Balancing forces: architectural control of mechanotransduction

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Abstract | All cells exist within the context of a three-dimensional microenvironment in which they are exposed to mechanical and physical cues. These cues can be disrupted through perturbations to mechanotransduction, from the nanoscale-level to the tissue-level, which compromises tensional homeostasis to promote pathologies such as cardiovascular disease and cancer. The mechanisms of such perturbations suggest that a complex interplay exists between the extracellular microenvironment and cellular function. Furthermore, sustained disruptions in tensional homeostasis can be caused by alterations in the extracellular matrix, allowing it to serve as a mechanically based memory-storage device that can perpetuate a disease or restore normal tissue behaviour.

Kartagener's syndrome
A developmental disorder in which a disruption to mechanotransduction signalling by cilia-driven fluid flow results in the mirror-image reversal of internal organs.

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From simple bacteria and archaea to higher-level eukaryotes, survival is based upon the ability of the organism to respond to environmental pressures, including diverse sets of mechanical force. Virtually all organisms have evolved specific structures that are tailored to respond to physical force. For instance, single-celled organisms, such as bacteria, and complex multicellular eukaryotes, such as plants and animals, all express stretch-activated ion channels[1,2]. Cell surface receptors such as integrins, which transmit forces from the external environment across the cell membrane, are also evolutionarily conserved molecules. Indeed, integrin heterodimers span a diverse array of life forms, ranging from sponges to humans, and their complexity has increased with organism evolution owing to variants and functional redundancy[3]. Consistently, the complexity of the Tyr phosphorylation of adhesion plaque-associated proteins, an indicator of the adhesion complex's capacity to engage in signal transduction in focal adhesions, has also developed concomitantly with integrin-mediated adhesion, suggesting a close evolutionary link between the diversity of signal transduction and mechanical interactions[4].

Cells in multicellular tissues are subjected to a myriad of forces, including compressive, tensile, fluid shear stress and hydrostatic pressure, each of which plays an intricate part in the shaping, development and maintenance of the tissue. Importantly, the manner in which cells interact with these forces, and hence respond to them, is largely dictated by the physical properties of the cells, their adjacent cells and the extracellular matrix (ECM), which is the principal extracellular component of all tissues and organs (BOX 1). Biological materials, including cells, exhibit the characteristics of both solids and liquids, in that they are both elastic and viscous. They are thus viscoelastic, meaning that they will deform in a time-dependent manner upon applied force (that is, they partially flow) and can return towards their initial form following removal of the applied stress.

Diseases such as atherosclerosis, arthritis, deafness, osteoporosis and cancer, and a number of developmental disorders, including Kartagener's syndrome and Hutchinson–Gilford progeria syndrome, commonly result from an abnormal physiological response to extrinsic (applied) or intrinsic (cell-generated) cues[5]. Thus, the physical basis for disease can be a product of either altered tensional homeostasis, owing to, for example, altered cellular-level or tissue-level forces and material properties, or the perturbed cellular response to mechanical stimuli. Consequently, it is important to understand the functional link between the sensing of mechanical cues and the subsequent biochemical response, a process termed mechanotransduction, as this relationship is important for the maintenance of tensional homeostasis and for normal tissue structure and function. Despite the importance of this relationship, and although much is known about how biochemical signalling can direct cell behaviour, relatively little is known about how forces inherent to the cell and the cellular and non-cellular microenvironment contribute to the regulation of tissue fate and function (for reviews, see REFS 6–9).

Accordingly, the broad objective of this Review is to describe coordinated mechano-responsiveness in the context of tissues and the cellular microenvironment. We begin with a discussion of how mechanical signals are sensed and interpreted through the molecular machinery

Box 1 | **Structure and composition of the extracellular matrix**

The extracellular matrix (ECM) is the principal extracellular component of all tissues and organs. It provides the scaffold that gives physical support to cells and regulates intercellular biochemical and biomechanical signalling. As a result, it has a role in a number of cellular processes, including adhesion, migration, apoptosis, proliferation and differentiation. The molecular components of the ECM include collagens, elastins, proteoglycans, fibronectin and laminin. At the molecular level, the ECM is capable of binding, integrating and controlling the presentation of growth factors and other ligands to cells[104]. The organization of the ECM is not static; it is a dynamic structure with varying composition and distribution between different tissues and also during the stages of development. Remodelling of the ECM occurs through an altered balance between matrix deposition and its degradation by matrix metalloproteinases, and also through enzymes, such as lysyl oxidase, that crosslink collagen and elastin[16]. The diversity of ECM composition and organization lends itself to a wide range of forms and functions, ranging from solid structures found in bones and teeth to the elastic and pliable matrix found in cartilage and tendons[105]. Disruptions and perturbations to this network result in a loss of cell and tissue homeostasis and lead to a number of diseases, including cancer.

that mediates mechanotransduction, such as integrins (which are present at focal adhesions) and cadherin complexes (which are present at adherens junctions). We next describe how mechanical cues are integrated into tissues and illustrate, using examples, the importance of mechanical context as a critical regulator of cell behaviour and a key determinant during tissue-specific development. We also describe how the ECM is able to direct the behaviour of cells by regulating tensional homeostasis over many length scales and timescales through a process termed mechanoreciprocity. Finally we discuss how, in certain instances, the ECM may act as a memory-storage device that is capable of orchestrating and perpetuating disease states that may regulate cancer relapse and the progression to metastasis.

Integrating mechanical cues in cells

The translation of local extrinsic mechanical events into global changes in cellular function is dependent upon cells possessing integrated machinery that is capable of sensing and responding to mechanical force. A number of sensory elements and mechanisms exist whereby cells are able to probe and detect external forces through a process termed mechano-sensing. This force-sensing can occur through force-induced conformational or organizational changes in cellular molecules or structures, such as stretch-sensitive ion channels[10], cadherin complexes in cell–cell adhesions[11], G protein-coupled receptors, Tyr kinase receptors[12–14] and integrins[3]. When the mechanical cue has been received, the signal is amplified and propagated through a series of force-dependent biochemical reactions, whereby intracellular signalling pathways become sequentially activated through mechanotransduction[15]. For example, in response to elevated tension within focal contacts, increases in integrin clustering and in the phosphorylation of focal adhesion kinase (FAK) ensue, and these molecular changes initiate a cascade of signalling events. This cascade includes the activation of Rho-family GTPases, such as RhoA, which stimulates actin remodelling, induces protein phosphorylations to promote cell survival, and alters the levels and activity of transcription factors to regulate gene expression. Another integrin-dependent signalling pathway that is activated in response to mechanical force is the mitogen-activated protein kinase–extracellular signal-regulated kinase (MAPK–ERK) pathway, which has been implicated in a number of cancers and regulates cell proliferation and differentiation to influence tissue development[5,16].

