Bidirectional extracellular matrix signaling during tissue morphogenesis

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Abstract

Normal tissue development and function are regulated by the interplay between cells and their surrounding extracellular matrix (ECM). The ECM provides biochemical and mechanical contextual information that is conveyed from the cell membrane through the cytoskeleton to the nucleus to direct cell phenotype. Cells, in turn, remodel the ECM and thereby sculpt their local microenvironment. Here we review the mechanisms by which cells interact with, respond to, and influence the ECM, with particular emphasis placed on the role of this bidirectional communication during tissue morphogenesis. We also discuss the implications for successful engineering of functional tissues ex vivo.

Keywords

mechanotransduction; dynamic reciprocity; pattern formation

1. Introduction

The extracellular matrix (ECM) arguably influences almost every decision made by the cells it contacts. ECM provides mechanical support and biochemical signals, both of which can affect cytoskeletal structure, chromatin organization, and gene transcription. Cells, in turn, reorganize and remodel the ECM and thus play an active role in sculpting their surrounding environment and in directing their own phenotypes. This concept of bidirectional communication from the ECM to the cytoskeleton to the nucleus and back again was put forth almost at the same time as the community recognized the likely existence of ECM receptors [1,2] and appears to play a fundamental role in tissue development, differentiation, homeostasis, and disease progression.

ECM and its receptors are highly conserved. ECM proteins, especially components of the basement membrane, are found in the tissues of nearly all multicellular animals. Basement membrane forms at the blastula stage very early during mammalian embryonic development. Embryos with homozygous null mutations in laminin genes that prevent assembly of the basement membrane die early after implantation (reviewed in [3]). Dynamic reciprocal communication between cells and ECM is now considered to be essential during tissue morphogenesis and wound healing. Here, we review the mechanisms by which cells respond

to and remodel the ECM, with a specific focus on implications for the development and morphogenesis of native and engineered tissues.

2. ECM signaling to the nucleus – biochemical

Cells interact with the ECM via transmembrane receptors, including members of the integrin and dystroglycan families. Integrins comprise a large conserved family of α and β subunits which combine to form heterodimers that bind selectively to different ligands. Each subunit has a large extracellular domain, a single transmembrane domain, and a short cytoplasmic tail which contains well-defined motifs that act as binding sites for various structural and signaling proteins. ECM adhesion sites are thus multiprotein complexes that connect the ECM to the cytoskeleton and serve as platforms for key signaling proteins. Over 150 distinct proteins have been found at the cytoplasmic face of integrin-mediated adhesions [4]. Integrins and their downstream signaling are critical for development, as knockout mice for most integrin subunits are embryonic (or perinatal) lethal [5].

Integrin engagement leads to several downstream signaling events that can ultimately alter cell fate (Figure 1). Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that is activated by integrin signaling. Autophosphorylation of FAK creates high-affinity binding sites for Src-homology 2 (SH2) domains, leading to an interaction with Src-family kinases. Src in turn activates the Ras-extracellular signal-regulated kinase (ERK) signaling cascade, which modulates the contractile properties of the cell by regulating myosin dynamics through myosin light chain kinase. ERK also relays extracellular information by translocating to the nucleus, where it stimulates gene expression by phosphorylating transcription factors, including ternary complex factors (TCFs) such as Elk-1, which regulate genes by binding to serum response elements (SREs). In addition to signaling through the Ras-ERK pathway, integrin engagement leads to signaling through several other kinase pathways, including phosphatidylinositol 3' kinase (PI3K)/Akt, integrin-linked kinase (ILK), and Jun-N-terminal kinase (JNK). FAK, ILK, and JNK are all required for normal development: FAK, ILK, and JNK1/JNK2 knockout mice are embryonic lethal [6–8]. The final outcomes of these kinase signals generated by adhesion complexes often depend in part on the mechanical properties of the ECM (see below).

2.1 Signaling to cell survival pathways

In most types of adherent cells, detachment from the ECM induces a specific apoptotic pathway called anoikis [9]. Avoidance of anoikis leading to adhesion-independent cell growth is thought to be a key step in neoplastic transformation. In untransformed cells, detachment causes pro-apoptotic proteins to translocate to the mitochondrial membrane, leading to mitochondrial depolarization, release of cytochrome C into the cytoplasm, and activation of caspases (reviewed in [10]). Activated caspase-3 initiates a proteolytic cascade, causing cleavage of signaling molecules such as FAK and structural proteins such as nuclear lamins. The caspase cascade also induces specific enzymes responsible for DNA fragmentation, including caspase-activated DNase. The anoikis response is highly regulated throughout development and homeostasis, and can be ECM-specific in certain cell types. For example, basement membrane proteins, but not interstitital collagens, prevent apoptosis of mammary epithelial cells [11]. Integrin-mediated signaling through FAK, ILK, Src, PI3K, and ERK have all been shown to protect against anoikis (reviewed in [12]).

