HHS Public Access

Author manuscript

Nat Metab. Author manuscript; available in PMC 2022 February 22.

Published in final edited form as:

Nat Metab. 2021 April ; 3(4): 456–468. doi:10.1038/s42255-021-00384-w.

Reciprocal regulation of cellular mechanics and metabolism

Tom MJ Evers[1], **Liam J. Holt**[2], **Simon Alberti**[3], **Alireza Mashaghi**[1,*]

[1]Medical Systems Biophysics and Bioengineering, Leiden Academic Centre for Drug Research, Faculty of Science, Leiden University, 2333CC Leiden, The Netherlands

[2]Institute for Systems Genetics, New York University Langone Health, New York, NY 10016, USA

[3]Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany

Abstract

Metabolism and mechanics are intrinsically intertwined. External forces, sensed through the cytoskeleton or distortion of the cell and organelles, induce metabolic changes in the cell. The resulting changes in metabolism, in turn, feedback to regulate every level of cell biology including the mechanical properties of cells and tissues. Here, we examine the links between metabolism and mechanics, highlighting signaling pathways involved in the regulation and response to cellular mechanosensing. We consider how forces and metabolism regulate one another through nanoscale molecular sensors, micron-scale cytoskeletal networks, organelles, and dynamic biomolecular condensates. Understanding this crosstalk will create diagnostic and therapeutic opportunities for metabolic disorders such as cancer, cardiovascular pathologies and obesity.

In 1910, James B. Herrick reported mechanical deformations in the red blood cells (RBCs) of a patient with severe anaemia[1], a condition we now know as sickle-cell anaemia (SCA). Forty years later, Linus Pauling elucidated the mechanism of SCA, revealing the molecular causes of a disease for the first time. In their paper entitled “Sickle Cell Anaemia, a Molecular Disease[2]“, Pauling and colleagues determined that it was the abnormalities in haemoglobin from SCA patients that led to disruption of cellular morphology and mechanics.[3] In fact, a single point mutation causes sickle cell beta-haemoglobin (Hb) to polymerize in an oxygen-dependent manner.[4] These polymers can exceed the diameter of red blood cells, distorting these cells to a characteristic sickle shape (figure 1).

Amazingly, recent work has echoed these findings, showing that single mutations are sufficient to cause protein polymerization, especially if protein complexes are symmetric multimers, as is the case with many metabolic enzymes.[5] Throughout the course of evolution, there have been many cases of enzymes transitioning to proteins with structural roles. For example, crystallins, the structural proteins in vertebrate eyes, derive from different enzymes in different species. Human alpha-crystallin is a small heat-shock protein,

*Corresponding author: Alireza Mashaghi (a.mashaghi.tabari@lacdr.leidenuniv.nl).

Author Contributions

AM conceived and supervised the project. TMJE performed the literature search and analysis. TMJE, LH, SA, and AM contributed to writing of the initial draft and the subsequent revisions.

Competing interests

The authors declare no competing financial interests.

while argininosuccinate lyase has been co-opted as the delta 2 crystallin in ducks.[6] Furthermore, many metabolic enzymes have been shown to polymerize in response to changing nutrient status[7]. Hexokinase shares a common fold with actin, and has been shown to polymerize in response to glucose and ATP.[8]

In light of these findings, an emerging hypothesis postulates that the modern cytoskeleton evolved from metabolic enzymes. Initially, polymerization may have served to regulate metabolic activity, but then the polymers themselves were co-opted to perform mechanical work. Thus, metabolism, polymerization, and mechanical processes could have been intrinsically connected throughout evolution. In what follows, we discuss the emerging links between cellular mechanical and metabolic processes. We organize our essay spatially, initially moving from the outside of the cell in towards the centre of the nucleus, and then in terms of scale, from the molecules to organelles to tissues. Finally, we discuss these issues in the context of disease.

Basic concepts in cellular mechanics and metabolism

The *eukaryotic* cytoskeleton is comprised of polymerizing proteins, including actin filaments (AF), microtubules (MT) and intermediate filaments (IF). The latter include nuclear lamins, which can dominate cellular stiffness and serve as a crucial focal point of mechanical sensing.[9] To perform their functions, the cytoskeletal components integrate with multiple proteins (e.g. motor proteins) and cellular organelles. Of these, actin and tubulin are highly dynamic, and their assembly is regulated by binding to and hydrolysis of ATP and GTP respectively. Thus, actin and tubulin can be assembled (polymerization) and disassembled (depolymerization) to form different networks that organize cellular contents, connect the cell physically and biochemically to the external environment, generate coordinated forces that enable the cell to move, enable cells to adopt different shapes, and segregate chromosomes and organelles during cell division.[10] The functions and properties of the cytoskeleton have been the focus of numerous excellent reviews[11–13]. The fundamental fact that actin and tubulin are enzymes (that hydrolyse phosphorylated nucleotides) represents an obvious coupling of mechanical and metabolic processes.

Cellular and tissue mechanics are determined by many factors beyond the cytoskeleton. At the most fundamental level, the high concentration of macromolecules found in cells creates unusual mechanical properties. Just like traffic suddenly grinding to a halt when a lane of the freeway is closed, dense colloids become non-Newtonian and highly viscous as they approach the “jamming transition”.[14] Try making oobleck (2:1 corn-starch:water). The mechanical properties of this mix are liquid-like at rest, but become solid-like when sheered forcefully. Concentrated polymers can undergo gel transitions as they become entangled at high concentrations (this is why your car needs oil changes). Of course, the cell is a complex mixture of particles and polymers that all interact[15]. Crucially, this cellular milieu is also “active matter”; ATP consuming processes (e.g. molecular motor activity) constantly stir the cell.[16] This crucial dependence upon energy-consuming processes to support the material properties of the cell highlights a fundamental biophysical connection between metabolism and mechanics. A dramatic illustration of this fact is that *E. coli* cells undergo a glass transition and become solid-like upon metabolic poisoning and associated macromolecular

crowding.[17] As discussed below, it is now thought that related phase transitions of proteins from particles to complexes have been co-opted as a central organizing principle of cell biology.

When we begin to consider the interrelationship between the interior and exterior of cells, all organisms rely on turgor pressure as a crucial determinant of mechanics. Thus, the flow of water into and out of cells, driven by active ion pumps and osmotic pressure, is key.[18] Pumping ions is perhaps the one of the most energetically demanding task that cells undertake. For example, 20% of the ATP of a typical mammalian cell is used just to pump two ions: sodium and potassium.[19] These monovalent ion fluxes, as well as proton gradients help control cell volume, and generate outward forces in the form of turgor pressure.[20] These forces can range up to megapascals (> 100 psi); as a consequence, plants can exert sufficient force to grow through concrete.

In many organisms, the cell wall is crucial for mechanics. In this review, we focus on mammalian cells, which don't have a cell wall, but nevertheless, the surface glycocalyx (a layer of glycoproteins and glycolipids that surrounds the cell) is a crucial determinant of the mechanical properties of cells[21], and is highly responsive to metabolic cues. In addition to the glycocalyx, the plasma membrane (PM) is an important contributor to cell mechanics[22] that is intrinsically linked to metabolism, as it provides a key platform for lipid biophysics to translate into metabolic cues.[23] The PM is composed of a lipid bilayer that provides elastic rigidity to the cell; when a mechanical force is exerted on the membrane, either by stretching or bending, the lipid bilayer is slightly deformed, eliciting a mechanical restoring force that contributes to sculpting a stable membrane curvature.[24] At the same time, the PM hosts mechanosensors (ion channels, junctional proteins and cell adhesion elements) that detect extracellular forces and initiate signaling cascades that culminate in rewiring of metabolism, as discussed below. Furthermore, the mechanical properties of *metazoan* tissues are determined to a large degree through the extracellular matrix (ECM). Importantly, the ECM has also been shown to regulate metabolic processes, such as glycolysis, as discussed below.[25]

Over the last two decades, research into metabolism has been reinvigorated, and the mechanisms by which metabolites regulate myriad cellular process have been slowly elucidated.[26] For the purpose of exposition, we focus here on the mTORC1 and AMPK kinases, both essential energy sensors that have been frequently linked to mechanical processes. mTORC1 activation alters the phosphorylation state of many downstream targets to coordinate the transition from a resting state to growth by promoting anabolic processes, including protein[27], lipid[28] and nucleotide synthesis[29], and by suppressing catabolic processes such as proteasome-dependent proteolysis and autophagy.[30] Disturbances in any of these metabolic signaling pathways have been implicated in diseases such as metabolic syndrome,[31] cancer[32] and neurodevelopmental disorders.[33]