Sensing and integrating force through focal adhesions. A rich diversity of mechano-sensing mechanisms is present across virtually all biological systems. Among the most-studied mechano-sensory complexes are focal adhesions (reviewed in REF. 17). These dynamic protein complexes consist of integrins and a multitude of adaptor and signalling proteins, including vinculin and talin, which, as an integrated unit, provide the mechanical link between the actomyosin cytoskeleton and the ECM. This bridging between cellular components and the ECM enables the focal adhesion to serve as the conduit through which signal transduction occurs in response to physical force. The application of external force can directly influence the shape, size and composition of focal adhesions, thereby demonstrating a direct correlation between force and biochemical signal generation[18] (FIG. 1). Proteins within the focal adhesion, including the integrins themselves[19] and plaque proteins such as talin[20] and p130Cas (also known as BCAR1)[21], undergo conformational changes in response to applied force. These force-dependent conformational changes can either stabilize protein–protein interactions, as is the case for the conformational change that occurs in an integrin when it binds to the ECM component fibronectin, or they can unravel the molecule to reveal cryptic binding sites, as in the case of talin. Talin undergoes a force-dependent unfolding in response to picoNewton forces, which results in the exposure of otherwise inaccessible vinculin-binding sites[20,22]. The net result of talin–vinculin binding in response to force is an increase in the clustering of integrins and the nucleation of adhesion plaque proteins, which facilitates the cell–substrate junction to activate signal transduction molecules at the intracellular face of the adhesion. Similarly to talin, ECM proteins such as fibronectin can also unfold in response to mechanical stress to reveal cryptic binding sites that reinforce integrin adhesions and promote focal adhesion complex assembly[16].

To date, the most-studied mechanosensing mechanisms involve protein sensors that undergo conformational changes in response to a physical force. However, focal adhesions are theorized to have force-dependent behaviours through mechanisms that do not rely solely on protein switches[23]. In these strain-based mechanisms, forces on protein complexes may change intermolecular distances that ultimately lead to altered cellular function. Applying the concepts from this simple scenario to focal adhesions, there is a direct relationship between the intermolecular distances separating individual components within an adhesion and the rates at which signal-transducing adaptor proteins are recruited to the adhesion[23]. Strain on the complexes depends on the applied force and resistance provided by the ECM. In this model, focal adhesion assembly is naturally linked to ECM compliance and force[24].

Hutchinson–Gilford progeria syndrome
A disorder that is characterized by the rapid and dramatic appearance of ageing. The disease results from a genetic condition in which an abnormal version of the lamin A protein is produced, resulting in a highly unstable nuclear envelope. This is hypothesized to result in a disruption to mechanotransduction in vascular cells, contributing to arteriosclerosis, the leading cause of death for patients with this disease.

Mechanotransduction
The process through which cells sense and respond to their mechanical environment, such as the extracellular matrix, adjacent cells or external stresses. During mechanotransduction, mechanical signals are sensed and activate intracellular biochemical signalling pathways.

Actomyosin
Actin is one of the principal components of the cytoskeleton and forms a network of filaments with a class of molecular motors called myosins. The actomyosin network is best known for its role in contractility and force generation.

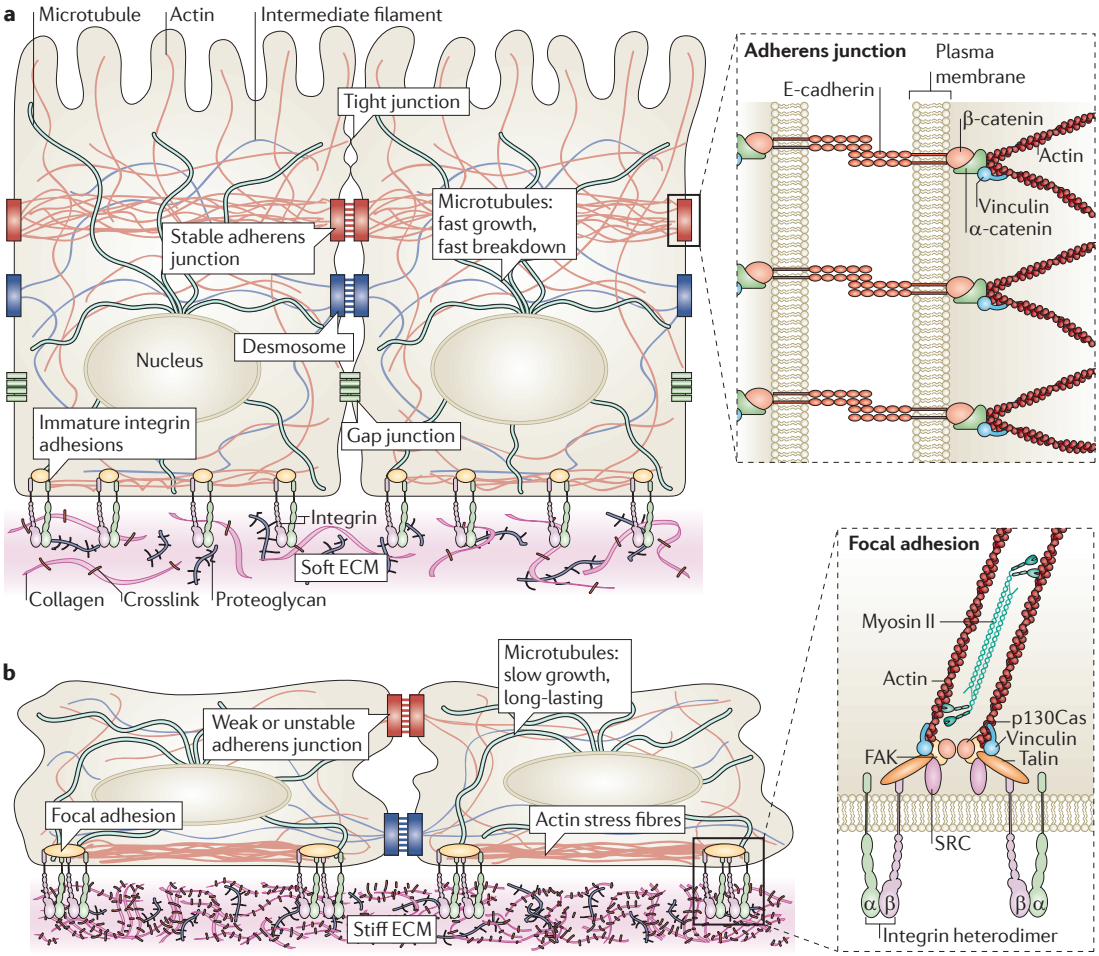

Figure 1 | **The mechanical network. a** | Tissues are mechanically integrated structures, the physical behaviour of which is defined by interconnected networks of cell–cell junctions, cell–matrix adhesions, intracellular filament networks (of actin, microtubules and intermediate filaments) and the extracellular matrix (ECM). Embedded throughout the network are mechanotransducing machines that convert mechanical stimuli into biochemical signals. This process, termed mechanotransduction, enables cells and tissues to sense and respond to their physical surroundings. The ECM controls network connectivity and tension on the network, thereby regulating sites of mechanotransduction. **b** | Cell–matrix adhesion complexes containing integrins can also directly sense the physical properties of the ECM. These complexes contain specialized protein sensors, including talin, p130Cas (also known as BCAR1), and integrins themselves, that undergo force-dependent conformational changes to elicit downstream signalling responses. The physical properties of the ECM are determined by its composition, the organization of its components, and their degree of intramolecular and intermolecular crosslinking. Interactions between the cell and ECM are dynamic, interwoven and reciprocal. Transcellular tension transmitted across adherens junctions affects ECM remodelling, which in turn regulates cell–matrix and cell–cell adhesions. Increased ECM stiffness owing to remodelling can result in changes in cell and nuclear shape, chromatin organization, assembly of cell–matrix adhesions (called focal adhesions), formation of actin stress fibres, destabilization of cell–cell adhesions, and changes in microtubule dynamics. FAK, focal adhesion kinase.

