PORTLAND PRESS

Review Article

Conformationally active integrin endocytosis and traffic: why, where, when and how?

Giulia Mana[1,2], Donatella Valdembri[1,2] and ⓘ Guido Serini[1,2]

[1]Department of Oncology, University of Torino School of Medicine, 10060 Candiolo, Torino, Italy; [2]Candiolo Cancer Institute - Fondazione del Piemonte per l'Oncologia (FPO) Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), 10060 Candiolo, Torino, Italy

Correspondence: Guido Serini (guido.serini@ircc.it) or Donatella Valdembri (donatella.valdembri@ircc.it)

OPEN ACCESS

Spatiotemporal control of integrin-mediated cell adhesion to the extracellular matrix (ECM) is critical for physiological and pathological events in multicellular organisms, such as embryonic development, angiogenesis, platelet aggregation, leukocytes extravasation, and cancer cell metastatic dissemination. Regulation of integrin adhesive function and signaling relies on the modulation of both conformation and traffic. Indeed, integrins exist in a dynamic equilibrium between a bent/closed (inactive) and an extended/open (active) conformation, respectively endowed with low and high affinity for ECM ligands. Increasing evidence proves that, differently to what hypothesized in the past, detachment from the ECM and conformational inactivation are not mandatory for integrin to get endocytosed and trafficked. Specific transmembrane and cytosolic proteins involved in the control of ECM proteolytic fragment-bound active integrin internalization and recycling exist. In the complex masterplan that governs cell behavior, active integrin traffic is key to the turnover of ECM polymers and adhesion sites, the polarized secretion of endogenous ECM proteins and modifying enzymes, the propagation of motility and survival endosomal signals, and the control of cell metabolism.

Introduction

Metazoan cells attach to extracellular matrix (ECM) proteins via integrin αβ heterodimers that, through a series of cytosolic adaptor proteins, physically connect to the actin cytoskeleton and modulate the enzymatic activity of kinases, phosphatases, and small GTPases [1–3]. Overall, the complex protein network associated with integrin-based adhesion sites is known as the adhesome [2,3]. Twenty-four integrin heterodimers exist which promiscuously allow the interaction with hundreds of ECM proteins in different tissues and organs [4]. On the cellular surface, integrin receptors are in an allosteric equilibrium between a bent/inactive and an extended/active conformation that respectively interact at a low and high affinity with ligands [1]. The percentage of surface integrins adopting the extended/active conformation vary significantly depending on the adhesion and spreading degree of cell types, being for example ∼0.2% in poorly adherent K562 leukemia cells [5] or 10% in widely spread endothelial cells (ECs) [6]. Active integrin conformers are stabilized by the four-point-one, ezrin, radixin, moesin (FERM) domain of talin [7,8] and kindlin [8,9] adaptor proteins with a membrane proximal and distal Asn-Pro-X-Tyr (NPXY) motif in the cytodomain of integrin β subunits, respectively. Both talins and kindlins are required for integrin conformational activation, to which they seem to contribute differently by allowing the vinculin-mediated perception of mechanical forces (talins) and triggering biochemical signaling pathways (kindlins) [8], e.g. through paxillin and focal adhesion kinase (FAK) [10–12]. It has long been thought that the appearance of integrins and associated proteins allowed the evolutionary transition from unicellular to multicellular organisms [13]. Yet, over the last decade, independent studies proved that genes encoding for integrins [14,15] and most adhesome proteins, such as talins [15], but not kindlins [16], were already present in unicellular

ancestors of animals that exploited them in the aggregation phase of their life cycle [13]. Hence, it is conceivable that the emergence of kindlins had been important for the development of multicellular organisms [16].

The dynamic control of integrin conformational activation is central in different physiological and pathological settings, such as tissue and organ development, platelet aggregation, leukocyte extravasation, autoimmune diseases, fibrosis, and cancer [1,17–19]. In these contexts, the small GTPase Rap1 is a main driver of integrin conformational activation. Indeed, talin exists in an autoinhibited state that is relieved by its direct [20–24] or indirect (e.g. through Rap1-interacting adapter molecule — RIAM) [25] binding to Rap1-GTP, allowing the FERM domain of talin to bind and promote integrin activation. Both the fulfillment of complex morphogenetic programs [26,27] as well as the normal functioning of platelets and leukocytes [1,25,28] rely on a fine spatiotemporal modulation of integrin activation by Rap1 and talin. In this scenario, chemoattractant ligands, e.g. C-X-C motif chemokine 12 (CXCL12) [29] or vascular endothelial growth factor-A (VEGF-A) [30], promote Rap1 activation via G protein coupled receptors (GPCRs) or tyrosine kinase receptors (TKRs) coupled to downstream Rap1 guanine nucleotide exchange factors (GEFs) [31]. Conversely, chemorepulsive ligands such as semaphorins (SEMAs) signal through the cytosolic GTPase activating protein (GAP) of Plexin receptors to inhibit Rap1 GTP loading [32–34] and integrin activation [17,18]. The evidence that both talin autoinhibition [27] and Plexin-mediated SEMA inhibition of Rap1 [17,34,35] are required for morphogenesis, in diverse experimental systems, supports the concept that conformational integrin inactivation is as crucial as activation for the accomplishment of complex shaping programs in animal tissues, organs and systems [17,36–39].

In addition to integrin conformational activation, cell-to-ECM adhesion contact dynamics critically relies on integrin traffic [19,40–43]. While it has long been thought that detachment from ECM ligands [44,45] and conformational inactivation [46] were necessary to allow integrin internalization, in the last decade, thanks to the availability of monoclonal antibodies detecting activation dependent epitopes [47], different laboratories showed that conformationally active integrins can also be endocytosed and trafficked under the control of molecular machineries and signaling pathways distinct from those regulating inactive integrin traffic. Here, we will review our current understanding of the rationale and the mechanisms by which cells traffic conformationally active integrins in both normal and cancer cells.

Active integrin traffic controls ECM adhesion site turnover and cell metabolism

Integrin binding to soluble monomeric ECM proteins, such as fibronectin (FN), results in traction force-dependent unmasking the otherwise cryptic protein–protein interaction sites that promote ECM polymerization in insoluble protein networks [48,49]. Polymerized FN act as a scaffold on which collagen I molecules are deposited to form fibrils and fibers via repeated cycles of integrin-mediated contraction and relaxation [50]. Subsequently, matrix metalloproteinases (MMPs) cleave ECM fibrils into fragments that get endocytosed and degraded, thus requiring the replenishment with freshly synthesized ECM molecules (Figure 1). Thus, ECM meshworks are unstable objects whose dynamic remodeling is crucial for tissue morphogenesis and healing as well as cancer cell invasion and dissemination [51]. Additionally, it was found, in keratinocytes, that microtubules anchored by cytoplasmic linker associated proteins (CLASPs) in proximity of ECM adhesions allow the targeted transport of secretory vesicles, which locally deliver membrane-type 1 MMP (MT1-MMP) [52] to degrade the ECM [53]. Moreover, it has been shown, in fibroblasts, that, upon MT1-MMP dependent cleavage [54], FN fragments bound to active α5β1 get endocytosed, trafficked and degraded into lysosomes [55]. As a result, in the absence of such constant FN synthesis, secretion and polymerization, FN fibrils disappear, being degraded [56]. Integrins have been reported to mediate the internalization and turnover of other ECM proteins, e.g. type I collagen [57] in fibroblasts, vitronectin [58] and laminin 111 [59] in cancer cells. Therefore, active integrins participate to the control of ECM turnover on the cell surface by mediating the endocytosis of MMP-cleaved ECM fragments in different cell types.

