Review TRENDS in Cell Biology Vol.16 No.4 April 2006

Substrate rigidity and force define form through tyrosine phosphatase and kinase pathways

Grégory Giannone[1,2] and Michael P. Sheetz[1]

[1]Department of Biological Sciences, Columbia University, New York, NY10027, USA
[2]CNRS, UMR 5091, Universite Bordeaux 2, 33077 Bordeaux, France

Cell forces define cell morphology, alterations in which are caused by tyrosine kinase and phosphatase mutations, which implies a causal linkage. Recent studies have shown that phosphotyrosine signaling is involved in force sensing for cells on flat surfaces. Early force-dependent activation of Src family kinases by phosphatases or cytoskeleton stretch leads to the activation of downstream signaling. In addition, force generation by cells depends on a feedback mechanism between matrix rigidity or force generation and myosin contractility. Components of the force-sensing pathway are linked to the integrin–cytoskeleton complex at sites of force application and serve as scaffolds for signaling processes. Thus, early events in force detection are mechanically induced cytoskeletal changes that result in biochemical signals to mechanoresponsive pathways that then regulate cell form.

Introduction

Architectural remodeling and changes in tissue tension are common in living tissues (Box 1). There are local tension changes during the addition or removal of cells, cell movements linked to morphogenesis, muscle contraction and relaxation, as well as during bone compression and decompression. Therefore, cell–extracellular matrix (ECM) and cell–cell contacts are subjected to force fluctuations and adjust to changes in tension. Studies demonstrate that mechanical factors affect cellular functions. At the level of cell growth and viability, normal cells require a rigid substrate or internal cytoskeletal tension for growth, whereas transformed cells have lost this requirement [1,2]. ECM rigidity and shear flow can alter migration [3,4], tyrosine kinase activities [5–7], gene expression [8,9] and cellular differentiation [10,11] in various cell types. Force transducers involved in mechanosensation are ion channels that convert mechanical force into an electrical or chemical signal [12]. However, the detailed mechanisms of force transduction in responses to the ECM during adhesion, migration or cell differentiation have yet to be identified.

Corresponding author: Sheetz, M.P. (ms2001@columbia.edu).
Available online 10 March 2006

Integrins are important plasma membrane proteins that bind to the ECM, and antibodies directed against them can block cellular adhesion and migration. Integrin binding to the ECM induces the clustering and recruitment of scaffolding proteins that connect integrins to the actin cytoskeleton [13,14], activation of tyrosine kinase and phosphatase signaling, and the coupling of cell-generated forces with the ECM (reviewed in [15]). As transmembrane linkers that are involved in adhesion and motility, integrins are involved in the transduction of ECM rigidity to modifications of cellular morphology. Forces applied to ECM–integrin–cytoskeleton connections, which can be generated by internal actin or external ECM motion, induce maturation of adhesion sites to focal adhesions, which are coupled to bundles of actin called stress fibers [16,17] (Box 2). Conversely, loss of force triggers the disassembly of stress fibers and adhesion sites [16]. These observations indicate that structural and signaling functions of ECM–integrin–cytoskeleton molecular complexes are modified depending on the magnitude of the forces.

Early evidence that forces exerted by the ECM proteins and rigidity are sensed through integrin adhesions was acquired using magnetic and optical tweezers in endothelial cells [18] and fibroblasts [14]. Beads coated with integrin ligands were twisted or restrained at the cell surface, mimicking the forces that are generated during adhesion-site formation. Local generation of forces caused cytoskeletal stiffening or the mechanical reinforcement of integrin–cytoskeleton linkages, which increased in direct proportion to the applied stress. Although general changes in the cytoskeletal rigidity have been described as a result of external forces [19], adhesion site initiation and maturation occur locally where the forces are applied in fibroblasts [14,16,17,20–22] or in the direction of shear flow in endothelial cells [4]. Additional studies showed that forces affect integrin-dependent adhesion properties [23], linkage to the actin cytoskeleton [14] and recruitment and activation of signaling proteins [6].

Rigidity responses are difficult to understand because local, transient mechanical perturbations can be converted to distinct biochemical signals that have either limited or global effects on cellular functions. These temporally and spatially restricted signals depend on tyrosine kinase and/or phosphatase activities in the adhesion sites at the junction between integrins and the

 0962-8924/$ - see front matter doi:10.1016/j.tcb.2006.02.005

Box 1. 2D versus 3D matrices

Although most studies of cell adhesion, migration and rigidity responses have been performed in a two-dimensional (2D) environment, several studies performed in three-dimensional (3D) environments have demonstrated that adhesion sites, actin cytoskeleton structures, integrins signaling and cell morphology are altered compared with 2D [69,70]. Furthermore, the rigidity of the 2D environment, usually glass or plastic, used in most studies is higher than the rigidity of 3D matrices encountered in normal tissue [24]. Therefore, one should be careful extrapolating from 2D to 3D matrices. For example, mature focal adhesions and flat lamellipodia, which are a hallmark of cells migrating on 2D matrices, do not have an equivalent in 3D matrices [69,70]. However, some cell behavior is seen in both. Cells migrate towards a more rigid substrate [3,69,71] and this migration involves cycles of contraction and relaxation, and of extension and retraction [49,50].

cytoskeleton, that is, at the first intracellular molecular complex that will be subjected to the application of force. Local deformation can be either mechanically propagated to remote parts of the cell by the connecting cytoskeleton or converted to a global contraction or relaxation signal and therefore dictate cell shape. However, recent studies have found that confined rigidity response events that are dependent on phosphotyrosine signaling control locally and temporally the formation, stabilization and disassembly of adhesion sites and associated cytoskeletal structures. Consequently, we suggest that the integration of local changes in contractile state and motility behavior results in major effects on cell morphology.

Alteration of tyrosine kinase activity affects rigidity-dependent growth, migration and cell morphology

Early observations linked transformation to uncontrolled cellular growth and to profound alterations in cell shape and migration. Transformation is often characterized by deregulation of tyrosine kinase and phosphatase activity. The first defined oncogene, vSrc, encodes an early recognized tyrosine kinase. In most studies on tumor cells, changes in morphology, but not cytoskeletal dynamics, have been reported. However, changes in environmental factors (i.e. changes in ECM rigidity) and internal force generation (i.e. inappropriate rigidity responses) might be key factors in determining transformed cell morphology and the malignant phenotype [24] (Box 3). Certain transformed cells in culture have an altered morphology (they are typically rounder) and are more refractive than are normal cells (Figure 1a). In transformed cells, the macromolecular structures that are responsible for cell morphology and migration are affected. Focal adhesions can be replaced by podosomes and stress fibers can be absent [25,26] (Figure 1b,c). Transformed cells acquire anchorage independence, that is, they can grow without attachment to a substrate, suggesting rigidity response deregulation [1,2]. For example, transformed cells generate weak, poorly coordinated traction forces [27]. However, transformation is also associated with increased contractility (Box 3).

Cell motility depends on substrate density and rigidity and, therefore, also on the processes that respond to rigidity (Box 4) [28]. Many of the proteins involved in the rigidity response have been linked to motility disorders, including cancer, malformations in development and neuronal connectivity. Src family kinases (SFKs) [29,30], focal adhesion kinase (FAK) [31,32], the SH2 domain-containing phosphatase SHP-2 [32] and receptor-like protein tyrosine phosphatases (RPTPs) [22] are important components of the force-dependent signal transduction pathways that lead to the assembly of adhesion sites. The force-dependent initiation of adhesion sites, named reinforcement, occurs in protruding portions of cells, where adhesion sites can transmit cell propulsive forces [20,27]. In extending regions of the cell, forces are generated on integrins by actin rearward flow rather than stress fibers. At the trailing end of the cell, mature focal adhesions create passive resistance during cell migration. To overcome this resistance, high forces must be generated by nascent adhesion sites [20]. However, in some static cells, higher forces are correlated with mature

Box 2. From nascent ECM–integrin–cytoskeleton connections to fibrillar adhesion sites

Recruitment of integrin-associated proteins to nascent adhesion sites is hierarchical. Integrin aggregation or occupancy can be controlled using micrometer-sized beads coated with ECM proteins or antibodies, possibly in combination with soluble integrin ligand [13,14]. Forces can be applied to these coated-beads by optical or magnetic tweezers. Binding of specific cytoplasmic proteins to integrin adhesions can be triggered by integrin aggregation, a combination of aggregation and matrix ligand binding, and aggregation and/or occupancy in addition to force generation [13,14,21]. After integrins interact with the clustered ECM, scaffolding proteins connect integrins to the actin cytoskeleton in motion. Nascent ECM–integrin–cytoskeleton connections develop to focal complexes, which are defined as early adhesion sites and are localized at the cell periphery [15]. Force generated by myosin II is responsible for the maturation of focal complexes to focal adhesions [17,46], which require sustained forces for their stabilization [16] and are connected to stress fibers. Further maturation leads to the transition from mature focal adhesions to fibrillar adhesion sites [62]. The nomenclature of adhesion sites is mainly derived from studies of Rho GTPases [72]. Excessive Rac activity is associated with the presence of focal complexes and the absence of stress fibers. However, elevated Rho activity is characterized by the presence of focal adhesions linked to stress fibers [72]. Antagonistic activities between Rac and Rho are responsible for the transition from focal complexes to focal adhesions [72,73].

