GIVING OFF MIXED SIGNALS – DISTINCT FUNCTIONS OF $\alpha_5\beta_1$ AND $\alpha_V\beta_3$ INTEGRINS IN REGULATING CELL BEHAVIOUR

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Abstract

The formation, maturation and dissolution of focal adhesions are basic prerequisites of cell migration and rely upon the recruitment, signalling and endocytosis of integrins. In many instances, extracellular matrix molecules are recognised by a number of integrins, and it is the sequential involvement of different integrins that allows establishment of cell polarity and migration toward a matrix stimulus. In this review we consider both the similarities and differences between two key fibronectin receptors: $\alpha_V\beta_3$ and $\alpha_5\beta_1$ integrin. By considering the GTPase and kinase signalling and trafficking of two such closely-related receptors we begin to understand how cell migration is coordinated.

Keywords

Integrin; signalling; trafficking; endocytosis; focal adhesion; migration

1. Introduction

Intercellular communication in metazoa relies not only on autocrine, paracrine and exocrine signalling systems, but also on the structural and positional information encoded in extracellular matrices (ECM). Cell-ECM interactions act as sites of mechanotransduction, transmitting short-range tensile and elastic force across the plasma membrane, and interpreting long-range alterations in tissue flow. They also regulate chemical signalling by controlling the spatiotemporal assembly of enzymes and adaptors into dynamic complexes. At the cellular level, cell-ECM contact sites are elaborated as clusters of adhesion receptors, principally integrins, interacting extracellularly with ECM polymers and intracellularly with cytoskeletal and signalling components. The close integration of the external tissue structure with the internal signalling machinery enables exquisite environmental sensing. Fundamental cellular processes, including survival, division, differentiation and migration are reliant upon effective cell-ECM associations. For this reason, there has been much interest in defining the mechanisms of adhesion receptor-ligand binding and signalling. Understandably, for such an important protein assembly, the cell-ECM junction is complex, containing at least several hundred protein components. Currently, our insight into the molecular composition of adhesion complexes is limited; we understand which molecules and pathways are possible, but not the stoichiometry, turnover and dynamic relationships of molecules in real adhesion complexes. A number of recent reports have impacted significantly on these questions by opening up new avenues of research. We will draw together these diverse findings and predict directions that the field will take, with a

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particular view to understanding the spatial and temporal control of the mechanisms described and the molecular complexity associated with these processes.

The adhesion contacts of cultured cells have been broadly divided into three categories according to their molecular composition. Focal complexes (FX) are nascent adhesion plaques that are rich in phosphotyrosine, talin and $\alpha_v\beta_3$ integrin (1); focal adhesions (FA) develop from FX upon membrane retraction and contain $\alpha_5\beta_1$ integrin and zyxin, in addition to the FX components (1,2); fibrillar adhesions (FB) develop as FA translocate centripetally across the ventral surface of the cell and have a limited molecular composition that includes $\alpha_5\beta_1$ integrin and tensin, but lacks $\alpha_v\beta_3$ integrin, paxillin and phosphotyrosine (2,3). The sequential recruitment of adhesion contact components means that we must be careful not to over-interpret the partition into FX, FA and FB, and the cell-specific composition of the different contacts is still disputed, but the labels do reflect the molecular heterogeneity and dynamic relationship of integrin complexes. Integrin activation and the maturation of adhesion plaques is heavily influenced by the transmission of force through the actin cytoskeleton (4,5), which depends on both the rigidity of ECM and contraction of the cytoskeleton itself. Cytoskeletal reorganisation and contraction are driven by activation of the small GTPases Rac1 and RhoA (6), to the extent that activation of Rac1 drives FX formation, while suppression of RhoA blocks the transition to FA (7). Release of RhoA inhibition then drives FA formation and stress fibre contractility.

During biological processes such as cell migration and matrix fibrillogenesis, the heterodimer-specific functions of $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins are precisely co-ordinated. When considering the relative roles of $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins, there appears to be a dynamic interaction between the processes of FA formation/maturation, transduction of heterodimer-specific signalling and spatially restricted cell-surface expression of specific integrin heterodimers. In this review, we will consider these phenomena in turn and highlight mechanisms by which they influence one another. A recurring theme will be the concept of dynamic cross-regulation of these processes and how it is regulated by the extracellular environment. Specifically, how the spatiotemporal regulation of these processes, and their influence on one another, must be precisely co-ordinated to permit efficient cell migration.

