Trends in Cell Biology

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Review

Mechanical Regulation of Transcription: Recent Advances

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Mechanotransduction is the ability of a cell to sense mechanical cues from its microenvironment and convert them into biochemical signals to elicit adaptive transcriptional and other cellular responses. Here, we describe recent advances in the field of mechanical regulation of transcription, highlight mechanical regulation of the epigenome as a key novel aspect of mechanotransduction, and describe recent technological advances that could further elucidate the link between mechanical stimuli and gene expression. In this review, we emphasize the importance of mechanotransduction as one of the governing principles of cancer progression, underscoring the need to conduct further studies of the molecular mechanisms involved in sensing mechanical cues and coordinating transcriptional responses.

Highlights

Cells and tissues sense and respond to mechanical stimuli and the physical properties of their environment by engaging specific biochemical pathways and gene expression programs.

Recent technological advances in microscopy, genomics, and 3D cell culture systems have demonstrated that mechanotransduction is an integral part of transcription regulation.

Mechanical cues lead to epigenetic regulation through direct changes in chromatin accessibility.

Spatiotemporal analysis of the transcriptome *in vivo* in response to mechanical cues will improve our understanding of mechanoreciprocity (the interconnectedness of the cell with its local environment) and will aid the study of complex biological processes such as development, tissue homeostasis, and disease.

Cells and Tissues Respond to the Physical Environment

Cells in the human body are subject to a wide variety of mechanical stimuli acting at multiple scales. At the single molecule level, receptors on immune cells such as T and B cells leverage force to discriminate between ligands, enabling efficient recognition of antigen [1]. At the single-cell level, mechanical cues guide cell fate decisions in stem cells [2] and migration strategies of cancer cells [3]. Matrix stiffness alters the force generation capability of cancer cells, which scales with metastatic potential [4]. Finally, collective processes such as wound healing, tumorigenesis, and tissue homeostasis are intimately linked with the physical microenvironment [5,6]. In order to engage in functional responses appropriate to both passive mechanical stimuli, such as stiffness or topographic features of the cellular environment, and active ones, such as forces generated by cells and tissues, cells must be able to sense and measure mechanical perturbations. Different elements of the cell act in concert to maintain structural integrity and coordinate cellular sensing of external forces and mechanical stimuli. These stimuli are subsequently transmitted to the nucleus leading to broad changes in chromatin structure and accessibility (Figure 1, Key Figure) [7]. While we have come to appreciate the role of mechanical forces in shaping the genome, the molecular mechanisms involved in **mechanotransduction** (see Glossary) remain an enigma, with potential long-term implications for physiology, disease, and therapeutics. The focus of this review is to highlight recent advances in understanding the interplay between **mechanosensing** and transcription, with a particular emphasis on tumorigenesis and cancer progression (Figure 1).

The Cellular Mechanosensing Apparatus

The structural mechanosensing machinery can be broadly classified into two groups: (i) proximal mechanosensing apparatus consisting of cell surface receptors, **focal adhesion (FA)** complexes, cell–cell junctions, and the actomyosin cytoskeleton, and (ii) proteins of the nuclear envelope. In adherent cells, **integrins** link the cell to the **extracellular matrix (ECM)** through FAs (Figure 2). Under applied forces, integrins undergo conformational changes that result in stronger catch bonds with the ECM [8], leading to the formation and maturation of FAs, which

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Key Figure

Dynamic Interplay between the Physical Environment, Cellular Mechanosensing, and Gene Transcription