Detachment from ECM is not a universal death sentence, however, as recent studies have revealed specific survival pathways that are induced upon cell detachment. Autophagy is a highly regulated lysosomal self-digestion process that is stimulated upon cell detachment and promotes cell survival, in part by delaying induction of anoikis. For example, lack of integrin engagement has been shown to induce autophagy during cavitation of the lumen of mammary epithelial acini [13,14]. A similar process has been observed for lumen cavitation during

embryoid body formation [15], suggesting a conserved role during tissue development. The signaling pathways between the ECM and induction of autophagy remain to be elucidated, but involve increased expression of autophagy-related genes (reviewed in [16]).

2.2 Signaling to cell proliferation

Cell adhesion to the ECM elicits the same types of signals as do mitogens, and growth factor receptors are often found within adhesion complexes [17]. It is not surprising, then, that the proliferation of most adherent cell types involves cooperation between ECM receptors and growth factor or hormone receptor signaling. In non-transformed cells, progression through the cell cycle depends on regulated expression of cyclins, which form complexes with cyclin-dependent kinases (CDKs). Cyclin D1 is critical for progression through G1 phase, and its expression requires signaling from both the ECM and growth factors (reviewed in [18]). For example, coordinated signaling through the ERK cascade stimulates the expression of AP-1 transcription factors; an AP-1 binding site is found in the promoter of the cyclin D1 gene. Crosstalk is also important for hormone-mediated regulation of cell proliferation. Proliferation of mammary epithelial cells during pregnancy is regulated by estrogen and progesterone, but progression of these cells through the cell cycle requires simultaneous adhesion to basement membrane proteins [19,20]. This may be due in part to upregulation of estrogen receptor expression by the basement membrane [21]. Promoter regions of several ECM-regulated genes have been found to contain an ECM-response element, but the signaling between ECM to these regulatory sites is still an active area of investigation (reviewed in [22]).

2.3 Signaling to differentiation

ECM provides contextual signaling in the functional differentiation of cells from a number of organs, including those from the mammary gland, lung, and liver. Although lactogenic hormones such as prolactin induce mammary differentiation and milk protein secretion, sustained signaling through the prolactin receptor requires anchorage of β1-integrins [23]. This is due in part to specific integrin-mediated signals as well as to integrin-mediated establishment of basolateral polarity, which exposes the prolactin receptor to its ligand [24]. Type II alveolar epithelial cells, the cuboidal cells of the lung responsible for secreting surfactant, are also highly regulated by ECM composition; in the absence of appropriate cues, these cells differentiate into type I cells. This switch between phenotypes appears to be regulated in part by the degree of heparin sulfation within the surrounding ECM [25]. The differentiated function of hepatocytes has been explored in depth using ECM microarrays, which revealed that adhesion to collagen IV positively regulates albumin production [26].

A similar role for ECM is being uncovered in regulation of stem cell fate. Stem cells reside within niches, specialized microenvironments that promote both self-renewal and asymmetric division. Exit from the niche induces stem cell differentiation. Circumstantial evidence suggests that ECM adhesion may be a defining feature of stem cells: high levels of β1-integrin are a marker of stem cells of the mammary epithelium [27], the hair follicle and epidermis [28], and the central nervous system [29]. The niche, therefore, would be expected to include specific ECM proteins depending on the tissue or organ. This appears to be true, as neural stem cells within the subventricular zone of the adult brain reside in regions enriched for laminin-containing ECM [30] and epidermal stem cells lie adjacent to the basement membrane [31]. Similarly, LaBarge et al used combinatorial microenvironments to identify laminin-111 as a major putative component of the niche surrounding progenitor cells of the human mammary gland [32]. The signaling pathways downstream of ECM adhesion that regulate stem cell fate are an active area of investigation.

3. ECM signaling to the nucleus – mechanical

3.1 Mechanotransduction

The multitude of cells and tissues within multicellular organisms function as a single coordinated living unit by communicating signals, which in addition to biochemical, can be mechanical in origin. The mechanical state of a tissue is maintained and modulated by the cytoskeletal contractile activity of the cells along with the surrounding ECM. A growing body of evidence supports the notion that the mechanical properties of the ECM, in addition to providing structural support to cells, furnish instructive signals, which cells sense, integrate, and interpret to effect response, a process termed mechanotransduction [33].