Central to the ability of cells to transduce integrin heterodimer-specific intracellular signals in a spatially and temporally co-ordinated manner, are precisely regulated mechanisms to control cell surface localisation of receptors, including adhesive contact maturation and vesicular trafficking. The centripetal translocation of $\alpha_5\beta_1$, but not $\alpha_v\beta_3$, is a defining characteristic of the maturation of adhesive contacts. A consequence of this is ligation of specific heterodimers in a spatially-restricted manner. Signalling as a consequence of ligand engagement of these different integrin heterodimers, particularly via the Rho family small GTPases and non-receptor tyrosine kinases, has a key role in regulating many of the cellular processes responsible for adhesion maturation and vesicular trafficking. Moreover, we will survey the present evidence that heterodimer-specific cytoplasmic interactions regulate integrin endocytosis and therefore cell surface availability/accessibility of receptors.

2. Alternative signals from fibronectin receptors

Heterogeneity in the integrin composition of adhesion plaques could feasibly lead to variation in the signalling cascades initiated in response to ECM engagement, and integrin-specific signalling pathways have been investigated extensively in both fibroblasts and epithelial cells. One prominent example is the regulation of Rho family GTPases. Engagement of either $\alpha_5\beta_1$ or $\alpha_v\beta_3$ integrin is sufficient for the suppression of RhoA activity during the early stages of cell spreading on fibronectin (8,9), but while engagement of $\alpha_5\beta_1$ integrin, found in FA and FB, is capable of reactivating RhoA, engagement of $\alpha_v\beta_3$

integrin, found in FX and FA, is not, reflecting the role of RhoA in the FX-FA transition (9) (Fig. 1). The morphologies of cells expressing specific integrins reflect these signalling differences as $\alpha_v\beta_3$-expressing cells have low RhoA activity and form broad lamellipodia, while cells expressing $\alpha_5\beta_1$ integrin have high RhoA activity and form actin stress fibres. Pharmacological or dominant-negative inhibition of RhoA switches $\alpha_5\beta_1$-expressing cells to an $\alpha_v\beta_3$-like morphology (10), demonstrating the key role of RhoA in mediating integrin-specific behaviour. The $\alpha_5\beta_1$ integrin-RhoA link also explains some of the features of fibronectin fibrillogenesis, which requires both translocation of $\alpha_5\beta_1$ integrin (3) and activation of RhoA (11). Cells expressing $\alpha_v\beta_3$, rather than $\alpha_5\beta_1$, fail to organise fibronectin into fibrils but can be partially rescued by expression of dominant active RhoA (9,12). This is not to suggest that $\alpha_v\beta_3$ is simply an inert version of $\alpha_5\beta_1$, as $\alpha_v\beta_3$ can mediate fibronectin fibrillogenesis, by an unknown alternative mechanism, in the developing embryo or when cells are cultured on laminin-coated surfaces (13). The alternative mechanisms of fibronectin fibrillogenesis are just one example of the molecular redundancy between integrins, and highlight the difficulties of drawing simple, linear connections between molecules that are frequently context-specific.

Unlike RhoA, Rac1 activation is influenced by both $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrin. Clustering of cytoplasmic tails of either β_1 or β_3 integrin causes Rac1 activation (14) and steady-state activity levels are also similar between $\alpha_5\beta_1$- or $\alpha_v\beta_3$-expressing cells (9). The regulation of Rac1 is complicated as engagement of $\alpha_5\beta_1$ integrin causes membrane recruitment (15), while simultaneous engagement of the transmembrane proteoglycan, syndecan-4, causes GTP-loading (16). It is still not entirely clear whether $\alpha_v\beta_3$ integrin relies on cooperation with syndecans to the same extent as $\alpha_5\beta_1$. Unlike $\alpha_5\beta_1$, $\alpha_v\beta_3$-mediated adhesion appears to induce FA formation (9) and fibronectin fibrillogenesis (13) in the absence of syndecan engagement. However, other experiments have demonstrated that syndecan-1 plays an important role in the activation of $\alpha_v\beta_3$ integrin (17) and that reduction of syndecan-4 expression alters the morphology and migration of cells adhering through $\alpha_v\beta_3$ (12,16). As syndecans are understood to act as complementary receptors that fine-tune integrin function, it is logical that they should exert different influences over the different integrins, and resolving the subtle interplay between receptors will be an important step toward understanding how cells precisely regulate signalling cascades, both spatially and temporally.

