Adhesion dynamics at a glance

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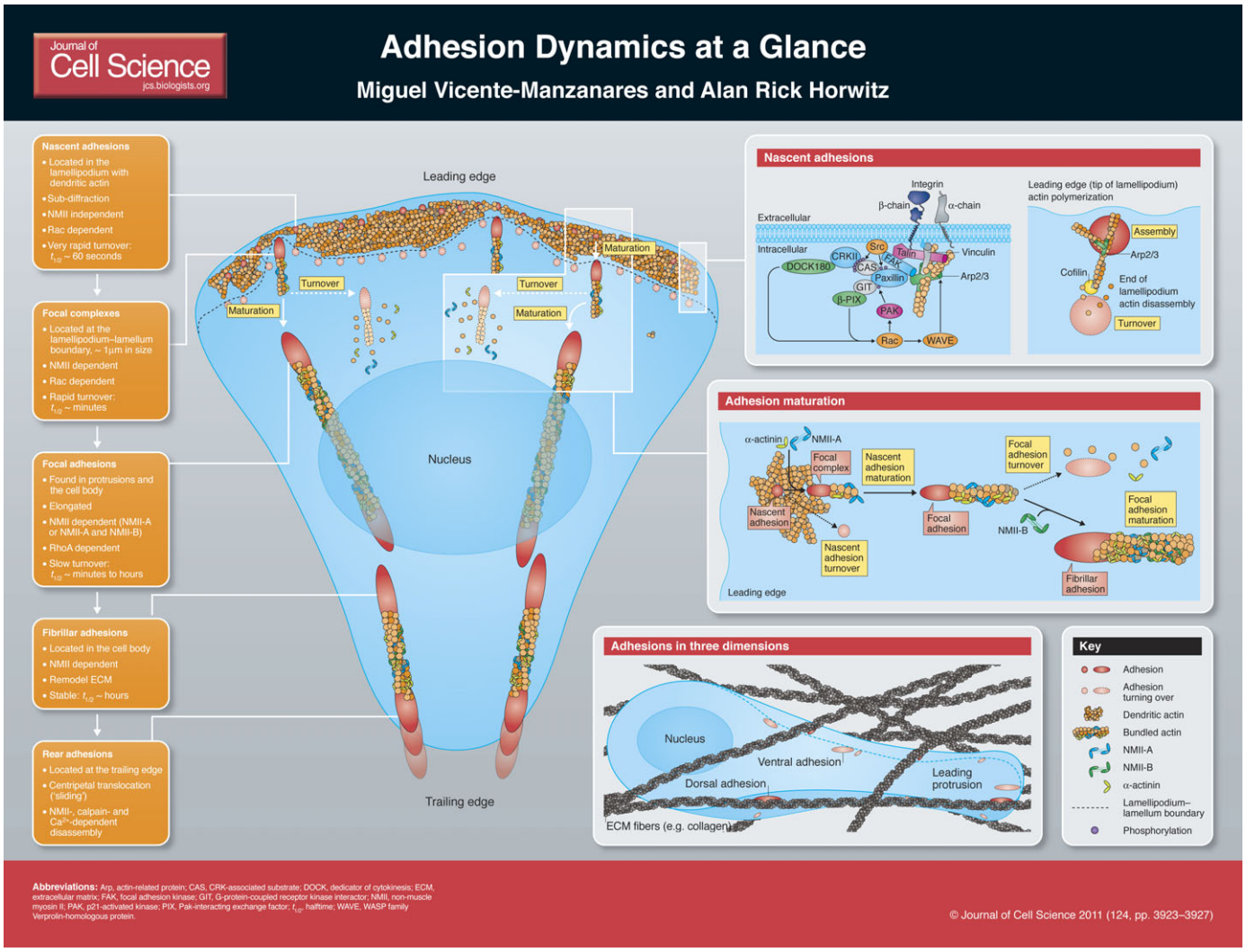
Both prokaryotic and eukaryotic cells establish complex adhesion-based relationships with their microenvironment. Single-cell bacteria (myxobacter) and amoeba, for example, display social behaviors that include adhesion to substrata and each other (Hodgkin and Kaiser, 1979; Swaney et al., 2010). In eukaryotic multicellular organisms, cells adhere to each other and migrate as sheets during the gastrulation phase of early embryogenesis, as well as later in development. They also adhere to various substrata and migrate as single cells during the transition from their site of origin to the sites of terminal differentiation that occur throughout development. Examples of this include neural crest cells, somite-derived cells and ventricle-derived cells in the brain (Aman and Piotrowski, 2010). In addition, fibroblasts form strong adhesions, which they use to remodel their matrix environment, but these cells also undergo rapid migration in response to tissue damage and other extracellular cues (Shaw and Martin, 2009). Such changes in cell adhesion and migration accompany many other processes including epithelial–mesenchymal transition (EMT), responses to wounding, cell differentiation, the mobilization of progenitor cells from their niches and many more (Laird et al., 2008; Revenu and Gilmour, 2009; Ridley et al., 2003). Specialized cell–cell contacts, for example at neuronal and immune synapses, enable specific and localized signal activation and communication processes (Fooksman et al., 2010; Yuste and Bonhoeffer, 2004). Adhesion also has crucial roles in the pathology of several diseases. For example, abnormal leukocyte adhesion and recruitment in most autoimmune diseases, such as asthma, rheumatoid arthritis or multiple sclerosis, result from changes in cell adhesion (Chavakis et al., 2009). In addition, altered cell–cell adhesion and migration are prominent during tumor invasion and metastasis (Mayor and Carmona-Fontaine, 2010; Sahai, 2007).

