The structural and mechanical complexity of cell-growth control

Sui Huang* and Donald E. Ingber*†

**Departments of Pathology and Surgery, Children's Hospital and Harvard Medical School, Boston, Massachussetts 02115, USA*
†e-mail: ingber@a1.tch.harvard.edu

Tight control of cell proliferation is required to ensure normal tissue patterning and prevent cancer formation. The analysis of cultured cells has led to an explosion in our understanding of the molecules that trigger growth and mediate cell-cycle progression. However, the mechanism by which the local growth differentials that drive morphogenesis are established and maintained still remains unknown. Here we review recent work that reveals the importance of cell binding to the extracellular matrix, and associated changes in cell shape and cytoskeletal tension, to the spatial control of cell-cycle progression. These findings change the paradigm of cell-growth control, by placing our understanding of molecular signalling cascades in the context of the structural and mechanical complexity of living tissues.

Over the past decade, enormous advances have been made in our understanding of the molecules that mediate the control of cell proliferation. Soluble mitogens, insoluble extracellular matrix (ECM) molecules, cell-surface growth-factor receptors, integrins, signal-transducing molecules and proteins that form the central core of the cell-cycle machinery have all been identified, isolated and sequenced, and their encoding genes cloned. With the rapid progress of the Human Genome Project, more growth activators and inhibitors will no doubt be uncovered in the near future. However, the questions that are central to studies of both morphogenesis and cancer formation are not just how cell growth is turned on and off, but also where and when this happens. A more thorough understanding of the intricate workings of the molecular cascades that mediate cell-cycle progression inside individual cells will not answer this question of spatial control. In this review, we focus on recent work that reveals the importance of cellular adhesion to the ECM, cell shape and mechanical tension in the cytoskeleton for local control of cell-cycle progression. These results provide new insight into morphogenetic regulation and emphasize the need to develop new approaches to confront the structural complexity of biological systems.

Back to basics

The three-dimensional forms of specialized tissues, such as branching capillary networks and multilobular secretory epithelia, result from the establishment of local growth differentials over distances that sometimes extend across just a few cell diameters (Fig. 1a)[1,2]. Reiteration of this simple building rule over time and space causes similar localized sites of growth acceleration to be established along the sides of newly formed buds or branches, thereby creating the fractalized forms that are exhibited by almost all living tissues (Fig. 1b). Localized production of growth factors, such as members of the fibroblast growth factor (FGF) family, has been shown to promote tissue expansion and to guide branching in certain developing tissues[3]. However, although localized production of soluble chemoattractants and mitogens may determine the general position at which future tissue branches form, they do not explain how these branches are created. For example, the existence of soluble gradients of mitogens alone cannot explain how the sharp growth differentials that determine tissue patterning can be established on the micrometre scale (for example, between adjacent cells). Localized growth differentials are also observed in microenvironments that are known to be saturated with numerous soluble mitogens, such as during wound healing or angiogenesis as well as in *in vitro* morphogenesis models. In fact, careful analysis of capillary development in living embryos reveals that growing sprouts, quiescent differentiated tubes and regressing capillaries can all co-exist in the same

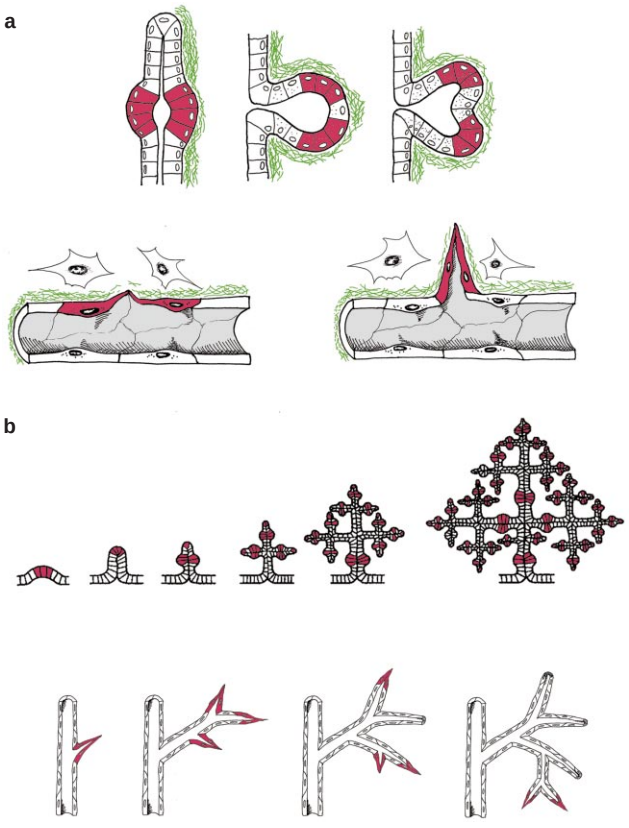

Figure 1 **How local growth differentials drive normal tissue patterning during epithelial morphogenesis and angiogenesis.** Epithelial morphogenesis is shown at the top of each panel, and angiogenesis at the bottom. **a**, A higher magnification view showing how growth is constrained to small groups of cells (red) under which lie regions of the basement membrane (green) that have become thin as a result of accelerated rates of ECM turnover. Outward budding and branching result from mesenchymal influences and because neighbouring cells along the same basement membrane remain quiescent (white cells). **b**, A lower magnification view showing how reiteration of this simple building rule over time and space results in creation of complex tissue architecture with characteristic fractal-like forms.

chemical milieu[4]. Thus, some form of local control of cell sensitivity to growth stimuli must exist to produce and maintain these growth differentials that are so critical for normal tissue patterning. Importantly, progressive loss of this spatial control leads to the disorganization of normal tissue architecture that is the hallmark of neoplastic transformation[5–7]. Cancer is not caused simply by uncontrolled growth in the sense of increased rates of cell proliferation; malignancy results when cells grow at times and in locations where proliferation is normally suppressed, that is, when they become autonomous of the normal controls that spatially constrain growth within living tissues.

How are local growth differentials established in developing tissues and maintained in the adult? Work in the past has revealed that local changes in ECM remodelling (synthesis and degradation) are central to this process, with matrix turnover rates being highest in regions of most rapid cell proliferation[2]. Most cultured cells also require anchorage to an ECM-coated substrate to grow, whereas development of anchorage independence is a fundamental feature of malignant transformation. In fact, changes in the ECM play a key part during the earliest stages of neoplastic transformation[5], and studies of transgenic mice show that deregulated ECM remodelling can induce tumour formation *in vivo*[8]. Because of these observations, recent attention has focused on the role of ECM molecules and their cell-surface integrin receptors in anchorage-dependent growth control.

Adhesion-dependent control of cell-cycle progression

Although our understanding of signal transduction and cell-cycle regulation was first defined through analysis of soluble mitogens, it is now clear that the ECM is an equally important growth regulator. Cell-surface integrin receptors promote cell attachment to the ECM and transduce biochemical signals to the nucleus by activating the same intracellular signalling pathways that are used by growth-factor receptors[9]. Integrin occupation and clustering leads to stimulation of multiple early mitogenic events associated with transition from the G0 to G1 phase of the cell cycle, including expression of immediate early growth response genes.

The emerging theme in the field of anchorage-dependent growth control is that integrin-mediated adhesion to the ECM activates the mitogen-activated protein kinase (MAPK)/extracellular-signal-regulated kinase (ERK) pathway that has been shown to be central to growth control by linking activation of growth-factor receptors to the cell-cycle machinery[10–12]. This pathway involves a progressive cascade of signal transduction from receptor-dependent activation of the small G protein Ras, to the kinase Raf, to MAPK/ERK kinase (MEK), and finally to the downstream MAPKs, ERK1 ($p44^{Erk1}$) and ERK2 ($p42^{Erk2}$)[13]. ERK/MAPK activation leads to the induction of cyclin D1, which triggers a series of nuclear events that leads to passage through the late-G1 restriction point and entry into S phase[14].