Sensing and integrating forces beyond focal adhesions. The majority of cell surface receptors that have been identified to date are characterized by their ability to respond to chemical factors. Despite the prevalence of these receptors, only a relatively small set (that is, the integrins and cadherins) appear to be capable of responding to mechanical cues. Given the importance of regulating and sensing forces across multiple length scales, from the molecular level to the tissue level, it stands to reason that a versatile set of force sensors must exist that have yet to be fully described. One explanation for the elusiveness of these types of force-sensors lies in the fact that many proteins fall into multiple receptor categories, such that they are capable of responding to mechanical strains and biochemical cues. Accordingly, a broadened view of the role of cell surface receptors is currently being developed that includes their ability to work across length scales and encompasses their generalized role in maintaining tissue structure and function.

There are multiple examples in which cell surface receptors serve to modify cell fate and tissue behaviour through the coupling of biochemical and mechanical cues. For instance, tumour cells can generate autologous gradients of chemokine (C-C motif) ligand 21 (CCL21) and

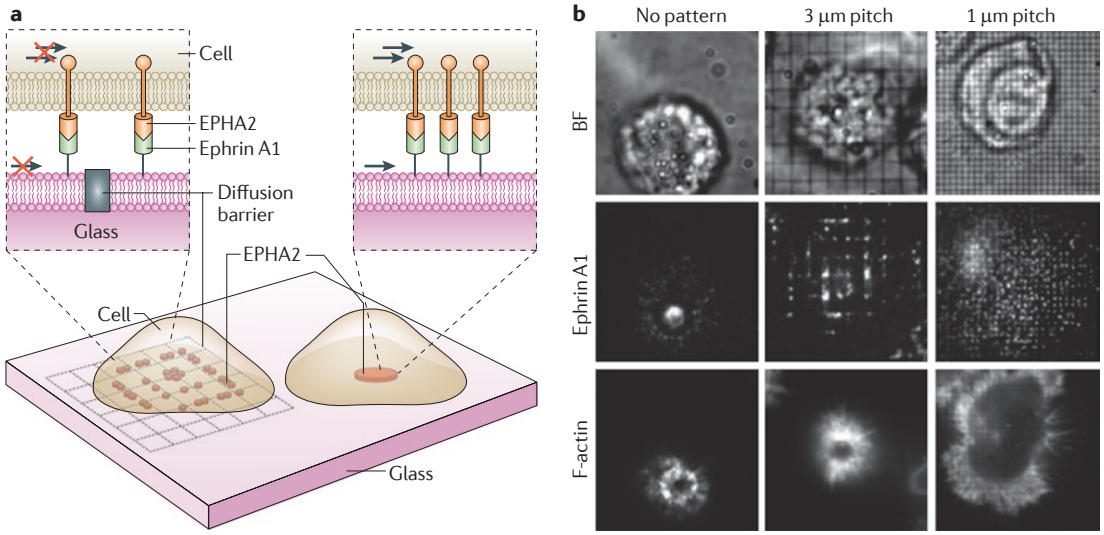

Figure 2 | **Spatio-mechanical regulation of signalling pathways.** Evidence suggests that the mechanically induced spatial organization and clustering of cell surface receptors by the physical properties of materials, including the extracellular matrix (ECM), cell membrane, and cytoskeleton, can regulate a number of signal transduction pathways[24,29,30]. Juxtacrine signalling is one example in which physical contact between two cells is required for signalling, owing to the receptor (for example, ephrin A receptor 2 (EPHA2)) and ligand (for example, ephrin A1) pairs being presented on apposed cell membranes. **a**,**b** | To demonstrate how the mechanics of the microenvironment regulate EPHA2–ephrin A1 signalling in mammary epithelial cells, nanofabricated substrates, consisting of 10 nm high and 100 nm thick chromium lines arranged in a grid pattern (unrestricted (no pattern), 3 µm pitch and 1 µm pitch barriers), were used as a support for a membrane functionalized with laterally mobile (arrows) and fluorescently labelled ephrin A1 ligand, as schematically depicted (**a**). Representative bright field (BF) and epifluorescence images are also shown (**b**). When unimpeded, EPHA2 forms clusters with filamentous actin (F-actin) localized at the cell periphery, and is transported radially towards the centre of the cell upon ligand binding in an actomyosin-dependent mechanism[29] (**b**, no pattern). However, the grid pattern creates diffusion barriers to receptor–ligand complexes, impeding their movement and resulting in their accumulation at barrier boundaries (**b**, ephrin A1: 3 µm and 1 µm pitch). Distinct cluster patterns yielded different signalling responses and changes in cytoskeletal morphology, which demonstrated a functional link between mechanical environment, receptor organization, signal activation and cell phenotype (**b**, F-actin). Images are modified, with permission, from REF. 29 © (2010) AAAS.

CCL19 in response to interstitial flow. This force-mediated chemical gradient can promote lymph-node metastasis by increasing the frequency with which these ligands bind to chemokine (C-C motif) receptor 7 (CCR7), which is expressed on the tumour cell[25,26]. The implication of this mechano-chemical coupling is that tumour cells may utilize low-magnitude shear forces to create and amplify autologous transcellular chemokine gradients that permit the tumour cell to move towards the draining lymphatic vessels by chemotaxis. It is plausible that, by using such an aggressive mechano-chemical coupling mechanism, tumour cells would be able to exploit even a weak autocrine chemokine circuit to promote their directed migration and subsequent metastasis. Another illustration of the synergistic relationship between biochemical signalling and mechanical force is demonstrated by the mechanical-force model for Notch-signalling activation, which is referred to as the lift-and-cut model. The Notch pathway has a key role in regulating pre-existing developmental programmes by directing cell proliferation and cell death, promoting specific cell fates and activating distinct differentiation programmes. Fundamentally, Notch signals enable short-range communication between adjacent cells, and they are normally triggered by a ligand-induced activation mechanism. Prior to activation, Notch is in a metalloprotease-resistant conformation, which effectively blocks the site that must be cleaved by a metalloprotease for Notch activation. It seems reasonable to suggest that a mechanical force facilitates the substantial conformational changes deemed necessary to uncover the cleavage site required to activate Notch. Given that the ligand-binding domain of Notch is in close proximity to the protective Lin12–Notch repeat (LNR) modules, this coincides with data showing that ligand-binding confers sufficient force to peel away the LNR modules, providing the 'lift' that exposes the metalloprotease cleavage site where the 'cut' required for activation occurs[27,28].

The local mechanical environment also plays a critical part in the organization and clustering of specific surface receptors, the functions of which are directly linked to their coordinated spatial organization[29] (FIG. 2). Ephrin A receptor 2 (EPHA2) binds to membrane-bound ephrin A1 ligand that is presented on adjacent cells and, when stimulated, regulates cell growth, migration and adhesion, and is also associated with tumour angiogenesis and metastasis. Mechanically impeding the lateral movement of EPHA2s altered their spatial organization and clustering. Specific spatial assemblies elicit distinct signal responses associated with the ERBB, p53, integrin and MAPK pathways and an invasive phenotype, demonstrating that ephrin signalling is sensitive to spatio-mechanical cues from the local environment[29].

Interstitial flow
Present in all living systems, this type of fluid flow produces small currents through tissues and the extracellular matrix and is driven by dynamic stress.

