Focal Adhesions: A Nexus for Intracellular Signaling and Cytoskeletal Dynamics

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INTRODUCTION

Focal adhesions (FAs) are specialized sites of cell attachment to the extracellular matrix (ECM) where integrin receptors link the ECM to the actin cytoskeleton. Integrins cluster into supramolecular complexes with structural, cytoskeletal proteins like talin, vinculin, and α-actinin, as well as numerous signaling molecules, including c-Src, FAK, p130cas, and paxillin [1]. The composition and molecular architecture of FAs have been reviewed elsewhere [2–4] and are beyond the scope of this brief review. FAs serve at least two significant cellular functions: to transmit force or tension at adhesion sites to maintain strong attachments to the underlying ECM and to act as signaling centers from which numerous intracellular pathways emanate to regulate cell growth, survival, and gene expression [5, 6].

FAs are dynamic structures that assemble, disperse, and recycle (turnover) as cells migrate or enter into mitosis. Recent evidence reveals the complexity of these processes. Assembly/disassembly involves the coordinate regulation of Rho family GTPases through cross talk between integrins and numerous adhesion receptors (cadherins, cell adhesion molecules (CAMs), selectins, and syndecans), G-protein-coupled receptors (GPCRs), and receptor tyrosine kinases (RTKs), as well as the interplay between microtubules and actin. It is also apparent that FAs are themselves motile and heterogeneous in composition. Finally, turnover of FAs entails communication with components of vesicle trafficking pathways and microtubules. This review highlights recent findings relating to FA assembly, dynamics, and turnover.

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FOCAL ADHESION ASSEMBLY

Contractility

FAs have long served as a model system for the study of cell–matrix interactions. These structures are prominent in many adherent cell types grown in culture, but are rarely observed *in vivo.* Several features of the tissue culture environment promote FA assembly [1]. FAs form during spreading or migration on flat, rigid substrates to which ECM components become adsorbed. The assembly of FAs in response to adhesion to the ECM is gradual, usually occurring within 1 to 2 h after cell attachment. Initially, nascent cell–matrix adhesions, or focal complexes, form at the cell periphery as a cell spreads or at the leading edge as a cell migrates. Focal complexes mature into FAs as cells become stably attached to their substrates and tension is exerted on these sites of adhesion. Actin filaments are indirectly tethered to integrins at FAs [4, 7]. In migrating cells, FAs can provide traction on the substrate over which cells crawl, although some cells can migrate without FAs and large FAs retard motility due to excessive adhesion [8].

Additionally, cells in culture are grown under conditions that mimic a wound environment, frequently in the presence of serum factors such as lysophosphatidic acid (LPA). Normally, LPA is secreted into a wound to stimulate cell contraction, contributing to wound closure. However, in tissue culture, contraction is opposed by adhesion to a rigid substrate. This generates isometric tension between sites of strongest adhesion. In turn, the isometric tension results in alignment of bundles of actin filaments (stress fibers) and the clustering of integrins, giving rise to FAs (Fig. 1). An experimental system often utilized to study FA formation uses nonmigratory, adherent fibroblasts that have become quiescent as a result of being serum-starved. Under this condition, FAs and stress fibers are disassembled, despite contact with the underlying ECM. Quiescent
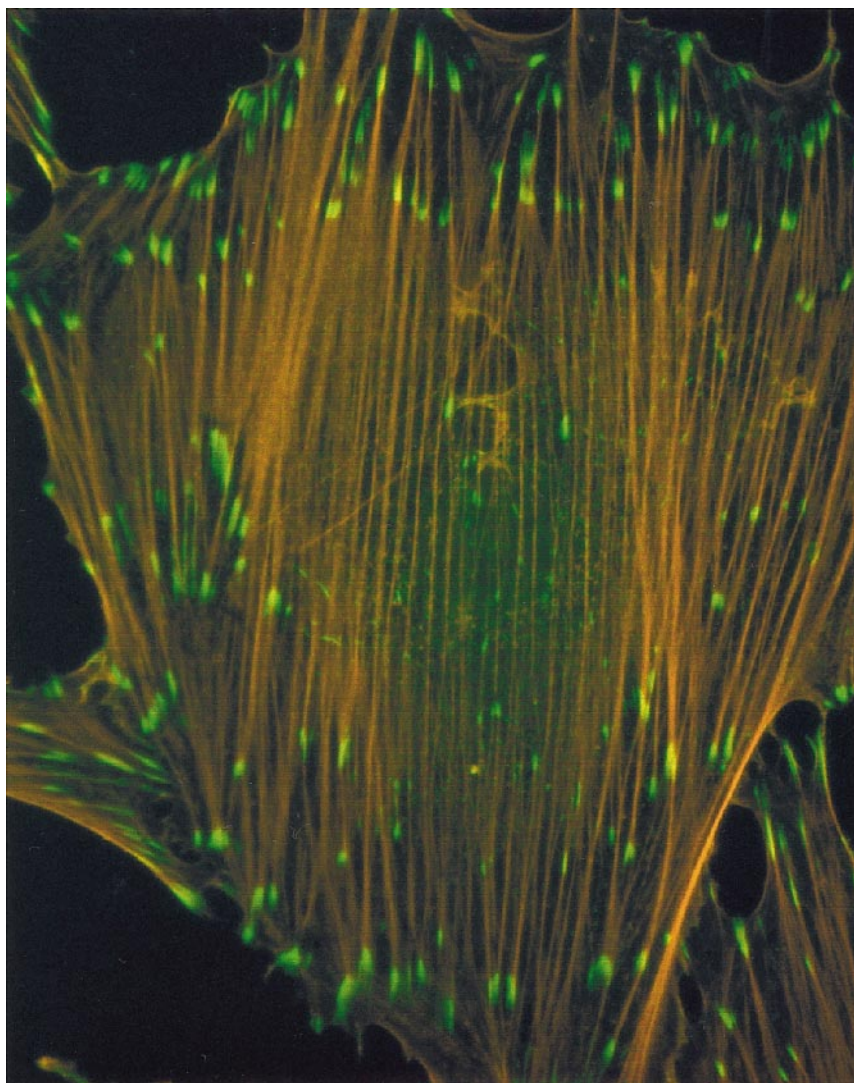
FIG. 1. Focal adhesions localize at the ends of actin stress fibers. A rat embryo fibroblast is immunostained for the focal adhesion protein vinculin (green) and for actin stress fibers (red). Strong adhesion to the substrate mediated by integrin receptors and stimulation of contractility by soluble serum factors generate isometric tension resulting in alignment and bundling of actin filaments to form stress fibers and clustering of integrins and associated proteins, like vinculin, to form focal adhesions.