Whereas mTORC1 shifts the metabolic balance towards anabolism, AMP-activated protein kinase (AMPK) does the reverse.[34] By phosphorylating downstream targets, AMPK inhibits energy-consuming, growth-promoting pathways and promotes catabolism of fatty acids and

other bioenergetic fuels.[35] Additionally, AMPK has been shown to regulate other cellular processes including autophagy, mitochondrial homeostasis and cell polarity.[34,36]

Mechanics and metabolism from the outside in

Junctional mechanics and regulation of metabolism

The outermost detection of cell-cell and cell-matrix forces is through cell surface protein assemblies such as cadherins at cell–cell junctions[37], and integrins in large dynamic protein structures called focal adhesion complexes, which link the cell to the ECM. Upon activation, both integrins and cadherins form clusters and recruit a repertoire of proteins to initiate signaling cascades that culminate in the intracellular activation of the RhoA GTPase pathway (figure 2). RhoA modulates actin cytoskeleton remodelling, intracellular contractile forces and growth of the associated adhesion complex, allowing a cell that is under tension to respond to extracellular forces.[38]

Recent studies have uncovered reciprocal regulation between integrins and critical controllers of cell metabolism, including mTORC1 and AMPK. In general, integrins control cell metabolism by ligand-induced modulation of the PI3K-Akt-mTORC1 signaling cascade, resulting in the upregulation of many metabolic pathways. Ligand-engaged integrins undergo internalization via the endo/exocytic pathway before being recycled back to the plasma membrane. For example, internalization and recycling of the α5β1 integrin, controlled by the Arf4 GTPase, was shown to be regulated by amino acid availability in an mTORC1-dependent manner.[39] In turn, Arf4-mediated integrin internalization is required for mTORC1 lysosomal recruitment and activation, indicating the two-way regulatory interaction between mTORC1 and integrin trafficking. In addition, growth factor starvation in human mammary epithelial cells induces upregulation and endocytosis of β4-integrin along with its matrix substrate, laminin.[40] Internalized laminin is degraded in lysosomes, resulting in increased intracellular levels of essential amino acids required for mTORC1 signaling and cell survival. In contrast, high nutrient conditions inhibit integrin activity through AMPK-dependent suppression of the integrin binding protein tensin.[41] Loss of AMPK results in enhanced integrin activity, adhesion complex formation, and cell spreading, and enables cells to generate higher traction forces and increase fibronectin fibre formation.

Integrin signaling further interacts with metabolism by coupling mechanics to transcription. Forces are transduced to the nucleus through cytoskeletal connections to DNA via the LINC complex and nuclear lamina (see below)[42] or via activation of mechanosensors in the cytosol.[43] Either way, the transmission is facilitated by remodelling of the cytoskeleton, resulting in activation of transcription factors that regulate cell-type specific gene expression programs (figure 2).[44]

The cadherins are another crucial family of cell surface adhesion molecule that relay mechanical information and feed into metabolic decisions. Application of force directly to E-cadherin junctions between epithelial cells was shown to stimulate liver kinase B1 (LKB1) to activate AMPK.[45] This in turn stimulates actomyosin contractility, glucose uptake and ATP production (see figure 2). The increase in ATP provides energy to reinforce the

adhesion complex and actin cytoskeleton so that the cell can resist forces, which is required for efficient formation of an epithelial barrier.

Cytoskeletal regulation of metabolism

Many metabolic enzymes are known to interact with cytoskeletal proteins[46] (e.g. F-actin, tubulin), but only recently have studies begun to unravel their role linking metabolic activity to cytoskeletal organization. The dynamic interactions of metabolic enzymes with microtubules and actin filaments supports the localization of these enzymes to areas of the cell with specific metabolic needs, allowing the cytoskeleton to respond to metabolic activity and ensure ATP production at cellular localities where demand is high. For instance, lung epithelial cells show locally increased actin cytoskeleton assembly and increased levels of phosphofructokinase (PFK), one of the key regulatory enzymes in glycolysis, on stiffer substrates[47]. PFK shows high-affinity for F-actin (linear polymers of globular actin subunits that occur as microfilaments in the cytoskeleton) and thus may provide a localized source of glycolytically derived energy. In addition, TRIM21, an E3 ubiquitin ligase, that regulates PFK degradation, is sequestered by F-actin rendering it inactive thus increasing PFK activity (figure 2). In this way, actin polymerization drives local ATP production to support cytoskeletal remodelling during mechanical stress.

In addition to its function as glycolytic enzyme, alpha-enolase constitutes a receptor for plasminogen on the cell surface, facilitating metastatic cancer invasion by promoting plasminogen activation into plasmin, a serine-protease involved in ECM degradation. In the presence of alpha-enolase, actin assembled into filaments localized closely to the cell surface. Silencing of alpha-enolase was shown to inhibit adhesion, invasion, and metastasis of pancreatic cancer cells, due to alterations in actin cytoskeleton organization, adhesion proteins, and integrin expression.[48] Cancer cells having silenced alpha-enolase showed reduced actin filament organization, and actin relocalised adjacent to the nucleus, suggesting a profound and reorganization of the actin cytoskeleton by alpha-enolase.

Full activation of glycolysis by the phosphoinositide 3-kinase (PI3K)-pathway requires downstream activation of the serine/threonine kinase Akt as well as Rac-dependent actin remodelling (figure 2).[49] Binding of insulin or growth factors to their receptors on the cell membrane activates PI3K resulting in generation of phosphatidylinositol-3,4,5-trisphosphate (PIP3) and recruitment and activation of GTP-Rac. GTP-Rac promotes actin fibre disassembly, causing the release of actin filament-associated metabolic enzyme aldolase, which catalyses the conversion of 6-carbon fructose molecules to the 3-carbon molecules glyceraldehyde and dihydroxyacetone, ultimately accelerating glycolysis (figure 2).[49] Moreover, in response to stiff ECM, the small GTPase Rac controls the activity of YAP/TAZ[50], crucial mechanosensors that are discussed below.

Although major emphasis has been placed on the interaction of actin with metabolic enzymes, a recent study suggests an important role for tubulin. Mechanical cues were shown to precisely coordinate glutamine metabolism with microtubule (MT) glutamylation, which modulates MT lattice stability to adjust the stiffness of the cytoskeleton and thereby adapt cell mechanics to the mechanical load of the environment.[51] The metabolic enzyme tubulin glutamylase TTLL4 was identified as the major mediator of tubulin glutamylation

in response to mechanical cues and directly interacts with microtubules by binding to the C-terminal tubulin tails.[52] Depletion of TTLL4 enhanced MT dynamics, cell compliance and contractility, thereby impacting cell spreading, proliferation and migration, providing a clear link between metabolism and mechanics through MT dynamics.[51]

Overall, these studies demonstrate that the redistribution of metabolic enzymes in response to mechanical and/or metabolic signaling achieves rapid coordination of cytoskeletal dynamics and metabolic fluxes, while avoiding the time- and energy-consuming signaling cascades, transcriptional activation and biosynthesis of new enzyme molecules.

YAP/TAZ at the crossroad of mechanics and metabolism

Some of the best described integrators of mechanical and metabolic information are YAP and TAZ, which act as transcriptional co-activators for the TEA domain transcription factor (TEAD) family of transcription factors and play a key role in the transduction of mechanical signals from actin to the nucleus (figure 2).[43,53] YAP/TAZ are effectors of the Hippo pathway, a signaling pathway that controls organ size in animals through the regulation of cell proliferation and apoptosis, and are regulated by several upstream kinases including MST1/2 and LATS1/2. MST1/2 are activated to phosphorylate and activate LATS1/2, which, in turn, phosphorylate YAP/TAZ promoting their degradation through the ubiquitin-proteasome system. When Hippo signaling is suppressed, YAP and TAZ are dephosphorylated leading to their stabilization and translocation to the nucleus, thus initiating transcriptional programs.

