REVIEW ARTICLE

Extracellular matrix and integrin signalling: the shape of things to come

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The extracellular matrix (ECM) and integrins collaborate to regulate gene expression associated with cell growth, differentiation and survival. Biochemical and molecular analyses of integrin signalling pathways have uncovered several critical cytoplasmic proteins that link the ECM and integrins to intracellular pathways that may contribute to anchorage-dependent growth. A large body of evidence now indicates that the non-receptor protein kinases focal adhesion kinase (FAK) and specific members of the mitogen-activated protein kinases (MAPKs), including the extracellular-signal-regulated kinases (ERKs), mediate these ECM- and integrin-derived signalling events. However, little is known about how FAK and MAPKs contribute to biological processes other than cell proliferation or migration. In addition, remarkably little is known concerning the signalling events that occur in cells that adhere to complex multivalent extracellular matrices via multiple integrin receptors. Given the stringent requirement for attaining a proper morphology in ECM/integrin-directed cell behaviour, it is still not clear how cell shape and tissue architecture impact upon intracellular signalling programmes involving FAK and MAPKs. However, the recent discovery that members of the Rho family of small GTPases are able to regulate ECM/integrin pathways that modulate both cell shape and intracellular signalling provides new insights into how cell morphology and signal transduction become integrated, especially within three-dimensional differentiated tissues.

Key words: anchorage-dependent growth, cell shape, focal adhesion kinase, integrins, mitogen-activated kinase.

SPECIFICITY AND REDUNDANCY OF CELL–EXTRACELLULAR-MATRIX (ECM) INTERACTIONS

It is now firmly established that the ECM profoundly influences the major cellular programmes of growth, differentiation and apoptosis. For example, promotion or suppression of growth by the ECM is associated with either stimulation or inhibition of key cell-cycle mediators, including cyclins and early-response genes [1–4]. The ECM regulates the transcription of genes associated with specialized differentiated functions, exemplified by both induction and repression of β-casein in mammary epithelial cells [5–7]. Alterations in the ECM also modulate the expression of genes associated with apoptosis [8–11]. Deciding which of these programmes a cell will elect is ultimately determined by the composition of the surrounding ECM. Several reviews have highlighted the regulation, expression and functions of various ECM proteins and integrins [12–16]. We therefore refer readers to these articles for in-depth discussions of these topics. Here we will focus on how cells perceive and react to complex extracellular environments, and how integrin-dependent activation of intracellular signalling mediators, such as focal adhesion kinase (FAK), mitogen-activated protein kinases (MAPKs) and Rho-family GTPases, leads to alterations in cell shape, gene expression and cell behaviour within three-dimensional tissues.

The integrin transmembrane receptors are heterodimeric molecules composed of α and β subunits, with extracellular domains which bind to the ECM, and cytoplasmic domains which associate with the actin cytoskeleton and affiliated proteins, including vinculin, talin and α-actinin [17,18]. Thus, as their name implies, integrins create an 'integrated' link between the outside and the inside of the cell. At least 16 α and eight β subunits have been identified, which could potentially generate 22 distinct $\alpha\beta$ heterodimeric receptors. To date, however, only a dozen or so $\alpha\beta$ combinations have been found *in vivo*.

Integrin receptors exhibit considerable overlap in their ligand-binding specificities. For example, the $\alpha v\beta 3$ 'vitronectin' receptor shows strong binding affinity for fibronectin, collagen, tenascin-C, thrombospondin and fibrinogen [19]. Furthermore, particular ECM components can bind to more than one integrin. In addition to the $\alpha 5\beta 1$ receptor, fibronectin also binds at least seven other integrin heterodimers. Similarly, laminin binds $\alpha 3\beta 1$, $\alpha 6\beta 1$ and $\alpha 6\beta 4$ integrins with high affinity. This apparent redundancy may suggest that, in addition to mediating attachment to a particular ECM ligand, different integrins perform specialized signalling functions. Indeed, recent work with integrin knock-out mice has helped to define distinct specialized roles for multiple laminin receptors with similar binding affinities. For example, keratinocytes use both $\alpha 3\beta 1$ and $\alpha 6\beta 4$ integrins to adhere to a laminin-rich basement membrane. In contrast with wild-type animals, mice lacking $\alpha 3\beta 1$ integrin exhibit a poorly organized basement membrane, but the ability of keratinocytes to adhere is maintained through the $\alpha 6\beta 4$ integrin. These findings suggest that the primary function of the $\alpha 3\beta 1$ laminin receptor is to maintain the organization of the basement membrane, whereas the $\alpha 6\beta 4$ integrin functions primarily to mediate stable adhesion in hemidesmosomes [20].

COMMON INTRACELLULAR SIGNALS ACTIVATED BY INTEGRINS

Specialized cells are surrounded by different combinations of ECM proteins and express an array of tissue-specific integrin receptors. This diversity may represent one way to generate

Abbreviations used: ECM, extracellular matrix; FAK, focal adhesion kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MEK, MAPK/ERK kinase; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; NGF, nerve growth factor.

