Cellular Mechanosensing of the Biophysical Microenvironment: A Review of Mathematical Models of Biophysical Regulation of Cell Responses

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Abstract: Cells *in vivo* reside within a complex microenvironment composed of both biochemical and biophysical cues. The dynamic feedback between cells and their microenvironments includes an important role for biophysical cues in regulating critical cellular behaviors. Understanding this regulation from sensing to reaction to feedback is therefore critical, and a large effort is afoot to identify and mathematically model the fundamental mechanobiological mechanisms underlying this regulation. This review provides a critical perspective on recent progress in mechanosensitive mathematical models, with a focus on different biophysical cues in cellular microenvironment, including dynamic strain, osmotic shock, fluid shear stress, mechanical forces, matrix rigidity, microchannel and matrix shapes. The review highlights key successes and failings of existing models, and discusses future opportunities and challenges in the field.

Keywords: Cellular mechanosensing, mathematical modeling, focal adhesion, stress fibers, signaling pathway, mechanobiology, biomechanics

1. Introduction

Cells *in vivo* are situated in a complex microenvironment that is rich in biochemical and biophysical cues [1-7]. The interactions between biochemical and biophysical cues are the focus of intense research [8-14], and new mathematical models are becoming available to predict how cells respond to the biophysical cues (*e.g.*, matrix rigidity, mechanical stretch and fluid shear stress) of their microenvironments. These factors have been shown to regulate critical cellular behaviors such as spreading, migration and differentiation that underlie important pathologies such as metastasis and fibrosis [15-25] **(Fig. 1)**. A great many of these models trace their genesis to efforts to explain the observation that the lineage of mesenchymal stem cells (MSCs) is strongly affected by the modulus of the substratum upon which they are cultured [26-29]. For example, matrices that mimic the compliance of brain or fat maximize neurogenesis or adipogenesis and matrices with rigidity like muscle or bone are optimum for the myogenesis or osteogenesis, respectively [26]. However, the molecular mechanisms remain elusive. The field has grown substantially to integrate a broad range of integrated intracellular protein structures and signaling systems. The goal of this review is to highlight some important breakthroughs and critically assess the capability of existing models to capture the breadth of mechanobiological responses known to govern the behavior of animal cells.

The focus of the review is how cellular mechanosensitivity arises from the range of biophysical sensing modes inside the cells. Protein structures at the interface of the cell and extracellular matrix (ECM), including those that comprise focal adhesions, are known to sense ECM rigidity and tension. For example, chemomechanical signal conversion at the cell-ECM interface can arise through force-induced conformational or organizational changes in proteins or structures near the transmembrane domains that can reveal cryptic domains and stabilize adhesions [17, 19, 30-34]. Stress-activated ion channels are known to sense membrane tension [35, 36]. Cytoskeletal elements connect to the LINC (linker of nucleoskeleton and cytoskeleton) complex and possibly enable mechanical forces to affect gene expression and

transcription directly via nuclear deformation [37, 38]. All of these result in signals transduced to activate intracellular signaling [39-42] and enable cells to respond to microenvironmental biophysical cues [43, 44]. As the mechanisms underlying functional relationships between biophysical microenvironment and cellular behaviors begin to be revealed themselves, a range of predictive models are becoming available.

Advances in biomaterials, especially hydrogels that mimic ECM and micro/nano technologies, have enabled a wealth of cellular mechanosensing phenomena to be characterized experimentally [3, 17, 45-48]. Many of these phenomena seem tuned by cells to enable distinct, cell type-specific behaviors. For example, dorsal root ganglion neurites show maximal outgrowth when cultured on substrata with a rigidity analogous to that of brain parenchyma, approximately 1 kPa [49]. Several sets of technologies have proven particularly informative for quantifying how cellular behaviors and their underlying molecular interactions depend upon the cell microenvironment. The first is two-dimensional substrata with defined mechanical properties [50-52]. The second is micropost arrays with tunable flexural and material rigidity [53]. The third is three-dimensional tissue constructs with defined ECMs [54, 55]. The fourth is the external loads applied to cells by micropipette, magnetic or optical tweezers and atomic force microscopy (AFM) [56-58]. These systems have been used to quantify behaviors at the whole cell level, such as traction force distribution, spreading area, migration rate, and force regulation [59-64]. However, integrated models are required for gaining insight into the molecular mechanisms of mechanosensing, and molecular probes (*e.g.*, fluorescence resonance energy transfer, FRET) are required to quantify protein conformation changes and probe receptor-ligand rupture events [65-67].

Despite the large number of experimental developments, many fundamental features of cellular mechanosensing are still not fully understood, including the complex relationship of matrix rigidity, integrin clustering and biochemical signal activation [68, 69]. Mathematical models have been central to reconciling different experimental

observations into simpler and universal sets of principles describing how different component processes cooperate to produce mechanosensing [70-73]. A key model highlighted in this review is the "molecular clutch model", which helped uncover talin-vinculin binding dynamics as pivotal to matrix rigidity sensing across cell types [10, 74, 75]. Emerging models seek to explain how cellular mechanosensing integrates not only interactions between biomolecules but also a dynamic interplay across wide temporal and spatial scales in the cell microenvironment [76-78]. Mathematical models based on the associated biochemical and biophysical processes are needed to further unravel the biophysics of cellular mechanosensing.