It is still unknown how mechanical signals are transmitted to the Hippo pathway. MST1/2 and LATS1/2 can bind to or colocalize with F-actin, suggesting that these kinases monitor cytoskeletal integrity.[54] Mechanical signals may also directly affect LATS activity. In addition, Hippo-independent YAP/TAZ signaling occurs in many cellular contexts, such as cell migration and expansion[55,56]. Nuclear relay of mechanical signals through YAP/TAZ requires Rho GTPase activity and tension of the actomyosin cytoskeleton, but is independent of the Hippo/LATS cascade.[57] In contact-inhibited cells (*i.e.* low mechanical stress), YAP/TAZ are inhibited by F-actin-capping and severing proteins (*e.g.* CapZ, Cofilin) resulting in diminished YAP/TAZ nuclear localization, transcriptional activity, and proliferation.[58] This suggests that YAP/TAZ inhibition by cell mechanics entails a remodelling of the F-actin cytoskeleton distinct from LATS-induced YAP/TAZ inhibition, which requires a mechanically competent cytoskeleton. Direct physicochemical regulation of YAP/TAZ may occur as changes in molecular crowding favour liquid-liquid phase separation of YAP/TAZ[59,60] (see below), and cellular mechanics (including cell spreading on stiff substrates[61] or mechanical compression[62]) both drive changes in intracellular molecular crowding.

YAP and TAZ have been shown to couple mechanics to metabolic changes in multiple contexts. YAP/TAZ nuclear localization and activity is proportional to ECM stiffness, but is also regulated by multitude of other mechanical signals, such as cell-cell contact and cell geometry. In the context of pulmonary vascular stiffening, YAP/TAZ induces the metabolic enzymes glutaminase 1 (GLS1), pyruvate carboxylase (PC), and lactate dehydrogenase (LDHA) to promote glycolysis and glutaminolysis, thereby driving cell proliferation and

providing energy during migration.[63] Similarly, matrix stiffening activates YAP/TAZ to induce LDHA, GLUT1, and hexokinase II, which enhance aerobic glycolysis during the migration of hepatocellular carcinoma cells.[64] Glycolysis can in turn sustain YAP/TAZ transcriptional activity by facilitating the interaction of YAP/TAZ with transcriptional co-activators, such as PFK1, which binds the YAP/TAZ TEADs and stabilizes their interaction with YAP/TAZ, thereby further reinforcing YAP/TAZ activation on a stiff ECM.[65]

YAP/TAZ mechanosignaling has also been found to regulate metabolism in both cancer cells and cancer-associated fibroblasts (CAFs) by increasing glutamine metabolism in response to a stiff ECM.[66] It was shown that YAP/TAZ upregulates glutaminase to generate glutamate from glutamine, as well as the aspartate/glutamate SLC1A3 transporter, to exchange amino acids between cancer cells and CAFs. In this case, the cancer cells export glutamate in exchange for uptake of CAF-derived aspartate to fuel precursors for nucleotide synthesis, whereas CAFs import cancer cell-derived glutamate in order to sustain their ECM remodelling activity, exporting aspartate. This YAP/TAZ-dependent glutamate/aspartate crosstalk highlights an intercellular metabolic network in the tumour niche.

Recent evidence also indicates a role for YAP/TAZ in coupling of mechanical cues to autophagy; a process that can promote cell survival by generating important metabolites through recycling of macromolecules during conditions of metabolic stress.[67] Cells plated at high densities are subject to contact-inhibition of proliferation, a fundamental property whereby cells cease proliferation and cell division upon reaching confluence.[68] In these cells, YAP/TAZ fail to co-transcriptionally regulate the expression of actomyosin genes, resulting in the loss of F-actin stress fibres, which impairs autophagosome formation.[69]

Together, these findings unravel a complex interplay between YAP/TAZ and metabolism, in which YAP/TAZ actively coordinate intracellular metabolic programs by regulating expression of metabolic enzymes and nutrient transporters, whereas, in turn, YAP/TAZ activity is affected by metabolic cues, such as glucose, and metabolites with signaling activity. However, details remain to be elucidated and an integrated molecular understanding of YAP/TAZ, as central effectors of mechanotransduction being called into action via both Hippo pathway-dependent and -independent routes, is an important black box to elucidate in mechanobiology.

Organelle scale integration of mechanics and metabolism

Above we discuss molecular scale regulation, but cells also couple larger-scale, more global mechanical stresses to transcription. Changes in cell shape translate to altered membrane tension, which is coupled to ion fluxes through stretch-activated channels such as Piezo both on the plasma membrane and the ER.[70,71] It was recently found that some force-activated transcription programs critically depend on nuclear shape.[72] Force transmission leads to nuclear flattening, which stretches nuclear pores, reduces their mechanical resistance to molecular transport, and increases transcription factor nuclear import.[55] Alterations to nuclear morphology also induce changes in the spatial organization of chromosomes, promoting their intermingling, and cells under tension reveal increased transcription of genes at the intermingling regions.[73]

The Golgi apparatus is also mechanosensitive and capable of transducing mechanical forces into biochemical signaling.[74] Actomyosin relaxation, either by plating cells on soft substrates or by inhibiting the activity of myosins, inhibits lipin-1 activity, a phosphatidic acid phosphatase (PAP) in the Golgi that converts phosphatidic acid (PA) to diacylglycerol (DAG). Decreased DAG content in the Golgi membrane limits trafficking between the Golgi and endoplasmic reticulum, promoting the synthesis and accumulation of SREBPs in the Golgi. Protease-mediated cleavage of SREBP facilitates their translocation to the nucleus where they induce a transcription program leading to lipid synthesis.

Mitochondria, the key metabolic organelles in human cells, are structurally regulated by physical forces and the cytoskeleton.[75,76] Very recently, it was found that cytoskeletal tension can induce a specific mitochondrial stress response, termed mitohormesis, which serves to activate oxidative stress resilience (OxSR) via solute carrier family 9 member A1 (SLC9A1) and (heat shock factor 1) HSF1.[77] Mechanosensation by SLC9A1 and HSF1 was shown to stimulate mitochondrial reorganization (fragments with toroidal shapes) and metabolic reprogramming by increasing mitochondrial protein turnover and limiting respiration. These data indicate that cytoskeletal tension regulates mitochondrial function and suggests that mechanical forces influence tissue behaviour by modulating mitochondrial metabolism.

Linking metabolism and mechanics via phase separation

An important “organelle scale” coupling between mechanics and metabolism occurs through the biophysical process of phase separation, the process by which molecules with distinct chemical properties spontaneously demix. This physical organization of proteins and protein complexes into membrane-less compartments has received increasing attention over the last decade.[78] Phase separation of cytoplasmic or nuclear macromolecular components results in the formation of condensates including gels, glasses (see *E. coli* above) and viscous lipid droplets. Here we focus on liquid-liquid phase separation, which is analogous to the separation of oil and water in a salad dressing.[79] The interactions driving phase separation are often weak and distributed (e.g. electrostatic or dipole-dipole) and often mediated by intrinsically disordered proteins/regions (IDPs/IDRs) or other multivalent interactions. Importantly, when condensates assemble, they enrich certain molecules, while they exclude others.[80,81] This allows condensates to dynamically organize and regulate myriad biochemical reactions.

Phase separation can dramatically affect molecular dynamics in cells, but can also perform mechanical work. Synthetic optogenetic platforms that use light to manipulate matter inside living cells, have recently been leveraged to elucidate how proteins assemble into different liquid and solid-like states.[82,83] A CRISPR/Cas9-based optogenetic technology, CasDrop, revealed that growing nuclear condensates can mechanically pull genomic loci together, while excluding background chromatin. These condensates grow within softer genomic regions, due to lower mechanical energy cost of droplets deforming low-density genomic regions. Additionally, it has previously been shown that an elastic nuclear F-actin scaffold mechanically stabilizes ribonucleoprotein droplets (nucleoli and histone locus condensates)

against gravitational forces in large cells.[84] These studies begin to establish a link between intracellular mechanics and condensate formation.

