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Adhesion protein networks reveal functions proximal and distal to cell-matrix contacts

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Cell adhesion to the extracellular matrix is generally mediated by integrin receptors, which bind to intracellular adhesion proteins that form multi-molecular scaffolding and signalling complexes. The networks of proteins, and their interactions, are dynamic, mechanosensitive and extremely complex. Recent efforts to characterise adhesions using a variety of technologies, including imaging, proteomics and bioinformatics, have provided new insights into their composition, organisation and how they are regulated, and have also begun to reveal unexpected roles for so-called adhesion proteins in other cellular compartments (for example, the nucleus or centrosomes) in diseases such as cancer. We believe this is opening a new chapter on understanding the wider functions of adhesion proteins, both proximal and distal to cell-matrix contacts.

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Introduction

The extracellular matrix (ECM) forms an essential part of the cellular microenvironment; adhesion of cells to the ECM is critical for much of metazoan development, and its perturbation contributes to disease. The composition of ECM is highly diverse, containing proteins, glycoproteins and proteoglycans that interact to form a complex milieu [1]. It provides a structural support for cells to enable tissue formation and mechanosensing, and it binds soluble ligands and cell-surface receptors to trigger and coordinate cellular signalling [2]. Cells also use cell-surface adhesion receptors to sense the topology and stiffness of the pericellular ECM [3]. Mechanical information is transmitted via receptor-associated proteins to, and from, the actin cytoskeleton. Thus, adhesion receptors integrate and process biochemical and biophysical cues to control many aspects of cell behaviour, including differentiation, proliferation and migration.

The proteins that mediate adhesion signalling have been studied for decades. Recently, progress has been made in cataloguing the components of adhesions in various cell types, revealing that adhesion signalling is complex and diverse, both in terms of the number of components and the interrelations between them in signalling networks. Furthermore, the spatial restriction of this signalling is thought to drive emergent properties of multicellular systems in a way that is not yet fully understood [4]. Working out how cell adhesion systems function at a holistic network level is currently under intense scrutiny.

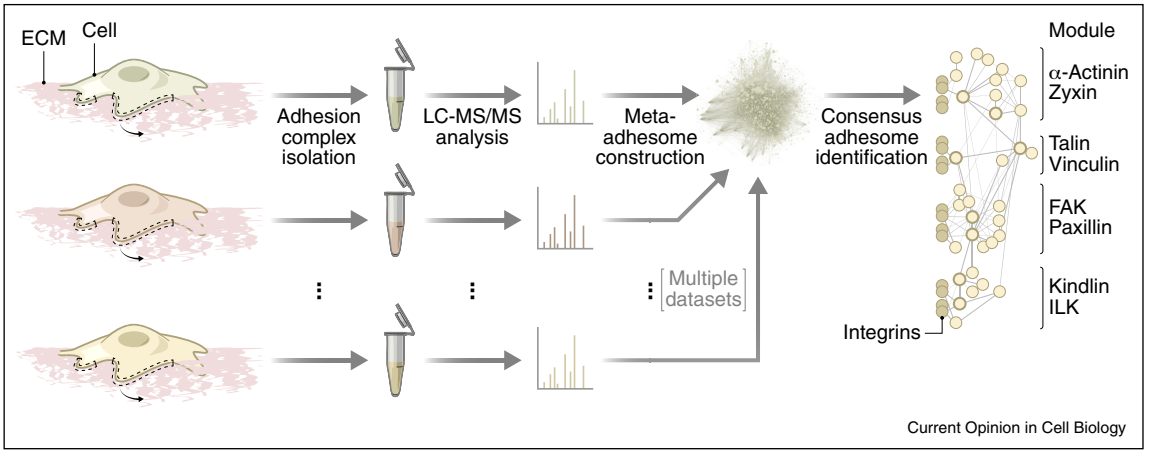
Here, we review recent progress in the elucidation of adhesion protein networks that mediate cell adhesion and provide the downstream effector signalling mechanisms. We also highlight new studies that have uncovered wider roles for adhesion protein signalling downstream of — and distal from — cell-ECM receptors. These studies suggest important new roles for adhesion proteins in diverse cellular locales.