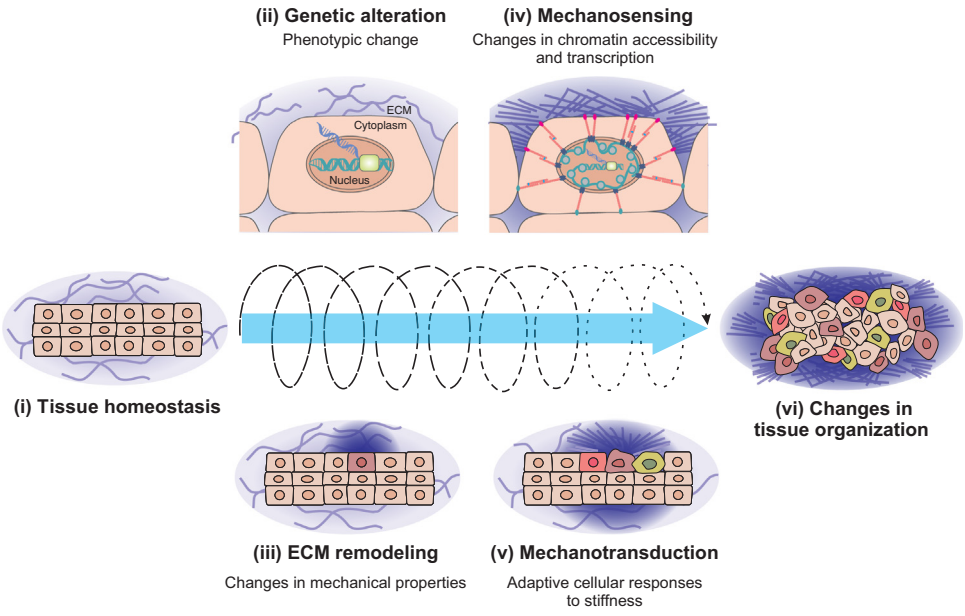


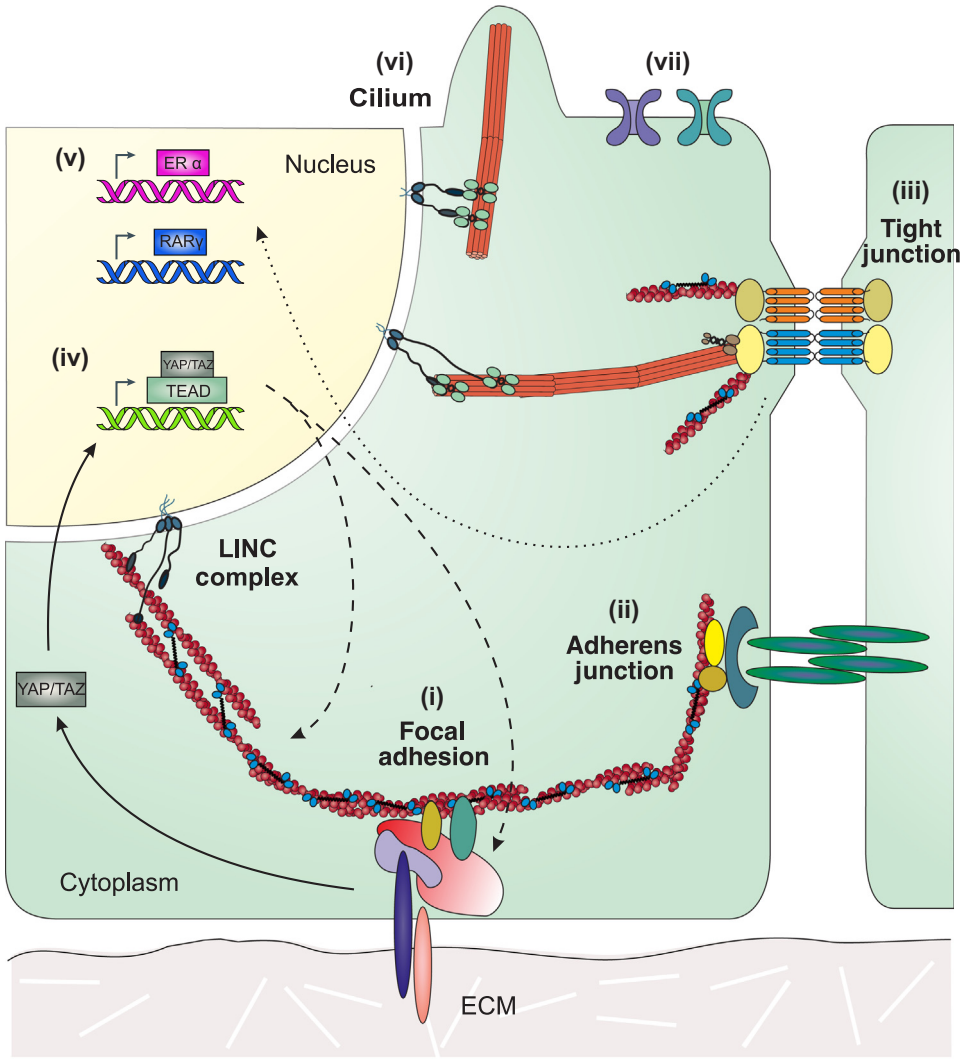
Figure 1. (i) Cells sense and respond to the physical properties of the extracellular matrix (ECM) and their neighboring cells. (ii) Oncogenic activation triggers reprogramming of a cell. (iii) Changes in the transcriptome result in secretion of ECM proteins and crosslinkers, leading to changes in the rheology of the ECM. (iv) These physical changes in the ECM are sensed by neighboring cells, where changes in the physical environment and the forces exerted on the cell are transmitted to the nucleus, eliciting biochemical and transcriptional responses. (v) Changes in the cell and ECM rheology are transmitted to neighboring cells through cell-cell junctions, leading to adaptive alterations of these cells. (vi) This feed-forward loop further changes the organization of the tissue.

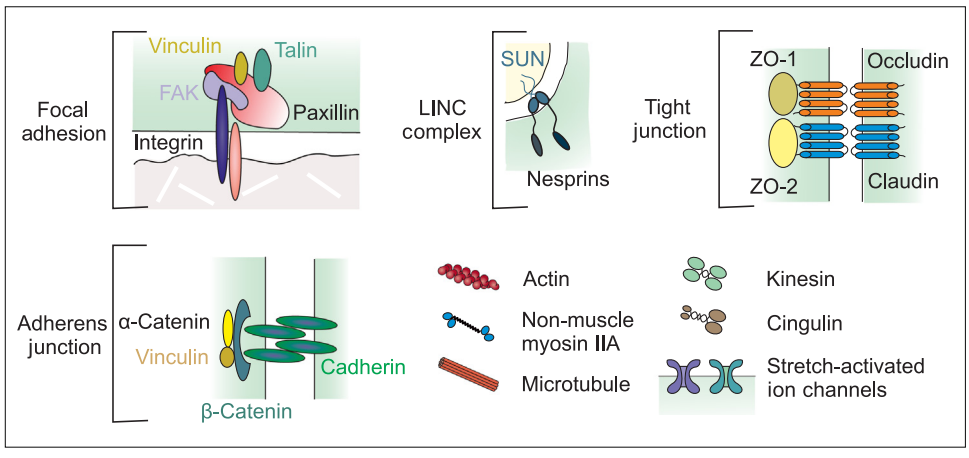
are composed of linker proteins, kinases, and other signaling modules. Through adaptor proteins such as α-actinin, talin, and vinculin, FAs connect to the actin cytoskeleton, which forms the major load-bearing element of the cell. Cell–cell junction complexes such as adherens junctions (AJs) and tight junctions (TJs) also serve as mechanosensors (Figure 2). AJs consist of transmembrane proteins of the cadherin family, which interact with actin via catenins. α-Catenin unfolds under tension, revealing a cryptic vinculin-binding domain that recruits vinculin to the AJ, facilitating actin binding [9]. TJs form via transmembrane proteins of the claudin and occludin families, which are linked to actin and microtubules through adaptor proteins (Figure 2). The actin cytoskeleton is itself a highly dynamic, polymorphic structure that rapidly reorganizes itself in response to biomechanical stimuli [10].

The nucleus, while being the largest and stiffest organelle in the cell, is not isolated from the cytoskeleton and the forces thereof. The linker of nucleoskeleton and cytoskeleton (LINC) complex, consisting of nesprins, and Sad1 and UNC-84 (SUN) proteins, connects the nucleus to the cytoskeleton, regulating both mechanical force transduction and gene expression [11]. Nesprins, which contain a Klarsicht, ANC-1, Syne homology (KASH) domain, span the outer nuclear membrane (ONM) and interact with the cytoskeleton on the cytoplasmic face of the

Glossary

Amoeboid: cells whose motility is driven by cytoplasmic projections called pseudopodia (e.g., leukocytes in humans).
Cancer plasticity: the ability of cancer cells to shuttle between a differentiated and undifferentiated state.
Cellularity: the number and condition of the cells present in a mass.
Contact-inhibition-of-proliferation: cell–cell contacts leading to abrogation of locomotion and/or cell growth; absent in cancer cells.
Dynamic reciprocity: (mechanoreciprocity); feedback mechanism by which cells and surrounding tissues affect each other's biomechanical properties.
Epigenome: the set of chemical modifications on DNA and histones that regulate gene expression without altering the DNA sequence.
Extracellular matrix (ECM): 3D network of proteins and biopolymer filaments that form the scaffold and biochemical niche for surrounding cells.
Fluorescence *in situ* hybridization (FISH): technique that uses fluorescently labeled DNA probes which bind (hybridize) to sequences of RNA or DNA with high complementarity for quantification using light microscopy.
Focal adhesion (FA): multiprotein complex that serves as the mechanical and biochemical signaling interface between the cell and ECM.
H3K27me3: trimethylation of histone H3 at lysine residue 27; indicates downregulation of nearby genes.
H3K9me2,3: di(tri)-methylation of histone H3 at lysine residue 9; normally associated with heterochromatin.
Integrins: proteins that span the cell membrane, linking the ECM to the cytoskeleton; part of the focal adhesion complex.
Mechanical stress: the force applied to a material per unit cross-sectional area.
Mechanosensing: recognition of mechanical properties of the microenvironment.
Mechanotransduction: conversion of mechanical cues to biochemical signaling and gene expression.
Nuclear receptor: transcription factors that are regulated by ligands (steroids or hormones).
Organoid: *in vitro* minimalistic 3D cellular aggregate that mimics *in vivo* organ structure.