fibroblasts respond rapidly to LPA stimulation, with FA assembly occurring within a few minutes [9].

The pioneering work of Ridley and Hall established the small GTP-binding protein, RhoA, as a cornerstone for FA assembly [10]. Activation of RhoA is essential for FA assembly in response to both integrin-mediated adhesion [11–13] and LPA stimulation [9]. The mechanism by which RhoA drives FA assembly has recently been elucidated. RhoA stimulates actin–myosin contractility [14] via a kinase cascade leading to the phosphorylation of the regulatory light chain of myosin II [15]. A downstream effector of RhoA, Rho kinase, can directly phosphorylate myosin light chain [16] and can also inhibit myosin phosphatase [17], both of which

result in enhanced light chain phosphorylation and hence increased contractility. Increased actin–myosin contractility results in bundling of actin filaments to generate stress fibers and clustering of integrins and associated proteins to form FAs [14, 18].

The role of actin–myosin contractility in FA assembly was initially demonstrated using pharmacological inhibitors of myosin ATPase or myosin light chain kinase [14]. Further evidence for FA assembly being regulated by contractility has come from studies with caldesmon, an endogenous inhibitor of actin–myosin interaction. Caldesmon is an actin-binding protein that inhibits myosin ATPase activity in the absence of calcium. Overexpression of caldesmon was observed to

inhibit cell contractility as evidenced by a decrease in wrinkling of silicone rubber substrates upon which cells had been cultured [19]. In parallel, there was a decrease in the size and number of FAs. Finally, caldesmon overexpression resulted in increased cell spreading and membrane extensions, also a sign of decreased tension. Caldesmon acts downstream of RhoA, since it blocked FA assembly when coexpressed with activated forms of RhoA. It will be interesting to determine the physiological conditions under which caldesmon functions to affect FA assembly.

Activation of RhoA

Progress has also been made regarding the activation of RhoA. Early work suggested that a tyrosine kinase acted upstream of RhoA and was essential for FA assembly [20]. The identity of this kinase remains elusive. It is clear, however, that many factors can regulate RhoA activity, including integrin signaling, other adhesion receptors, soluble factors like LPA, receptor tyrosine kinase signaling, and components of the microtubule cytoskeleton (Fig. 2) [10, 21]. Using an affinity precipitation assay to directly measure RhoA activity, Ren *et al.* observed that integrin-mediated adhesion leads to a biphasic response in RhoA activity [22]. Attachment to ECM initially suppresses RhoA activity and this is followed by a modest activation phase. This activation is enhanced significantly in the presence of LPA. Thus LPA is a more potent stimulator of RhoA than is integrin-mediated adhesion to the ECM. This study also showed that adhesion is required to attenuate LPA-induced RhoA activity, since in suspended cells, LPA stimulation led to sustained RhoA activity.

Microtubule depolymerization was shown many years ago to stimulate cell contractility and the assembly of stress fibers [23]. Not surprisingly, subsequent work demonstrated that microtubule depolymerization also resulted in the assembly of FAs [24–28]. Microtubule depolymerization was shown to elevate the level of myosin light chain phosphorylation [29], whereas the formation of stress fibers and FAs was blocked by the inhibitor of RhoA, *Botulinum* C3 exoenzyme [26, 28]. These findings suggested that microtubule depolymerization stimulates increased actin–myosin contractility by activating RhoA. This was confirmed by direct measurement of RhoA activity [22].