Transcellular
Whereas 'paracellular' delineates processes occurring between cells, transcellular describes processes occurring through cells. One example is in transcellular transport, where molecules are moved through an epithelial cell layer.

The strong correlation between spatial organization and function that has been demonstrated for EPHA2s is likely to be shared with other cell surface receptors, such as members of the ERBB family of receptors, which includes epidermal growth factor receptor (EGFR)[30]. In a recent study by Chung *et al.*[31], EGFR dimers, which are formed before signalling, were found to be enriched in the cell periphery in an actin-dependent manner, resulting in a peripheral enhancement of EGF-induced signalling that theoretically enabled the cells to respond to growth factors in a polarized manner. The authors showed that receptor diffusion, and hence the spatial organization of receptors, was ultimately determined by actin organization at the membrane[32,33]. In this respect, it is important to note that nearly all forces, including shear stress, axial stress and cellular contractile forces, will induce actin reorganization when applied to the cell, and may affect receptor organization as a result[34–37].

In general, distinct classes of biomolecules can reciprocally influence one another's spatial organization. It therefore follows that changing the spatial organization of transmembrane receptors should ultimately influence the organization of lipids in the plasma membrane, and that modifying lipid domain formation will alter receptor distribution[38]. Force-induced reordering of biomolecules, including receptors, lipids and actin, can thus be propagated from one class of molecule to another, suggesting a global, interwoven regulation of cellular systems by intrinsic and extrinsic forces. Predictably, this global sensation of force can ultimately synergize with local mechano-sensory mechanisms, such as protein unfolding, to dictate the cellular response to force.

Integrating mechanical cues in tissues

Tissue function arises from the coordinated behaviour of cells in both time and space. Consequently, a cell's response to external stimuli is largely dictated by its interactions with the ECM, neighbouring cells and soluble cues from the microenvironment. As a result, cells *in vivo* do not function independently, but act in an integrated manner that depends strongly upon the mechanical and spatial context of their surroundings and their intrinsic mechano-sensory mechanisms. Although the study of molecules and signalling out of context (that is, in a classic two-dimensional (2D) monolayer culture first) is useful for establishing the basic structure–function relationships of molecular interactions, these systems must ultimately be considered within the context of the multicellular tissues in which they reside.

The importance of mechanical context. Multiple examples demonstrate how the study of cells is highly context-dependent and that changing the local environment of the cell can have dramatic effects on cellular behaviour and phenotype. For example, mammary epithelial cells (MECs) incorporated into polarized, 3D tissue structures are resistant to myriad death cues, including chemotherapy drugs such as taxol and etoposide, as well as tumour necrosis factor (TNF) ligand and TNF-related apoptosis-inducing ligand (TRAIL). Conversely, MECs grown in 2D monolayers are exquisitely sensitive to cell death induced by these stimuli[39]. Furthermore, the position of epithelial cells in 3D engineered mammary epithelial tubules determines whether they undergo branching morphogenesis in response to EGF or hepatocyte growth factor (HGF), where branches are initiated exclusively from the ends of the tubules and not from the sides[40]. Differences between 2D and 3D have also been observed in cultured osteoblasts, in which changes in gene expression under static conditions and cellular responses to shear stress vary dramatically depending upon the extracellular environments[41]. The spatial location of epithelial–mesenchymal transitions induced in a 3D living tissue seems to coincide with, and may even be directed by, differences in cell shape and tissue geometry; this might be because these spatial cues reflect gradients of mechanical stress[42]. Indeed, experiments utilizing micromechanical devices to move cells into physical contact illustrate the critical role of cell–cell contacts as key regulators of the responsiveness of a cell to soluble factors and tissue signalling. Collectively, such studies emphasize how the nature of a cell's response can be dramatically modified by its physical and chemical environment[43].

Mechanical context is derived from the collective relationships between cells (mediated by cell–cell adhesions), between the cell and its ECM (mediated by cell–matrix adhesions) and between both types of adhesion and the intracellular-filament network (FIG. 1). The distribution of tension, and hence the sites of mechanotransduction, depends on the spatial connectivity and emergent material properties of the tissue network[44]. Tension develops on these networks owing to actomyosin contractility[45,46]. Analogously to the cellular effects of morphogen gradients, changing tissue geometry alters tension gradients, sites of mechanotransduction and the location of the proliferating, migrating and differentiating cells within a tissue[47,48]. Even small local changes in cell–cell or cell–ECM connectivity can have dramatic consequences for global tissue structure and function. In a recent demonstration of this principle, Martin *et al.*[49] found that reducing the number of adherens junctions between cells, using a partial loss-of-function mutant or by laser-ablating small portions of the actomyosin network in tissues, resulted in tissue-wide epithelial tears during *Drosophila melanogaster* embryogenesis. On the single-cell level, the ablation of even a single actin fibre is able to induce a change in cell shape, alter cytoskeletal organization and modify focal adhesion assembly[50,51]. The connectivity in tissue networks is not static, but is dynamically rearranged as the need arises. For example, as a cell polarizes, the adaptor protein vinculin is recruited from sites of cell–matrix adhesion to sites of cell–cell adhesion, and stress fibres connected to focal adhesions reorganize into cortical bundles that run parallel with cell–cell contacts[52] (FIG. 1). This adaptability in mechanical connectivity, coupled with mechanotransduction mechanisms, facilitates the coordinated spatial and temporal control of signal transduction, gene expression and cell behaviour, and delineates a hierarchy of communication in tissues that is formed by mechanical and biochemical connections.

Epithelial–mesenchymal transitions
Instances of a developmental programme that is hypothesized to be activated in metastasis and proliferation. This transition is characterized by an enhanced migratory capacity, loss of cell adhesion, the downregulation of E-cadherin, and a malignant phenotype.

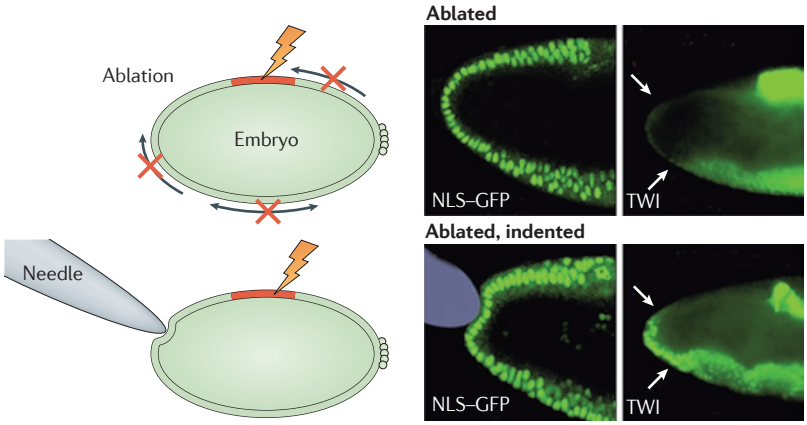

Figure 3 | **The extracellular matrix and tensional homeostasis in development.** In tissue and organ morphogenesis, mechanical forces generated by morphogenetic movements play an important part in gene expression by activating developmental biochemical signalling cascades[47]. Embryogenesis provides an example of a multistage process that requires mechanical force, the subsequent localization of proteins, and biochemical signalling to work together. In this process, the cytoskeleton of the cells themselves has a key role in generating the contractile forces required for invagination, gastrulation, proliferation and differentiation[44,106]. During the onset of gastrulation, an early phase of embryonic development in *Drosophila melanogaster*, actin filaments are contracted by non-muscle myosins. Compressive forces result in the ectopic expression of the transcription factor Twist (TWI)[54,107], which then directs significant changes in the shape of the developing embryo. When compressive forces (depicted by arrows in the schematic) are disrupted through the laser-ablation of dorsal cells (red region), there is a corresponding reduction in the level of mechanically induced TWI expression in the stomodeal primordium (white arrows) (ablated). Cell nuclei are visualized with a nuclear localization signal-tagged green fluorescent protein (NLS–GFP) and immunofluorescence shows the distribution of TWI. Upon gentle compression of the stomodeal cells using a needle, TWI expression is restored in a mechanically induced mechanism (ablated, indented). Figure is modified, with permission, from REF. 54 © (2008) Elsevier.

