oligodendrocyte expansion, maturation and turnover, will inform research directions aimed at treating devastating myelin-related disorders.

COMPETING FINANCIAL INTERESTS
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Job-splitting among integrins

Ronen Zaidel-Bar

How different integrin receptors for the same extracellular ligand transduce distinct cellular responses is unclear. The characterization of the class-specific adhesomes of β_1 and α_V integrins now shows that whereas α_V integrins promote unbranched actin polymerization, β_1 integrins induce myosin-II-dependent contractility, and both integrin subtypes synergistically mediate rigidity sensing.

Integrins are transmembrane receptors that bind specific extracellular matrix proteins or other receptors outside the cell, and connect with the cytoskeleton inside the cytoplasm through adaptors bound to their intracellular tails[1]. Integrins cluster in *cis* to form cell adhesion structures that are essential for cell spreading, migration, matrix remodelling and degradation[2]. As well as forming a physical link between the cell and its environment, integrin-mediated adhesions serve as signalling hubs, harbouring hundreds of adaptor and regulatory proteins that together control cytoskeletal and adhesion dynamics in response to external or internal cues[3].

The functional unit of integrins is a heterodimer made of α and β chains. Early Metazoans possessed only a few integrin genes, and even the nematode *Caenorhabditis elegans* has only two α and a single β integrin. In contrast, the mammalian genome encodes 18 α and 8 β integrin genes, which can form 24 different heterodimers. This diversity enables mammalian cells to distinguish between different extracellular matrix ligands, such as laminin, collagen and fibronectin. However, multiple heterodimers also bind the same ligand. For example, fibronectin is recognized by 8 integrin heterodimers, including $\alpha_5\beta_1$ and $\alpha_V\beta_3$. Fässler and colleagues now provide insights into how integrins binding the same ligand can achieve functional diversity, by analysing the protein interaction networks and downstream responses of the α_V and β_3 subtypes[4].

Previous studies indicated that different integrins are not functionally redundant and may have unique roles. Loss of function of either α_5 or α_V in mice is embryonic lethal, but with different phenotypes, neither of which is as severe as the genetic loss of their common ligand fibronectin[5]. Furthermore, studies of cultured fibroblasts demonstrated that $\alpha_5\beta_1$ is enriched in fibrillar adhesions[6], adhesive sites at central regions of the cell, which are associated with fibronectin fibrils[2]. In contrast, $\alpha_v\beta_3$ is confined to focal complexes at the base of cellular protrusions, and to focal adhesions, which anchor contractile stress fibres at the periphery of the cell[2]. This segregation was later shown to be dynamic, due to actomyosin-dependent pulling of $\alpha_5\beta_1$ out of the rear end of focal adhesions and into fibrillar adhesions, whereas $\alpha_v\beta_3$ remains stationary[7,8]. Several studies have also pointed to differences in signalling downstream of each class of integrin. For example, they differ in their ability to activate the GTPase RhoA and to recruit tyrosine (Src, FAK) and serine/threonine (CamKII, PKC) kinases (reviewed in ref. 9). Finally, *in vitro* reconstitution experiments have shown that $\alpha_5\beta_1$ integrins determine adhesion strength through their catch-bond binding to fibronectin, whereas $\alpha_v\beta_3$ integrins mediate force-induced reinforcement of the adhesion site through their connection with the actin cytoskeleton[10].

Integrin signalling is the outcome of the many protein interactions within the integrin adhesome[3]. The differences in signalling between $\alpha_5\beta_1$ and $\alpha_v\beta_3$ are probably the result of differences in their adhesomes, which at the most basic level stem from distinct affinities for proteins binding to their tails. Such tail-specific differences are responsible for ERK1 and protein kinase D1 binding to β_3 and not β_1 (refs 11,12), but the full repertoire of tail-specific binding partners and their ramifications for the integrin-specific functions have not yet been addressed. The study by Fässler and colleagues investigates both the β_1 and β_3 tail-specific binding and the $\alpha_5\beta_1$- and $\alpha_v\beta_3$-specific adhesomes[4]. This work was facilitated by recent advances in quantitative mass spectrometry (reviewed in ref. 13), and by the genetic engineering of three mouse cell lines expressing on the background of pan-integrin knockout (pKO), either $\alpha_5\beta_1$ (pKO-β_1 cells), $\alpha_V\beta_3$ and $\alpha_V\beta_5$ (pKO-α_V cells), or $\alpha_5\beta_1$, $\alpha_V\beta_3$ and $\alpha_V\beta_5$ (pKO-α_V/β_1 cells). Adhesomes were analysed in all three cell lines under various conditions, including myosin inhibition, and phosphoproteomics analyses were performed to identify over 1,000 phosphorylation events on 150 proteins. This immense data set will serve as a valuable resource for investigating many aspects of integrin-specific signalling. In light of the phenotypes of the integrin-specific cell lines (Fig. 1), the authors further focused on the regulation of actomyosin contractility.

