Integrin traffic

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Accepted 15 July 2006
Journal of Cell Science 119, 3723-3731 Published by The Company of Biologists 2006
doi:10.1242/jcs.03216

Summary

Cell adhesion, migration and the maintenance of cell polarity are all processes that depend on the correct targeting of integrins and the dynamic remodelling of integrin-containing adhesion sites. The importance of the endo/exocytic cycle of integrins as a key regulator of these functions is increasingly recognized. Several recent publications have provided mechanistic insight into how integrin traffic is regulated in cells. Increasing evidence suggests that small GTPases such as Arf6 and members of the Rab family control integrin internalization and recycling back to the plasma membrane along microtubules. The fine tuning of these trafficking events seems to be mediated by specific guanine-nucleotide-exchange factors (GEFs) and GTPase-activating proteins (GAPs). In addition, several kinases regulate integrin traffic. The identification of their substrates has demonstrated how these kinases regulate integrin traffic by controlling small GTPases or stabilizing cytoskeletal tracks that are crucial for efficient traffic of integrins to the plasma membrane.

Key words: Integrin, Migration, Trafficking, Rab GTPases

Introduction

Integrins are the major cell surface adhesion receptors for ligands in the extracellular matrix. They are heterodimeric proteins consisting of an α- and a β-chain and are involved in the transmission and interpretation of signals from the extracellular environment into various signaling cascades (Hynes, 2002). Ligation of integrin by matrix proteins such as fibronectin is important for cell migration and leads to assembly of focal adhesion proteins at the site of attachment. An essential protein for focal adhesion assembly and disassembly is focal adhesion kinase (FAK), which phosphorylates various signaling and adaptor proteins, such as Src, CAS, paxillin, α-actinin and phosphatidylinositol kinases. Controlled phosphorylation of these proteins is an important regulator of focal adhesion turnover (Mitra et al., 2005).

The role of integrin endo/exocytic cycle in the regulation of cell adhesion, spreading and motility is becoming increasingly recognized. The basic idea has been that exocytosis at the advancing edge assists cell locomotion by providing fresh adhesion receptors and that these trafficking receptors are internalized by endocytosis at the retracting end of the cell. Early studies showed that the transport of integrins to the cell surface is adhesion dependent in fibroblasts (Dalton et al., 1995). In addition, several studies on the fibronectin receptor of CHO cells (integrin α5β1) highlighted more than a decade ago that this adhesion receptor is constantly endocytosed and that the function of this internalization is to recycle rather than degrade the receptor (Bretscher, 1989; Bretscher, 1992; Raub and Kuentzel, 1989; Sczekan and Juliano, 1990). We have also known for some time that integrins regulate matrix turnover by endocytosis. Integrin αVβ5 is internalized in an active, vitronectin-bound form (Panetti and McKeown-Longo, 1993a; Panetti and McKeown-Longo, 1993b), through clathrin-coated pits (Memmo and McKeown-Longo, 1998), and recycled back to the membrane; the vitronectin is targeted for degradation. Endocytosed β1 integrins have also been shown to remain in an active conformation and colocalize with fibronectin and collagen (Ng et al., 1999), which suggests that integrin traffic provides the cell with a constant supply of 'refreshed' receptors that can bind ligand.

Several recent studies have provided mechanistic insight into the mechanisms governing integrin traffic. Different kinases, modulators of the actin cytoskeleton and members of the Rab- and Arf GTPase families have been implicated in experiments using different techniques, cell types and stimuli. Here, we discuss some recent advances in our understanding of the molecular mechanisms involved in integrin traffic.

Integrin cytoplasmic domains in regulation of traffic

The cytoplasmic domains of integrins play a pivotal role in integrin function. Thus far, many studies on the endo/exocytic cycle of integrins have focused on the cytoplasmic domains of the β subunits. Sequences involved in integrin traffic have been identified in the β1-, β2- and β3-tails (Fabbri et al., 2005; Parsons et al., 2002; Woods et al., 2004). The role of the β2- and β3-tails have been reviewed elsewhere (Caswell and Norman, 2006), and so we do not discuss them in detail here. Briefly, 14 C-terminal amino acids of the cytoplasmic domain of β3 integrin, including a crucial unphosphorylated tyrosine (Y759), are involved in binding to protein kinase D1 (PKD1, Fig. 1). This interaction promotes fast recycling of αvβ3 integrin from recycling endosomes to the plasma membrane upon growth factor stimulation (Woods et al., 2004). Similarily, a membrane-proximal YRRF motif in β2 integrin was found to mediate recycling of αLβ2 integrin in CHO cells (Fabbri et al., 1999) (Fig. 1). Mutagenesis of this motif decreases the ability of cells to migrate on the αLβ2 substrate ICAM-1 but not on fibronectin.