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unique intracellular signals that give rise to tissue-specific phenotypes. However, at present little is known regarding tissue-specific signalling pathways. Instead, the majority of signalling molecules implicated in ECM–integrin interactions appear to be rather ubiquitous mediators of signal transduction. For example, Miyamoto et al. [21] showed that at least 20 different proteins, including Rho GTPases, Raf, Ras, FAK, and MAPKs such as extracellular-signal-regulated kinases (ERKs), can be recruited to the ECM ligand/integrin-binding site. In an attempt to understand how these intracellular mediators may contribute to specialized patterns of gene expression and cell behaviour, we will focus primarily on FAK and on the ERK/MAPK pathway.

FAK

Early studies on integrin-dependent cell adhesion and signalling demonstrated that cell ligation to the ECM was accompanied by integrin aggregation, and that this clustering could trigger increased tyrosine phosphorylation of a number of intracellular proteins [22–25]. Additional work demonstrated that integrin clusters are concentrated in specialized organelles known as focal adhesions, which are also enriched with bundles of actin and associated cytoskeletal proteins, including vinculin, talin, paxillin and tensin [17,26]. Since inhibitors of tyrosine kinase activity could block the formation of focal adhesions, and since integrins lack intrinsic tyrosine kinase activity, focal adhesions were dissected carefully to identify potential tyrosine kinases that could initiate focal adhesion assembly and intracellular signalling pathways [24,27]. A predominant protein in focal adhesions which was shown to undergo rapid tyrosine phosphorylation following integrin ligation and clustering is a 120 kDa non-receptor tyrosine kinase known as FAK [28,29]. Although FAK appears to be incapable of phosphorylating other substrates directly, integrin-dependent autophosphorylation of FAK allows it to interact with docking or adaptor proteins, including paxillin, tensin and Grb2/Son of Sevenless ('SOS'), which in turn are able activate downstream signalling mediators previously implicated in growth control, including Src, Ras and Raf [30,31].

A number of observations strongly suggest that activation of FAK by integrins plays a central role in initiating many of the signals that regulate growth. For example, mutation of tyrosine residues critical for FAK autophosphorylation prevents integrin-mediated proliferation [32]. Also, oncogenic transformation of cells, which abolishes the requirement for anchorage-dependent growth, activates FAK [33]. Consistent with this, introduction of constitutively active FAK leads to cell transformation, anchorage-independent growth and the suppression of apoptosis [34].

CROSS-TALK BETWEEN INTEGRINS AND RECEPTOR TYROSINE KINASES

FAK can be activated by certain soluble growth factors, indicating that integrin and growth factor signalling pathways may converge at FAK [35]. In support of this, recent studies have demonstrated that, following integrin clustering, growth-factor receptors are also recruited into focal adhesion complexes. For example, treatment of endothelial cells with beads coated with an Arg-Gly-Asp (RGD) tripeptide or with fibronectin leads to co-aggregation not only of β1 integrins and FAK, but also of high-affinity receptors for basic fibroblast growth factor (bFGF) in the newly assembled focal adhesions [36,37]. Using a similar experimental approach with fibroblasts, Miyamoto and colleagues [38] have extended these findings to show that integrins interact with a more extensive repertoire of growth-factor receptors, including those for epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), as well as bFGF. In this instance, recruitment of growth-factor receptors to focal adhesions has been shown to result in enhanced phosphorylation and activation of the growth-factor receptor, accumulation of downstream substrates, and ultimately an enhanced growth response to exogenously added mitogens [38,39]. Similarly, in vascular smooth-muscle cells, ligation of the $\alpha v\beta 3$ integrin by tenascin-C alters cell morphology and promotes aggregation of EGF receptors to focal adhesions that are also enriched with tyrosine-phosphorylated proteins [40].

How integrin ligation leads to the recruitment of receptor tyrosine kinases to the focal adhesion site is not fully understood, yet the fact that high-affinity EGF receptors can directly bind actin, which in turn enhances EGF-dependent autophosphorylation and activation of downstream substrates, indicates a critical role for the actin cytoskeleton in co-ordinating signalling between integrins and growth factors [41–43]. Not surprisingly, then, disruption of the actin cytoskeleton with agents such as cytochalasin D inhibits not only focal adhesion formation, but also activation of growth factor receptors, including those for PDGF and EGF, which in turn attenuates cellular growth responses, resulting in G_0 arrest [44,45]. Thus the actin cytoskeleton and associated proteins may act as a solid-state scaffold which spatially and biochemically co-ordinates cross-talk between integrins and growth-factor-receptor tyrosine kinases.

Gene targeting experiments in mice have further revealed the critical role of FAK in mediating ECM-dependent cell behaviour [46]. The phenotype of FAK-deficient mice is embryonic lethality, characterized by delayed embryonic migration, impaired organogenesis and vascular defects. This phenotype is also reminiscent of fibronectin- or α5-integrin-deficient mice, further supporting the notion that ECM, integrins and FAK are intimately linked [47–50]. Surprisingly, cells isolated from the FAK-null mice are still capable of assembling focal adhesions, and display an enhanced ability to adhere to the ECM substrates in tissue culture. This may explain why the FAK-deficient cells are unable to migrate *in vivo* [46]. Taken together, these results suggest that FAK may act primarily as a temporal mediator which limits adhesion to the ECM, in a way that would allow cells to adopt a position or shape that is permissive for migration and proliferation. However, whether activated FAK is subsequently required for ECM-dependent gene expression associated with differentiated tissues is not clear. One recent report noted, both in culture and *in vivo*, that levels of FAK were significantly lower in differentiated prostatic epithelium cells as compared with their proliferative counterparts [51]. In contrast, the highest levels of FAK observed *in vivo* are found in the brain, a highly differentiated, largely non-proliferative tissue [52].