The roles of mechanical cues in driving development. Although the genetic control of morphogenesis remains in vogue, a more comprehensive view of tissue development is beginning to be appreciated. This incorporates the large-scale coordination of cell shape and movement that is facilitated by mechanical force. Cell shape and motility are ultimately the products of a cell's material properties, its contractile and protrusive forces, and the magnitude, direction and duration of the external forces that it is subjected to. Mechanical forces are thus the primary determinants in the organization of cells and embryos and, consequently, they are a key element in many of the fundamental processes that operate during development, including cell sorting, differentiation and compartmentalization[53]. Although several biochemical signals, including EGF, Notch and Hedgehog, are necessary for regulating myosin organization and activity during development, recent evidence demonstrates that mechanical feedback also has an essential role in regulating the contractile systems that drive embryogenesis. For instance, the deformation of cells by morphogenetic movement during *D. melanogaster* gastrulation induces the expression of the transcription factor Twist[54] (FIG. 3). Twist and its transcriptional target Fog in turn stabilize apical myosin II, which promotes the formation of supracellular networks of contractile myosin fibres that are joined end-to-end at adherens junctions[49,55]. Twist, through regulation of G protein signalling and the transmembrane protein T48, controls the organization of, and tension on, the contractile network, and recruits adherens junctions to the sites of apical constriction, thereby facilitating the dynamic and precise control of cell shape[56]. In a positive feedback loop, force itself controls the recruitment of myosin to the apical cortex. Applying force to a cell within an embryo with a micropipette is sufficient to recruit myosin in 1–3 minutes to the apical cortex, whereas relieving tension with laser-ablation leads to a rapid loss of myosin from the cortex[55,57]. Remarkably, cutting a single cell–cell boundary in the amnioserosa of a *D. melanogaster* embryo with laser microsurgery rapidly terminates contractility in neighbouring cells, clearly demonstrating that the forces driving morphogenesis are sensitive to changes in the local mechanical environment[58]. Collectively, the rapid response of the embryo to force reflects robust and overlapping mechanotransduction mechanisms that are embedded throughout the tension network. The specific molecular details of these mechanisms remain unresolved, but an explosion of recent interest portends rapid progress.

The roles of the ECM in driving development. The ECM is a crucial structural component that defines the mechanical behaviour of tissues. In gastrulation, for example, the assembly of fibronectin in the ECM into more complex fibrillar structures supports cell movement and shape changes that are essential for epiboly and radial intercalation[59]. The ECM also physically delineates tissue boundaries, enabling the compartmentalization of developing tissues[60]. Cells in the embryo also remodel the matrix as they pass over it, and this ECM remodelling commences coincidently with the initiation of morphogenetic movement. Indeed, the integrin-dependent assembly of the fibronectin matrix in the blastocoel roof of *Xenopus laevis* embryos probably requires cadherins, cortical actin fibres and myosin contractility[61]. Fibrillar assembly of the fibronectin matrix is also functionally linked to the forced unfolding of matrix protein domains by integrin receptors[62,63]. These results suggest that tension developed in the supracellular actomyosin network in the developing organism is transmitted through integrins to physically assemble the matrix, where biochemical–mechanical feedback again prevails. For example, in reconstituted ECMs the physical state and material rigidity of the ECM regulate the activation of Rho GTPases, increase myosin activity, enhance the formation of actin stress fibres and induce tissue tension concomitantly with ECM stiffening[64]. Furthermore, ephrin signalling controls the spatial organization of α5β1 integrin clusters along cell boundaries and thus also the spatiotemporal deposition of the fibronectin matrix[65]. As ephrin signalling is sensitive to the local mechanical environment, this system could allow cells to spatially coordinate matrix assembly using contextual information encoded in the pushes and pulls of neighbouring cells[29].

The ability of the ECM to control cell fate and material properties is another dramatic example of the intricate, interwoven nature of mechanical regulation in

Stomodeal primordium
In *Drosophila melanogaster*, the stomodeal primordium separates the anterior midgut from the middle germ layer, the mesoderm.

Gastrulation
A developmental change that is characterized by a large-scale movement of cells, from which the embryo first begins to take shape, transitioning from a spherical mass of cells into an organized multilayered structure establishing the three primary germ layers.

Amnioserosa
An extraembryonic epithelial tissue present in *Drosophila melanogaster* that is required for dorsal closure.

developing tissues. The physical properties of the ECM direct the differentiation of stem cells down specific lineages[66]. As cells differentiate, they generally become stiffer in a cell-type specific manner and further tune their stiffness to the properties of the local materials that make up their ECM, progressively stiffening on more rigid material[67]. These changes in cell stiffness can in turn directly affect mechanotransduction. In response to applied stress, stiff differentiated cells deform less than compliant stem cells, resulting in attenuated levels of mechanotransduction[67]. This mechanotransduction mechanism seems ideally suited to allowing undifferentiated cells in the embryo to respond in a highly sensitive manner to coordinated mechanical cues, while ensuring that differentiated cells remain buffered against acute mechanical perturbations in homeostatic tissues. The material properties of cells are also used in other developmental processes. The level of cortical tension (stiffness, as measured by atomic force microscopy) is different in cells that make up the zebrafish ectoderm, mesoderm and endoderm[68]. Both experimental and theoretical models suggest that these differences in cell stiffness may support cell-sorting behaviours and could function in conjunction with differential cell adhesion to promote the formation of the three germ layers[69].

The ECM in force homeostasis and disease

Mechanotransduction occurs on very fast timescales that can exceed the speed of signalling through soluble factors by orders of magnitude[70–72]. Forces can propagate through a wave-like mechanism across the cell body, along cytoskeletal filaments, in just 2 μs, and the subsequent activation of signal transduction networks occurs on the order of seconds[73] (FIG. 4). These fast response times are crucial for highly dynamic cell processes. When the direction of motility is controlled by the physical stiffness of ECM, a process termed durotaxis, cells migrate from softer to stiffer regions. This probably requires fast dynamic coordination between the mechano-sensors (integrins) and response elements (actin cytoskeleton) that direct motility[74]. Dynamic processes such as these can contribute to pathology; for example, it has been proposed that metastatic cells disseminate from tumours by crawling along stiff fibres or following tension gradients. It is important to note, however, that most diseases linked with altered mechanotransduction, such as atherosclerosis and cancer, progress over months or even years, and therefore require sustained disruption to tensional homeostasis[64,75]. A prime candidate for achieving this type of sustained mechano-perturbation is the ECM.