The short cytoplasmic domains of integrin β subunits also

contain three highly conserved motifs – cyto1, cyto2 and cyto3 – implicated in the regulation of targeting of integrins to focal adhesions (Reszka et al., 1992) (Fig. 1). Cyto2 and cyto3 contain NPxY motifs similar to those known to act as internalization signals for other membrane receptors, although their role in the endocytic and/or exocytic trafficking of integrins is somewhat different. Replacement of the tyrosines in the NPxY motifs of the cyto2 and cyto3 domains with serines impairs targeting of integrins to focal adhesions. However, internalization of α5β1 integrin is not impaired by these mutations to the β1 tail despite the fact that it is constitutively internalized in CHO cells (Bretscher, 1989; Raub and Kuentzel, 1989; Sczekan and Juliano, 1990). The cyto2 and cyto3 domains nevertheless play an important role in the internalization of β1-integrin. PKCα binds to these regions of β1-integrins, and its kinase activity regulates their endocytosis (Ng et al., 1999). The interaction involves the V3 hinge region in PKCα, and efficient binding requires both of the conserved NPxY motifs (Parsons et al., 2002). This interaction is important in regulation of directional cell motility of breast cancer cells up an EGF gradient, which suggests a role for the interaction in integrin traffic. However, direct evidence showing that binding of PKCα to β1 integrin is required for PKC-activity-dependent integrin traffic is still missing. In addition, it would be interesting to further define the exact amino acids crucial for the PKCα–β1-integrin interaction and compare these with the crucial amino acids identified in other β-integrin tails (Caswell and Norman, 2006).

There are several examples of signaling mediated by the integrin α subunit (Ivaska et al., 1999; Klekotka et al., 2001a; Klekotka et al., 2001b; Klekotka et al., 2001c; Mattila et al., 2005; Wary et al., 1996), and the α-chain has a role in integrin internalization as well. In polarized cells, integrins are segregated to discrete domains of the plasma membrane and need to be redistributed to allow differentiation and cell migration to occur. Integrin internalization might be involved in such redistribution. Early studies showed that, upon detachment, keratinocytes internalize basally located membrane domains containing α6β4 integrin. The internalization route does not follow the classical receptor-mediated endocytic route and may involve intermediate filaments (Poumay et al., 1993). The integrin α6 cytoplasmic domains can promote internalization of chimeric CD8–α6-integrin proteins, which suggests that this process is mediated by the α6 cytoplasmic domain. The integrin α2 subunit can also direct specific internalization of α2β1 integrin from membrane microdomains in other cell types (Upla et al., 2004).

More recently, the conserved membrane-proximal GFFKR segment (Fig. 1) shared by the majority of integrin α subunits has been shown to be important in integrin traffic (Pellinen et al., 2006). The small GTPase Rab21 interacts with integrin α subunits to induce endocytosis and recycling to the membrane. The association requires the conserved arginine residue (Fig. 1) in the integrin α subunit and seems to depend on the conformation of the membrane-proximal segment. Rab21 promotes cell adhesion and migration by inducing integrin trafficking, and mutagenesis of this conserved arginine abrogates its ability to induce adhesion.

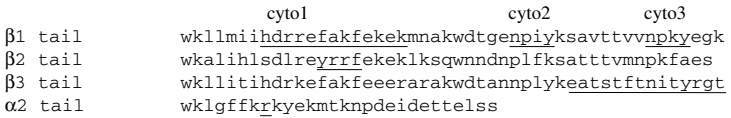

Fig. 1. Integrin cytoplasmic motifs involved in integrin trafficking. The cyto1, cyto2 and cyto3 regions of β integrins are underlined in the β1-integrin sequence. The cyto1 sequence is conserved in all β integrins except β4, but the cyto2 and cyto3 signals (NxxY) are found in all β integrins. All three cyto motifs are important for focal adhesion localization. Cyto2 and cyto3 are involved in endocytosis of β1 integrins (see text). The underlined β2-integrin tail motif YRRF, the C-terminal β3-integrin tail and the Arg1161 in α2 integrin are involved in trafficking of αLβ2, avβ3 and α2β1, respectively.

Rab-proteins and Arf6 in regulation of β1-integrin traffic

Rab and Arf family GTPases regulate membrane traffic in the exo- and endocytotic pathways. Both families are important regulators of the structure and dynamics of intracellular membranes, associating with membrane lipids and recruiting various effector proteins. Rab GTPases are involved in tethering and fusion of membrane vesicles, as well as in transport of them and associated cargo proteins through interactions with the cytoskeleton and motor proteins (Zerial and McBride, 2001). Arf GTPases promote the recruitment of coat proteins from the cytosol to membranes and regulate the formation of coated vesicles and cargo selection (D'Souza-Schorey and Chavrier, 2006).