Trends in Cell Biology

Transcription factor: protein that binds to specific DNA sequences and regulates transcription.
Transcriptional coregulator: protein that does not bind to DNA but regulates gene expression through interactions with transcription factors.
Transcriptome: set of all (coding and noncoding) RNA transcripts.
Transformation: process through which cells are able to overcome nonproliferative signals and grow outside their normal microenvironment.
Viscoelasticity: property of materials to be both elastic and viscous. As compared with elastic materials, viscoelastic materials deform under force and do not fully return to their original state once the force is removed.

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ONM (Figure 2) [12]. On the nucleoplasmic face of the ONM, nesprins bind to SUN proteins that tether to the inner nuclear membrane (INM) and bind to the nuclear lamina. The lamina consists of A and B type lamins, expression levels of which can alter nuclear stiffness, which, in turn, scales with ECM stiffness [13] and cell geometry [14]. In addition to sensing mechanical forces, the nucleus rapidly adapts to applied stress in order to protect the genome. Direct force application to isolated nuclei through the LINC complex induces the redistribution of emerin, a nuclear membrane protein, from the INM to the ONM, resulting in nuclear stiffening [15]. When subjected to compressive forces, the nucleus acts as a gauge, the shape of which determines cellular responses. Compression increases nuclear membrane tension, resulting in the release of calcium stored in the nuclear envelope and perinuclear endoplasmic reticulum, and downstream activation of the phospholipase cPLA2, leading to stimulation of actomyosin contractility [16,17]. A similar calcium release mechanism in cells subjected to mechanical stretch results in nuclear softening mediated by the loss of heterochromatin [18]. The role of nuclear membrane-associated proteins as intermediaries in force transduction has been reviewed in [19]. The actin cytoskeleton and nucleus thus act in unison to sense mechanical forces and allow cells to adapt to these forces. There are other mechanosensing proteins such as stretch-activated ion channels (Figure 2, reviewed in [20]) but these are beyond the scope of this review.

Mechanical Regulation of Transcription and Chromatin Landscape

Cell-Matrix Interactions Modulate Gene Expression

FAs form the proximal mechanosensing apparatus in cells adhering to ECM. In addition to serving as physical links between the cell and ECM, they also regulate expression of mechanosensitive genes (reviewed in [21]). Kinases and other signaling molecules such as LIM domain proteins are sequestered at FAs under high mechanical tension. For example, four and a half LIM domains 2 (FHL2) associates with FAs upon actin stress fiber formation and links FAs to gene expression. In cells experiencing low levels of **mechanical stress** (e.g., on soft substrates), FHL2 translocates to the nucleus where it accumulates at the p21 promoter, causing cell cycle arrest (Figure 3A) [22].

Passive and active mechanical stimuli such as fluid shear stresses, extracellular stiffness, and topography lead to cytoskeletal rearrangement. The Hippo signaling proteins yes-associated protein 1 (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), which are important for organ size control, are key mechanosensitive **transcriptional coregulators** that alter their subcellular localization in response to actin cytoskeletal dynamics induced by extracellular cues [23–25]. For instance, cells that experience increased mechanical stress (in high shear or high

Figure 2. Physical Links between the Extracellular Matrix (ECM) and Nucleus. The cell responds to external mechanical stresses through mechanosensitive transmembrane junctions. (i) Integrins connect the cell cytoskeleton to the ECM through vinculin, talin, paxillin, and other adaptor proteins within focal adhesions. Vinculin and focal adhesion kinase (FAK) interact with actin nucleators to control actin polymerization and depolymerization. (ii,iii) Mechanical forces between neighboring cells are transmitted to the cell cytoskeleton through cell–cell junctions. (ii) Adherens junctions comprise transmembrane cadherins, which link the actin cytoskeleton of neighboring cells through catenins and vinculin. (iii) Tight junctions (TJs) comprise claudins and occludins binding actin via zonula occludens (ZO) proteins and microtubules through the adaptor cingulin. (iv,v) Cell–matrix and cell–cell junctions transmit external forces via the cell cytoskeleton to the nucleus through the linker of nucleoskeleton and cytoskeleton (LINC) complex. (iv) Besides connecting ECM to the actin cytoskeleton, integrins also control yes-associated protein 1 (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) phosphorylation, which regulates YAP/TAZ nuclear translocation and subsequent transcriptional regulation of the Hippo pathway through the TEAD family of transcription factors (unbroken arrows). YAP/TAZ in turn modulate the expression of actin regulators and ECM proteins (broken arrows). (v) Cell–cell junctions have been shown to activate Src family kinases (SFKs), which regulate nuclear receptors such retinoic acid receptor γ (RARγ) and estrogen receptor α (ERα) (dotted arrows, see also, Figure 3). (vi) Microtubules can also sense and transmit physical forces through kinesin motors and the LINC complex. (vii) Stretch-activated ion channels such as Piezo 1 and Piezo 2 are important mechanosensors in the cell membrane.

