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Signal transduction via integrin adhesion complexes

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Abstract

Integrin adhesion complexes (IACs) have evolved over millions of years to integrate metazoan cells physically with their microenvironment. It is presumed that the simultaneous interaction of thousands of integrin receptors to binding sites in anisotropic extracellular matrix (ECM) networks enables cells to assemble a topological description of the chemical and mechanical properties of their surroundings. This information is then converted into intracellular signals that influence cell positioning, differentiation and growth, but may also influence other fundamental processes, such as protein synthesis and energy regulation. In this way, changes in the microenvironment can influence all aspects of cell phenotype. Current concepts envisage cell fate decisions being controlled by the integrated signalling output of myriad receptor clusters, but the mechanisms are not understood. Analyses of the adhesome, the complement of proteins attracted to the vicinity of IACs, are now providing insights into some of the primordial links connecting these processes. This article reviews recent advances in our understanding of the composition of IACs, the mechanisms used to transduce signals through these junctions, and the links between IACs and cell phenotype.

IAC components

Cells adherent to ECM proteins assemble a range of IACs that are focally distributed [1,2]. Detailed functional and morphological analyses have defined several major forms of IAC, including focal complexes, focal adhesions and fibrillar adhesions [2]. Each type of IAC is formed sequentially and disrupted as cells migrate and, based on immunofluorescence analyses, each has a distinct composition. Most recently, a new class of adhesion, which contains high levels of integrin $\beta 5$, clathrin and endocytic adaptors, has been identified by a range of laboratories and variously termed flat

clathrin lattices, clathrin plaques or reticular adhesions [3-6]. The extent to which these reports describe the same type of adhesion complex is currently unclear. All IACs are connected to the actomyosin cytoskeleton via adaptor proteins such as talin and vinculin (except reticular adhesions [6]), but they also serve as hubs that link integrin-containing plasma membrane regions to the cytoplasmic signalling network.

IACs are located at the membranous junction between ECM-bound, plasma membrane-intercalated integrins and intracellular, actin-based cytoskeletal filaments. Isolation of such a structure away from all adjacent cellular material is currently not possible, but in the last decade techniques have been developed to purify ventral IACs from cells in 2D culture, together with material that is found in close apposition to the junction [7-9]. Since the function of IACs is to convert spatiotemporal information about the extracellular environment into signals with wide-ranging consequences, the composition of these purified IACs, and their associated material, is highly relevant.

Although a range of cell types has been investigated, most IAC preparations have been isolated from cells attached a fibronectin substrate. Compilation of seven of these datasets led to the definition of a fibronectin-induced 'meta adhesome' of over 2400 proteins, which was further refined to 60 core proteins most frequently identified in IACs, termed the 'consensus adhesome' [10]. The consensus adhesome is likely to represent the core cell adhesion machinery, centred round potential axes that link integrins to actin (e.g. ILK-PINCH-parvin-kindlin, FAK-paxillin, talin-vinculin and α -actinin-zyxin-VASP; Figure 1). This core connection network has recently been integrated with a 'literature-curated adhesome', generated *in silico* by Geiger and colleagues [11,12], and has proven valuable for filtering large datasets and identifying potential candidates for follow-up experimentation [13-21]. A recent meta analysis suggests nearly 20% of the human kinome is found in the adhesome, supporting the view that this region of the cell is a general signalling hub [22].

Since publication of the meta adhesome, IAC preparations have been generated from other cell types, such as endothelial cells [23], but in the future proteomic analysis of IACs isolated from a wider range of cell types in various cellular contexts (e.g. different ligands, in 3D culture, or *in vivo*) will undoubtedly broaden our understanding of adhesome composition and regulation. Similarly, current IAC preparations are isolated from thousands of cells, and therefore comprise a range of different types of IAC, found in different subcellular locations. Future improvements in mass spectrometric sensitivity will enable more focused analyses of the composition of IACs in space and time. In recent years, new techniques for IAC analysis have been developed, including the use of mass spectrometry in conjunction with the proximity-dependent biotinylation technique, BioID [20]. By using this method with paxillin and kindlin baits, a number of well-established adhesome components were identified, but in addition several new associations were revealed, including Kank2 (for paxillin) and liprin- β (for kindlin). In parallel studies, another Kank family member, Kank1, was localised to the periphery of mature IACs through its ability to bind talin. At these sites, Kank1 coordinated the formation of cortical microtubule stabilisation complexes (containing ELKS, liprins, KIF21A, LL5 β and

CLASPs), which in turn led to the destabilisation of IACs [24,25]. Thus, although marginally failing to meet the criteria for inclusion in the original consensus network [10], Kank proteins now appear central to integrin regulation and should be considered core adhesome components. Additional key functional interactions within the consensus adhesome that have been elucidated recently include a role for kindlin-2 dimerisation in integrin activation and clustering [26], direct kindlin-actin binding [27], and a role for the kindlin-paxillin axis in activation of Rac1 and recruitment of Arp2/3 to early IACs [28].