In fibroblasts, ERKs are rapidly activated upon cell adhesion to fibronectin or upon crosslinking of integrin β1 with an antibody in the absence of soluble growth factors[15–21]. Integrin binding appears to promote signalling to ERK through multiple pathways that already diverge at the receptor level[15]; these signals can impinge on the canonical Ras/ERK cascade either upstream[18,19] or downstream of Ras activation[17,20,21]. As well as signalling directly, integrin binding is also required for efficient propagation of signals from growth-factor receptors to Raf and, subsequently, to the downstream growth-associated kinases, MEK and ERK1/2 (refs 20–22).

Thus, despite minor variations in molecular signalling in different cells, the common finding is that adhesion-dependent G1-phase progression requires joint regulation of the ERK/MAPK pathway by integrins and growth-factor receptors. Sustained activation of ERK/MAPK is thought to be essential for G1 progression[23–25], and so these results have led to the view that anchorage-dependent growth control results from biochemical signals that are elicited in response to integrin binding. However, many studies have shown that activation of ERK/MAPK is not sufficient for passage through the late-G1 checkpoint. For example, when activated Raf is expressed in suspended lung fibroblasts using an inducible promoter, strong and sustained activation of ERK/MAPK is produced, but cyclin D1 levels remain low and cells fail to hyperphosphorylate the retinoblastoma protein (pRb)[26], a requirement for passing through the late-G1 restriction point. Overexpression of MEK1 in adherent NIH3T3 cells also fails to promote efficient pRb phosphorylation or S-phase entry in the absence of growth factors, even though it induces expression of cyclin D1 and promotes assembly of complexes containing cyclin D1 and cyclin-dependent kinase (CDK) 4 (ref. 12). These results indicate that another critical governor of cell-cycle progression must exist in the cell that is distinct from MAPK/ERK.

Mitogen-dependent control of progression through later stages of G1 (that is, downstream of ERK/MAPK) is orchestrated by cyclins and CDKs; the activities of the latter proteins depend on cyclin binding, phosphorylation state, and interactions with CDK-inhibitory proteins, such as $p27^{Kip1}$ (p27) and $p21^{Cip1}$ (p21)[27]. Cyclins D and E control progression through the late-G1 restriction point by assembling with their catalytic CDK partners and, when activated, phosphorylating and thereby inactivating the inhibitory pRb protein. This cell-cycle gate in late G1 marks the end of a requirement for stimulation by external growth factors[27,28].

Studies using fibroblasts that exhibit anchorage-dependent growth have revealed that cell adhesion regulates the transition through the late-G1 restriction point by altering the cyclin-dependent cell-cycle machinery[29,30]. Unanchored fibroblasts remain arrested in mid-G1 phase, whereas the same cells pass through this restriction point and enter S phase when allowed to reattach and spread on an ECM substrate. This anchorage-dependent G1 arrest correlates with a marked reduction in cyclin E–CDK2 kinase activity in suspended cells, and has been attributed to increased levels of the CDK inhibitors p27 and p21, although the particular CDK inhibitor responsible varies in different studies[31–35]. Some authors have also observed a decrease in the levels of cyclin D1 in suspended cells; this could lead to redistribution of p21 and p27 and thus to enhanced inhibition of CDK2 (refs 31,35,36). In one mutant cell line, a later anchorage-dependent checkpoint involving cyclin A has been described at the G1-to-S transition; this checkpoint can apparently be dissociated from the late-G1 restriction point[37]. However, the failure of normal fibroblast cells to induce cyclin A when placed in suspension appears to be secondary to arrest at the restriction point in the absence of anchorage[32,33,38].

These studies on the cell cycle have further cemented the idea that anchorage-dependent growth control is governed by integrin binding and associated receptor signalling events. However, in developing epithelial and endothelial tissues, all cells remain anchored to their ECM (basement membrane) whether growing or not; loss of ECM contact triggers apoptosis and tissue regression in many of these tissues (for example, mammary epithelium and blood capillaries[39,40]) and terminal differentiation in others (such as skin[41]). Moreover, *in vitro* studies show that although many integrin signalling events can be induced in suspended cells by allowing the cells to bind to ECM-coated microbeads[42–44], these cells never enter S phase[45] and, in the case of endothelial cells, undergo apoptosis[46]. This leads to a key question: if mere adhesion to the ECM and activation of integrin signalling alone are not sufficient to explain how the local growth differentials are established during morphogenesis, then how does the ECM exert this effect?

Shape and tension dependence

Another major difference between anchored cells and suspended cells besides integrin binding is their shape: cells spread and flatten on rigid planar ECM substrates but remain round in suspension. Tight coupling between cell shape and growth has been recognized in anchorage-dependent cells for over 20 years[47] and oncogenic