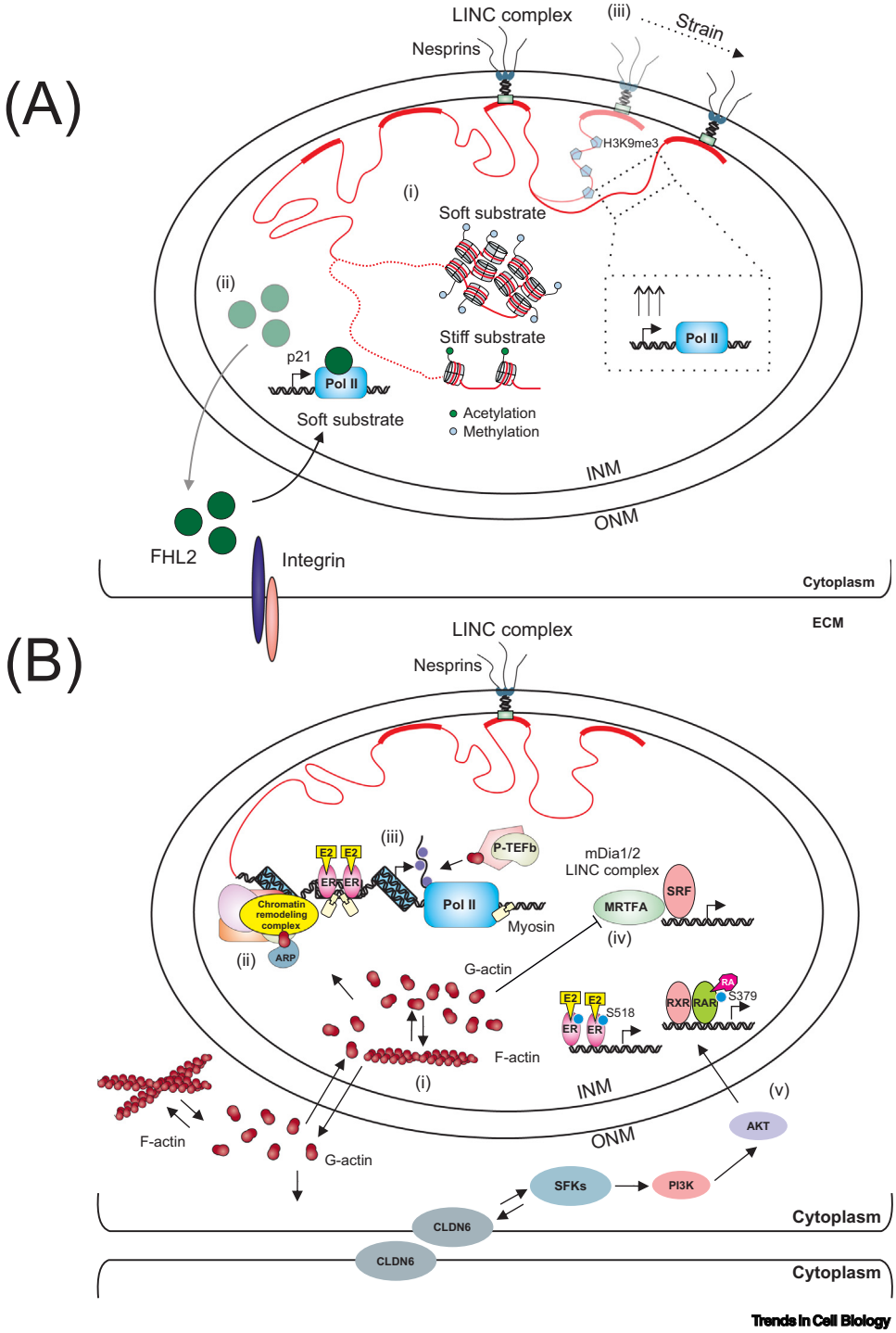

Figure 3. Changes in Chromatin Landscape and Gene Activity in Response to Mechanical Stimuli. (A) Mechanical stress and chromatin accessibility. Extracellular matrix (ECM) stiffness regulates overall chromatin accessibility in the nucleus affecting gene expression. (i) Cells grown on matrices of increasing stiffness present increasing levels of histone acetylation and decreasing levels of histone methylation. (ii) Mechanical properties of the ECM affect protein translocation through focal adhesion proteins such as the adaptor four and a half LIM domains 2 (FHL2), which

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stiffness environments), tend to present nuclear YAP/TAZ, which serve as coregulators for several **transcription factors** that induce cell proliferation, organ growth, and tumorigenesis [26]. Upon integrin binding to fibronectin in the ECM, focal adhesion kinase (FAK) regulates YAP nuclear localization via the FAK/Src/PI3K pathway [27]. YAP/TAZ reinforce FAs and the actin network by controlling the expression of FA proteins [28] and actin regulators [29]. YAP/TAZ regulation has been extensively reviewed [30] and YAP/TAZ nuclear translocation is now often used as a reporter of mechanotransduction (Figure 2).

Signaling from Cell–Cell Junctions

Cell–cell junctions also provide essential mechanical cues, which promote cell fate decisions such as **contact-inhibition-of-proliferation**. This signaling occurs through junction proteins such as cadherins and claudins (Figures 2 and 3). E-cadherin is known to regulate the activity of transcriptional coregulators such as catenins and YAP (Figure 2). Under biaxial mechanical stretch, YAP and β-catenin, a component of the cadherin complex, induce cell cycle entry in an E-cadherin-dependent manner [31]. The TJ protein claudin-6 has been shown to activate Src-family kinases (SFKs), which trigger activation of the **nuclear receptors** retinoic acid receptor γ (RARγ) and estrogen receptor α (ERα) through AKT-mediated phosphorylation, independent of their cognate ligands (Figures 2 and 3B) [32].

Nuclear receptors in general and steroid hormone receptors in particular are an important class of transcription factors that play a crucial role in several types of cancers. Importantly, endocrine signaling pathways have recently been implicated in YAP/TAZ signaling. For instance, induction of the glucocorticoid receptor (GR) leads to increased YAP mRNA levels, nuclear YAP localization, and YAP-luciferase reporter activity [33]. This is accompanied by increased fibronectin deposition, further implicating the aforementioned FAK/Src/PI3K axis in regulating YAP. The Hippo signaling pathway is also involved in estrogen receptor (ER) and androgen receptor (AR) signaling, which are important molecular targets for breast and prostate cancer, respectively [34,35].

Nuclear Actin and Myosin

Apart from their role in force generation, actin, actin binding proteins (ABPs), and myosins have come under scrutiny for their role in regulating transcription through various mechanisms (reviewed in [36]). Monomeric actin is actively imported into the nucleus by Importin-9 in a complex with the small ABP, cofilin [37], and is exported as a complex with profilin by Exportin-6 [38]. In the nucleus, monomeric actin forms a part of the pre-initiation complex (PIC) [39] and participates in transcription elongation [40–42]. Actin interacts with P-TEFb [42], which phosphorylates the RNA polymerase II (Pol II) C terminal domain, promoting productive transcription [43]. Actin also directly modulates subcellular localization of transcription factors. For example, myocardin-related transcription factor A (MRTF-A), a transcriptional coactivator of serum response factor

translocates to the nucleus in cells grown on soft substrates and regulates the p21 gene promoter. (iii) Active mechanical strains like pulling and pushing on a cell membrane are transmitted to chromatin through the linker of nucleoskeleton and cytoskeleton (LINC) complex, increasing chromatin accessibility by lowering H3K9me3 and thereby regulating transcription by RNA polymerase II (Pol II). This decrease in H3K9me3 also serves to soften the nucleus and protect the genome. (B) Mechanotransduction and transcription regulation. (i) G-actin is actively transported in and out of the nucleus and under certain conditions may polymerize there. (ii) Actin-related proteins (Arps) and G-actin are found in chromatin remodeling complexes. (iii) Monomeric actin interacts with P-TEFb and together with nuclear myosins can associate with RNA Pol II. Myosin VI may also be recruited to DNA through its binding to estrogen receptor (ER). (iv) The nuclear localization and activity of myocardin-related transcription factor A (MRTF-A), a coactivator of the serum response factor (SRF), are inhibited when in a complex with G-actin. Actin polymerization promotes dissociation of MRTF-A from G-actin leading to SRF activation and transcription upregulation upon serum stimulation. (v) Cell adhesion signals initiated by the tight-junction protein claudin-6 (CLDN6) regulates the activity of nuclear receptors. CLDN6/SFK/PI3K/AKT axis targets the AKT phosphorylation sites S379 in the retinoic acid receptor γ (RARγ) and S518 in the estrogen receptor α (ERα) and stimulates their activities. Abbreviations: INM, inner nuclear membrane; ONM, outer nuclear membrane.

(SRF), interacts with monomeric actin (Figure 3B). Serum stimulation leads to actin polymerization, dissociation of monomeric actin from MRTF-A, its subsequent nuclear import, and association with SRF (Figure 3B) [44]. This pathway depends on mDia1/2 formins and the coupling between cytoplasmic and nuclear actin networks via the LINC complex [45,46]. Moreover, nuclear myosin I and myosin VI (MVI) form a complex with Pol II [47,48] and MVI is recruited to regulatory sites via binding to ER (Figure 3B) [49]. These studies indicate a dual role for cytoskeletal proteins as signaling molecules involved in transcription regulation as well as in the transmission of and response to cellular forces.

