Molecular complexity and dynamics of cell-matrix adhesions

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Summary

Currently >50 proteins have been reported to be associated with focal contacts and related ECM adhesions. Most of these contain multiple domains through which they can interact with different molecular partners, potentially forming a dense and heterogeneous protein network at the cytoplasmic faces of the adhesion site. The molecular and structural diversity of this 'submembrane plaque' is regulated by a wide variety of mechanisms, including competition between different partner proteins for the same binding sites, interactions triggered or suppressed by tyrosine phosphorylation, and conformational changes in component proteins, which can affect their reactivity. Indeed, integrin-mediated adhesions can undergo dynamic changes in structure and molecular properties from dot-like focal complexes to stress-fiber-associated focal contacts, which can further 'mature' to form fibronectin-bound fibrillar adhesions. These changes are driven by mechanical force generated by the actin- and myosin-containing contractile machinery of the cells, or by external forces applied to the cells, and regulated by matrix rigidity.

Key words: Focal contacts, Focal adhesions, Cytoskeleton, Adhesion-mediated signalling, Extracellular matrix

Introduction

Structurally defined adhesion sites between cultured cells and the extracellular matrix (ECM) were initially described about 30 years ago in studies using interference-reflection microscopy and electron microscopy (Abercrombie and Dunn, 1975; Abercrombie et al., 1971; Izzard and Lochner, 1976; Izzard and Lochner, 1980). These studies revealed that matrix adhesion occurs at many specialized, elongated small regions (usually a few microns in length) along the ventral plasma membrane, which are tightly connected with the substrate and leave a gap of only ~10-15 nm. Moreover, these sites, which were termed focal contacts or focal adhesions, are associated with actin microfilaments at their cytoplasmic aspects and apparently play an important role in the regulation of actin organization, thereby affecting cell spreading, morphogenesis and migration.

Complexity

Immunofluorescence and immunoelectron microscopy revealed that focal contacts contain a surprisingly large number of proteins. The major transmembrane ECM receptors in these sites belong to the integrin family (Fig. 1). Integrins are heterodimers of α and β subunits that contain a large extracellular domain responsible for ligand binding, a single transmembrane domain and a cytoplasmic domain (Hynes, 1992; Schwartz et al., 1995). There are several α- and β-subunit isoforms, and the exact subunit combination of these dictates the binding specificity of the integrin to different ECM components. A specific ECM molecule can nevertheless be bound by different types of integrin, and specific integrins can bind to different types of ECM molecule. In addition to integrins, several membrane molecules were recently reported to localize to focal contacts, including proteoglycans (Woods and Couchman, 1994; Woods and Couchman, 1998; Woods et al., 2000; Zimmermann and David, 1999), glycosaminoglycan receptors (Bono et al., 2001; Borowsky and Hynes, 1998), as well as signaling molecules (Myohanen et al., 1993; Tang et al., 1998; Wei et al., 1999; Yebra et al., 1999); however, the role of these components in mediating or regulating adhesion is unclear.

A current molecular inventory of focal contacts is schematically presented in Fig. 1 (area boxed in green) and the accompanying Cell Science at a Glance poster in this issue (Zamir and Geiger, 2001). In fact, the molecular complexity of this site is probably considerably greater, since many of the components are still unknown, and others can be post-translationally modified and/or proteolytically processed, undergo conformational changes and exist as a series of splice variants. Sorting this group of proteins according to their presumed functions reveals cytoskeletal proteins (e.g. tensin, vinculin, paxillin, α-actinin, parvin/actopaxin and talin), tyrosine kinases (Fig. 1, red-colored proteins; e.g. Src, FAK, PYK2, Csk and Abl), serine/threonine kinases (Fig. 1, purple-colored proteins; e.g. ILK, PKC and PAK), modulators of small GTPases (Fig. 1, pink-colored proteins; e.g. ASAP1, Graf and PSGAP), tyrosine phosphatases (Fig. 1, orange-colored proteins; e.g. SHP-2 and LAR PTP) and other enzymes (Fig. 1, dark-yellow-colored proteins; e.g. PI 3-kinase and the protease calpain II). Some of these proteins can directly bind, cap, bundle or nucleate actin filaments (e.g. vinculin, tensin, α-actinin, VASP, parvin/actopaxin and ERM-proteins) and/or directly bind to the cytoplasmic tails of integrins (e.g. talin, tensin, FAK, ILK and α-actinin). Moreover, many of the components shown in Fig. 1 and their variants are expressed in a cell-type restricted fashion, introducing yet another level