In addition to the polymerization of metabolic enzymes discussed in the introduction, it has become apparent in the last decade that many metabolic enzymes and other regulators of metabolism form condensates in response to stresses (figure 3). One example is condensation of the translation initiation factor Ded1p, which promotes a switch from translation of housekeeping protein to translation of stress proteins.[85] In *S. cerevisiae* and human hepatocarcinoma cells, hypoxia induces coalescence of glycolytic enzymes into 'G bodies'.[86] Cells containing G bodies showed increased glucose consumption, whereas cells unable to form G bodies exhibited growth defects and produce inviable daughter cells during cell division. These observations suggest that condensation of enzymes may help cells to survive and grow under low oxygen conditions.

Glucose withdrawal in budding yeast triggers assembly of TORC1 into large assemblies.[87] Assembly inactivates TORC1, suggesting a mechanism to preserve energy during glucose deprivation. Conversely, TORC1 activity has been shown to generally regulate condensate assembly in cells. Tracking of genetically encoded multimeric nanoparticles (GEMs) provided a rapid assay to quantify macromolecular crowding in cells. GEMs are homomultimeric scaffolds fused to a fluorescent protein that self-assemble into bright, stable particles of defined size and shape. Using GEMs together with genetic and pharmacological perturbations led to the discovery that mTORC1 controls macromolecular crowding inside cells by tuning ribosome concentration.[88] Macromolecular crowding favours phase separation, therefore mTORC1 activity couples metabolic sensing to condensation in cells. Extrapolating from these studies, mTORC1-dependent changes in molecular crowding may also impact the dynamics of many processes, including cytoskeletal elements, through the physicochemical mechanisms of depletion attraction and viscosity effects. Indeed, it has been shown that microtubule dynamics strongly depend upon crowding both in vitro[89], and in cells.[90]

Stress-induced phase transitions can propagate to the whole-cell scale in yeast. Similar to the glass transition in *E. coli*, the widespread assembly of higher order structures in starved yeast cells has additional remarkable consequences for cell mechanics: the cytoplasm of starved cells transitions from liquid to solid.[91,92] This solid-like state makes yeast cells so rigid that they can keep their shape in the absence of a cell wall. These material changes may protect cells from mechanical stress, or may be a mechanism for energy conservation. The assembly of higher-order structures through condensation is an emerging explanation for how cells rapidly adapt metabolism as well as the mechanical properties to sudden changes in the environment.

Genome folding and metabolic reprogramming

Finally, mechanics and metabolism are integrated at the level of genome folding and chromatin state. At the micron scale, genome organization is orchestrated by multiple mechanisms, including association with the nuclear lamina, which connects to the

cytoskeleton and thus the extracellular environment via the LINC complexes[93], and perhaps by phase separation of heterochromatin.[94,95]

The global mechanical properties of the nucleus have been shown to be strongly dependent on chromatin state. During the earliest stages of stem-cell differentiation, nuclei exhibit highly unusual auxetic mechanics where compression in one axis drives compression in an orthogonal axis rather than spreading, and compression-induced stiffening. This auxetic behaviour is driven by histone acetylation, as inhibition of histone deacetylases (HDACs) causes stem cells to adopt the auxetic phenotype.[96] On the other hand, increased methylation and heterochromatin formation leads to softening of the nucleus. In epithelial cells, oscillatory stretch leads to opening of Piezo-1 channels in the ER, which activates histone methylases, thus changing the mechanical properties of the nucleus. This softening of the nucleus is necessary to prevent DNA damage during oscillatory stress (*e.g.* in beating cardiomyocytes).[70] The metabolic state of the cell, in turn, can also directly impact chromatin state and mechanical properties of the nucleus by supplying co-factors for histone-modifying enzymes, such as histone acetyl transferase (HAT) and HDAC enzymes, which depend on acetyl-Co-enzyme A and NAD+ cofactors respectively.[97]

The genome is a dramatic example of the importance of “active matter” introduced above. The metabolic activity of ATP and metabolite production impacts the mechanics of chromatin through an intricate system of molecular motors and mechanical constraints. Transcription is one of the most significant contributors to the active dynamics of chromatin. RNA-polymerase holoenzymes are some of the most powerful and energy-demanding molecular motors, generating larger forces than kinesin- or myosin-type motors.[98] Polymerase activity repositions nucleosomes and generates torsional stress behind the transcription bubble.[99] These mechanical effects drive genomic spatial organization and dynamics, which in turn can regulate metabolic transcription programs. For example, CCCTC-binding factor (CTCF) is a key architectural protein that forms complexes at metabolic promoters to create DNA loops, organizing protein interactions and regulating epigenetic marks.[100] Metabolic factors, in turn, can regulate CTCF mediated DNA looping.[101,102]

Together, these findings indicate that mechanics and metabolism are integrated at the genome level, whereby genomic spatial organization and dynamics regulate metabolic transcription programs, and metabolic states, in turn, influence genomic organization in normal and pathogenic gene programming states.

Mechanics and metabolism in disease

Cancer

The field of cancer metabolism is enormous and an intense area of ongoing research[103,104]. Here, we highlight a few key areas of dysregulation at the mechano-metabolic interface, again stepping from the outside toward the centre of the cell.

Tissue-scale mechanical changes and metabolic reprogramming—During cancer progression, the ECM becomes deregulated and disorganized[105] leading to tissue

stiffening. Changes in osmolytes and vasculature can increase interstitial fluid pressure, while excess growth within a confined space cause the build-up of mechanical solid compressive stress.[106] In addition to hyperproliferation of tumour cells, tumour infiltrating cells (e.g. fibroblasts and immune cells) further remodel the tumour microenvironment leading to dramatic mechanical changes. Cancer cells must adapt both mechanically and metabolically to survive, proliferate and disseminate in this environment. Metabolic reprogramming is part of this adaptation and also feeds back to modify the ECM and the mechanical environment.

In fibrotic pancreatic cancers, the stiff tumour ECM has been shown to stimulate YAP-mediated upregulation of cytoplasmic creatine kinase B (CKB), as well as directing arginine-derived carbon into creatine biosynthesis and away from the urea cycle.[107] These processes promote creatine-phosphagen ATP-recycling, in which CKB regulates the phosphorylation of creatine to phosphocreatine, which in turn transfers phosphate to ADP to regenerate ATP, thus providing a non-mitochondrial energy source for cancer cells. In non-small-cell lung carcinoma (NSCLC), ECM stiffening induces proline metabolism and consequently cell proliferation.[108] Kindlin-2, a focal adhesion component that is essential for cell–ECM signaling, was found to localize to mitochondria and stimulate proline synthesis by upregulating pyrroline-5-carboxylate reductase 1 (PYCR1). As proline is the main building block for collagen, the kindlin-2-PYCR1 axis could play a crucial role in collagen synthesis and ECM remodelling, and consequently lung tumour progression.[108] Uptake of pyruvate in breast cancer drives the TCA cycle to enhance the production of α-ketoglutarate.[109] This metabolite enhances the activity of the enzyme collagen prolyl-4-hydroxylase, which promotes ECM remodelling. Thus, tissue stiffening can reprogram metabolism, which in turn can further perturb tissue mechanics in a vicious cycle, a mechanochemical axis that remains a crucial underexplored aspect of oncogenesis.

Mechanical effects on migration and metastasis—The most dangerous feature of cancer is the progression to metastasis, leading to cancer dissemination beyond the primary site. Metastasis requires both reprograming of cells to attain a migratory phenotype, typically through the epithelial to mesenchymal transition (EMT), and metabolic and mechanical adaptation of cells to facilitate migration within the dense, stiff tumour microenvironment.

Multiple studies have shown that mechanical perturbations can drive EMT. In pancreatic cancer, ECM matrix rigidity promotes elements of EMT, including downregulation of E-cadherin expression and increased nuclear localization of YAP and TAZ, driving cells towards a mesenchymal phenotype.[110] Independent of YAP/TAZ, integrin-dependent mechanosensation events in breast cancer lead to release of the mechanosensor TWIST1 from its cytoplasmic anchor G3BP2, to enter the nucleus and drive transcriptional events of EMT.[111] These examples suggest that multiple independent mechanosensation pathways can drive EMT in response to the altered mechanical properties of the tumour microenvironment.

