Review

Integrating an integrin: a direct route to actin

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Abstract

Integrins were so named for their ability to link the extracellular and intracellular skeletons. Now almost 20 years into integrin research, numerous questions remain as to how this interaction is accomplished and how it is modified to achieve a desired phenotype. As the cell adhesion and actin assembly fields are merging in combined approaches, novel actin assembly mechanisms are being uncovered. Some of the earliest identified cytoplasmic linker molecules, believed to mediate integrin-actin binding, are once again the subject of scrutiny as potential dynamic mediators of cell anchorage. It seems plausible that each unique cellular morphology occurs as the result of activation of distinct actin assembly systems that are either stabilized by unique bundling and linker proteins or modified for progression to a new phenotype.

While this research initiative is likely to continue rapidly in a forward fashion, it remains to be clarified how integrins assemble the most stable and basic cytoskeletal phenotype, the adherent cell with prominent stress fibers. Recent investigations point towards a shift in the current model of anchoring at the cell periphery by providing both mechanisms and evidence for de novo actin assembly orchestrated by the adhesion site. Lacking a complete pathway from integrin ligation to an integrated extracellular–intracellular skeleton in any single system, this review proposes a simple model of integrin-mediated stress fiber integration by drawing from work in multiple systems.

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1. Introduction

The principal means of cell attachment to substrate utilizes receptors of the integrin family [1]. While extensive study of cell adhesion has uncovered a multitude of variables involved in this process, their integration into a complete mechanism remains to be determined [2]. The basic steps toward cell adhesion can be visualized in most experimental systems; cell encounter with substrate, integrin ligation, cell spreading, formation of adhesive contacts, and generation of actin stress fibers. These basic events do not substantively differ between cell types or with the identity of the integrin–ligand pairing. However, the variables within each system and the degree to which they modulate adhesion differ greatly. The inability to simultaneously factor all variables within a single system has restricted the evolution of a consensus working hypothesis of cell adhesion.

It has been recognized for some time that cellular morphology reflects the current cellular task. Migrating cells adopt an elongated phenotype. Cells in the presence of chemoattractants exhibit filopodia. Mitotic cells contract. These morphologies have been characterized by the temporal dominance of particular Rho GTPases [3,4]. For example, activation of cdc42 results in the appearance of filopodia while activation of RhoA induces robust stress fibers and cell spreading. In many systems, adhering cells naturally progress through several phenotypes; for example filopodia, lamellipodia, and cell spreading, controlled by the GTPases cdc42, Rac, and Rho, respectively. With recognition of these phenotypes, a new approach to studying cell adhesion was begun [5]. Expression of active or dominant inactive forms of these GTPases arrested cells in one of the stages of adhesion. This permitted biochemical characterization of system variables at each of these stages. However, a potential complication results from this approach that limits interpretation. It is recognized that the nature of the actin cytoskeleton largely defines the cell morphology, and we now know that the activation status of the Rho GTPase family members defines the cytoskeletal phenotype. Recent evidence suggests that control of these GTPases as well as their cytoskeletal effector molecules lies within the adhesion site [6,7]. Thus, exoge-
nous control of GTPase activity experimentally is driving the mechanistic pathway in reverse. In current models of cell adhesion, where actin is assembled independent of the adhesion site, the assumption is made that the adhesion site will accommodate all forms of actin filament structure, as dictated by the prevailing GTPase \[8\]. It seems unlikely that GTPase-driven actin assembly occurs independent of the adhesion site as depicted in many current models. If, as recent data suggest, the assembly of actin emanates from the adhesion site, then it is likely that different forms of actin are assembled and utilized by the adhesion site as the progression of the integrin adhesion responds to environmental cues \[9\]. If so, driving this system in reverse will not accommodate all forms of actin filament structure, as suggested in many current models. If, as recent observations suggest that Arp2/3 may participate in reinforcement of adhesion sites. First, initial study of Arp2/3 nucleated actin demonstrated a unique branched morphology to the resultant actin fibers \[13\]. This branching was visually similar in actin polymerized by purified Arp2/3 and that seen at the periphery of cellular cytoskeletons \[14\]. As a result of these studies, Arp2/3-polymerized actin was interpreted as capable of broad membrane deformation seen in structures such as lamellipodia. It seemed unlikely that Arp2/3 could produce the thickened actin filaments known as stress fibers in the adherent cell. Recently, it was demonstrated that deletion of actin capping protein could alter the phenotype of Arp2/3-generated actin fibers, producing more linear, non-branched structures \[15\]. This finding permits easier integration of Arp2/3 into a model of cell adhesion wherein progressive recruitment of modifying variables, such as capping proteins, could modify the actin structures produced sufficiently enough to account for the varying cellular phenotypes seen during the process of cell adhesion.