Chromatin Landscape Is Modulated by Mechanical Stimuli

While significant work has delineated the contributions of the cytoskeleton and nuclear envelope proteins, the interplay between the ECM and chromatin landscape is less well understood. This is especially important in pathological settings, where extracellular stiffening triggers reprogramming of normal cells into tumor precursors. Recent advances in fabrication and polymer chemistry have allowed direct examination of how mechanical cues regulate the global epigenetic state and transcriptional output of the cell. Cells grown on hydrogels with linear stiffness gradients tune the expression of tissue-specific transcription factors to peak at the appropriate tissue stiffness [50]. Topographic cues act as epigenetic modifiers, leading to widespread changes in histone acetylation and methylation [51]. Using photoconvertible (photo-softening [52], photo-stiffening [53]) hydrogels, researchers have shown that human mesenchymal stem cells grown on stiff matrices undergo chromatin remodeling that is manifest in increased histone acetyltransferase (HAT) and reduced histone deacetylase (HDAC) levels. These changes require an intact LINC complex, reinforcing the notion that nucleo-cytoskeletal coupling is essential for chromatin remodeling [52,53] and correlates with increased acetylated chromatin and YAP nuclear localization. Interestingly, upon chronic culture on stiff substrates, cells are unable to remodel their nuclear architecture in response to photo-softening, underscoring the importance of physiological stiffness for cell culture [52]. By contrast, in epidermal progenitor cells subjected to mechanical stretch, redistribution of emerin to the ONM from the INM leads to a reduction in **H3K9me2,3** and an increase in Polycomb repressive complex 2 (PRC2) mediated **H3K27me3**, resulting in global transcriptional silencing (Figure 3A). Emerin in the ONM binds actin, leading to the formation of a perinuclear actin cap [54] and modulates other F-actin structures [15]. This reduces nuclear G-actin levels and Pol II activity (Figure 3B) [54]. The loss of H3K9me3 marked heterochromatin also results in actin-independent nuclear softening, which allows cells to dissipate strain energy while protecting the genome. On longer timescales, cells reorient their cytoskeleton and AJs to redistribute the strain energy, preventing nuclear force transduction and allowing the cell to return to its native epigenetic state [18].

In addition to substrate rigidity, and compressive and tensile forces, cellular geometry also modulates nuclear organization, chromatin structure, and gene expression. When grown on small circular islands, fibroblast nuclei display increased chromatin and nuclear membrane dynamics [14], changes in interchromosomal contacts [55], and increased nuclear HDAC3 levels [56] as compared with fibroblasts on elongated rectangular islands. As on stiff matrices, chronic culture of fibroblasts on laterally confined islands leads to de-differentiation and reprogramming [57], which can be leveraged to rejuvenate ageing fibroblasts [58]. These findings suggest that mechanical cues come in different flavors and can differentially regulate the **epigenome** in a cell type-specific manner with important consequences for physiology and therapeutics.

Measuring Transcription in Response to Direct Mechanical Force

In the studies discussed so far, mechanical stimuli have been intimately linked to biochemical signaling pathways through FAs, cell–cell junctions, or YAP/TAZ. How physiological forces physically alter chromatin organization and modulate biochemical signaling pathways and gene

expression is an active area of research. To probe direct transcriptional responses to mechanical perturbations, cells were subjected to a controlled amount of force via magnetic twist cytometry while chromatin stretching and transgene expression were monitored using **fluorescence *in situ* hybridization (FISH)** [59]. The study showed that application of physiological levels of force can stretch chromatin and upregulate both transgenes and endogenous mechano-responsive genes. This upregulation depends on H3K9me3 at gene promoters and suggests a correspondence between gene proximity to the nuclear envelope, degree of H3K9me3, and subsequent force-dependent upregulation (Figure 3A) [59,60]. While conceptually powerful, these studies leave open questions about how mechanical forces alter chromatin accessibility of endogenous loci to regulate gene expression.

Mechanical Stimuli in Physiology

Cancer as Unbalanced Mechanoreciprocity

Thus far, we have looked at how mechanical cues can alter gene expression. These changes in gene expression profiles in turn modulate the microenvironment by changing cellular contractility and adhesion, secreting structural proteins, and regulating surrounding cells via biochemical signals to create a feedback loop between the cell **transcriptome** and the mechanical properties of the environment (Figures 1 and 4). These reciprocal interactions are finely tuned and contribute to tissue development, homeostasis, and regeneration. Disruption of this balance can cause tissue deformation and the onset of pathological states (Figure 4B,C) [61]. For example, during the early stages of tumorigenesis, genetic alterations lead to ECM stiffening in the stroma, which facilitates signaling pathways that, through enhanced integrin-mediated mechanotransduction, lead to proliferation and **transformation**, shifting the balance away from tissue homeostasis (Figure 4B) [62,63]. Increasing evidence suggests that tumor initiation requires both alteration of intrinsic cellular state and external mechanical cues [64,65]. We refer the reader to recent reviews for additional details on the subject [66,67].

Biochemical and Biophysical Process of Tissue Stiffening

ECM stiffening during tumorigenesis is a complex biochemical and biophysical process involving various types of cells, structural proteins, enzymes, and physical forces. Tumor cells carrying damaged DNA secrete inflammatory cytokines and matrix remodeling enzymes that recruit fibroblasts and immune cells to the tumor initiation site [68]. Under physiological conditions, fibroblasts are involved in organ development and wound healing by depositing and remodeling ECM components. However, cancer-associated fibroblasts (CAFs), which are activated by biophysical and biochemical stimuli in the tumor microenvironment, deposit excess ECM components, deregulate proliferation of surrounding cells, and contribute to an imbalance in tissue homeostasis [69]. Cancer-associated immune cells induce inflammatory signaling befitting cancer's description as 'wounds that do not heal' [70]. The hypoxic environment created due to locally elevated cell density and metabolism facilitates lysyl oxidase (LOX) expression, which leads to elevated collagen crosslinking, thereby creating a dense ECM (Figure 4B) [71]. The stiffened ECM triggers phenotypic changes of tumor cells through epithelial–mesenchymal transition (EMT) [72]. In addition to morphological changes such as loss of polarity, cell–cell adhesion, and acquisition of mobility, changes in gene expression during EMT trigger deposition of ECM components, including fibronectin and fibrillin, thus contributing to ECM rigidity [72,73]. Actomyosin-driven cell contractility can further enhance ECM stiffness, leading to mechanical feedback between cells and ECM and increased tension in the tissue [74–76].

