Cell adhesion molecules, signal transduction and cell growth
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Signals from dynamic cellular interactions between the extracellular matrix and neighboring cells ultimately input into the cellular decision-making process. These interactions form the basis of anchorage-dependent growth. Recent advances have provided the mechanistic details behind the ability of integrins, and other cell adhesion molecules (CAMs), to regulate both early signal transduction events initiated by soluble factors and downstream events more proximally involved in cell cycle progression. These actions appear to depend on the ability of CAMs to initiate the formation of organized structures that permit the efficient flow of information.

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Abbreviations
APC adenomatous polyposis coli
CAM cell adhesion molecule
CKI cyclin-dependent kinase inhibitor
ECM extracellular matrix
ERK extracellular-signal-regulated kinase
FAK focal adhesion kinase
GPCR G-protein-coupled receptor
GSK-3β glycogen synthase kinase-3β
ILK integrin-linked kinase
JNK c-Jun amino-terminal kinase
LEF lymphocyte enhancer binding factor
MAPK mitogen-activated protein kinase
MEK MAPK kinase
PAK p21-activated kinase
PKA cAMP-dependent protein kinase
PKC protein kinase C
pRb retinoblastoma protein
RTK receptor tyrosine kinase
TCF T-cell-specific factor

Introduction
Cells detect their extracellular milieu through interactions employing a variety of cell adhesion molecules (CAMs). They interact with extracellular matrix (ECM) components via integrins and syndecan molecules, and with adjacent cells via members of the cadherin, selectin and Ig-CAM families. Initial research in the adhesion field focused on the structural capabilities of these molecules with an emphasis on their ability to link to the actin cytoskeleton. Importantly, these molecules have also recently been discovered to participate in transduction events, either by directly eliciting signals upon engagement, or in a collaborative manner by modulating the efficiency of signaling pathways initiated by soluble factors. Currently, of particular interest is the manner in which CAMs co-ordinate these structural and signaling functions. Such regulation may occur either through actin-based and/or focal adhesion-like structures, or through one of a number of docking and scaffolding molecules that are being discovered. This review focuses on the effects of integrins on signal transduction, but certain effects of other CAMs are also discussed.

Integrins and receptor signaling
Early signaling events regulated by integrin-mediated adhesion were first described for events downstream of receptor tyrosine kinases [1•]. Collaboration between integrins and G-protein-coupled receptors (GPCRs) has been recently and convincingly demonstrated ([2,3]; reviewed in [4•]). The paradigm of this phenomenon is that the normal cellular response to a soluble mitogen can be dramatically altered by changes in cell adhesion.

While the basic phenomenon of integrin–mitogen collaboration continues to be demonstrated in many cell types and for many ECM ligands, the cutting edge research in this area is focused on elucidating the mechanisms through which this collaboration occurs. Work thus far has shown that regulation of signaling by integrin-mediated adhesion is complex, in that it can occur at the level of the receptor or further downstream along a signaling pathway. Integrins can modulate receptor activity through direct interaction with the receptors [5•], upon integrin-mediated adhesion [6], or through regulation of receptor expression [7,8,9•]. An intriguing recent report demonstrates that integrins may also modulate receptor activation by binding that receptor’s ligand, thereby restricting or localizing the cellular response [10].

Currently, less is known about regulation of downstream signaling components. Perhaps the most intensely studied adhesion-mediated signaling pathway is the mitogen-activated protein kinase (MAPK) pathway. The level at which adhesion regulates mitogen activation of the MAPK pathway has been mapped to either MAPK kinase (MEK) [11] or Raf [12] (Figure 1). This variability may reflect a pleiotropic mechanism of regulation in which several potential levels of regulation co-exist and are required under different circumstances. Recent observations support this view. There is evidence that forced activation of focal adhesion kinase (FAK) in non-adherent cells can allow efficient, anchorage-independent MAPK activation [4•]. In our laboratory, it was found that suppression of cAMP-dependent protein kinase (PKA) activity transiently permits anchorage-independent activation of MAPK by growth factors, through a mechanism that appears to involve regulation of p21-activated kinase activity (PAK) (AK Howe, RL Juliano, unpublished data). Integrins may also directly regulate cytoplasmic signaling modules, such