MAPKs

Activation of the MAPK signal-transduction pathway provides a common route leading to transcriptional regulation of genes that are crucial for cell growth and differentiation. Family members that are sequentially activated following transient activation of Ras GTP-binding proteins via receptor tyrosine kinases include MAPK/ERK kinase (MEK; also known as MAPKK) and ERK1(p44)/ERK2(p42) [53]. In early studies, the activity of this MAPK pathway was found to be elevated following exposure of cells to soluble mitogens; consistent with this, direct overexpression of MEK resulted in transformation and anchorage-independent cell growth in the absence of exogenous mitogens [54]. Subsequently it was shown that MEK-

dependent phosphorylation of ERK1 and ERK2 results in their translocation to the nucleus, where they phosphorylate and activate a number of transcription factors associated with early-response genes [53,55].

In addition to growth factors, adhesion of cells to ECM proteins, including fibronectin, vitronectin, collagen, tenascin and laminin-5, via ligation of $\alpha5\beta1$, $\alpha v\beta3$, $\alpha2\beta1$, $\alpha v\beta6$ and $\alpha6\beta4$ integrins respectively, also leads to activation of the MAPK pathway [32,56–58]. Recent evidence also suggests that integrin activation of MAPK may be independent of FAK or Ras, therefore implying the use of alternative or parallel pathways [30]. In certain cases, it is believed that FAK-related proteins, such as CAKβ and PYK2/RAFTK, may compensate for FAK, thereby linking integrins to MAPK via alternative effectors [59–61]. To date, however, little is known about how or when cells may use these alternative routes.

Another unresolved issue regarding integrin-mediated signalling is whether integrins themselves actually initiate signals, or whether they simply modulate or enhance signals generated by soluble mediators such as growth factors. Although growth factors appear to co-operate with integrins in a synergistic fashion to activate FAK and MAPK, the similarities and intimate association of signals initiated by soluble mitogens on the one hand and by integrins on the other has made it difficult to establish the precise contributions of each of these players to cell proliferation. As mentioned above, whereas growth factors are dependent upon Ras for activating MAPK, integrins are not [62]. In keeping with these dissociable effects of growth factors and integrins, Zhu and colleagues [63] have demonstrated that the kinetics of MAPK activation by soluble mitogens differ from those observed on integrin ligation. However, these tissue-culture studies have not unequivocally resolved whether activation of MAPK attributed to integrins may in fact arise from an accumulation of endogenously produced mitogens. Although these measurements were performed in serum- and growth-factor-free media, the majority of cells cultured on two-dimensional ECMs synthesize endogenous mitogens in quantities sufficient to modulate the expression of early-response genes associated with the G_0–G_1 transition, the very same genes that are targets of MAPKs [1,2,53,63]. Nonetheless, it is clear that both integrins and MAPKs are essential for eliciting specific cellular responses.

INTEGRIN-DEPENDENT GROWTH INHIBITION

Based on the evidence linking MAPK activation to cell proliferation, it might then follow that growth arrest and subsequent differentiation would require that MAPK activity be suppressed. Recent evidence, however, suggests that activation of MAPKs may also be required for growth arrest and differentiation. For example, constitutive expression of MEK in MDCK or erythroleukaemia cells results in the attenuation of cell proliferation [64,65]. In 3T3 cells, MAPK is required for both proliferation and differentiation of adipocytes [66]. In addition, while nerve growth factor (NGF) treatment of PC12 or NIH 3T3 cells expressing the trkA/NGF receptor normally induces cell-cycle arrest accompanied by increased levels of p21, this process could be prevented by inhibiting MAPK activity [67–69].

Whether ECM/integrin-mediated growth arrest and differentiation also depend upon MAPK has not been directly addressed, but this possibility raises some intriguing questions with respect to integrin and cell-type-specific responses. For example, while previous reports have shown that ligation of $\alpha5\beta1$ or $\alpha6\beta4$ integrins by fibronectin or laminin respectively will activate ERK and stimulate cell proliferation [38,56,57], expression of the $\alpha5\beta1$ receptor in transformed CHO cells promotes adhesion to fibronectin, but induces growth [70]. Similarly, introduction of the $\alpha6\beta4$ integrin into the colon carcinoma cell line RKO permits adhesion to laminin, leading to growth inhibition and the induction of the cyclin kinase inhibitor p21 [71]. A comparative analysis of MAPK activity following ligation of a particular integrin by a given ligand in cells with opposite biological responses might help to clarify two unresolved questions. (1) Does a particular ECM/integrin receptor combination activate the MAPK pathway universally, independent of cell type? (2) Is there a cell-type-specific response to MAPK activation? Recent evidence in yeast suggested that distinct MAPK isoforms are used to generate specific responses, such as mating or filamentation and invasion [72]. Whether proliferation or differentiation in higher eukaryotic cells are mediated by distinct MAPKs awaits future investigations.