Several β1 integrins, as well as αvβ3, α6β4 and αLβ2, have been shown to recycle back to the plasma membrane via the Rab11-positive perinuclear recycling compartment (Caswell and Norman, 2006). This long-loop recycling pathway has been suggested to play a general role in recycling, because many membrane proteins associate with this compartment en route to the plasma membrane. In addition to Rab11, Arf6 GTPase has been implicated in the recycling of β1 integrins from the perinuclear recycling compartment. Nucleotide exchange on Arf6 and Rab11, in addition to actin remodeling by Arf6, is crucial for β1-integrin traffic, because mutants lacking these functions abrogate the recycling of this integrin from the perinuclear compartment to the plasma membrane (Powelka et al., 2004). Upon stimulation by growth factors, αVβ3 integrin, but not α5β1 integrin, is diverted to a short-loop trafficking pathway dependent on Rab4 (Roberts et al., 2001). Inhibition of Rab4 function by a dominant-negative Rab4 mutant (Rab4-GDP) inhibits integrin recycling and compromises mouse 3T3 fibroblast cell adhesion and spreading on the αVβ3 ligand vitronectin. The growth-factor-induced faster recycling pathway depends on the direct interaction of PKD1with the β-integrin tail (Woods et al., 2004).

Recent studies of Rab5 and Rab21 suggest an essential role for Rab5 family GTPases in the regulation of the endocytosis and recycling of β1 integrins as well (Fig. 2). Rab5 family GTPases are central regulators of endocytosis and movement of endocytic vesicles along microtubules (Simpson and Jones, 2005). Rab5 and Rab21 associate with the membrane-proximal part of the cytoplasmic tail of integrin α subunits pairing with β1 integrin. Expression of a Rab21 mutant locked in the GDP-bound conformation induces the accumulation of active β1 integrins in large focal adhesions, which partially colocalize