The ECM directs cell behaviour on two length scales. As a structural material, the ECM controls spatial organization in the tissue across broad length scales, ranging from the nanoscale to the microscale and larger. On the nanoscale level, the ECM affects the organization of receptors on the cell surface and the sequestering of soluble factors. The nanoscale organization of the ECM can affect how growth factors are presented to their receptors (some can be tethered to the matrix) and how morphogens diffuse through tissue. Arrays of titanium dioxide (TiO_2) nanotubes ranging in diameters from 22 nm to 300 nm, and in lengths of up to 1 mm, can affect cellular proliferation and motility while simultaneously changing the expression level of molecules associated with inflammation and coagulation[76]. These nanotubes are comparable in size to cell receptors and proteins and serve as a general example of how mechanical and topographical cues alone can affect cell behaviour.

On a more specific level, the spatial presentation of ECM ligands, such as fibronectin, vitronectin, laminin and collagen, and the nanotopography of the ECM, control integrin organization, adhesion assembly, and signal transduction to direct cell behaviour[77,78]. As shown through the use of functionalized gold dots on nanopatterned surfaces, differences in average ligand spacing of as little as ~10 nm seem to be capable of dictating whether integrins are able to assemble into focal adhesions[79]. This indicates that there is a critical threshold of ligand density that is required for integrin clustering and focal adhesion assembly. Cells are also capable of sensing and responding to gradual changes in the spacing of ECM ligands of as little as 1 nm over the entire length of a cell body[80]. Additionally, such relatively small gradients are able to direct migration and the alignment of cells and their cytoskeleton[80]. Because the cellular response to the spatial organization of matrix components also depends on the material properties of the matrix, this raises the important distinction that chemistry is not independent of mechanics. Matrix stiffness ultimately controls the cellular response to ligand presentation and matrix organization. Thus, when cells are cultured on ECM substrates of varying stiffness, focal adhesions fail to assemble below a critical stiffness even when matrix ligands are presented to the cell at saturated levels[81].

At the microscale level and larger, the ECM controls cell shape and tissue boundaries. Matrix dimensionality is a dramatic example of how cell behaviour can be controlled at the microscale[82]. For instance, cells cultured in 1D, 2D and 3D show pronounced differences in their motility[83,84], morphology and cytoskeletal organization[85,86], as well as in the composition and function of their adhesions[85], their viability[86], and their response to soluble factors[39]. Spatial modifications in collagen organization at this length scale can additionally promote the metastatic behaviours of tissues, possibly by enhancing the viability and motility of disseminated cells. In this regard, multiphoton microscopy has been used to directly visualize cancer cells and macrophages exploiting collagen fibres as veritable 'highways' that seem to facilitate their invasion into the interstitial matrix and their rapid travel through the stroma[87,88]. As collagen fibres in the tumour are often tethered to blood vessels, these observations also raise the possibility of a direct route for tumour cell dissemination into the bloodstream[89].

In summary, nanotopological features and larger-scale organization of the ECM control the motility and positioning of cells, their geometry, and their mechanical connectivity within the surrounding cellular and non-cellular

Epiboly
Formally defined as a growing of one part over another, epiboly is a coordinated movement occurring during gastrulation that is characterized by the thinning and spreading of a multilayered cell sheet.

Radial intercalation
A tissue-rearrangement process during development in which the cells in the deep germ layers of a developing embryo move towards the outer layers.

Blastocoel
A fluid-filled cavity that the embryo develops as it forms. It is the central region of a blastocyst.