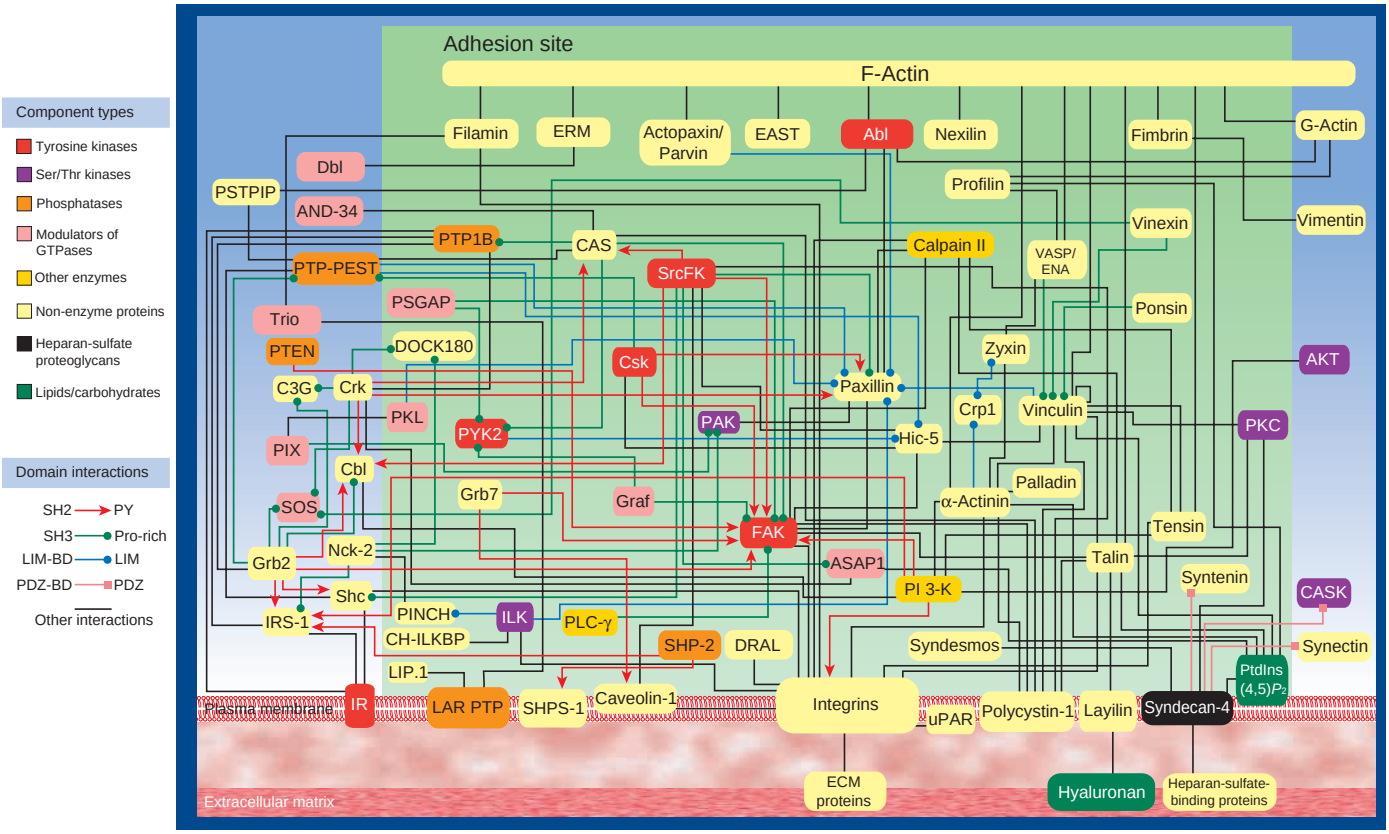

Fig. 1. A scheme summarizing known interactions between the various constituents of cell-matrix adhesions. Components that were found to be associated with cell-matrix adhesion sites are placed inside the internal green box, whereas additional selected proteins that affect matrix adhesions but were not reported to stably associate with them are placed in the external blue frame. The general property of each component is indicated by the color of its box, and the type of interaction between the components is indicated by the style and color of the interconnecting lines, as indicated at the legend. For further details about this scheme see Cell Science at a Glance in this issue (Zamir and Geiger, 2001).

of complexity. Does this list include the full repertoire of focal contact molecules? Probably not, since the search for such molecules until now has been neither systematic nor comprehensive, and the discovery of new molecules has usually been based on fortuitous immunolabeling, biochemical or immunochemical binding assays or two-hybrid screens. Looking towards the future, we imagine that the development of tagged cDNA libraries of all the proteins encoded in the human genome, together with the development of high-throughput microscopy for visual screening of transfected cells, will help to extend this list.