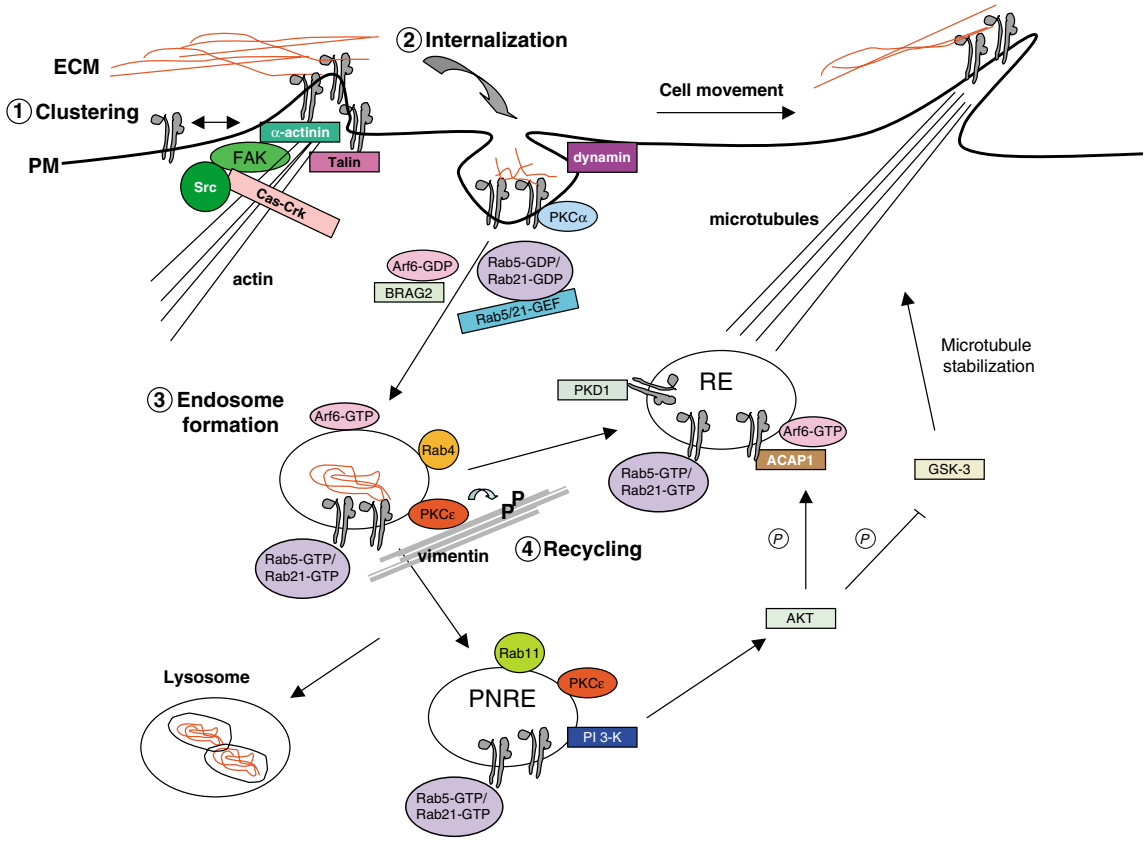

Fig. 2. A model for integrin traffic. Integrin-ECM interaction leads to clustering of integrins and formation of focal contacts (FC), where integrins form connections to the actin cytoskeleton through the FAK-Src complex and their substrates (Mitra et al., 2005). Integrin internalization could be coupled to FC disassembly, in which the connection to actin is lost and microtubules are targeted to dynamin-dependent internalization sites (Burridge, 2005; Ezratty et al., 2005). β1 integrins and ECM proteins are internalized with the help of activated PKCα, which binds directly to β1 integrin cytoplasmic tails (Ng et al., 1999). Internalization of β1 integrin has been shown to be regulated by Rab5/Rab21 and microtubules (Pellinen et al., 2006), as well as active Arf6. Arf6 is activated by Arf6 GEF (BRAG2), promoting integrin endocytosis (Dunphy et al., 2006), and a similar requirement for GEF activity is likely to exist for Rab5/Rab21-regulated integrin internalization (see text). In the endosomal compartments, β1 integrins associate with Rab21-GTP in an α-subunit cytoplasmic-tail-dependent manner. From these compartments and the perinuclear recycling endosomes (PNRE), the long-loop recycling of β1 integrins back to the PM has been shown to be regulated by Rab11, Rab21 and Arf6 GTPase activities (Powelka et al., 2004; Roberts et al., 2004). Upon growth-factor stimulation β3 integrins are diverted to a short-loop recycling pathway involving Rab4 and PKD1 (Woods et al., 2004). The Arf6-dependent recycling of integrins is further regulated by the GTPase-activating protein (GAP) ACAP, which in turn is positively regulated by Akt-mediated phosphorylation. In fibroblasts, the exit of β1 integrins from recycling endosomes (RE) is also regulated by PKCε-mediated phosphorylation of vimentin (Ivaska et al., 2005). Increased transport of α6β4 integrin to the plasma membrane (PM) has recently been shown to be facilitated by Akt-GSK3β-dependent stabilization of microtubules (Yoon et al., 2005). The return of integrins to the PM is also dependent on the activity of the PI-3-K–AKT–GSK3 pathway (Roberts et al., 2004).

with Rab21-GDP. A Rab21 mutant locked in the GTP-bound conformation associates more strongly with β1 integrin and causes accumulation of β1 integrins in endocytic vesicles. GDP-locked Rab21 thus somehow blocks the endocytosis of β1 integrins, and nucleotide exchange allows integrins to internalize into Rab21-GTP-positive vesicles. This activity of Rab21 could be regulated by focal adhesion proteins, because β1-integrin internalization is preceded by integrin activation and clustering (Gao et al., 2000; Panicker et al., 2006; Sharma et al., 2005; Upla et al., 2004; Wong and Isberg, 2005). Notice that Rab5 has been shown to organize actin structures – together with PI 3-K and Rac1 but also independently of them (Lanzetti et al., 2004; Spaargaren and Bos, 1999).

A guanine nucleotide exchange factor (GEF) for Rab21 must drive its conversion to the GTP-bound form similarly to the recently described Arf6 GEF BRAG2, which also contributes to the internalization of β1 integrins (Dunphy et al., 2006). Of the Rab5 GEFs (Rabex-5, Als2, Als2CL, Rap6, and Rin1, Rin2 and Rin3) only Rabex-5 has been confirmed as a Rab21 GEF (Delprato et al., 2004; Otomo et al., 2003; Tall et al., 2001). The ankyrin-repeat protein Varp is also a Rab21 GEF (Mosavi et al., 2004). Ectopically expressed Rin1, however, co-immunoprecipitates with both Rab21 and β1 integrins, which indicates that Rin1 could work as a GEF for Rab21 (Pellinen and Ivaska, unpublished observations). Rin1 is activated by binding to activated Ras through Ras-binding domain. This leads to Rab5 activation and enhanced endocytosis of EGFR (Tall et al., 2001). A similar mechanism could drive integrin endocytosis as well. Binding of Ras-GTP to Rin1 also stimulates the tyrosine-kinase-activation function of Rin1,

which can bind and activate ABL kinases (Hu et al., 2005). This leads to phosphorylation of CRK/L, resulting in remodeling of the actin cytoskeleton, enhanced adhesion and migration. Thus, cytoskeletal remodeling through ABL kinases could be coupled to the endocytotis of EGFR and integrins.