'INSIDE-OUT' SIGNALLING AND MAPKs

Although a role for MAPKs in mediating integrin-induced differentiation remains to be established, evidence is emerging to suggest that they may participate by creating an extracellular environment which supports the differentiated phenotype. For example, the MAPK-dependent differentiation of PC12 cells is accompanied by up-regulation of the $\alpha1\beta1$ collagen/laminin receptor, which helps to establish an elongated morphology that is often associated with differentiation of this cell type. Similarly, the MAPK-dependent differentiation and growth arrest of erythroleukaemia cells is accompanied by up-regulation of the platelet receptor $\alpha IIb\beta3$, the expression of which is impaired by inhibiting MAPK. Also, the integrin-dependent activation of ERK1/2 appears to be essential for the transcription of ECM proteins, including tenascin [73]. In addition, the activity of the Ets transcription factor PEA3 (polyoma enhancer activator) appears to be a direct downstream target of ERK [74]. This factor has also been shown to be essential for activation of the transcription of a number of integrin genes, including αIIb and αv integrins [16]. Furthermore, PEA3 is critical for the expression of ECM-degrading proteases, including urokinase plasminogen activator, collagenase and stromelysin [75–77]. Thus ERK may be intimately involved in regulating the transcription of genes which profoundly alter the extracellular environment, including ECM proteins and the integrin receptors that interact with this environment.

More intriguing are the observations by Hughes et al. [78] showing that activation of MAPK leads to suppression of high-affinity ligand binding by a number of integrins, including $\beta1$, $\beta3$ and $\alpha6$. Interestingly, in contrast with other ERK-dependent events, this 'inside-out' function was not dependent on *de novo* gene transcription. Another striking example of non-transcriptional MAPK-dependent events is increased cell motility and phosphorylation of myosin-light-chain kinase [79]. Whether increased motility is also be linked to decreased integrin ligand binding is not known, but together these studies suggest that, like FAK, MAPK may play a role in modulating cell adhesion to the ECM, and thereby contributes to the morphological reorganization required for mitosis, migration or differentiation. Furthermore, MAPK-dependent attenuation of integrin binding affinities may well contribute to growth arrest and differentiation by attenuating the generation of growth-promoting signals induced following integrin ligation. One striking example where reduced integrin activity can lead directly to differentiation is in transformed mammary epithelial cells. Despite a number of genotypic abnormalities, blocking $\beta1$ integrin allows the cells to become quiescent and resume their differentiated function, which