Two kin theories aimed at explaining the phenomenon whereby the mechanical environment and perturbations therein can elicit cellular response are Ingber's tensegrity [34–36] and Bissell's dynamic reciprocity [1,22] models, which appeared around the same time. Both of these models postulate the existence of physical continuity within tissues, extending from the ECM to the nucleus itself (Figure 2). It is this architectural network that allows external mechanical information to be transmitted to the interior of the cell and the nucleus, where it could affect structural rearrangements ultimately resulting in changes in gene expression. Experimental studies have confirmed many aspects of these theories. It is now widely recognized that the actin cytoskeleton is mechanically coupled to ECM proteins at focal adhesions comprised of integrins and other structural and signaling proteins [37]. The physical continuity at the opposite end – between the cytoskeleton and the nucleus – is still largely speculative. Nevertheless, a number of molecular candidates that bridge chromatin and the cytoskeleton have been identified (reviewed in [38]). Actin fibers bind the KASH domain of nesprins, large proteins that span the outer nuclear membrane and project into the cytoplasm [39], which in turn interact with the SUN domain of proteins integral to the inner nuclear membrane, providing a mechanical bridge between the cytoskeleton and the nuclear membrane [40]. Furthermore, the nuclear lamina, a thin mesh-like structure associated with the inner nuclear membrane, forms scaffolds which bind the SUN/KASH complex [41]. Finally, the nuclear lamina is coupled to chromatin either directly or indirectly through associated proteins [38]. The molecular sequence described here forms a physical link that spans the distance between ECM and chromatin, theoretically allowing for outside-in force transmission. The concept of ECM-originating force being channeled to the nucleus is confirmed by "intracellular" traction force microscopy studies, where force applied locally to apical integrins is transmitted to basal focal adhesions and the nucleus [42,43]. Other studies demonstrate that exerting force on integrins by manipulating bound magnetic microbeads leads to cytoskeletal rearrangements, organelle movement, nuclear shape changes, and nucleolar rearrangements [44,45].

3.2 Tension-mediated signaling

How does the physical continuity between the ECM and nucleus enable mechanical signaling? One hypothesis is that the cytoplasm is a site of solid-state biochemistry: Many of the molecules that mediate DNA synthesis, transcription, protein synthesis, and signal transduction do not diffuse freely in the cytoplasm, but rather are immobilized on cytoskeletal fibers [36,46]. Thus, any mechanical perturbations taking place in the ECM can propagate to the cell's interior and cause structural rearrangement of the cytoskeleton and the immobilized proteins, potentially altering their activity. Studies of molecular mechanics reveal a number of structural motifs that respond to applied force (reviewed by [33]). Mechanical forces can expose cryptic protein sequences that are otherwise unavailable in the unstressed state for interaction with cell surface receptors. For instance, contractile force generated by cells is sufficient to partially unfold fibronectin [47]. In addition, many enzymes exhibit altered activity in response to mechanical

stress (reviewed in [48,49]), enabling another mechanism for transduction of mechanical into biochemical signals.

Mechanotransduction may proceed through direct mechanical impingement upon chromatin, initiated by cues transmitted from the cell exterior. The work of Dalby et al demonstrates that changes in substratum adhesion of fibroblasts leads to changes in the position of chromosomes and altered gene expression [50–52]. One could speculate that, in analogy to force-mediated protein unfolding, mechanical stresses experienced by chromatin may induce unraveling of certain DNA motifs, making them more accessible to transcription factors and thus influencing gene expression. Physical forces open mechanosensitive ion channels that are tethered to ECM or cytoskeletal filaments, regulating ionic transport into the cell [53,54]. Applying sustained nanonewton-level force to integrins results in calcium influx [55], and applying shear to integrins triggers the cAMP signaling cascade, which activates transcription of genes containing multiple cAMP-response elements [56]. In these studies, force applied to other transmembrane receptors does not influence signaling, indicating that the response is specific to integrins. Perturbing the cell mechanical environment by increasing ECM stiffness activates the small GTPase Rho [57]. Active Rho promotes actomyosin stress fiber assembly [58], rendering the cell mechanically more tensed [59]. The balance between two effectors of Rho, mDia and Rho kinase, regulates the G1/S-phase transition via the degradation of p27, a CDK inhibitor [60]. Stiffer matrices have also been shown to disrupt cadherin-based intercellular junctions, leading to loss of tissue architecture and altered junctional localization of β-catenin [57].