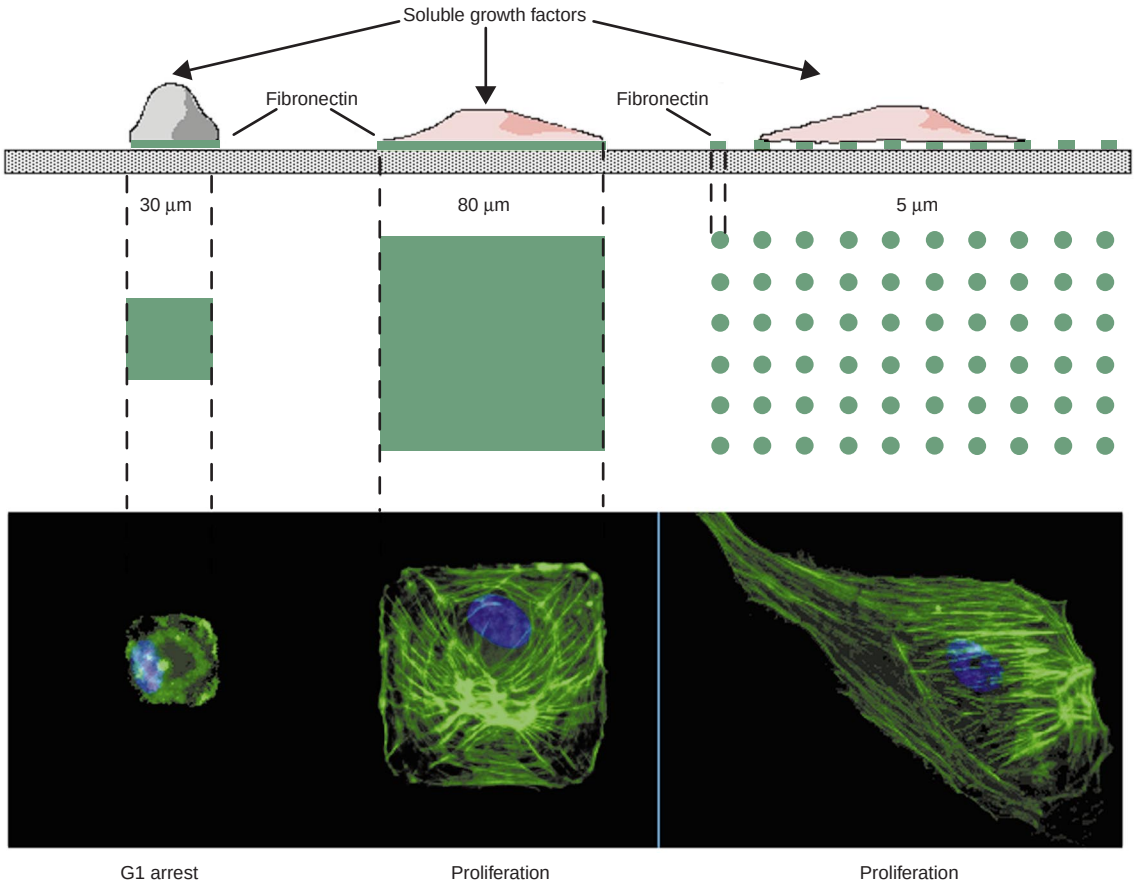

Figure 2 **Control of cell shape independently of the total cell–ECM contact area, studied using micropatterned adhesive substrates.** Top, side view of a cell-culture substrate containing adhesive islands of defined shape and size on the micrometre scale; these islands were coated with a saturating density of fibronectin (green) and separated by intervening non-adhesive regions coated with polyethylene glycol using a self-assembly-based microfabrication method[46]. Middle, a view from above of the same micropatterned substrates. Bottom, immunofluorescence micrographs of human endothelial cells cultured on the islands shown and stained for actin microfilaments with fluorescein-isothiocyanate–phalloidin (green) and for DNA with 4,6-diamidino phenylindole (blue). Note that cells remain small and fail to form actin bundles on the small adhesive island (left), but spread and fully reorganize their cytoskeleton when cultured on many small adhesive dots (right), even though the total area of ECM directly bound by the cell is identical in the two cases. Cells that spread on large squares (centre) or across many dots (right) pass through the late-G1 checkpoint when stimulated by growth factors, whereas cells that are restricted in their spreading never enter S phase[58].

transformation has been shown to be accompanied by a progressive loss of shape sensitivity[48]. In these early studies, standard tissue-culture substrates were precoated with varying thicknesses of a non-adhesive polymer to progressively interfere with cell contact with the substrate, and hence to prevent cell spreading in serum-containing medium. However, the role of the ECM in shape-dependent growth control could not be directly determined in these studies because it was not possible to control the chemistry of the substrates or to rule out changes in cell sensitivity to different growth factors in the medium. More recent studies carried out under more defined conditions confirm that the ability of various ECM molecules, including laminin, types I, III, IV and V collagens, and different fibronectin isoforms, to stimulate growth correlates with their ability to promote cell spreading in several cell types[49–54], although the nature of the integrin subtype activated by the ECM ligands may also be important[19]. Similar tight coupling between cell shape and proliferation has been shown by preventing cell spreading through reducing the density of the ECM molecule coated on otherwise non-adhesive dishes[45,55,56] or using a peptide containing the amino-acid sequence RGD, a cell-binding peptide from fibronectin[57]. Nevertheless, the interpretation of these results is complicated because the variations in the ECM substrate used to control cell shape can also affect integrin signalling. In other words, it was impossible to distinguish the effects of cell attachment *per se*, which triggers immediate integrin signalling events, from subsequent integrin-mediated changes in cell shape and cytoskeletal organization.

This issue has been resolved directly by use of microfabricated culture substrates containing ECM-coated adhesive islands with defined shape, size and position on a micrometre scale[46,58]. When the spreading of human endothelial cells was restricted by culturing them on small adhesive islands (of 30 μm^2), entry into S phase was restricted compared with cells on larger islands (of 80 μm^2), even though the surface chemistry and the density of ECM molecules were identical (Fig. 2). Furthermore, by causing cells to spread over many small, fibronectin-coated adhesive islands (dots of 3–5 μm in diameter), separated by intervening non-adhesive regions of varying sizes, cell shape could be controlled over a wide range while holding the total area of cell–ECM contact constant (Fig. 2). When the cells were grown on these micropatterned substrates, their ability to enter S phase in the presence of mitogens depended directly on the degree to which they were allowed to distend physically, and not on their level of ECM binding. Cells that were bound to ECM but prevented from spreading failed to increase cyclin D1 levels and downregulate the cell-cycle inhibitor p27, and thus mimicked the

effects of detachment from substrate, even though local integrin binding was high and early signalling events, including activation of ERK1/2, occurred completely[58]. Using this micropatterning method, local growth differentials could be established *in vitro* in the presence of soluble mitogens by plating cells on differently sized adhesive islands in the same dish (Fig. 3), thereby emulating the tight spatial control of cell growth that is observed during normal development (Fig. 1a).

Studies of endothelial cells, fibroblasts and many other cell types also show that maintenance of the integrity of the actin cytoskeleton, which determines cell shape and is key to cell spreading, is critical for G1 progression. Disruption of the actin cytoskeleton using cytochalasin inhibits S-phase entry when added before, but not after, the late-G1 checkpoint[58–61]. In fact, actin disruption produces effects on the cell-cycle machinery that are similar to those of cell rounding or detachment, including downregulation of cyclin D1, upregulation of p27, and inhibition of pRb hyperphosphorylation, even though the cells remain adherent to the ECM[58,61].

Most interestingly, the same cell-cycle block in late G1 can be induced without altering cytoskeletal integrity or cell shape in endothelial cells, by inhibiting cytoskeletal-tension generation using an inhibitor of the ATPase activity of the motor protein myosin[58]. Decreasing cytoskeletal tension by inhibiting the small G protein Rho with lovastatin in the presence of farnesol similarly prevents S-phase entry without altering cell shape in prostate tumour cells[62]. G1 progression can also be inhibited by decreasing the ECM's ability to resist cell-generated tensile forces (for example, by increasing its mechanical compliance or decreasing its adhesivity) and thereby dissipating cell tension[63,64]. However, this response may be biphasic, as greatly overincreasing cell contractility by overexpressing constituitively active myosin-light-chain kinase inhibits both cell spreading and growth in fibroblasts[65]. Taken together, these results indicate that the molecular machinery that controls the late-G1 restriction point is itself governed by the mechanical and structural context in which it acts.

Cadherin-mediated cell–cell contacts can also modulate p27 (refs 66,67) and thus may have a similar role in cell-cycle control to that of cell–ECM contacts. However, the results obtained with micropatterned surfaces show that local control of growth by ECM and cell shape occurs in the absence of cell–cell contact formation. The growth arrest observed when cells crowd into a monolayer (that is, density-dependent inhibition of growth) can also be explained by associated changes in cell shape (compaction and rounding), independently of cell–cell contacts[47]. Thus, it appears that the organism possesses redundant mechanisms to prevent uncontrolled growth, including restriction of growth factors, stabilization of cell–cell and cell–ECM contacts, and maintenance of cell shape and tissue mechanics. However, regulation by cell shape and mechanical forces is of particular physiological importance in the tissue context, as adherent cells form contacts with ECM and with their neighbours *in vivo*, and many receive growth-factor signals, yet normal morphogenesis and tissue homeostasis both require that only a subset of these cells proliferate in distinct locations.

The mechanics of cell-cycle control

Where do we go from here? How do we link mechanics with cell-cycle biochemistry? As described above, the early ERK/MAPK signalling pathway downstream of Ras appears to be insufficient for S-phase entry, yet overexpression of Ras can overcome anchorage dependence in fibroblasts[13,68]. Successful progression into S phase requires sustained Ras activity until late G1, beyond the period of ERK/MAPK activity[69–71]. Interestingly, Ras is required for downregulation of p27 in fibroblasts, and this effect does not appear to be mediated by ERK/MAPK as dominant-negative ERK does not interfere with growth-factor-induced downregulation of p27 (ref. 11). Furthermore, overexpression of MEK is unable to downregulate p27 in the absence of mitogenic stimulation[12]. These observations are consistent with the finding that cell shape and the actin cytoskeleton control p27 expression independently of MAPK/ERK activation in endothelial cells[58].