After EMT, the process of migration is energetically costly, especially in the perturbed tumour microenvironment.[112] The cytoskeletal dynamics that generate and relay the forces

that propel cell migration have been estimated to consume up to 50% of the cell's ATP budget.[113] Some of the metabolic reprogramming described above may help supply the requisite ATP. There is consensus that motile aggressive mesenchymal cells exhibit increased aerobic glycolysis[114], and that the Warburg Effect, which is a hallmark of many cancer cells[115], may induce migratory or invasive behavior.[116] For instance, mesenchymal prostate cancer cells exhibit higher glycolytic activity relative to their epithelial precedents. This higher glycolysis is associated with increased rates of cytoskeletal remodeling, greater cell traction forces and higher migration speeds.[117]

Together, these studies indicate that metastatic cancer cells reprogram various metabolic pathways in order to migrate to and invade secondary tissues. However, even in non-cancerous migratory processes such as cellular unjamming and EMT in the neural crest, the migrating cell layers exhibit increased glycolytic flux in much of the same manner as migrating mesenchymal cells.[118]

Cellular mechanics—Altered mechanics are a hallmark of metastatic cells: more than two-thirds of metastatic cells exhibit lower stiffness[119]. The origins of these mechanical changes are the subject of ongoing investigation, and are likely to be a consequence of a combination of altered cytoskeletal activity, glycocalyx composition and organelle properties, including chromatin state (see below), all of which are strongly impacted by metabolic state.[120,121] A crucial, often underappreciated, determinant of the mechanical properties of cells and tissues is the cell surface glycocalyx. This incredibly dense network of glycoproteins and sugar polymers that is secreted into the ECM and presented at the cell surface dramatically alters the physical properties of tumours and cancer cells. For example, cell surface mucins such as Muc1, Muc4 or Muc16 are highly overexpressed in most breast, pancreatic and ovarian cancers, especially in metastatic cells.[122] These large, highly glycosylated proteins can extend 200 nm from the cell surface and generate large entropic pressures that drive increased membrane curvature and tubulation (figure 4). Mucin transcription is stimulated by mechanical perturbation and their glycosylation state is highly sensitive to the metabolic state of the cell. Due to their crucial role in cell surface interaction with the environment, mucins are regulated by multiple metabolites[123] and are therefore a crucial mechanism linking cell mechanics to metabolism.

Cytoskeleton—Hyperactivation of PI3K as a consequence of constitutively active receptor tyrosine kinases (RTKs)[124] results in increased cytoskeleton remodelling, which in turn regulates metabolic pathways. Filamentous actin usually sequesters aldolase. Therefore, increased actin dynamics can cause excess free, cytoplasmic aldolase that drives glycolysis, promoting cancer cell proliferation (figure 4).[49] Cells growing on relaxed matrices usually target PFK for proteasome-mediated degradation leading to downregulation of glycolysis, but this mechanism is overridden in cancer cells transformed with oncogenic KRAS, and MYC or silenced TP53.[47] Thus, perturbed crosstalk between the matrix, cytoskeleton and key metabolic enzymes may explain how cancer cells maintain high glycolytic rates despite continuous mechanical alterations to the tumour tissue.

Nuclear mechanics—The nucleus is the stiffest organelle in the cell, partly due to the mechanically robust lamina. The stiffness of nuclei has been shown to decrease as

cancer cells progress to more metastatic states.[121,125] These mechanical changes facilitate migration in constricted 3D environments but also lead to frequent nuclear rupture[121,126] and DNA damage that drives genome instability.[120] Apart from their crucial mechanical roles, lamins also directly organize the genome and coordinate transcriptional regulation of many genes, including metabolic enzymes. For example, 12-lipoxygenase expression is increased in cancer cells, resulting in conversion of polyunsaturated fatty acid to pro-carcinogenic metabolites.[127] Mutations in lamins also cause dysregulation of mTORC1, autophagy, and adipogenesis.[128] mTORC1 and its downstream targets are over-activated in cells derived from patients suffering from metabolic laminopathies, resulting in an abnormal autophagy, and downregulation of genes that regulate adipogenesis. In this way, lamins couple nuclear mechanical properties and metabolic states and act as mechanosensors.

Clinical prospects—Mechano-metabolic coupling in cancer cells is emerging as an important concept in drug development. Although several different approaches have been explored, only a small number of effective mechanical and metabolic inhibitors have been developed so far for use in cancer therapy.[129,130] Moreover, altered cell and ECM mechanical properties are thought to contribute to drug resistance.[131] More holistic studies of the interplay between mechanical and metabolic features of the tumour microenvironment are likely to suggest therapeutic strategies and perhaps explain the variable success of current treatments. For example, surgical intervention has dramatic mechanical impacts – how might the changes in tissue mechanics, inflammation, cellular metabolism and scarring impact the likely effects of subsequent chemotherapy?

Cardiovascular disease

The interplay between cell mechanics and metabolism has been extensively studied in cardiovascular pathologies. Atherosclerotic plaque formation frequently initiates at branches and bends in arteries that are exposed to irregular, disturbed patterns of blood flow.[132] Irregular shear stress has been shown to upregulate Rac1 activity, which mediates both cytoskeletal alignment as well as the upregulation of intercellular cell adhesion molecule 1 (ICAM-1), the latter through activation of nuclear factor-kappa-B (NF-κB). NF-κB activation requires metabolic generation of reactive oxygen species (ROS).[133]

Non-uniform shear stress also stimulates integrin-mediated upregulation of RhoA and subsequent YAP activation. Similar to ROS, YAP enhances JNK signaling and upregulates pro-inflammatory genes such as NF-κB.[134] NF-κB is a central inflammatory mediator that plays a major role in the progression of atherosclerosis by inducing upregulation of pro-inflammatory cytokines, chemotactic factors and focal enrichment of adhesion molecules, thereby promoting monocyte recruitment into the arterial wall of an atherosusceptible site.[135]

In ischemia, shear stress causes endothelial plasma membrane depolarization via ATP-sensitive K+ channel closure, inducing PI3K/Akt-mediated signaling transduction cascade resulting in NADPH oxidase activation and ROS production.[136] Recently, application of mechanical fluctuations to vascular smooth muscle cells was shown to regulate mitochondrial bioenergetics by tuning the structure of mitochondrial network, ensuring

ATP production via the electron transport chain and minimizing ROS production.[137] Pathological high or low mechanical strain reduced ATP production and increased ROS production, promoting oxidative stress and metabolic disturbances. These data indicate the importance of optimal mechanical homeostasis in the prevention of metabolic disturbance and cardiovascular disease.

Connective tissue diseases

Fibrosis in human tissues is observed in response to hypoxia as well as mechanical shear, indicating the importance of both metabolism and mechanosensing in these pathologies. Impaired YAP/YAZ mechanotransduction is implicated in fibrotic diseases, including viral hepatitis induced liver fibrosis or Lupus-induced lung and kidney fibrosis. YAP/TAZ promote TGFβ signaling, thereby inducing kidney and liver tissue fibrosis, which then further activates YAP/TAZ in a viscous cycle. Liver damage caused by viral hepatitis results in increases in collagen deposition and smooth muscle actin expression, which are associated with the generation of myofibroblasts, key players in fibrotic disease. Associated with these mechanical alterations, dysregulated glycolysis has been reported in experimental models of lung, kidney and liver fibrosis.[138] Upregulation of glutaminolysis and enhanced fatty acid oxidation are also drivers of fibroblast activation. For example, glutaminolysis is increased in TGFβ-stimulated fibroblasts. This leads to enhanced conversion of glutamine to glutamate which promotes the stabilization of collagen via mTORC1 signaling; inhibition of glutamine conversion ameliorates TGFβ-induced lung fibrosis. Verteporfin, a small-molecule that inhibits YAP/TAZ and TGF-β/Smad crosstalk, illustrates promising mechano-interference efficacy in preclinical kidney and hepatic fibrosis models[139,140]Also, Rho/ROCK is an upstream regulator of YAP/TAZ and the therapeutic effects of ROCK inhibitors in preclinical fibrosis models may reflect, in part, reduced YAP/TAZ activation[141,142] The processes that link metabolism and mechanics thus appear as promising targets for future drugs to manage fibrosis.