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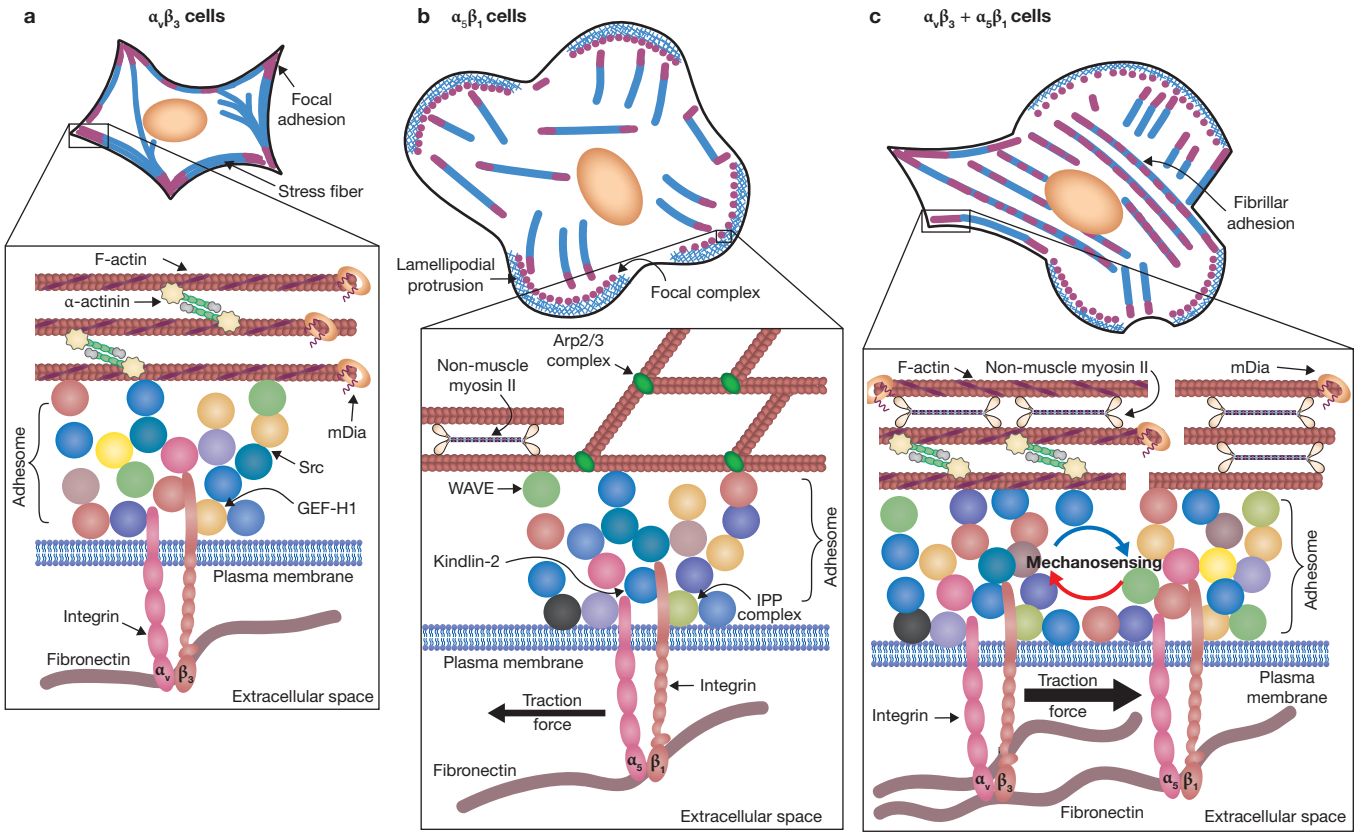

Figure 1 Unique phenotypes of cells expressing $\alpha_v\beta_3$, $\alpha_5\beta_1$ or both heterodimers are explained by their integrin-specific adhesomes. (**a**) Fibroblasts expressing only $\alpha_v\beta_3$ do not spread well, and although they have robust focal adhesions and stress fibres, they are not contractile and do not exert traction forces on the matrix. Enriched in the $\alpha_v\beta_3$ adhesome (enlarged box) are proteins that induce the formation of actin stress fibres. (**b**) Fibroblasts expressing only $\alpha_5\beta_1$ are very well spread, and form numerous lamellipodial protrusions and focal complexes, but very few mature focal adhesions. Nevertheless, they are contractile and exert moderate traction forces on the matrix. The $\alpha_5\beta_1$ adhesome (enlarged box) is enriched in proteins that induce the branched actin network that pushes the lamellipodia forward, and activators of myosin contractility. (**c**) Fibroblasts expressing both $\alpha_v\beta_3$ and $\alpha_5\beta_1$ embody both phenotypes as they have focal complexes and protrusions as well as large focal adhesions and stress fibres. They also have fibrillar adhesions. An emergent property of the combined adhesome (enlarged box) is the ability to sense the rigidity of the extracellular matrix and respond by reinforcing the actomyosin structure.