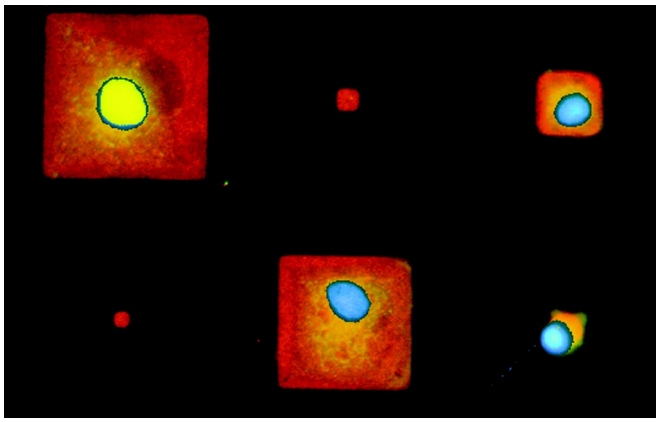

Figure 3 **A pseudocolour image showing establishment of local growth differentials in the presence of soluble mitogens *in vitro*.** Endothelial cells were cultured in the same dish on square fibronectin islands of varying sizes (5–50 μm^2) and fluorescently labelled with antibodies against fibronectin to visualize the islands (red), 4,6-diamidino phenylindole to stain all nuclei (blue), and antibodies against bromodeoxyuridine to label nuclei in S phase (yellow/green). Note that only the highly spread cell progressed through the cell cycle to S phase.

The small G protein Rho may be important in shape- and tension-dependent growth control, as it regulates cytoskeletal contractility[72,73] and G1 progression[74], mediates changes in cytoskeletal organization and cell morphology[75], and is downstream of Ras[13,76]. Activation of newly synthesized RhoA by geranylgeranylation is necessary for p27 degradation[77], and ectopic activation of RhoA stimulates p27 degradation and CDK2 activation[78] as well as growth that is free of anchorage, but not of serum[79]. Conversely, inhibition of RhoA results in increased p27 levels and late-G1 arrest[11,80]. Future studies will be required to determine whether Rho produces its effects on p27 and cell-cycle progression by altering cytoskeletal tension. At the same time, increases in cell tension may feed back to activate Rho, as inhibition of Rho using botulinum C3 exoenzyme inhibits focal-adhesion formation and actin reorganization induced by exogenous mechanical stresses exerted by stretching the cell's adhesive substrate[81]. Thus, in addition to mediating growth-factor-induced changes in cell morphology, Rho may also be involved in the mechanism by which altering the balance of mechanical forces across the cell's ECM adhesions produces the changes in internal cell structure that govern cell-cycle progression.

Integration of chemistry and mechanics

These results, combined with information obtained from work on soluble growth factors, alters the way in which we think about growth control and morphogenetic regulation. Analysis of cell-cycle regulation has revealed that growth control can no longer be defined solely in terms of the presence or absence of specific mitogens or different interactions of integrins. Soluble growth factors, insoluble ECM molecules and mechanical forces or cell distortion all contribute to control of G1 progression and each can be rate-limiting for the others (Fig. 4). But is this new understanding of growth control consistent with what is observed during morphogenesis *in vivo*? It has in fact been known for over a century that whereas soluble factors drive tissue development, mechanical forces dictate tissue pattern; in the case of bone, this governing principle even has a name ('Wolff's law'). The importance of cell tension for morphogenetic control has also been shown in living tissues ranging from skin to brain[82,83]. Analysis of epithelial morphogenesis in

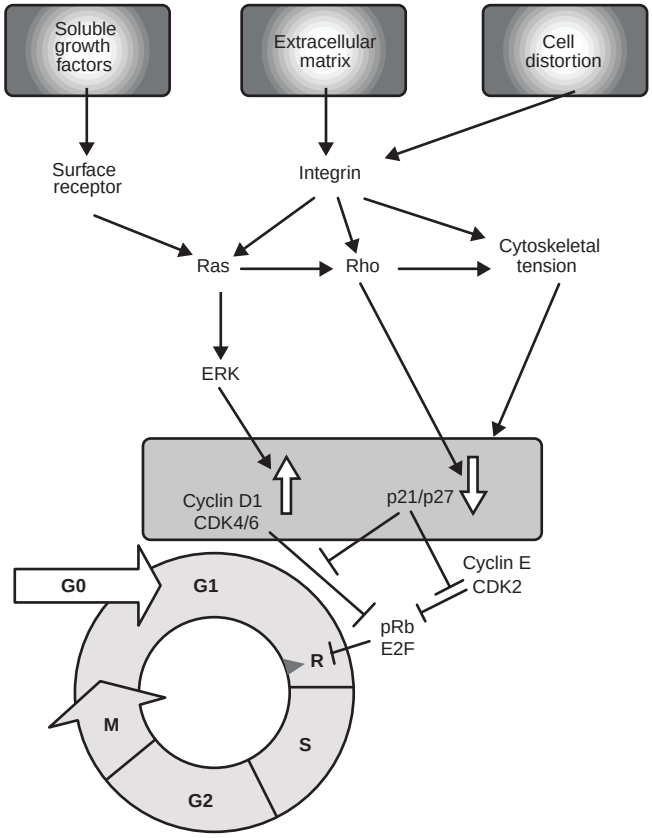

Figure 4 **Working model for regulation of G1 progression by growth factors, adhesion to ECM and cell distortion.** R, restriction point.

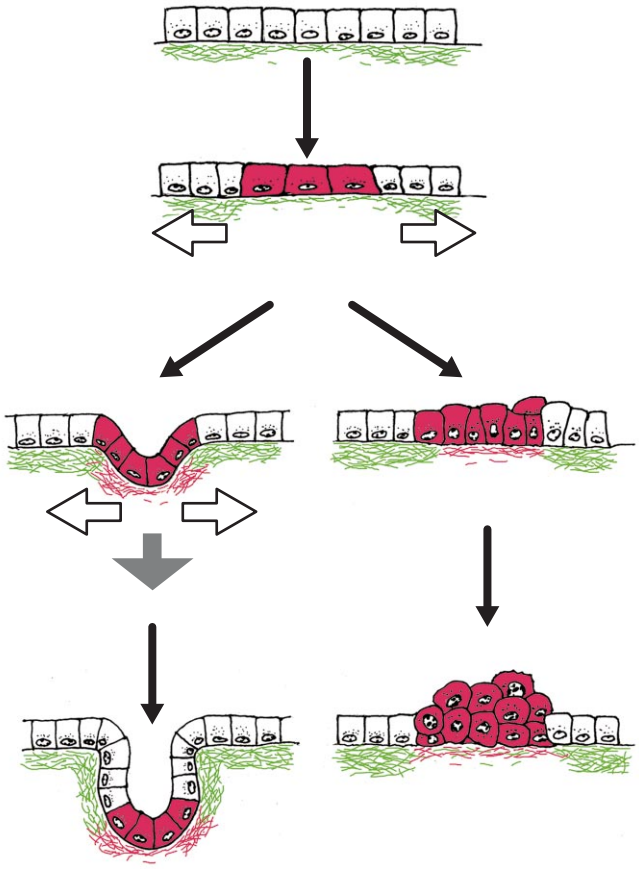

Figure 5 **Model for tension-driven tissue remodelling during normal morphogenesis and its deregulation during tumour formation.** Left, normal epithelial morphogenesis. Local increases in ECM turnover result in the formation of focal defects in the basement membrane (green) which stretches and thins because of the contraction and pulling of neighbouring adherent epithelium (white arrows) and underlying mesenchyme (grey arrow). Cells adherent to the basement membrane in this extending region will distort or experience changes in isometric tension within the cytoskeleton and thus become preferentially sensitive to growth stimuli. Cell division is accompanied by deposition of new basement membrane (red), and therefore cell-mass expansion and ECM extension are tightly coupled, leading to bud formation in this localized region. Right, neoplastic disorganization of tissue architecture through deregulation of tension-driven remodelling. Increasing ECM turnover in an adult epithelium may lead to basement-membrane thinning, changes in cell mechanics, and an increase in the sensitivity of adjacent cells to growth stimuli, much as is observed during epithelial bud formation (left). Because cell division is not coupled to basement-membrane extension, piling up of disorganized epithelial cells results. Rapid and complete dissolution of the basement membrane will result in cell apoptosis and involution of most epithelial and endothelial tissues and thereby self-restrict further tissue disorganization. If these more subtle changes in tissue structure and mechanics are sustained over time, this continued growth stimulus could lead to selection of anchorage-independent cells with malignant features.