Following the biochemical tradition, many research groups have attempted to characterize the interactions between the different focal contact molecules, hoping that such information might help us understand how this molecular ensemble works. Such in vitro binding studies have revealed a multitude of protein-protein interactions that might take place in these contact sites (Fig. 1). Some of the interactions within focal contacts are mediated by known binding motifs, such as SH2 and SH3 domains, which serve as specific docking sites for tyrosine-phosphorylated proteins (which can be regulated by kinases and phosphatases) and proline-rich domains, respectively. For example, FAK contains tyrosines that upon phosphorylation can bind to the SH2 domains of several molecules, including Src kinases, Csk, PTEN, Grb2, Grb7 and PI 3-kinase, and proline-rich domains that can bind to Cas, Graf, PSGAP and PLC-γ. Obviously, FAK cannot be engaged with all these molecules simultaneously, and the mechanism by which it selects its partners is obscure. Equally unclear are the specificity and susceptibility to external regulation of the other types of molecular interaction (direct binding or other regulatory interactions) that might occur in focal contacts (interconnecting lines in Fig. 1).

Since most components of focal adhesions contain multiple binding sites for other components, the molecular ensemble can, theoretically, assemble in numerous alternative ways, thus giving rise to many different supramolecular structures. Therefore, the regulation of the various interactions between the components in vivo plays a key role in defining the structure and function of focal contacts. To illustrate the significance of regulating the binding activities of different sites in these multidomain proteins, we would like to discuss the properties of one of the most prominent residents of focal contacts, namely vinculin (Fig. 2). Electron microscopy indicated that vinculin contains a globular head and a long flexible tail (Milam, 1985; Molony and Burridge, 1985; Winkler et al., 1996). The head region contains binding sites for α-actinin (Kroemker et al., 1994; Wachsstock et al., 1987) and talin (Burridge and Mangeat, 1984; Johnson and Craig, 1994), as well as an intramolecular binding site for the vinculin tail (Johnson and Craig, 1994; Miller et al., 2001; Weekes et al., 1996). The vinculin tail can bind not only to the vinculin