Although the mechanisms by which integrins exert their influence on intracellular signals are still poorly resolved, heterodimer-specific associations with a number of protein kinases have been characterised. A range of tyrosine kinases including Hck, Lyn and c-Yes complex with the cytoplasmic domains of both β_1 and β_3 integrins, but the potential for alternative signalling pathways relies primarily on c-Src, which binds to, and is activated by, the clustered β_3 cytoplasmic tail (18,19). Despite the role of Src in achieving full activation of another tyrosine kinase, focal adhesion kinase (FAK), the critical autophosphorylation of FAK on tyrosine-397 is delayed in $\alpha_v\beta_3$-expressing compared with $\alpha_5\beta_1$-expressing cells (9). The recruitment of FAK to FA is accelerated in Src/Yes/Fyn-null cells (20), which suggests a model of Src recruitment to $\alpha_v\beta_3$-containing FX that precedes FAK recruitment to $\alpha_5\beta_1$-containing FA. In support of this model, Src-independent autophosphorylation of FAK is blocked by a β_1 fragment that inhibits cell spreading (21), FAK is enriched in FA compared to FX (1) and also enriched in $\alpha_5\beta_1$-containing compared to $\alpha_v\beta_3$-containing pseudopodia (22). The reason for the enrichment of FAK in FA is unclear, as one of the recruitment pathways of FAK is by association with talin, which binds equally well to β_1 and β_3 cytoplasmic domains (23). The second method by which FAK might be recruited is through association with the adapter molecule paxillin. Like FAK, paxillin is enriched in FA over FX (1), but the paxillin that is found in FX is recruited before $\alpha_5\beta_1$ is visibly clustered

(24), which would argue that the differences in paxillin abundance are not entirely due to integrin specificity.

Heterodimer-specific relationships with serine/threonine kinases have also been characterised and often regulate integrin localisation, rather than mediating signals downstream of integrin engagement. The alpha and epsilon isoforms of protein kinase C (PKC) regulate trafficking of β_1 integrin by binding to the cytoplasmic domain, either directly or in complex with the scaffolding protein RACK1, respectively (25-27). By contrast, β_3-integrin interacts exclusively with PKCβ in a complex that again includes RACK1 and, like the β_1-PKC complex, regulates cell migration (28). Although this study focused on integrin $\alpha_{IIb}\beta_3$ rather than $\alpha_v\beta_3$, it does suggest that β_1 and β_3 integrins are linked to similar, yet distinct pathways, though isoform-specific interactions. Of even greater interest is the potential for regulatory crosstalk between signals downstream of the different integrins. Ligation of $\alpha_5\beta_1$ causes activation of calmodulin-dependent kinase II (CamKII) that is necessary for $\alpha_5\beta_1$-mediated migration toward fibronectin (29). Not only is $\alpha_v\beta_3$-mediated migration independent of CamKII activity (30), but engagement of $\alpha_v\beta_3$ even inhibits CamKII activity to block $\alpha_5\beta_1$-mediated migration (29). Interestingly, $\alpha_5\beta_1$ does not exert a similarly antagonistic influence on $\alpha_v\beta_3$. Engagement of $\alpha_5\beta_1$ and $\alpha_v\beta_3$ each make critical contributions toward suppression of PKA activity to the extent that engagement of $\alpha_5\beta_1$ by low abundance ligand is necessary for efficient migration over the $\alpha_v\beta_3$-specifc ECM, vitronectin (30). Other integrin-specific interactions include the association of $\alpha_v\beta_3$ with ERK1 (31) and protein kinase D1 (PKD1) (32). ERK1 and PKD1 bind respectively to the central and membrane-distal NITY motifs of the β_3 cytoplasmic domain, which are distinct from the talin-binding NPXY motif, and not conserved in β_1. Disruption of the association of $\alpha_v\beta_3$ with either ERK1 or PKD1 blocks the initiation of FX formation and directionally persistent migration, by inhibiting Rab4-dependent recycling of $\alpha_v\beta_3$ as described below (Fig. 2).