In agreement with the fact that active integrins act as receptors for ECM internalization, several key regulators of endocytosis were found to localize at adhesion sites and regulate the rate at which their molecular components are renewed both *in vitro* and *in vivo*. The small GTPase Rab5 concentrates at myotendinous junctions (MTJs) of *Drosophila* embryos, where it promotes β position-specific (βPS) integrin turnover allowing MTJ remodeling in developing skeletal muscles [60]. Of note, internalization rather than lateral diffusion in the plasma membrane is the main mechanism responsible of βPS integrin dynamics in *Drosophila* MTJs [60]. In
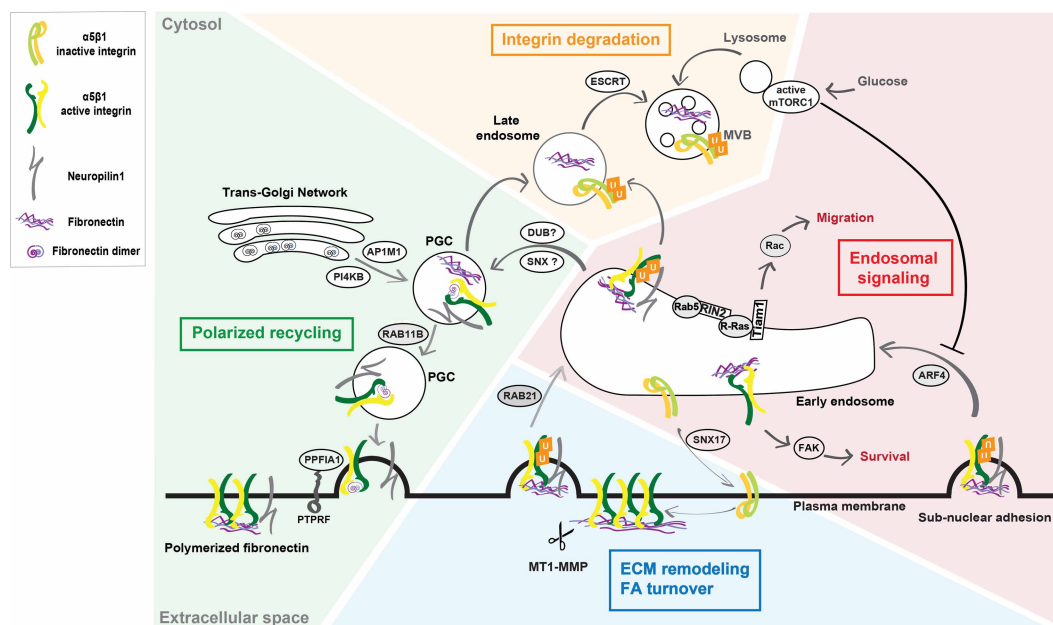
Figure 1. Active integrin internalization and trafficking pathways.

Upon MMP-dependent cleavage of polymerized FN fibrils, the endocytic receptor NRP1 interacts on the cell surface with FN fragment-bound ubiquitinated α5β1 active-integrin that is internalized within early endosomes (EEs) through either Rab21, or Rab5, or Afr4 small GTPase, depending on the internalization site and cell type (see text and Figure 2). In EEs, active α5β1 integrin may activate different signaling pathways such as: (i) Rac1 to promote integrin-dependent cell spreading and migration on FN; (ii) FAK to suppress anoikis in normal cells or promote cancer cell anchorage-independent growth. Moreover, in Rab25 overexpressing ovarian cancer cells Arf4-dependent active α5β1 integrin is required for correct lysosome positioning and activation of mTORC1, whose kinase activity is also regulated by glucose levels. From the EE compartment, inactive integrins can move back to the plasma membrane via sorting nexin 17 (SNX17) and the retriever complex [124]. A fraction of FN fragment-bound active α5β1 integrins is sorted into multivesicular bodies (MVBs) and degraded into lysosomes; this degradative fate depends on α5β1 integrin ubiquitination and on endosomal sorting complexes required for transport (ESCRT) protein complex. Another fraction of FN fragment-bound active α5β1 integrin instead traffics to PGCs; it is still unclear if this latter step relies on deubiquitinase (DUB) and/or SNX activities. It is posited that, in PGCs, endocytosed old FN may be separated from active α5β1 integrin that would then be free and able to bind freshly synthesized FN originating from TGN cisternae in a PI4KB and AP-1A-dependent manner. Through a RAB11B-dependent endosomal recycling pathway, vesicles containing newly synthesized FN-bound active α5β1 integrins are next directed to the basolateral side of the cell surface, where the PTPRF/PPFIA1 complex support their docking, likely via PPFIA1-mediated interaction with the β1 integrin subunit cytodomain.

adhesion sites, integrins withstand retrograde actin flow-driven traction [61], exist in stationary ECM-bound subpopulations [62], and active integrins form tightly ordered nanoclusters [63]. MMP-assisted endocytosis may hence represent an efficient strategy to allow the turnover of ECM-bound active integrins at adhesion sites. The Rab5 GEF Ras and Rab interactor 2 (RIN2) [64] and the Rab5 GAP USP6NL (also known as RN-Tre) [65,66] respectively localize in nascent adhesions and focal adhesions to promote or inhibit active β1 integrin endocytosis and motility of ECs and fibroblasts (Figure 2). In migrating cells, small, round, and peripheral nascent adhesions initially form at leading edge lamellipodium and later either disassemble or, due to actomyosin contractility, mature into elongated and stable focal adhesions [67]. It is tempting to speculate that, by exerting opposite effects on Rab5 GTP-loading and active β1 integrin internalization, RIN2 [64] and RN-Tre [65,66] may co-operate in funneling the conversion of nascent adhesions into focal adhesions in migrating cells. In fibroblasts, FN-bound α5β1 integrin slides outside focal adhesions and translocates along stress fibers giving rise to elongated fibrillar adhesions and FN fibrils [67] that are not influenced by Rab5 activity [65]. At least in ECs [6], active α5β1 integrin endocytosis at fibrillar adhesions and FN fibril turnover are promoted
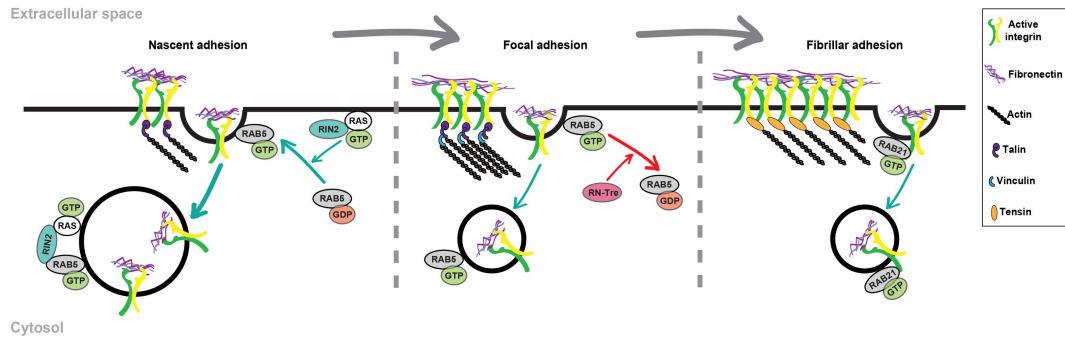
Figure 2. Working model for Rab5/Rab21 small GTPase interplay in active α5β1 integrin endocytosis in non-cancer cells.
The Rab5 GEF RIN2 and Rab5 GAP RN-Tre/USP6NL are respectively located in nascent and focal adhesions. Due to their enzymatic antagonistic activity on Rab5 GTP-loading and active α5β1 integrin internalization, RIN2 and RN-Tre might co-operate to foster the evolution of nascent into focal adhesions. Moving along stress fibers, active α5β1 integrins generate fibrillar adhesions and FN fibrils, which are not influenced by Rab5. Indeed, in ECs active α5β1 integrin endocytosis at fibrillar adhesions and FN fibril turnover are promoted instead by Rab21.

instead by Rab21, which localizes to adhesion sites [68,69] and was previously reported to stimulate integrin internalization [68,70,71] (Figure 2).

Differently from ECs [6], Arf4, rather than Rab21, was implicated in the endocytosis of FN-bound active α5β1 integrin from FN fibrils specifically localized in the subnuclear of area of A2780 ovarian cancer cells over-expressing Rab25 (A2780-Rab25) [72] (Figure 1), which was previously found to co-operate with chloride intra-cellular channel protein 3 (CLIC3) to allow the recycling from late endosomes/lysosomes of endocytosed FN-bound active α5β1 integrin in this cell line [73]. Interestingly, in A2780-Rab25 ovarian cancer cells, upon endocytosis of FN-bound active α5β1 integrin and its delivery to late endosomes/lysosomes, likely due to the increased lysosomal concentration of amino acids caused by FN degradation, the master regulator of cell metabolism and growth mechanistic target of rapamycin complex 1 (mTORC1) gets activated [72] (Figure 1). Similarly, in starving conditions, mammary epithelial cells up-regulate α6β4 integrin-mediated laminin endo-cytosis and lysosomal degradation thus resulting in mTORC1 activation [74]. In addition, pancreatic ductal adenocarcinoma cells internalize and degrade type I and type IV collagens as source of proline that fuels tri-carboxylic acid cycle metabolism under nutrient limited conditions [75]. Finally, the metabolic sensor adeno-sine monophosphate activated protein kinase (AMPK), which is activated when AMP level raises during energy stresses, was discovered to inhibit the transcription of the α5β1 integrin-specific adaptors and activators tensin1 and 3, thus impairing α5β1 integrin activation and FN fibrillogenesis [76].