It is not clear whether transition from nascent ECM–integrin–cytoskeleton connections to focal complexes requires the generation of forces on those linkages. Focal complexes are less tension dependent than focal adhesions and accumulate along the cell edge following treatment with inhibitors of actomyosin contractility [42]. Forces applied to these nascent ECM–integrin–cytoskeleton connections induce strengthening of the integrin–cytoskeleton interactions [14], a phenomenon that might mimic focal complex initiation and stabilization [21]. Focal complexes are dissociated by inhibitors of myosin II-dependent contractility, but not by an inhibitor of Rho kinase [73]. Accumulation of adhesion site proteins, paxillin and vinculin, around ECM-coated beads is dependent on Rac but not Rho activity and is inhibited by a myosin light chain kinase inhibitor, indicating that physical forces are involved in the initiation of focal complexes [21].

Box 3. Transformation: increase or decrease contractility?

Transformed cells have an altered rigidity homeostasis. It is not yet clear whether transformation is associated with enhanced contractility or impaired contractility. The current model is that contractility is impaired in malignant cells because malignant cells are associated with migration, which is enhanced when focal adhesions and stress fibers are destabilized. Transformed cells are less able to sense and respond to different surface rigidities compared with normal cells [2] and are less efficient at generating forces [27]. However, other studies demonstrate that rigid tumors are formed by transformed cells and uncontrolled growth is promoted by increased contractility [24]. Nevertheless, both unregulated growth and migration, which characterize transformed cells, could be explained by a decoupling of the cell response to the rigidity of its environment.

focal adhesions [16]. At the cell rear, traction stresses induce the disassembly instead of the reinforcement of focal adhesions and linked stress fibers; this is dependent on mechanosensitive ion channels and calcium signaling in keratocytes and astrocytoma cells [33–35]. SFKs, FAK and PEST domain-enriched tyrosine phosphatase (PTP-PEST) are also crucial factors in adhesion site disassembly [26,29,34,36]. This suggests that different modalities of force generation and rigidity response at the cell front and rear correlate with position-dependent regulation of phosphotyrosine signaling, and that different mechanisms of rigidity responses based on phosphotyrosine signaling can independently direct cell morphology.

At the subcellular level, the actin cytoskeleton appears to be the major site for force transduction. Cytoskeletal stretching correlates with the recruitment of adhesion-complex proteins, especially tyrosine kinases and their substrates, both in intact [17,21] and triton-treated fibroblasts, which are devoid of membrane [37,38].

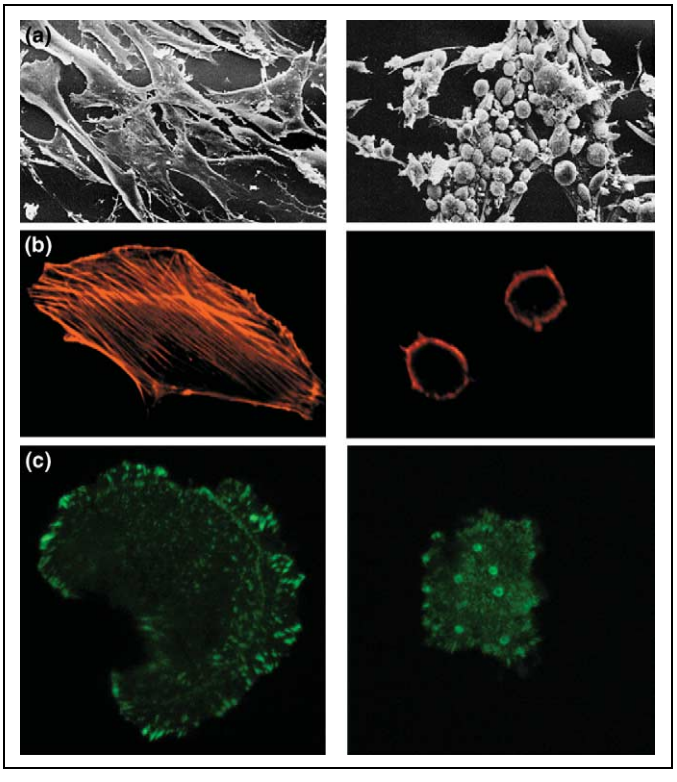

Figure 1. Cell transformation: a tight link between change in morphology and tyrosine kinase activity. **(a)** The distinct morphology of normal (left) and transformed fibroblasts (right). **(b)** Fluorescent staining of the actin cytoskeleton showing its disorganization in transformed (right) compared with normal (left) fibroblasts. **(c)** Fluorescent staining of adhesion sites illustrates the replacement of mature focal adhesions in normal fibroblasts (left) by podosomes in transformed fibroblasts (right); green fluorescence corresponds to paxillin–GFP. Adapted, with permission, from [77] (a) and [26] (b).

Furthermore, cytoskeleton stretching triggers specific biochemical signals, particularly tyrosine kinase and phosphatase pathways [38]. Thus, mechanical alterations of cytoskeletal proteins probably underlie rigidity sensing. All of these observations point to tight links between morphology, migration, rigidity responses and tyrosine kinase activity.

Force sensor elements at adhesion site–cytoskeleton junctions

The rigidity of an object is defined by the amount of force that is required for a given deformation. Therefore, rigidity responses require an element that will be deformed by the force (force sensor) and an element that will convert this into a biochemical signal (signal generator) (Figure 2a). Cells bind to specific ECM molecules through specific integrins and forces are focused on these sites by macromolecular complexes that connect the integrin cytoplasmic domains to the actin cytoskeleton (reviewed in [15]). For complexes to sense rigidity, they must contain proteins that: (i) mechanically link ECM–ligand integrins and actin; and (ii) signal to the rest of the cell in response to mechanical stimuli while bound to actin and/or integrin (including tyrosine signaling) (Table 1). The mechanical link is a force sensor that can be deformed by changes in force that are generated by the ECM and the cell cytoskeleton; these changes are then translated into a biochemical signal by a signal generator. Therefore, the force sensor and signal generator should be co-localized at integrin–cytoskeleton junctions. Although components of the cytoskeleton are also involved in rigidity responses, the most extensively studied rigidity response mechanisms involve elements at adhesion sites. Forces applied to integrin–ECM bonds only cause a deformation in the intracellular and ECM elements when the ECM is connected to a rigid support. Cellular force moves the ECM in proportion to its rigidity as well as modifying the force-sensitive elements in the integrin–cytoskeleton linkage (Figure 2a). The ECM is modified by force (reviewed in [39]). Thus, forces generated from outside and inside the cell will lead to deformation of force-sensitive linkages, which are probably localized at the intracellular side of adhesion sites.

Application of external forces using fluid flow [4], ECM stretching [31,37], laser and magnetic tweezers [14,18] and pipette deformation [17] lead to the initiation and expansion of adhesion–cytoskeleton complexes. The site of the greatest response correlates with the site of greatest applied force, indicating that the rigidity response process is locally activated by force. This might explain why cells move towards a more rigid substrate, which induces

Box 4. Definition of mechanotransduction and rigidity response pathways

The broad definition of mechanotransduction is the conversion of changes in mechanical factors into changes in protein activity. Forces applied to a protein can cause conformational modifications ranging from distortion to domain unfolding. Work done by the force could lower or increase the energy barrier between the bound and free states, for slip bonds [52] or catch bonds [74], respectively. Deformation induced by force could mask or expose binding sites [39,75]. In addition, the application of force to an enzyme could change its activity [6,76] or the opening behavior of an ionic channel [12].