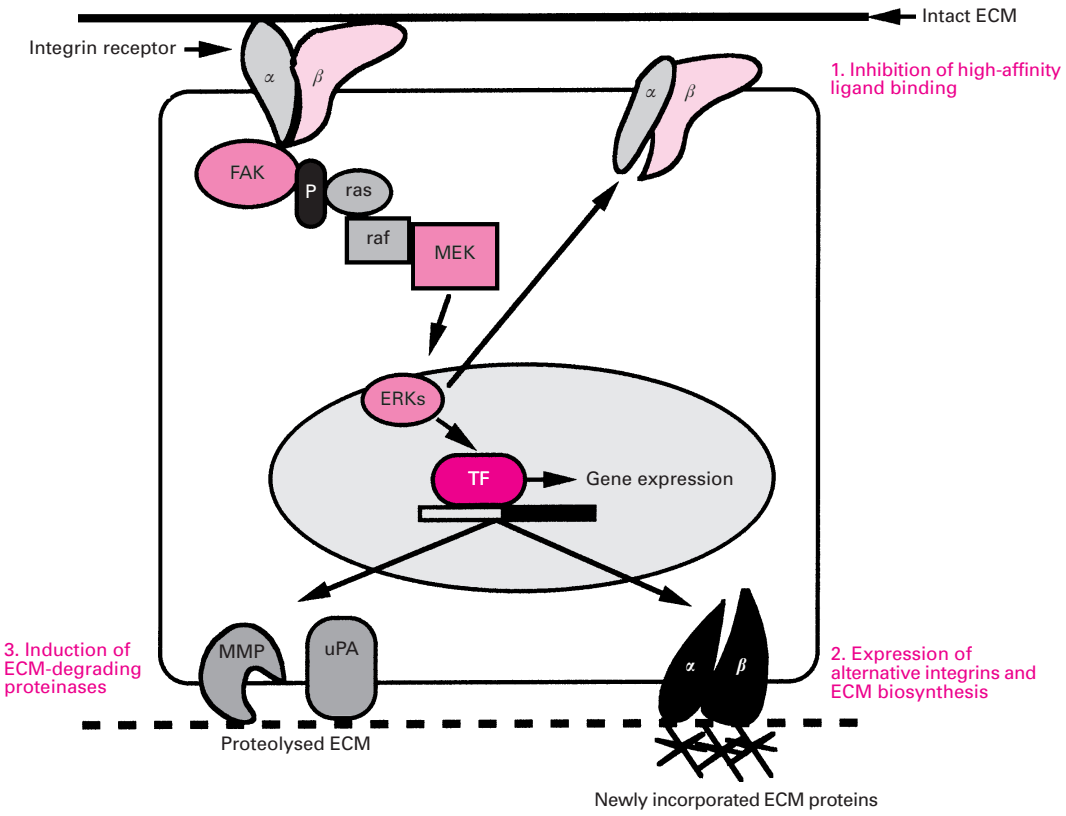

Figure 1 'Inside-out' functions of FAK and MAPKs

Representation of how integrin-mediated activation of the FAK/MAPK (ERK) signal-transduction pathway may limit, or modify, integrin–ECM interactions from the inside-out. Activation of the FAK/MAPK pathway leads to inhibition of integrin binding to the ECM in a manner that is independent of *de novo* gene transcription (1). In addition, activation of the MAPK pathway may lead to the transcription of target genes that modify cell–ECM interactions. These include integrins, ECM proteins (2) and ECM-degrading proteinases such as urokinase plasminogen activator (uPA) and matrix metalloproteinases (MMPs) (3). Abbreviations: TF, transcription factor; P indicates phosphorylation.

is accompanied by a morphological reorganization into structures resembling normal mammary acini [80].

Given that cells exist in a dynamic state where they must constantly perceive, respond to and modify their microenvironment, the potential of MAPKs to modulate ECM composition and integrin-receptor expression or activity suggests that these enzymes are likely to play a role in the 'dynamic reciprocity' model of cell–ECM interactions proposed by Bissell and colleagues nearly two decades ago [81]. These additional roles for MAPK also raise the possibility that the constitutive activation of MAPKs observed in many transformed cell lines may, in certain instances, represent an attempt to re-establish homoeostasis. In these instances, the inability to arrest growth is related not so much to enhanced MAPK-dependent proliferation, but rather to an inability to respond appropriately to MAPK and to create an environment that supports differentiation. Given the role of FAK in limiting cell adhesion, and the established and potential roles for MAPK in modulating adhesion, through reduced binding, altered integrin expression and increased ECM biosynthesis/catabolism, these signalling mediators appear to display characteristics more consistent with 'inside-out' functions (Figure 1), rather than the 'outside-in' signalling paradigm initially ascribed. It is worth noting that integrin-linked kinase, a mediator of integrin-dependent proliferation, also exhibits 'inside-out' signalling, whereby its activation leads to decreased adhesion to the ECM [82].

CELL SHAPE AS AN INTEGRAL COMPONENT OF ECM/INTEGRIN SIGNALLING

It is well appreciated that alterations in ECM–integrin interactions cause changes in cell shape and behaviour [40,83–85]. Many investigators have attempted to segregate the effects of the ECM on morphology from integrin- and growth-factor-derived biochemical signalling and concomitant changes in gene expression. However, recent studies have demonstrated that ECM-dependent changes in cell shape and three-dimensional tissue architecture determine cell function by modulating integrin signalling pathways. For example, when cultured on an exogenous basement membrane, normal mammary epithelial cells adopt a polarized cuboidal morphology, become quiescent and express high levels of β-casein. Although expression of β-casein depends upon basement-membrane laminin interacting with β1 integrins and activation of a tyrosine phosphorylation signalling cascade, if mammary epithelial cells are forced to spread on laminin, while maintaining their interaction with β1 integrins, expression of β-casein is suppressed [84]. A role for cell shape impacting upon integrin-dependent signalling pathways has also been demonstrated in angiogenic endothelial cells, which degrade their existing basement membrane and proliferate in response to bFGF. The proliferation and subsequent survival of the endothelial cell depends upon adhesion to a provisional ECM via the αvβ3 integrin, as blockade of αvβ3 integrins results in a

failure to proliferate due to unscheduled apoptosis [86]. However, occupation and ligation of $\alpha v\beta 3$ integrins using anti-integrin antibodies fails to support endothelial-cell proliferation and survival if the cells are prevented from acquiring a spread morphology [87]. It could be postulated, then, that cellular rounding leads to apoptosis due to decreased integrin ligation, and therefore less signalling. However, by culturing endothelial cells on a defined concentration and area of different ECM ligands, while altering the extent of cell spreading, Chen et al. [85] demonstrated that cell spreading alone was conducive to proliferation, whereas cellular rounding was associated with apoptosis. Thus cell shape appears to profoundly modulate the processing of signals generated by identical ECM–integrin interactions.

HOW DOES CELL SHAPE INFLUENCE INTRACELLULAR SIGNALLING?

Given that integrin-dependent functions can be dramatically altered by cell shape, it might be expected that changes in morphology modulate either the magnitude or the duration of integrin-activated signalling mediators, such as MAPKs. Indeed, induction of MAPK activity and cell proliferation occur in fibroblasts only when cells adhere to and spread on tenascin using $\alpha v\beta 3$ and $\alpha 9\beta 1$ integrins, whereas adhesion to tenascin via the $\alpha v\beta 6$ integrin, which does not support cell spreading, fails to induce MAPK activity and cell proliferation [50]. Similarly, although growth factors can activate the upstream mediators Ras and Raf in non-transformed cells cultured in suspension, the subsequent activation of ERK2 occurs only when cells adhere to fibronectin [88]. Together these results support the hypothesis that ECM-directed cell morphology and associated cytoskeletal changes may spatially co-ordinate cell receptors and intracellular signalling molecules, thereby permitting upstream effectors, including focal adhesion proteins such as FAK, Ras and Raf, to couple with their downstream targets, including MAPKs (Figure 2).