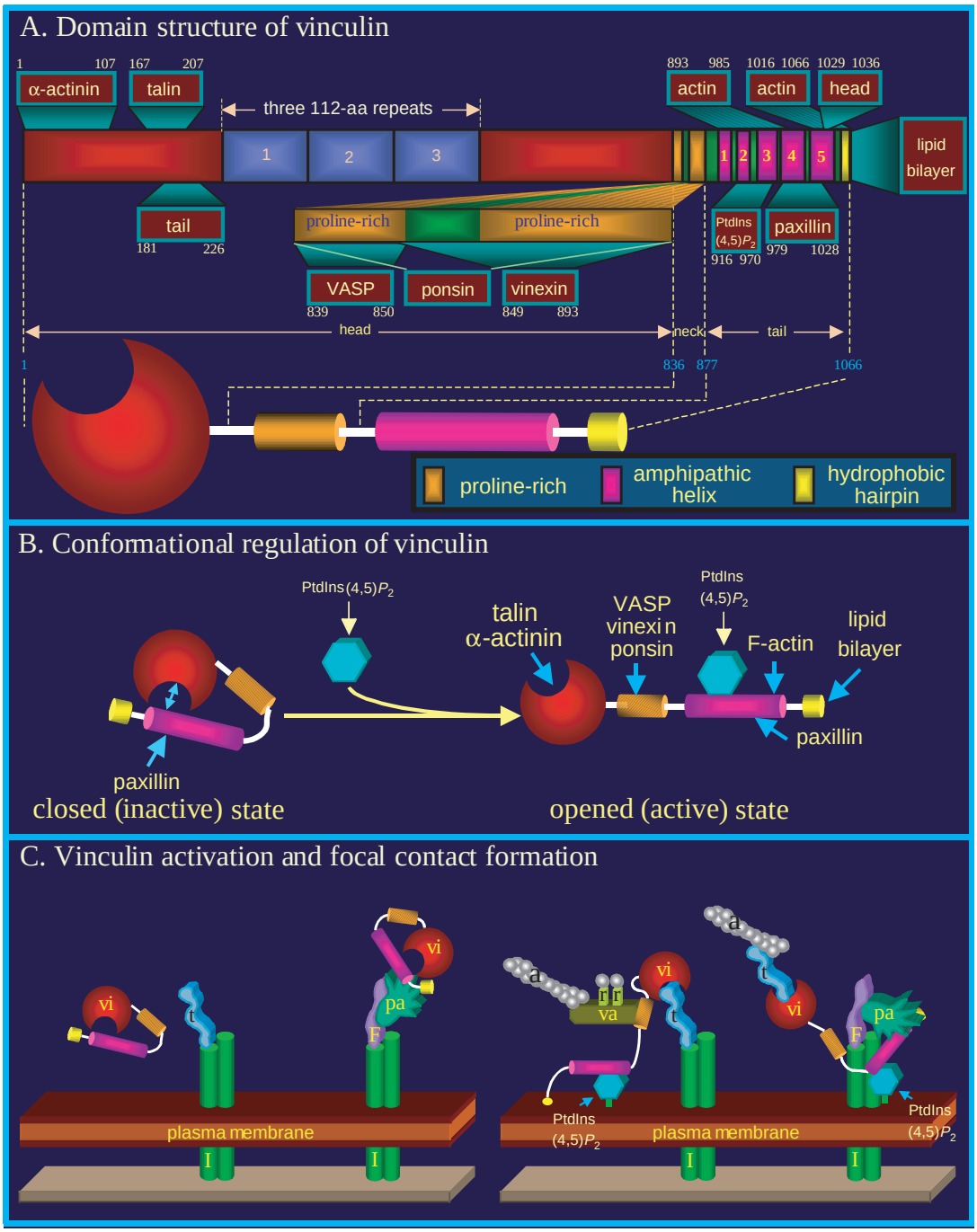

Fig. 2. The proposed domain structure and possible post-translational regulation of vinculin. (A) Scheme showing the primary structure of vinculin, annotated with the various binding domains and some secondary and tertiary structural information. Numbers near component boxes indicate the locations of specific binding sites along the polypeptide chain (starting from the N-terminus). (B) The regulation of vinculin-binding activities by conformational changes. The intramolecular interaction between the head and tail of vinculin leads to a 'closed' conformation and masks many of the binding sites. The interaction of vinculin with PtdIns(4,5)P_2 may release this inhibitory head-tail interaction and unmask different binding sites. (C) A hypothetical model for the effect of vinculin activation on the formation and assembly of focal contacts. Inactive vinculin (left) cannot crosslink the various molecular partners. Upon activation by PtdIns(4,5)P_2 (right), vinculin can bind to actin filaments and other focal contact components. Abbreviations: a, actin; F, FAK; I, integrins, pa, paxillin; r, profilin; t, talin; va, VASP; vi, vinculin.

head but also to paxillin (Turner et al., 1990; Wood et al., 1994), F-actin (Huttelmaier et al., 1997; Jockusch and Isenberg, 1981; Wilkins and Lin, 1982), phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2 (Fukami et al., 1994; Johnson et al., 1998; Niggli and Gimona, 1993; Sechi et al., 2000)] and the lipid bilayer proper (Bakolitsa et al., 1999; Johnson et al., 1998) (Fig. 2A). The head and tail of vinculin are connected through a proline-rich neck, which can bind to VASP (Brindle et al., 1996; Reinhard et al., 1996), ponsin (Mandai et al., 1999) and vinexin (Kioka et al., 1999) (Fig. 2A). Interestingly, the intramolecular interaction between the head and the tail of vinculin masks the binding sites for α-actinin (Kroemker et al., 1994), talin (Johnson and Craig, 1994), F-actin (Johnson and Craig, 1995) and VASP (Huttelmaier et al., 1998), and therefore prevents vinculin from binding to these proteins (Fig. 2B). Transition from a 'closed' to an 'open' conformation is induced by the binding of PtdIns(4,5)P_2 to the vinculin tail (Gilmore and Burridge, 1996; Weekes et al., 1996) (Fig. 2B). Thus, upon activation by PtdIns(4,5)P_2, vinculin appears to facilitate the assembly of focal contacts by crosslinking and recruiting its various partners (Fig. 2C). Such PtdIns(4,5)P_2-mediated activation of vinculin might be induced for example by Rho, which activates the PI4P5-kinase that catalyzes the synthesis of PtdIns(4,5)P_2.

We provide these examples not to further confuse the reader but to make the point that the structure and dynamics of focal contacts are way too complex to be determined simply on the basis of a comprehensive list of their components and biochemical data about the potential interactions between them. Thus 'wiring diagrams' such as the one shown in Fig. 1 and similar models, which are based on biochemical information only, should not be regarded as faithful structural models of focal contacts. To get a closer understanding of the actual structure of these sites and their assembly, we need additional, high-resolution (both spatial and temporal) information.