3.3 Stem cells and morphogenesis

Increasing evidence implicates the tensional state of a tissue, to a great extent dictated by the ECM, as a key regulator of differentiation, morphogenesis, homeostasis, and disease progression. Cellular geometry and spreading control the transition of cells between proliferation and apoptosis [61], as well as the ability of a cell to undergo epithelial-mesenchymal transition (EMT) [62]. The mechanical properties of the microenvironment control the differentiation of stem cells: Human mesenchymal stem cells differentiate down lineages depending upon matrix stiffness [63]. By micropatterning endothelial and epithelial cells on adhesive islands of controlled geometry, Nelson et al found that patterns of cell proliferation correlate with patterns of mechanical tension within tissues [64]. These mechanically-regulated basic cellular processes (proliferation, differentiation, apoptosis) are finely orchestrated within the developing embryo to give rise to a complex multicellular organism. Thus, one could imagine a role for physical force in the control of development on a larger scale. Indeed, a number of recent studies implicate mechanics as a regulator of embryogenesis (reviewed in [65]). Mesoderm and notochord stiff enough to resist buckling are needed during *Xenopus laevis* gastrulation [66,67], and actomyosin contractility is required for dorsal closure in the *Drosophila melanogaster* embryo [68,69]. Twist – a master regulator of cell shape changes during gastrulation – has been implicated as a major player in the mechanical control of embryogenesis. Applying both tensile and compressive force on *Drosophila* embryos results in increased Twist expression [70,71], whereas laser ablation of dorsal epithelium leads to reduced levels of Twist [70].

Tissue contractility has also been implicated in organogenesis during embryonic development. Down-modulating Rho-mediated tension inhibits branching morphogenesis of the embryonic lung [72] and kidney [73], whereas increasing tension promotes branching [74]. The mechanotransduction pathways that regulate branching are unclear. Moore et al observed that branch initiation is preceded by local thinning of the basement membrane and speculated that the budding process is foremost mechanical – ECM degradation physically enables the adjacent pre-stressed epithelium to invade, analogous to a "run in a stocking" [72]. The events that

prompt the initial ECM degradation, however, are unknown. One could also speculate that the mechanical differentials originate in the epithelium rather than the stroma. Based on results showing that cells exposed to elevated mechanical stress exhibit high proliferation rates [64] and higher responsiveness to growth factors [75], one could imagine that epithelial regions experiencing higher stress could acquire a more invasive motile phenotype and thereby undergo branching.

4. ECM remodeling – biochemical

The cell responds to its microenvironment, but also plays an active role in sculpting the surrounding ECM. During tissue development, differentiation, and homeostasis, the ECM is continuously remodeled by its resident cells. The ECM is comprised of a large variety of proteins, proteoglycans, and hyaluronan, and the synthesis of each molecule depends on organ, disease status, and age. In addition, many of the protein constituents of the ECM are regulated by alternative splicing. At least 20 different isoforms of fibronectin are expressed in humans, with higher expression of specific isoforms during embryonic development, wound healing, angiogenesis, and fibrosis (reviewed in [76]). Hyaluronan also shows age-dependent expression patterns, with high expression during embryogenesis and decreasing expression as the organism ages [77]. Hyaluronan synthase-2 is required for embryonic development; null embryos die of severe heart defects midway through gestation, including a lack of heart valves due to endocardial cushion cells failing to undergo EMT [78]. Our basic understanding of tissue development and morphogenesis would be significantly enhanced by revelation of the signals and mechanisms that differentially and temporally regulate ECM expression.

The structure and biochemical properties of existing ECM polymers are altered via proteolytic cleavage. Several families of proteases, including matrix metalloproteinases (MMPs), serine proteases, and cysteine proteases are actively secreted by cells. The MMP family consists of more than 20 structurally related zinc-dependent proteases that can degrade ECM proteins, cell surface receptors, and other molecules. Most family members are secreted from the cell, including collagenase-1/MMP1 (which was identified in studies of tadpole tail metamorphosis [79]), but others are associated with the cell membrane and provide localized proteolysis. Cells use MMPs to remove steric barriers during migration, to produce cleavage fragments with unique biological activities, and to remodel intercellular junctions during morphogenesis (reviewed in [80]). Despite their broad usage and highly regulated expression and activation, MMP knockout mice survive to birth, suggesting either that other proteases dominate embryonic remodeling or that MMPs are highly functionally redundant. There is also ample crosstalk between members of the different protease families during ECM remodeling. Intracellular serine proteases, such as furins, cleave and activate the transmembrane MMP, MMP14. Urokinase plasminogen activator (uPA), also a serine protease, activates plasmin, which can cleave ECM proteins as well as activate MMPs. Misregulation of protease function is a hallmark of diseases including chronic inflammation and cancer.