The coordinated assembly and disassembly of IACs must be tightly regulated for effective cell adhesion and migration. A highly multiplexed, high resolution imaging approach revealed that as IACs were assembled, molecular noise was reduced, suggesting that despite the large number, and extensive binding capabilities, of adhesome components, IAC assembly is tightly regulated [29]. Further studies have identified novel roles for well-known adhesome components in the maintenance of integrin activation during integrin endocytic recycling prior to IAC assembly. FAK, talin, and PIPK γ 2 were shown to associate with endocytosed integrins, maintaining their active state, and priming them for rapid IAC formation at the leading edge of migrating cells [30].

Mechanotransduction via IACs

A major challenge is to elucidate the mechanisms whereby information is transduced through IACs. It is now firmly established that the ability of cells to sense force, generated externally via the ECM or internally by actomyosin-based contractility, and convert that information into biochemical signals, plays a key role in differentiation and proliferation [31,32]. Altered cell and tissue stiffness modifies transcriptional programming [33], thereby affecting lineage decision-making, and is also associated with diseases such as cancer and fibrosis [34,35]. Our understanding of mechanosensing has increased substantially in recent years and has been reviewed in depth [36-38]. Here, we focus on recent conceptual and mechanistic advances.

Integrin-actin connection via a molecular clutch

Force sensing is transmitted by integrin receptors and their associated adhesion signalling complexes via a molecular clutch, whereby cell-ECM bonds dynamically engage and disengage with the cytoskeleton [39]. By using nanopatterned substrates of defined ligand separation distances, the clutch was shown to function by recruitment of additional integrins to allow the distribution of force over multiple connections [40]. This model demonstrated how low ligand spacing promotes IAC formation on soft substrates, but leads to collapse on stiff substrates. In this way, both ECM rigidity and molecular spacing determine an optimal threshold for IAC formation to coordinate with downstream signalling [40]. Additionally, the molecular clutch model has been shown to be relevant to ligand mobility changes (surface viscosity) [41] and membrane tension [42]. Using nanopatterning to create aligned

ECM resulted in spatially constrained IAC orientation, presumably via spatial restriction of the organisation of the molecular clutches, that resulted in anisotropic traction forces and facilitated cell orientation and directional migration [43].

Force-dependent changes in composition

Analysis of force-dependent changes in the consensus adhesome revealed that the majority of components were reduced or lost when actomyosin contractility was inhibited [44]. The turnover of talin and vinculin, but not FAK and paxillin, was increased by extracellular stiffness [45], suggesting that these proteins are specifically responsible for mechanotransduction. Moreover, while phosphorylation of paxillin and FAK was not responsible for force-sensitive changes in vinculin dynamics, it was required for subsequent Rac1-dependent signalling processes [45]. These data are consistent with studies that demonstrated migration and proliferation induced by FAK- and Src-dependent phosphorylation occurs independently of IAC composition [21] and demonstrate possible segregation of signalling and mechanosensing functional modules within IACs.

Further analysis of the links between composition, organisation, turnover and signalling at IACs may be complicated by their molecular heterogeneity and differential response to force modulation [46]. For example, tracking the Rho-associated kinase (ROCK)-induced changes of IAC proteins using four-colour live cell imaging of zyxin, FAK, vinculin and paxillin revealed an unexpectedly diverse pattern of responses, with the identity of the strongest responder varying between individual IACs [47]. Similarly, the nanoscale organisation of IAC components such as vinculin, paxillin and talin underwent a non-random reorganisation upon myosin inhibition suggesting force-dependent regulation of IAC organisation [48]. Furthermore, while genetic ablation of myosin II in *Drosophila* embryonic muscles did not cause loss of IACs, the stoichiometry of adhesion sites did depend on contractility, as IAC components were differentially affected by different alterations to muscle contraction [49]. Taken together, all of these findings argue that force might produce rearrangements of proteins within IACs without effecting major changes in composition.

Relay mechanisms

Mechanotransduction can take several forms, including activation of ion channels, use of catch and slip bonds, and force-induced conformational changes in sensory proteins that lead to the exposure of cryptic binding sites for other proteins. Evidence is accumulating that all of these mechanisms contribute to signal relaying via IACs.