It is unknown how polymerizing actin filaments actually exert force on the cell membrane. In bacteria, proteins such as Act A serve as actin anchors at the membrane and transmit force from growing actin fibers to the membrane \[16\]. In mammalian cells it is thought that VASP family proteins might serve this function, although the membrane component with which they interact is unknown \[17,18\]. The actual force of actin-dependent membrane protrusion may come from ratcheting of the actin fiber as new monomers are inserted \[19\]. In this model, Arp2/3 activation leads to binding of the Arp2/3 complex on the side of a preexisting filament—leading to nucleation of a new filament at a characteristic 70° angle to the parent filament \[14\]. Logistically problematic with this model, the adhesion site must generate signals to initiate actin assembly, transmit these signals to the cytosol and then receive polymerized actin fibers to support adhesion. Importantly, this model has not yet provided clues to the anchoring of actin filaments at the membrane.

Several laboratories have now reported localization of Arp2/3 to adhesion sites during integrin-mediated cell adhesion \[9,20,64\]. For some time it has been recognized that podosomes, small adhesion structures found in leukocytes and transformed fibroblasts, contain a core of actin surrounded by integrins and integrin-associated proteins.

3. New findings demand integration of fields

The Arp2/3 protein complex was among the earliest functional units described with inherent actin nucleation capabilities (see Ref. \[12\] for review). Extensive and thorough study in model systems has described not only its basic function, but also its activation and some regulatory events, primarily from the viewpoint of its role in motility. Recent observations suggest that Arp2/3 may participate in reinforcement of adhesion sites. First, initial study of Arp2/3 nucleated actin demonstrated a unique branched morphology to the resultant actin fibers \[13\]. This branching was visually similar in actin polymerized by purified Arp2/3 and that seen at the periphery of cellular cytoskeletons \[14\]. As a result of these studies, Arp2/3-polymerized actin was interpreted as capable of broad membrane deformation seen in structures such as lamellipodia. It seemed unlikely that Arp2/3 could produce the thickened actin filaments known as stress fibers in the adherent cell. Recently, it was demonstrated that deletion of actin capping protein could alter the phenotype of Arp2/3-generated actin fibers, producing more linear, non-branched structures \[15\]. This finding permits easier integration of Arp2/3 into a model of cell adhesion wherein progressive recruitment of modifying variables, such as capping proteins, could modify the actin structures produced sufficiently enough to account for the varying cellular phenotypes seen during the process of cell adhesion.