Adhesion signalling complexes: defining the players

The best-characterised family of cell-surface ECM receptors is the integrins, members of which interact with a range of ligands in the extracellular milieu [5]. Upon ligand binding, intracellular adhesion proteins are recruited to clustered integrin heterodimers at the plasma membrane, forming adhesion complexes [6,7]. These consist of signalling and structural proteins that connect integrins to the actin cytoskeleton, the sum of which has been termed the 'adhesome' [8]. The latest literature-curated adhesome database contained 232 proteins derived from studies using multiple cell types and experimental conditions [9••].

Until recently, the comprehensive, global analysis of adhesomes was restricted by the challenges of purifying the labile, membrane- and cytoskeleton-linked adhesion complexes. The development of biochemical methodologies to isolate integrin-associated proteins, coupled with advances in proteomics and informatics, has largely overcome the earlier major challenges, thus enabling the characterisation of adhesion complexes by quantitative mass spectrometry [10–14]. Computational integration of multiple adhesion-site proteomes yielded an experimentally defined 'meta-adhesome', from which a core set of 60 frequently identified proteins — a 'consensus' adhesome — was identified [15••] (Figure 1).

Figure 1



Definition of a consensus adhesome. Adhesion complexes induced by the integrin ligand fibronectin were stabilised and purified (curly arrows) and their proteomes were characterised by quantitative mass spectrometry (LC–MS/MS) in multiple studies using different cell types. Integration of these datasets generated a meta-adhesome, from which a core consensus adhesome was established [15••]. Network nodes (circles) represent interacting proteins; thick node borders indicate proteins that define the axes of emergent consensus adhesome modules (labelled, right).

It was clear from the first mass spectrometric analyses of isolated adhesion complexes that the number of proteins in these assemblies was greater than previously appreciated [16–18]. This showed that integrin-mediated adhesions are sites of considerable molecular complexity and diversity, and it is likely that they are sophisticated signalling hubs with physical and functional links to the cytoskeleton and to other organelles and cellular processes. Moreover, adhesion complexes induced by different extracellular ligands, or recruited to different integrin receptors, contain both common and condition-specific subsets of proteins [16,19,20]. Therefore, understanding the precise and context-dependent relationships between multiple adhesion proteins, and the mechanisms by which they control cell behaviour, have become important future priorities.

Adhesion signalling close to integrins: mechanosensing the microenvironment

The assembly and disassembly of adhesion complexes are tightly and dynamically regulated. However, the precise interactions of adhesion proteins are poorly defined in both space and time. A recent fluorescence correlation microscopic analysis of tagged adhesion proteins led to a model of hierarchical protein recruitment to integrins at early (nascent) adhesions [21•]. This proposed initial binding of kindlin-2 to α5β1 integrin, a role for α-actinin in nucleation of adhesions and subsequent association of talin and vinculin in response to myosin II activation (Figure 2). Talin forms a complex with vinculin before it associates with integrin [21•], as appears to be the case for several other adhesome components [22•]. Active myosin II generates mechanical forces that can change the conformation of proteins, including talin [23]. In filopodial and lamellipodial protrusions, talin links integrin to RIAM, which can promote actin polymerisation [24]. Focal adhesion kinase (FAK) may also accumulate at adhesion sites at the front of cells before paxillin [25], while some molecules, such as zyxin and tensin, are generally absent from nascent adhesions [26]. However, the temporal sequence of events may be cell and context specific. Proteomic quantification of assembly and disassembly of isolated adhesion complexes has revealed distinct temporal profiles of protein recruitment [15••]. These proteomic studies support the early recruitment of α-actinin and the later appearance of zyxin at adhesion sites (Figure 2). Moreover, adaptor proteins are apparently lost from adhesion complexes more rapidly than actin-binding proteins during disassembly, suggesting a relatively late disruption of the integrin-actin connection during adhesion turnover [15••].

Despite the remarkable consistency of very early adhesion assembly, regardless of ligand density, rigidity or intracellular tension [27,28•], the stability and growth of nascent adhesions are regulated by physical links to the cytoskeleton and are influenced by actin-associated proteins (for example, formins, septins and synaptopodins [29–31]). Microtubules also influence adhesion complex composition and dynamics [32•,33–36], with their targeting to adhesion sites being regulated by integrin activation state [37•].

The interactions of vinculin with talin and actin probably form the major mechanosensory module that controls adhesion site composition, organisation and stability [38,39•,40,41], with a role also for FAK [42–44]. Proteomics experiments have identified many proteins, including a