In summary, the pair-wise analysis of molecular interactions reveals a number of differences between $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins (Fig. 1). However, these studies are united by one common feature; namely that interfering with integrin-specific interactions consistently results in compromised cell migration rather than complete inhibition of cell adhesion. The subtlety of this phenotype emphasises the importance of the nucleation and maturation of adhesion plaques in coordinating cell behaviour, and as we go on to consider the consequences of the spatial localisation and appropriate trafficking of the different integrins this becomes increasingly apparent.

3. Receptor trafficking determines integrin function

Adhesion receptor trafficking is a precisely controlled mechanism that regulates the subcellular localisation of signalling cascades and cell-matrix traction. By targeting specific integrin heterodimers to discrete regions of membrane, the cell controls integrin accessibility and co-ordinates heterodimer-specific signalling. Additionally, integrins can be co-trafficked with other adhesion and growth factor receptors, with the result that the molecular processes that coordinate recycling of integrins may also regulate cell-surface delivery of receptors capable of modulating integrin function. In this section we will consider the role of adhesion receptor recycling as a means of differentially regulating the intracellular signalling of $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins (Fig. 2), and discuss the role of this regulation in adhesion plaque formation and directional cell migration.

Adhesion receptor internalisation occurs through both clathrin-dependent and clathrin-independent endocytosis and internalisation of these endocytic vesicles can occur in a dynamin-dependent or independent manner (33-35). Following endocytosis, receptors are

trafficked to early endosomes, after which they face three possible fates: they can be recycled back to the membrane via the short-loop pathway, shuttled to the perinuclear recycling compartment (PNRC) before being returned to the membrane via the long-loop pathway, or alternatively, the receptors can be transported to late endosomes for degradation.

Cholesterol-enriched membrane microdomains (CEMMs), which include caveolae, are ordered regions of membrane that, as a result of their lipid composition, spatially restrict the distribution of, and signalling from, adhesion receptors (Fig. 3). Loss of integrin engagement results in internalisation of CEMMs due to the release of phosphorylated caveolin-1 and dynamin-2 from integrin clusters (36). Conversely, clustering of β_1 integrin leads to the membrane localisation of CEMM markers and Rac1 (15), while direct clustering of the CEMM component, GM1, results in the membrane-recruitment of Rac1 and activation of its downstream effector, PAK (15). Anchorage-dependent regulation of CEMM membrane-targeting and Rac1 activation is mediated by microtubules and the vesicular trafficking-associated small GTPase, Arf6 (37). Interestingly, recent work by Palamidessi et al (2008) suggests that Rab5- and clathrin-dependent endocytosis is required for GTP-loading of Rac1 (38). Consistent with the role of Arf6 in CEMM recycling, Arf6 regulates membrane localisation of endocytically-regulated active Rac1 (37,38) (Fig. 3).

When considering the endocytosis of integrins themselves, it is clear that the coordination of a number of protein kinases is necessary for the trafficking of different integrin heterodimers. Using high-throughput screening, Pelkmans et al (2005) identified large numbers of kinases involved in different types and stages of endocytic recycling, and demonstrated that specific subsets of kinases have opposing effects on different modes of endocytosis (39). The processes that regulate endocytosis of integrins in a heterodimer-specific manner are still poorly understood but, as many of the kinases associated with internalisation of integrins can be differentially regulated by engagement of specific integrin heterodimers or their co-receptors, it is possible to postulate a number of heterodimer-specific recycling mechanisms. For example, caveolin-1 is phosphorylated on Y14 by Src (40), and phosphorylation of this residue is required for caveolin-mediated internalisation of CEMMs (36) and p190RhoGAP-dependent suppression of Rho activity (41). The differential regulation of Src by $\alpha_5\beta_1$ and $\alpha_v\beta_3$ may provide a means by which integrins can influence endocytosis in a heterodimer-specific manner. Interestingly, in studies using cytoplasmic mutants of $\alpha_{IIb}\beta_3$ integrin, the distal NITY motif, which is unique to the β_3 cytoplasmic domain, allows internalisation of β_3. Substitution of this domain or mutation of tyrosine-759, which is a substrate for Src family kinases, suppresses endonexin-mediated uptake of β_3 (42,43). Likewise, the isoform-specific interactions of PKCs, described above, make a major contribution to integrin trafficking. Stimulation of PKCα increases dynamin-dependent endocytosis and recycling of β_1 integrin and, as a consequence, increases migration of carcinoma cells (26). In fibroblasts, PKCϵ, which binds to the cytoplasmic domain of β_1 integrin, in complex with RACK1, phosphorylates the intermediate filament protein, vimentin, on the surface of integrin recycling endosomes (44). Phosphorylation of vimentin mediates the dissociation of PKCϵ from β_1 integrin, and is essential for trafficking of β_1 integrin back to the surface.