The signal transduction pathways leading to RhoA activation by LPA, integrins, RTKs, or microtubule depolymerization are intense areas of investigation. A schematic diagram of proposed pathways regulating RhoA activation is shown in Fig. 2. The immediate upstream activators of RhoA are guanine nucleotide exchange factors (GEFs), which promote the exchange of GDP for GTP, thus inducing an active conformation of RhoA (or other family members) [30, 31]. The pathway by which LPA elevates RhoA–GTP levels may be the best characterized at present. LPA binds to a heptahelical G-protein-coupled receptor and activates $G\alpha12/13$ or $G\beta\gamma$ subunits, which then associate with GEFs for RhoA. Introduction of $G\alpha12/13$ or $G\beta\gamma$ subunits into quiescent fibroblasts on their own stimulates FA assembly [32, 33]. In the case of $G\alpha12/13$ subunits, p115 RhoGEF [34] or PDZ RhoGEF [35] appear to be direct targets. It has also been suggested that the tyrosine kinase PYK2 (CAKβ, CADTK, RAFTK) may play a role in GPCR signaling to RhoA [36].

The regulation of RhoA activity by integrins is complex. Integrins can either stimulate or inhibit RhoA activity depending on the cell type, engagement of specific integrins, and time course of engagement [22, 37, 38]. This duality may reflect the role of integrin-mediated signals in promoting membrane extensions, a condition in which low RhoA activity is desirable, versus the role of integrins in establishing strong attachments across which tension is transmitted, for which higher RhoA activity is needed. As discussed above, in fibroblasts, integrin engagement initially inhibits RhoA activity but later activates it, correlating with the completion of cell spreading [22], during which time integrin-mediated activation of Rac1 is high [39]. Barry and colleagues found that addition of RGD peptides to quiescent fibroblasts stimulated FA and stress fiber assembly [11]. In adenocarcinoma cells, which are epithelial in origin, crosslinking of $\alpha6\beta4$ integrin stimulates RhoA activity, whereas crosslinking of $\beta1$ integrins inhibits RhoA [37]. Arthur and colleagues identified a pathway by which integrin engagement results initially in a decrease in RhoA activity [38]. It was found that incubation of fibroblasts with integrin ligands, such as RGD peptides, caused a rapid drop in RhoA activity, but that this did not occur in cells deficient in the Src family tyrosine kinases (Src, Fyn, and Yes). This integrin-mediated drop in RhoA activity was restored if c-Src was reexpressed in these cells. Downstream from c-Src, p190RhoGAP was identified as a target that is phosphorylated and activated, in response to integrin engagement. Interestingly, v-Src expression in fibroblasts has long been known to disrupt FAs and stress fibers (see below).

The pathway by which integrin-mediated adhesion results in long-term activation of RhoA has not been determined. It seems likely that the initial activation of p190RhoGAP must be switched off, but whether there is an additional activation of a RhoA-specific GEF remains to be elucidated. Thus far, numerous GEFs for the Rho family of GTPases have been discov-

ered [30, 31, 40, 41] but little information exists concerning how these GEFs become activated by integrin signaling. One exception is Vav1, a hematopoietic GEF for Rho family GTPases. Vav1 is tyrosine phosphorylated and activated in response to integrin engagement or clustering [42–45].

Similar to integrins, receptor tyrosine kinases transmit both positive and negative signals to RhoA. Initial stimulation (within minutes) with growth factors such as PDGF or EGF promotes the formation of membrane extensions through activation of Rac1 while suppressing RhoA [46]. RhoA is likely inhibited via Src-dependent activation of p190 RhoGAP [47]. Furthermore, recruitment of p190 RhoGAP to RTKs may be regulated by PYK2 [48]. Prolonged addition of PDGF (more than 2 h) results in FA assembly, suggesting that growth factors can activate RhoA. Recently, a GEF that may mediate growth factor-dependent regulation of RhoA has been identified. Vav2, a widely expressed relative of Vav1, is tyrosine phosphorylated and activated in response to EGF or PDGF stimulation [49–51]. Vav2 activates several Rho family members, including Rac1 and RhoA [49–53].

Finally, how might depolymerization of microtubules lead to enhanced RhoA activity? It has been suggested that intact microtubules may sequester GEFs for RhoA that are released upon microtubule depolymerization [26]. In support of this idea, several GEFs have been found to bind tubulin or microtubules. For example, in hematopoietic cells the exchange factor Vav1 binds tubulin, although whether this affects GEF activity has not been determined [54]. The association of more widely expressed Vav family members, Vav2 or Vav3, with microtubules has not been fully investigated. GEFH1, which is specific for RhoA, has recently been shown to bind microtubules [55]. The existence of multiple GEFs for RhoA reflects either a functional redundancy or that specific GEFs act on RhoA in response to distinct stimuli.