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Arp2/3 has also been localized to the podosome core. In WASp-deficient macrophages, loss of polarization and migration has been attributed to a failure of Arp2/3 to localize to the podosome core. Disruption of WASp signaling can disrupt Arp2/3 localization as well. As WASp-deficient macrophages exhibit chemotactic, but not chemokinetic deficiencies, it implies that localization of actin nucleation machinery to the adhesion site is important for directed cell progression toward an adhesive phenotype [20]. In a separate system, De Mali et al. [9] demonstrated a vinculin-dependent recruitment of Arp2/3 that is required for deformation of the cell membrane at the leading edge. If accepted that cell spreading increases the number of contacts, i.e. avidity, this finding suggests that adhesion-site-based actin nucleation is important for the early steps in cell adhesion as well as for cell migration. In both of these reports, Arp2/3 localization was involved in membrane extension at the leading edge of cells. As cell phenotype progression was not monitored until stress fibers were achieved, it is not apparent what role Arp2/3 plays in this event. In our own studies, Arp2/3 localizes to macrophage podosomes early in the adhesion process. This localization precedes the formation of stress fibers and, in accordance with data from WASp-deficient cells, occurs during a period of cdc42 dominance within the cell [64]. In a model system of αvβ3 podosomes, Arp2/3 localization to the podosome is dependent upon β3 tyrosine phosphorylation as well as recruitment and activation of Pyk2 [64]. In these examples, localization of Arp2/3 to adhesion sites was observed when cells were exhibiting what is commonly considered a Rac dominant or cdc42 dominant phenotype. Certainly, modification of actin fibers post-formation through bundling proteins, or modification during assembly by capping protein, may account for changes in the actin cytoskeletal phenotype seen in the spread, firmly adherent cell. However, investigation of other actin nucleation-promoting proteins suggests that individual actin phenotypes may result from distinct mechanisms.

Formins are conserved, multidomain proteins involved in dynamic rearrangement of the actin cytoskeleton (see Ref. [22] for review). Only recently has it been recognized that formins are capable of nucleating actin directly [23,24]. Initially studied in model systems such as yeast, flies, and worms, more than a half-dozen human isoforms have now been identified. Understanding of formin activation, regulation, and function lags behind that of the Arp2/3 complex; however, several consensus themes have emerged, particularly among the diaphanous related formins. Two such themes are of particular interest to cell adhesion biologists. First, many formins contain a GTPase-binding domain that, when occupied, causes autoinhibition of formin activity [22]. Several formins were identified in screens for GTPase binding partners [25,26]. While a complete profile of formin-GTPase interactions is unavailable, at least 10 such interactions have been described, including those for Rho and Rac. A second theme emerging from formin study is their production of a phenotypically unique actin filament. In contrast to the highly branched actin produced by purified Arp2/3, formins produce a longer and more linear actin filament [23]. While the significance of these actin structures remains to be determined, their similarity to stress fibers in adherent cells suggests that different actin nucleating proteins may be responsible for the unique actin structures seen in the progression of cell adhesion phenotypes. Early studies of formins in mammalian cells are highly suggestive of a role for formins in the production of actin filaments anchored in adhesive structures [65,66]. In adherens junctions, formin localization is dependent upon alpha-catenin [65]. Disruption of the catenin–formin association limited the assembly of linear radial actin fibers typical of adherens junctions and disturbed intercellular adhesions. This is consistent with earlier reports demonstrating incorporation of monomeric actin at adherens junctions [66].

Regulation of small GTPases via integrin signaling has been observed for some time and several mechanisms have been proposed including transactivation of growth factor receptors [27], aggregation of membrane domain-anchored signaling molecules [28], and recruitment and activation of cytosolic scaffold and signaling proteins to the integrin tail [7]. As a result of the diversity of cell adhesion systems and experimental environments in which integrin-mediated GTPase activation has been studied, there is no clear consensus linking specific integrin–ligand combinations to certain GTPases. However, there is general agreement on the phenotype cells exhibit when under control of specific GTPases and that cells progress through several of these phenotypes in the process of adhesion. Progression through phenotypic stages likely accounts for apparent discrepancies wherein a particular integrin reportedly increases or decreases the activity of a given GTPase in different experimental conditions. For example, both activation and suppression of Rho by αvβ3 have been recently reported [6,7]. Despite limited information, the observations that GTPase activity is regulated by integrins and GTPases physically associate with the adhesion complex are supportive of an adhesion-site-based model of actin polymerization.

By supplying the requisite signaling and physical translocation mechanisms to co-localize actin polymerization machinery, the integrin adhesion site could serve as a focus for actin nucleation. A model where orchestration of the actin cytoskeleton resides within the adhesion site provides a logically sound mechanism for the protrusion of cell membranes in migrating and spreading cells, the production of stress fibers in the anchored cell, and other cytoskeletal phenotypes occurring in the progression toward cell adhesion.