Rigidity response is a case of mechanotransduction in which the variations in the compliance of the ECM are converted into changes in protein activity. Greater rigidity results in less integrin movement for a given cell-generated force. Thus, not only are the forces and their site of application crucial, but the time and/or position-dependent changes in force are also important for cellular function. Therefore, here we have focused on mechanisms of force transduction, which can be time and position dependent.

greater formation of adhesion sites and force generation [3,31]. The assembly of components at adhesion sites has been linked to early phosphotyrosine signals. Force-dependent activation of SFKs by phosphatases [22] or by cytoskeleton stretch [38,40] is an upstream event in the signaling pathways that leads to activation of small G proteins [38] and MAP kinases [41]. Small GTPases can be activated by force [9] (and in the case of Rap1 downstream of the tyrosine kinases) through the activation of guanine exchange factors (GEFs) at appropriate sites [38]. Thus, we suggest that tyrosine kinases and phosphatases are directly involved in the force sensing mechanism. Rapid activation of the SFKs occurs in response to local forces at the cell edge [6]. In all of these cases, the details of the

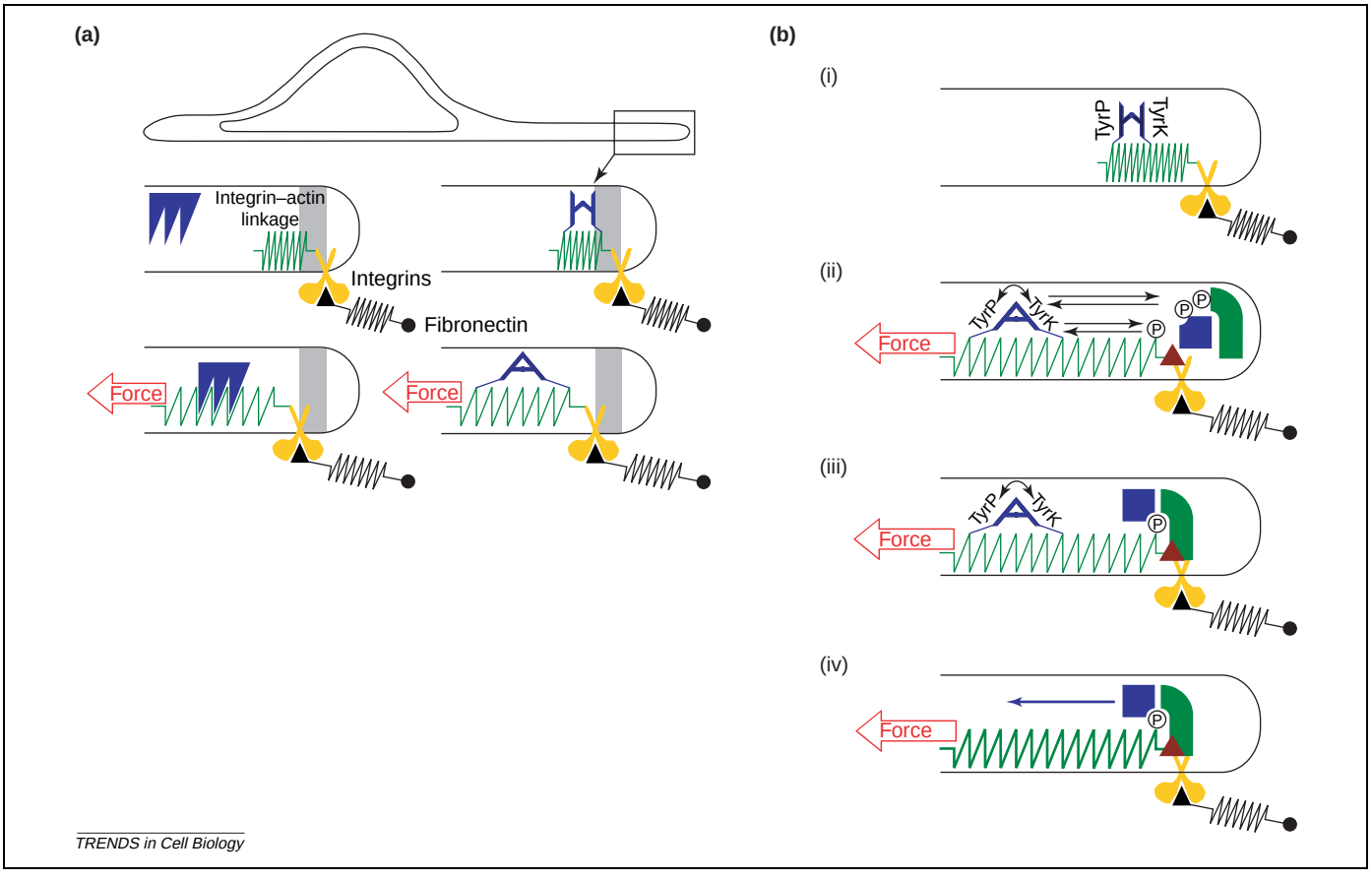

Figure 2. Rigidity detection and phosphotyrosine signaling. **(a)** The basic elements of the rigidity response machinery can be represented by a force sensor element (green spring) and a signal generator element (blue shapes). Extracellularly, the interaction between the transmembrane integrins (yellow) and proteins of the ECM (fibronectin, black triangle) provide one anchor point. The black spring represents the rigidity of the ECM. The green spring represents the actin-associated proteins that are regrouped into an integrin–cytoskeleton complex (composed of scaffolding, signaling and structural proteins), which couples the actin filaments to the adhesion protein. Intracellularly, the actin filaments are pulled away by motors (generally myosin), generating forces on the integrin–cytoskeleton complex and therefore on the ECM (red arrow). The signal generator (blue triangles), which is not directly experiencing force, can bind to a deformed force-sensor component (green spring) of the integrin–cytoskeleton complex and be activated or inhibited (left). Or, the signal generator (H, inactivated; A, activated) might be incorporated into the force-sensor element and be directly activated or inhibited by force generation (right). **(b)** Stretching of the integrin–cytoskeleton complex is the primary transducer of force into biochemical changes that involve changes in phosphotyrosine signaling (tyrosine phosphorylation and dephosphorylation). (i) Without force, the signal-generator element (blue shapes) is inactive. (ii) Some structural and/or scaffolding proteins (brown triangle) connect integrins and the cytoskeleton through phosphotyrosine-independent processes that enable the accumulation of force. Force generated by the cytoskeleton activates tyrosine kinases and/or phosphatases (transition of the blue H to the blue A). Balance of phosphotyrosine signaling (double arrows) defines the level of substrate phosphorylation. Substrates can also be tyrosine kinases and/or phosphatases. Structural (green solid) and signaling (blue solid) proteins, which are substrates of phosphotyrosine signaling, are either cytosolic or part of the force-sensor elements. (iii) The binding of structural and signaling proteins to phosphotyrosine and/or phosphotyrosine signaling induces changes in protein binding and/or catalytic activity. (iv) Binding and/or changes in activity induce modification of the strength of integrin–cytoskeleton linkage (shown by thickening of the green spring) and downstream signaling (blue arrow).

Table 1. Tyrosine kinases and phosphatases and their function in rigidity sensing

Protein	Function	Target in rigidity response	Localization	Rigidity response	Assay	Reinforcement	Cytoskeleton stretch	Effects on AS	Cell type	Refs
FAK	Tyr K, IBP	α-actinin?	AS	Y	SS, SF	Y inhibition?	Recruitment	Assembly Disassembly	Fibroblast Astrocytoma Endothelial	[31,32,37]-[34] [4]
RPTP-α	Tyr P, IBP	Src, Fyn activation	AS?, CM	Y	OT	Y activation		Assembly	Fibroblast	[22]
SHP-2	Tyr P	FAK inhibition	AS	Y	OT	Y activation		Assembly	Fibroblast	[32,47]
Src	Tyr K	Paxillin? Vinculin?	AS	Y	FRET, OT, SS	Y inhibition	Recruitment	Disassembly	Fibroblast Endothelial	[21,22,29, 30,37] [6,26]
Fyn	Tyr K	Paxillin? Vinculin?	AS	Y	OT	Y activation		Assembly	Fibroblast	[22]
PTP-PEST	Tyr P							Disassembly	Fibroblast	[36]
PECAM-1	Scaffolding Tyr Signal	Cadherin VEGFR2 Integrin		Flow sensing	SF MT			Assembly	Endothelial	[7]
p130Cas	Scaffolding Tyr Signal		AS, CM	Y	SS	Y?	Recruitment		Fibroblast	[38]
Paxillin	Scaffolding		AS (early)	Y	OT, SS, PS	Y recruitment	Recruitment		Fibroblast	[17,22,37]
Vinculin	ABP		AS (early)	Y?	OT, SS, PS	Y? recruitment	No effect		Fibroblast	[16,17,21,-22,37]
Zyxin			AS (late)						Fibroblast	[20]
α-Actinin	ABP, IBP		AS (late)	Y	OT	Y recruitment			Fibroblast	[32]
Tensin	ABP, IBP		AS						Fibroblast	[62]
Talin-1	ABP, IBP, scaffolding	Paxillin, Vinculin, Integrin	AS (early)	Y	OT	Y recruitment			Fibroblast	[52,53]
Filamin	ABP, IBP		AS, CM	Y	OT	N			Fibroblast	[53]
Integrin $\alpha_v\beta_3$	Adhesion protein	RPTPα, Talin-1	AS, CM	Y	OT, SF	Y			Fibroblast Endothelial	[22,52,53] [7,9,54]

ABP: actin-binding protein; AS: adhesion site; CM: cell margin; IBP: integrin-binding protein; MT: magnetic tweezers; N, No; OT: optical tweezers; PS: pipette stretch; SF: shear flow; SS: substrate stretch; Tyr K: tyrosine kinase; Tyr P: tyrosine phosphatase; Y, Yes. A question mark indicates that the hypothesis is not yet confirmed.

assembly by force and disassembly by relaxation have yet to be clarified but there is clearly a mechanical regulation of kinase and phosphatase activities.