the mammalian lung reveals that localized distortion of the growing epithelium in the sites of the newly forming buds actually precedes growth, as measured by S-phase entry[84]. Actin-filament-dependent changes in cell shape are tightly coupled to local cell proliferation in the same system[85]. In other words, epithelial budding is not initiated by increased cell mass because of local cell growth in response to mitogenic stimulation; instead, local changes in cell shape and cytoskeletal distortion associated with bud extension allow the cell to respond to the mitogen by progressing through the cell cycle and proliferating locally, thereby further propagating tissue expansion in these regions. Placed in the context of recent genetic studies on growth factors in morphogenesis[3], these findings indicate a mechanism for growth control by which localized production of soluble factors drives tissue growth and governs the sites of formation of new branches at the tissue level, whereas localized changes in ECM turnover and cytoskeletal mechanics determine precisely which cells will respond to these factors as well as how differently shaped extensions (pulmonary versus capillary branches) can be created.

How could cytoskeletal tension change within cells that are surrounded by neighbouring cells and bound to ECM? Again, analysis of regions of the growing buds within developing tissues, such as lung, salivary gland and capillaries, has helped: such analysis reveals that the basement membrane thins specifically beneath the sites of the most rapid cell proliferation, as a result of accelerated ECM turnover[1,8,86]. More recently, a secreted matrix-degrading protease has been found to be essential for organ expansion during gonadal organogenesis in *Caenorhabditis elegans*[105]. All tissues exist in a state of isometric tension because of the contraction of their constituent cells and the resistance of the surrounding ECM scaffolds[5,87,88]. If the basement membrane is under tension because of the contractility of adherent cells, then local thinning of the ECM scaffold would be expected to cause spreading of this weakened region, much as a run in a stocking spreads from a small local defect to cover a large space. In a similar manner, a local decrease in ECM thickness (and increase in compliance) could lead to spreading of adjacent adherent cells or a change in cytoskeletal tension. As suggested by *in vitro* studies[45–47,58], this change in cell shape and mechanics would increase the cell's ability to respond to surrounding mitogens by passing through the restriction point and entering S phase (Fig. 5). Neighbouring cells on thicker, intact basement membrane that did not feel this pull or experience distortion would remain quiescent,

although confronted by the same soluble stimuli.

This tension-driven remodelling hypothesis for morphogenetic regulation[6] is consistent with both *in vivo* and *in vitro* data, as summarized above, and with the finding that pharmacological inhibition of tension generation inhibits morphogenesis in developing salivary gland[87]. Interestingly, variations in the pattern-directing behaviour of different mesenchymal tissues correlate with their ability to generate mechanical tension[89]. This is important because the mesenchyme determines tissue-specific pattern formation in epithelia[90]. Perhaps it is because of this reliance on local distortion for spatial control of cell growth that the expression of high stromal levels of growth factors in transgenic mice increases branching of mammary epithelium, rather than producing proliferation in all cells and amorphous tissue growth[91]. This is consistent with a view of tissue regulation in which the function of soluble growth factors is to regulate overall tissue and organ size, while their effect is spatially restricted in a tissue-specific manner to generate distinct histological patterns, much like phyllotaxis in plants. The important point here is that this spatial restriction may involve localized changes in ECM and cell mechanics as well as localized production of distinct positive and negative growth modulators[3].

Conversely, loss of this form of tension-driven structural remodelling may lead to disorganization of normal tissue patterns[5,6]. Tissue boundaries, such as the basement membrane in epithelium, commonly remain physically intact during early stages of tumour formation (that is, before malignant transformation and invasion); however, a reduction in the thickness of the basement membrane or subtle decreases in the levels of certain ECM constituents can be detected[5,92,93]. This situation is analogous to what is observed in regions of rapid growth in normal embryonic tissues, except that the oncogenic changes are not normally restricted in space or time and disorganization of tissue structure results (Fig. 5, compare right and left). In fact, it is often the loss of tissue pattern that is first recognized by the pathologist as abnormal[7]. Sustained changes in ECM structure and compliance could promote distortion or increase cytoskeletal tension locally and hence increase the sensitivity of adjacent adherent cells to mitogenic stimuli, much as is observed in the embryo. However, a piling up of cells would result because of failure to produce a commensurate increase in basement-membrane extension (that is, expansion of available adhesive area to match increases in cell number), as is normally observed in developing tissues (Fig. 5). Sustained growth stimulation over many years could result in selection of cells that grow free of anchorage *in vivo*, just as continued culturing of normal cells may lead to spontaneous transformation *in vitro*. Cell growth and survival free of contact with the basement membrane are sufficient to explain the disorganization of normal cell–cell relations that is observed during early stages of neoplastic transformation[5].

Studies involving culture of normal mammary epithelial cells and their transformed counterparts on basement-membrane gels *in vitro* show that the ability of cells to form three-dimensional organotypic structures and maintain normal cell shapes may suppress expression of the malignant phenotype[94,95]. This may, in part, be due to reciprocal modulation of integrin and growth-factor-receptor signalling through the ERK/MAPK pathway; such modulation requires growth in three-dimensional ECM gels and does not occur in monolayer culture. Furthermore, targeting an autoactivating form of the matrix-degrading metalloproteinase stromelysin-1 to the mammary epithelium of transgenic mice promotes malignant transformation in this tissue[8]. Again, in early phases of this process, before full expression of the invasive phenotype, enhanced ECM degradation is observed although the basement membrane does not fully disrupt[96]. Taken together, these results indicate that the local structural and mechanical context of the cell may represent a critical epigenetic safeguard against neoplasia *in vivo*, as well as guiding normal developmental patterning. Loss of this spatial growth control at the level of tissue architecture may therefore represent one step in the multistep process of tumorigenesis, along with genetic alterations in the cell.

Conclusions

We must begin to deal with complexity. Cells as the basic building blocks of tissues represent autonomous agents endowed with programmes that allow them to sense their chemical and mechanical context, and to divide when they sense space is available. Such self-organizing behaviour that drives morphogenesis appears to be achieved by coupling cell division to cell distortion and even to changes in the balance of mechanical forces within the cell[6,97,98]. The discovery of the importance of cell shape and cytoskeletal tension for control of cell-cycle progression requires that we place what we have learned about biochemical mechanisms of cell-growth regulation within a larger frame of reference that also takes into account cellular architecture, micromechanics and structural complexity. Most important, we must begin to invoke models of biological regulation that are based on more than just changes in molecular binding events. This will be difficult because it requires that we devise new theoretical paradigms and analytical methodologies to handle highly complex systems, including approaches not used at present by experimental biologists. These approaches will need to deal with the dynamics, mechanics, network properties and hierarchical complexity that characterizes living systems[99,100].