Do Arf6 and Rab5/Rab21 function independently or together in integrin traffic? Rab5 and Arf6 family members regulate membrane dynamics via activation of the lipid kinases phosphatidylinositide 3-kinase (PI 3-K) and phosphatidylinositol-4-phosphate 5-kinase (PIP5K), respectively. Both kinases are important for the regulation of integrin-mediated pathogen uptake (see below), cell motility (Cantley, 2002; Ling et al., 2006; Shaw et al., 1997) and focal adhesion turnover (Mitra et al., 2005). The PIP5K product PtdIns(4,5)P_2 regulates actin remodeling through its binding to PH domains of Rho GEFs and other actin-binding focal adhesion proteins, such as talin (Di Paolo et al., 2002), vinculin (Chandrasekar et al., 2005) and ezrin (Barret et al., 2000; Shin et al., 2005). Expression of GTP-locked Arf6 results in the accumulation of plasma membrane proteins including β1 integrin in PtdIns(4,5)P_2-containing macropinocytic vesicles (Brown et al., 2001) and overexpression of PIP5Kα has the same effect. This indicates that formation and loss of PtdIns(4,5)P_2 are essential in the recycling process. Rab5 binds to and activates PI 3-Ks (hVps34 and PI 3-Kβ) as well as phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase and phosphatidylinositol-3,4-bisphosphate 4-phosphatase to regulate the turnover of various phosphoinositides (Shin et al., 2005). The PI 3-K hVps34 generates PtdIns(3)*P* on the early endosome and recruits FYVE-containing proteins such as EEA1 and Rabenosyn-5, which are important in early endocytosis (Nielsen et al., 2000; Simonsen et al., 1998). Thus, the distinction between Arf6 and Rab5/Rab21 actions in integrin trafficking could be made by the recruitment of different effectors by the different phosphoinositides generated.

Microtubules and other cytoskeletal tracks

Activation of Akt in response to external stimuli regulates cell migration by mechanisms that may be pro- (for a review, see Cheng et al., 2005) or anti-migratory (Irie et al., 2005; Liu et al., 2006). The pro-migratory effect of Akt involves the inactivation of GSK3β and subsequent effects on integrin traffic. The endo/exocytic cycle of integrins α5β1 and αVβ3 through the endosomal pathway to the perinuclear recycling compartment is independent of GSK3β activity. However, their recycling via the so-called long-loop route (Caswell and Norman, 2006) is Rab11 dependent, and requires Akt-mediated phosphorylation and inactivation of GSK3β (Roberts et al., 2004). A recent study provides further insight into this. Inhibition of GSK3β by hypoxia leads to dispersal of Rab11 vesicles throughout the cytoplasm and enhanced transport of α6β4 integrin to the plasma membrane along stabilized microtubules. GSK3β inactivation leads to the accumulation of detyrosinated, stabilized tubulin and correlates with increased invasion of breast cancer cells through matrigel. GSK3β phosphorylates several microtubule-associated proteins and decreases their ability to stabilize microtubules (Akhmanova et al., 2001; Goold et al., 1999; Lovestone et al., 1996; Sanchez et al., 2000; Zumbrunn et al., 2001), which suggests that the effects of GSK3β on integrin traffic may involve some of these effectors.

Stable, detyrosinated microtubules are a hallmark of migrating cells (Gundersen and Bulinski, 1988) and the localized stabilization of microtubules is observed at the leading edge (Gundersen and Bretscher, 2003). This is controlled by integrins, because integrin-mediated activation of FAK is required for microtubule stabilization by the Rho-mDia signaling pathway in mouse fibroblasts (Palazzo et al., 2004). Recent data further underscore the role of microtubules in integrin traffic. Overexpression of Rab21 induces localization of active β1 integrins to endocytic vesicles and the long-distance bi-directional movements of these Rab5/Rab21-positive vesicles depend on microtubules (Pellinen et al., 2006). Rab5 regulates motility of early endosomes along microtubules (Nielsen et al., 1999) and its is possible that the same applies to Rab21, a member of Rab5 family.

Integrins have been shown to be located in detergent-resistant microdomains (Fabbri et al., 2005), and integrin α2β1 is known to be internalized into caveolae (Upla et al., 2004). Interestingly, the trafficking of caveolin-bearing vesicles is also dependent on microtubules (Mundy et al., 2002), which suggests that they may be tracks for trafficking of integrins internalized by different mechanisms.

Matrix-bound integrins are closely associated with actin filaments in focal adhesions (Fig. 2) and the turnover of these structures is an essential part of integrin traffic and important for cell motility (Ezratty et al., 2005; Wells et al., 2005). Integrins are also actively transported along actin filaments. Several integrin β-subunit cytoplasmic domains bind directly to the actin-based motor myosin X (Zhang et al., 2004). Myosin-X-binding integrins are transported along actin to the tips of filopodia, where they are involved in the stabilization of these structures. Furthermore, this interaction is important for the initial spreading and adhesion of cells. It is currently unknown whether the integrin transported in filopodia by myosin X is associated with endocytic vesicles or how these vesicles might be linked to the integrin trafficking mechanisms, but future work should clarify this.