4. A proposed linear model of anchorage

As a paradigm for integrin activation, the β3 integrin family has been studied extensively in multiple cell types

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and has provided many advances to our knowledge of adhesion [29,30]. Not coincidentally, it has also been an early focus for the study of adhesion-dependent control of actin cytoskeletal assembly [31]. For the convenient incorporation of these fields, we will use $\beta_3$ integrins as an example in the following proposed model.

As shown in Fig. 1, integrins exist in the membrane associated with other molecules, including integral and transmembrane proteins and lipids [1,2]. Other proteins associate either directly with the integrin cytoplasmic tails or with complexes that anchor the integrin to cortical cytoskeletal elements. Upon binding Arg-Gly-Asp ligand, $\beta_3$ integrins undergo two distinctive biochemical modifications; the order and co-dependence of which appear independent. First, the integrin undergoes a change in conformation, resulting in a higher affinity for ligand as well as an increase in projection from the lipid bilayer [32–35]. The conformational change and subsequent change in affinity for ligands are subject to regulation by integrin-associated molecules [36,37]. Second, the $\beta_3$ integrins become phosphorylated upon tyrosines in the cytoplasmic tail [38]. In platelets and macrophages, this phosphorylation is required for firm adhesion; however, genetic preclusion of phosphorylation does not affect normal development [39,40].

The conformational change and subsequent increase in affinity as well as the cytoplasmic tyrosine phosphorylation appear to be the requisite events for the assembly of a $\beta_3$ adhesion complex capable of supporting cell adhesion. The increased affinity, perhaps in combination with src activity, affects the mobility of integrins within the membrane and may regulate the formation of receptor clusters [41,42]. Tyrosine phosphorylation, together with the conformational change, results in the recruitment of proteins to the cytoplasmic tail [7]. Phosphorylation of the remarkably conserved NPXY domains of integrin cytoplasmic tails has been implicated in regulating protein association with $\beta_3$, $\beta_1$ and $\beta_7$ integrins. As this motif is also present in $\beta_5$ integrins, it is likely that phosphotyrosine-binding domain interaction with integrin cytoplasmic tails is a conserved activation mechanism among these integrin families [67]. The protein complex sequestered as a result of these two biochemical modifications appears to contain all the necessary components for actin nucleation from the clustered integrins [2,7,20,31].

A minimal complement of integrin-associated proteins sufficient to orchestrate actin assembly could be comprised of a nucleator, a GTPase to activate the nucleator, a guanine exchange factor (GEF) to activate the GTPase, an anchor for the nascent actin filament and some means to attach this complex to the integrin cytoplasmic face. Cell adhesion research has implicated numerous candidates for many of these processes, but a complete pathway in any single system remains indeterminate. As outlined above, the limited data available support Arp2/3 as the actin nucleator in mammalian cells. However, existing evidence implicates Arp2/3 in cdc42 and Rac-directed events [9,20]. As formins both produce actin filaments more closely resembling stress

Fig. 1. Schematic of adhesion-site directed actin assembly following integrin activation. Integrin receptors exist in a resting conformation in the membrane in association with various molecules that regulate their activation. Upon binding of Arg-Gly-Asp (RGD) containing ligands, $\beta_3$ integrins undergo activating biochemical modifications including a conformational change in structure and tyrosine phosphorylation of the $\beta$-subunit cytoplasmic tail. Multivalent ligands and diffusion within the membrane facilitate clustering of integrins. Active integrin complexes recruit and activate actin nucleation machinery to produce de novo actin filaments anchored to the adhesion complex at the growing end. As dictated by environmental stimuli, the adhesion site is either reinforced through stabilization of actin filaments resulting in cell spreading and adhesion or remodeled to suit progressive cellular phenotypes.
fibers and in some cases are directly activated by Rho, they serve in this model as the actin nucleator in a cell with an adherent, spread morphology.