Although integrins and GPCRs are a major focus in upstream regulation of RhoA, other adhesion receptors also promote FA assembly, likely via the activation of RhoA. Recently, a role for syndecans in FA assembly has been demonstrated. Syndecan-4 is a transmembrane member of the heparan sulfate proteoglycan family that localizes in FAs [56]. Its overexpression in cultured fibroblasts increases FAs and stress fibers [57, 58], whereas syndecan-4 null cells have impaired FAs [59]. Additionally, FA assembly on the cell binding domain of fibronectin is promoted by antibody ligation of syndecan-4 in a RhoA-dependent manner [60]. A particularly intriguing system in which multiple adhesion receptors, including selectins, integrins, and other CAMs, cooperate to potentially regulate RhoA is the interaction of leukocytes with endothelial cells during inflammation. Adhesion of monocytes to endothelial cells induces the assembly of stress fibers and presumably FAs in the endothelial cells. Crosslinking of the endothelial cell adhesion molecules VCAM-1, ICAM-1, or E-selectin, but not ICAM-2 or ICAM-3, also stimulates stress fiber formation [61]. It will be important to determine whether these effects are due to increased RhoA activity. In contrast to the above-mentioned cell adhesion molecules, the crosslinking of which promotes FA assembly, the formation of adherens junctions of the cadherin type tends to inhibit FA assembly. The mechanism of this inhibition has not been determined. Recent work, however, has identified a cadherin-binding protein, p120 catenin, as a regulator of Rho family GTPases [62, 63]. Overexpression of p120 catenin disrupts focal adhesions and stress fibers and decreases RhoA activity in cells. In addition, p120 catenin elevates the activity of Rac1 and Cdc42 and binds to Vav2, a Rho family GEF [62].

FOCAL ADHESION DISASSEMBLY

FAs disassemble or disperse under a number of physiological situations. For example, adhesions to the ECM are released at the rear of a migrating cell and this is accompanied by a disruption of FAs. Another instance of FA disassembly occurs during mitosis, during which cells lose their attachments to the ECM and adopt a round morphology. Finally, in oncogenically transformed fibroblasts, FA integrity is often compromised [64]. Since FA assembly involves both the activation of RhoA and the stimulation of contractility, loss of FAs would ostensibly involve mechanisms that counteract these pathways. The role of integrin signaling in FA disassembly has recently been reviewed [65]. Here we highlight some novel and significant observations.

GAPs

GTPase-activating proteins, or GAPs, which promote hydrolysis of GTP to GDP, are immediate upstream inhibitors of RhoA. Indeed, introduction of several GAPs into cultured cells leads to a loss of FAs and actin stress fibers and also causes cell rounding. As discussed above, p190RhoGAP suppresses RhoA activity in response to integrin ligation [38] as well as growth factor stimulation [47]. Other GAPs for RhoA which may participate in FA disassembly have also been identified, including GRAF, or GAP for Rho associated with FAK [66], and p122GAP, which blocks LPA-induced FA and stress fiber assembly [67]. Interestingly, a novel RhoGAP that acts on RhoA in mitotic cells has recently been described [68].

Interplay of Rho and Ras Family GTPases

Our understanding of FA disassembly is further complicated by recent evidence that additional Rho family GTPases can affect FA organization. It has long been noted phenomenologically that the actions of Rac and RhoA are functionally antagonistic. Rac1 promotes membrane extension, whereas in many situations RhoA induces membrane retraction. Such a reciprocal relationship is most clearly seen in migrating fibroblasts or in growth cones of neurons. In epithelial cells, activation of Rac promotes the assembly of cell–cell junctions and blocks FA formation, while activation of RhoA promotes a fibroblastic phenotype [46, 69–71]. Rottner *et al.* have recently shown that expression of a dominant negative Rac1 mutant in quiescent fibroblasts induces FA assembly [72]. This effect could be attributed to regulation of contractility by Rac1 effectors downstream of RhoA [73]. However, using affinity precipitation assays to measure RhoA activity, Sander *et al.* have shown that activation of Rac1 can itself suppress RhoA activity [46]. This was shown either by expression of TIAM-1, an exchange factor for Rac1, or by expression of activated mutants of Rac1 in NIH3T3 fibroblasts. Cdc42 expression also antagonized RhoA activity. The mechanism by which Rac1 (or Cdc42) inhibits RhoA is currently unknown. A signaling complex that links Rac1 effectors to GAPs for RhoA would be one potential mechanism.

A newly identified Rho family member, RhoE/rnd, also disrupts FAs and stress fibers [74, 75]. RhoE/rnd was identified as a binding partner for p190RhoGAP [76]. Unlike RhoA, which cycles between GDP- and GTP-bound states, RhoE is constitutively active in the GTP-bound state and is insensitive to GAPs. It is unclear how RhoE functions. One possibility is that it titrates away downstream effectors from RhoA. It is also possible that RhoE can inhibit RhoA activity, although this has not been directly demonstrated. The function of RhoE within cells remains undetermined.