Finally, as discussed below, the intermediate filament protein vimentin regulates integrin traffic in mesenchymal cells, and tethering of endocytosed integrins to vimentin filaments regulates the recycling of these receptors by a phosphorylation-dependent mechanism. Therefore, it seems that microtubules, actin and intermediate filaments can all function as tracks for trafficking integrins. Use of these different cytoskeletal elements probably differs between cell types and may even be integrin specific in some cases.

Kinases in integrin traffic

Several kinases regulate cell migration and integrin traffic. These include serine-threonine kinases of the PKC family, Akt (or PKB) and PKD1, as mentioned above. Stimulation of PKC isotypes increases cell migration irrespective of the matrix proteins recognized by the cells (Rigot et al., 1998), but overexpression of specific isoforms induces distinct motility responses. PKCα stimulates both random motility and directional motility (haptotaxis) in epithelial cells (Ng et al., 1999; Parsons et al., 2002), whereas PKCε seems to contribute solely to haptotaxis – at least in mesenchymal cells (Ivaska et al., 2002b). PKCα controls constitutive integrin traffic by regulating internalization and recycling of the receptor (Ng et al., 1999), whereas PKCε activity is required for the return of

the constitutively endocytosed β1 integrin to the plasma membrane (Ivaska et al., 2005). Akt acts via its downstream target GSK3β to regulate the traffic of α5β1 and αVβ3 integrins through the perinuclear recycling compartment in unstimulated cells (Roberts et al., 2004). PDGF-induced recycling of αVβ3 integrins, by contrast, is regulated by PKD1 – phospholipase C and PKC being upstream triggers (Woods et al., 2004). PKCε has been shown to activate PKD1 and associate with an αVβ3-integrin–PKD1 complex involved in the PDGF-induced traffic of αVβ3 (Woods et al., 2004). Although PKCε plays an important role in this process, PKD1 is not likely to be a regulator of the PKCε–β1-integrin vesicular compartment involved in regulation of constitutive integrin traffic in fibroblasts (Ivaska, 2002b).

Kinases have been known for some time to regulate endocytosis (Woodman et al., 1992), and recently an unexpectedly large number of kinases (over 35% of the human kinome) were shown to regulate clathrin- and caveolin-mediated endocytoses (Pelkmans et al., 2005). Several kinases regulate these distinct routes differentially and thus may control the balance between them. Furthermore, kinases that have well-established roles in integrin signaling also regulate endocytosis. Integrin trafficking and signaling are probably therefore tightly coupled. Indeed, signaling might also occur in endocytic vesicles bearing integrins and be different from that at the classical adhesions.

In spite of the relatively abundant data on kinases that regulate integrin traffic, the relevant substrates have remained unidentified in most cases, and thus the molecular details remain to be clarified. Two recent papers identify kinase substrates relevant for integrin traffic and cell migration (Ivaska et al., 2005; Li et al., 2005). Since Akt regulates cell motility in response to various stimuli, the finding that Akt-mediated phosphorylation of ACAP1 – an Arf6 GAP (Donaldson and Jackson, 2000) that is an effector in endosomal cargo sorting – is required for β1-integrin recycling, is important. Li and co-workers (Li et al., 2005) showed that stimulation-induced phosphorylation of ACAP1 at S554 by Akt regulates ACAP1–β1-integrin interactions in endosomes. siRNA-mediated silencing of either ACAP1 or Akt impairs the interaction and inhibits integrin recycling. These data are thus relevant to the earlier findings that serum-stimulation-dependent recycling of β1 integrin involves Arf6 and Rab11 (Powelka et al., 2004).

The recycling of endocytosed β1 integrin in fibroblasts requires PKCε-dependent phosphorylation of vimentin (Ivaska et al., 2005). PKCε regulates the passage of endocytosed integrin from intracellular vesicles back to the plasma membrane, and phosphorylation of vimentin triggers integrin recycling possibly through the release of a mechanical tether. Vimentin dissociates from integrin-containing membrane structures following PKCε-dependent phosphorylation at several N-terminal serine residues. An earlier study suggested that intermediate filaments play a role in the internalization and recycling of α6β4 integrin from the basal side of keratinocytes (Poumay et al., 1993). More recently, vimentin has been shown to associate with integrin-containing focal adhesions (Gonzales et al., 1999) and to interact with α2β1 integrin (Kreis et al., 2005).

Mesenchymal cells may thus employ integrin endo/exocytosis mechanisms different from those in epithelial cells, in which integrins appear to traffic along microtubules and actin (see above). It is currently unclear whether vimentin plays an active role in cell transformation by accelerating integrin traffic and inducing cell motility. Differences in integrin traffic between different cell types have not been analysed systematically, but fundamental alterations in integrin traffic may well occur during cell transformation, and these may be key to the loss of cell polarization and increase in motility and invasion seen following malignant transformation.