Force sensing has been linked to ion channel opening and cytoskeleton stretch. Although there is strong evidence for ionic movements in some force responses [12,33,35], a completely cytoskeleton-dependent mechanism has been observed in several cases [37]. Thus, the force sensor might be incorporated into the cytoskeleton (i.e. as a force-sensitive element). For example, forces applied to the cytoskeleton can cause protein deformation (unfolding or distortion) and induce a direct change in protein activity (indicating that the signal generator directly senses force). Alternatively, the signal generator could bind to a deformed component or elastic element in the cytoskeleton (the force sensor) and be activated or inhibited (indicating that the signal generator senses force indirectly) (Figure 2a) (Box 4). In the case of the rigidity response mechanism, no definitive force sensor or signal generator has been identified; however, a tyrosine-kinase dependent phosphorylation of p130Cas has been found in stretched cytoskeletons [38]. Strikingly, adhesion sites under tension [42] and the lamellipodium [43], which are structures that are involved in rigidity responses at the cell edge, contain proteins that have high concentrations of phosphotyrosine. Thus, we favor cytoskeleton stretch rather than ion movements as the primary transducer of force from the environment into biochemical changes that involve changes in tyrosine phosphorylation levels by substrate, kinase and/or phosphatase modification (Figure 2b).

Spatio-temporal aspects of force sensing

A migrating cell on a soft substrate that encounters a border with a rigid substrate will cross towards the rigid substrate [3]. This process, known as durotaxis, indicates that rigidity responses are based on localized and dynamic processes, which can locally and temporally change cell contractility, motility and shape [31]. In these dynamic processes, adhesion sites and the actin cytoskeleton are assembled, stabilized, moved and disassembled on a time frame of seconds to minutes [44,45]. In addition, the molecular components of these complexes are rapidly exchanging on a timescale of seconds, even if the macromolecular structures are relatively immobile in space. Cycles of tyrosine phosphorylation and dephosphorylation are implied in both of these dynamic processes.

Adhesion-site dynamics

Treatments that increase or decrease tyrosine phosphorylation promote enhancement or inhibition of adhesion-site formation, respectively [46]. For example, the non-specific tyrosine phosphatase inhibitor PAO induces the formation

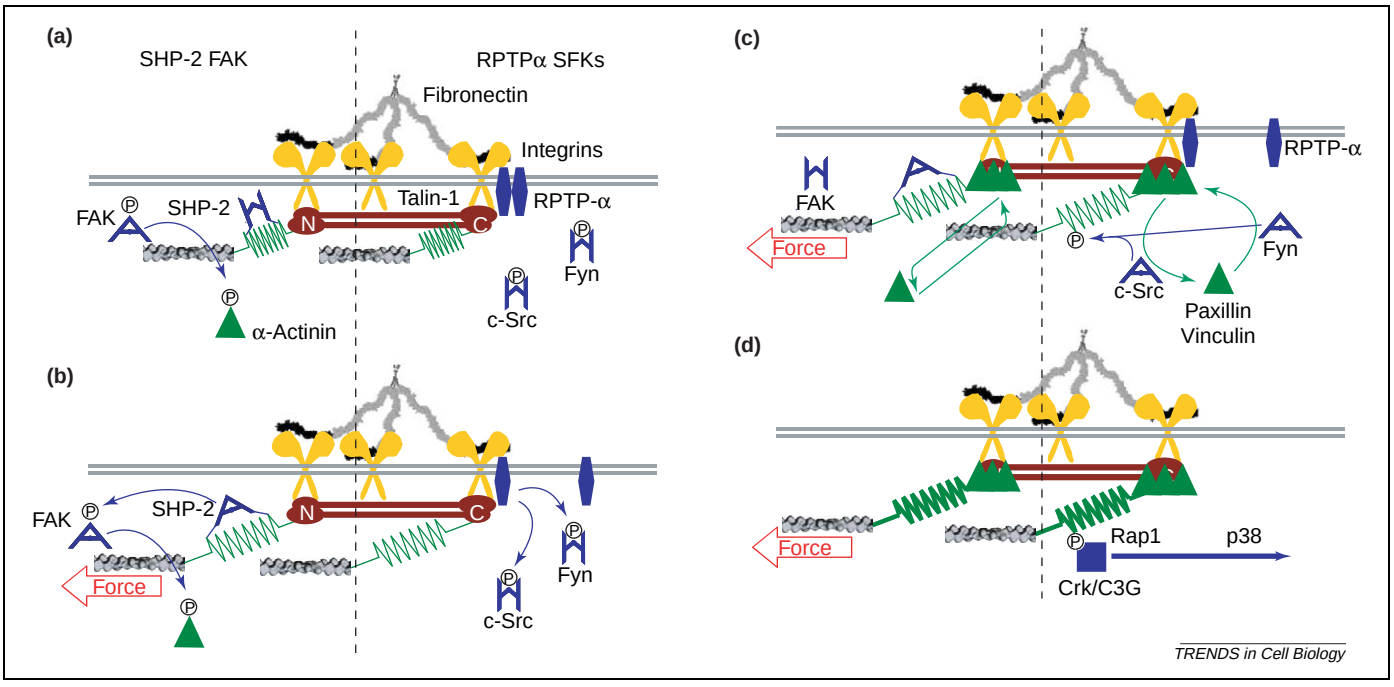

Figure 3. Two particular cases of rigidity responses involving phosphotyrosine signaling, SHP-2 and FAK (left) and RPTP-α and SFKs (right). The left side illustrates the reinforcement of integrin–cytoskeleton linkage after the force-dependent activation of SHP-2. **(a)** Without force, active FAK phosphorylates α-actinin (green triangle) decreasing its interaction with the integrin–cytoskeleton complex. **(b)** Force induces activation of SHP-2, which dephosphorylates and inhibits FAK. **(c)** FAK inhibition decreases α-actinin tyrosine phosphorylation, increasing its interaction with the integrin–cytoskeleton complex. **(d)** Increased association of α-actinin induces reinforcement of the integrin–cytoskeleton complex. The right side illustrates the reinforcement of integrin–cytoskeleton linkage after the force-dependent activation of the tyrosine phosphatase, RPTP-α, and the phosphotyrosine signaling cascade activated by SFKs after cytoskeleton stretch. (a) Without force, RPTP-α and SFKs are inactive. Integrins are linked to the actin cytoskeleton through talin-1. (b) Force induces RPTP-α activation, which dephosphorylates and activates SFKs. (c) SFKs activity induces association (through the activity of Fyn) or dissociation (through the activity of c-Src) of structural proteins (green triangle) to the integrin–cytoskeleton complex (through scaffolding proteins, e.g. talin-1). The activity of SFKs in stretched cytoskeleton induces tyrosine phosphorylation of integrin–cytoskeleton complex proteins (e.g. p-130Cas). (d) Recruitment of structural proteins (such as paxillin and vinculin) correlates with reinforcement of the integrin–cytoskeleton complex. Recruitment of signaling proteins (such as Crk and C3G) on phosphotyrosine site (perhaps on p-130Cas) induces activation of downstream signaling cascades.

of stable adhesion sites on soft substrates, where such sites are normally irregularly shaped and highly dynamic, inducing an inappropriate response to rigidity [5]. However, FAK-deficient fibroblasts exhibit a reduced rate of cell motility that is associated with long-lasting adhesion sites, suggesting that FAK-mediated tyrosine phosphorylation events are involved in adhesion-site turnover. SHP-2 and PTP-PEST maintain FAK dephosphorylation and inactivation but they are involved in maturation and disassembly of adhesion sites, respectively [36,47]. Furthermore, tyrosine phosphatases are involved in the reinforcement of integrin–cytoskeleton linkages [14] and the membrane tyrosine phosphatase RPTPα, which is required to dephosphorylate the inhibitory phosphotyrosine residue of SFKs, is involved in the activation of Fyn that is required for early reinforcement [22] (Figure 3). To reconcile these observations, it might be necessary to view the phosphotyrosine signaling of integrin-associated proteins within the context of a dynamic process that involves repeated cycles of phosphorylation and dephosphorylation of specific substrates at defined times and subcellular locations in the life cycle of an adhesion site.