5. ECM remodeling – mechanical

In addition to biochemical signals, the ECM is remodeled by mechanical force (Figure 3). Early experiments in which cells were plated on silicone rubber substrata [81], as well as later studies using techniques such as traction force microscopy [82,83] and subcellular-scale microarrays of posts [84] all demonstrated that cells pull on their substrata with nanonewton-scale forces. It is these forces, generated at integrin-ligand interaction points, which actively remodel the fibrous three-dimensional (3D) matrices in which cells reside *in vivo*. Confocal reflection microscopy studies revealed that migrating cells such as fibroblasts and tumor cells radially align surrounding matrix fibers towards the cell body in structures ranging from 50 to 200 µm in length [85]. Fibroblasts embedded in 3D collagen gels can deform and compact their matrix

by approximately two orders of magnitude [86]. Mechanical remodeling of the ECM is dependent on the contractile activity of the cells. Activation of the Rho pathway and the subsequent assembly of stress fibers results in integrin clustering, focal adhesion maturation, and elevated traction forces at the cell-matrix interface [57,63]. This contractility leads to assembly of fibronectin matrix: Inhibition of actomyosin stress fiber formation results in reduced fibronectin fibril assembly, whereas stimulation of Rho- or myosin light chain kinase-mediated contractility promotes assembly [87]. Inhibiting cellular contractility also abolishes collagen gel contraction [88].

Mechanical remodeling of the ECM is often self-regulated and the result of cell-matrix crosstalk. Mechanical stiffening of the ECM itself can lead to Rho activation [57], which, as discussed above, results in mechanical remodeling of the matrix through focal adhesion maturation and increased force generation by the cell. These processes ultimately lead to increased matrix traction and compaction, further elevating local ECM stiffness. This positive feedback is balanced by negative regulation, in the form of mechanically-induced biochemical matrix remodeling. The mechanical state of the ECM can elicit a complex proteolytic response in the cell through altered production levels of MMP2 and MMP9, responsible for matrix turnover. In particular, it has been demonstrated that the levels of both proteases dramatically increase upon mechanical loading of soft matrices, whereas mechanical loading of stiff matrices leads to an increase in MMP9 production only [89]. Thus, two mechanical parameters that characterize the ECM – stiffness and load – both control the extent of matrix remodeling through biochemical degradation, which ultimately results in altered mechanical properties. This tightly regulated cell-matrix crosstalk could be an essential mechanism by which tissues finely tune their mechanical state, thus adapting to changes during development, disease progression, or aging.

6. ECM in engineered tissues

Functional engineered tissues, whether for use in regenerative medicine or in studies of basic biology, must include both the cells and their surrounding ECM. As discussed above, cells within native tissues are constantly communicating with and influencing their microenvironments; the same is true for those in man-made tissues. One challenge in the field is to build tissues containing ECMs that recapitulate both the biochemical and mechanical properties of native ECMs in vivo. For tissues to undergo proper development, differentiation, and homeostasis, the ECM necessarily contains signaling moieties and cleavage sites to enable appropriate remodeling. The ECM is also not a homogeneous mixture of proteins – it follows the heterogeneity of the tissue and itself is heterogeneous on the micrometer length scale.

Strategies to mimic native ECM have been reviewed extensively elsewhere [90–92]. Efforts are currently biased towards synthetic ECMs in which a biologically inert polymer, such as polyethylene glycol, is modified to contain sites that support cell adhesion and/or proteolytically-mediated degradation [93]. The advantages of this strategy are the high degree of control and batch-to-batch consistency; one major disadvantage is the inherent presumption that we currently understand all of the signaling properties endowed by any one particular ECM molecule within a given tissue. An orthogonal strategy is to use chemically modified natural ECM polymers, such as derivatives of hyaluronan [94]. Finally, unmodified natural ECM molecules, including type I collagen and fibrin, have been used extensively in studies aimed at recapitulating native tissue structure. Aside from maintaining known and undiscovered adhesion moities, natural ECMs can be manipulated to achieve microscale heterogeneities, including channels and networks [95–97], which permit engineering of functional microscale tissues.