Catch bond behaviour has been demonstrated previously for integrins [50], and now through the use of single molecule optical trapping, vinculin has been shown to form a polarised, force-dependent catch bond with actin [51]. Modelling suggests that the directionality of the vinculin-actin catch bond may have long-range effects on the actin cytoskeleton relevant to migration [51]. Albeit through a different mechanism,

the application of fluorescence polarisation microscopy to analysis of GFP-tagged integrins has demonstrated a role for directional actin retrograde flow in receptor alignment in IACs [52,53]. Modelling also suggests that a combination of adaptor binding and cytoskeletal force application is required for integrin extension and activation [54].

There are several classes of force-sensitive ion channels, but the Piezo family appears to be functionally relevant for adhesion-related signalling (in addition to its canonical roles in touch sensing and proprioception). Through the use of atomic force microscopy, an ECM dependence to the activation of Piezo1 has been demonstrated [55], calcium flickers generated by Piezo1 have been linked directly to actomyosin-based traction force (<https://www.biorxiv.org/content/early/2018/04/03/294611>), and activation of Piezo1 in epithelial sheets shown to induce cell division via calcium-dependent activation of the MAPK pathway [56]. L-type calcium channel activity is also regulated by integrin activity and results in maturation of filopodia-associated complexes to IACs [57].

Substantial evidence has accumulated to indicate key roles in mechanotransduction for talin, vinculin and p130Cas [58-63]. In recent years, new tension sensors have been developed for talin and used to probe the role of different regions of the molecule in mechanosensing and IAC formation [64,65]. Tension on talin was higher in peripheral IACs than central IACs, a phenomenon that required the talin actin-binding site 3 (ABS3), while tension sensing on soft versus stiff substrates was mediated by the ABS2 site in conjunction with vinculin [64]. These findings argue for spatial differences in the organisation of IACs. Combined structural and biophysical analyses have now elucidated the conformational changes that take place in vinculin during activation [66].

It is established that the Hippo pathway effectors YAP and TAZ drive transcriptional programmes in response to mechanosensing [31]. Force has now been shown to trigger YAP nuclear entry via a relay involving cortical actin, nucleus-associated cytoskeleton, nuclear membrane flattening and nuclear pore opening [67,68]. As might be predicted, direct roles for core IAC adhesome components such as FAK, kindlin and β -PIX are now being uncovered [69-71]. Intriguingly, there is growing evidence that many adhesome components shuttle between the cytoplasm and nucleus (reviewed in [22]). When combined with recent interest in the role of nuclear actin in controlling DNA repair [72,73], it is likely that analyses of the structural anatomy and functional importance of the nuclear adhesome will become a major focus.

Distal consequences of signalling via IACs

Signalling via IACs controls a wide range of cellular functions, including migration, proliferation and differentiation. Ontological analysis of the adhesome, however,

suggests connections to other cell fate decisions [74]. Here, we review recent advances in our understanding of one established and one novel functional link.

Cell cycle

2018 marks the 50-year anniversary of the confirmation of anchorage-dependent growth and the finding that suspension of normal cells results in cell cycle arrest prior to DNA replication [75]. Subsequent research has identified key signals that link adhesion to the G1 checkpoint [76] and led to the concept of anchorage-dependent survival, or anoikis [77], but despite the major changes in shape that take place during division, other connections between the cell cycle engine and IACs have not been made.

CDK1, the master controller of the cell cycle, primarily pairs with cyclins A2 (outside of mitosis) and B1 (for mitosis) and drives complex morphological programs through phosphorylation of more than a hundred targets [78,79]. These targets include a number of cytoskeletal/adhesion proteins and recent phosphoproteomic analysis of IACs suggested the existence of many more [80]. Inhibition of CDK1 perturbed IACs in interphase cells, implying a role for the enzyme in promoting adhesion when paired with cyclin A2 [81]. Analysis in synchronised cells demonstrated that the loss of IACs prior to mitosis begins in early G2, well before cell rounding [81], and consistent with changes in traction force [82,83]. The trigger for this decrease was Wee1-dependent phosphorylation and inhibition of CDK1 activity, which was itself induced by cyclin B1 expression. Thus, a ubiquitous feature of the cell cycle provides a primordial connection between the cell cycle and adhesion. This finding also suggests that there may be links between adhesion and cell cycle checkpoints other than that in G1. In this context, ECM stiffness is an environmental cue that promotes mutant p53 stabilisation via mevalonate-RhoA-mediated mechanosignalling [84]. Furthermore, over-expression of $\beta 1$ integrin correlated with stabilisation of RAD51, which is a key component of the homology-directed DNA repair pathway, by preventing its ubiquitination by RING1 and subsequent degradation, thus increasing cell survival [85]. Taken together, these findings might imply functional links between IACs and the nucleus, and recent evidence suggests nuclear decoupling in response to rapid changes in mechanical loading (<https://www.biorxiv.org/content/early/2018/05/09/317404>) and evidence of nuclear envelope rupture during confined migration [86].