A wealth of experiments and mathematical models exist for this purpose, and a range of excellent reviews can be found of both experiments (synthesis and measurement) [37, 45] and mathematical models [44, 79, 80]. A critical review of mechanistic and kinetic mathematical models for cellular mechanosensing is still lacking, however, and this review aims to fill this void. The review categorizes models into different biophysical cues based on the current biotechnology methods, *i.e.*, dynamic strain, osmotic shock, fluid shear stress, external mechanical forces, matrix rigidity, microchannel and matrix shapes. We critique these modeling efforts, highlight their strengths and limitations, then conclude with a perspective on important open challenges to understanding how cell mechanosensing affects cell physiology.

2. Cellular mechanosensing in response to dynamic strain

Many types of cells are subjected to dynamic deformation in physiological processes and pathologies, such as cardiac myocytes in the heart beating, endothelial cells on the pulsating vessel wall and even cancer cells bearing a compression deformation in the tumor [81]. Numerous studies have been done to understand why and how cells could sense and respond to external dynamic strain, in hopes of finding potential ways to cure diseases associated with blood vessels and heart in the future.

2.1 Cellular reorientation and cytoskeleton remodelling

It has now been shown that cells could actively sense the dynamic strain of their environments, resulting in cytoskeleton remodelling, *i.e.*, cellular reorientation. For example, skeleton myocytes tend to align parallel to the stretching direction for a static or quasi-static stretch [82] **(Fig. 2A)**. Many tissue cells (*e.g.*, fibroblasts and endothelial cells) prefer to align perpendicular to the direction of applied cyclic strain, especially at high frequency (>1 Hz, note that the stretch is static or quasi-static at very low frequencies) and larger stretching magnitude (>5%) [83] **(Fig. 2B)**. Very recently, it has been shown that cyclic stretching reorients cells along two mirror-image angles [84] **(Fig. 2C)**. More interestingly, it has been shown that fibroblasts align themselves in the direction of applied stretch in 3D environments [85, 86]. Not only cell reorientation, transient stretch-compress maneuvers often cause cell fluidization, *i.e.*, disassembly of stress fibers (SFs), along the direction of deformation [87]. It also has been shown that signals (*e.g.*, Rho and JNK) have an important effect on cellular mechanosensing in response to dynamic strain. For example, inhibition of Rho will abolish the cell reorientation in response to cyclic stretch [88]. There are plenty of experimental observations and the underlying mechanism remains elusive. So a completed mathematical model will help us understand the cellular mechanosensing in response to dynamic strain **(Fig. 2D)**.

2.2 Chromatin condensation and remodeling

In addition to cellular reorientation, Mauck *et al.* found that nuclear configuration (*e.g.*, chromatin condensation and remodeling) is also regulated by dynamic stretch [89-93]. Recently, it has been found that ATP and Ca^{2+} will be released into cytoplasm through hemichannels (maybe membrane-tension-mediated ion channel) in respond to external mechanical force or dynamic stretch, and then ATP-dependent purinergic signals (*e.g.*, G protein coupled P2Y receptors) can reinforce the SFs in the cytoplasm (*e.g.*, the reorientation of SFs perpendicular to the stretch direction), finally leading to chromatin condensation. Transforming growth factor beta (TGF-β) also can enhance the mechanical dependent chromatin condensation, maybe through the interactions of TGF-β/Smad signaling and Rho signaling which can enhance the cell traction. More

interestingly, it takes 10-20 sec to release the ATP while 10 min to change the chromatin structure with a 1 Hz dynamic stretch, suggesting that mechanical cues may be firstly converted into chemical signals (*e.g.*, ATP, Ca^{2+} and Rho) at cell-ECM surface other than directly transmitted into nucleus in this process. However, it has also been found that mechanical forces transmitted directly through the actin cytoskeleton from cell-ECM surface to the nuclear envelope are also important for cellular mechanosensing [92]. But now, there is still rarely mathematical model in this fresh field.

2.3 The mathematical models

2.3.1 One-dimensional stress fiber model

Qian *et al.* proposed a viscoelastic-sarcomere-adhesion (VISA) model to describe the cell reorientation in response to cyclically stretched substrate [94]. The model mainly includes four parts: substrate stiffening, adhesion bond dynamics, stress fiber (SF) assemble/disassemble dynamics, and cell rotational diffusion. The rigidity of matrix usually increases with cyclic stretching, *i.e.*, strain stiffening. Therefore, the effective stiffness of adhesion bond (adhesion molecule-fibronectin-substrate bond) increases with increasing stretching amplitude, in turn, resulting in a decrease in adhesion bond dissociation rate. Then, the adhesion bond dynamics is connected to SF dynamics by a couple of first order kinetic equations implying that the density of SFs is proportional to the adhesion bond density:

$$\frac{dc_a}{dt} = k_+(c_0 - c_a) - k_- c_a \tag{1}$$

$$\frac{\mathrm{d}c_f}{\mathrm{dt}} = k_+^f c_a - k_-^f c_f \tag{2}$$

where k_+ (s^{-1}) and k_- (s^{-1}) are on and off-rates of receptor-ligand bonds; c_a is the bond density; k_+^f (s^{-1}) and k_-^f (s^{-1}) are the assembly and disassembly rates of SFs; c_f is the density of SFs. Finally, the stretching amplitude and frequency are sensed by SFs (SFs are constituted by a parallel configuration of viscoelastic and contraction elements; note that some experiments support that a tandem configuration [95]) and transmitted to adhesion plaque, in turn, influencing the adhesion bond dissociation

rate. When cells cannot develop the stable adhesions and SFs, they will undergo the rotational diffusion to explore new orientations until stable adhesions and SF structures are formed. This integrated mechanochemical model could explain a broad range of experimental observations of cell reorientation on cyclic stretch substrate, such as cells tend to align perpendicular to the stretching direction at a high cyclic stretch frequency (>1Hz) and stretching magnitude (5%~6%). Besides, Chen *et al.* also proposed an elastic-sarcomere-adhesion (ELSA) model (SFs are constituted by linear elastic sarcomeres), which suggested that catch bonds in adhesions and two intrinsic clocks of the stress fiber, *i.e.*, localized activation (homogeneous activation) of sarcomere units in the SFs at low (high) stretching frequency, play an important role in cell reorientation induced by cyclic stretch [96].