7. Perspective

Cells and the ECM communicate in a dialogue that is dynamic and constantly evolving over the lifetime of the tissue. Cell and developmental biologists are actively trying to understand the signals conveyed by the ECM and how those signals are translated into functional cellular responses. Engineers, on the other hand, are trying to mimic the ECM half of the conversation. Advances in building engineered tissues will require designer ECMs, either synthetic or natural, that not only "speak" to cells, but also "listen" to them.

Abbreviations

CDK, cyclin-dependent kinase
ECM, extracellular matrix
EMT, epithelial-mesenchymal transition
ERK, extracellular signal-regulated kinase
FAK, focal adhesion kinase
ILK, integrin-linked kinase
PI3K, phosphatidylinositol 3' kinase
JNK, Jun-N-terminal kinase
MMP, matrix metalloproteinase
SH2, Src-homology 2
3D, three-dimension(al)

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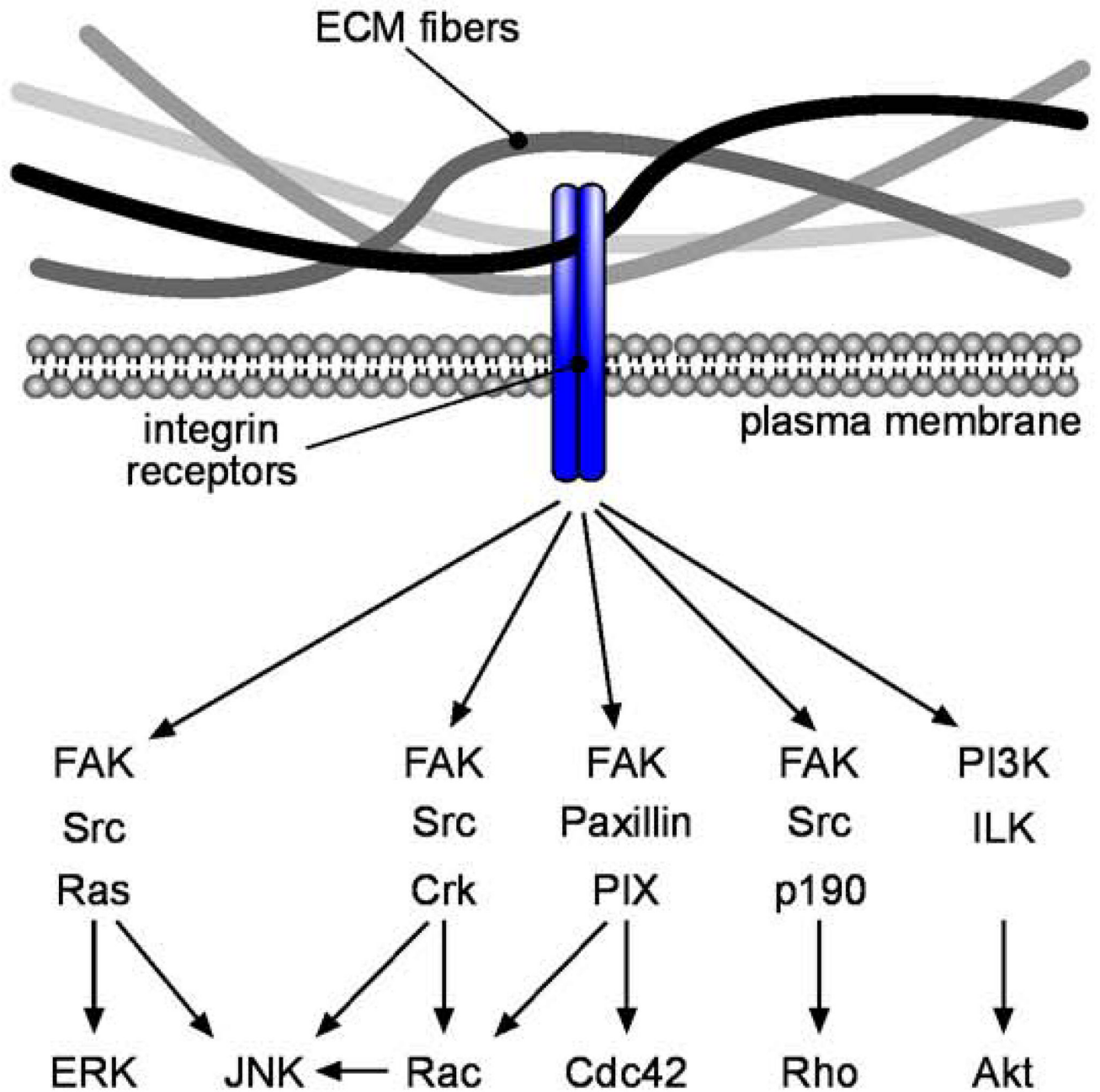
Figure 1. Biochemical signaling from ECM to the nucleus
Integrin signaling activates a large number of pathways, many of which induce cytoskeletal alterations. Kinase signaling cascades lead to gene transcription changes in the nucleus.
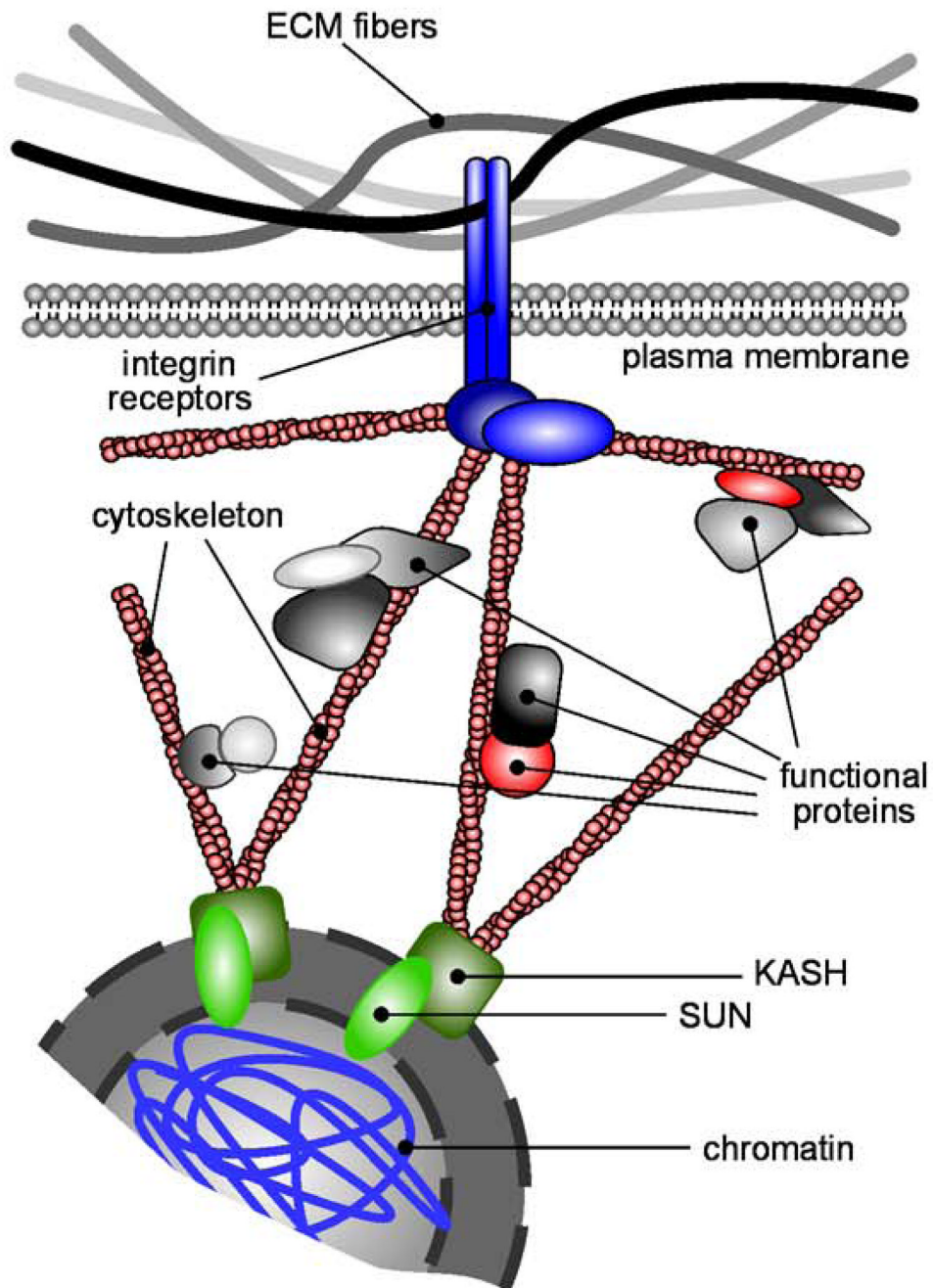
Figure 2. Mechanical signaling from ECM to the nucleus