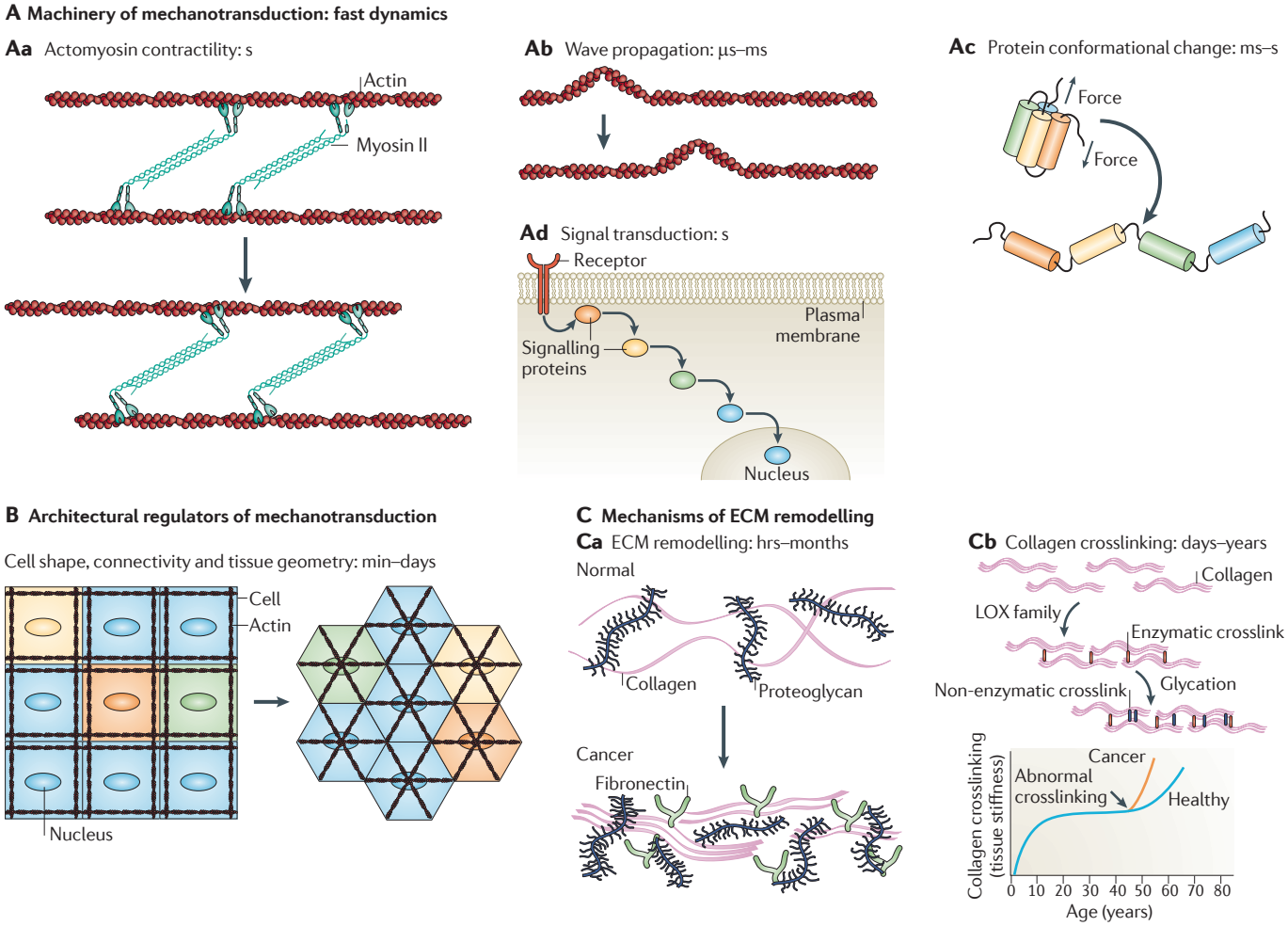

Figure 4 | **Sustaining mechanotransduction. A** | Mechanotransduction occurs on very fast timescales. Actomyosin cables contract at a rate of ~1 μm s^{-1} (**Aa**), and stress waves generated by contractility can rapidly propagate across cytoskeletal networks and structural materials (**Ab**). Stress on mechanically sensitive proteins (mechano-sensors) can induce conformational changes, such as protein unfolding, on the order of milliseconds to seconds for picoNewton- level forces (**Ac**). During mechanotransduction, these changes in protein conformation activate biochemical signalling networks (**Ad**), through which signals originating at the cell membrane can travel to the nucleus in tens of seconds. Mechanical signalling thus occurs rapidly on timescales of seconds or less. **B** | Diseases that are linked to altered mechanical signalling require perturbations to be sustained. This can be achieved by genetically controlling the steady-state levels of mechanotransduction machinery and associated regulators. Alternatively, modifying the tissue architecture might sustain mechanical signalling perturbations. The distribution of forces throughout the tissue, and hence of the local sites of mechanotransduction, depend on cell shape, position and connectivity, as well as the intracellular organization of the cytoskeleton and other structural elements. **C** | The extracellular matrix (ECM), which largely determines tension in tissues, is remodelled during development and disease, which results in altered patterns of tension that can persist for hours, months or even longer (for example, in scar tissue) (**Ca**). Remodelling enzymes, including the crosslinking enzyme lysyl oxidase (LOX), alter the mechanical properties and spatial topology of the ECM (**Cb**). Enzymatic (LOX family; red) and non-enzymatic (glycation-mediated; blue) crosslinking in mature collagen filaments is particularly stable and can persist for decades in healthy adults. However, the enhanced activity of enzymes such as LOX in disease can enhance crosslinking, contribute to tissue stiffening, and alter mechanotransduction for prolonged durations.

microenvironment. Such physical rearrangements would be expected to occur on the order of hours and days, and are likely to persist at steady-states dictated by the ECM for considerably longer time durations of months and perhaps years. Such ECM topological reorganizing thereby provides for long-term patterning within the tissue that could elicit profound physiological changes through modifications of tissue-level mechanical forces and cellular mechanotransduction.

The ECM as a memory-storage device. Evidence linking ECM remodelling to disease progression is drawn from a compelling body of evidence implicating the status of the ECM (that is, its composition, organization and post-translational modification state) in cancer incidence and disease progression. Patients with cancer who present with high levels of fibrillar collagen show enhanced incidence of metastasis, and women with increased mammographic density, which is characterized by

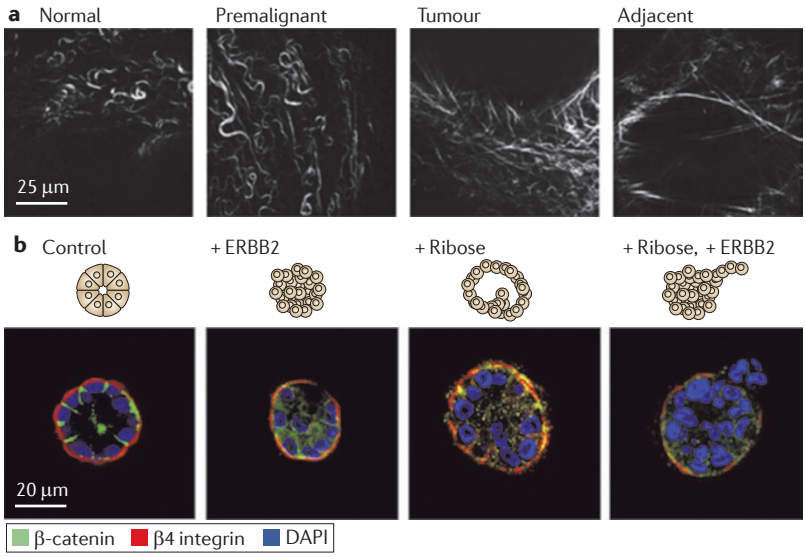

Figure 5 | **Tensional homeostasis in tumour progression.** Extracellular matrix (ECM) stiffness has been shown to be a potent regulator of cellular behaviour by affecting growth, survival, motility and differentiation. Tensional homeostasis requires a balance between the forces exerted on cells by the ECM and the reciprocal forces generated by cells themselves. When this balance is disrupted by stiffening the ECM, a number of signalling pathways can be adversely affected[90]. **a** | The collagen crosslinking enzyme lysyl oxidase (LOX) mediates collagen crosslinking and remodelling during breast tumour progression, which results in ECM stiffening. Second-harmonic generation imaging, a type of nonlinear microscopy, was used to show that the collagen in regions adjacent to epithelial lesions in mouse mammary glands undergoes significant morphological modifications, becoming progressively more linear during tumour progression. This remodelling of the ECM was also found to correlate with increased collagen crosslinking and the amount of the crosslinking enzyme LOX, suggesting an association with ECM stiffness, tissue fibrosis and breast tumorigenesis. **b** | However, matrix remodelling alone is insufficient to promote tumour invasion. Mammary epithelial cells cultured in three-dimensional gels assembled spherical acini, as shown with immunofluorescence of basally oriented β4 integrin and β catenin localized at cell–cell junctions (control). Acini that only had the oncogene ERBB2 activated failed to produce an invasive phenotype (+ ERBB2), as did stiffening by nonspecific ribose crosslinking (+ Ribose). Invasion only occurred when stiffening was accompanied with oncogene activation (+ Ribose, + ERBB2). This suggests that matrix remodelling and oncogene activation synergistically induce metastatic progression and that inhibiting LOX and LOX-like enzymes could reduce tumour metastasis by modifying cell–ECM interactions. DAPI, 4′,6-diamidino-2-phenylindole. Image is modified, with permission, from REF. 75 © (2009) Elsevier.

increased collagen and ductal and lobular epithelium, exhibit a greater risk of developing breast cancer[75,90]. Whether mammographic density predicts elevated ECM stiffness, and if and how fibrillar collagen could enhance tumour metastasis, awaits further clarification. Nevertheless, such provocative findings present an enticing argument that the status of the ECM could dictate tumour phenotype by modifying ECM tension. In this regard, one plausible molecular mechanism whereby modifications to the ECM could promote cancer progression is illustrated by recent data demonstrating strong associations among ECM stiffening, collagen crosslinking and expression, and the activity of the crosslinking enzyme lysyl oxidase (LOX) during breast tumour progression. These studies demonstrated that enhancing LOX-dependent collagen crosslinking stiffened the tissue and enhanced tumour progression, whereas inhibiting LOX-mediated collagen crosslinking not only prevented tissue stiffening but also delayed tumour progression and significantly reduced tumour incidence[75] (FIG. 5). Indeed, many tumours show elevated expression of LOX and LOX-like enzymes, and data suggest that inhibiting LOX activity could reduce tumour metastasis by modifying tumour cell–ECM interactions or by altering the metastatic site (niche)[74,75,91,92]. This type of tissue and matrix remodelling is tissue-wide and systemic, and necessarily occurs over long timescales spanning many months and even years, and it will, by its very nature, result in non-uniform matrix remodelling and topological changes that could easily facilitate tumour invasion. In addition to directly fostering a disease state, the ability of the ECM to sustain perturbations over long periods of time may enable it to play a part in disease recurrence (FIG. 4c). For instance, it was demonstrated that tumours are self-seeded by circulating tumour cells and that tumours can recur at the site of removal of the primary tumour. Remodelling of the ECM could function as a type of retention mechanism, whereby the physical microenvironment of the diseased state is preserved so that circulating tumour cells in tissues adjacent to the site of the primary tumour promote disease recurrence[93].