More targets downstream of these kinases are likely to be identified, and many may be proteins that function in other cellular activities involving endocytosis. Identifying these will thus provide insight into how different endosomal pathways integrate extracellular signals and coordinate their activities in different situations.

Integrin-guided pathogen invasion as model for studying integrin endocytosis and traffic

Many viruses and bacteria exploit the endocytic machinery of the host for invasion. Pathogens associate with and are internalized with numerous mammalian cell receptors. Integrins can be used either as primary attachment receptors or as co-receptors in the entry process (Greber, 2002; Marsh and Helenius, 2006; Pizarro-Cerda and Cossart, 2006), and pathogen attachment triggers host-cell signaling pathways that follow binding of the endogenous ligand to integrins (see Table 1) (see also Brakebusch and Fassler, 2003; Wozniak at al., 2004). Furthermore, the mechanisms regulating pathogen entry seem to overlap significantly with the known endocytotic pathways for integrins. Pathogen entry mechanisms may therefore provide important insights into the mechanisms regulating integrin traffic.

The most explored integrin-mediated mechanism for bacterial endocytosis is the uptake of *Yersinia*, which depends on conserved tyrosine and threonine residues in the β1A integrin cytoplasmic tail as well as Rac1 activation (Gustavsson et al., 2002; Wong and Isberg, 2003; Wong and Isberg, 2005). The upstream regulators of Rac1 include phosphoinositide 3-kinase class I and a Cas-Crk complex, both of which are regulated by the FAK-Src complex (Welch et al., 2003; Chodniewicz and Klemke, 2004). The requirement for Rac1 can be bypassed by overexpression of PIP5K or Arf6 (Wong and Isberg, 2003). The importance of Arf6 in β1-integrin-mediated bacterial uptake could reflect its role in the endocytosis and recycling of β1 integrin (Dunphy et al., 2006; Powelka et al., 2004).

Several viruses also use integrins for their internalization (see Table 1). Echovirus1 and rotavirus enter cells via α2β1-integrin-mediated endocytosis, regulated by dynamin-dependent mechanisms (Pietiainen et al., 2004; Sanchez-San Martin et al., 2004). Adenoviral entry via αV integrin is dependent on Rab5 (Rauma et al., 1999). The rotavirus spike protein Vp4 is able to bind both to the extracellular domain of α2 subunit and to Rab5 in the cytosol (Enouf et al., 2003; Graham et al., 2003). It could therefore bypass the requirement for integrin-Rab5/Rab21 association by carrying its own Rab5-binding domain and, in that way, promote its own trafficking along microtubules.

The signaling originating from focal adhesions is crucial for subsequent actin and membrane dynamics and pathogen trafficking. The pathways involved are probably similar to

Table 1. Integrins in pathogen entry

Pathogen (integrin receptor)	Route	Kinase/adaptor	GTPase	Reference
Rotavirus (α2β1, αXβ2, αVβ3)	Non-clathrin, non-caveolin		Dynamin, Rab5	Ciarlet et al., 2002; Enouf et al., 2003; Graham et al., 2003; Lopez and Arias, 2004; Sanchez-San Martin et al., 2004
EV1 (α2β1)	Caveolae/raft	p38, ERK1/2, PKCα	Dynamin II	Huttunen et al., 1998; Pietiainen et al., 2004
Adenovirus group C: ad2, ad5 (αVβ1, αVβ3, αVβ5)	Clathrin, microtubules	PI3K, CAS, PKA, p38	Rab5, dynamin, Rac1, Cdc42	Li et al., 2000; Rauma et al., 1999; Li et al., 1998; Suomalainen et al., 2001; Meier and Greber, 2004
AAV-2 (αVβ5)	Clathrin	PI3K	Dynamin I, Rac1	Duan et al., 1999; Sanlioglu et al., 2000
KSHV (α3β1)	Clathrin, microtubules	FAK, Src, PI3K, ERK, PKCζ-MEK-ERK	RhoA, Cdc42	Akula et al., 2003; Akula et al., 2002; Krishnan et al., 2006; Naranatt et al., 2003, Sharma-Walia et al., 2004; Sharma-Walia et al., 2005; Naranatt et al., 2005
HCMV (α2β1, α6β1, αVβ3)	Clathrin	FAK, Src, PI3K		Tugizov et al., 1999; Wang et al., 2005; Feire et al., 2004
FMDV (αVβ1, αVβ3, αVβ6, αVβ8, α5β1), VP1 protein	Clathrin	AKT deactivation, GSK3 dephosphorylation		Berinstein et al., 1995; Jackson et al., 2002; Jackson et al., 2000; Berryman et al., 2005; O'Donnell et al., 2005, Peng et al., 2004; Jackson et al., 2004
Yersinia pseudotuberculosis and *Y. enterocolitica* (β1 integrins, collagen)		FAK, Src, Paxillin, Arp2/3, Cas, Crk, Pyk2, PI3K, Ras, ERK1/2, PIP5K, NF-κB, p38, ILK	Rac1, Arf6, Cdc42h, Rac1, Rho	Alrutz and Isberg, 1998; Andersson et al., 1996; Alrutz et al., 2001; Bruce-Staskal et al., 2002; Weidow et al., 2000; Eitel et al., 2005; Wiedemann et al., 2001; Wong and Isberg, 2003; Grassl et al., 2003; Wang et al., 2006
Shigella flexneri (α5β1)	Clathrin	FAK, paxillin, Src, cortactin, PKC	Rho, Cdc42, Rac, Rho	Clerc and Sansonetti, 1989; Watarai et al., 1996; Watarai et al., 1997; Dehio et al., 1995; Mounier et al., 1999
Staphylococcus aureus (FN,α5β1)	Clathrin	FAK, Src, cortactin, ILK		Ellington et al., 1999; Agerer et al., 2005; Wang et al., 2006
Group A *Streptococcus spp.*	Caveolae	FAK, Src, paxillin, ILK, PI 3-K	Cdc42, Rac1, Ras	Ozeri et al., 2001; Wang et al., 2006; Rohde et al., 2003; Purushothaman et al., 2003

