically processed into 150 kDa and 85 kDa subunits); Laylin, 55 kDa; LIP-1, LAR-interacting protein 1, 160 kDa; Nck-2, 83 kDa; Nexillin, 72-78 kDa; PAK, p21 activated kinase, 85-60 kDa; Palladin, 90-92 kDa; Paxillin, 68 kDa; PI 3-K, phosphoinositide 3-kinase, 85 kDa and 110 kDa subunits; PINCH, particularly interesting new Cys-His protein, 35-40 kDa; PtdIns(4,5)P2, phosphatidylinositol 4,5-bisphosphate; PIX, PAK-interacting exchange factor, 85 kDa; PKC, protein kinase C, 78 kDa; PKL, paxillin kinase linker, 95 kDa; PLC-γ, phospholipase C-γ, 148 kDa; Polycystin-1, the product of the polycystic kidney disease gene-1 (PKD1), 462 kDa; Ponsin (SH3P12 and CAP), 93 kDa; Profilin, 14 kDa; PSGAP, PH-
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