Durotaxis, preferential migration towards higher stiffness gradients [77], would then drive migrating cells to enhance **cellularity** and chemical signaling of the tumor microenvironment. Stiffness measurements of human breast biopsies revealed that the periphery of the tumor is stiffer than the core

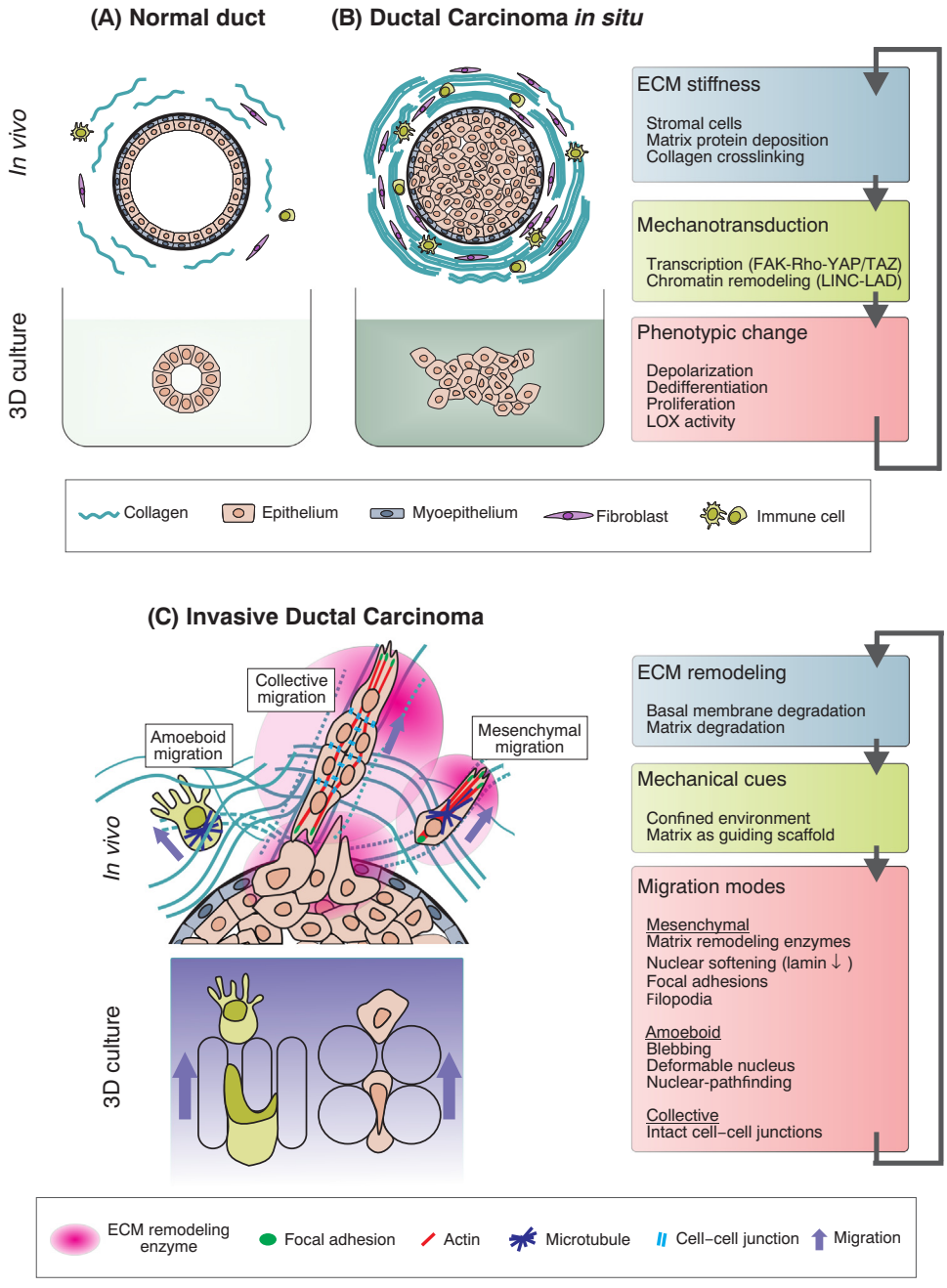

Figure 4. 3D Cell Culture Systems That Facilitate Mechanoreciprocity Studies. (Depicted here: breast cancer development as a model.) (A) Mammary duct in a physiological state. (Top) Epithelial and myoepithelial cells form a layer beneath the basal membrane and structure a mammary duct. The ducts are surrounded by soft connective tissue that consists of fat, extracellular matrix (ECM) proteins, and stromal cells. (Bottom) Epithelial cells in 3D culture using substrates with physiological stiffness form acinar structures. (B) Ductal carcinoma *in situ*. (Top) Genetic mutations in a subpopulation of epithelial cells cause secretion of tumorigenic signals and local inflammation leading to accumulation of activated stromal cells at the site. Together with mutated epithelial cells, cancer-associated stromal cells secrete excessive ECM proteins such as collagen. The elevated density of cells produces a hypoxic environment that stimulates lysyl oxidase (LOX) expression, which crosslinks the collagen and contributes to the rigid microenvironment. Disturbed mechanotransduction alters gene

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[78], suggesting that the gradual increase of stiffness towards the edge facilitates tumor cell invasion of the surrounding tissue [72]. The collective cell durotaxis model, where a sheet of cells migrates towards higher stiffness gradients while maintaining cell–cell junctions, implies a systematic invasion of tumor cells into the stiffened ECM (Figure 4C) [79,80]. Since the tumor edge is stiffer than the surrounding healthy tissue, the durotaxis model alone cannot explain how cancer cells disseminate to their metastatic sites [81]. When subjected to competing influences of 3D confinement and integrin-mediated adhesion, cells can spontaneously switch between mesenchymal and **amoeboid** phenotypes, leveraging actomyosin contractility to drive cell polarization [82,83] and metastatic potential. To test how tumor cells and cancer-associated stromal cells utilize mechanical cues from the environment, novel experimental tools have been developed to mimic the heterogeneous physical features observed *in vivo* (Figure 4).

Physical Cues and Cellular Transformation: Advances in 3D Culture Systems

Cells adjust to the physical features of the local environment and take advantage of these mechanical cues to evolve survival strategies. Transformation is a process whereby a normal differentiated cell acquires **cancer plasticity** [61]. Key open questions include the mechanisms that link the local mechanical environment with gene expression leading to transformation. Recent advances in 3D culture systems such as **organoid** cultures have informed us about cell physiology and gene expression patterns in a more naturalistic yet controlled setting, allowing the study of lineage decision processes [84], heterogeneity of tissues in disease [85,86], and drug responses (Figure 4) [87]. Decellularized ECM culture further reveals detailed mechanisms of cancer plasticity [88]. For example, a recent study explains the long debated 'obesity as cancer risk' precept from a physical perspective, demonstrating that increased myofibroblast content from obese mice changes collagen structure and increases local ECM stiffness, promoting tumorigenesis through enhanced mechanotransduction [89]. Since natural tissue tends to be viscoelastic, **viscoelasticity** is emerging as an important determinant of cell fate. Recent work has shown that mesenchymal stem cells, when grown on viscoelastic gels with the same initial elastic modulus but different stress relaxation times, undergo enhanced osteogenic differentiation on gels with the fastest stress relaxation [90].