In sum, the turnover of the different types of cell adhesion structures relies on distinct pro-endocytic small GTPases and regulatory proteins that differentially modulate in space and time the internalization of ECM-bound active integrins. Furthermore, a direct link between ECM-bound active integrin traffic and nutri-ent signaling exists. To support their proliferation rate, cancer cells exploit active integrin-mediated ECM endo-cytosis as an effective strategy to directly acquire nutrients from the extracellular environment.

Active integrin traffic role in establishing cell polarity

Polarized epithelial cells, neurons, vascular ECs, and directional migrating cells are characterized by spatially and functionally distinct plasma membrane areas defined by PAR proteins, the CRB complex, and phosphatidylinositol-phosphates (PIPs) [77]. β1 integrin-mediated adhesion to the ECM elicits biochemical signals aimed at establishing and maintaining apico-basal polarity both in epithelial cells [78] and ECs [79]. Specifically, β1 integrin functions upstream of PAR polarity proteins in the signaling cascade that defines EC apico-basal axis, driving vascular morphogenesis and lumen formation [79] in response to FN [80,81]. On the contrary, basement membrane proteins, such as laminin, are alternative β1 integrin ligands that inhibit vascular morphogenesis, while maintaining the stability of mature blood vessels [80,81]. During blood vessel formation, once apico-basal axis is defined, FN-bound active α5β1 integrin signals to keep directing the secretion of

freshly synthesized endogenous FN towards the abluminal basolateral plasma membrane of ECs [82], giving rise to a self-sustaining polarity signaling cascade.

In ECs, apart from its ability to extracellularly interact with SEMA3A or VEGF-A [83], the transmembrane glycoprotein neuropilin 1 (NRP1) localizes at adhesion sites [66,69,84–86], where it promotes FN-bound active α5β1 integrin endocytosis [84] (Figure 1). The binding of extracellular NRP1 b1 domain to the C-terminal basic motif of SEMA3A [34,83] or C-end rule (CendR) peptides [87] fosters the internalization of NRP1 [88] and associated membrane receptor cargos, such as active α5β1 integrin [34,84]. NRP1-dependent endocytosis largely relies on its short cytodomain [84,88] that, via its C-terminal Ser-Glu-Ala (SEA) motif, binds the PSD95-DLG1-ZO1 (PDZ) domain of the endocytic adaptor GAIP interacting protein C terminus, member 1 (GIPC1, also known as synectin) that associates to myosin VI (MYO6) motor to allow the transport of early endosomes through the cortical actin network [89]. Arterial branching morphogenesis is substantially impaired in knock-in mice lacking NRP1 cytodomain [90] and, albeit at lower extent, in GIPC1 [91] and MYO6 [92] knock-out animals. In contrast, knock-in mice expressing a b1 domain NRP1 mutant unable to bind VEGF-A only do not display any vascular defect [93]. Altogether, these findings support a model in which, VEGF-A-independent NRP1/GIPC1/MYO6-driven internalization of transmembrane cargos, such as integrins, promote FN-dependent branching [94] and vascular [95] morphogenesis.

Along with the fact that, upon NRP1-driven internalization, active α5β1 integrins are returned back to the EC surface [84], the observation that, via its cytodomain, NRP1 also promotes endothelial FN fibrillogenesis [84] suggests that these integrins may, either by signaling or by direct binding or both, favor the exocytosis of newly synthesized FN from perinuclear *trans*-Golgi network (TGN) to replace MMP-cleaved FN fibrils. This hypothesis is also in agreement with the observation that endocytosed active α5 [6] and β1 [96] integrins are recycled, likely from vesicular compartments laid closer to the nucleus and farther from the plasma membrane [97], with considerably slower kinetics compared with their inactive counterparts in different cell types. Indeed, in ECs, Rab21, which may interact with NRP1 via adaptor protein containing a PH domain, PTB domain, and leucine zipper motif 1 (APPL1) and GIPC1 [42], promotes the internalization in early endosomes of FN fragment-bound active α5β1 integrins, which subsequently traffic to post-Golgi carrier (PGC) vesicles stemmed from the TGN in a phosphatidylinositol 4-kinase, catalytic, beta (PI4KB) and clathrin adaptor protein complex-1A (AP-1A)-dependent manner and containing freshly synthesized FN [6] (Figure 1). Albeit further studies are needed to elucidate this step, once in PGCs, active α5β1 integrins might exchange exhausted and to-be-degraded FN fragments for newly synthesized FN dimers (Figure 1). Whether internalized NRP1 reaches the TGN/PGC compartment and participates in the recycling of active α5β1 integrins is still open issue. Next, RAB11B routes PGCs containing newly synthesized FN-bound active α5β1 towards basolateral side of ECs, where a complex formed by protein tyrosine phosphatase receptor type f polypeptide (PTPRF, otherwise identi- fied as leukocyte common antigen related — LAR protein) and PTPRF interacting protein a1 (PPFIA1), also known as liprin-α1, concentrates in close proximity of fibrillar adhesions [6] (Figure 1). Intriguingly, in neuro- nal pre-synapses PTPRF and PPFIA1/liprin-α1 synchronize endo-exocytic traffic and mediate the docking of neurotransmitter vesicles at the plasma membrane [98,99]. In addition, similarly to kindlins, the emergence of liprins has also been implicated in the rise of multicellular organisms [16]. Analogously to its neuronal function and due to its binding to the β1 cytodomain of active α5β1 integrin, in ECs PPFIA1/liprin-α1 promotes the docking of PGCs containing newly synthesized FN and active α5β1 integrins [6]. The fusion of these PGCs with the plasma membrane may bolster the local release of newly synthesized FN and active α5β1 integrins and the exchange of old for new fibrillar FN at the abluminal side of endothelium [6]. Of note, PPFIA1/ liprin-α1 silencing also affects subendothelial FN deposition and vascular morphogenesis in developing *Zebrafish* embryo [6]. Supporting its role in defining EC polarity [6], PPFIA1/liprin-α1 was discovered to control the basolateral secretion of at least three additional ECM components that are pivotal regulators of vas- cular ECM organization and remodeling, namely lysyl oxidase, multimerin 2, and dystroglycan-1 [100]. Thus, ECs employ a synaptic-like machinery to control the coupling of active α5β1 integrin endo-exocytosis and the replenishment of degraded FN fibrils with the basolateral secretion of TGN-derived newly synthesized FN [6], along with proteins involved in FN rehandling [100]. It has been for long known that, during embryogenesis, dynamically remodeling angiogenic blood vessels develop in a FN-rich ECM, which, during the subsequent vascular maturation and stabilization phase, is substituted with a laminin-rich basement membrane [81,101]. To this matter, the discovery that PPFIA1/liprin-α1 also controls the basolateral secretion of the basement membrane organizing protein dystroglycan-1 [100] suggests that PPFIA1/liprin-α1 may participate in orches- trating the transition from angiogenic to quiescent blood vessels [81].