Integrins are the major mediators of contacts between cells and the extracellular matrix (ECM). They are heterodimers comprised of an α- and a β-chain. A large number of different αβ-heterodimers have been described, which reflect the redundancy in these interactions as



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well as the variety of extracellular molecules with which integrins can interact (Hynes, 2002). Integrins are part of multi-molecular complexes that connect them to the actin cytoskeleton and generate signals that regulate diverse cellular processes. Several components of these complexes regulate adhesive strength either through changes in the conformation of the integrin (affinity) or through integrin clustering (avidity) (Carman and Springer, 2003). In addition, these adhesion-associated cytoplasmic protein complexes generate signals that control cell proliferation and survival, gene expression, cytoskeletal organization and migration. The ultrastructure of adhesions and the sub-structural distribution of their components are only beginning to be elucidated (Kanchanawong et al., 2010; Patla et al., 2010).

Recent progress has pointed to a signaling loop that links actin polymerization and organization with adhesion. On the one hand, these actin properties appear to orchestrate the architecture and dynamics of adhesions (Alexandrova et al., 2008; Choi et al., 2008; Gupton and Waterman-Storer, 2006; Hotulainen and Lappalainen, 2006). On the other hand, signals generated by adhesions regulate the polymerization and organization of actin. This Cell Science at a Glance article will focus on the bi-directional relationship between actin and cell–ECM adhesions, the role of actin as a scaffold for adhesion formation and maturation, and the signals that link adhesion and actin polymerization and organization.

Initial adhesion formation: nascent adhesions

Adhesions initially form primarily near the leading edge (~1–2 μm away from the plasma membrane) at the front of spreading and migrating cells (Alexandrova et al., 2008; Choi et al., 2008; Nayal et al., 2006; Zaidel-Bar et al., 2003) in a region called the lamellipodium (Cramer et al., 1997; Ponti et al., 2004). This is a region of active actin polymerization that functions to drive membrane protrusion. Although there is some controversy about the organization of the actin filaments in this region, they are thought to adopt a branched morphology, at least initially (Svitkina and Borisy, 1999; Urban et al., 2010). Whereas actin polymerizes rapidly near the front of the lamellipodium, it depolymerizes at locations more distal to the leading edge (Pollard and Borisy, 2003).

Nascent adhesions are small, dot-like and transient adhesions with relatively short lifetimes (Alexandrova et al., 2008; Choi et al., 2008; Nayal et al., 2006; Zaidel-Bar et al., 2003). Although they are not yet characterized in detail, they contain many well-studied adhesion molecules, such as integrins, actin linkage molecules, like talin and vinculin, and signaling molecules, like focal adhesion kinase [FAK, also known as protein tyrosine kinase 2 (PTK2)] and p130CAS [CRK-associated substrate, officially known as breast cancer anti-estrogen resistance 1 (BCAR1)] (Alexandrova et al., 2008; Choi et al., 2008; Galbraith et al., 2007; Zaidel-Bar et al., 2003). Many of these molecules appear to enter nascent adhesions at approximately the same time (Choi et al., 2008). One key feature of nascent adhesions is that they are largely immobile, despite being surrounded by lamellipodial actin that undergoes rapid retrograde flow (Alexandrova et al., 2008; Brown et al., 2006; Hu et al., 2007).