Most cells round at mitosis, having remodelled or lost their IACs, but do not detach. Since frustrated rounding leads to aberrant division, this also implies cell cycle-dependent regulation of IACs. Although most IAC components are lost during mitotic rounding, integrins remain in place and mediate adhesion to the substrate via retraction fibres [87] and a new type of adhesion complex that has been termed reticular adhesions (<https://www.biorxiv.org/content/early/2017/12/14/234237>). These modified IACs provide a template for daughter cell re-spreading following mitotic exit, which may equate to a molecular memory of the parental cell microenvironment and is required for accurate division [87].

Integrins are also important for mitosis, particularly for correct orientation of the mitotic spindle [88-91]. Recent work suggests that the IAC components lost at cell rounding relocate at the lateral regions of the cell cortex where they co-localise with active $\beta 1$ integrin [92]. This association was ligand-independent but force-dependent, with the force generated by integrin-anchored retraction fibres. Although inconsistent with a role for ligand-dependent anchorage in controlling the division axis, it has been proposed that the formation of this lateral cortical mechanosensory complex determines orientation of the mitotic spindle irrespective of cell microenvironment [92].

Energy and metabolism

In a teleological sense, it is plausible that signal transduction via IACs controls all cellular functions that need to respond to changes in the extracellular environment. Energy regulation has little historical precedent, but current interest in the tumour microenvironment has raised awareness [93]. Ontological analysis has confirmed the presence of metabolism-related proteins in the proteome of IACs [74,94], one of which is the key metabolic sensor AMPK. AMPK is activated in response to energy insufficiency and drives cell surface changes leading to enhanced nutrient uptake. Pharmacological activation of AMPK reduced expression of receptors associated with adhesion, including $\beta 1$ integrin, possibly to conserve energy expended during migration [95]. Conversely, inhibition of AMPK upregulated the expression of tensins, which bind directly to, and activate, integrins at fibrillar adhesions [96]. Tensins have been implicated previously in Arp4-dependent integrin turnover at fibrillar adhesions [97], where endocytosis of integrins and ECM was required for recruitment and activation of mTOR.

CAIX, a hypoxia-induced carbonic anhydrase upregulated in cancer cells, is an additional metabolism-related component of IACs. Analysis of the CAIX interactome by BioID under hypoxic conditions identified integrins, CD98 (also an adhesome component) and MMP14 co-located in pseudopodia [98]. MMP14 was also co-localised with Tks5 and cortactin in invadopodia. While not reported to be part of the adhesome, the metabolic pathway co-activator PGC-1 α is part of a transcriptional program that down-regulates the expression of adhesion molecules, including integrins, via TCF4 transcription factor inactivation [99]. Conversely, down-regulation of PGC-1 α increased expression of integrins and FAK [100]. Together, these findings are suggestive of a co-ordination between ECM turnover and energy metabolism.

Outlook

Our understanding of the composition and structure of IACs, and the mechanisms by which signalling via IACs links to cell fate decisions, has improved markedly over the past decade, and the most significant contributions over the past two years have been reviewed above. In the near future, the application of techniques such as proximity biotinylation, in conjunction with mass spectrometry, will enable networks

to be built that will complement current models generated from IACs in 2D culture. These studies will provide a means to annotate the adhesome with spatial information, as well as enable investigations of IAC composition in 3D models and *in vivo*. Improvements in microscopy techniques will also drive investigations of the substructure of IACs, together with analyses of the dynamic changes in location, conformation and post-translational modification of components during signal relaying. Finally, multidisciplinary approaches will continue to link these membrane-proximal events to the plethora of functions controlled by adhesion.

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Figure legend

Figure 1. Signal relaying via the consensus adhesome. A schematic representation of proteins most commonly found in IACs [10]. The diagram shows the interactions between adhesome proteins. Interactions with the highest confidence, based on the literature, have greater thickness. Proteins with a thick black node border are members of the literature-curated adhesome protein. Recent evidence suggests the left half of the network transduces signals that regulate phosphorylation and small GTPases [21,28], while the right half is primarily responsible for mechanosensitive connections to actin filaments [45]. Together, these outputs are integrated to influence many aspects of cell phenotype.

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