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as the MAPK cascade, through interaction with caveolin-1, a transmembrane protein, and recruitment of the SH2-domain adaptor protein, Shc [13]. Oddly, while some researchers have reported that loss of caveolin function impairs integrin-mediated MAPK activation and anchorage-dependent growth [13], others have found that downregulation of caveolin can actually enhance MAPK activity and induce anchorage-independent growth [14]. Nevertheless, the potential importance of integrin–caveolin interaction is underscored by recent work that shows that depletion of caveolin inhibits Src recruitment to β1 integrins which diminishes FAK tyrosine phosphorylation, focal adhesion formation, and cell adhesion [15].

Research into adhesion-mediated signaling has gained much from delineating the fine specificity of the regulatory mechanisms. Within the previously mentioned references are excellent examples [5•,8,9•]. These studies demonstrate the importance of the specific interactions between adhesive substrate and integrin, and illustrate that the regulation of growth by integrins is not necessarily always positive regulation. It is not simply attachment, but attachment to a physiologically relevant and conformationally correct matrix that generates an environment permissive for growth. Conversely, attachment to a foreign matrix may provide a point of purchase but cannot provide the full complement of permissive cues to allow productive signaling. Our studies have demonstrated that a variety of integrins are able to support adhesion-dependent growth factor activation of MAPK, indicating that this pathway remains intact in the presence of different matrix conditions. These findings indicate a requirement for integrin-mediated pathways, in addition to MAPK, for permissive growth (AE Aplin, SM Short, RL Juliano, unpublished data).

It is also clear from the work of several groups that, in addition to adhesion, the specific architecture of the
cytoskeleton is fundamentally important for efficient signaling [2,16] and cell cycle traverse [17*,18]. This is not surprising given the significant evidence that intracellular tension and cellular shape can so dramatically affect cell function [19,20]. The cytoskeletal elements required for efficient growth factor signaling appear to be punctate focal complexes and/or cortical actin structures regulated by the Rho family member Cdc42 [16] (Figure 1). Interestingly, constitutive Cdc42 activity is able to partially rescue growth factor activation of MAPK in non-adherent cells, despite the absence of integrin activation. Work on direct activation of MAPK by integrin-mediated adhesion has also found Cdc42 to be important for this pathway [21]. A similar requirement for actin architecture and focal complexes in adhesion-dependent signaling through GPCRs has been demonstrated [2], although the degree to which this signaling depended on actin organization varied with the cell type. A recent report [22**], however, suggests that actin can impinge more directly on growth related pathways. Sotiropoulos et al. [22**] demonstrated activation of the serum response factor, an immediate-early gene transcription factor, by LIMK, an actin regulatory kinase, through the regulation of levels of G-actin.

**Integrins and cell cycle**

At present, a direct role for integrin-modulation of early signaling events has not been linked in a causative manner to integrin-modulation of cell cycling. Indeed, there are reports that clearly separate the two areas of regulation [17*,23]. There are also reports, however, that make a substantive correlation between control of upstream signaling and the success of G1/S transition [13,24]. In both of these reports, the inability of integrin-mediated adhesion to recruit and activate the She adapter protein, after depletion of caveolin-1 [13] or lack of α1 subunit expression [24], correlated with diminished MAPK activation and cell proliferation. Other reports indicate that a potential effector through which regulation of cell cycle progression by ECM/cytoskeleton conformation might occur is FAK [25,26]. The control of FAK activity by cell adhesion and cytoskeleton dynamics is well documented [27], and recent reports have established that FAK activity is required for successful transit into S phase [25,26], possibly through modulation of upstream components including extracellular signal-regulated kinase (ERK) [4*,25] and c-Jun amino-terminal kinase (JNK) [26].