Other studies suggest that morphological influences on MAPK activation may be more complex. For example, whereas PDGF can transiently and rapidly elevate ERK activity in fibroblasts, cell spreading induced by adhesion to fibronectin resulted in a more gradual and sustained activation of ERK, which was then permissive for proliferation [3]. Similarly, Eliceiri et al. [89] showed that, whereas bFGF could induce a rapid increase in ERK phosphorylation in endothelial cells *in vivo*, sustained ERK activity was dependent on endothelial-cell adhesion to the ECM via the $\alpha v\beta 3$ integrin. Whether this sustained activation of MAPK reflects the gradual recruitment of growth-factor receptors into focal adhesions and enhanced MAPK activation is not known. Increasing evidence in both normal and transformed mammary epithelial cells also suggests that morphological changes evoked by the ECM and integrins can influence the duration of MAPK activity. In the absence of basement membrane, mammary epithelial cells display a flattened, spread morphology and exhibit prolonged MAPK activation. However, when these cells are cultured on basement membrane, they adopt a rounded morphology, and this is accompanied by transient activation of MAPK and growth arrest (C. Roskelley, personal communication). In each of these cases, rounded cells exhibit relatively short bursts of MAPK activity and do not proliferate, which would be consistent with the idea that MAPK must be suppressed or attenuated in order for adherent cells to differentiate. This may be due to the recently reported reciprocal cross-modulation between $\beta 1$ integrins and EGF-receptor-dependent MAPK activity that can only be achieved when mammary epithelial cells are allowed to attain a three-dimensional tissue organization within basement membrane material [90].

The duration of MAPK signalling may therefore determine whether a given cell will grow or differentiate [91]. As described above, basement-membrane-dependent mammary epithelial cell differentiation is associated with short bursts of MAPK signalling activity, whereas NGF-mediated cell-cycle withdrawal and neuronal differentiation in PC12 cells appears to require prolonged ERK signalling. In contrast, EGF treatment of PC12 cells elicits transient ERK activity and cellular proliferation. Woods et al. [92] have also demonstrated that low levels of Raf are accompanied by induction of cyclin D1 and proliferation, whereas higher, sustained levels of Raf lead to induction of p21 and cell-cycle arrest. Thus, although it appears that the duration of MAPK signalling determines whether a specific cell type will grow or differentiate, it is still not clear how specialized cell morphologies may influence these activities. This idea is further complicated by the fact that, in certain cell types, activation of MAPK or other signalling mediators is seemingly unaffected by changes in cellular morphology. For example, PDGF-treated human arterial smooth-muscle cells display DNA synthesis only when attached and spread on fibronectin. Blocking the $\alpha 5\beta 1$ integrin prevents cell spreading and attenuates PDGF-induced

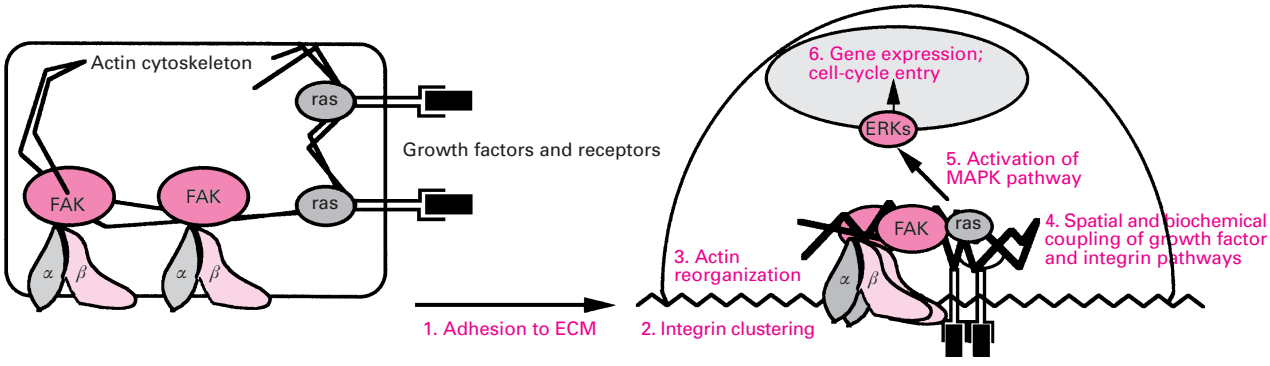

Figure 2 Cell-shape-dependent coupling of the ECM/integrin and growth-factor-receptor tyrosine kinase pathways

Hypothetical model showing the influence of integrin-dependent changes in cell shape on MAPK signalling and downstream cellular functions. Cell adhesion to the ECM via integrins activates FAK, leading to reorganization of the actin cytoskeleton, and subsequently to changes in cell shape. In turn, cytoskeletal-associated proteins, including integrins and growth-factor receptors, couple within focal adhesions, thereby interacting with and activating the ERK signal-transduction pathway.