Conclusion and future perspectives

Interest in the reciprocal regulation of cellular mechanics and metabolism has grown extensively over the last decade. We now know that metabolism is interwoven with cellular mechanics at multiple scales. Nanoscale molecular sensors at the cell surface relay mechanical information through the cytoskeleton and transcriptional programs, and feed into metabolic pathways to increase energy production, allowing cells to resist and generate mechanical forces. At the micron-scale, cytoskeletal networks facilitate local enrichment of metabolic enzymes to provide high rates of metabolic activity where demand is high, while deformation of organelles, such as the nucleus, Golgi apparatus and mitochondria, induces transcriptional changes that rewire metabolism. The resulting alterations in metabolites, in turn, regulate every level of cell biology including the mechanical properties of cells and tissues.

Much remains to be discovered. One frontier is the single-cell analysis of mechano-metabolic coupling. Many current approaches homogenize complex tissues, with a consequent loss of information about the unique behaviour of individual cells, including

cellular metabolism, growth, and proliferation.[143,144]. Cutting edge technological advances are beginning to address these issues. For example, combining single-cell metabolic modalities with single-cell force spectroscopy (SCFS) techniques enables rapid and automatic linking of metabolic profiles to multiple mechanical parameters (figure 5). Single-cell mass spectrometry-based approaches also show great promise: recently, researchers were able to measure metabolic changes in response to a drug in a single cell.[145] SCFS techniques such as atomic force microscopy and optical tweezers have proven crucial for providing physical insight into the mechanical properties of cells[146–148] and offer a unique opportunity to unravel inter- and intracellular heterogeneity.[149] Understanding the crosstalk between mechanics and metabolism at the singe-cell level will likely create diagnostic and therapeutic opportunities for metabolic disorders such as cancer, cardiovascular pathologies and obesity.

Conceptually, an intriguing emerging field is that of phase separation. Although several groups have shown that membrane-less condensates perform mechanical work, and that metabolic enzymes and other regulators of metabolism can undergo condensate formation, very little else is known. This field is likely to be a productive area of research in the near future. Furthermore, the study of the material properties of cells as soft-condensed matter, far from equilibrium will yield deeper insights. We have made amazing progress since the first description of sickle-cell anaemia as a molecular disorder at the intersection of metabolism and mechanics. The coming years are sure to yield many more exciting discoveries at the intersection of metabolism and mechanosensing.

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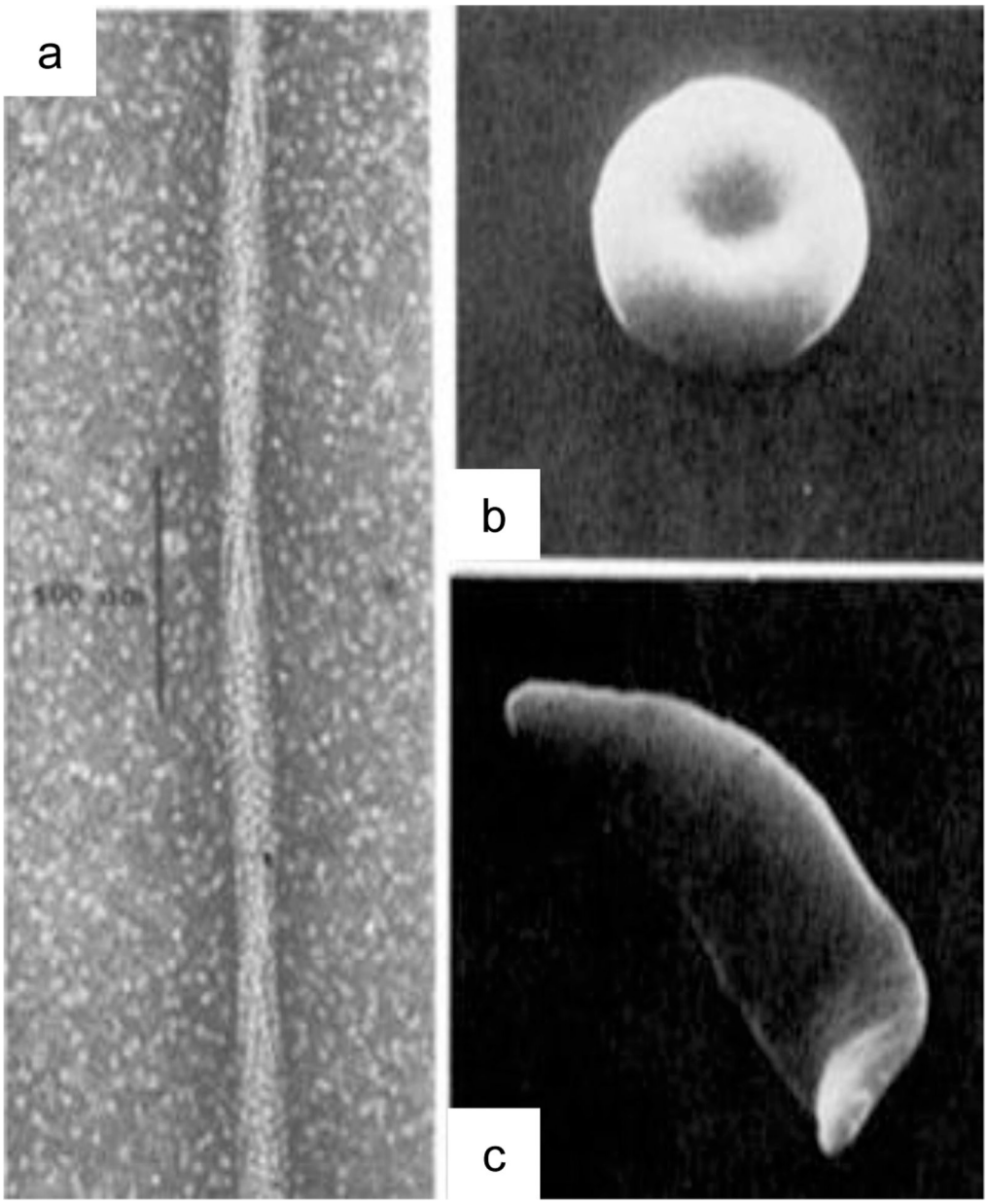

Figure 1.

Polymerization of haemoglobin (Hb) drives sickle cell formation. (a) a single point mutation causes Hb to convert to a polymer in an oxygen and pH dependent manner. (b) normal shaped red blood cell. (c) Distortion of a red blood cell into a sickle shape. Reproduced with permission from Dykes, G., Crepeau, R. H. & Edelstein, S. J. Three-dimensional reconstruction of the fibres of sickle cell haemoglobin. Nature (1978) doi:10.1038/272506a0[4], and Eaton, W. A. & Hofrichter, J. Sickle Cell Hemoglobin Polymerization. Adv. Protein Chem. (1990) doi:10.1016/S0065-3233(08)60287-9.[150]