Regulation of early signaling events, however, is clearly not the last opportunity between G0 and S phase for regulation of cell division by integrin-mediated adhesion. Here we highlight a few recent advances and trends in cell cycle control by integrins; for a more detailed review see [28]. As mentioned above, integrin-mediated enhancement of early signal transduction pathways, though necessary, has been shown to be insufficient to allow progression through the cell cycle [17*,25]. In one case, events fundamental to cell cycle progression, namely phosphorylation of the retinoblastoma protein (pRb) and increased cyclin D expression, did not occur in non-adherent cells, despite the ectopic expression of hyperactive form of Raf and the subsequent induction of MAPK activity [23]. Similarly, hypophosphorylated pRb, diminished cyclin D expression and persistence of the cyclin-dependent kinase inhibitor (CKI) p27 were observed in cells that were prevented from spreading by restriction of the size of the adhesive substrate or by treatment with inhibitors of actin filament assembly or actinomyosin-based tension, despite normal MAPK activation [17*]. Further studies will clarify the links between cell adhesion, shape and tension, and enhancement of signal transduction and cell cycle progression.

As mentioned previously, integrins can also negatively regulate cell cycle progression. A particularly striking example of this comes from observations that the alternately spliced β1c integrin subunit potently inhibits cell growth [29]. Interestingly, growth suppression occurred even when the β1c cytoplasmic domain was expressed as a soluble protein [30]. Although the exact mechanism for this growth arrest has not been defined, there is evidence implicating p27 as an effector [31].

**Signal modulation involving other CAMs**

The emerging theme is that CAMs tether the actin cytoskeleton as well as modulating the signaling that impinges on cellular events. Although much less is known about the regulation of receptor-mediated signaling events by cell–cell adhesion than by cell–matrix adhesion, there are several reports linking intercellular adhesion and cell cycle control. The regulation of cell cycle progression by cell–matrix and cell–cell contact, however, is quite distinct. Perhaps the most accessible example of this is that adherent cells in culture grow in the presence of serum until reaching confluence, when contact inhibition of growth quells cell division, despite the presence of mitogens and matrix attachment. Thus, it is apparent that certain CAMs have the ability to negatively influence signal transduction pathways initiated by factors in the external environment. Such mechanisms may be used as preventative measures to forestall aberrant activation of pathways. Here, we concentrate our attention mainly on cadherin-based signaling in cell–cell adhesion signaling events. Recently, however, other work has elucidated signaling roles for N-CAMs and selectins [32,33].

Recently, the CKI p27 has been established as an effector for contact inhibition of growth mediated by both E-cadherin and N-cadherin [34*,35]. Interestingly, functional inhibition of another CKI p16 INK4a can overcome contact inhibition of growth, implicating another mechanism of regulation [36]. The involvement of CKIs, in contact inhibition of growth points to regulation of pRb phosphorylation as a principal mechanism through which cell–cell adhesion regulates cell growth, although other mechanisms are sure to exist. Interestingly, one study has demonstrated that the combined loss of pRb and CKI p21
function, although able to induce anchorage-independent growth, could not overcome contact-inhibition [37]. In contrast, another study has patently shown that the pRB–E2F interaction is actually required for contact inhibition of growth [38]. Given the growing understanding of signals arising from cell–cell adhesion, elucidation of the mechanisms through which this aspect of the extracellular environment controls cell growth is on the horizon.

Levels of E-cadherin are often downregulated in malignant cells, and re-expression of E-cadherin in such cells has a tumor suppressor effect. Indeed, E-cadherin expression can inhibit the proliferation of dermal fibroblasts [39]. This role of E-cadherin has been proposed to be mediated through the modulation of the Wnt signaling pathway (reviewed in [40]). Moreover, β-catenin is a signaling entity in the Wnt pathway (Figure 2) and is predominantly found at sites of cell–cell contact where it links cadherin molecules to the actin cytoskeleton through the binding of α-catenin. Under conditions that the Wnt pathway is unstimulated, β-catenin is downregulated through its association within a complex containing the adenomatous polyposis coli (APC) protein, glycogen synthase kinase-3β (GSK-3β) and axin. Phosphorylation by GSK-3β within this complex targets β-catenin for degradation by the ubiquitin-proteasome pathway. Conversely, Wnt binding to its receptor results in inhibition of GSK-3β and consequently to the accumulation of cytoplasmic β-catenin. This unsuppressed accumulation of β-catenin leads to its translocation into the nucleus where it co-operates with lymphocyte enhancer binding factor-1 (LEF-1)/T-cell factor (TCF) in transcriptional events [40].