pKO-α_V cells have few protrusions, do not spread well, migrate slowly but persistently, and have very large focal adhesions. In contrast, pKO-β_1 cells are very protrusive, have numerous focal complexes and migrate the fastest, albeit least persistently. pKO-α_V/β_1 cells have both focal complexes and focal adhesions, and migrate at an intermediate velocity and persistence. These phenotypes are in line with what would be expected on the basis of previous studies. However, when the authors examined the contractility of these cells by looking at phosphorylated (active) myosin light chain (pMLC) and by direct traction force measurements, they observed that pKO-α_V cells with their large focal adhesions actually had the lowest level of pMLC and produced the smallest traction forces. pKO-β_1 cells had intermediate levels of contractility, and the highest levels of pMLC and traction forces were found in pKO-α_V/β_1 cells. These counterintuitive results are nonetheless consistent with another recent force measurement study[14], and the notion of decoupling between the actin content of stress fibres and tension, as seems to be the case in pKO-α_V cells, was recently demonstrated in cells in which formins were optogenetically activated[15]. Interestingly, although it does not produce forces on its own, α_V integrin clustering is myosin-dependent and accumulates in focal adhesions under tension. In contrast, β_1 integrin, which can produce contractility, is not enriched in focal adhesions under high tension and its clustering is tension-independent.

These findings suggest that mammalian cells need more than one receptor for fibronectin, probably because functions that were once performed by a single integrin diverged during evolution and are now carried out by distinct integrins, which must cooperate to produce the desired outcome. For example, $\alpha_5\beta_1$ clusters independently of myosin and induces contractility, followed by $\alpha_v\beta_3$, which subsequently responds to the tension and provides cytoskeletal reinforcement. A striking demonstration of this need for cooperation emerged from traction force measurements carried out on substrates with varying rigidity. Mechanosensing required both $\alpha_5\beta_1$ and $\alpha_v\beta_3$, as only pKO-α_V/β_1 cells were able to adjust their contractility to the substrate rigidity, whereas pKO-β_1 cells maintained an equal level of contractility on all substrates.

The authors analysed their proteomic data to identify signalling modules that are selectively recruited and activated by either integrin subtype. They showed that integrin $\alpha_5\beta_1$ selectively recruited Kindlin-2 and the ILK/PINCH/Parvin complex as well as WAVE and Arp2/3 complexes, which are known to drive the dendritic actin polymerization necessary for lamellipodia formation. In contrast,

integrin $\alpha_v\beta_3$ selectively recruited RhoGEF-H1 and associated with the RhoA effector mDia1, which generates the long unbranched actin filaments necessary for stress fibres. Another effector of RhoA is Rho kinase, which is responsible for activating myosin light chain and thereby increases contractility. Therefore, the authors' observation that pKO-α_V cells, which have very low pMLC and contractility, also have the highest level of active RhoA, was somewhat unexpected. This active Rho was shown not to activate Rho kinase, as transfection of pKO-α_V cells with a constitutively active form of Rho kinase rescued their contractility. How active RhoA in pKO-α_V cells differs from active RhoA in pKO-β_1 or pKO-α_V/β_1 cells is a question for future research. Fässler and colleagues also uncovered other potentially important differences in pMLC regulation: myosin phosphatase-1 (Mypt1) was highly phosphorylated (and therefore inactivated) only in pKO-β_1 and pKO-α_V/β_1 cells, which also had high levels of phospho-ERK2. Importantly, expression of an active form of MEK1, which phosphorylates ERK2, was able to rescue the pMLC levels in pKO-α_V cells.

By comprehensively characterizing each integrin subtype alone and in combination, the authors took a significant step toward delineating the unique pathways that characterize them and the many ways in which the two separate integrin networks combine to create an adhesome with novel emergent properties. These interesting findings notwithstanding, the full picture of how each integrin contributes to adhesion and actomyosin dynamics is still far from complete. Another major remaining question is how the subcellular localization of each integrin type is determined. One possibility is that the localization pattern of different integrins reflects the biophysical nature of their interactions with the extracellular matrix and dynamic cytoskeleton[8,16]. Integrin localization may also be regulated by subtype-specific trafficking pathways, and both mechanisms could be at play in the cell. Clues to answer this and other questions regarding the regulation and functions of different integrins are certain to be hiding in the trove of proteomic data provided in this study.

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