For example, tyrosine dephosphorylation of FAK by SHP-2 induces the stabilization and maturation of the early focal complexes to focal adhesions [32] (Figure 3), although the formation of paxillin-positive immature adhesion sites is not affected by SHP-2. Fyn and c-Src have opposite effects in the force-dependent reinforcement of integrin–cytoskeleton linkages. Similar to FAK, c-Src is involved in the turnover of adhesion sites [26] and, accordingly, force-dependent reinforcement is increased by the loss of c-Src [21,29], whereas loss of the closely related kinase Fyn inhibits reinforcement [22]. We suggest that time- and position-dependent processes account for the specificity of SFKs in reinforcement and rigidity responses (Figure 4). For example, Fyn and c-Src could have a different threshold of force-dependent activation, leading to a temporal shift in their activity. Alternatively, Fyn activity could be higher close to the cell edge, whereas c-Src activity could be predominant further back, leading to a spatial difference in the activities of these proteins.

Most components of the integrin–cytoskeleton complex have fast exchange rates with half-lives ranging from a few seconds to a few minutes [32,34,48]. Only integrins have molecular dynamics sufficiently slow to support the half-life of adhesion sites [44]. Of particular interest, tyrosine dephosphorylation of FAK by SHP-2 controls FAK-dependent tyrosine phosphorylation of α-actinin and modulates the association of α-actinin with the integrin–cytoskeleton complex. A SHP-2-dependent decrease in FAK activity increases the association of α-actinin and force-dependent strengthening of integrin–cytoskeleton linkages [32] (Figure 3). At the cellular level, this stable association enables the maturation of focal complexes. Therefore, adhesion-site molecular dynamics are regulated during adhesion formation. The disassembly of focal adhesions is also regulated by the residency time of FAK

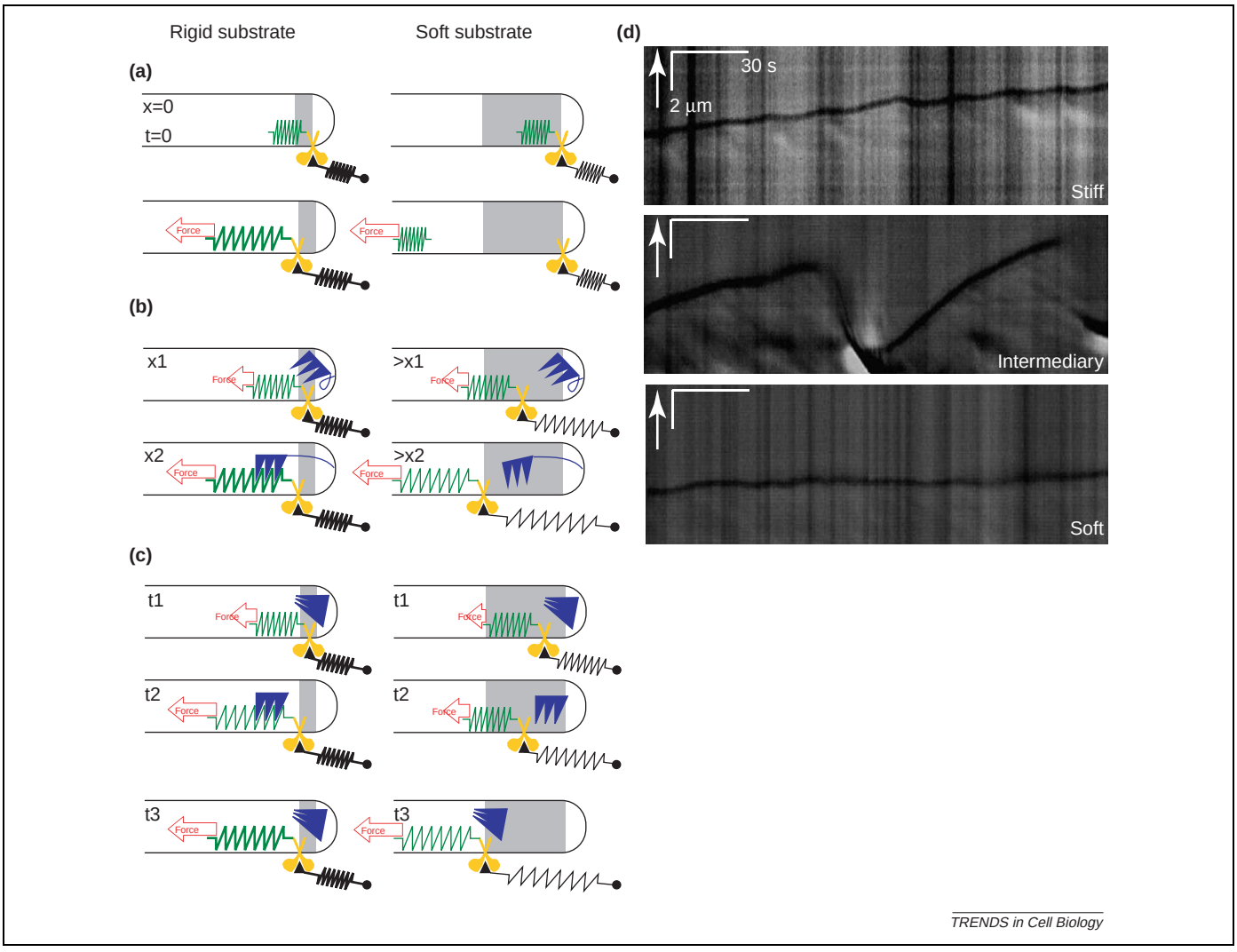

Figure 4. Spatial and temporal aspect of rigidity responses. Possible mechanisms are shown for the transduction of motile and force-dependent events that are important in defining cell morphology and are restricted in space and time. **(a)** On a rigid substrate (black spring, left) force rises after a small displacement (grey shading represents displacement) and time period, leading to the initiation and maturation of adhesion sites. The reinforcement of the integrin–cytoskeleton linkage is represented by thickening of the green spring. However, on a soft substrate (right), a larger displacement and time period are required to produce the same force. This inappropriate force generation prevents the formation of adhesion sites because dissociation of integrin–cytoskeleton linkage (known as a slip bond) occurs before stabilization of the connection. **(b)** Position-dependent rigidity responses. For a rigid substrate, the position of the unfolded or distorted force-sensor element (green spring) is co-localized with the signal generator (blue triangles), which leads to rigidity responses. The conformation of the force-sensor element after unfolding and/or distortion corresponds to the conformation that binds to the signal generator. For a soft substrate, a larger displacement of the ECM is required to attain the same force-sensor distortion (force) so the force sensor will not be co-localized with the signal generator and no rigidity responses will occur. (x1 and x2 refer to the position of the force-sensor element) **(c)** Time-dependent rigidity responses. The signal generator (blue triangles) is activated for a limited amount of time (shown in t2, which refers to the time when the signal generator is active). For a rigid substrate, the time necessary to unfold or deform the force-sensor element (green spring) corresponds to this activation window leading to rigidity responses. For a soft substrate, because a longer time is required to attain the same force-sensor distortion (and therefore the same force), the signal generator will be no longer activated and no rigidity response will occur. **(d)** Motile cells are characterized by the generation of periodic contractions (period of 25 s here). The three panels are kymographs that are used to visualize movements (*y* axis) of the cell edge (black) as function of time (*x* axis). The arrows indicate the direction of cell edge protrusion. These kymographs are generated from cells spreading on polyacrylamide gels of different rigidity. This phenomenon of periodic contractions occurs mostly on rigid substrates (top panel) and only poorly on intermediate to soft substrates (middle and lower panels). This process illustrates important characteristics of the rigidity responses mechanism. It is a positive feedback mechanism between forces that are generated by the cell and substrate rigidity. Initiation and sustained cycling requires myosin II activity and a rigid substrate. It is a time-dependent process, every 25 s the cell is probing the substrate rigidity by a contraction of the cell edge, and is position dependent, the contraction and probing occur on a limited portion of the cell edge. Scale bars: vertical = 2 μm; horizontal = 30 s. Adapted, with permission, from [49].

at focal adhesions. Prolonged association of FAK within focal adhesions is correlated with increased FAK activity and focal adhesions disassembly [34].