Complexity sciences[101–103] and tensegrity architecture[6,100,104] are two examples of such approaches that may be useful in this context. For example, in the treatment of complex dynamic systems, patterns are thought to emerge as distinct self-stabilizing states ('attractors') starting with a wide range of different initial inputs. The finding that a generalized stimulus, such as cell distortion, induces the same distinct biochemical pattern in cells as that produced by soluble messengers that bind to specific cell-surface receptors (such as changes in p27 and cyclin D1) is therefore consistent with the existence of a robust attractor in the network of cell-cycle-regulatory pathways (S.H. and I.D.E., unpublished observations). Use of tensegrity architecture by cells, which provides an engineering basis for integrating cell tension and cytoskeletal structure[5,94], may offer a mechanism by which changes in mechanical forces can influence thermodynamics and kinetics within load-bearing structures within the cell, and hence regulate specific molecular biochemistry[97,98]. It also will be necessary to develop techniques to alter the structure and mechanics of cells and of specific multimolecular networks controllably or, at least, to provide ways for experimentalists to hold these structural variables constant. In general terms, cell biology must remodel itself to make the transition from molecular reductionism to cellular realism; only then will we answer the fundamental questions that first launched us on the exciting adventure in which we participate today. □

1. Ausprunk, D. H. & Folkman, J. Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. *Microvasc. Res.* **14,** 53–65 (1997).
2. Bernfield, M. R. & Banerjee, S. D. in *Biology and Chemistry of Basement Membranes* (ed. Kefalides, N.) 137–148 (Academic, New York, 1978).
3. Metzger R. J. & Krasnow, M. A. Genetic control of branching morphogenesis. *Science* **284,** 1635–1639 (1999).
4. Clark, E. R. & Clark, E. L. Microsopic observations on the growth of blood capillaries in the living mammal. *Am. J. Anat.* **64,** 251–301 (1938).
5. Ingber, D. E., Madri, J. A. & Jamieson, J. D. Role of basal lamina in neoplastic disorganization of tissue architecture. *Proc. Natl Acad. Sci. USA* **78,** 3901–3905 (1981).
6. Ingber, D. E. & Jamieson, J. D. in *Gene Expression During Normal and Malignant Differentiation* (eds Anderson, L. C., Gahmberg, C. G. & Ekblom, P.) 13–32 (Academic, Orlando, 1985).
7. Clark, W. H. Jr The nature of cancer: morphogenesis and progressive (self)-disorganization in neoplastic development and progression. *Acta Oncol.* **34,** 3–21 (1995).
8. Lochter, A. *et al.* Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. *J. Cell Biol.* **139,** 1861–1872 (1997).
9. Kumar, C. C. Signaling by integrin receptors.*Oncogene* **17,** 1365–1373 (1998).
10. Lavoie, J. N., L'Allemain, G., Brunet, A., Müller, R. & Pouysségur, J. Cyclin D1 expression is regulated positively by the p42/p44MAPK and negatively by the p38/HOGMAPK pathway. *J. Biol. Chem.* **271,** 20608–20616 (1996).