Finally, the Ras pathway is suggested to play a role in FA turnover. Active Ras is required for the turnover of FAs during cell migration [77]. Interestingly, the duration of Ras activation affects the activation state of RhoA. Transient expression of activated Ras in epithelial cells results in activation of Rac1 and inhibition of RhoA activity [71]. In contrast, sustained Ras activation promotes enhanced RhoA activity [71] as well as a fibroblastic morphology [70, 71]. Knockout cell lines lacking p120 RasGAP, an upstream inhibitor of Ras, are unable to adopt a polarized morphology [78] or turn over FAs. This effect of p120 RasGAP is independent of Ras regulation. Instead, p120 RasGAP regulates cell polarity, and presumably FA turnover, via its interaction with p190 RhoGAP.

Tyrosine Phosphorylation

As mentioned earlier, signaling from a tyrosine kinase is necessary for FA assembly. It was thought that Src family kinases or perhaps FAK may be required for FAs to form. However, through the analysis of knockout cell lines, it is apparent that Src family kinases play a role in FA disassembly or turnover [80]. As discussed above, c-Src is required for inhibition of RhoA by integrins [38]. Furthermore, overexpression of v-Src leads to FA disruption, while the expression of kinase-inactive v-Src in normal cells leads to formation of exaggerated FAs [81]. FAK is also likely to play a role in FA turnover or disassembly. FAK-null cells possess abnormally large FAs and are unable to migrate [82]. In contrast, overexpression of FAK stimulates motility [83]. Consistent with this finding is the observation that in permeabilized fibroblasts, an increase in tyrosine phosphorylation accompanies FA disruption in response to ATP [79].

Significant progress has also been made in identifying protein tyrosine phosphatases that may promote FA disassembly or turnover. Knockout cell lines of either SHP-2 or PTP-PEST exhibit enhanced FAs [84, 85]. The mechanism of this phenotype remains to be determined. However, one or more PTPases may act upstream of RhoA. Using calpeptin, an inhibitor originally designed for the Ca^{2+}-dependent protease calpain, it was recently demonstrated that this inhibitor stimulates FA assembly in quiescent fibroblasts through inhibition of a PTPase [86]. Calpeptin-induced FA assembly was blocked by C3 exoenzyme, indicating that the PTPase acts upstream of RhoA. In addition two transmembrane PTPases, LAR and RPTPα, have been shown to localize in FAs under restricted conditions [87, 88]. Finally, PTPases may play a major role in regulation of FA disassembly during mitosis. Comparison of interphase versus mitotic cells shows that FAK, p130cas, and paxillin are dephosphorylated on tyrosine residues but phosphorylated on serine and threonine in mitosis [89]. These proteins are rapidly tyrosine phosphorylated in response to adhesion [3, 5, 18]. In mitotic extracts, FAK kinase activity is decreased and its associations with p130cas and paxillin are disrupted. The disruption of this complex is thought to prevent integrin signaling until the completion of cytokinesis. It will be interesting to determine which tyrosine phosphatases and which serine–threonine kinases act on these proteins in mitosis.