EMT, along with expression of ECM remodeling enzymes, prepares cancer cells for dissemination from the primary tumor site. When migrating through compressed tissue, cells can undergo nuclear swelling and rupture (Figure 4C) [91] due to an influx of cytoplasmic proteins into the nucleus caused by an increase in confinement-induced RhoA-mediated actomyosin contractility [92]. Alterations in chromatin compaction change nuclear rigidity, which allows it to dissipate external forces and prevent rupture [18,93]. Microfluidic culture systems enable monitoring of dynamic changes in abundance, localization, and structure of molecules and organelles that protect the nucleus during migration through constrained spaces (Figure 4C). During constrained migration of cancer cells, non-muscle myosin IIB localizes to the perinuclear region, generating forces that push the nucleus through the constriction [94]. In fibroblasts migrating through pores, nesprin-2 accumulates at the anterior edge of the nucleus, where it tethers to the actomyosin cytoskeleton. Contractile cytoskeletal forces then pull the nucleus through the constriction [95]. Amoeboid cells find the path of least resistance by positioning their nuclei to the cell anterior

expression of epithelial cells, resulting in acquired plasticity, lost polarity, and unregulated proliferation, further increasing ECM stiffness. (Bottom) 3D culture with a rigid substrate causes deformation of acinar structures. (C) Invasive ductal carcinoma. (Top) The transformed epithelial cells overexpress ECM remodeling enzymes such as matrix metalloproteinases that degrade basal membrane and fibrous ECM proteins. The acquired mobility through epithelial–mesenchymal transition facilitates cellular migration through the confined environment. (Bottom) Microfluidics-based culture systems allow scientists to investigate different migration modes of cells. Abbreviations: FAK, focal adhesion kinase; LINC, linker of nucleoskeleton and cytoskeleton; TAZ, transcriptional coactivator with PDZ-binding motif; YAP, yes-associated protein 1.

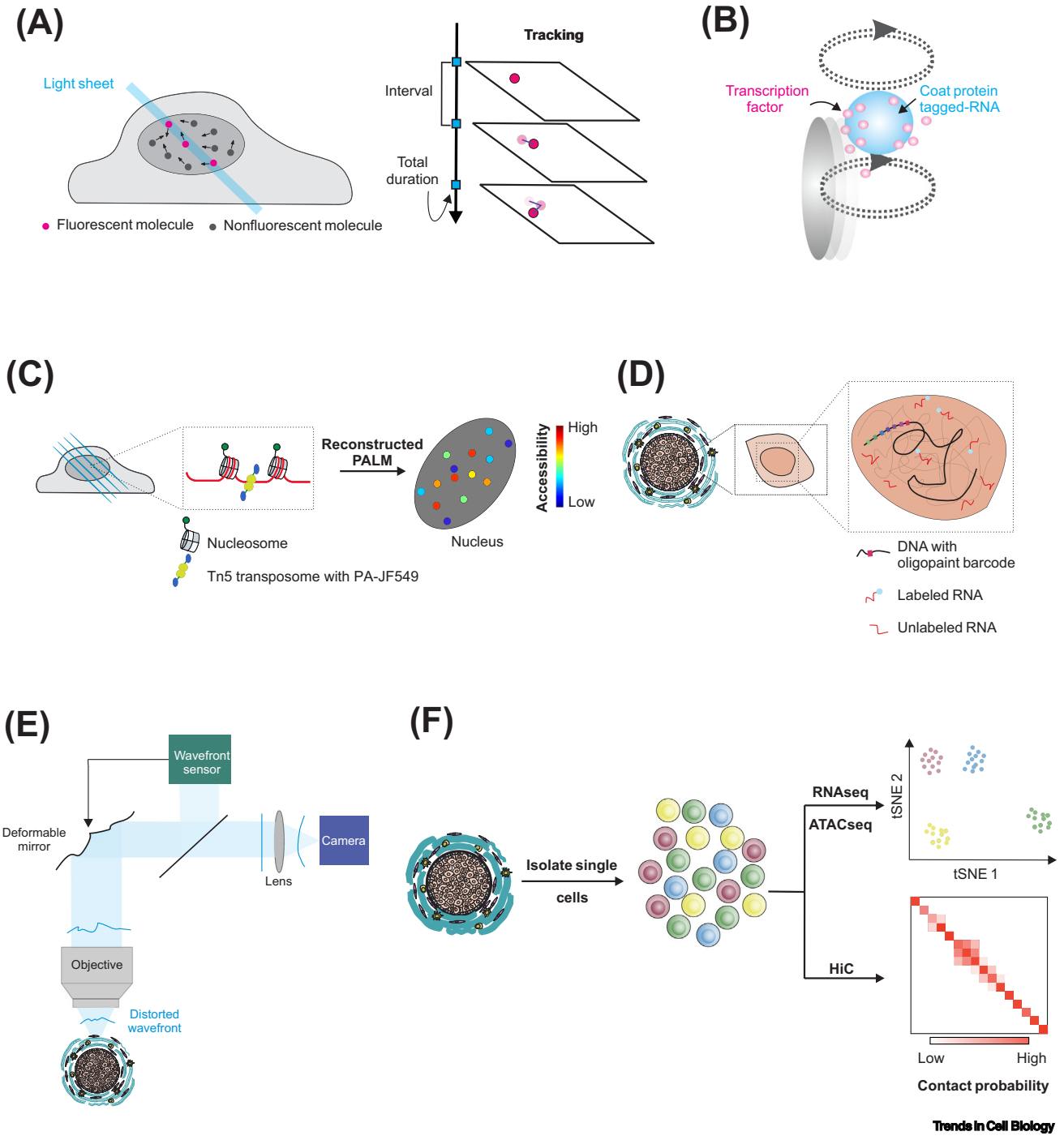

Figure 5. Key Technologies in Mechanobiology. (A) Single molecule tracking: schematic of a single molecule tracking experiment with a light sheet microscope. The thin light sheet illuminates a small section of the nucleus, reducing background signal and photobleaching. This allows tracking the dynamics of single molecules with high signal to noise ratio. (B) 3D orbital tracking: this technique 'locks-in' to fluorescently labeled targets (single molecules or organelles) using circular scanning rather than conventional raster scanning. The intensity of fluorescence along the orbit is used to localize the target and continuously recenter the orbit to the target position. Fluorescently labeled transcription factor molecules are shown in magenta and RNA labeled with a fluorescent coat protein in cyan. In this example, the laser illumination swept the transcription site four times in two z-planes. (C) 3D assay for transposase-accessible chromatin with photoactivated localization microscopy

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(Figure 4C) [96]. To navigate complex spatial geometries, cancer cells go through epithelial, mesenchymal, and amoeboid states as the disease progresses. Developing single-cell tools that incorporate genomics and live-cell imaging as cells in 3D culture undergo transformation and diverse modes of motility will provide mechanistic understanding of cancer from tumorigenesis to metastasis.

Key Technologies to Integrate Mechanical Force and Chromatin Organization in Live Cells

Mechanical cues affect gene expression at multiple scales, from cell to tissues. Many current assays rely on large numbers of cells, making it difficult to directly discern these responses at the single-cell level. Understanding how mechanical cues alter patterns of gene expression ('outside-in' transduction) and how in turn the rheological properties of cells influence surrounding tissue ('inside-out' transduction) requires methods to study transcription at the level of single cells while simultaneously manipulating their mechanical environment.