11. Weber, J. D., Hu, W, Jefcoat, S. C. Jr, Raben, D. M. & Baldassare, J. J. Ras-stimulated extracellular signal-regulated kinase 1 and RhoA activities coordinate platelet-derived growth factor-induced G1 progression through the independent regulation of cyclin D1 and p27. *J. Biol. Chem.* **272,** 32966–32971 (1997).
12. Cheng, M., Sexl, V., Sherr, C. J. & Roussel, M. F. Assembly of cyclin D-dependent kinase and titration of p27Kip1 regulated by mitogen-activated protein kinase kinase (MEK1). *Proc. Natl Acad. Sci. USA* **95,** 1091–1096 (1998).
13. Vojtek, A. B. & Der, C. J . Increasing complexity of the Ras signaling pathway. *J. Biol. Chem.* **273,** 19925–19928 (1998).
14. Sherr, C. J . Cancer cell cycles. *Science* **274,** 1672–1677 (1996).
15. Morino, N. *et al.* Matrix/integrin interaction activates the mitogen-activated protein kinase, p44erk-1 and p42erk-2. *J. Biol. Chem.* **270,** 269–273 (1995).
16. Schlaepfer, D. D & Hunter, T. Integrin signalling and tyrosine phosphorylation: just the FAKs? *Trends Cell Biol.* **8,** 151–157 (1998).
17. Chen, Q., Lin, T. H., Der, C. J. & Juliano, R. L. Integrin-mediated activation of MEK and mitogen-activated protein kinase is independent of Ras. *J. Biol. Chem.* **271,** 18122–18127 (1996).
18. Clark, E. A. & Hynes, R. O. Ras activation is necessary for integrin-mediated activation of extracellular signal-regulated kinase 2 and cytosolic phospholipase A2 but not for cytoskeletal organization. *J Biol. Chem.* **271,** 14814–14818 (1996)
19. Wary, K. K., Manieri, F., lsakoff, S. J., Marcantonio, E. E & Giancotti, F. G . The adaptor protein Shc couples a class of integrins to the control of cell cycle progression. *Cell* **88,** 573–575 (1997).
20. Lin, T. H., Chen, Q., Howe, A. & Juliano, R. L. Cell anchorage permits efficient signal transduction between ras and its downstream kinases. *J. Biol. Chem.* **272,** 8849–8852 (1997).
21. Renshaw, M. W., Ren, X. D. & Schwartz, M. A. Growth factor activation of MAP kinase requires cell adhesion. *EMBO J.* **16,** 5592–5599 (1997).
22. Short, S. M., Talbott, G. A. & Juliano, R. L. Integrin-mediated signaling events in human endothelial cells. *Mol. Biol.Cell* **9,** 1969–1980 (1998).
23. Meloche, S., Pages, G. & Pouyssegur, J. Functional expression and growth factor activation of an epitope-tagged p44 mitogen-activated protein kinase, p44mapk. *Mol. Biol. Cell* **3,** 63–71 (1992).
24. Zhu, X. & Assoian, R. K. Integrin-dependent activation of MAP kinase: a link to shape-dependent cell proliferation. *Mol. Biol. Cell* **6,** 273–282 (1995).
25. Weber, J. D., Raben, D. M., Phillips, P. J. & Baldassare, J. J . Sustained activation of extracellular-signal-regulated kinase 1 (ERK1) is required for the continued expression of cyclin D1 in G1 phase. *Biochem. J.* **326,** 61–68 (1997).
26. Le Gall, M., Grall, D., Chambard, J. C., Pouyssegur , J. & Van Obberghen-Schilling, E. An anchorage-dependent signal distinct from p42/44 MAP kinase activation is required for cell cycle progression. *Oncogene* **17,** 1271–1277 (1998).
27. Sherr, C. J. & Roberts, J. M. Inhibitors of mammalian G1 cyclin-dependent kinases. *Genes Dev.* **9,** 1149–1163 (1995).
28. Weinberg, R. A. The retinoblastoma protein and cell cycle control. *Cell* **81,** 323–330 (1995).
29. Guadagno, T. M. & Assoian, R. K. G1/S control of anchorage-independent growth in the fibroblast cell cycle. *J. Cell Biol.* **115,** 1419–1425 (1991).
30. Assoian, R. K. & Zhu, X. Cell anchorage and the cytoskeleton as partners in growth factor dependent cell cycle progression. *Curr. Opin. Cell Biol.* **9,** 93–98 (1997).
31. Zhu, X., Ohtsubo, M., Bohmer, R. M., Roberts, J. M. & Assoian, R. K. Adhesion-dependent cell cycle progression linked to the expression of cyclin D1, activation of cyclin E-cdk2, and phosphorylation of the retinoblastoma protein. *J. Cell Biol.* **133,** 391–403 (1996).
32. Fang, F., Orend, G., Watanabe, N., Hunter, T. & Ruoslahti, E . Dependence of cyclin E-CDK2 kinase activity on cell anchorage. *Science* **271,** 499–502 (1996).
33. Schulze, A. *et al.* Anchorage-dependent transcription of the cyclin A gene. *Mol. Cell Biol.***16,** 4632–4638 (1996).
34. Kuzumaki, T. & Ishikawa, K. Loss of cell adhesion to substratum up-regulates p21Cip1/WAF1 expression in BALB/c 3T3 fibroblasts. *Biochem. Biophys. Res. Commun.* **238,** 169–172 (1997).
35. Resnitzky, D. Ectopic expression of cyclin D1 but not cyclin E induces anchorage-independent cell cycle progression. *Mol. Cell Biol.***17,** 5640–5647 (1997).
36. Radeva, G.*et al.* Overexpression of the integrin-linked kinase promotes anchorage-independent cell cycle progression. *J.Biol. Chem.* **272,** 13937–13944 (1997).
37. Kang, J. S. & Krauss, R. S. Ras induces anchorage-independent growth by subverting multiple adhesion-regulated cell cycle events. *Mol. Cell. Biol.* **16,** 3370–3380 (1996).
38. Guadagno, T. M., Ohtsubo, M., Roberts, J. M. & Assoian, R. K. A link between cyclin A expression and adhesion-dependent cell cycle progression. *Science* **262,** 1572–1575 (1993).
39. Wicha, M. S., Liotta, L. A., Vonderhaar, B. K. & Kidwell, W. R. Effects of inhibition of basement membrane collagen deposition on rat mammary gland development. *Dev. Biol.* **80,** 253–266 (1980).
40. Ingber, D. E., Madri, J. A. & Folkman, J. A possible mechanism for inhibition of angiogenesis by angiostatic steroids: induction of capillary basement membrane dissolution. *Endocrinology* **119,** 1768–1775 (1986).
41. Adams, J. C. & Watt, F. M. Regulation of development and differentiation by the extracellular matrix. *Development* **117,** 1183–1198 (1993).
42. Schwartz, M. A., Lechene, C. & Ingber, D. E . Insoluble fibronectin activates the Na/H antiporter by clustering and immobilizing integrin alpha 5 beta 1, independent of cell shape. *Proc. Natl Acad. Sci. USA* **88,** 7849–7853 (1991).
43. Plopper, G. E, McNamee, H. P, Dike, L. E., Bojanowski, K. & Ingber, D. E. Convergence of integrin and growth factor receptor signaling pathways within the focal adhesion complex. *Mol. Biol.Cell* **6,** 1349–1365 (1995).
44. Miyamoto, S. *et al.* Integrin function: molecular hierarchies of cytoskeletal and signaling molecules. *J. Cell Biol.* **131,** 791–805 (1995).
45. Ingber, D. E. Fibronectin controls capillary endothelial cell growth by modulating cell shape. *Proc. Natl Acad. Sci. USA* **87,** 3579–3583 (1990).
46. Chen, C. S., Mrksich, M., Huang, S., Whitesides, G. M. & Ingber, D. E. Geometric control of cell life and death. *Science* **276,** 1425–1428 (1997).
47. Folkman, J. & Moscona, A. Role of cell shape in growth control. *Nature* **273,** 345–349 (1978).
48. Wittelsberger, S. C., Kleene, K. & Penman, S. Progressive loss of shape-responsive metabolic controls in cells with increasingly transformed phenotype. *Cell* **24,** 859–866 (1981).
49. Ingber, D. E., Madri, J. A. & Folkman, J. Endothelial growth factors and extracellular matrix regulate DNA synthesis through modulation of cell and nuclear expansion. *In Vitro Cell Dev. Biol.* **23,** 387–394 (1987).
50. Junker, J. L. & Heine, U. I . Effect of adhesion factors fibronectin, laminin, and type IV collagen on spreading and growth of transformed and control rat liver epithelial cells. *Cancer Res.* **47,** 3802–3807 (1987).
51. Vitale, M. *et al.* Integrin binding to immobilized collagen and fibronectin stimulates the proliferation of human thyroid cells in culture. *Endocrinology* **138,** 1642–1648 (1997).
52. Koyama, H., Raines, E. W., Bornfeldt, K. E., Roberts, J. M. & Ross, R. Fibrillar collagen inhibits arterial smooth muscle proliferation through regulation of Cdk2 inhibitors. *Cell* **87,** 1069–1078 (1996).
53. Manabe, R., Oh-e, N. & Sekiguchi, K. Alternatively spliced EDA segment regulates fibronectin-dependent cell cycle progression and mitogenic signal transduction. *J. Biol.Chem.* **274,** 5919–5924 (1999).
54. Zanetti, N. C., Dress, V. M. & Solursh, M. Comparison between ectoderm-conditioned medium and fibronectin in their effects on chondrogenesis by limb bud mesenchymal cells. *Dev . Biol.* **139,** 383–395 (1990).
55. Mooney, D. J. *et al.* Switching from differentiation to growth in hepatocytes: control by extracellular matrix. *J. Cell Phys.* **151,** 497–505 (1992).
56. Ingber, D. E. *et al.* Control of intracellular pH and growth by fibronectin in capillary endothelial cells. *J. Cell Biol.* **110,** 1803–1811 (1990).
57. Hansen, L. K., Mooney, D. J., Vacanti, J. P. & Ingber, D. E . Integrin binding and cell spreading on extracellular matrix act at different points in the cell cycle to promote hepatocyte growth. *Mol. Biol. Cell* **5,** 967–975 (1994).
58. Huang, S., Chen, S. C., Whitesides, G. M. & Ingber, D. E. Control of cyclin D1, p27(Kip1), and cell cycle progression in human capillary endothelial cells by cell shape and cytoskeletal tension. *Mol. Biol. Cell* **9,** 3179–3193 (1998).
59. Iwig, M. *et al.* Growth regulation by cell shape alteration and organization of the cytoskeleton. *Eur. J. Cell Biol.* **67,** 145–157 (1995).
60. Ingber, D. E., Prusty, D., Sun, Z., Betensky, H. & Wang, N. Cell shape, cytoskeletal mechanics and cell cycle control in angiogenesis. *J. Biomech.* **28,** 1471–1484 (1995).
61. Bohmer, R. M., Scharf, E. & Assoian, R. K . Cytoskeletal integrity is required throughout the mitogen stimulation phase of the cell cycle and mediates the anchorage-dependent expression of cyclin D1. *Mol. Biol. Cell* **7,** 101–111 (1996).
62. Ghosh, P. M. *et al.* Role of RhoA activation in the growth and morphology of a murine prostate cell line. *Oncogene* **18,** 4120–4130 (1999).
63. Ingber, D. E. & Folkman, J. How does extracellular matrix control capillary morphogenesis? *Cell* **58,** 803–805 (1989).
64. Mochitate, K., Pawelek, P. & Grinnell, F. Stress relaxation of contracted collagen gels: disruption of actin filament bundles, release of cell surface fibronectin, and down-regulation of DNA and protein synthesis. *Exp.Cell Res.* **193,** 198–207 (1991).
65. Cai, S. *et al.* Regulation of cytoskeletal mechanics and cell growth by myosin light chain phosphorylation. *Am. J. Phys.* **275,** C1349–C1356 (1998).
66. St Croix, B. *et al.* E-Cadherin-dependent growth suppression is mediated by the cyclin-dependent kinase inhibitor p27(KIP1). *J. Cell. Biol.* **142,** 557–571 (1998).
67. Levenberg, S., Yarden, A., Kam, Z. & Geiger, B. p27 is involved in N-cadherin-mediated contact inhibition of cell growth and S-phase entry. *Oncogene* **18,** 869–876 (1999).
68. Yang, J. J., Kang, J. S. & Krauss, R. S . Ras signals to the cell cycle machinery via multiple pathways to induce anchorage-independent growth. *Mol. Cell Biol.* **18,** 2586–2595 (1998).
69. Aktas, H., Cai, H. & Cooper, G. M. Ras links growth factor signaling to the cell cycle machinery via regulation of cyclin D1 and the Cdk inhibitor p27KIP1. *Mol. Cell Biol.* **17,** 3850–3857 (1997).
70. Taylor, S. J. & Shalloway, D. Cell cycle-dependent activation of Ras. *Curr. Biol.* **6,** 1621–1627 (1996).
71. Takuwa, N. & Takuwa, Y. Ras activity late in G1 phase required for p27kip1 downregulation, passage through the restriction point, and entry into S phase in growth factor-stimulated NIH 3T3 fibroblast. *Mol. Cell Biol.* **17,** 5348–5358 (1997).
72. Kimura, K. *et al.* Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science* **273,** 245–248 (1996).
73. Amano, M. *et al.* Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). *J. Biol. Chem.* **271,** 20246–20249 (1996).
74. Olson, M. F., Paterson, H. F. & Marshall, C. J. Signals from Ras and Rho GTPases interact to regulate expression of p21Waf1/Cip1. *Nature* **394,** 295–299 (1998).
75. Hall, A. Rho GTPases and the actin cytoskeleton. *Science* **279,** 509–514 (1998).
76. Qiu, R. G., Chen, J., McCormick, F. & Symons, M. A role for Rho in Ras transformation. *Proc. Natl Acad. Sci. USA* **92,** 11781–11785 (1995).
77. Noguchi, Y. *et al.* Newly synthesized Rho A, not Ras, is isoprenylated and translocated to membranes coincident with progression of the G1 to S phase of growth-stimulated rat FRTL-5 cells. *J. Biol. Chem.* **273,** 3649–3653 (1998).
78. Hu, W., Bellone, C. J. & Baldassare, J. J. RhoA stimulates p27(Kip) degradation through its regulation of cyclin E/CDK2 activity. *J. Biol. Chem.* **274,** 3396–3401 (1999).
79. Schwartz, M. A, Toksoz, D. & Khosravi-Far, R. Transformation by Rho exchange factor oncogenes is mediated by activation of an integrin-dependent pathway. *EMBO J.* **15,** 6525–6530 (1996).
80. Hirai, A. *et al.* Geranylgeranylated rho small GTPase(s) are essential for the degradation of p27Kip1 and facilitate the progression from G1 to S phase in growth-stimulated rat FRTL-5 cells. *J. Biol. Chem.* **272,** 13–16 (1997).
81. Yano, Y., Saito, Y., Narumiya, S. & Sumpio, B. E. Involvement of rho p21 in cyclic strain-induced tyrosine phosphorylation of focal adhesion kinase(pp125FAK), morphological changes and migration of endothelial cells. *Biochem. Biophys. Res. Commun.* **224,** 508–515 (1996).
82. Squier, C. A. The stretching of mouse skin *in vivo*: effect on epidermal proliferation and thickness. *J. Invest. Dermatol.* **74,** 68–71 (1980).
83. Van Essen, D. C . A tension-based theory of morphogenesis and compact wiring in the central nervous system. *Nature* **385,** 313–318 (1997).