Microtubule targeting to adhesive contacts mediates FA disassembly, but it is not yet clear what role integrin endocytosis may play in this process. However, microtubule-dependent FA dissociation is dynamin-dependent (45) and microtubules regulate both internalisation and subsequent recycling of CEMMs (37), so it is likely that adhesion receptor endocytosis will play a significant role in microtubule-dependent FA disassembly.

As a result of recent advances in our understanding of vesicular trafficking, the processes regulating heterodimer-specific membrane-delivery of $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins are far better understood than those regulating heterodimer-specific endocytosis (Fig. 2). In serum-free conditions, fibroblasts and ovarian carcinoma cells recycle both $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins are recycled through the Rab11- and Arf6-dependent long-loop pathway, under the regulation of PKB/Akt and GSK-3β (32,46,47) (Fig. 2a). However upon platelet-derived growth factor (PDGF) stimulation, $\alpha_v\beta_3$ integrin, but not $\alpha_5\beta_1$, is trafficked back to the membrane from early endosomes via the Rab4-mediated short-loop pathway (Fig. 2b). Short-loop recycling of $\alpha_v\beta_3$ depends upon direct association of the $\alpha_v\beta_3$ cytodomain with PKD1 (PKCμ) to the extent that substitution of the NITY motif, expression of a catalytically inactive mutant of PKD1, substitution of the autophosphorylated serine-916 of PKD1, or suppression of endogenous PKD1 expression, inhibits PDGF-stimulated short-loop recycling of $\alpha_v\beta_3$ (32,48). Significantly, disruption of the PKD1-$\alpha_v\beta_3$ interaction not only compromises short-loop recycling of $\alpha_v\beta_3$, but also increases long-loop recycling of $\alpha_5\beta_1$, as does suppression of $\alpha_v\beta_3$ expression (48). The increase in $\alpha_5\beta_1$ recycling, in response to inhibition of $\alpha_v\beta_3$, depended upon association of $\alpha_5\beta_1$ with Rab-coupling protein (RCP), which in turn binds to Rab11 (46). These experiments provide a powerful example of direct antagonism between integrin heterodimers.

Integrins colocalise with other ECM and growth factor receptors in recycling endosomes. Perturbation of syndecan recycling by mutation of the PIP$_2$-binding site of the scaffolding protein, syntenin, or expression of a dominant negative mutant of Arf6, leads to accumulation of syndecans, β1 integrin, and FGF receptor in long-loop recycling vesicles (49). Furthermore, clustering of syndecan-4, or FGF2 stimulation, regulates redistribution of syndecan-4 to CEMMs and promotes internalisation (35,50), raising the intriguing possibility that integrin co-receptors might influence heterodimer-specific recycling of integrins.