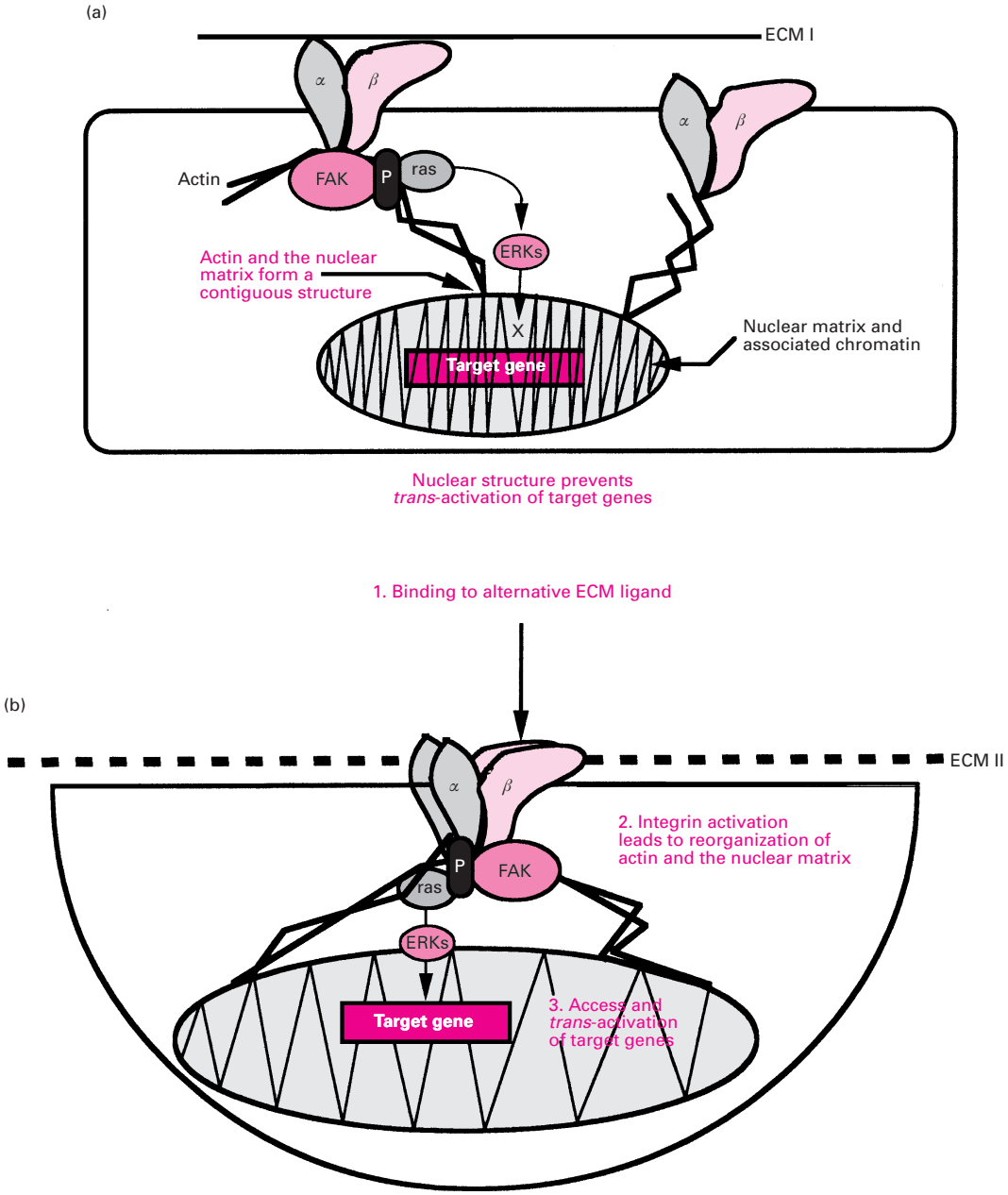

Figure 3 ECM- and nuclear-matrix-dependent regulation of gene expression

Hypothetical model showing how altered cell adhesion to the ECM via integrins may regulate the access of transcription factors to target genes. On the first ECM substrate (**a**), transcription factors are present within the cell, but they are physically unable to interact with their target genes due to condensation of chromatin. However, following ligation of cells to alternative integrins (**b**), cell morphology changes at the level of the actin cytoskeleton. In turn, reorganization of the nuclear matrix and associated chromatin, which is contiguous with the actin cytoskeleton, allows access of transcription factors to target genes.

DNA synthesis, but has no influence on the magnitude of ERK signalling or its translocation to the nucleus [93]. Also, adhesion to fibrillar polymerized collagen via the $\alpha 2\beta 1$ integrin suppresses the proliferation of smooth-muscle cells, while adhesion to denatured monomeric collagen via this same integrin induces proliferation and changes in morphology, independent of the level of MAPK activity [94]. In both of these examples, it was suggested that smooth-muscle cells are likely to use MAPK-independent pathways to undergo integrin-dependent proliferation; however, it was not determined whether inhibition of existing MAPK activity in the longer term might interfere with this response.