More recently, it has been reported that the other TGN-associated clathrin adaptor protein, known to participate together with AP-1 [102] in the secretory pathway, namely Golgi-localized gamma ear-containing Arf-binding protein 2 (GGA2) [102], is also involved in active β1 integrin recycling in human MDA-MB-231 breast cancer cells [103]. The association of GGA2 with active β1 integrin is stabilized by the small GTPase RAB13, which also supports the return of internalized active β1 integrin to the cell surface. Moreover, both GGA2 and RAB13 are required for efficient cancer cell migration and invasion [103]. Of note, along with RAB13, proximity biotinylation analyses also identified PTPRF as a GGA2 interactor [103]. In addition, since PTPRF was previously reported to drive active α5β1 integrin recycling in ECs [6,104], it may hence function as a multipurpose docking receptor that, localizing at ECM adhesion sites [105], allows the polarized and targeted recycling of active β1 integrins via different TGN-connected trafficking pathways. Indeed, GGAs and AP-1 TGN-associated clathrin adaptors may have overlapping [102], as well as distinct functions, GGAs, but not AP-1, transporting ubiquitinated protein cargos [106,107]

Internalized active integrins elicit endosomal signaling pathways

Rac1-stimulated actin branched polymerization drives the formation of plasma membrane extensions, known as lamellipodia, which need to be confined at the leading edge of migrating cells to effectively allow directional motility [108]. In this context, integrin-ECM engagement at the cell front triggers a self-sustaining Rac1-activating positive feedback loop that supports lamellipodium-driven cell motility [109]. In addition, Rac1 endocytosis, from and recycling to, the plasma membrane represents a strategy to selectively restrain and polarize, in space and time, the signaling of this small GTPase [110]. Early endosomes have been identified as further subcellular sites for Rac1 activation in addition to the plasma membrane [110]. Indeed, the major Rac1 GEF T-lymphoma invasion and metastasis-inducing protein 1 (TIAM1) was found to reside on early endosomes of HeLa cancer cells [110], likely because, via its pleckstrin homology (PH) domain [111], TIAM1 binds PI3P [112], a phospholipid produced by the key Rab5 effector PI3KC3, also known as VPS34 [113] (Figure 1). The small GTPase R-Ras, one of the master regulators of integrin function [114], is highly expressed in vascular ECs [64], localizes in lamellipodia-associated nascent ECM-adhesions, where it recruits RIN2 as a result of the interaction with its Ras association (RA) domain [64]. In ECs, the binding to R-Ras converts RIN2 from a Rab5 GEF to an adaptor that first interacts at high affinity with Rab5-GTP to selectively promote ECM-bound/active β1 integrin endocytosis and next causes R-Ras repositioning on early endosomes [64]. After the active β1 integrin/Rab5/RIN2-dependent transfer on early endosomes, R-Ras contacts the RA domain of TIAM1, thus promoting the GTP loading of Rac1 [64] (Figure 1) followed by its polarized relocation to the plasma membrane, likely via the small GTPase Arf6 [110,115]. In sum, it appears that, at the leading edge of migrating ECs, the endocytosis of active β1 integrins co-ordinates the RIN2-dependent translocation of R-Ras on early endosomes, where it triggers, via TIAM1, a self-sustaining Rac1-activating positive feedback loop that drives directed cell motility. Along this line, it was previously reported that in cancer cells Rab5 gets activated upon integrin-mediated cell spreading on FN [116] that, indeed, depends on a focal adhesion kinase (FAK)-Rab5-Rac1 signaling pathway that critically acts downstream of the mechanosensing protein vinculin [117]. More recently, it has been proposed that FAK may favor Rab5 GTP loading by binding and inhibiting the Rab GAP activity [118] of the p85α subunit of PI3K [119]. Furthermore, upon GTP loading, Rac1 elicits additional phosphorylation of FAK that in turn promotes further Rac1 activation, giving rise to a positive feedback mechanism [117]. Altogether these data support a model in which active integrin endocytosis is key in promoting the GTP loading of Rac1 to enable cell spreading.

FAK activation at the plasma membrane regulates cell proliferation, survival, migration, and invasion [120]. However, it has been proposed that endosomal FAK signaling may also support the resistance of normal cells to anoikis as well as breast cancer cell anchorage independent growth and metastatic dissemination [121]. In the cytoplasm, FAK exists in an autoinhibited conformation and the binding of its FERM domain to PI(4,5)P2, which at the plasma membrane is generated in close proximity to ECM-bound integrins, elicits FAK conformational activation [120]. Consistently, the FERM domain is sufficient to recruit FAK to active integrin containing endosomes through still unknown mechanisms [121] (Figure 1). While conformational activation is not required for vesicular targeting, FAK endosomal signaling strictly depends on its tyrosine kinase activity. Similarly to what observed at the plasma membrane [122], FAK, once activated, may promote talin recruitment and tension-independent activation on endosomes. Mechanistically, it has been proposed that FAK phosphorylates

and activates type I phosphatidylinositol phosphate kinase (PIPKIγi2), generating PI(4,5)P2 promoting talin recruitment [123]. The presence of talin on endosomes may facilitate the maintenance of integrin active conformation during its recycling to the plasma membrane, even in the absence of ligands [123].

Perspective

- Differently from what previously hypothesized, integrins do not need to be conformationally inactivated to be endocytosed. Dedicated transmembrane and cytosolic proteins contribute to ECM-bound active integrin internalization and recycling to the cell surface, along with the secretion of newly synthesized ECM.
- Active integrin endocytosis and traffic control ECM and adhesion site turnover, metabolism, polarity, and endosomal signaling supporting motility and survival both of normal and cancer cells.
- It will be crucial to identify and thoroughly dissect the molecular mechanisms responsible for those signaling aspects of active integrin traffic still poorly understood, such as those involved in the control of ECM-containing PGCs from the TGN or in the targeting of FAK on endosomes.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

Author Contribution

All authors contributed substantially to the conception and design of work leading to this review, provided content and wrote the article. All authors have approved the final version for publication.

Acknowledgements

Abbreviations

AMPK, adenosine monophosphate activated protein kinase; AP-1A, adaptor protein complex-1A; CLASPs, cytoplasmic linker associated proteins; CLIC3, chloride intracellular channel protein 3; CXCL12, C-X-C motif chemokine 12; ECM, extracellular matrix; ECs, endothelial cells; FAK, focal adhesion kinase; FERM, four-point-one, ezrin, radixin, moesin; FN, fibronectin; GAP, GTPase activating protein; GEFs, guanine nucleotide exchange factors; GGA2, Golgi-localized gamma ear-containing Arf-binding protein 2; MMPs, matrix metalloproteinases; MT1-MMP, membrane-type 1 MMP; MTJs, myotendinous junctions; MYO6, myosin VI; NRP1, neuropilin 1; PGC, post-Golgi carrier; PI4KB, phosphatidylinositol 4-kinase, catalytic, beta; PPFIA1, PTPRF interacting protein a1; RA, Ras association; TGN, *trans*-Golgi network; TIAM1, T-lymphoma invasion and metastasis-inducing protein 1; VEGF-A, vascular endothelial growth factor-A; βPS, β position-specific.

References

1   Bachmann, M., Kukkurainen, S., Hytönen, V.P. and Wehrle-Haller, B. (2019) Cell adhesion by integrins. *Physiol. Rev.* **99**, 1655–1699 https://doi.org/10.1152/physrev.00036.2018

2   Byron, A. and Frame, M.C. (2016) Adhesion protein networks reveal functions proximal and distal to cell-matrix contacts. *Curr. Opin. Cell Biol.* **39**, 93–100 https://doi.org/10.1016/j.ceb.2016.02.013

3   Humphries, J.D., Chastney, M.R., Askari, J.A. and Humphries, M.J. (2019) Signal transduction via integrin adhesion complexes. *Curr. Opin. Cell Biol.* **56**, 14–21 https://doi.org/10.1016/j.ceb.2018.08.004

4   Hynes, R.O. (2012) Evolution: the evolution of metazoan extracellular matrix. *J. Cell Biol.* **196**, 671–679 https://doi.org/10.1083/jcb.201109041

5   Li, J., Su, Y., Xia, W., Qin, Y., Humphries, M.J., Vestweber, D. et al. (2017) Conformational equilibria and intrinsic affinities define integrin activation. *EMBO J.* **36**, 629–645 https://doi.org/10.15252/embj.201695803

6   Mana, G., Clapero, F., Panieri, E., Panero, V., Böttcher, R.T., Tseng, H.Y. et al. (2016) PPFIA1 drives active α5β1 integrin recycling and controls fibronectin fibrillogenesis and vascular morphogenesis. *Nat. Commun.* **7**, 13546 https://doi.org/10.1038/ncomms13546

7   Goult, B.T., Yan, J. and Schwartz, M.A. (2018) Talin as a mechanosensitive signaling hub. *J. Cell Biol.* **217**, 3776–3784 https://doi.org/10.1083/jcb.201808061