Diversity

The apparent complexity of cell-matrix adhesions raises compelling questions concerning the molecular diversity of these sites. Are all adhesions structurally equivalent? Or are there distinct classes of matrix adhesion, each consisting of a distinct subset of proteins, exhibiting a characteristic subcellular distribution and participating in different signaling events. Theoretically, huge heterogeneity is possible through combinatorial assembly of different proteins; yet, under in vivo conditions, which are usually exquisitely regulated, the diversity might be restricted.

To gain insight into this issue, we used quantitative fluorescence microscopy to explore variations in the structure and molecular composition of cell-matrix adhesions (Katz et al., 2000; Zamir et al., 1999). In cultured fibroblasts, we noted striking differences between 'classical' focal contacts - oval, peripheral structures enriched with $\alpha_v\beta_3$-integrin, paxillin, vinculin and tyrosine-phosphorylated proteins - and 'fibrillar adhesions', which are elongated or dot-like, central structures containing $\alpha_5\beta_1$-integrin, tensin and parvin/actopaxin and attached to fibronectin fibrils (Fig. 3) (Katz et al., 2000, Olski et al., 2001; Zamir et al., 1999). Beside the sharp differences between focal contacts and fibrillar adhesions, there are more subtle variations in their molecular compositions. This is manifested by variations in the relative fluorescence labeling intensities for different proteins, evident through 'fluorescence ratio imaging' (Katz et al., 2000; Zamir et al., 1999). Further characterization of matrix adhesion heterogeneity, to determine its structural and functional significance, is therefore necessary.

Additional forms of integrin-associated matrix adhesion are the so-called focal complexes - small, dot-like adhesions present at the edges of lamellipodia (Clark et al., 1998; Nobes and Hall, 1995; Rottner et al., 1999). The formation of focal complexes is induced by the small Rho-family GTPase Rac (Nobes and Hall, 1995; Rottner et al., 1999). The differences between the molecular compositions of focal complexes and focal contacts have not been quantitatively determined, although the former were reported to be enriched in activated $\alpha_v\beta_3$ integrin (Kiosses et al., 2001) and to apply stronger traction forces to the substrate during cell migration (Beningo et al., 2001). Paradoxically, focal complexes in stationary cells are significantly less tension dependent than focal contacts and tend to accumulate along the cell edge following treatment with inhibitors of actomyosin contractility (Zamir et al., 1999).

Focal complexes normally develop into focal contacts as a consequence of the activation of Rho (Clark et al., 1998; Rottner et al., 1999) or following the application of external force (Riveline et al., 2001). Active Rho has multiple targets (Bishop and Hall, 2000), but the combined action of just two of them, Rho kinase and Diaphanous (Dia1), appears to induce