those induced by ligation of integrin by ECM. Notice, however, that pathogen entry is a complicated process that is also regulated by enzymes and signaling molecules secreted by the pathogen, and so one must be cautious about generalizing too far.

Moving integrins

The view that integrin traffic moves adhesion receptors from the back to the front of migrating cells is largely based on the ground-laying reviews by Bretscher (Bretscher, 1989; Bretscher, 1992; Bretscher, 1996a; Bretscher, 1996b). There are reports suggesting that αvβ3-integrin-mediated (Lawson and Maxfield, 1995) and α5β1-integrin-mediated (Pierini et al., 2000) adhesions are released at the retracting edge of crawling neutrophils and trafficked via intracellular vesicles to the leading edge, thus facilitating cell motility. In addition, there are numerous studies describing intracellular vesicular integrins (Fabbri et al., 2005; Ivaska et al., 2002b; Ivaska et al., 2005; Laukaitis et al., 2001; Li et al., 2005; Ng et al., 1999; Powelka et al., 2004; Regen and Horwitz, 1992; Roberts et al., 2001; Woods et al., 2004; Yoon et al., 2005). However, in many of these studies there is no direct evidence that these vesicles transport endocytosed receptors from the rear of the cell to the front. Instead, GFP-tagged α5-integrin-containing vesicles have been detected moving from the rear of the cell into the perinuclear region by time-lapse epifluorescence microscopy (Laukaitis et al., 2001). In addition, α2- and α5-bearing vesicles can be detected moving from the perinuclear area towards the front of the cell (Ivaska et al., 2005). Numerous highly motile vesicles bearing Rab21 (and probably β1 integrin) have also been detected close to the leading edge in freshly plated, actively spreading cells, and their number decreases significantly following prolonged adhesion (Pellinen et al., 2006). Further investigations employing total internal reflection (TIRF) microscopy demonstrated that the vesicles move rapidly from the plasma membrane into the cytosol and back.

Integrin traffic has not been studied in detail in cells migrating within 3D-collagen, but similar receptor recycling mechanism are probably employed. Primary fibroblasts migrate rather poorly inside collagen and their adhesion to 3D-collagen inactivates Akt and activates GSK3β via the activation of PP2A (Ivaska et al., 2002a). By contrast, fibrosarcoma cells that exhibit deficient PP2A function (Li et al., 2003) exhibit constitutive mesenchymal-type movement (Wolf et al., 2003) inside collagen.

Current data thus seem to support a model in which increased localized movement and recycling of integrins close to the leading edge of the cell or between the perinuclear recycling compartment and the lamellipodia are needed to support migration. Advances in microscopy techniques and fluorescent probes are likely to provide more definite answers to where trafficking integrins are endocytosed and how their exocytosis is spatially targeted to facilitate motility. It is likely that integrin trafficking differs significantly between cell types, and alterations in proteins regulating integrin traffic could well be key players in human disease. Furthermore, motile cells are known to employ diverse migration strategies during cancer invasion in tissues and 3D in vitro model systems (Friedl et al., 2004). Studies addressing integrin traffic in environments other than tissue culture plastic could provide totally new insights

into how integrin trafficking is regulated and how this contributes to cell motility. Finally, receptor tyrosine kinases have been shown to signal differently following endocytosis (e.g. Kermorgant et al., 2004), As suggested above, it is likely that in addition to the integrin signaling taking place at the cell surface, the endocytosed trafficking integrin may also signal via associated proteins also present in the vesicles. Studies investigating this possibility might provide important information on the spatial regulation of integrin signaling complexes during cell motility.

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