Because of its capability to perpetuate either the healthy or diseased state, it is instructive to equate the ECM to a biological memory-storage device. On a basic level, memory devices store information for extended periods of time and allow systems to 'write' and 'read' this information. DNA is a classic example: it is an unusually stable molecule, the functional state of which is written by replication machinery and epigenetic regulators and is read by the cell's transcriptional machinery. The ECM, although not widely appreciated as a storage device, shares analogous features to DNA. Major components of the ECM are incredibly stable and are further subjected to covalent modifications that change their functional properties. An illustrative example is provided by collagen, the most abundant molecule in the ECM. The half-life for collagen before turnover through degradation by matrix metalloproteinases is 2–4 years in bone, 10–15 years in skin and ~100 years in tendon[94]. Even in tumours in which ECM remodelling is considered to be extremely fast, long-term intravital microscopy studies suggest that individual collagen fibres are remarkably stable and persist for at least 4 days, the maximum duration of these studies[95]. Ultimately, the topological and mechanical features of the ECM are read by cellular systems, including integrin adhesions. For collagen, these properties are imprinted by crosslinking enzymes, such as LOX, in a tissue-specific manner. Enzyme-catalysed, intermolecular and intrafibrillar crosslinks control the bundling of collagen molecules into fibres, dictate the mechanical properties of the fibres, and slow the turnover of collagen[96]. The crosslinks themselves are essentially irreversible and their quantity changes very little, from a steady-state perspective, over durations of decades in healthy adults[94]. Elastin, which provides elasticity to the ECM, is similarly stabilized by enzymatic crosslinking[97].

Strong experimental evidence supports a link between tissue integrity and the cellular hardware that modifies, or writes, information to the ECM, including crosslinking enzymes, as discussed above, and matrix metalloproteinases. Altered proteolysis of the ECM by matrix metalloproteinases in cancer, a corruption of the information stored by the ECM, leads to unregulated tumour growth, inflammation and metastasis[98]. It also stands to reason that sustained physiological perturbation can be achieved by disruption of the primary cellular hardware that relays, or reads, information from the ECM. Indeed, cancer is frequently linked to altered integrin expression and activity, as well as to perturbations in key integrin-associated signalling proteins, such as SRC and FAK, and to changes in the levels and activity of actin-remodelling enzymes that are linked to mechanotransduction, including Rho and Rac GTPases. Appropriate matrix stiffness can support healthy tissue growth and homeostasis, but a cell's perception of stiffness depends on the functional state of its adhesion machinery. For example, the β1 integrin- and actin-binding protein filamin A is necessary for cells to pull on collagen fibrils and remodel collagen matrices. Absence of filamin A impairs morphogenesis even in compliant matrices that normally support the process, whereas upregulation of filamin A allows cells to contract stiff collagen gels and undergo morphogenesis in an otherwise non-permissive microenvironment[99].

How integrins perceive the ECM depends on their local micromechanical environment and, consequently, integrin–ECM interactions are precisely regulated by the physical and material properties of not only the ECM but also the glycocalyx, the cell membrane and the cell cortex[24]. This implies that any number of physical perturbations occurring at the cell surface could fundamentally change how cells read information from the ECM. In particular, physical perturbation of the cellular glycocalyx is likely to reflect a common but underappreciated disease mechanism. Drastic alterations to cell surface glycans are associated with many diseases, especially cancer, and rigorous theoretical models, some dating back more than 25 years, predict a strong dependence of integrin function on the physical properties of the glycocalyx[24]. The large, heavily glycosylated transmembrane protein mucin 1 (MUC1) is a major structural component of the tumour cell glycocalyx, being overexpressed in 95% of breast cancers and 70% of solid tumours[100]. MUC1 can extend more than 100 nm from the cell surface, which is considerably farther than the ~20 nm that integrins can maximally extend, and consequently MUC1 can alter the interaction of integrins with the ECM[100]. Similarly, the proteo-glycan hyaluronic acid forms thick coats on the exterior of tumour cells and is upregulated in a number of cancers, including pancreatic, colon and gastric cancers, and glioblastoma[101–103]. Abnormal levels of glycosylated molecules on the cell surface have been functionally linked to all stages of tumorigenesis and metastatic progression and are largely implicated in altering normal communication between cells and their tissue environment.

Multiple overlapping perturbations to the machinery that writes to and reads from the ECM are clearly demonstrated in diseases such as cancer. This raises the possibility that perturbations must accumulate in order to finally compromise the robustness of the storage system. The role of the ECM as a storage device that can promote and perpetuate disease states or even restore healthy tissue behaviour is underdeveloped. Yet, the accumulation of evidence implicating the ECM in sustained disruption of mechanical and chemical signalling argues for the importance of ongoing and future studies.

Concluding remarks and future directions

Sustained and coordinated tissue-level responses and reciprocal ECM-remodelling events that occur over long periods of time, and that span multiple length scales from the subcellular to the tissue level, are likely to contribute to the progression and initiation of diseases like cancer. Yet, although much effort has been exerted towards understanding the molecular details of pathobiology, more effort must be directed towards understanding how nano-, cell- and tissue-scale material properties are altered in disease and how these modifications impact signalling. Formulating an integrated mechano-chemical perspective can lead to the effective development of tractable translational and clinical methods to control the way in which cells interact with their local physical and soluble microenvironment to effectively treat diseases, including cancer. To achieve this, there is an urgent need to extend our understanding of mechanotransduction beyond what is known for focal adhesions and ion channels, to discover new and alternate mechano-regulatory mechanisms that promote normal cell and tissue behaviour, and to study these networks in the correct tissue-like context. There is also a need to understand how biochemical alterations, such as the mutation of kinases, synergize with mechanical perturbations to drive disease progression. As is so dramatically presented in the case of development, there exists a clear role for tissue organization in mechanically dependent cell signalling. However, little is known about the specific mechanisms that operate to synergize and coordinate these integrated responses.

Any mutation or perturbation that causes defects in organization on the molecular, cellular, or whole-tissue level can alter cellular mechano-sensing and will therefore conceivably contribute to disease. Much of the previous work in this field has focussed on soluble factors as being the mechanistic determinants that drive and coordinate tissue and matrix remodelling. However, it seems logical to predict that there is also a mechanism on the larger scale whereby mechanically altered cell and whole-tissue properties drive the upregulation of growth factors and chemokines, and that the perturbation of this might promote disease. As a more complete understanding and appreciation of the link between mechanics and biochemistry develops, the future challenge will be to take what is known from *in vitro* single-cell experiments and extend those principles to the tissue level *in vivo*. This is now becoming necessary because although much has been learned by studying the way in which single components interact with well-defined components of the ECM, it is now apparent that a host of long-range synergistic phenotypes exist that only manifest at the tissue and organ level, and these now urgently need our attention.

Glycocalyx
The carbohydrate-enriched coating, consisting of proteoglycans and glycoproteins, of the plasma membrane of eukaryotic, bacterial and archaeal cells. It has a number of functions, including roles in cell adhesion, mechanotransduction, vascular physiology, pathology, and guiding cell movement during development.

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