The formation of nascent adhesions requires actin polymerization; however, they form and persist in the absence of non-muscle myosin II (NMII) activity (Alexandrova et al., 2008; Choi et al., 2008; Vicente-Manzanares et al., 2007). Whereas it is unclear how actin polymerization or its nucleators control adhesion assembly, possible links between these two events include: (1) the Arp2/3 complex, which interacts with vinculin and FAK in vitro (DeMali et al., 2002; Serrels et al., 2007); (2) vasodilator-stimulated phosphoprotein (VASP), which binds to the barbed end of actin and associates with vinculin and zyxin (Bear and Gertler, 2009); (3) vinculin and talin themselves, which bind directly to actin (Critchley, 2009; Ziegler et al., 2006); and (4) α-actinin, an actin cross-linker that is also present in lamellipodia (Sjoblom et al., 2008).

Nascent adhesions display active adhesive signaling, and several adhesion components, including FAK, Src, paxillin, p130CAS, vinculin and others, are heavily phosphorylated on tyrosine (as well as serine and threonine) residues (Ballestrem et al., 2006; Kirchner et al., 2003; Nayal et al., 2006). These phosphorylation events create docking sites for the recruitment of additional signaling proteins to nascent adhesions and might promote actin polymerization. One example is the activation of Rac by dedicator of cytokinesis 180 (DOCK180, also known as DOCK1) and β-Pak-interacting exchange factor (β-PIX). DOCK1 is recruited to the leading edge through its interaction with the CRKII–p130CAS complex (Kiyokawa et al., 1998), which is mediated by FAK- and Src-dependent paxillin phosphorylation on Tyr31 and Tyr118 (Petit et al., 2000). By contrast, β-PIX is recruited to the leading edge through its interaction with the adaptor GIT1 (for G-protein-coupled receptor kinase interactor 1), whose interaction with paxillin is regulated by phosphorylation on Ser273 by p21-activated kinase 1 (PAK1), which in turn also associates with β-PIX (Nayal et al., 2006). GIT2 has a similar role to GIT1, and its phosphorylation by FAK and Src is required for its localization to adhesions (Brown et al., 2005). β-PIX can also be recruited to the leading edge through its interaction with parvin (PARV, also known as affixin) (Matsuda et al., 2008).

When nascent adhesions are approached by the rear of the lamellipodium, which is an area of actin disassembly, they either disassemble or begin to elongate and grow (i.e. mature). The lifetime of nascent adhesions is thus determined by the rate of protrusion: the faster the cells protrude and move, the faster the nascent adhesions turn over, and therefore, they are mostly short-lived ($t_{1/2}$ of ~60 seconds) (Alexandrova et al., 2008; Choi et al., 2008). Presumably, branched actin in the lamellipodium provides a physical scaffold for the nascent adhesions, whereas actin depolymerization by cofilin, for example, decouples the nascent adhesions from actin and leads to adhesion disassembly (Oser and Condeelis, 2009).

Adhesion maturation

Nascent adhesions that do not disassemble enlarge instead. They grow initially at the rear of the lamellipodium. The maturation process can continue over a relatively long time period, as the adhesions remain in place while the continued forwards motion of the protrusion (and cell) moves over them. Despite the continuous nature of the maturation process, some discrete stages have been identified.

Focal complexes represent an initial maturation step and are particularly abundant in cells that express activated Rac1 (Ridley et al., 1992; Rottner et al., 1999). They differ from nascent adhesions by their location near the boundary of the lamellipodium and lamellum, their larger size, and their NMII dependence; however, their molecular constituents are similar to those found in nascent adhesions (Choi et al., 2008). In general, the focal complex is a transient entity: as the lamellipodium moves forward, they mature into larger, elongated focal adhesions. As focal adhesions form, they serve as physical platforms that slow down the retrograde movement of the lamellipodial actin filament bundles that comprise the lamellum through the action of NMII-A (Burnette et al., 2011).