An equally attractive idea is that cell morphology may alter the accessibility of ERK to its downstream target genes, and thus prevent activation of the cell-cycle machinery. We hypothesized previously that the structural continuity that exists between the ECM, integrins, the cytoskeleton and the nuclear matrix may regulate the access of transcription factors to their target genes, perhaps through modulation of histones and chromatin structure [5,13]. Consistent with this notion, in mammary epithelial cells, where $\beta 1$ integrin ligation, tyrosine kinase signalling and morphological changes induced by basement membrane are all required for transcriptional activation of the milk protein β-casein, access of transcription factors to the β-casein gene depends

ultimately upon chromatin organization [95]. Therefore it is likely not only that cell shape permits the interaction of upstream effectors, such as Ras and Raf, with downstream mediators including ERKs, but that cell shape also influences the interaction of these downstream mediators with their growth- or differentiation-specific target genes at the level of nuclear structure (Figure 3).

In order to understand further how such ECM/integrin-dependent 'cell shape' models operate to control cell behaviour, it will clearly be necessary to identify additional proteins that are able to co-ordinate both intracellular signalling pathways and cell shape. To this end, the recent discovery that the Rho family of small GTPases are able to relay integrin-derived signals, as well as organize the actin cytoskeleton [96–99], suggests that these proteins are well poised to integrate cell shape and function.

Rho GTPases INTEGRATE CELL SHAPE AND FUNCTION

Like all members of the Ras superfamily, Rho proteins act as molecular switches that cycle between the active GTP-bound and inactive GDP-bound states. This cycling is controlled by GDP/GTP exchange factors, GTPase-activating proteins and guanine nucleotide dissociation inhibitors.

The ability of Rho proteins to organize the actin cytoskeleton has been well demonstrated in microinjection experiments. Introduction of activated Rho protein into fibroblasts causes bundling of actin filaments into stress fibres, Rac induces the formation of membrane ruffles and lamellipodia, and Cdc42 induces the formation of filopodia [100]. In addition, Rho GTPases control other actin-dependent cellular processes, including thrombin-induced retraction in neurites [101]. In human endothelial cells, Rho is involved in the maintenance of their barrier function, whereas Rac participates in cytoskeletal remodelling induced by thrombin [102]. In each case, the actin structures specified by each of these Rho GTPases are associated with clustering of integrins and other proteins within focal adhesion complexes. Indeed, the assembly of integrin complexes requires both the ECM and intracellular Rac and Rho GTPases [103]. Furthermore, cross-talk between different Rho proteins has also been observed, such that Cdc42 can activate Rac, which in turn can activate Rho [100]. How Rho GTPases regulate the actin cytoskeleton has been the subject of intensive research. PRK2, a Ser/Thr kinase related to protein kinase C, has been shown to be a potential effector target of Rho and Rac GTPases, and is able to regulate organization of the actin cytoskeleton [104]; and citron kinase, which regulates the actin-dependent process of cytokinesis, has been shown to be a downstream target of Rho [105]. In addition, GTP-bound Rho and Rac interact with and activate PtdIns4*P* 5-kinase, and in this way may increase PtdIns(4,5)P_2 production and its associated actin-polymerizing functions [106].

In addition to these effects on the actin cytoskeleton, activated Rho GTPases also control cell proliferation [107] and gene transcription via their effects on integrin-dependent intracellular signalling pathways [106]. Fibroblast spreading on fibronectin, or on an anti-β1-integrin antibody, results in activation of a p21-activated kinase (PAK) kinase, a downstream target of Rac and Cdc42 [108–111]. Furthermore, disruption of actin with cytochalasin D, or microinjection of dominant-negative mutants of Rac and Cdc42, inhibits cell spreading, indicating that Rac and Cdc42 are required for this process [108]. Regulation of the collagenase-1 gene in synovial fibroblasts by the $\alpha5\beta1$ integrin receptor is dependent upon cell shape changes induced by Rac [112]. Also, activation of Cdc42 or Rac has been shown to disrupt mammary epithelial morphogenesis and functional differentiation [113], a process that is wholly dependent upon integrins [13].

CONCLUSION

In order to understand how the ECM and soluble factors affect cell morphology and biochemical signalling pathways in a co-ordinate manner to influence cellular behaviour, it must be emphasized that, although blocking or distorting each of these variables alone is sufficient to impair the biological response in many cases, ECM ligands and receptors, biochemical signals or cell or tissue morphology in isolation are not sufficient to elicit an appropriate biological response. Generation of signals without the proper morphology, or acquiring a particular morphology without the appropriate signals, more often than not results in cells undergoing apoptosis. Thus it is critical that these factors be co-ordinated, a role which is fulfilled by tissue-specific ECMs. The multivalent nature of complex tissue-specific ECMs provides an array of ligands to interact with multiple cell-surface integrins, which, in addition to generating biochemical phosphorylation cascades utilizing many similar mediators, also supports distinct, unique morphologies which can then properly interpret and direct these incoming responses.

We thank Sean Egan, Connie Myers and Calvin Roskelley for their helpful comments during the preparation of this review.

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