Throughout this review we have discussed the signalling consequences and trafficking mechanisms of $\alpha_5\beta_1$ integrin, in comparison with $\alpha_v\beta_3$ integrin, and identified striking differences between the two. In this final section we consider the biological consequences of differential integrin signalling. Integrin endocytosis and exocytosis exerts a significant influence over cell migration, consistent with the role in regulating FX formation, stability, turnover, and spatial localisation. Phosphorylated caveolin-1 has a polarised distribution in migrating endothelial cells (51) and Src-family kinase-mediated caveolin-1 phosphorylation regulates directionally-persistent migration and cell polarisation. Moreover, caveolin-deficient fibroblasts, which are incapable of internalising CEMMs upon loss of integrin engagement, exhibit reduced directional persistence (36,41). Endocytosis mediated by the association of β_1 integrin and PKCα regulates carcinoma cell migration (26,27) and recently, it has been demonstrated that direct association of the β_1 cytoplasmic domain with the epithelial-specific Rab11 homologue, Rab25, restricts delivery of $\alpha_5\beta_1$ to the leading edge and promotes tumour cell invasion of 3D matrices (52). Cell type-specific expression of molecules that regulate integrin trafficking is an area of research that requires further investigation and could eventually provide a strategy for targeting metastatic tumour cells without affecting the surrounding tissue. It is now established that activation of RhoA and its downstream effector, ROCK, promotes random, amoeboid migration (53). In the same way, suppression of $\alpha_5\beta_1$-recycling, triggered by PDGF-stimulated $\alpha_v\beta_3$ short-loop recycling, promotes directionally persistent migration as a result of reduced ROCK and cofilin activity (48). This is consistent with the role of $\alpha_5\beta_1$, but not $\alpha_v\beta_3$, in activating Rho upon ligand engagement (9) and the fact that cells over-expressing $\alpha_v\beta_3$, but not $\alpha_5\beta_1$, migrate in a directionally-persistent manner (10). Danen et al. also showed that expression of $\alpha_v\beta_3$, rather than $\alpha_5\beta_1$, reduced FA translocation and the turnover of FA components (10). In order for a cell to migrate efficiently it is necessary for FA stability to be actively regulated,

so that FA stabilisation occurs only at points requiring static cell-ECM interactions to provide traction, and that FA formation, sliding and disassembly occur in other regions to allow cellular translocation. As such, it is arguable that precisely regulated migration can only be achieved when heterodimer-specific integrin signalling and recycling, and FA maturation, stabilisation and disassembly are completely integrated.

4. Conclusions

Although progress towards an understanding of cell adhesion complex assembly and turnover has been rapid, a number of major gaps remain. Defining the proteomes of receptor-specific adhesion complexes using mass spectrometry and sophisticated imaging techniques will provide insights into the extent that complexes vary. Systems analysis of signalling modules and networks will shed light on the molecular mechanisms responsible for controlling assembly and maturation of adhesion complexes. Such approaches will also delineate the cross-regulation between different integrin heterodimers, and explain how adhesion complexes initiate long-range effects on cell differentiation. In this context, analyses of the convergence of signalling by other receptor families, such as growth factor, chemokine and cytokine receptors, will lead to an explanation of the cell's sensory functions that are modulated by adhesion to the ECM.