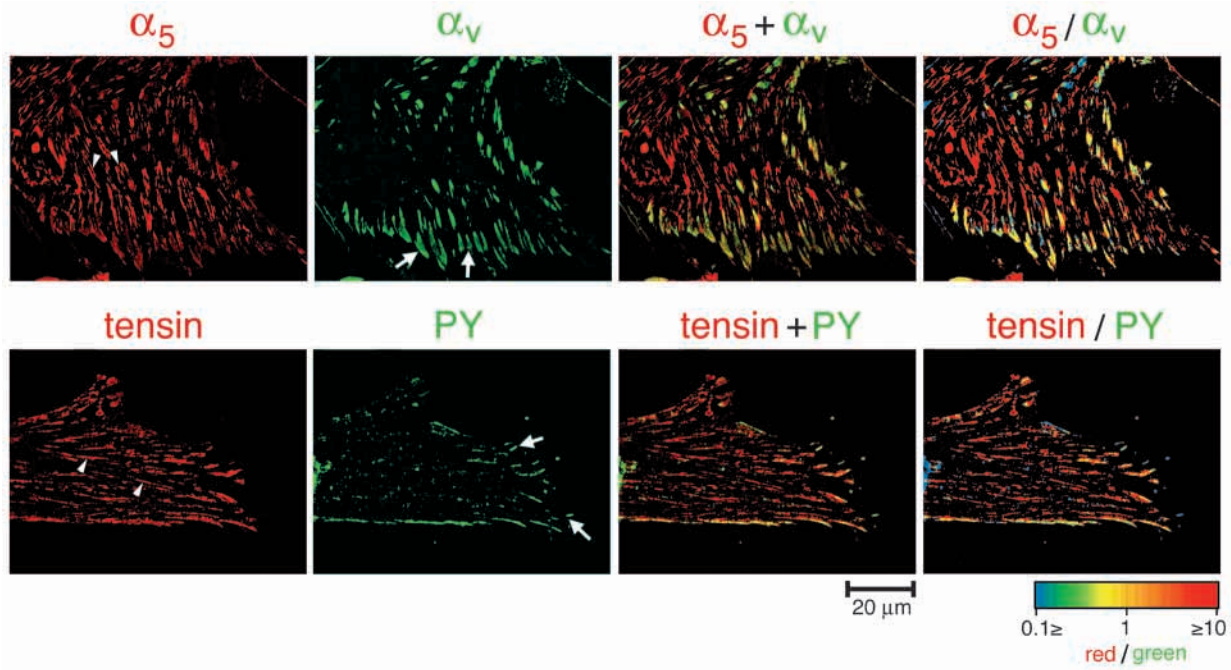

Fig. 3. Molecular diversity of focal contacts and fibrillar adhesions in human fibroblasts. The cells were fixed 24 hours after plating and double labeled for α_5-integrin and α_v-integrin or for tensin and phosphotyrosine (PY). The right-hand images show, in a spectrum scale, the ratio between the two labeled components, calculated as previously described (Zamir et al., 1999). Note the contrast between the high α_5/α_v and tensin/PY ratios in the fibrillar adhesions (indicated by their red color in the ratio image) and the lower ratio values in the focal contacts (indicated by their yellow color). Arrows and arrowheads indicate examples of focal contacts and fibrillar adhesions, respectively.

the transition of focal complexes into focal contacts. This conclusion is based on their capacity to restore stress fiber and focal contact formation in cells expressing Botulinum C3 transferase, which specifically inactivates Rho (Watanabe et al., 1999).

Podosomes are another form of integrin-mediated adhesion (David-Pfeuty and Singer, 1980; Marchisio et al., 1984; Tarone et al., 1985). They were first described as aberrant matrix adhesions formed in Rous-sarcoma-virus-transformed cells. They are small (~0.5 μm) cylindrical structures containing an actin core surrounded by tyrosine phosphorylated proteins and several typical focal contact proteins, such as vinculin and talin. The precise molecular composition and organization of podosomes is unclear. Podosomes are present in a variety of normal cells, such as monocytes and macrophages, in which they are apparently involved in cell motility, and osteoclasts, in which they aggregate in the sealing zone at the periphery of the cell and play a role in bone resorption (Duong et al., 1998; Lakkakorpi et al., 1999; Wesolowski et al., 1995). The development of podosomes appears to be regulated by a variety of signaling and cytoskeletal systems, including the microtubular system (Linder et al., 2000) and those involving dynamin (Ochoa et al., 2000), PI 3-kinase (Lakkakorpi et al., 1997) and RhoA (Chellaiah et al., 2000).

The presence of structural variants of matrix adhesions, which have distinct morphologies, compositions and dynamics, may provide important clues to the molecular basis for the variations in structure, assembly and function of the different forms of adhesions. It may also shed light on the interplay between the structure of matrix adhesions and their capacity to activate or respond to specific signaling pathways. Of particular interest are the dynamic processes involved in the formation and transformation of matrix adhesions from one form to the another, which raises several questions. How is Rac involved in assembly of focal complexes, and how is Rho involved in their development into focal contacts? How does activated $pp60^{src}$ convert focal contacts into podosomes? How does mechanical force stimulate focal contact growth, and how do the molecular and structural variations, evident in different adhesions, affect their differential involvement in cell motility, invasion, matrix assembly and growth?

Dynamics

Despite the fact that most of the information on matrix adhesion structure is based on static immunofluorescent images, it was always realized that these sites are in fact dynamic. This is manifested by their assembly, disassembly and translocation, which occur during cell spreading, polarization, migration and division. The tagging of proteins with the green fluorescent protein (GFP) or its derivatives has facilitated the study of cell-matrix adhesion dynamics. In a recent study, a GFP-tagged β_1-integrin subunit was used to follow and compare focal contact dynamics in motile and non-motile fibroblasts at a molecular level (Smilenov et al., 1999).