The cytoskeleton physically links the ECM to chromatin and functional proteins in the cytoplasm. Mechanical perturbations in the ECM can propagate to the interior of the cell, structurally rearranging proteins and DNA tethered to the cytoskeleton and thus altering biochemical signaling.
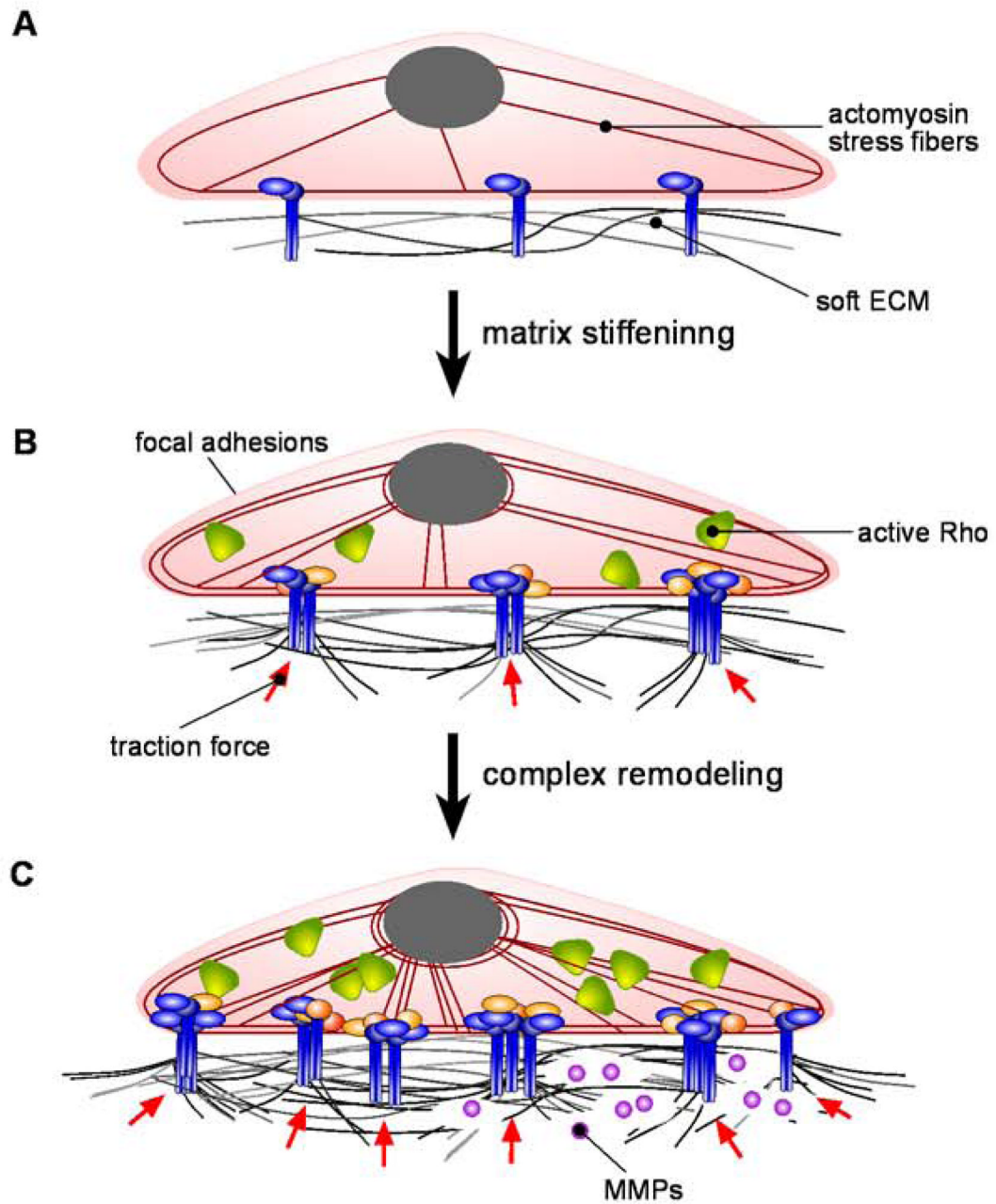
Figure 3. Mechanical remodeling of the ECM
(A) Cells residing within soft matrices are minimally mechanically active. (B) ECM stiffening causes Rho activation, stress fiber formation, integrin clustering and force generation. (C) These events can elicit a complex response: positive mechanical feedback resulting in further matrix compaction and stiffening, or proteolytic degradation of the ECM through increased MMP production.