Rigidity responses involve force sensing and distance or time sensing

The biological rigidity-sensing mechanism must have means of transforming the force and displacement (i.e. distance and time information) into a biochemical signal (Figure 4b,c). Cells display complex patterns of successive extensions and retractions that seem to be part of an active rigidity-response mechanism. In one case, the cell periodically probes the rigidity of its surroundings during spreading and migration [49] (Figure 4d). Also, the forces that are applied on a collagen filament are oscillating [50], implying that a rigidity response might involve a threshold force that is reached after a defined distance and during a limited time. From the study of rigidity responses using laser tweezers during fibroblast migration, the movements involved are calculated to be over distances of ~100 nm and durations of ~1 s [51]. Consequently, time and distance are plausible parameters

to be sensed in the rigidity response process. Changing the ECM rigidity affects these probing cycles (on softer substrates, none or fewer periodic probing events have been observed) suggesting that a feedback mechanism exists between ECM rigidity and the cellular architecture, and motile activities that are involved in the probing mechanism (Figure 4d). More studies are required to define the exact mechanism of force and displacement sensing involved in detecting rigidity and whether external forces can regularly activate the same signaling pathways.

Position dependence and rigidity responses

The position dependence of rigidity responses (Figure 4b) is exemplified by the fact that structural and signaling proteins that are necessary for rigidity responses are placed at strategic locations, for example, at the cell edge during protrusive events and at early adhesion sites. Many proteins involved in rigidity responses and/or phosphotyrosine signaling, including talin [52,53], integrins ($\alpha v\beta 3$) [52,54], paxillin [22], α-actinin [32,49], RPTPα [22], Rap 1 [55] and p130Cas [56], are localized at the leading edge of the cell, ready to respond to any contraction generated by the cell or by the ECM. There is a position-dependent binding-and-release cycle of fibronectin–integrin–cytoskeleton interactions, with preferential binding occurring at active edges of motile fibroblasts and release at 0.5–3 μm back from the edge [57]. Interestingly, this position-dependent binding correlates with the efficiency of the reinforcement process in the rigidity response [51]. Forces generated at single ECM–integrin–cytoskeleton connections can lead to breaking or reinforcement of the integrin–cytoskeleton linkage [51,52] (Figure 4a). With rigid optical tweezers holding fibronectin beads, contact reinforcement is favored over breaking of bead–cytoskeleton contacts at the cell edge but not at 2 μm back, whereas no differences are observed using soft optical tweezers [51]. At the molecular level, the reinforcement of integrin–cytoskeleton interactions are limited to linkages that have experienced force and not those nearby (<1 μm) [14].

Forces exerted on a macromolecular complex might affect the specific distances required to assemble that complex. The density and clustering of integrin ligand RGD affects cell adhesion [58], the establishment of integrin–cytoskeleton linkages [52] and the formation of focal adhesions and stress fibers [59]. These processes might be dependent on a crucial minimum distance between occupied integrins. In agreement, the distance between RGD peptide sites must be <70 nm for the formation of stress fibers and focal adhesions [58,59]. This might be the length of the molecule that bridges and clusters integrins during the formation of integrin–cytoskeleton complexes [52]. These data indicate that the distances between occupied integrins within an adhesion site can act as a rigidity response that responds to tension-dependent changes in integrin density [44]. At the cellular level, the periodic contractions of the lamellipodium involved in ECM probing are confined to a segment of the cell edge and involve local activation of a contractile signal that directs cell probing [49]. Therefore, at the cellular and molecular levels, rigidity response is a localized phenomenon.

Time dependence and rigidity responses

The time dependence of rigidity responses (Figure 4c) might involve the precise order in which components of the integrin–cytoskeleton complex bind and detach during the life cycle of an adhesion site [45]. The formation of the elementary connection between integrin and the cytoskeleton, and its reinforcement, depend on talin, which is probably one of the first proteins that enter adhesion sites [52,53]. α-Actinin and zyxin enter the adhesion site during its maturation [60,61]. The transition from mature focal adhesion to fibrillar adhesion is characterized by the segregation of tensin and specific integrins [62]. Because the ECM–integrin–cytoskeleton connection is a viscoelastic material (i.e. it is not purely elastic) [63], the time required to reach the threshold force for rigidity responses probably differs depending on the stiffness of the ECM. Accordingly, a soft optical trap could mimic the effects of a rigid trap on the stabilization of the integrin–cytoskeleton linkages if externally applied forces rise rapidly [51]. In lamellipodia, the cytoskeletal-dependent radial transport of a contractile signal directs the timing of contraction and, probably, adhesion site initiation to stabilize protrusive events [49]. Consequently, formation of cell contacts with the ECM is not a continuous process, but involves cycles of contraction and relaxation. Furthermore, in a tissue, contractile activity and external forces should also produce a rigidity response. The limited time of contraction, during which the cell locally probes the ECM composition and physical properties, might reduce the time window for the activation of a rigidity response.

Steady-state morphology of cells

Cells in suspension usually adopt a spherical shape. After they contact a surface with suitable chemical (ECM proteins) and physical (rigidity) properties, cells spread and migrate through a series of motile events. Detailed analyses of cell movements on short timescales show that they move discontinuously, with a seemingly random mixture of brief extensions, retractions and quiet periods [49,64,65] (Figure 5a). However, motile events (extension and retraction) can be precisely defined in their geometry and dynamics by the structure and dynamics of the underlying actin cytoskeleton. Ena/VASP-capping activity, for example, determines the angle between actin filaments and the cell edge, thereby affecting the speed of protrusion [64]. The lamellipodial width is correlated with the periodicity of contractions that are generated by the cell edge during motility, indicating that the transport of a contractile signal by the rearward flow of actin controls the time between contractions [49]. Therefore, the periodicity, frequency and amplitude of extension and retraction events can be precisely defined by cell signaling pathways, and depend on external factors, for example, the nature of the ECM proteins and rigidity (Figure 4d). Many events are involved in defining cell shape because individual extension and