But above models still cannot explain some experimental observations, *e.g.*, the cell fluidization mechanism (the disassemble of the cytoskeleton) [87], chemical molecules (such as Rho pathway) activation [88], inhomogeneous SFs contraction [97]. Recently, Wu *et al.* proposed a Kelvin-Voight-myosin (KVM) model, which couples assemble-disassemble of myosin motors with the viscoelastic Kelvin-Voigt element (single stress fiber) [98]. Their model successfully explains that tension-regulated myosin detachment is the main reason for cell fluidization in response to transient-compress other than compress-transient dynamic strain.

2.3.2 Two-dimensional stress fiber model

In addition to one-dimensional (1D) SF models (VISA, ELSA and KVM), a two-dimensional SF-networks (2D-SN) model based on constrained mixture theory has been proposed by Kaunas and colleagues to study the SF reorganization in response to the cyclic uniaxial stretch [99-102]. The model concludes that SFs tend to dissociate in the direction of stretch and reach a stable configuration in the direction of lowest stretch at high frequencies. The cyclic stretches are translated to the deformation gradient (α_i) of SFs using finite elasticity theory (*i.e.*, a cyclic stretch with special magnitude and frequency can be approximated by a series of incremental

stretches). As a result, matrix strains will change the distance between focal adhesions and the lengths of the associated SFs. Thus, the disassembly probability of a single SF (depending on the difference between current and initial deformation) is described as

$$P = \left(k_0 + k_1 \left(\frac{\alpha_i - \alpha_0}{\alpha_0} \right)^2 \right) \quad (3)$$

where k_0 (s^{-1}) is the intrinsical stress fiber dissociation rate without tension; k_1 (s^{-1}) is the SF dissociation rate with fiber prestretch; α_i and α_0 are the current and initial levels of stretch of the SFs. Interestingly, SFs would only dissociate during the compression other than stretching phase of the cyclic strain in KVM model as described in section 2.3.1. Recently, a multiscale mechano-chemical (MMC) model is proposed by Ji and colleagues, which suggests that a biphasic relationship between cell reorientation time and stretching frequency may be caused by the competition between instability of adhesions and reassembly of SFs [103]. In order to solve the same question, an elastic force-dipoles (EFD) model (note that this is 1D SF model) is proposed by Safran and colleagues, which considers that cells would prefer to maintain constant local stress and strain in contact with their surrounding matrix by adjusting a force dipoles which characterize the contraction force of cells [104-106].

The SF networks are also modeled as the discrete rod structures which are more consistent with their intrinsical states *in vivo*. For example, a two-dimensional SF-network (2D-SN) model based on coarse grained Monte Carlo approach has been proposed by Puskar and colleagues [107]. Similarly, a cytoskeletal tensegrity system (CTS) model is proposed, which consists of four struts (representing the longitudinal SFs and lateral actin network) and eight strings (denoting the microfilaments) [108]. Their study concludes that the lateral struts (actin network) play a vital role in regulating the cellular orientation.

2.3.3 Rho-regulated mechanochemical stress fiber model

The stress fibers are usually regulated not only by mechanical forces but also by chemical signals, such as Rho and ROCK. Therefore, the mathematical models which

could integrate both mechanical factors and chemical factors are still needed. A Rho-regulated mechanochemical (RMC) model was proposed by Schwarz and colleagues for inhomogeneous stress fiber contraction [109]. In their model, mechanical forces could trigger Rho signals (*e.g.*, Rho/ROCK/MLCP/myosin), leading to adhesion reinforcement and increasing contraction force in SFs (interestingly, other studies suggest that forces caused by mechanical stretch would reduce the stability of adhesion clusters [110]). The mechanosensing process (*e.g.*, the conversion of mechanical force into biochemical signals at focal adhesions) is treated as an enzyme in the framework of Michaelis-Menten kinetics:

$$\frac{\partial ROCK(t)}{\partial t} = \frac{r_1 F_b(t)(ROCK_{tot} - ROCK(t))}{K_1 + (ROCK_{tot} - ROCK(t))} - \frac{V_{-1} ROCK(t)}{K_{-1} + ROCK(t)} \quad (4)$$

where $F_b(t)$ (pN) is the mechanical force applied on focal adhesion depending on SF deformation; $ROCK_{tot}$ (nM) and $ROCK(t)$ (nM) are the concentrations of total and active ROCK; K_1 (nM) is the Michaelis-Menten constant. The second term accounts for the degradation of active ROCK with maximum velocity V_{-1} (nM s^{-1}) and Michaelis-Menten constant K_{-1} (nM). The mechanical forces acted on focal adhesions lead to a position-dependent feedback loop for adhesion maturation. The myosin motors and biochemical signals are described by a system of reaction-diffusion equations and myosin-filaments are modeled as a viscoelastic contractile actin bundle (a serious of viscoelastic-contraction elements). With this model, it was found that the contraction force of SFs displays spatial gradients corresponding to the deformation pattern of SFs, *i.e.*, upon stimulation of contraction, only the sarcomeres in the cell edge shorten while those in the center elongate.