HETEROGENEITY OF CELL–MATRIX ADHESIONS

The molecular architecture of FAs and the nature of specific protein interactions have been reviewed elsewhere [1–4] and will not be dealt with in detail here. It
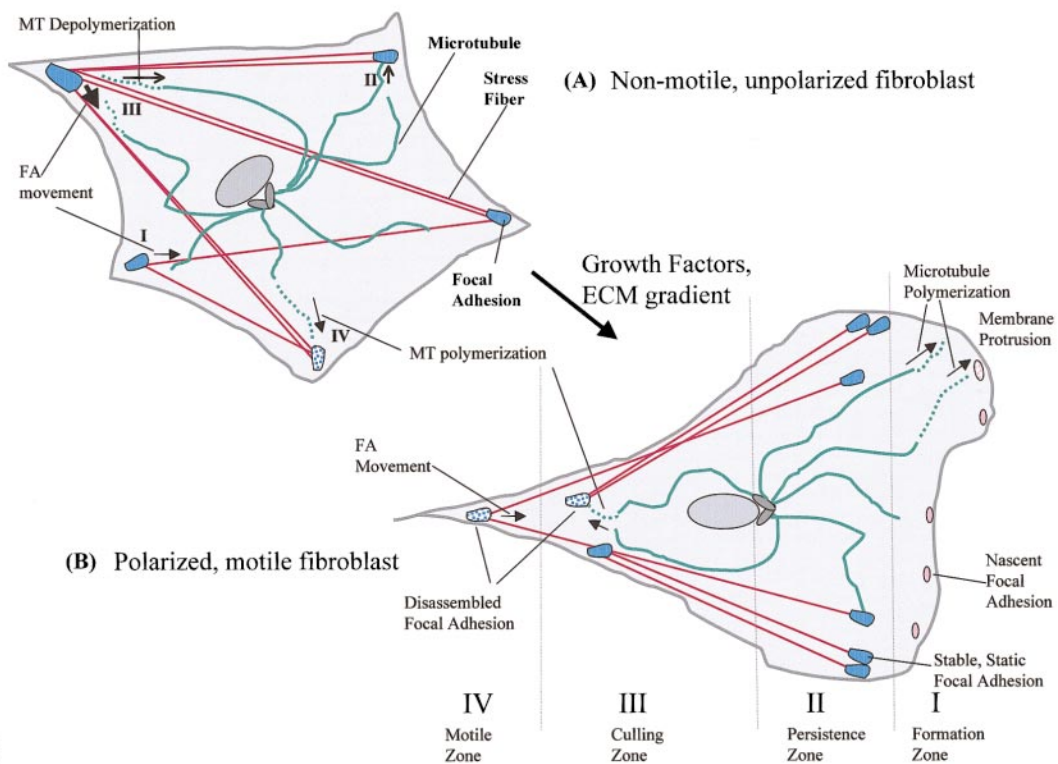
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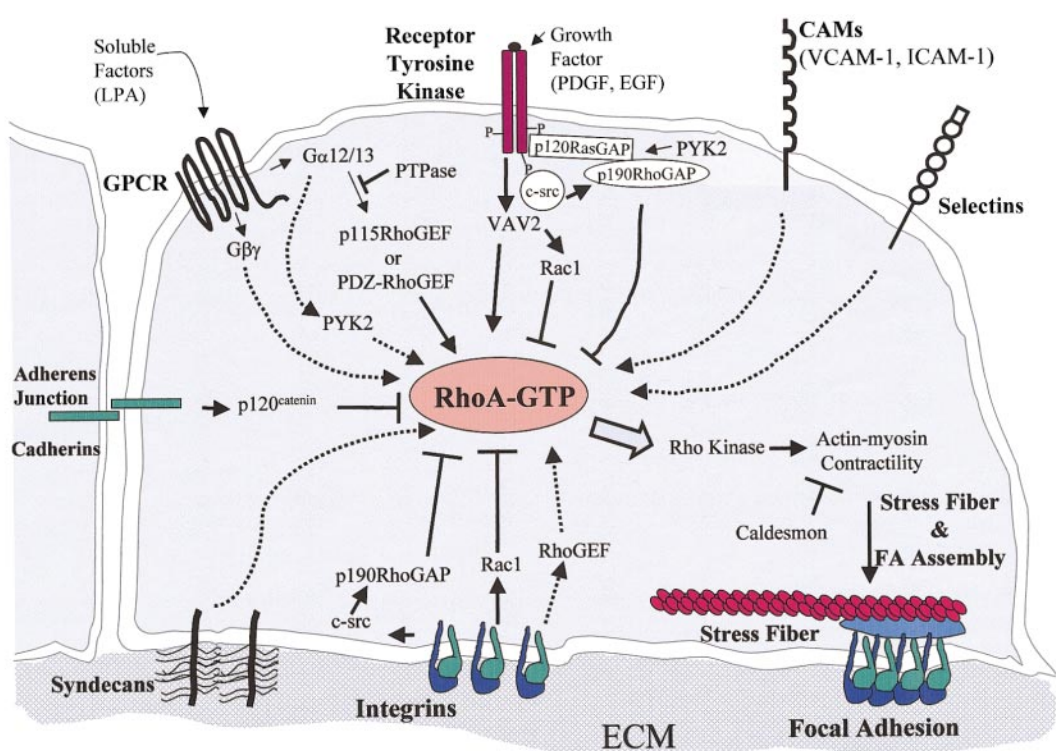
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FIG. 2. Multiple signaling pathways control focal adhesion assembly by coordinately regulating the activation of the small GTP-binding protein RhoA. Soluble serum factors, like LPA, signal via G-protein-coupled receptors (GPCRs) to activate guanine nucleotide exchange factors (GEFs) for RhoA. Growth factors such as PDGF or EGF, acting through receptor tyrosine kinases, transmit both stimulatory and inhibitory signals to RhoA. c-Src-dependent activation of p190 RhoGAP inhibits RhoA. Vav2 is a growth factor-stimulated GEF for Rac1 and RhoA. RhoA activity is also controlled by cell adhesion. Crosslinking of cell adhesion receptors like VCAM-1, ICAM-1, E-selectin, or

is noteworthy, however, that recent observations point to considerable molecular and structural diversity among FAs in a single cell and also within individual FAs. Using fluorescence ratio imaging, the distribution of several FA proteins was compared. This analysis has identified at least three structurally distinct types of adhesion sites whose molecular compositions differ. Classical FAs are large, spearheaded or ellipsoid in shape, and located at the cell periphery and contain vinculin, paxillin, phosphotyrosine, and $\alpha v\beta 3$ integrins [90]. In contrast, fibrillar adhesions are elongated, centrally located, and contain tensin, $\alpha 5\beta 1$ integrins, and fibronectin with little or no phosphotyrosine, vinculin, or paxillin. Finally, "mosaic" FAs are morphologically similar to "classical" FAs, but their content is variable. Interestingly, the assembly of these distinct adhesions depends on several critical factors. The first is contractility. The assembly of classical FAs but not fibrillar adhesions was sensitive to contractility inhibitors [90]. The second is the physical state of the ECM. A nonimmobilized fibronectin matrix that is adsorbed to the substrate promotes fibrillar adhesions, while an immobilized matrix, crosslinked to the substrate, leads to the formation of classical FAs [91]. The third factor is the type of integrin involved. Classical FAs typically contain $\alpha v\beta 3$ integrins and fibrillar adhesions contain $\alpha 5\beta 1$ [90]. Using GFP-tagged c-Src, Felsenfeld and coworkers noted that c-Src selectively localizes in phosphotyrosine-rich FAs formed by $\alpha v\beta 3$ integrins, but not those formed by $\alpha 5\beta 1$ integrins [92]. This type of analysis has been performed only on some FA components. A more comprehensive survey will be informative and may reveal additional complexities.