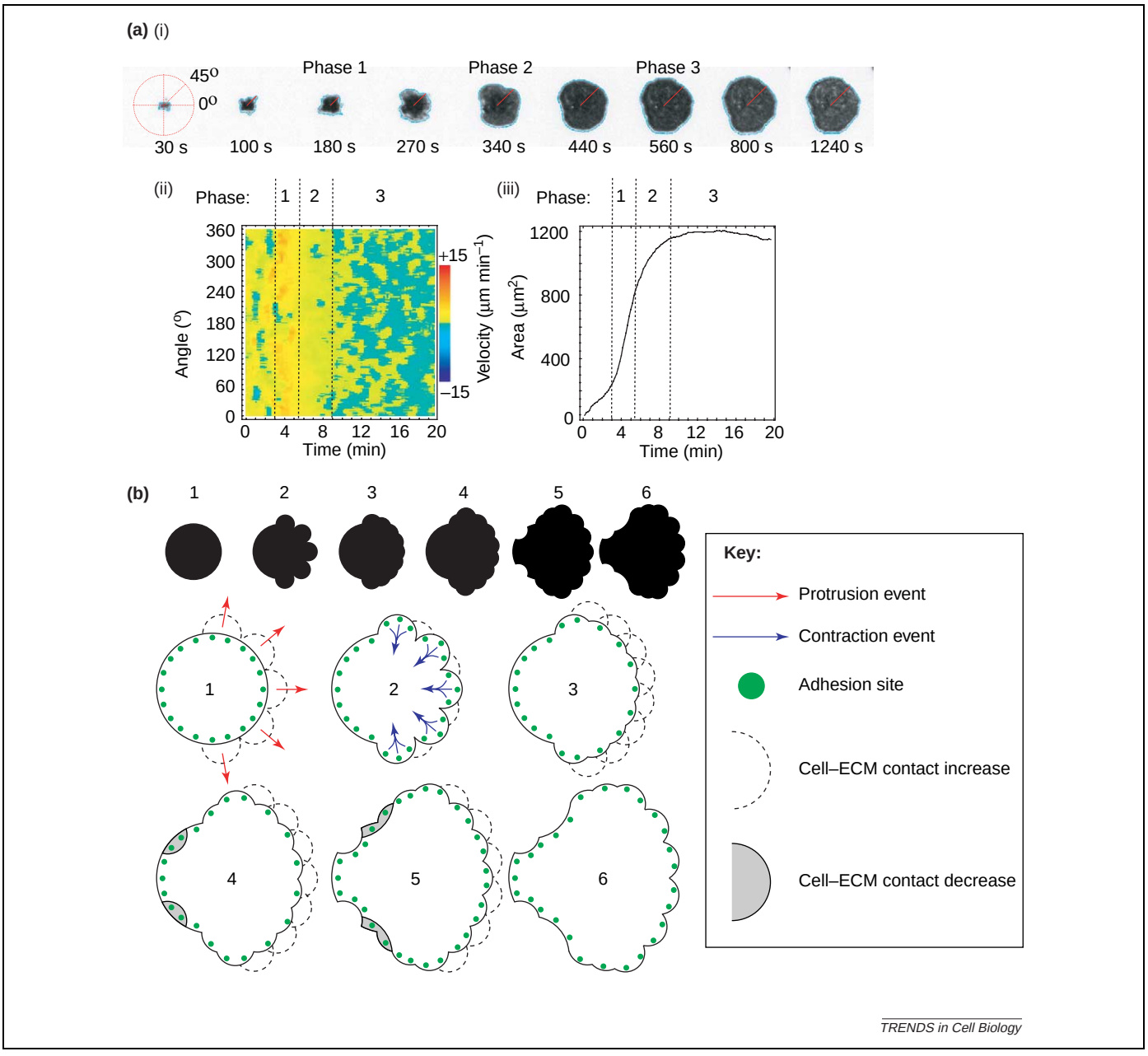

Figure 5. Force sensing and generation control cell motility and cell morphology. **(a)** Total internal reflection fluorescence microscopy enables the measurement of the cell–ECM contact during cell spreading (i). Detailed analysis of cell movements on short timescales (ii) shows that the cell moves discontinuously, with a mixture of brief extensions (yellow to red) and retractions (light blue to dark blue) (as seen in phase 3). The final surface contact of the cell with the substrate (iii) and its morphology are defined by the frequency, duration and direction of protrusion and contraction events. Cell movement is also characterized by transitions between different modes of protrusion and retraction dynamics. In this example, a fibroblast is spreading through initiation (Phase 1), a fast spreading phase (Phase 2, which has low activity of myosin II) and a contractile phase (Phase 3, which has high activity of myosin II). Adapted, with permission, from [49]. **(b)** The final morphology of a cell is the integral of many individual rigid-substrate responses and the dynamics of adhesion sites and associated cytoskeletal structures. The black outlines of the cells show the evolution of the cell-surface contact with the ECM. Each increase in the contact area involves an individual and localized protrusion event followed by a contraction event (blue arrow) that leads to the formation of adhesion sites (green structures), which stabilize the protrusion on the ECM. Dissociation of stable interactions is represented as a gray area. Protrusion and contraction events are based on the dynamics of the actin cytoskeleton, protrusion events by actin polymerization and contraction events by myosin motor activities. Forces generated by the cytoskeleton stabilize its interaction with adhesion sites. Thus, if the adhesion is to a rigid substrate, a stable adhesion site will be formed.

retraction events only last for 20–90 s and involve 0.2–4 μm of the cell edge.

The precise timing and localization of the forces generated on different actin structures during motility will direct the amplitude and localization of forces that are applied on integrins and further adhesion-site initiation. Therefore, after the extension of the cell edge, the sensing of matrix rigidity is one of the earliest events in the formation of adhesion sites that leads to the stabilization of the extension on the ECM.

Small movements can cause large changes in morphology. Therefore, the final morphology is the integral of many individual rigid substrate responses and the dynamics of the focal contacts and associated cytoskeletal structures (Figure 5b). Accordingly, cell spreading on soft substrates, which fails to stabilize cell protrusion [49], results in a greatly decreased cell area compared with cells on a rigid substrate [2,24,51]. In addition to this local effect of forces, the orientation of stress fibers (the axis where the internal forces are higher) also directs the

protrusive activity of the cell [66]. Therefore, by sensing force and generating force in the appropriate areas, cells can control their motility as well as their morphology.

The number of different motile activities is limited. For example, cellular spreading can be described by a few characteristic phases [67] (Figure 5a). Thus, only nine basic morphologies are produced by the expression of active small GTPases [68]. For a complete description of the morphology, all factors must be included as each influences the overall dynamics. Changes in cell composition influence the duration and extent of individual processes, which, in turn, influences the overall behavior at the cellular level.

Concluding remarks

The morphology of a cell is the result of discrete rigidity response events that are integrated over time. These events are defined by the localized rigidity of the ECM that controls the local deformation of the force sensor at the integrin–cytoskeleton junction and, therefore, the activation of a signal generator, which involves tyrosine signaling. Rigidity response events and motile events are based on the same molecular processes and are highly interconnected. The important task now is to define the distinct phases of motility and the dynamics of the cellular motile processes to obtain an overall working model of morphological changes that can then be tested by altering the activities and dynamics of contributing factors.

References

1 Huang, S. and Ingber, D.E. (1999) The structural and mechanical complexity of cell-growth control. *Nat. Cell Biol.* 1, E131–E138
2 Wang, H.B. *et al*. (2000) Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. *Am. J. Physiol. Cell Physiol.* 279, C1345–C1350
3 Lo, C.M. *et al*. (2000) Cell movement is guided by the rigidity of the substrate. *Biophys. J.* 79, 144–152
4 Li, S. *et al*. (2002) The role of the dynamics of focal adhesion kinase in the mechanotaxis of endothelial cells. *Proc. Natl. Acad. Sci. U. S. A.* 99, 3546–3551
5 Pelham, R.J., Jr. and Wang, Y. (1997) Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc. Natl. Acad. Sci. U. S. A.* 94, 13661–13665
6 Wang, Y. *et al*. (2005) Visualizing the mechanical activation of Src. *Nature* 434, 1040–1045
7 Tzima, E. *et al*. (2005) A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature* 437, 426–431
8 D'Addario, M. *et al*. (2002) Interaction of p38 and Sp1 in a mechanical force-induced, β 1 integrin-mediated transcriptional circuit that regulates the actin-binding protein filamin-A. *J. Biol. Chem.* 277, 47541–47550
9 Tzima, E. *et al*. (2002) Activation of Rac1 by shear stress in endothelial cells mediates both cytoskeletal reorganization and effects on gene expression. *EMBO J.* 21, 6791–6800
10 Sikavitsas, V.I. *et al*. (2003) Mineralized matrix deposition by marrow stromal osteoblasts in 3D perfusion culture increases with increasing fluid shear forces. *Proc. Natl. Acad. Sci. U. S. A.* 100, 14683–14688
11 Engler, A.J. *et al*. (2004) Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. *J. Cell Biol.* 166, 877–887
12 Sukharev, S. and Corey, D.P. (2004) Mechanosensitive channels: multiplicity of families and gating paradigms. *Sci. STKE* 2004, re4
13 Miyamoto, S. *et al*. (1995) Synergistic roles for receptor occupancy and aggregation in integrin transmembrane function. *Science* 267, 883–885
14 Choquet, D. *et al*. (1997) Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages. *Cell* 88, 39–48
15 Geiger, B. *et al*. (2001) Transmembrane crosstalk between the extracellular matrix–cytoskeleton crosstalk. *Nat. Rev. Mol. Cell Biol.* 2, 793–805
16 Balaban, N.Q. *et al*. (2001) Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. *Nat. Cell Biol.* 3, 466–472
17 Riveline, D. *et al*. (2001) Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. *J. Cell Biol.* 153, 1175–1186
18 Wang, N. *et al*. (1993) Mechanotransduction across the cell surface and through the cytoskeleton. *Science* 260, 1124–1127
19 Ingber, D.E. (2003) Tensegrity I. Cell structure and hierarchical systems biology. *J. Cell Sci.* 116, 1157–1173
20 Beningo, K.A. *et al*. (2001) Nascent focal adhesions are responsible for the generation of strong propulsive forces in migrating fibroblasts. *J. Cell Biol.* 153, 881–888
21 Galbraith, C.G. *et al*. (2002) The relationship between force and focal complex development. *J. Cell Biol.* 159, 695–705
22 von Wichert, G. *et al*. (2003) RPTP-α acts as a transducer of mechanical force on αv/β3-integrin-cytoskeleton linkages. *J. Cell Biol.* 161, 143–153
23 Boettiger, D. *et al*. (2001) Activation of α(v)β3-vitronectin binding is a multistage process in which increases in bond strength are dependent on Y747 and Y759 in the cytoplasmic domain of β3. *Mol. Biol. Cell* 12, 1227–1237
24 Paszek, M.J. *et al*. (2005) Tensional homeostasis and the malignant phenotype. *Cancer Cell* 8, 241–254
25 Linder, S. and Aepfelbacher, M. (2003) Podosomes: adhesion hot-spots of invasive cells. *Trends Cell Biol.* 13, 376–385
26 Frame, M.C. *et al*. (2002) v-Src's hold over actin and cell adhesions. *Nat. Rev. Mol. Cell Biol.* 3, 233–245
27 Munevar, S. *et al*. (2001) Traction force microscopy of migrating normal and H-ras transformed 3T3 fibroblasts. *Biophys. J.* 80, 1744–1757
28 Janmey, P.A. and Weitz, D.A. (2004) Dealing with mechanics: mechanisms of force transduction in cells. *Trends Biochem. Sci.* 29, 364–370
29 Felsenfeld, D.P. *et al*. (1999) Selective regulation of integrin–cytoskeleton interactions by the tyrosine kinase Src. *Nat. Cell Biol.* 1, 200–206
30 Volberg, T. *et al*. (2001) pp60(c-src) and related tyrosine kinases: a role in the assembly and reorganization of matrix adhesions. *J. Cell Sci.* 114, 2279–2289
31 Wang, H.B. *et al*. (2001) Focal adhesion kinase is involved in mechanosensing during fibroblast migration. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11295–11300
32 von Wichert, G. *et al*. (2003) Force-dependent integrin–cytoskeleton linkage formation requires downregulation of focal complex dynamics by Shp2. *EMBO J.* 22, 5023–5035
33 Lee, J. *et al*. (1999) Regulation of cell movement is mediated by stretch-activated calcium channels. *Nature* 400, 382–386
34 Giannone, G. *et al*. (2004) Calcium rises locally trigger focal adhesion disassembly and enhance residency of focal adhesion kinase at focal adhesions. *J. Biol. Chem.* 279, 28715–28723
35 Doyle, A. *et al*. (2004) Calcium transients induce spatially coordinated increases in traction force during the movement of fish keratocytes. *J. Cell Sci.* 117, 2203–2214
36 Angers-Loustau, A. *et al*. (1999) Protein tyrosine phosphatase-PEST regulates focal adhesion disassembly, migration, and cytokinesis in fibroblasts. *J. Cell Biol.* 144, 1019–1031
37 Sawada, Y. and Sheetz, M.P. (2002) Force transduction by Triton cytoskeletons. *J. Cell Biol.* 156, 609–615
38 Tamada, M. *et al*. (2004) Activation of a signaling cascade by cytoskeleton stretch. *Dev. Cell* 7, 709–718
39 Vogel, V. *et al*. (2001) Structural insights into the mechanical regulation of molecular recognition sites. *Trends Biotechnol.* 19, 416–423
40 Han, B. *et al*. (2004) Conversion of mechanical force into biochemical signaling. *J. Biol. Chem.* 279, 54793–54801
41 Sawada, Y. *et al*. (2001) Rap1 is involved in cell stretching modulation of p38 but not ERK or JNK MAP kinase. *J. Cell Sci.* 114, 1221–1227