It has also been shown that the activity of JNK can be upregulated by reassembly of SFs (uniaxial stretch induces the transient activation of JNK by formation of new adhesion bonds) [111]. A JNK-regulated mechanochemical (JMC) model is proposed by Kaunas and colleagues. In their model, the JNK activation rate is expressed by the first-order kinetics,

$$\frac{dC_{PJNK}}{dt} = k_1 \left(\sum \frac{d\varphi}{dt} + u \right) C_{JNK} - k_2 C_{PJNK} \quad (5)$$

where k_1 (s^{-1}) and k_2 (s^{-1}) are the rates for activation and deactivation of JNK; C_{PJNK} (mol) and C_{JNK} (mol) are the concentrations of the activation and deactivation forms of JNK, respectively; u (s^{-1}) is the formation rate of integrin bond independent of dynamics of SFs; $\sum \frac{d\varphi}{dt}$ is the formation rate of integrin bond because of assembly of new stress fibers (this key value is calculated from the matrix stretch patterns by 2D-SN model). Thus, matrix stretch and chemical factors are exquisitely incorporated into the SF network dynamics which make cells sense and adapt to their cyclic stretch matrix.

2.3.4 Minimum energy principle stress fiber model

Another mathematical model based on the minimum energy principle (MEP) is proposed to understand the cell reorientation in response to dynamic stretch [112, 113]. Realignment of SFs in the cells can be explained by the follow factors (steps) in this model: 1) normal substrate strain could be converted into the deformation of individual SF; 2) individual SF has a basal-strain-energy (BSE) in its initial state without stretching; 3) individual SF would disassemble when their strain energy are zero or larger than the twice of BSE. Thus, the model suggests that SFs prefer to orient in the direction where their basal-strain-energy is minimally changed. But caution is used here, the model mainly bases on the elasticity of SFs without a time dependent change. So the steady state distribution of SFs other than time-dependent cell reorientation process can be got from the model. Recently, an improved 2D SF-actin network-FA (SAF) model based on MEP is proposed to understand that why and how cells reorient themselves along two mirror-image angles. This improved model can explain the temporal evolution of SFs in response to various of cyclic frequency [84].

3. Cellular mechanosensing in response to osmotic shock

Cells can sense the osmotic shock (*e.g.*, cells are subjected to a hypotonic shock and then a hypertonic shock) by activation of mechanosensitive ion channels, which are

regulated by the mechanical force balance at the cell membrane-cortex surface, resulting in homeostatic values of cell volume and membrane tension (**Fig. 3E-F**) [71]. To understand the underlying mechanism, a corresponding chemomechanical model including the Rho signaling pathway which is activated by mechanosensitive ion channels was proposed by Tao & Sun [71]. There are two main assumptions in this model. The first assumption is that the model considers a spherical (*e.g.*, a suspended cell) or cylindrical cell (*e.g.*, a cell between plat cantilevers) to keep the cell geometry simple and remove complexities from cell adhesion, *i.e.*, no adhesions or fixed adhesion area. The second assumption is that the actomyosin cortical layer coupled to membrane is modeled as an active viscoelastic gel-like fluid. The ion channel chemomechanical model mainly contains three parts: force balance at the cell edge, membrane tension mediated myosin activation, and cell volume change that depends on local traction force. Force balance at the cell surface, *i.e.*, the osmotic pressure difference, is balanced by tension in the membrane and mechanical stress in the cortex;

$$\Delta P\, R/2 \;= A + \sigma h \tag{6}$$

where ΔP (Pa) is the hydrostatic pressure difference, R (nm) is the radius of a spherical cell, A (pN) is the tension in the membrane, σ is the active stress tensor, and h (nm) is the cortical layer thickness. An increase in membrane tension and mechanical stress in the cortex leads to an increase in myosin contraction tension via the Rho signals. Most importantly, the authors use a function $\Lambda(A)$ to model the Rho activation probability that depends nonlinearly on membrane tension, A. The functional form of Λ is essentially the same as a Michaelis-Menten type of enzymatic kinetics. Later, the concentration of myosin can be calculated as,

$$\frac{d\rho}{dt} = a_1\Lambda(T)(1-\rho) - d_1\rho \tag{7}$$

$$\frac{dM}{dt} = a_2(1-M)\rho - d_2 M \tag{8}$$

$$\sigma = K_{max}M \tag{9}$$

where ρ and M are percentages of activated Rho and myosin, respectively; a_1 (s^{-1}) and d_1 (s^{-1}) are activation and deactivation rates of Rho, respectively; a_2 (s^{-1}) and d_2 (s^{-1})

are the myosin assembly and disassembly rates of myosin, respectively; K_{max} (nN/μm) is the maximum contractile stress. Finally, in addition to active regulation of myosin contraction, cells can also adjust its internal osmotic pressure (mainly by regulation of water and ion fluxes), leading to cell-volume change,

$$\frac{dn}{dt} = A(J_1 + J_2) \tag{10}$$

$$\frac{dV}{dt} = -\alpha A(\Delta P - \Delta \Pi) \tag{11}$$

where $\Delta \Pi$ (Pa) is the osmotic pressure difference; n (mol) is the total osmolytes in the cell; V (μm^3) and A (μm^2) are cell volume and surface area, respectively; J_1 (mol/m^{2}s) is the ion flux out of the cell through passive membrane channels; J_2 (mol/m^{2}s) is the ion flux through active ion pumps. The mechanosensitive ion channel model successfully explains the mechanism of cell mechanosensing to the osmotic shock for a variety of environmental perturbations. Most importantly, the Rho signal is integrated into a typical volume-osmotic mechanical model for the first time, which is the key step for the cell to maintain a homeostatic volume and membrane tension.