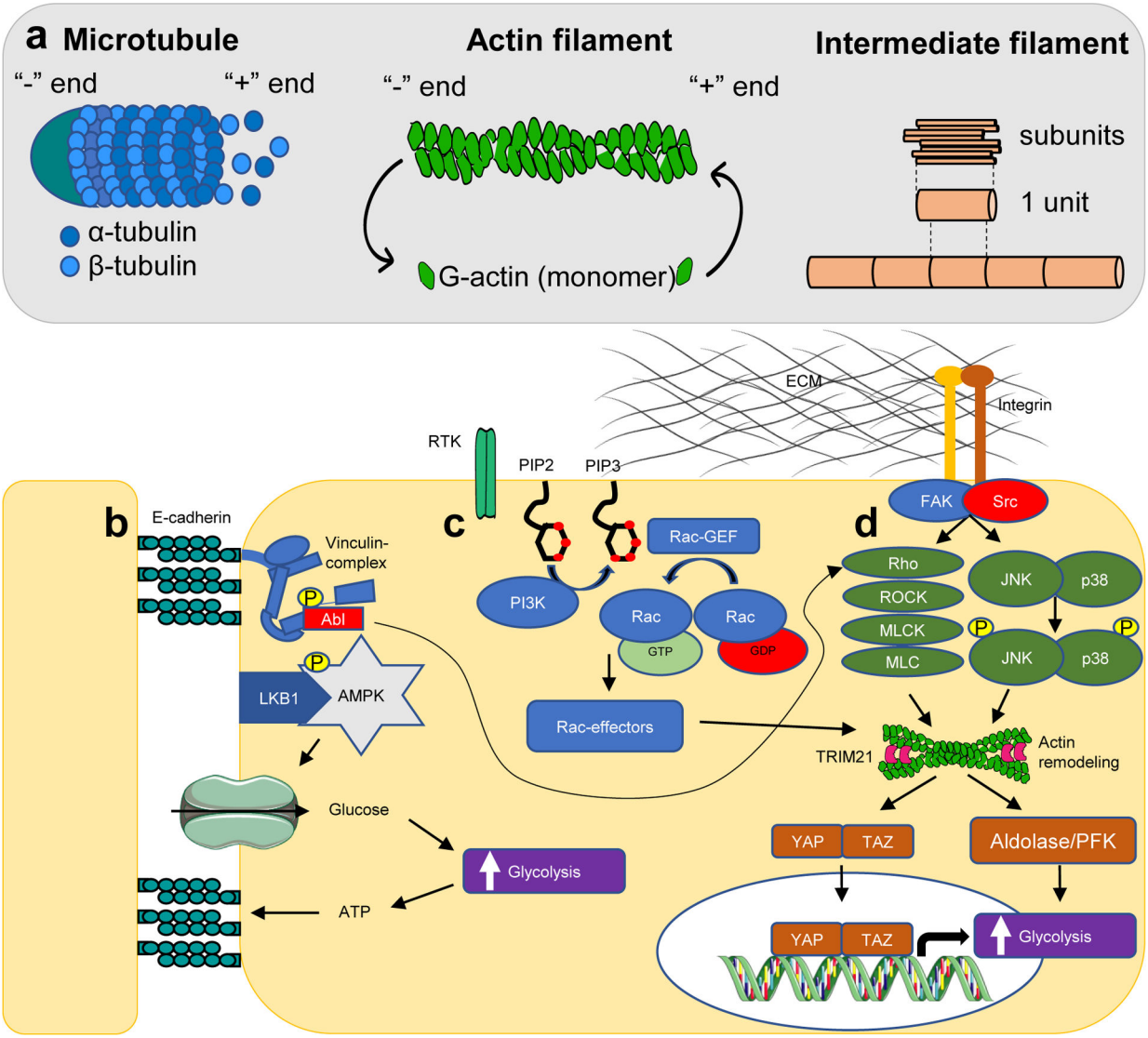

Figure 2.

Intracellular mechanosensation pathways that accelerate glycolysis. (a) Cytoskeleton filaments and their dynamics. (b) Cell-cell adhesion forces are sensed through E-cadherin, which forms a complex with and activates liver kinase B1 (LKB1) as well as AMPK. AMPK and the membrane-cytoskeletal protein vinculin are phosphorylated by LKB1 and tyrosine-protein kinase Abl, respectively. Vinculin activity enhances actin remodelling through the Rho/ROCK pathway, whereas AMPK promotes glucose uptake and ATP production in order to maintain energy supply to adhesions. (c) Stimulation of receptor tyrosine kinases by growth factors activates PI3K, resulting in generation of PIP3, and recruitment and activation of GTP-Rac, which promotes actin fibre remodelling. (d) Cell-matrix forces are sensed through integrins in focal adhesion complexes, which promote actin remodelling through Rho/ROCK and JNK/p38 signaling. Actin fibre remodelling, integrates all three signaling pathways, and enhances transcriptional activity of YAP/TAZ as well as release of glycolytic enzymes in the cytosol. The cytoskeleton also sequesters TRIM21 from the

cytosol, preventing proteasomal-mediated degradation of PFK. These processes culminate in acceleration of glucose metabolism.

FAK, focal adhesion kinase; MLC, myosin light chain; MLCK, myosin light-chain kinase; p38, P38 mitogen-activated protein kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; ROCK, Rho-associated kinase; RTK, receptor tyrosine kinase; Src, proto-oncogene tyrosine-protein kinase.

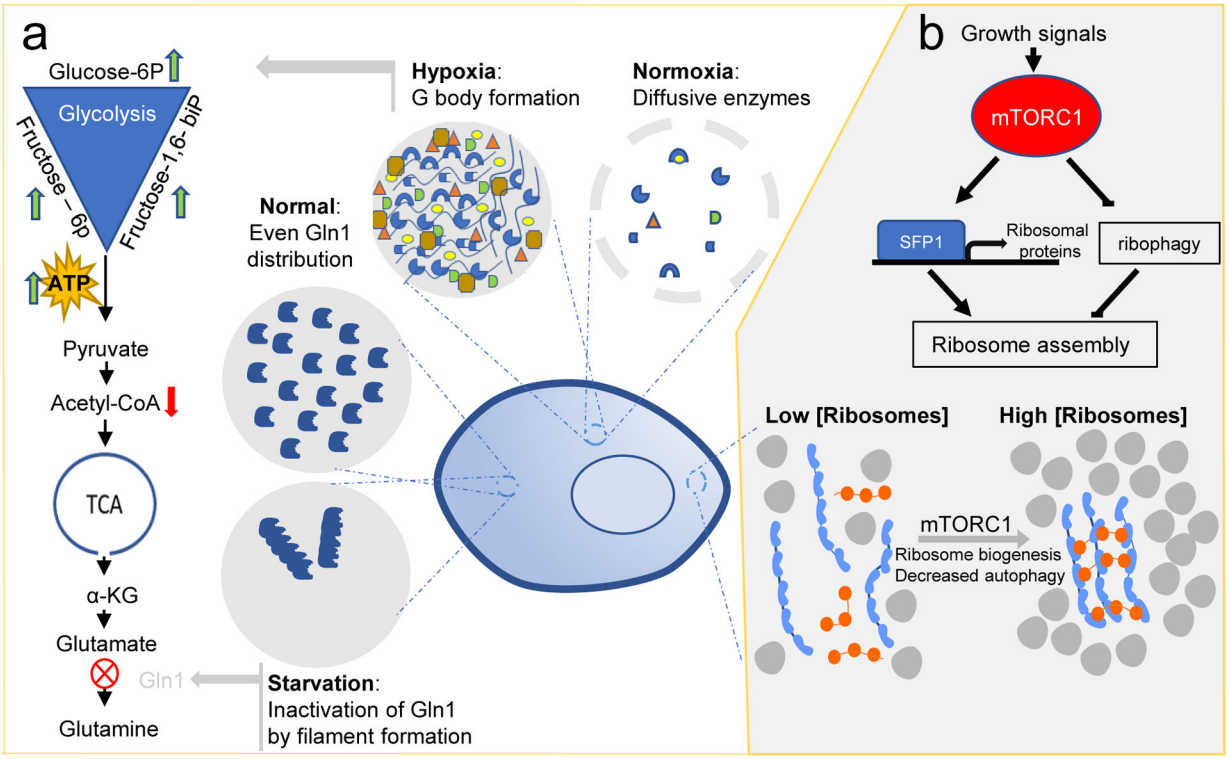

Figure 3.

Reciprocal regulation of phase separation and cellular metabolism. (a) Hypoxia induces condensation of glycolytic enzymes into ‘G bodies’. G bodies promote cycling through the entire glycolytic pathway, reflected by increased levels of glucose-6-phosphate, fructose-6-phosphate and fructose-1,6-bisphosphate, and decreased levels of acetyl-CoA. Under normoxia, glycolytic enzymes remain diffusive in the cytoplasm. Starvation induces filament formation of glutamine synthetase (Gln1) and promotes its inactivation and storage in the cytoplasm. In the presence of nutrients, Gln1 is evenly distributed through the cytoplasm. (b) In response to growth signals, mTORC1 (blue) tunes macromolecular crowding by modulating ribosome concentration (grey) in the cytoplasm. Higher crowding favors phase separation and molecular assembly. In the absence of growth signals, ribosome concentration is decreased by ribophagy. glucose-6P, glucose-6-phosphate; fructose-6P, fructose-6-phosphate; fructose-1,6-biP, fructose-1,6-bisphosphate; acetyl-CoA, acetyl coenzyme-A; α-KG, α-ketoglutarate; SFP1, transcription factor SFP1.