8   Sun, Z., Costell, M. and Fässler, R. (2019) Integrin activation by talin, kindlin and mechanical forces. *Nat. Cell Biol.* **21**, 25–31 https://doi.org/10.1038/s41556-018-0234-9

9   Rognoni, E., Ruppert, R. and Fässler, R. (2016) The kindlin family: functions, signaling properties and implications for human disease. *J. Cell Sci.* **129**, 17–27 https://doi.org/10.1242/jcs.161190

10   Theodosiou, M., Widmaier, M., Böttcher, R.T., Rognoni, E., Veelders, M., Bharadwaj, M. et al. (2016) Kindlin-2 cooperates with talin to activate integrins and induces cell spreading by directly binding paxillin. *eLife* **5**, e10130 https://doi.org/10.7554/eLife.10130

11   Klapproth, S., Bromberger, T., Türk, C., Krüger, M. and Moser, M. (2019) A kindlin-3-leupaxin-paxillin signaling pathway regulates podosome stability. *J. Cell Biol.* **218**, 3436–3454 https://doi.org/10.1083/jcb.201903109

12   Zhu, L., Liu, H., Lu, F., Yang, J., Byzova, T.V. and Qin, J. (2019) Structural basis of paxillin recruitment by kindlin-2 in regulating cell adhesion. *Structure* **27**, 1686–1697.e5 https://doi.org/10.1016/j.str.2019.09.006

13   Sebé-Pedrós, A., Degnan, B.M. and Ruiz-Trillo, I. (2017) The origin of metazoa: a unicellular perspective. *Nat. Rev. Genet.* **18**, 498–512 https://doi.org/10.1038/nrg.2017.21

14   King, N., Westbrook, M.J., Young, S.L., Kuo, A., Abedin, M., Chapman, J. et al. (2008) The genome of the choanoflagellate monosiga brevicollis and the origin of metazoans. *Nature* **451**, 783–788 https://doi.org/10.1038/nature06617

15   Sebé-Pedrós, A., Roger, A.J., Lang, F.B., King, N. and Ruiz-Trillo, I. (2010) Ancient origin of the integrin-mediated adhesion and signaling machinery. *Proc. Natl Acad. Sci. U.S.A.* **107**, 10142–7 https://doi.org/10.1073/pnas.1002257107

16   Paps, J. and Holland, P.W.H. (2018) Reconstruction of the ancestral metazoan genome reveals an increase in genomic novelty. *Nat. Commun.* **9**, 1730 https://doi.org/10.1038/s41467-018-04136-5

17   Serini, G., Valdembri, D., Zanivan, S., Morterra, G., Burkhardt, C., Caccavari, F. et al. (2003) Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. *Nature* **424**, 391–397 https://doi.org/10.1038/nature01784

18   Valdembri, D., Regano, D., Maione, F., Giraudo, E. and Serini, G. (2016) Class 3 semaphorins in cardiovascular development. *Cell Adh. Migr.* **10**, 641–651 https://doi.org/10.1080/19336918.2016.1212805

19   Moreno-Layseca, P., Icha, J., Hamidi, H. and Ivaska, J. (2019) Integrin trafficking in cells and tissues. *Nat. Cell Biol.* **21**, 122–132 https://doi.org/10.1038/s41556-018-0223-z

20   Zhu, L., Yang, J., Bromberger, T., Holly, A., Lu, F., Liu, H. et al. (2017) Structure of Rap1b bound to talin reveals a pathway for triggering integrin activation. *Nat. Commun.* **8**, 1744 https://doi.org/10.1038/s41467-017-01822-8

21   Camp, D., Haage, A., Solianova, V., Castle, W.M., Xu, Q.A., Lostchuck, E. et al. (2018) Direct binding of talin to Rap1 is required for cell-ECM adhesion in *drosophila*. *J. Cell Sci.* **131**, jcs225144 https://doi.org/10.1242/jcs.225144

22   Bromberger, T., Klapproth, S., Rohwedder, I., Zhu, L., Mittmann, L., Reichel, C.A. et al. (2018) Direct Rap1/Talin1 interaction regulates platelet and neutrophil integrin activity in mice. *Blood* **132**, 2754–2762 https://doi.org/10.1182/blood-2018-04-846766

23   Gingras, A.R., Lagarrigue, F., Cuevas, M.N., Valadez, A.J., Zorovich, M., McLaughlin, W. et al. (2019) Rap1 binding and a lipid-dependent helix in talin F1 domain promote integrin activation in tandem. *J. Cell Biol.* **218**, 1799–1809 https://doi.org/10.1083/jcb.201810061

24   Bromberger, T., Zhu, L., Klapproth, S., Qin, J. and Moser, M. (2019) Rap1 and membrane lipids cooperatively recruit talin to trigger integrin activation. *J. Cell Sci.* **132**, jcs235531 https://doi.org/10.1242/jcs.235531

25   Lagarrigue, F., Kim, C. and Ginsberg, M.H. (2016) The Rap1-RIAM-talin axis of integrin activation and blood cell function. *Blood* **128**, 479–487 https://doi.org/10.1182/blood-2015-12-638700

26   Chrzanowska-Wodnicka, M. (2013) Distinct functions for Rap1 signaling in vascular morphogenesis and dysfunction. *Exp. Cell Res.* **319**, 2350–2359 https://doi.org/10.1016/j.yexcr.2013.07.022

27   Ellis, S.J., Goult, B.T., Fairchild, M.J., Harris, N.J., Long, J., Lobo, P. et al. (2013) Talin autoinhibition is required for morphogenesis. *Curr. Biol.* **23**, 1825–1833 https://doi.org/10.1016/j.cub.2013.07.054

28   Hogg, N., Patzak, I. and Willenbrock, F. (2011) The insider's guide to leukocyte integrin signalling and function. *Nat. Rev. Immunol.* **11**, 416–426 https://doi.org/10.1038/nri2986

29   Strazza, M., Azoulay-Alfaguter, I., Peled, M., Smrcka, A.V., Skolnik, E.Y., Srivastava, S. et al. (2017) PLCε1 regulates SDF-1α-induced lymphocyte adhesion and migration to sites of inflammation. *Proc. Natl Acad. Sci. U.S.A.* **114**, 2693–2698 https://doi.org/10.1073/pnas.1612900114

30   Chrzanowska-Wodnicka, M. (2017) Rap1 in endothelial biology. *Curr. Opin. Hematol.* **24**, 248–255 https://doi.org/10.1097/MOH.0000000000000332

31   Gloerich, M. and Bos, J.L. (2011) Regulating Rap small G-proteins in time and space. *Trends Cell Biol.* **21**, 615–623 https://doi.org/10.1016/j.tcb.2011.07.001

32   Wang, Y., He, H., Srivastava, N., Vikarunnessa, S., Chen, Y.B., Jiang, J. et al. (2012) Plexins are GTPase-activating proteins for Rap and are activated by induced dimerization. *Sci. Signal.* **5**, ra6 https://doi.org/10.1126/scisignal.2002636

33   Bos, J.L. and Pannekoek, W.J. (2012) Semaphorin signaling meets rap. *Sci. Signal.* **5**, pe6 https://doi.org/10.1126/scisignal.2002913

34   Gioelli, N., Maione, F., Camillo, C., Ghitti, M., Valdembri, D., Morello, N. et al. (2018) A rationally designed NRP1-independent superagonist SEMA3A mutant is an effective anticancer agent. *Sci. Transl. Med.* **10**, eaah4807 https://doi.org/10.1126/scitranslmed.aah4807

35   Worzfeld, T., Swiercz, J.M., Sentürk, A., Genz, B., Korostylev, A., Deng, S. et al. (2014) Genetic dissection of plexin signaling in vivo. *Proc. Natl Acad. Sci. U.S.A.* **111**, 2194–2199 https://doi.org/10.1073/pnas.1308418111

36   Serini, G. and Bussolino, F. (2004) Common cues in vascular and axon guidance. *Physiology (Bethesda)* **19**, 348–354 https://doi.org/10.1152/physiol.00021.2004

37   Bussolino, F., Valdembri, D., Caccavari, F. and Serini, G. (2006) Semaphoring vascular morphogenesis. *Endothelium* **13**, 81–91 https://doi.org/10.1080/10623320600698003