TURNOVER AND DYNAMICS OF FOCAL ADHESIONS

Up to this point, much of this review has dealt with potential pathways leading to FA formation or disassembly. These are active, dynamic processes intimately associated with turnover or recycling of FAs. Information from knockout cell lines and biochemical screening has led to the identification of potential regulators of FA turnover. Additionally, the use of elegant imaging techniques to visualize cell–substrate contact dynamics in live cells has contributed to our knowledge of how FAs undergo remodeling and turnover during cell spreading and motility.

Movement of Focal Adhesions

FAs that form at the front of a migrating cell generally remain fixed relative to the substrate as the cell moves over them. FAs then disperse at the cell tail [93, 94]. Stationary FAs maintain stable attachments to the ECM to resist actin–myosin contraction that propels the cell forward. However, in some situations FAs move relative to the substrate. A recent study found that static FAs occur primarily in motile cells. Using GFP-tagged $\beta 1$ integrin subunits, Smilenov *et al.* compared FA movement in stationary (nonmotile) and migrating fibroblasts [95]. Using time-lapse imaging of live cells and overlaying of sequential images, they found that FAs in nonmotile cells are not static. Instead, FAs in stationary cells were observed to move linearly toward the cell center. This movement depended on actin–myosin contractility since FA movement was not observed in the presence of BDM, a myosin inhibitor. Microtubule disassembly, which en-

syndecan-4 stimulates focal adhesion and stress fiber assembly likely through activation of RhoA (dotted arrows). The formation of adherens junctions, containing cadherins, antagonizes focal adhesion and stress fiber formation, possibly via p120 catenin, whose overexpression inhibits RhoA activity. Finally, integrins transmit both positive and negative signals to RhoA. Initially, engagement of integrins with ECM inhibits RhoA. Integrins activate p190 RhoGAP through a c-Src-dependent mechanism. Integrins also activate Rac1 as a cell spreads, which can antagonize RhoA activity. As stable adhesions form, integrins activate RhoA, most likely through an integrin-dependent Rho GEF. Downstream of RhoA, actin–myosin contractility stimulates actin stress fiber formation and clustering of integrins and associated proteins to form focal adhesions. Actin–myosin contractility is positively regulated by RhoA effectors, like Rho kinase, and negatively regulated by caldesmon, an actin-binding protein.

FIG. 3. Focal adhesion (FA) dynamics and turnover in nonmotile and motile fibroblasts is partly controlled by cross talk between adhesion sites and the microtubule cytoskeleton. (A) A nonmotile, unpolarized fibroblast. (I) FA movement, relative to the substrate, toward the cell center depends on actin–myosin contractility. Stress fibers under isometric tension contract, pulling FAs centripetally inward. (II) Growing microtubules target FAs. FAs capture and stabilize microtubules by capping microtubule ends. (III) Microtubule depolymerization enhances FA assembly through activation of RhoA. FA movement toward the cell center is enhanced due to increased actin–myosin contractility. (IV) Active assembly of microtubules induces FA disassembly and turnover. Microtubules deliver local "relaxation" signals to FAs; presumably these factors regulate RhoA activity or contractility downstream of RhoA. (B) In a polarized, motile fibroblast, FA movement is generally not observed but FAs have distinct behaviors in different regions of the cell. (I) Formation zone: nascent FA (focal complexes) form at the leading edge. Microtubule polymerization activates Rac1 and stimulates membrane protrusions. Growing microtubules target selected FAs at the leading edge and induce their disassembly. (II) Persistence zone: a fraction of nascent FAs matures into stable, static FAs in which contractile stress fibers exert tension on the substrate. Capture and capping of microtubules by FAs leads to increased FA stability. (III) Culling zone: growth of microtubules toward FAs at the cell rear promotes FA disassembly and turnover. (IV) Motile zone: contractility during tail retraction may cause some FAs at the tail to move relative to the substrate toward the cell center. Ultimately, these posterior FAs release from the substrate and disassemble.

hances RhoA activity [22] and contractility [23, 29], increased the rate of FA movement. Interestingly, although FA movement was typically not observed in migrating cells, distinct zones of FA behavior were discerned. At the leading edge of a migrating cell is a formation zone; between the leading edge and the nucleus, a persistence zone exists in which stable FAs continue to grow and mature; between the nucleus and the tail a culling zone exists, where FAs turn over; at the cell tail is a small motile zone [95]. The different behaviors of FAs in both nonmigratory fibroblasts and polarized, migrating fibroblasts are depicted in Fig. 3. These findings have led to a molecular clutch model in which FAs transition between a motile and a nonmotile state in a contractility-dependent manner, to balance adhesive forces and migratory cues. How universal this behavior of FAs is remains to be determined.