Whether it be GTPase activation of Arp2/3 via WASp or direct activation of a diaphanous related formin, integrin-associated GTPase activity would be required at the adhesion site for a model of adhesion-site-directed actin assembly. Examples abound of integrin regulation of the Rho family of small GTPases [6,7,43]. In our studies, we find a large increase in the association of active RhoA with β3 integrins that occurs upon adhesion. This Rho activity is required for stress fiber formation, cell spreading and firm adhesion in macrophages [7]. GTPase activity is controlled by a balance between positive and negative regulators. Perhaps the most influential of these is the GEFs [44]. Among these, Vav1, a hematopoietic-specific GEF, can be found in association with β3 integrins [45]. Many aspects of Vav’s in vivo function remain to be determined, but it exhibits clear activation of Rho in vitro [46]. Translocation of Vav1 to β3 may be one means to affect the balance of GTPase activity and thus emphasizes the importance of integrin clustering in initiating interaction between the adhesion site and the actin cytoskeleton. Another factor to be determined is the target of PI3 kinase activity during cell adhesion. PI3 kinase activity and association with integrins is regulated by integrin adhesion [7]. Selective inhibitors of PI3 kinase have potent inhibitory effects on actin cytoskeleton-dependent events [7]. A likely mechanism for these effects is disruption of Vav GEF activity by inhibition of PI3 kinase-dependent Vav phosphorylation that alters its exchange activity in vitro [47–50]. This would also explain the effects of PI3 kinase inhibitors on GTPase activity in vivo [7].

In hematopoietic cell types Vav, PI3 kinase, Rho and Arp2/3 can all be localized to the integrin adhesion site, but it is unknown how these molecules interact with clustered integrin cytoplasmic tails. One potential mechanism is via Pyk2, a molecule related to FAK. Pyk2 binds directly to the cytoplasmic tail of the beta-subunit [31]. Pyk2 undergoes activating phosphorylation upon integrin ligation and clustering [31]. Both podosomal localization of Pyk2 as well as Pyk2 phosphorylation are required for formation of the osteoclast sealing zone, a β3 integrin- and actin-dependent structure [31,51]. Pyk2 also serves as a scaffold for the binding of both PI3 kinase and Vav [52,53]. Thus, Pyk2 may anchor the necessary elements for actin nucleation to the adhesion site. A recent report detailing defects in a Pyk2-deficient mouse supports a key role for this molecule in integrin-mediated assembly of the actin cytoskeleton [43]. Pyk2 null mouse macrophages exhibit impaired migration towards a stimulus and limited contraction of the actin cytoskeleton in lamellipodia. Importantly for the model proposed above, Pyk2 deficiency severely limited adhesion -dependent activation of Rho and PI3 kinase. While related to the more widely distributed FAK, the tissue-specific expression of Pyk2 and the apparent discrepancy between the integrin-binding domains of these two molecules fail to provide a mechanism that would be common to all cells. In this scenario, we have drawn heavily upon information from hematopoietic systems. In this regard, it is possible that the functional non-hematopoietic analogs of the proteins in the adhesion complex of non-hematopoietic cells work similarly, but interact through differing protein–protein interactions.

If all components for actin assembly are sequestered by the integrin adhesion site and filamentous actin is produced, how is that actin anchored? Early observations in integrin research identified several molecules with integrin and actin binding activity, including α-actinin and talin that tether vinculin to the adhesion site [54–56]. Recent evidence suggests that vinculin’s role may be to stabilize the growing end of actin filaments by its ability to bind integrins, actin filaments and Arp2/3 [9]. This provides a means for assembled actin to extend progressively outward from the adhesion site, rather than towards the adhesion site as is proposed in many current models. During progression towards an adherent phenotype, such as occurs during periods of active Rho dominance, newly formed actin filaments would be reinforced through actin bundling with continued aggregation of integrins into large adhesive complexes, establishing a firmly adhesive phenotype capable of withstanding shear forces.