38 Bouvard, D., Pouwels, J., De Franceschi, N. and Ivaska, J. (2013) Integrin inactivators: balancing cellular functions in vitro and in vivo. *Nat. Rev. Mol. Cell Biol.* **14**, 430–442 https://doi.org/10.1038/nrm3599

39 Bussolino, F., Caccavari, F., Valdembri, D. and Serini, G. (2009) Angiogenesis: a balancing act between integrin activation and inhibition? *Eur. Cytokine Netw.* **20**, 191–196 https://doi.org/10.1684/ecn.2009.0168

40 Bridgewater, R.E., Norman, J.C. and Caswell, P.T. (2012) Integrin trafficking at a glance. *J. Cell Sci.* **125** (Pt 16), 3695–3701 https://doi.org/10.1242/jcs.095810

41 Valdembri, D. and Serini, G. (2012) Regulation of adhesion site dynamics by integrin traffic. *Curr. Opin. Cell Biol.* **24**, 582–591 https://doi.org/10.1016/j.ceb.2012.08.004

42 Valdembri, D., Sandri, C., Santambrogio, M. and Serini, G. (2011) Regulation of integrins by conformation and traffic: it takes two to tango. *Mol. Biosyst.* **7**, 2539–2546 https://doi.org/10.1039/c1mb05066d

43 Santambrogio, M., Valdembri, D. and Serini, G. (2011) Increasing traffic on vascular routes. *Mol. Aspects Med.* **32**, 112–122 https://doi.org/10.1016/j.mam.2011.04.003

44 Bretscher, M.S. (1989) Endocytosis and recycling of the fibronectin receptor in CHO cells. *EMBO J.* **8**, 1341–1348 https://doi.org/10.1002/j.1460-2075.1989.tb03514.x

45 Lawson, M.A. and Maxfield, F.R. (1995) Ca^{2+}- and calcineurin-dependent recycling of an integrin to the front of migrating neutrophils. *Nature* **377**, 75–79 https://doi.org/10.1038/377075a0

46 Puklin-Faucher, E. and Sheetz, M.P. (2009) The mechanical integrin cycle. *J. Cell Sci.* **122** (Pt 2), 179–186 https://doi.org/10.1242/jcs.042127

47 Byron, A., Humphries, J.D., Askari, J.A., Craig, S.E., Mould, A.P. and Humphries, M.J. (2009) Anti-integrin monoclonal antibodies. *J. Cell Sci.* **122** (Pt 22), 4009–4011 https://doi.org/10.1242/jcs.056770

48 Schwarzbauer, J.E. and DeSimone, D.W. (2011) Fibronectins, their fibrillogenesis, and in vivo functions. *Cold Spring Harb. Perspect. Biol.* **3**, a005041 https://doi.org/10.1101/cshperspect.a005041

49 Vogel, V. (2018) Unraveling the mechanobiology of extracellular matrix. *Annu. Rev. Physiol.* **80**, 353–387 https://doi.org/10.1146/annurev-physiol-021317-121312

50 Humphrey, J.D., Dufresne, E.R. and Schwartz, M.A. (2014) Mechanotransduction and extracellular matrix homeostasis. *Nat. Rev. Mol. Cell Biol.* **15**, 802–812 https://doi.org/10.1038/nrm3896

51 Bonnans, C., Chou, J. and Werb, Z. (2014) Remodelling the extracellular matrix in development and disease. *Nat. Rev. Mol. Cell Biol.* **15**, 786–801 https://doi.org/10.1038/nrm3904

52 Stehbens, S.J., Paszek, M., Pemble, H., Ettinger, A., Gierke, S. and Wittmann, T. (2014) CLASPs link focal-adhesion-associated microtubule capture to localized exocytosis and adhesion site turnover. *Nat. Cell Biol.* **16**, 561–573 https://doi.org/10.1038/ncb2975

53 Wang, Y. and McNiven, M.A. (2012) Invasive matrix degradation at focal adhesions occurs via protease recruitment by a FAK-p130Cas complex. *J. Cell Biol.* **196**, 375–385 https://doi.org/10.1083/jcb.201105153

54 Shi, F. and Sottile, J. (2011) MT1-MMP regulates the turnover and endocytosis of extracellular matrix fibronectin. *J. Cell Sci.* **124** (Pt 23), 4039–4050 https://doi.org/10.1242/jcs.087858

55 Shi, F. and Sottile, J. (2008) Caveolin-1-dependent beta1 integrin endocytosis is a critical regulator of fibronectin turnover. *J. Cell Sci.* **121**, 2360–2371 https://doi.org/10.1242/jcs.014977

56 Singh, P., Carraher, C. and Schwarzbauer, J.E. (2010) Assembly of fibronectin extracellular matrix. *Annu. Rev. Cell Dev. Biol.* **26**, 397–419 https://doi.org/10.1146/annurev-cellbio-100109-104020

57 Shi, F., Harman, J., Fujiwara, K. and Sottile, J. (2010) Collagen I matrix turnover is regulated by fibronectin polymerization. *Am. J. Physiol. Cell Physiol.* **298**, C1265–C1275 https://doi.org/10.1152/ajpcell.00341.2009

58 Memmo, L.M. and McKeown-Longo, P. (1998) The alphavbeta5 integrin functions as an endocytic receptor for vitronectin. *J. Cell Sci.* **111** (Pt 4), 425–433 PMID:9443892

59 Leonoudakis, D., Huang, G., Akhavan, A., Fata, J.E., Singh, M., Gray, J.W. et al. (2014) Endocytic trafficking of laminin is controlled by dystroglycan and is disrupted in cancers. *J. Cell Sci.* **127** (Pt 22), 4894–4903 https://doi.org/10.1242/jcs.152728

60 Yuan, L., Fairchild, M.J., Perkins, A.D. and Tanentzapf, G. (2010) Analysis of integrin turnover in fly myotendinous junctions. *J. Cell Sci.* **123** (Pt 6), 939–946 https://doi.org/10.1242/jcs.063040

61 Hu, K., Ji, L., Applegate, K.T., Danuser, G. and Waterman-Storer, C.M. (2007) Differential transmission of actin motion within focal adhesions. *Science* **315**, 111–115 https://doi.org/10.1126/science.1135085

62 Rossier, O., Octeau, V., Sibarita, J.B., Leduc, C., Tessier, B., Nair, D. et al. (2012) Integrins β1 and β3 exhibit distinct dynamic nanoscale organizations inside focal adhesions. *Nat. Cell Biol.* **14**, 1057–1067 https://doi.org/10.1038/ncb2588

63 Spiess, M., Hernandez-Varas, P., Oddone, A., Olofsson, H., Blom, H., Waithe, D. et al. (2018) Active and inactive β1 integrins segregate into distinct nanoclusters in focal adhesions. *J. Cell Biol.* **217**, 1929–1940 https://doi.org/10.1083/jcb.201707031

64 Sandri, C., Caccavari, F., Valdembri, D., Camillo, C., Veltel, S., Santambrogio, M. et al. (2012) The R-Ras/RIN2/Rab5 complex controls endothelial cell adhesion and morphogenesis via active integrin endocytosis and Rac signaling. *Cell Res.* **22**, 1479–1501 https://doi.org/10.1038/cr.2012.110

65 Palamidessi, A., Frittoli, E., Ducano, N., Offenhauser, N., Sigismund, S., Kajiho, H. et al. (2013) The GTPase-activating protein RN-tre controls focal adhesion turnover and cell migration. *Curr. Biol.* **23**, 2355–2364 https://doi.org/10.1016/j.cub.2013.09.060

66 Dong, J.M., Tay, F.P., Swa, H.L., Gunaratne, J., Leung, T., Burke, B. et al. (2016) Proximity biotinylation provides insight into the molecular composition of focal adhesions at the nanometer scale. *Sci. Signal.* **9**, rs4 https://doi.org/10.1126/scisignal.aaf3572

67 Kechagia, J.Z., Ivaska, J. and Roca-Cusachs, P. (2019) Integrins as biomechanical sensors of the microenvironment. *Nat. Rev. Mol. Cell Biol.* **20**, 457–473 https://doi.org/10.1038/s41580-019-0134-2

68 Pellinen, T., Arjonen, A., Vuoriluoto, K., Kallio, K., Fransen, J.A. and Ivaska, J. (2006) Small GTPase Rab21 regulates cell adhesion and controls endosomal traffic of beta1-integrins. *J. Cell Biol.* **173**, 767–780 https://doi.org/10.1083/jcb.200509019