Focal adhesions are a heterogeneous class of adhesions with a spectrum of sizes that reflects the continuum of adhesion maturation (Geiger and Yamada, 2011; Vicente-Manzanares et al., 2011). Actin cross-linking, through α-actinin and NMII-A, for example, mediates initial maturation; however, the contractile activity of NMII-A appears dispensable for the initiation of this process (Choi et al., 2008). The adhesions associated with actin filament bundles

containing only NMII-A are also dynamic and turn over – albeit more slowly (a turnover rate of several minutes) (Webb et al., 2004) than nascent adhesions (a turnover rate of ~1 minute) (Choi et al., 2008) – as the thin (NMII-A) actomyosin bundles with which they associate turn over (Vicente-Manzanares et al., 2011). By contrast, adhesions associated with actin filament bundles containing NMII-B are very stable, as are the NMII-B-containing actomyosin bundles themselves. Focal adhesions can then mature further into fibrillar adhesions (as discussed in the following section).

The signals produced by adhesions presumably regulate their formation and turnover by regulating actin polymerization, crosslinking and filament contraction. The signaling properties of adhesions change as they mature. FAK and Src mediate the turnover of nascent adhesions, focal complexes and the smaller focal adhesions that are associated with NMII-A but not NMII-B in protrusions. However, tyrosine phosphorylation levels on molecules like FAK and paxillin tend to decrease as adhesions mature and move away from the lamellipodium (Ballestrem et al., 2006; Kirchner et al., 2003; Vicente-Manzanares et al., 2011). FAK and paxillin signaling appears to act primarily through Cdc42, Rac1 and Rho. Of these proteins, Cdc42 and Rac1 promote actin polymerization and adhesion formation and Rho promotes adhesion maturation, in part through its effect on NMII-A and NMII-B activity (Totsukawa et al., 2000). As the adhesions continue to mature, mechanical forces generated by NMII modulate the signals generated by adhesions. This arises from alterations in protein conformation and clustering due to contractile force and increased bundling driven by NMII, respectively. These effects can lead to changes in the phosphorylation and activation status of proteins associated with adhesions (Vicente-Manzanares et al., 2009), including Rho guanine-nucleotide-exchange factors (Rho GEFs), such as GEF-H1 and LARG (Guilluy et al., 2011). These, in turn, activate RhoA and promote the downstream activation of NMII (Matthews et al., 2006). Using mass spectrometry, two recent studies have revealed the key involvement of NMII in adhesion dynamics, as it modulates the composition of the adhesions (Kuo et al., 2011; Schiller et al., 2011). One study describes the recruitment of β-PIX to adhesions when NMII is inhibited (Kuo et al., 2011), whereas the other shows that LIM-domain-containing protein recruitment to adhesions is inhibited when NMII activity is blocked with blebbistatin (Schiller et al., 2011).

Some very rapidly migrating cells, like leukocytes, only have small dynamic adhesions and few, if any, large actin filament bundles that emanate from the lamellipodium (Smith et al., 2005). These small adhesions resemble focal complexes or immature focal adhesions and are likely to originate from the different actin organization in these cells (Insall and Machesky, 2009; Vicente-Manzanares and Sanchez-Madrid, 2004). In general, adhesion morphology and dynamics vary considerably among different cell types. These differences might arise from NMII activation and isoform composition, intrinsic differences in actin organization or cell-type-dependent variations in the molecular composition of the adhesions.

Fibrillar adhesions: large adhesions in protrusions and the cell body

Fibrillar adhesions represent the endpoint in terms of adhesion maturation. Their association with large stable NMII-B-decorated actin filament bundles results in large adhesions with a lifetime of several hours, and this class of adhesions has an active role in organizing the ECM (Geiger and Yamada, 2011; Zaidel-Bar et al., 2004). Most of the components present in nascent adhesions and focal complexes are also part of focal and fibrillar adhesions, although their distribution within adhesions may be different. For example, α-actinin is segregated to the distal part of the focal (and fibrillar) adhesion, where the adhesion overlaps more clearly with the actomyosin bundle than in other types of adhesions. Additional components that are not present in nascent adhesions or focal complexes but are present in fibrillar adhesions include tensin and zyxin (Zamir and Geiger, 2001a; Zamir and Geiger, 2001b). Signaling mediated by phosphorylated tyrosine residues is highly attenuated in fibrillar adhesions, which is thought to inhibit the signals that lead to protrusions in regions in which fibrillar adhesions are present (Ballestrem et al., 2006; Vicente-Manzanares et al., 2011).