As a basis for this model, we have inserted findings from both Arp2/3 and formin studies. Determination of which, if either, of these molecules assembles the actin cytoskeleton in mammalian cells will no doubt be a large focus of future work in adhesion biology. Current evidence implicates Arp2/3-mediated assembly in cells under the control of cdc42 and Rac, while early data on formin-mediated actin assembly suggest it would be better suited for production of the linear actin filaments seen in Rho-dependent stress fiber formation. This suggests that the various actin structures seen during cell adhesion may each be produced by unique actin nucleation systems. Whether this is accomplished by modification of molecules in the adhesion complex or complete exchanges of signaling molecules remains to be determined. Several possible mechanisms exist for modification of signaling platforms. Among them, changes in GEF specificity for GTPases is appealing. Alteration of GEF specificity through phosphorylation is suggested by conflicting reports on the selectivity of Vav Rho family GTPases [46]. Clustering of integrins, a hallmark of progression towards a Rho phenotype, results in PI3 kinase activation that has been shown to result in Vav phosphorylation [7,47]. A change in the dominant GTPase at the adhesion site could result in the activation of a different class of actin nucleation factors. For example, switching from active Rac to active Rho by changing GEF specificity may favor activation of formins, resulting in the production of stress fibers as seen in cells under the influence of active Rho. Another possible mechanism is modification of actin filaments as they are produced. A single example is available wherein a reduction in capping protein concentration...
alters the phenotype of nucleated actin from a branched to a non-branched form [15]. This may also result from aggregation of integrins at the adhesion site to the extent that sufficient actin assembly is occurring to overwhelm available capping proteins.

As an alternative to modifying the existing signaling apparatus, the integrin-associated complex may undergo more significant remodeling to effect cytoskeletal phenotype changes. Translocation of scaffolding proteins such as paxillin that can recruit powerful phosphatases as well as new complements of GTPase regulatory molecules are well suited for such a mechanism. Pyk2 also binds paxillin, providing a means for localization of remodeling complexes to the adhesion site.

5. Implications of an adhesion driven polymerization model

The model of adhesion-site-based actin nucleation described above utilizes a minimal complement of integrin-associated molecules to produce a cytoskeletal anchored adhesion. Lacking from this model is an integration of the majority of the dozens of proteins found associated with integrin receptors [2]. However, most of the described associating molecules regulate discrete steps in the assembly or maintenance of the adhesion complex. These steps could be categorized as activation, clustering, stabilization, and remodeling. Molecules affecting integrin activation would include those that alter integrin affinity ($\beta_3$ endonexin, CD98) as well as integrin phosphorylation (Src family kinases) [7,57,58]. Clustering of receptors is impacted by membrane-associated kinases such as PKC as well as by integrin-binding proteins associated with the cortical cytoskeleton such as talin [41,59]. The association of actin capping and bundling proteins including profilin and plasmin may determine stabilization of the nucleation complex and resultant actin fibers [24,60]. A large proportion of integrin-associated proteins may be involved in remodeling of the signaling complex to effect changes in cytoskeletal and cellular morphology, with paxillin described above as an example (see Ref. [61] for review). From this perspective, it is understandable why minor differences in the environment of different experimental systems yield conflicting data on the importance of adhesion-regulating molecules in each of these steps. It also underscores the potential misinterpretations that could result from studying signaling events proximal to integrin ligation in cells under the influence of exogenous GTPase activity.

In this abbreviated linear pathway, activation and recruitment of only a few molecules is necessary for the production of actin filaments by the adhesion site. If integrin ligation orchestrates assembly of the actin cytoskeleton through such a simplistic mechanism, the unresolved question remains of identifying the switch that initiates actin nucleation. Biochemical and empiric evidence both old and new suggest that clustering of integrins, and subsequent alteration in cytosolic concentration of signaling molecules, may bring about this dynamic. Recent technologic advances have improved the resolution of in vivo actin assembly during cellular phenotypic changes [62,63]. Application of these advances to cell adhesion research will determine whether actin assembly originating at the integrin complex controls the progression of cytoskeletal morphology seen during cell adhesion.

References