Cross Talk between Microtubules and Focal Adhesions

The state of the microtubule cytoskeleton can greatly influence the organization of FAs and actin stress fibers. An emerging view is that the relationship between microtubules and FAs is reciprocal, in which the organization of one affects the dynamics of the other (Fig. 3). As mentioned earlier, disruption of microtubules activates RhoA, leading to increased actin–myosin contractility, FAs, and stress fibers [23–29]. Conversely, elevated RhoA activity stabilizes microtubules [96]. In addition, Waterman-Storer and co-workers found that active microtubule polymerization was associated with increased Rac1 activity and membrane protrusions [97]. Given the antagonism between Rac1 and RhoA, these findings suggest that sites of microtubule growth would be associated with locally high concentrations of active Rac1 and decreased RhoA activity. Consequently, microtubule growth would be expected to promote focal adhesion disassembly. Indeed, Small and co-workers found that microtubule polymerization is associated with local destabilization of focal adhesions [98]. Small and his colleagues noted earlier that there is an association between the ends of microtubules and focal adhesions [98–100] and they speculated that this association might stabilize the adhesions. More recent work from this group, however, has shown that this association is antagonistic: the targeting of microtubules to focal adhesions causes the disassembly of these structures [101]. These investigators propose that growing microtubules negatively regulate focal adhesions by delivering a localized relaxing signal to this region. The evidence points to microtubule dynamics regulating the activity of Rho family GTPases in very localized regions of the cytoplasm, but proof of this awaits assays that will reveal the activity of these Rho family proteins at a subcellular level.

Delivery of Components to Focal Adhesions

An emerging concept is that some focal adhesion proteins are actively targeted to and from focal adhesions via a vesicle trafficking pathway. This also likely involves microtubules and may be another way in which microtubule behavior affects focal adhesion turnover. Several studies have implicated the ARF (ADP-ribosylation factor) family of GTPases in this targeting. ARFs have been shown to control intracellular membrane trafficking, including the delivery of membrane to sites of membrane protrusion [102]. They have also been shown to have a role in regulating cytoskeletal organization through an interplay with Rho family GTPases [103, 104]. ARF1 activity has been shown to be required for the recruitment of paxillin from the perinuclear compartment to focal adhesions [103]. Like Rho family GTPases, ARFs cycle between an inactive GDP-bound state and an active GTP-bound state. The mechanism by which paxillin is recruited to focal adhesions may lie in its interactions with ARF-GAPs. Paxillin has been shown to associate with a 95-kDa ARF-GAP, variously named PKL [105], PAG3/PAPα [106], or GIT [107]. This ARF-GAP acts on ARF6 [106] and is localized to focal adhesions [105]. Kondo *et al.* found that the activity of this ARF-GAP prevents paxillin recruitment to focal adhesions [106]. Another protein that is recruited to focal adhesions from the perinuclear regions is v-Src [108]. However, recruitment of v-Src to FAs does not require its kinase activity [81]. Whether this recruitment involves ARF activity has not yet been determined. An intriguing connection between ARFs and Src is suggested by the finding that ASAP-1, another ARF-GAP that localizes to FAs [109], is phosphorylated by Src family kinases [110]. Finally, some integrins associate with ARF-GEFs. For example, $\beta2$ integrin cytoplasmic domains associate with cytohesin-1, an ARF-GEF, and this interaction is implicated in the regulation of integrin affinity [111, 112]. The roles of ARFs and their regulatory GAPs and GEFs in focal adhesion turnover are only just beginning to be discovered. This promises to be an exciting area in the future.

CONCLUDING REMARKS

In this brief review of focal adhesions, we have tried to emphasize some of the recent developments. We anticipate that a number of emerging areas will continue to be developed in the next few years. The idea that FAs are heterogeneous bears further investigation. The relative temporal and spatial distributions of

many components have yet to be compared. The role of ARFs, along with their regulatory GEFs and GAPs, is currently enigmatic. The evidence for cross talk between ARFs and Rho family proteins is particularly intriguing. A great many factors influence the activity of Rho family proteins. In general, the effects of agents that stimulate or inhibit RhoA activity have been measured on whole populations of cells. In many situations, however, very localized, subcellular changes in RhoA activity are likely to be important. This is suggested by the studies on microtubule dynamics. The evidence indicates that the behavior of individual microtubules regulates the local activity of RhoA or Rac1, thereby affecting FA assembly and turnover in specific regions of the cell. The development of methods to determine subcellular changes in activity of Rho family proteins should yield a greater understanding of how various factors control FA dynamics.

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