84. Nogawa, H., Morita, K. & Cardoso, W. V. Bud formation precedes the appearance of differential cell proliferation during branching morphogenesis of mouse lung epithelium *in vitro*. *Dev. Dyn.* **213,** 228–235 (1998).
85. Goldin, G. V., Hindman, H. M. & Wessells, N. K. The role of cell proliferation and cellular shape change in branching morphogenesis of the embryonic mouse lung: analysis using aphidicolin and cytochalasins. *J. Exp. Zool.* **232,** 287–296 (1984).
86. Mollard, R. & Dziadek, M. A correlation between epithelial proliferation rates, basement membrane component localization patterns, and morphogenetic potential in the embryonic mouse lung. *Am. J. Respir. Cell Mol. Biol.* **19,** 71–82 (1998).
87. Ash, J. F., Spooner, B. S. & Wessells, N. K. Effects of papaverine and calcium-free medium on salivary gland morphogenesis. *Dev. Biol.* **33,** 463–469 (1973).
88. Banerjee, S. D, Cohn, R. H. & Bernfield, M. R. Basal lamina of embryonic salivary epithelia. Production by the epithelium and role in maintaining lobular morphology. *J. Cell. Biol.* **73,** 445–463 (1977).
89. Nogawa, H. & Nakanishi, Y. Mechanical aspects of the mesenchymal influence on epithelial branching morphogenesis of mouse salivary gland. *Development* **101,** 491–500 (1987).
90. Sakakura, T., Nishizura, Y. & Dawe, C. Mesenchyme-dependent morphogenesis and epithelium-specific cytodifferentiation in mouse mammary gland. *Science* **194,** 1439–1441 (1976).
91. Joseph, H., Gorska, A. E., Sohn, P., Moses, H. L. & Serra, R . Overexpression of a kinase-deficient transforming growth factor-beta type II receptor in mouse mammary stroma results in increased epithelial branching. *Mol. Biol. Cell* **10,** 1221–1234 (1999).
92. Tosios, K. I., Kapranos, N. & Papanicolaou, S. I. Loss of basement membrane components laminin and type IV collagen parallels the progression of oral epithelial neoplasia. *Histopathology* **33,** 261–268 (1998).
93. Henning, K., Berndt, A., Katenkamp, D. & Kosmehl, H. Loss of laminin-5 in the epithelium-stroma interface: an immunohistochemical marker of malignancy in epithelial lesions of the breast. *Histopathology* **34,** 305–309 (1999).
94. Petersen, O. W., Ronnov-Jessen, L., Howlett, A. R. & Bissell, M. J. Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. *Proc. Natl Acad. Sci. USA* **89,** 9064–9068 (1992).
95. Wang, F. *et al.* Reciprocal interactions between beta1-integrin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: a different perspective in epithelial biology. *Proc. Natl Acad. Sci. USA* **95,** 14821–14826 (1998).
96. Thomasset, N. *et al.* Expression of autoactivated stromelysin-1 in mammary glands of transgenic mice leads to a reactive stroma during early development. *Am. J. Pathol.* **153,** 457–467 (1998).
97. Ingber, D. E. Tensegrity: the architectural basis of cellular mechanotransduction. *Annu. Rev. Physiol.* **59,** 575–599 (1997).
98. Chicurel, M. E., Chen, C. S. & Ingber, D. E. Cellular control lies in the balance of forces. *Curr. Opin. Cell Biol.* **10,** 232–239 (1998).
99. Strohman, R. C. The coming Kuhnian revolution in biology. *Nature Biotechnol.*, **15,** 194–200 (1997).
100. Ingber, D. The architecture of life. *Sci. Am.* **278,** 48–57 (1998).
101. Kauffman, S. A. *The Origins of Order* (Oxford Univ. Press, New York, 1993).
102. Coffey, D. S. Self-organization, complexity and chaos: the new biology for medicine. *Nature Med.* **4,** 882–885 (1998).
103. Weng, G., Bhalla,U. S. & Iyengar, R. Complexity in biological signaling systems. *Science* **284,** 92–96 (1999).
104. Stamenovic, D., Fredberg, J. J., Wang, N., Butler, J. P. & Ingber, D. E. A microstructural approach to cytoskeletal mechanics based on tensegrity. *J. Theor. Biol.* **181,** 125–136 (1996).
105. Blelloch, R. & Kimble, J. Control of organ shape by a secreted metalloprotease in the nematode *Caenorhabditis elegans. Nature* **399,** 586–590 (1999).

ACKNOWLEDGEMENTS
This work was supported by grants from the NIH (CA58833, CA45548 & HL57669, to D.E.I.) and by a fellowship from the Schweizerische Stiftung für Medizinisch-Biologische Stipendien (to S.H.).