4. Cellular mechanosensing in response to shear stress

The endothelial cells are subjected to two kinds of mechanical cues in their surrounding microenvironment in the complex vascular system, including the shear stress caused by the blood and the cyclic stretch caused by the cardiac cycle [114]. The shear force can influence the signals activation, cytoskeleton realignment and gene expression. For example, cells prefer to align SFs along the direction of the fluid flow [114]. One of the advantages of SFs realignment is that it reduces the mechanical load applied to cells. A Rho GTPases signal-SF-adhesion coupling model was proposed to study the cellular mechanosensing in response to fluid shear stress [115]. The model assumes that actin cytoskeleton network inside the cell could transfer the shear stress to cell-adhesions on the bottom surface of the cell, leading to changes in adhesion dynamics. Subsequently, the concentration of Rho is transient decreasing, that results in disassembly in SFs. Because of decreasing traction force, there are more focal complexes other than focal adhesions, *i.e.*, the maturation of FAs is

inhibited. The increasing number of focal complexes could enhance the concentration of Rac, which in turn promotes the focal complexes assembly in a polarized manner, such as the downstream edge of the cell along the fluid flow. When the concentration of Rho increases back its baseline value, the SFs would assemble in new polarized location and then promote the maturation of focal complexes, thus successfully leading to a reorientation of SFs in response to shear force. In the model, SFs exhibit a rich dynamic behavior, including nucleating, shortening, merging, splitting and disappearing. Unfortunately, fluid shear stress is not explicitly introduced into their model as an input. Equivalently, they use a transient decrease of the Rho concentration to represent shear stress loading on the cell. Therefore, an actual and precise model which considers the interplay of fluid and actin cytoskeleton is still urgently needed.

5. Cellular mechanosensing in response to external forces

It has been shown that cells could sense and respond to external loads by reorientation of actin cytoskeleton and dynamics of focal adhesion [17]. There are many different ways to apply the external loads to cells, such as micropipette, magnetic or optical tweezers and atomic force microscopy (AFM). For now, several studies have experimentally and theoretically shown that distinct external loads can lead to different cellular mechanosensitive behaviors.

5.1 Multiscale cytoskeleton-myosin-membrane (MCMM) model

Although many studies have been placed on cell-adhesion complexes as the main mechanosensors, the mechanical cues can also be transmitted by the cortical cytoskeleton, a mechanosensitive system containing myosin II, actin cross-linkers and actin filaments. For example, myosin and α-actinin accumulation will enhance in the pipette tip while filamin accumulation will enhance in the neck region during micropipette aspiration [116] **(Fig. 3D)**. Robinson and colleagues proposed a multiscale cytoskeleton-myosin-membrane (MCMM) model deciphering the cortical cytoskeleton mechanosensing mechanism from molecular to cellular scales [116, 117].

Just like integrin-Fn bond, the dynamics of myosin-actin bond can also be described by catch-bond model, leading to the mechanosensitive accumulation (mechanical-stress-dependent) of myosin II (the effective off-rate of myosin motor from the actin filaments deceases with increasing applied force on myosin) [118],

$$k_{off} = k_{off}^{0} e^{\frac{-fx}{k_B T}} \tag{12}$$

where the k_{off} (s^{-1}) and k_{off}^{0} (s^{-1}) are the off-rate in the presence and absence of force; f (pN) is the mechanical force acting on myosin; x (nm) is the bond length. Besides myosin II as the force generator and actin cross-linker, other proteins such as α-actinin and filamin can also act as an important force transmitter from cell membrane to the actin cortex. Robinson and colleagues later suggested that myosin and α-actinin are sensitive to dilation stress (*i.e.*, assembly rates of the bipolar thick filament and α-actinin dimer can be changed with parallel actin filaments sliding during dilation) and filamin is sensitive to shear stress (*i.e.*, the off-rates for filamin from actin filaments can be changed with the angle between actin filaments during shear deformation) through a multi-scale simulation, including coarse-grained molecular dynamics simulation, force-dependent reaction-diffusion dynamics and viscoelastic model for the mechanical properties of actin cytoskeleton-membrane complexes.

5.2 Cable network model and tensegrity model

A two-dimensional cable network model has been proposed by Schwarz and colleagues to study the effect of an altered stress propagation inside the cell on the spatial distribution of focal adhesions [119] **(Fig. 3C)**. Such altered intracellular stress is provided by a shifted laterally microfabricated pillar contacted with actin cytoskeleton inside the adherent fibroblasts. The actin cytoskeleton is modeled as a 2D elastic cable network, which could be constituted by three different topologies respectively, *i.e.*, regular triangles, reinforced squares and random network topology. In cable network model, the cable (actin cytoskeleton) is stretchable like a linear spring, but does not show any mechanical resistance for compression. The cable

network is fixed by adhesion points that are immobile at its rim. The model finally shows that the size of focal adhesions in the front (back) of the pillar is decreasing (increasing), which is well consistent with experimental data. Recently, a multi-structural 3D finite element (FE) model based on tensegrity was proposed by Lacroix and colleagues to study the role of mechanical properties of the cytoskeleton (including actin cortex, stress fibers and microtubule) and interactions between cytoskeleton network, cell-adhesions and cytoplasm in cell response under external compressive loads by AFM [120-122] **(Fig. 3B)**. Recently, Zeng *et al.* proposed a 3D random network model of the actin cytoskeleton to study the nucleus deformation under micropipette pulling [123] **(Fig. 3A)**. This model assumes that nucleus deformation is mainly caused by actin cytoskeleton network that acts as a stress-transmission medium from the cell membrane directly to the nuclear membrane (mechanical stress will quickly dissipate when transmission into a viscous or viscoelastic cytoplasm). The 3D random network model concludes that nucleus deformation and displacements inside the cytosol increase with increasing concentration of actin filaments and then reach a maximal value at an optimal concentration of actin-binding proteins.