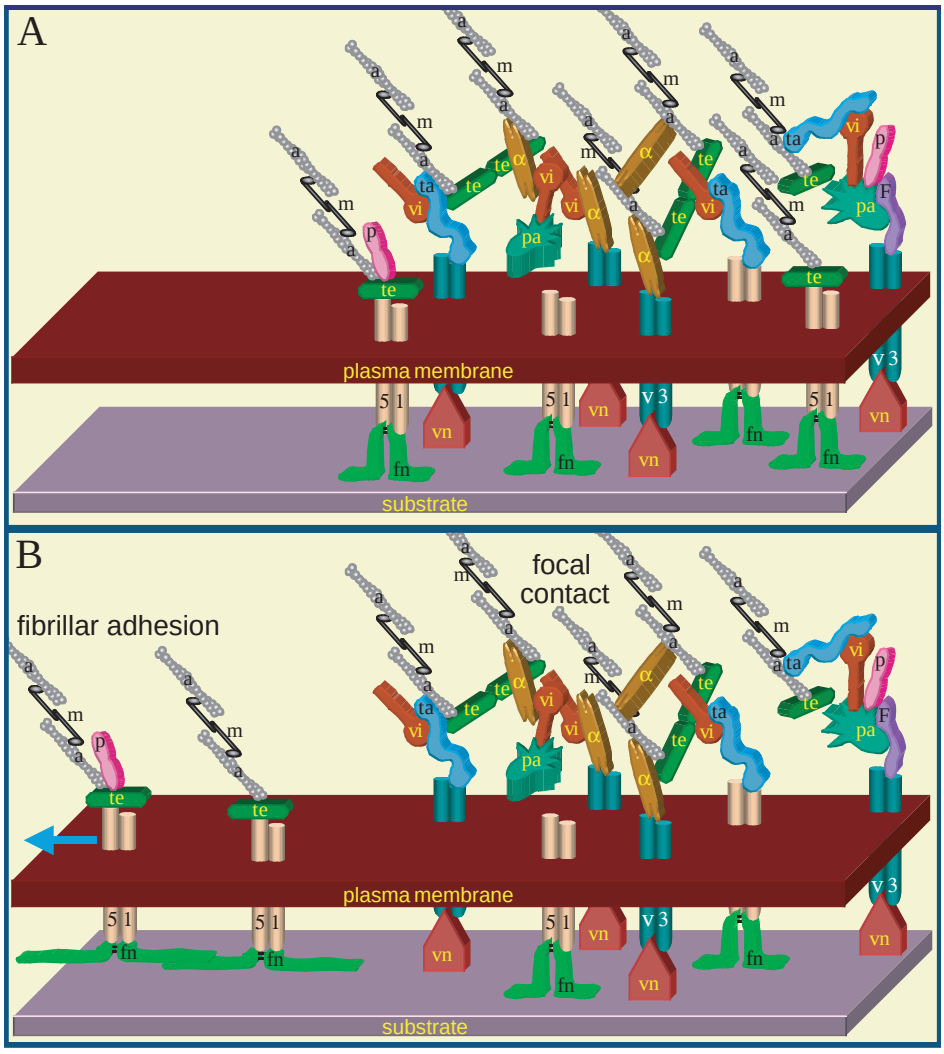

Fig. 4. A hypothetical molecular model depicting the segregation of focal contacts and fibrillar adhesions. (A) Initial adhesions contain both $\alpha_5\beta_1$ integrin (bound primarily to fibronectin) and $\alpha_v\beta_3$ integrin (bound primarily to vitronectin). Both integrins are associated through different proteins with actin filaments and are subjected to actomyosin-driven contraction forces. (B) Since substrate-attached vitronectin forms a rigid matrix, $\alpha_v\beta_3$ integrin remains immobile despite the applied contraction force. In contrast, $\alpha_5\beta_1$ integrin is bound to a relatively soft fibronectin matrix and thus translocates centripetally owing to the actomyosin-driven pulling. The translocation of the fibronectin receptor can also stretch the fibronectin matrix and promote fibrillogenesis. Abbreviations: a, actin; α, α-actinin, F, FAK; fn, fibronectin; m, myosin II; P, parvin/actopaxin; pa, paxillin; ta, talin; te, tensin; vi, vinculin; vn, vitronectin; 51, $\alpha_5\beta_1$ integrin; v3, $\alpha_v\beta_3$ integrin.

Interestingly, focal contacts were found to move centripetally in non-motile cells and to be rather stationary (relative to the substrate) in motile cells. In migrating cells, three distinct zones of focal contact behavior could be defined: a focal contact formation zone, between the leading edge and the nucleus; a persistence zone where focal contacts grow and mature, between the nucleus and the tail; and a culling zone, where focal contacts disassemble (Smilenov et al., 1999).