Fibrillar adhesions in both central regions of the cell and near the rear can undergo disassembly. Several mechanisms contribute to this process, including microtubule targeting (Kaverina et al., 1999), proteolytic activation (Cortesio et al., 2011; Chan et al., 2010; Franco et al., 2004), pH-dependent modulation of the interactions between actin and adhesion proteins (Srivastava et al., 2008) and NMII-based contraction (Chrzanowska-Wodnicka and Burridge, 1996). Central adhesions use microtubule- and FAK–dynamin-mediated endocytic mechanisms for disassembly (Ezratty et al., 2005). By contrast, calpain-based mechanisms appear to be particularly important for disassembly of adhesions that are found at the rear of the cell (Huttenlocher et al., 1997). For adhesion disassembly during retraction of the trailing edge, the adhesions at the rear often move centripetally and then disassemble, leaving integrin trails on the substratum (Ezratty et al., 2005; Laukaitis et al., 2001; Regen and Horwitz, 1992). This sliding appears to arise from a treadmilling mechanism during which components are released at the rear of the adhesion and enter new adhesions in the proximal region (Ballestrem et al., 2001; Digman et al., 2008).

Adhesions in three dimensions

Most of the mechanistic insight on adhesion formation and maturation, and the molecules that control them, has been obtained by studying cells on flat two-dimensional surfaces coated with ECM ligands. However, cells usually adhere in three dimensions, but visualizing adhesions in three dimensions has been challenging. More recently, however, adhesions have been studied using a variety of three-dimensional systems, including collagen and fibrin gels (Cukierman et al., 2001; Deakin and Turner, 2011; Kubow and Horwitz, 2011; Petroll and Ma, 2003; Takeda et al., 2003), as well as cell-free matrices that are generated by living cells, which are then removed by using a mild detergent (Hakkinen et al., 2011).

Adhesions are particularly difficult to visualize dynamically in three dimensions in living cells (Fraley et al., 2010). However, such adhesion dynamics have been described by several groups in different contexts (Cukierman et al., 2001; Deakin and Turner, 2011; Hakkinen et al., 2011; Kubow and Horwitz, 2011). The fluorescence background and the mislocalization of proteins generated by the overexpression of GFP-fusion constructs and the microenvironment of the cell are two major challenges in imaging these adhesions (Kubow and Horwitz, 2011; Fraley et al., 2010). On the basis of the few reported studies, adhesions in three dimensions tend to be small, although those associated with large collagen fibers can be highly elongated (Cukierman et al., 2001). These smaller adhesions are dynamic, NMII-dependent (Kubow and Horwitz, 2011) and appear to be similar to small focal adhesions. In three dimensions, adhesions are implicated in collagen fiber transport, reorientation and bundling when the matrix is pliable (Meshel et al., 2005). At this early point, two dominant interrelated contributions determine the properties of adhesions in three dimensions: the pliability of the microenvironment (i.e. the more pliable the environment, the smaller the adhesion) and the fibrillar nature of the matrix with which the cell associates.

Thus, although studies in two dimensions have and will continue to inform us about

physiological processes and reveal the palette of potential adhesion-generated cellular phenotypes, three-dimensional systems are closer to the in vivo setting and are revealing important cellular differences. Clearly, these studies are in a very early stage and future experiments are likely to produce some important surprises.

Conclusions

A large amount of literature on integrin-based adhesions has emerged. Much is now known about the different types of adhesions and their molecular composition, the mechanisms by which they form and disassemble, their linkage to actin and the signals they produce. Whereas this article has focused on the integrin-based focal adhesions that form in cells adhering to the ECM, cells also form adhesions that are associated with filopodia, invadopodia and podosomes, which we are only now beginning to study in this context. For focal-adhesion-like structures, it now appears that the polymerization and organization of actin, that is crosslinking and myosin-driven contraction, determines their assembly and structure. The adhesions themselves generate signals that regulate actin organization, polymerization and contraction, and thereby form a complex, and so far poorly understood, signaling loop. Future challenges include deciphering how biochemical and mechanical signals are interpreted and integrated by adhesions as a cell both adapts to and modifies its microenvironment, characterizing adhesions in living tissues, understanding how the microenvironment dictates cellular phenotypes, characterizing adhesive structures that differ from focal adhesions and determining the origins of the varieties in adhesions in different cell types.

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A high-resolution version of the poster is available for downloading in the online version of this article at jcs.biologists.org.

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