6. Cellular mechanosensing in response to matrix rigidity

Recently, it has been shown that matrix rigidity plays an important role in cell migration, shape and differentiation [45]. For example, fibroblasts display a cellular behavior known as “durotaxis”, *i.e.*, preferentially migration toward stiff substrate [124]. Neurons have more neurites on the soft substrate [49]. The MSC differentiation also can be determined by the matrix rigidity [26]. Next, the cellular mechanosensing components from cell-ECM interfaces to cell nuclear are described.

6.1 Cell-adhesion dynamics at cell-ECM interface

An effective cellular mechanosensitive system firstly needs mechanical sensing modes at the cell-ECM interface (*e.g.*, cell-adhesions) that could transform microenvironmental mechanical properties (*e.g.*, elasticity and viscoelasticity) to

intracellular signals [33]. The cell-adhesion sites are a group of highly dynamic structures which can directly connect extracellular matrix to intracellular components (*e.g.*, actin cytoskeleton). The cell-adhesions mainly have two roles in cellular mechanosensing: stress (strain) propagation and chemical signals activation. Cells sense the stress (strain) of the external matrix by forming a dynamic mechanical bond system (*e.g.*, slip/catch bond, sliding-rebinding/allosteric catch bond) involving hundreds of known adhesion proteins, such as integrin, talin and vinculin [125]. It has been shown that talin is a "force buffer", which means that all VBSs (vinculin binding sites) in talin can be unfolded within a relatively small force (5 ~ 10 pN), playing an important role of rigidity sensing under physiological conditions *in vivo* [126]. More interestingly, cells exhibit distinct behaviors for rigidity sensing because of the slight difference of talin unfolding threshold between talin isoforms (talin 1 and talin 2) [127]. Despite the complexity of focal adhesion components and dynamics, much of the mechanosensitivity of focal adhesions has been well described by the mathematical models based upon the 'molecular clutch' hypothesis of Mitchison and Kirschner [128], which postulates that engagement of a molecular clutch, now recognized to be the elements of focal adhesions [129], enables transmission of forces from the actin cytoskeleton to the ECM. This engagement reduces the retrograde flow rate of actin filaments, and downregulates both protrusion of the leading edge and activation of adhesion-mediated downstream signals.

Recently, a novel hypothesis, *i.e.*, integrin clusters are critical functional module for matrix rigidity sensing, is proposed according to experimental observations [30]. Sheetz *et al.* show that cell-adhesions are loose aggregates containing tight clusters of integrins with size of ~100 nm composed of ~20-50 molecules [130]. Interestingly, it has been concluded that clustered, but not individually distributed of plasma membrane proteins (*e.g.*, Ras nano-clusters) could recruit and activate their downstream signals (probably because of a higher local concentration of reactant, *i.e.*, higher probability of molecular collision) [131]. Thus, a single integrin cluster may act as a platform where chemical signals (*e.g.*, phosphatase and kinase) are activated

sequentially. Actually, activation of FAK (*e.g.*, phosphorylation of focal adhesion kinase on Y397) depends on integrin clustering and only happens inside the integrin clusters [132, 133]. Thus, a focal adhesion including many integrin clusters can be regarded as a highly dynamic mechanosensitive system immediately in response to altering of matrix rigidity. In spite of the large number of experimental findings, the steps and mechanisms of adhesion-dependent mechanosensing remain elusive. The computational models have been developed to simulate the protein clustering on the two-dimensional plane [134]. These models assume that protein clustering is mainly influenced by 1) confined diffusion resulting from special membrane structure, such as lipid raft or cortical cytoskeleton [135, 136], 2) and enhanced activation rate of integrin by talin [137]. However, the effects of real structure of integrin on the integrin clustering are still unclear. Besides, it is still unclear that whether assembly-disassembly of integrin clusters is influenced by matrix rigidity, and if so, are there some quantitative features of matrix rigidity that are related to integrin organization or intracellular signal activity.

6.2 Myosin-filament system in the cytoplasm

Mechanical cues at cell-ECM interface sensed by cells have to be transduced to the nucleus, a few microns away from sensing sites, to regulate gene expression and protein translation through mechanosensors in the cytoplasm [37]. There are two important components in the cytoplasm that respond to the mechanical cues at cell-ECM interface, one is myosin-filament system (a structural path) and the other is mechanical-regulated bio-signal network (a soluble path). The myosin-filament system can be classified two categories: actomyosin-based sarcomere-like contractile units (CUs) at the cell edge and stress fibers (SFs) which directly connect cellular membrane to cell nuclear membrane. The CUs test the matrix rigidity by pulling a couple of integrin clusters and reinforcing the links between actin cytoskeleton and integrin clusters by adding vinculin or α-actinin [30]. The SFs test the matrix rigidity by forming a special structure called “actin cap” in cells which are cultured on stiff substrate. The actin cap is formed above the nucleus which is in turn compressed by

tensed actin cap, leading to nuclear deformation and then chromatin compaction [138]. The arrangement of SFs at the bottom of the cells also depends on the matrix rigidity, for example, SFs display a random arrangement on soft substrate, but form an aligned arrangement on stiff substrate [72]. In contrast to myosin-filament system, mechanics-regulated bio-signal network mainly focuses on some key biomolecules which could respond to external mechanical cues, such as FAK, Src and Rho (e.g., FAK, Src and Rho activation rates are enhanced on stiff substrate) [139]. The corresponding downstream signaling events are then produced sequentially (e.g., FAK-RhoA-ROCK cascade), which also possibly crosstalk with other signaling pathways (e.g., TGFβ cascade and Hippo cascade), regulate nuclear events [43]. There are several differences between these mechanotransduction, one is signaling directionality, i.e., biochemical signals in network dynamic model depend on signals diffusion while mechanical signals in myosin-filaments system depend on physical displacement. Another difference is the relationship between signaling strength and transmission distance, where the strength of biochemical signals decreases with distance at a rate of $1/\text{distance}^2$, while mechanical signals do not lose their intensity with distance which depends on the mechanical properties of the transmitting substance.