69 Kuo, J.C., Han, X., Hsiao, C.T., Yates Iii, J.R. and Waterman, C.M. (2011) Analysis of the myosin-II-responsive focal adhesion proteome reveals a role for beta-Pix in negative regulation of focal adhesion maturation. *Nat. Cell Biol.* **13**, 383–393 https://doi.org/10.1038/ncb2216

70 Mai, A., Veltel, S., Pellinen, T., Padzik, A., Coffey, E., Marjomäki, V. et al. (2011) Competitive binding of Rab21 and p120RasGAP to integrins regulates receptor traffic and migration. *J. Cell Biol.* **194**, 291–306 https://doi.org/10.1083/jcb.201012126

71 Pellinen, T., Tuomi, S., Arjonen, A., Wolf, M., Edgren, H., Meyer, H. et al. (2008) Integrin trafficking regulated by Rab21 is necessary for cytokinesis. *Dev. Cell* **15**, 371–385 https://doi.org/10.1016/j.devcel.2008.08.001

72 Rainero, E., Howe, J.D., Caswell, P.T., Jamieson, N.B., Anderson, K., Critchley, D.R. et al. (2015) Ligand-occupied integrin internalization links nutrient signaling to invasive migration. *Cell Rep.* **10**, 398–413 https://doi.org/10.1016/j.celrep.2014.12.037

73 Dozynkiewicz, M.A., Jamieson, N.B., Macpherson, I., Grindlay, J., van den Berghe, P.V., von Thun, A. et al. (2012) Rab25 and CLIC3 collaborate to promote integrin recycling from late endosomes/lysosomes and drive cancer progression. *Dev. Cell* **22**, 131–145 https://doi.org/10.1016/j.devcel.2011.11.008

74 Muranen, T., Iwanicki, M.P., Curry, N.L., Hwang, J., DuBois, C.D., Coloff, J.L. et al. (2017) Starved epithelial cells uptake extracellular matrix for survival. *Nat. Commun.* **8**, 13989 https://doi.org/10.1038/ncomms13989

75 Olivares, O., Mayers, J.R., Gouirand, V., Torrence, M.E., Gicquel, T., Borge, L. et al. (2017) Collagen-derived proline promotes pancreatic ductal adenocarcinoma cell survival under nutrient limited conditions. *Nat. Commun.* **8**, 16031 https://doi.org/10.1038/ncomms16031

76 Georgiadou, M., Lilja, J., Jacquemet, G., Guzmán, C., Rafaeva, M., Alibert, C. et al. (2017) AMPK negatively regulates tensin-dependent integrin activity. *J. Cell Biol.* **216**, 1107–1121 https://doi.org/10.1083/jcb.201609066

77 Rodriguez-Boulan, E. and Macara, I.G. (2014) Organization and execution of the epithelial polarity programme. *Nat. Rev. Mol. Cell Biol.* **15**, 225–242 https://doi.org/10.1038/nrm3775

78 Datta, A., Bryant, D.M. and Mostov, K.E. (2011) Molecular regulation of lumen morphogenesis. *Curr. Biol.* **21**, R126–R136 https://doi.org/10.1016/j.cub.2010.12.003

79 Zovein, A.C., Luque, A., Turlo, K.A., Hofmann, J.J., Yee, K.M., Becker, M.S. et al. (2010) Beta1 integrin establishes endothelial cell polarity and arteriolar lumen formation via a Par3-dependent mechanism. *Dev. Cell* **18**, 39–51 https://doi.org/10.1016/j.devcel.2009.12.006

80 Iruela-Arispe, M.L. and Davis, G.E. (2009) Cellular and molecular mechanisms of vascular lumen formation. *Dev. Cell* **16**, 222–231 https://doi.org/10.1016/j.devcel.2009.01.013

81 Senger, D.R. and Davis, G.E. (2011) Angiogenesis. *Cold Spring Harb. Perspect. Biol.* **3**, a005090 https://doi.org/10.1101/cshperspect.a005090

82 Turner, C.J., Badu-Nkansah, K. and Hynes, R.O. (2017) Endothelium-derived fibronectin regulates neonatal vascular morphogenesis in an autocrine fashion. *Angiogenesis* **20**, 519–531 https://doi.org/10.1007/s10456-017-9563-8

83 Guo, H.F. and Vander Kooi, C.W. (2015) Neuropilin functions as an essential cell surface receptor. *J. Biol. Chem.* **290**, 29120–6 https://doi.org/10.1074/jbc.R115.687327

84 Valdembri, D., Caswell, P.T., Anderson, K.I., Schwarz, J.P., König, I., Astanina, E. et al. (2009) Neuropilin-1/GIPC1 signaling regulates α5β1 integrin traffic and function in endothelial cells. *PLoS Biol.* **7**, e1000025 https://doi.org/10.1371/journal.pbio

85 Ellison, T.S., Atkinson, S.J., Steri, V., Kirkup, B.M., Preedy, M.E., Johnson, R.T. et al. (2015) Suppression of β3-integrin in mice triggers a neuropilin-1-dependent change in focal adhesion remodelling that can be targeted to block pathological angiogenesis. *Dis. Model. Mech.* **8**, 1105–1119 https://doi.org/10.1242/dmm.019927

86 Schiller, H.B., Friedel, C.C., Boulegue, C. and Fässler, R. (2011) Quantitative proteomics of the integrin adhesome show a myosin II-dependent recruitment of LIM domain proteins. *EMBO Rep.* **12**, 259–266 https://doi.org/10.1038/embor.2011.5

87 Teesalu, T., Sugahara, K.N., Kotamraju, V.R. and Ruoslahti, E. (2009) C-end rule peptides mediate neuropilin-1-dependent cell, vascular, and tissue penetration. *Proc. Natl Acad. Sci. U.S.A.* **106**, 16157–16162 https://doi.org/10.1073/pnas.0908201106

88 Pang, H.B., Braun, G.B., Friman, T., Aza-Blanc, P., Ruidiaz, M.E., Sugahara, K.N. et al. (2014) An endocytosis pathway initiated through neuropilin-1 and regulated by nutrient availability. *Nat. Commun.* **5**, 4904 https://doi.org/10.1038/ncomms5904

89 O'Loughlin, T., Masters, T.A. and Buss, F. (2018) The MYO6 interactome reveals adaptor complexes coordinating early endosome and cytoskeletal dynamics. *EMBO Rep.* **19**, e44884 https://doi.org/10.15252/embr.201744884

90 Lanahan, A., Zhang, X., Fantin, A., Zhuang, Z., Rivera-Molina, F., Speichinger, K. et al. (2013) The neuropilin 1 cytoplasmic domain is required for VEGF-A-dependent arteriogenesis. *Dev. Cell* **25**, 156–168 https://doi.org/10.1016/j.devcel.2013.03.019

91 Chittenden, T.W., Claes, F., Lanahan, A.A., Autiero, M., Palac, R.T., Tkachenko, E.V. et al. (2006) Selective regulation of arterial branching morphogenesis by synectin. *Dev. Cell* **10**, 783–795 https://doi.org/10.1016/j.devcel.2006.03.012

92 Lanahan, A.A., Hermans, K., Claes, F., Kerley-Hamilton, J.S., Zhuang, Z.W., Giordano, F.J. et al. (2010) VEGF receptor 2 endocytic trafficking regulates arterial morphogenesis. *Dev. Cell* **18**, 713–724 https://doi.org/10.1016/j.devcel.2010.02.016

93 Gelfand, M.V., Hagan, N., Tata, A., Oh, W.J., Lacoste, B., Kang, K.T. et al. (2014) Neuropilin-1 functions as a VEGFR2 co-receptor to guide developmental angiogenesis independent of ligand binding. *eLife* **3**, e03720 https://doi.org/10.7554/eLife.03720

94 Wang, S., Sekiguchi, R., Daley, W.P. and Yamada, K.M. (2017) Patterned cell and matrix dynamics in branching morphogenesis. *J. Cell Biol.* **216**, 559–570 https://doi.org/10.1083/jcb.201610048

95 Astrof, S. and Hynes, R.O. (2009) Fibronectins in vascular morphogenesis. *Angiogenesis* **12**, 165–175 https://doi.org/10.1007/s10456-009-9136-6