Acknowledgments

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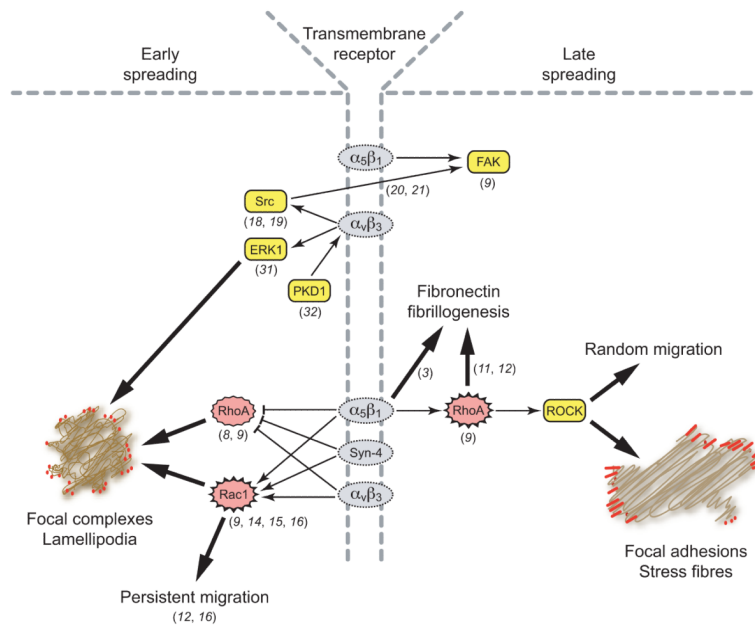
Fig. 1. $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins exert alternative influences over common signalling pathways
A broad range of signalling molecules that include Rho-family GTPases and kinases are regulated in response to integrin-engagement by the ECM. The subtle differences between signals downstream of $\alpha_5\beta_1$ and $\alpha_v\beta_3$ mean that changes in the integrin composition of adhesion complexes, as they mature, automatically trigger changes in the signals that regulate maturation itself. For example, the transition of $\alpha_v\beta_3$-rich FX into $\alpha_5\beta_1$-rich FA causes a switch from Rac1-dominated to RhoA-dominated GTPase signalling that in turn causes an increase in cytoskeletal tension and adhesion complex maturation.
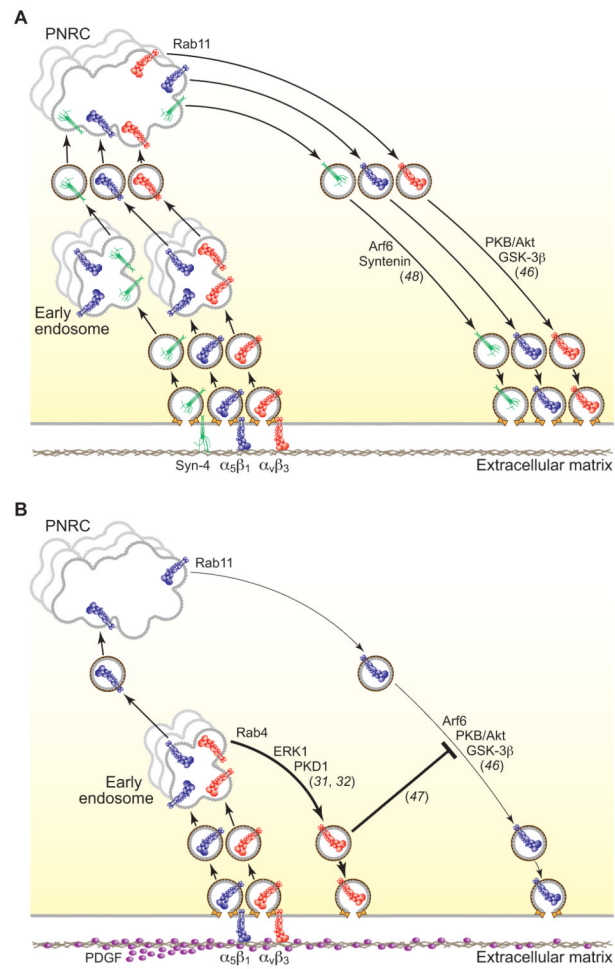
Fig. 2. Heterodimer-specific integrin recycling
A) In the absence of serum, both $\alpha_5\beta_1$ and $\alpha_v\beta_3$ are trafficked, via the PNRC, along the long-loop recycling pathway. Syndecan-4 is also recycled via the long-loop pathway in a syntenin-dependent manner. B) Stimulation with PDGF triggers membrane delivery of $\alpha_v\beta_3$ via the short-loop pathway and suppresses long-loop recycling of $\alpha_5\beta_1$; this allows spatial and functional compartmentalisation of the different heterodimers.
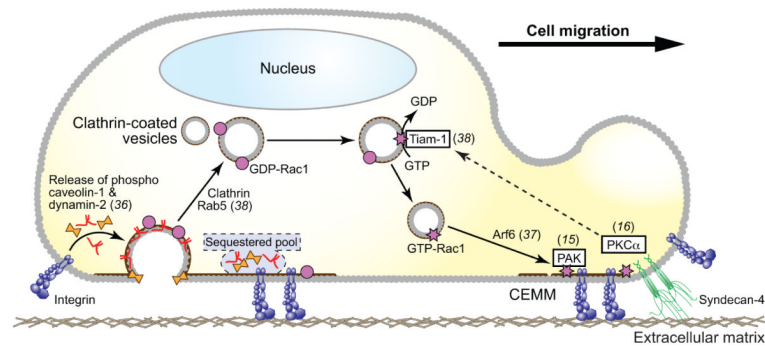
Fig. 3. Adhesion-dependent trafficking of Rac1

At the trailing edge of a cell, disengagement of integrins from the ECM causes the release of sequestered phosphorylated caveolin-1 and dynamin-2. Liberation of these endocytic regulators causes internalisation of CEMMs and associated Rac1. In response to syndecan-4 engagement, Rac1 is reloaded with GTP and then recycled to the leading edge where matrix engagement causes Arf6-dependent recruitment of CEMMs.