6.3 Matrix rigidity sensing by nuclear lamin-A

Recently, it has been found that nuclear structures, such as nuclear lamina, are mechanosensitive [11, 140] **(Fig. 6A, C)**. The level of nuclear lamin-A follows the power-law scaling versus matrix rigidity and the rates of phosphorylation (turnover) of lamin-A are inversely related with matrix rigidity [141]. From the viewpoint of dynamics, matrix rigidity promotes myosin-mediated cellular contraction formation and therefore enhances tension in nuclear lamin-A, thus inhibiting lamin-A disassociation and then forming a stiff nucleus. The level and conformation of nuclear lamin-A also regulate the location of proteins involved in gene expression (*e.g.*, nucleocytoplasmic shuttling of RARG and YAP) and thus lamin-A provides a potential mechano-chemical mechanism to explain the dependence of stem cell

differentiation on matrix with different rigidity. However, some questions still need to be addressed. For example, why and how tension could inhibit lamin-A degradation and exact rate constant should be experimentally measured with mechanical perturbations in future.

6.4 Matrix-rigidity-dependent intracellular signaling pathways

It has been demonstrated that matrix rigidity can significantly influence the cellular differentiation, though the mechanism of this mechanosensitive process remains elusive [26] **(Fig. 5A)**. Although matrix-rigidity-mediated cellular differentiation is a complex and multi-scale process, several studies focused on the mechanosensitive role of nucleocytoplasmic shuttling behaviors of transcriptional regulators (*e.g.*, YAP/TAZ, MAL/MRTF). For instance, increasing matrix rigidity or exerting a static stretch has been found to promote YAP/TAZ or MAL/MRTF nuclear translocation and its downstream transcription activity [142]. More importantly, it has been shown that actin cytoskeleton remodeling also plays an important role in this mechanosensing process. For example, the entry of MAL/MRTF into nucleus to interact with SRF transcription factor is regulated by the ratio of G-actin and F-actin, because G-actin could bind to MAL/MRTF to prevent it from binding to SRF [143]. Although YAP/TAZ has similar behavior to MAL/MRTF (*e.g.*, more YAP/TAZ in nucleus on stiffer substrate), their nucleocytoplasmic shuttling is regulated by a distinct way that appears to link to cellular contraction caused by SFs (*i.e.*, cell traction force) [144] **(Fig. 5C)**. Very recently, some studies aim to investigate the effect of crosstalk between chemical signaling pathways regulated by chemical factors (*e.g.*, growth factor, TGFβ) and mechanotransduction pathways regulated by matrix rigidity on cellular differentiation [145]. Although the details about individual pathway are well known, little is known about their interactions. Elucidating the mechanisms of conversion of mechanical signals to biochemical signals via adhesion molecules is a fundamental question of mechanobiology and offers many opportunities to understand the underlying mechanism by building mathematical models.

6.5 Mathematical models

6.5.1 Uniaxial molecular clutch model

The uniaxial molecular clutch model based upon the molecular clutch hypothesis is first proposed by Chan & Odde [146-148] **(Fig. 4D)**. In this model, stretchable adhesion proteins and a deformable substrate are modeled as a spring system loaded by mechanical traction from the flow of actin filaments. Three mechanical equations describe the system mathematically, accounting for equilibrium of contraction of the actin cytoskeleton (actomyosin sliding), stretching of the focal adhesion proteins, and deformation of the substrate. These relate to the effective dissociation rates of the mechanical bonds (*i.e.*, the weakest links in an adhesion) between the actin cytoskeleton and the ECM molecules, which are modeled by the Bell model:

$$k_{off} = k_{off}^{0}\, e^{(F/F_b)} \tag{13}$$

where k_{off} (s^{-1}) is the effective dissociation rate of the weakest bond in the cell-ECM interaction (*e.g.*, an integrin-fibronectin bond or an actin-talin bond, see reference [125] for more details), k_{off}^{0} (s^{-1}) is the intrinsic dissociation rate, F (pN) is the tension on the mechanical bond, and F_b (pN) is the characteristic bond breakage force. The contractile force originating from elongation of adhesion proteins and the substrate is represented by Hooke's Law:

$$F = k\,\delta x \tag{14}$$

where k (pN/nm) is the stiffness of adhesion proteins or substrate and δx (nm) is the elongation of adhesion proteins or substrate. A linear force-velocity relationship is used to relate the traction force and the actin flow rate:

$$v = v_0\left(1 - \frac{F}{F_{stall}}\right) \tag{15}$$

where v (nm/s) and v_0 (nm/s) are the effective actin flow rate and its force-free value, respectively, and F_{stall} (pN) is the stall force of myosin motors.