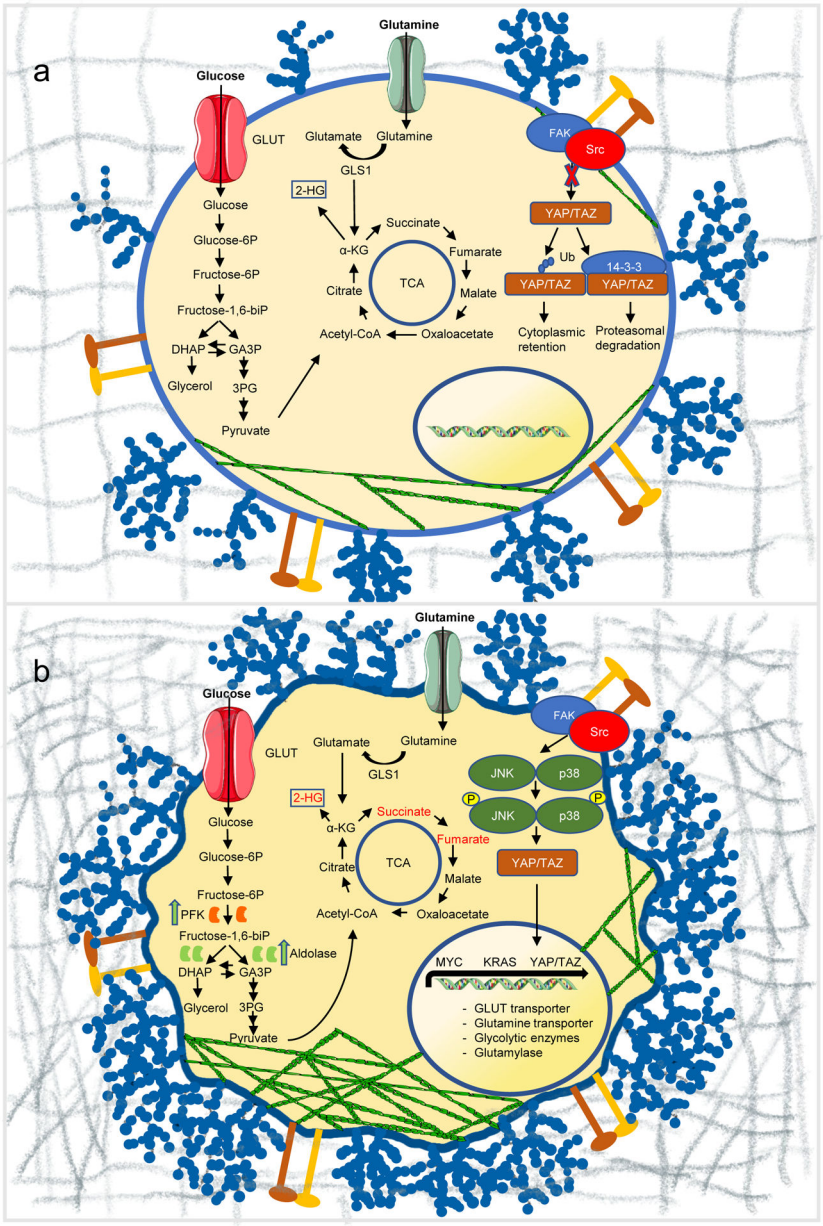

Figure 4.

Reciprocal regulation of mechanics and metabolism in cancer. (a) Under normal conditions, the compliant extracellular matrix suppresses JNK/p38 signaling, and promotes either retainment of inactive YAP/TAZ in the cytosol or its degradation by proteasomes. Cells show reduced actin cytoskeleton assembly and express normal levels of surface glycocalyx. (b) In cancer, extracellular matrix stiffening accelerates glycolysis through JNK/p38 and YAP/TAZ-mediated metabolic reprogramming, and induces cytoskeleton remodelling, resulting in the release of glycolytic enzymes in the cytosol, thereby promoting glucose metabolism. Transcriptional programs are perturbed, promoting metabolic reprogramming, including glucose and glutamine metabolism. Glutamine metabolic and glycolytic end-products feed into the TCA cycle, increasing the abundance of oncometabolites fumarate,

succinate, and 2-HG (red). The surface glycocalyx is frequently overexpressed generating large entropic pressures that drive increased membrane curvature and tubulation. GLUT, glucose transporter; GLS1, glutaminase 1; Ub, ubiquitin.

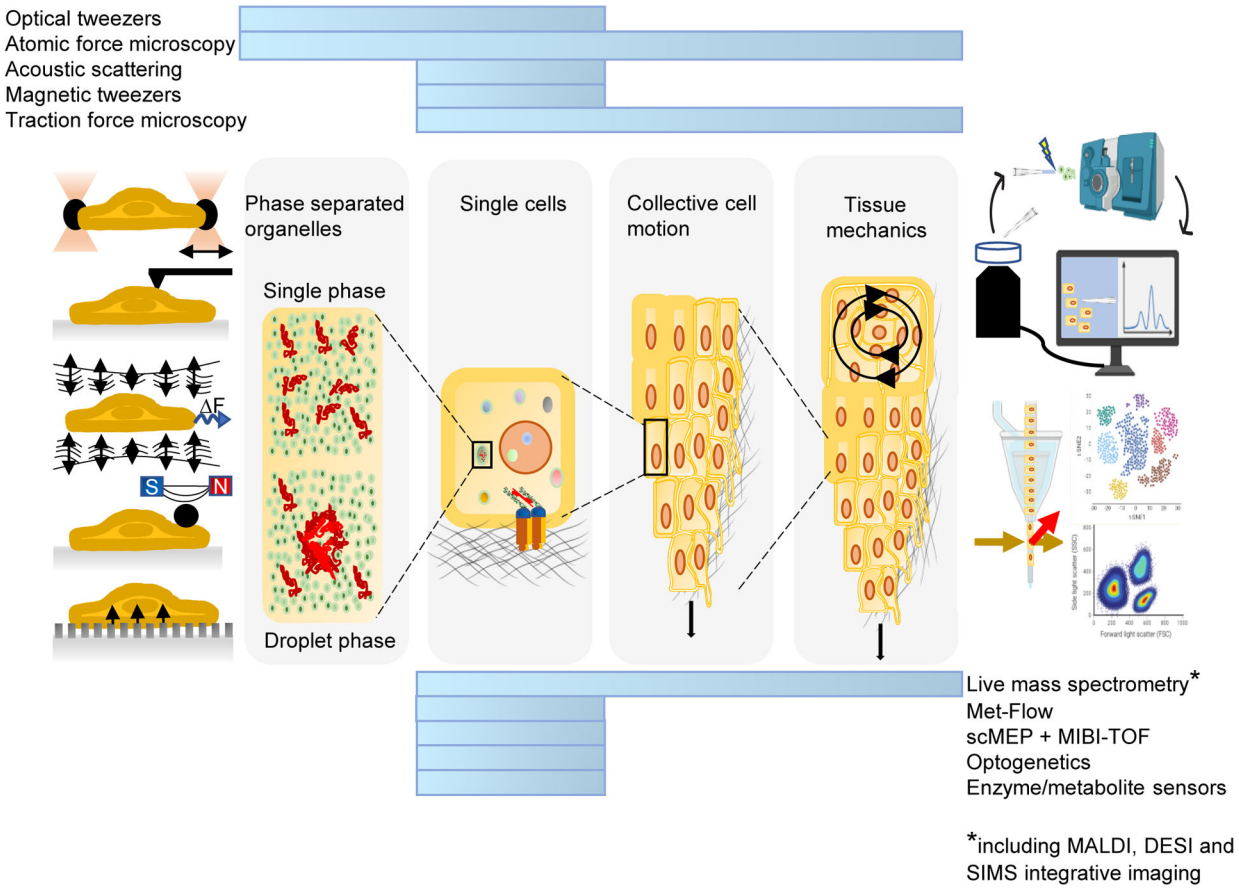

Figure 5.

Integration of metabolic technologies with force spectroscopy modalities correlate metabolic profiles to mechanics at different scales (blue bars) and in variable conditions. Future advances may improve the application of these techniques to single cells.

Met-Flow, a high-parameter flow cytometry method utilizing antibodies against metabolic proteins; scMEP, single-cell metabolic regulome profiling; MIBI-TOF, multiplexed ion beam imaging by time of flight; MALDI, matrix-assisted laser desorption/ionization; DESI, desorption electrospray ionization; SIMS, secondary ion mass spectrometry. Mass spectrometer and flow cytometry illustrations were generated using BioRender.