E-cadherin, APC and LEF-1 interactions with the ARM repeats of β-catenin are mutually exclusive, thus these proteins compete to partner β-catenin within the cell [41•]. Studies in Xenopus and Drosophila have previously implied a role for cadherins in the regulation of the Wnt pathway (reviewed in [40]). More recently, studies in E-cadherin null cells and colon carcinoma cells have shown that ectopic expression of E-cadherin or N-cadherin reduces nuclear localization and transcriptional activity of the free β-catenin [41•,42,43]. These findings demonstrate that cadherin interaction with β-catenin is not only important in linking cadherins to the actin cytoskeleton, but additionally acts as a regulatory sink to reduce a potentially deleterious build up of β-catenin (Figure 2).

Accumulation of uncomplexed cytoplasmic β-catenin, either by activation of the Wnt signaling pathway or, as is common in human colon carcinoma cells, by mutational inactivation of APC, contributes to tumor progression in several cell types. A recently discovered target of β-catenin/LEF-1 transactivation is the cyclin D1 gene [44••,45••]. High levels of cyclin D1 in colon carcinoma cells are dramatically reduced by overexpression of the
N-cadherin cytoplasmic domain [44••]. Thus, cadherin molecules may counteract tumor progression through the control of β-catenin's signaling potential. It is not known whether actin-containing structures at cell–cell contacts coordinate this role. Overexpression of α-catenin within cells, however, has a similar effect as overexpression of E-cadherin, indicating that stabilization of actin containing structures may be important [46]. Interestingly, the E-cadherin promoter contains a LEF-1 consensus sequence, raising the possibility that Wnt signaling, via β-catenin, directly modulates E-cadherin expression and leads to altered signaling [47].

Synergy and cross talk between adhesion receptors: connections to signaling pathways

Thus far we have focused on signaling events that are controlled by engagement of individual families of CAMs. In the multicellular environment, the concerted actions of multiple CAMs are required to maintain correct cell growth and function. This raises the notion of crosstalk and synergy between the various families of CAMs. A particularly striking example illustrating this point is the collaboration between integrins and syndecan-4 in the spreading of fibroblasts on fibronectin. The syndecans are a family of heparan-sulfate proteoglycans that link the ECM, lipoproteins and growth factors to the actin cytoskeleton (recently reviewed in [48]). Syndecan-4 binds to heparan-binding moieties in fibronectin and is localized to focal adhesions. Ligand binding to syndecan-4 synergizes with integrins, via binding to fibronectin RGD sequences, to promote cell spreading and focal adhesion formation [49•]. The mechanistic details of the signals that arise from syndecan-4 are still unclear, but appear to involve syndecan-4 binding to and activating protein kinase C (PKC) and a Rho component [49•,50]. However, this may also underline a general role for Rho in enhancing cell spreading via elevated actomyosin-based contractility [51].

Other studies have provided evidence of a ‘tug-of-war’ between different CAMs that affects cellular motility. In neural crest cells, interfering with β1- or β3-integrin-mediated interactions with the ECM blocks migration. This effect correlates with the enhanced localization of N-cadherin to adherens junctions and cell aggregation, indicating that integrin-mediated signals restrain the cellular distribution of N-cadherin [52]. Signals downstream of this repressive action of integrins involve calcium fluxes and the inhibition of serine/threonine kinases [52]. Thus, cell–matrix interactions influence cell–cell connections, attesting to the presence of signaling crosstalk. One possible mechanism concerning crosstalk between integrins and cadherins involves integrin-linked kinase (ILK) (Figure 2). This serine/threonine kinase interacts with the cytoplasmic domains of β1 and β3 integrins [53]. ILK directly phosphorylates GSK-3β and inhibits its activity [54]. Furthermore, epithelial cells overexpressing ILK show additional properties of Wnt signaling, such as β-catenin translocation to the nucleus and increased β-catenin/LEF-1 transcriptional activity [55•]. As noted above, one possible target of LEF-1 transcriptional activity is E-cadherin itself. Cells overexpressing ILK show dramatically reduced levels of E-cadherin and loss of cell–cell adhesion [55•,56]. This provides an interesting potential connection between integrins and cadherins, although the true importance of integrins in any such crosstalk remains to be confirmed.