96 Arjonen, A., Alanko, J., Veltel, S. and Ivaska, J. (2012) Distinct recycling of active and inactive β1 integrins. *Traffic* **13**, 610–625 https://doi.org/10.1111/j.1600-0854.2012.01327.x

97 Neefjes, J., Jongsma, M.M.L. and Berlin, I. (2017) Stop or go? endosome positioning in the establishment of compartment architecture, dynamics, and function. *Trends Cell Biol.* **27**, 580–594 https://doi.org/10.1016/j.tcb.2017.03.002

98 Haucke, V., Neher, E. and Sigrist, S.J. (2011) Protein scaffolds in the coupling of synaptic exocytosis and endocytosis. *Nat. Rev. Neurosci.* **12**, 127–138 https://doi.org/10.1038/nrn2948

99 Südhof, T.C. (2012) The presynaptic active zone. *Neuron* **75**, 11–25 https://doi.org/10.1016/j.neuron.2012.06.012

100 Wei, H., Sundararaman, A., Dickson, E., Rennie-Campbell, L., Cross, E., Heesom, K.J. et al. (2019) Characterization of the polarized endothelial secretome. *FASEB J.* **33**, 12277–12287 https://doi.org/10.1096/fj.201900262R

101 Risau, W. and Lemmon, V. (1988) Changes in the vascular extracellular matrix during embryonic vasculogenesis and angiogenesis. *Dev. Biol* **125**, 441–450 https://doi.org/10.1016/0012-1606(88)90225-4

102 Hirst, J., Borner, G.H., Antrobus, R., Peden, A.A., Hodson, N.A., Sahlender, D.A. et al. (2012) Distinct and overlapping roles for AP-1 and GGAs revealed by the "knocksideways" system. *Curr. Biol.* **22**, 1711–1716 https://doi.org/10.1016/j.cub.2012.07.012

PORTLAND PRESS

103 Sahgal, P., Alanko, J., Icha, J., Paatero, I., Hamidi, H., Arjonen, A. et al. (2019) GGA2 and RAB13 promote activity-dependent β1-integrin recycling. *J. Cell Sci.* **132**, jcs233387 https://doi.org/10.1242/jcs.233387

104 Hamidi, H. and Ivaska, J. (2017) Vascular morphogenesis: an integrin and fibronectin highway. *Curr Biol.* **27**, R158–R161 https://doi.org/10.1016/j.cub.2016.12.036

105 Serra-Pagès, C., Kedersha, N.L., Fazikas, L., Medley, Q., Debant, A. and Streuli, M. (1995) The LAR transmembrane protein tyrosine phosphatase and a coiled-coil LAR-interacting protein co-localize at focal adhesions. *EMBO J.* **14**, 2827–2838 https://doi.org/10.1002/j.1460-2075.1995.tb07282.x

106 Puertollano, R. and Bonifacino, J.S. (2004) Interactions of GGA3 with the ubiquitin sorting machinery. *Nat. Cell Biol.* **6**, 244–251 https://doi.org/10.1038/ncb1106

107 Scott, P.M., Bilodeau, P.S., Zhdankina, O., Winistorfer, S.C., Hauglund, M.J., Allaman, M.M. et al. (2004) GGA proteins bind ubiquitin to facilitate sorting at the trans-Golgi network. *Nat. Cell Biol.* **6**, 252–259 https://doi.org/10.1038/ncb1107

108 Krause, M. and Gautreau, A. (2014) Steering cell migration: lamellipodium dynamics and the regulation of directional persistence. *Nat. Rev. Mol. Cell Biol.* **15**, 577–590 https://doi.org/10.1038/nrm3861

109 Ridley, A.J. (2015) Rho GTPase signalling in cell migration. *Curr. Opin. Cell Biol.* **36**, 103–112 https://doi.org/10.1016/j.ceb.2015.08.005

110 Palamidessi, A., Frittoli, E., Garré, M., Faretta, M., Mione, M., Testa, I. et al. (2008) Endocytic trafficking of Rac is required for the spatial restriction of signaling in cell migration. *Cell* **134**, 135–147 https://doi.org/10.1016/j.cell.2008.05.034

111 Snyder, J.T., Rossman, K.L., Baumeister, M.A., Pruitt, W.M., Siderovski, D.P., Der, C.J. et al. (2001) Quantitative analysis of the effect of phosphoinositide interactions on the function of Dbl family proteins. *J. Biol. Chem.* **276**, 45868–45875 https://doi.org/10.1074/jbc.M106731200

112 Huotari, J. and Helenius, A. (2011) Endosome maturation. *EMBO J.* **30**, 3481–3500 https://doi.org/10.1038/emboj.2011.286

113 Bilanges, B., Posor, Y. and Vanhaesebroeck, B. (2019) PI3K isoforms in cell signalling and vesicle trafficking. *Nat. Rev. Mol. Cell Biol.* **20**, 515–534 https://doi.org/10.1038/s41580-019-0129-z

114 Lilja, J., Zacharchenko, T., Georgiadou, M., Jacquemet, G., De Franceschi, N., Peuhu, E. et al. (2017) SHANK proteins limit integrin activation by directly interacting with Rap1 and R-Ras. *Nat. Cell Biol.* **19**, 292–305 https://doi.org/10.1038/ncb3487

115 Goldfinger, L.E., Ptak, C., Jeffery, E.D., Shabanowitz, J., Hunt, D.F. and Ginsberg, M.H. (2006) RLIP76 (RalBP1) is an R-Ras effector that mediates adhesion-dependent Rac activation and cell migration. *J. Cell Biol.* **174**, 877–888 https://doi.org/10.1083/jcb.200603111

116 Torres, V.A., Mielgo, A., Barbero, S., Hsiao, R., Wilkins, J.A. and Stupack, D.G. (2010) Rab5 mediates caspase-8-promoted cell motility and metastasis. *Mol. Biol. Cell* **21**, 369–376 https://doi.org/10.1091/mbc.e09-09-0769

117 Atherton, P., Lausecker, F., Harrison, A. and Ballestrem, C. (2017) Low-intensity pulsed ultrasound promotes cell motility through vinculin-controlled Rac1 GTPase activity. *J. Cell Sci.* **130**, 2277–2291 https://doi.org/10.1242/jcs.192781

118 Arriagada, C., Silva, P., Millet, M., Solano, L., Moraga, C. and Torres, V.A. (2019) Focal adhesion kinase-dependent activation of the early endocytic protein Rab5 is associated with cell migration. *J. Biol. Chem.* **294**, 12836–12845 https://doi.org/10.1074/jbc.RA119.008667

119 Mellor, P., Marshall, J.D.S., Ruan, X., Whitecross, D.E., Ross, R.L., Knowles, M.A. et al. (2018) Patient-derived mutations within the N-terminal domains of p85α impact PTEN or Rab5 binding and regulation. *Sci. Rep.* **8**, 7108 https://doi.org/10.1038/s41598-018-25487-5

120 Kleinschmidt, E.G. and Schlaepfer, D.D. (2017) Focal adhesion kinase signaling in unexpected places. *Curr. Opin. Cell Biol.* **45**, 24–30 https://doi.org/10.1016/j.ceb.2017.01.003

121 Alanko, J., Mai, A., Jacquemet, G., Schauer, K., Kaukonen, R., Saari, M. et al. (2015) Integrin endosomal signalling suppresses anoikis. *Nat. Cell Biol.* **17**, 1412–1421 https://doi.org/10.1038/ncb3250

122 Lawson, C., Lim, S.T., Uryu, S., Chen, X.L., Calderwood, D.A. and Schlaepfer, D.D. (2012) FAK promotes recruitment of talin to nascent adhesions to control cell motility. *J. Cell Biol.* **196**, 223–232 https://doi.org/10.1083/jcb.201108078

123 Nader, G.P., Ezratty, E.J. and Gundersen, G.G. (2016) Talin and PIPKIγ regulate endocytosed integrin activation to polarize focal adhesion assembly. *Nat. Cell Biol.* **18**, 491–503 https://doi.org/10.1038/ncb3333

124 Cullen, P.J. and Steinberg, F. (2018) To degrade or not to degrade: mechanisms and significance of endocytic recycling. *Nat. Rev. Mol. Cell Biol.* **19**, 679–696 https://doi.org/10.1038/s41580-018-0053-7