Light microscopy provides several avenues to study transcriptional kinetics over different time and length scales: single molecule tracking allows monitoring of transcription factor binding kinetics in individual cells (Figure 5A) [97,98]; orbital tracking enables studying the interactions between transcription factor binding and RNA production (Figure 5B) [99,100]; 3D-ATAC PALM combines assay for transposase-accessible chromatin (ATAC) with photoactivated localization microscopy (PALM) to provide super-resolution maps of accessible chromatin, which can be used to study interactions between transcriptional regulators and accessible regions of chromatin (Figure 5C) [101]; Hi-M allows for simultaneous observation of chromatin interactions and gene expression by multiplexed barcoding and sequential imaging using oligopaint technologies (Figure 5D) [102]; adaptive optics with lattice light sheet microscopy makes it possible to image subcellular dynamics *in vivo* at high spatiotemporal resolution (Figure 5E) [103].

It is well established that most genes are transcribed discontinuously and RNA is synthesized in transcriptional 'bursts' with gene-specific 'on' and 'off' periods of activity (reviewed in [104]). It was suggested that cytoskeletal activity may influence transcriptional coactivation (co-bursting) of glucocorticoid-regulated gene reporters located near the nuclear periphery [100]. However, the bursting behavior of core mechanosensitive genes and how bursting responds to mechanical stimuli is yet to be examined. Moreover, the dynamics of *bona fide* mechanoresponsive transcription factors such as YAP, TAZ, and SRF as they interact with nuclear partners, chromatin, and DNA regulatory sites are also largely unknown. Combining imaging of real-time gene expression or chromatin accessibility, using the above techniques, with physical measurements of cell and tissue mechanical properties in both *in vitro* and *in vivo* contexts holds considerable promise for enhancing our understanding of transcriptional regulation and mechanosensing.

Concluding Remarks and Future Perspectives

The mechanical environment and active physical stimuli are emerging as key regulators of transcription and gene expression in diverse physiological contexts. Although we have discussed

Outstanding Questions

What are the common as well as cell type/tissue-specific principles of mechanical regulation of transcription?

What are the molecular players involved in individual mechanotransduction pathways?

What are the mechanisms of mechanical force-induced changes in chromatin structure and function and how dynamic are these changes?

Does mechanical force act simply as a rheostat for genes that are already expressed, or can it also activate silenced genes?

Which 2D and 3D cellular models should be used to properly recapitulate the physiology of mechanotransduction?

Which technologies can unravel the spatiotemporal relationship between transcription and the physical microenvironment?

How can a systematic study of mechanotransduction improve our understanding of cellular responses in health and disease in order to devise better treatment strategies?

(ATAC-PALM): this technique combines single molecule localization with lattice light sheet microscopy for imaging and genomic analysis of accessible chromatin regions. The Tn5 transposome conjugated with photoactivatable dye (here JF_{549}) binds to regions of accessible chromatin. Reconstructed PALM images show regions of differential accessibility. (D) Hi-M-seq: libraries of barcodes targeted towards specific genomic regions are used to label sites in whole organisms, organoids, or single cells. RNA transcripts are hybridized with digoxigenin-labeled RNA probes. Sequential multiplexed imaging allows for simultaneous visualization of transcriptional state and genomic overlap. (E) Adaptive optics: while imaging thick samples (tissues, organoids, zebrafish embryos), scattering of fluorescent light causes distortion of the propagating wavefront, leading to optical aberrations. The adaptive optics setup uses a wavefront detector to control a deformable mirror, which corrects the distorted wavefronts to enable high resolution imaging of cellular and subcellular dynamics. (F) Single-cell genomics: single cells are isolated from a heterogenous 3D sample. RNA-seq, ATAC-seq, or Hi-C are performed at the single-cell level. Downstream data analysis reveals single-cell heterogeneity in gene expression and accessibility [t-distributed stochastic neighbor embedding (tSNE) plot, top], or a sample contact map for a single cell (bottom).

several molecular pathways involved in mechanosensing, it is important to note that they are not isolated but are in fact deeply interconnected. FAs and cell–cell junctions, for example, share several kinases and cytoskeletal linker proteins, which simultaneously transmit mechanical forces while regulating the activity of transcription factors (e.g., ERα, RARγ) and coregulators (e.g., FHL2, YAP/TAZ, MRTF-A). These, in turn, modulate the expression of ECM proteins and actin regulators, creating a feedback loop between the cell and its microenvironment.

Despite recent advances, there remains a pressing need for further studies on chromatin topology, nuclear architecture, transcription factor kinetics, and gene regulation in response to mechanical forces in tissues (see Outstanding Questions). Several novel methods that could aid this pursuit exist but have not been used to study mechanotransduction. Complementary to microscopy techniques, genomics provides tools to study transcription on the timescale of hours. Techniques such as ChIP-seq, Hi-C, and DNase I hypersensitive sites sequencing (DNase-seq) provide insight into the organization of chromatin but are limited to 2D culture systems requiring large numbers of cells. ATAC followed by sequencing (ATAC-seq), which allows the mapping of accessible gene regions with as few as 500 cells, is especially suitable for mechanobiology assays to examine chromatin architecture in 3D cultures and organoids [105]. In a clinical setting, an optimized ATAC-seq protocol can enhance understanding of metastatic tropism and drug resistance in cancer cells [106].

In the context of cancer, mutations within a small subpopulation of cells initiate tumorigenesis. To understand disease progression, it is essential to learn how these small numbers of cells alter their transcriptional programs and development of methods of data acquisition and analyses at single-cell resolution will prove invaluable [107]. Single-cell omics approaches such as single-cell RNA-seq [108], single-cell ATAC-seq [109], and single-cell Hi-C [110] (Figure 5F), along with bioinformatics analyses, can be used in conjunction with mechanobiology assays to uncover the spatiotemporal context of gene expression patterns [111,112]. Concurrently, live cell imaging of transcription kinetics at various stages of disease progression will provide a more complete view of mechanotransduction in a physiologically relevant setting.

Studies beginning with Mina Bissel's pioneering work on **dynamic reciprocity** [113] have delineated differences between 2D plastic cultures (the norm in many laboratories) and *in vivo* conditions. Most of the aforementioned techniques have been developed for and applied to *in vitro* cell culture systems. However, a need for their application to cells in clusters, tissues, organs, or even *in vivo*, to allow measurements of *in situ* forces and stresses, has emerged in recent years. These approaches provide a spatiotemporal interrogation of intracellular dynamics and rheology in response to mechanical stimuli. As technologies continue to evolve, it is imperative that we pivot our research programs to involve culture systems, microscopy, and genomics tools that could better represent human physiology. The heterogeneity of the physical environment in the human body is one of the major causative factors in dynamic biological events such as organogenesis, tissue homeostasis, and disease development. Elucidating the mechanical regulation of transcription is a first yet critical step to obtain a complete understanding of how cells respond to physical cues.

Acknowledgments

This work was supported (in part) by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research. A.U. acknowledges support from the National Cancer Institute-University of Maryland (NCI-UMD) Cancer Technology Partnership, and the awards NSF PHY 1607645, NSF PHY 1806903, NSF PHY 1915534.

Declaration of Interests

The authors declare no competing interests.

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