It should be noted that the manner of such crosstalk is often cell type dependent. In some cases the expression levels of cadherins and integrins are linked. For example, overexpression of either α5 or β1 integrins (and presumably increased integrin signaling) leads to an increased expression of N-cadherin in primary myoblasts. Not surprisingly, these cells grow in aggregates and display retarded migration properties upon contact [57]. Similarly, down-regulation of E-cadherin levels in keratinocytes concomitantly decreases the expression of α2β1 and α3β1 integrins and increases cell motility [58]. There is growing indication of crosstalk between different CAM families. Mechanistic details underlying examples of crosstalk between different integrins are also being discerned. αvβ3-mediated effects on α5β1 migration in human erythroleukemia cells have been localized to Ser752 in the cytoplasmic domain of β3. Mutation of this site downregulates calcium/calmodulin-dependent protein kinase II [59•]. Evidence of crosstalk between adhesion receptors has particular relevance to malignant epithelial cells that show decreased levels of adhesion molecules and are highly motile and invasive.

Scaffolds and signaling

Now that it is abundantly clear that integrins and other CAMs can exert dramatic influences on a variety of signal transduction processes, the key issue has become the mechanistic basis for these phenomena. Although many of the details are lacking, it seems likely that CAMs facilitate signaling cascades by organizing cytoskeletal ‘scaffolds’ that allow efficient interactions between cytoplasmic signaling components. The idea that the cytoskeleton can help to localize and organize signaling components is not new [27,60], but the details of this process have recently become more developed, particularly in the context of signal transduction in lymphocytes [61].

The role of scaffolding and docking proteins in signal transduction in both mammalian cells and lower eukaryotes is emerging as an important general theme [62]. Several of the MAPK modules in yeast are organized by scaffolding proteins, including Ste5, which links the elements of the pheromone receptor cascade, and PBS2 [62,63], which docks elements of the osmolarity response pathway. In mammalian cells, JIP-1 acts as a scaffold, binding several components of the stress-activated pathway that leads to activation of JNK, while MP1 links MEK1 and ERK1 in the classic MAPK pathway [63,64]. There are also docking proteins for PKC isozymes (termed RACKs)
In the present context it is important to ask whether proteins known to serve a scaffolding function in signaling cascades link to CAMs and the cytoskeleton (Figure 1). Although the evidence thus far is fragmentary, several interesting examples have emerged. Thus, RACK-1, a docking protein for activated PKC, has been shown to bind directly to the cytoplasmic domains of several integrin β-subunits [67]. This interaction occurs via one of several WD repeats that occur in RACK-1. Interestingly, other WD repeat proteins have also been shown to bind to the cytoplasmic tails of integrin [68]. As the WD motif is found in other signaling molecules, notably the β-subunits of heterotrimeric G proteins [69], this hints that other important connections may emerge. Another fairly direct link between CAMs and docking proteins concerns the ICAM family of immunoglobulin-like cell–cell adhesion receptors. It has been known for some time that the cytoplasmic tails of ICAMs 1 and 2 bind to the cytosolic protein ezrin, which can then link to the actin cytoskeleton [70]. Ezrin itself is an AKAP, binding selectively to the type II regulatory subunit of PKA [71]. In addition, another sub-family of AKAPs (termed AKAP-KL) has been shown to tether the type II regulatory subunit of PKA to the actin-rich submembranous sites [72]. Thus, for both RACKs and AKAPs evidence for direct linkages with CAMs and/or the cytoskeleton is emerging. Perhaps the most impressive example, however, concerns p130(Cas), a protein that has been implicated in integrin-mediated cell adhesion and motility. This molecule associates with FAK, Src and paxillin as part of integrin-rich focal contact sites, and serves as a substrate for the FAK and Src tyrosine kinases. The tyrosine phosphorylation of p130(Cas) permits the binding of the adaptor protein Grk, which can then recruit components of the Rac–JNK signaling pathway [73]. In addition, p130(Cas) is phosphorylated at a serine in an integrin-dependent manner, leading to recruitment of 14-3-3ζ [74], which in turn can bind Raf-1 (Figure 1). Therefore, p130(Cas) may serve to link integrin-dependent focal contact assemblies to both the JNK and ERK signaling pathways. Thus a paradigm is beginning to emerge wherein CAMs interface with the actin cytoskeleton to form dense, highly organized submembranous structures that are specialized for both cell adhesion and for the assembly of signal transduction cascades. It seems likely that various scaffolding and docking proteins will directly or indirectly associate with CAM cytoplasmic domains or with cytoskeletal structural elements to allow coherent and efficient transmission of information.