42 Zamir, E. *et al*. (1999) Molecular diversity of cell-matrix adhesions. *J. Cell Sci.* 112, 1655–1669
43 Baisden, J.M. *et al*. (2001) The intrinsic ability of AFAP-110 to alter actin filament integrity is linked with its ability to also activate cellular tyrosine kinases. *Oncogene* 20, 6607–6616
44 Ballestrem, C. *et al*. (2001) Marching at the front and dragging behind: differential αVβ3-integrin turnover regulates focal adhesion behavior. *J. Cell Biol.* 155, 1319–1332
45 Webb, D.J. *et al*. (2004) FAK-Src signalling through paxillin, ERK and MLCK regulates adhesion disassembly. *Nat. Cell Biol.* 6, 154–161
46 Schoenwaelder, S.M. and Burridge, K. (1999) Bidirectional signaling between the cytoskeleton and integrins. *Curr. Opin. Cell Biol.* 11, 274–286
47 Yu, D.H *et al*. (1998) Protein-tyrosine phosphatase Shp-2 regulates cell spreading, migration, and focal adhesion. *J. Biol. Chem.* 273, 21125–21131
48 Edlund, M. *et al*. (2001) Dynamics of α-actinin in focal adhesions and stress fibers visualized with α-actinin-green fluorescent protein. *Cell Motil. Cytoskeleton* 48, 190–200
49 Giannone, G. *et al*. (2004) Periodic lamellipodial contractions correlate with rearward actin waves. *Cell* 116, 431–443
50 Meshel, A.S. *et al*. (2005) Basic mechanism of three-dimensional collagen fibre transport by fibroblasts. *Nat. Cell Biol.* 7, 157–164
51 Jiang, G. *et al.* Rigidity sensing at the leading edge through αvβ3 Integrins and RPTPα. *Biophys. J.* (in press)
52 Jiang, G. *et al*. (2003) Two-piconewton slip bond between fibronectin and the cytoskeleton depends on talin. *Nature* 424, 334–337
53 Giannone, G. *et al*. (2003) Talin1 is critical for force-dependent reinforcement of initial integrin–cytoskeleton bonds but not tyrosine kinase activation. *J. Cell Biol.* 163, 409–419
54 Kiosses, W.B. *et al*. (2001) Rac recruits high-affinity integrin αvβ3 to lamellipodia in endothelial cell migration. *Nat. Cell Biol.* 3, 316–320
55 Arthur, W.T. *et al*. (2004) Rap1 promotes cell spreading by localizing Rac guanine nucleotide exchange factors. *J. Cell Biol.* 167, 111–122
56 Di Stefano, P. *et al*. (2004) P130Cas-associated protein (p140Cap) as a new tyrosine-phosphorylated protein involved in cell spreading. *Mol. Biol. Cell* 15, 787–800
57 Nishizaka, T. *et al*. (2000) Position-dependent linkages of fibronectin–integrin–cytoskeleton. *Proc. Natl. Acad. Sci. U. S. A.* 97, 692–697
58 Koo, L.Y. *et al*. (2002) Co-regulation of cell adhesion by nanoscale RGD organization and mechanical stimulus. *J. Cell Sci.* 115, 1423–1433
59 Arnold, M. *et al*. (2004) Activation of integrin function by nanopatterned adhesive interfaces. *ChemPhysChem* 5, 383–388
60 Laukaitis, C.M. *et al*. (2001) Differential dynamics of α 5 integrin, paxillin, and α-actinin during formation and disassembly of adhesions in migrating cells. *J. Cell Biol.* 153, 1427–1440
61 Zaidel-Bar, R. *et al*. (2003) Early molecular events in the assembly of matrix adhesions at the leading edge of migrating cells. *J. Cell Sci.* 116, 4605–4613
62 Zamir, E. *et al*. (2000) Dynamics and segregation of cell-matrix adhesions in cultured fibroblasts. *Nat. Cell Biol.* 2, 191–196
63 Bausch, A.R. *et al*. (1998) Local measurements of viscoelastic parameters of adherent cell surfaces by magnetic bead microrheometry. *Biophys. J.* 75, 2038–2049
64 Bear, J.E. *et al*. (2002) Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility. *Cell* 109, 509–521
65 Dubin-Thaler, B.J. *et al*. (2004) Nanometer analysis of cell spreading on matrix-coated surfaces reveals two distinct cell states and STEPs. *Biophys. J.* 86, 1794–1806
66 Parker, K.K. *et al*. (2002) Directional control of lamellipodia extension by constraining cell shape and orienting cell tractional forces. *FASEB J.* 16, 1195–1204
67 Dobereiner, H.G. *et al*. (2004) Dynamic phase transitions in cell spreading. *Phys. Rev. Lett.* 93, 108105
68 Heo, W.D. and Meyer, T. (2003) Switch-of-function mutants based on morphology classification of Ras superfamily small GTPases. *Cell* 113, 315–328
69 Cukierman, E. *et al*. (2001) Taking cell–matrix adhesions to the third dimension. *Science* 294, 1708–1712
70 Beningo, K.A. *et al*. (2004) Responses of fibroblasts to anchorage of dorsal extracellular matrix receptors. *Proc. Natl. Acad. Sci. U. S. A.* 101, 18024–18029
71 Mandeville, J.T. *et al*. (1997) Dynamic imaging of neutrophil migration in three dimensions: mechanical interactions between cells and matrix. *J. Leukoc. Biol.* 61, 188–200
72 Nobes, C.D. and Hall, A. (1995) Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* 81, 53–62
73 Rottner, K. *et al*. (1999) Interplay between Rac and Rho in the control of substrate contact dynamics. *Curr. Biol.* 9, 640–648
74 Marshall, B.T. *et al*. (2003) Direct observation of catch bonds involving cell-adhesion molecules. *Nature* 423, 190–193
75 Zhong, C. *et al*. (1998) Rho-mediated contractility exposes a cryptic site in fibronectin and induces fibronectin matrix assembly. *J. Cell Biol.* 141, 539–551
76 Yin, H. *et al*. (1995) Transcription against an applied force. *Science* 270, 1653–1657
77 Alberts, B. *et al*. (1994) *Molecular Biology of the Cell*, Garland Publishing