Using GFP fusion proteins containing cytoplasmic components of focal contacts (e.g. paxillin) and fibrillar adhesions (e.g. tensin), we were able to differentially monitor the dynamics of these two structures in living cells (Zamir et al., 2000). Focal contacts, containing GFP-paxillin, grew, faded or translocated centripetally, which is consistent with previous studies. GFP-tensin, by contrast, continuously translocated from peripheral focal contacts towards the cell center, forming fibrillar adhesions (Fig. 4) (Zamir et al., 2000). Studies by Pankov et al. are consistent with this observation and showed, using an antibody-chase technique, that whereas $\alpha_v\beta_3$ integrin remains in focal contacts $\alpha_5\beta_1$ integrin translocates centripetally, which indicated that this process is instrumental in fibronectin fibrillogenesis (Pankov et al., 2000). The translocation of fibrillar adhesions is driven by actomyosin contractility and can be blocked by inhibitors such as H-7, ML-7 and latrunculin-A (Fig. 4) (Zamir et al., 2000). However, in a sharp contrast with focal contacts, which are strictly tension dependent, the maintenance of fibrillar adhesions does not depend on actomyosin contractility (Zamir et al., 1999; Zamir et al., 2000).

Thus, matrix adhesion dynamics appear to be tightly linked to matrix assembly and affected by the physical properties of the matrix. Indeed, development of fibronectin fibrils and formation of fibrillar adhesions occur when cells are plated on native fibronectin matrix, whereas cells plated on covalently immobilized fibronectin do not form fibrillar adhesions, and their peripheral focal contacts contain high levels of $\alpha_5\beta_1$ integrin and tensin (Katz et al., 2000). Early adhesions (probably focal complexes) might therefore contain different, non-sorted integrins associated with the matrix (Fig. 4A). Upon the development of actomyosin contractility, the two forms of matrix adhesion segregate; integrin-containing focal contacts grow and do not translocate, whereas $\alpha_5\beta_1$-integrin-containing fibrillar adhesions translocate centripetally. Matrix rigidity could act as a mechanical switch in this process (Fig. 4B). Implied by this model is a mechanosensory mechanism within matrix adhesions, which regulates the segregation of molecules between different adhesions and the tension-triggered growth of focal contacts. The nature of the mechanosensor is obscure, and several models can be considered, including perturbation and physical separation of molecules within the submembrane plaque, and even local conformational changes induced by the applied force (Geiger and Bershadsky, 2001).

An important potential element in the regulation of matrix adhesion reorganization is the fine-tuning of local tyrosine phosphorylation. As shown in Fig. 1, several molecular interactions in focal contacts depend on tyrosine-specific phosphorylation of different components of the submembrane plaque. Moreover, phosphorylation and dephosphorylation events can also regulate conformational states of molecules (e.g. $pp60^{src}$) by modulating SH2-phosphotyrosine interactions (Nada et al., 1991; Williams et al., 1997; Xu et al., 1997). A hint that such a mechanism is involved in the segregation of focal contacts and fibrillar adhesions emerged from a recent study on the organization of cell-matrix adhesions in Src-deficient cells (Volberg et al., 2001). Src-null cells exhibit considerably lower levels of phosphotyrosine in their matrix adhesion sites, compared with their wild-type counterparts, and strikingly, the level of tensin in classical focal contacts is very high. This suggests that the exit of tensin from the focal contacts and the formation of fibrillar adhesions depends on Src-mediated tyrosine phosphorylation.

Conclusion

In this commentary, we have discussed three related topics that appear to be crucial for understanding of the structure and function of matrix adhesions, including the molecular complexity of these sites, their heterogeneity and their dynamics. On all three fronts, we have seen major progress recently; the list of known constituents of matrix adhesions is expanding, and within the next few years this list will probably be complete. Instrumental in this regard will be comprehensive cell-based screens for new components that use epitope- or GFP-tagged cDNA libraries combined with high-throughput microscopy. To provide an insight into the local molecular architecture of adhesion sites, advanced ‘multi-dimensional microscopy’ is needed; this will allow the simultaneous localization of multiple components at a high spatial and temporal resolution (Kam et al., 2001). Imaging of molecular interactions using fluorescence resonance energy transfer (Harpur et al., 2001; Wouters et al., 1998), or other high-resolution spectroscopy methods, will be needed for studies of these complex molecular interactions in situ. Such approaches may help uncover not only the molecular architecture of adhesion sites but also the ways in which they function in cell motility, matrix rearrangement and adhesion-mediated signaling.

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