Conclusions

At the heart of multicellularity is the ability of every cell to successfully integrate signals arising from soluble factors, cell–matrix adhesion and cell–cell adhesion. Correct integration of these signals allows appropriate cellular growth, differentiation, and ultimately tissue morphogenesis, but incorrect integration contributes to pathologies such as tumor growth and metastasis. Because of the complexity of the in vivo situation, extensive studies have been carried out in culture based systems that are now giving rise to mechanistic details underlying CAM organization of signaling modules. Although this review has focused on the ability of interactions with the extracellular environment to regulate cell division, their contribution to the regulation of cell survival [75–77], movement [57,78], and differentiation [79,80] are also important.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

•• of outstanding interest


An excellent example of the specificity of integrin-mitogen collaboration. In endothelial cells, vascular endothelial growth factor (VEGF)-2 co-immunoprecipitates with β3, but not β1 or β5 integrin subunit. This correlates with a permissive effect of vitronectin, but not fibronectin or collagen, on VEGF signaling.


An excellent example of collaborative specificity, accentuated by the physiological relevance of the mammary epithelial cultures. Insulin, EGF and interferon show differing sensitivity to the adhesive substrate. In addition, insulin-stimulated tyrosine phosphorylation of the adaptor protein IRS-1 and...
activation of PI3-K was restricted to cells on the basement membrane, although both substrates allowed insulin-mediated MAPK activation.


17. Huang S, Chen CS, Inger DE: Control of cyclin D1, p27(Kip1), and cell cycle progression in human capillary endothelial cells by cell shape and cytoktoskeletal tension. Mol Biol Cell 1998, 9:3179-3193. This report utilizes the unique approach of altering cell morphology by replat- ing on microfabricated surfaces of differing size and shape. The findings sepa- rate mitogenic activation of MAPK from downstream cell cycle events, such as expression of cyclin D1 and pRb phosphorylation. Inhibition of cell spreading results in the inhibition of these later events.


23. Le Gall M, Grall D, Chambard JC, Pouyssegur J, Van Obberghen-Schilling E: An anchoradge-dependent signal distinct from p42/44 MAP kinase activation is required for cell cycle progression by cell anchorage and the cytoskeleton. Proc Natl Acad Sci USA 1998, 95:15339-15344. This paper clearly shows that E-cadherin, APC and LIF-1 compete for binding to β-catenin in vitro. Subsequently, the authors show that in cells containing high levels of cytoplasmic β-catenin, namely E-cadherin null cells or SW480 colon carcinoma cells, ectopic expression of E-cadherin pre- vents localization of β-catenin to the nucleus and its transcriptional activity.


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These experiments were carried out in fibroblast-null mouse fibroblasts to exclude the adhesion effects of endogenously synthesized and subse-
qiCytoskeleton and integrins in migrating neural crest cells.
quent secreted fibronectin. These cells attached but only partially spread on anti-β1 integrin antibody or the cell-binding domain of fibronectin, immo-

bilonized on a plate. Efficient spreading and focal adhesion formation was pro-
moted by antibody-mediated clustering of syndecan-4. This process was blocked by the action of the C3 exotransferase, implying that Rho acts downstream of syndecan-4.

This work reconstitutes αvβ3 integrin crosstalk to αβ1 in phagocytes in KB cells that only express the αβ1 integrin. Ecotypic expression of αvβ3 decreases migration of these cells to fibronectin. Functional analysis local-
izes the regulation to Ser752 of the β3 cytoplasmic domain, a site mutated in Glanzmann’s thrombasthenia. Mutation of this site impairs β3 inhibition of calcium/calmodulin-dependent protein kinase II and αvβ3 crosstalk to αβ1.
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These experiments establish that the focal contact protein p130(Cas) can bind 14-3-3 zeta in an adhesion-dependent manner.