The Chan-Odde model predicts a relationship between actin flow rate and matrix rigidity that is biphasic, consistent with experimental observations in filopodia in

neuronal growth cones [146] **(Fig. 4A)**. The model shows two regimes, one is "frictional slippage" with low traction force on stiff substrate and another is "load-and -fail" with higher traction force on the soft substrate. However, the model predictions are not consistent with observations of monotonic actin flow/matrix rigidity relationship in fibroblasts [10] **(Fig. 4B-C)**. To address this, the Bangasser-Odde group proposed the concept of ''optimum stiffness,'' where the actin cytoskeleton exhibits a minimal value in retrograde flow rate [147]. The Bangasser-Odde motor-clutch model suggests that a monotonic relationship between retrograde flow and stiffness would appear for experimentally accessible values mainly because of the shift of optimal stiffness, but the relationship essentially remains a biphasic curve over a wide range of stiffnesses. Recently, Roca-Cusachs and colleagues incorporated the molecular behaviors of talin and vinculin into molecular clutch model, which succeeded predicting how integrin-mediated actin flow relates to matrix rigidity in fibroblasts [10]. In their improved molecular clutch model, talin (acting as a clutch) can directly bind with actin and integrin to mediate force transmission between the actin cytoskeleton and ECM. Importantly, tension in talin can expose vinculin binding sites and lead to vinculin binding, thus resulting in adhesion reinforcement. Besides talin and vinculin dynamics, distinct bond dynamics of different integrin types have an important effect on the substrate stiffness/actin flow relationship in breast myoepithelial cells [25]. For example, $\alpha_v\beta_6$ (expressed in cancer cells) integrin has higher affinity with fibronectin than $\alpha_5\beta_1$ (constitutively expressed). Therefore, a molecular clutch model with force-dependent integrin recruitment predicts an additional third reinforcement regime, *i.e.*, the traction force will increase with substrate stiffness even on stiff substrate.

These two sets of models were unified under a single set of governing principles by Xu and co-workers in an integrated molecular clutch model that accounted for (1) the kinetics of adaptor proteins (*e.g.*, the talin exchange rate) and (2) the fact that "weakest link" in the dynamics of the adhesions can shift under certain circumstances from the actin-integrin bond to the integrin-fibronectin bond [125]. These two factors

are known to play an important role in adhesion dynamics and mechanosensing [149]. The integrated molecular clutch model predicts that both shift of weakest link location and the development of integrin clustering affect the actin flow/matrix rigidity relationship, cell spreading, and migration, and provide a mechanism by which different types of cells can differ in their mechanosensing. Later, Mooney and co-workers proposed a viscoelastic molecular clutch model which predicts that cells spreading area on soft substrate with stress relaxation has a similar value as cells on stiffer substrate [61]. This study obtains an important conclusion that both substrate stress relaxation and substrate stiffness can influence the cell behaviors (such as cell spreading area, traction force or location of YAP). Interestingly, Gardel *et al*. also found that the spatial distribution of retrograde flow rate and traction force is biphasic other than monotonic at the cell leading edge on the certain stiffness substrate [150] (later, an adhesion clutch model was proposed by Mogilner and co-workers to solve this problem [151]), which suggests that there are still many mechanisms of cellular mechanosensing that need to be further explored through both experimental and modeling approaches.

6.5.2 Two-dimensional molecular mechanical (TDMM) model

The molecular clutch model predicts cellular rigidity-sensing due to (1) a cell adhesion layer that is more easily deformed and strengthened on a stiff substrate, and (2) concentric actin flow that is inhibited by such adhesion. However, some experimental observations cannot be explained by the molecular clutch model, including substrate rigidity-mediated anisotropic growth of focal adhesions [15] and lateral interconnection and clustering of adhesion proteins [76]. To solve these issues, a two-dimensional molecular mechanical (TDMM) model was proposed by Walcott & Sun for describing rigidity-sensitive adhesion nucleation, growth and decay [152] **(Fig. 4E)**. In the TDMM model, integrin molecules are placed in a two-dimensional cell membrane plane. Single integrin can connect to four neighboring integrin molecules by the actin cytoskeleton. Below this plane, each adhesion site is attached to a substratum that is represented by a linear spring of stiffness k (pN/nm), and above

this plane, a force F_{app} can be applied. A typical simulation begins with such a force applied to a single integrin to initiate adhesion growth. The model predicts that, although substrate rigidity influences whether an adhesion is initially formed, the adhesion lifetime is independent of substrate rigidity. To make the model mechanosensitive to matrix rigidity, two important factors as adhesion molecular force-dependent state transition and strain-dependent binding to matrix are incorporated into the TDMM model.

The TDMM model idealizes an adhesion site as being comprised of adhesion molecules that shift between a resting "circle" state and a stretched "elliptical" state when loaded with sufficient force. The kinetics is approximated using a Bell model:

$$k_{CE} = k_{CE}^0 \exp\left(\frac{-kh(4z-3h)}{2k_BT} + i(G_{cc}-G_c) + j(G_{ce}-G_e)\right) \tag{16}$$

where k_{CE} (s^{-1}) is the circle-to-ellipse transition rate constant; h (nm) is the extension of the circle associated with the transition to an ellipse; k (pN/nm) is the effective stiffness of a linkage to the substratum; k_BT (pN·nm) is the product of temperature and Boltzmann's constant; i is the number of circular state neighbors that a bond has; j is the number of elliptical state neighbors that a bond has; G_c (k_BT) and G_e (k_BT) are the normalized free energies of the ellipse and circle bonds of the transition state, respectively; G_{cc} (k_BT) and G_{ce} (k_BT) are the free energy of circle-circle binding and circle-ellipse binding, respectively. Note that k_{CE}^0 (s^{-1}) is the reaction rate in a reference state when $z = 3h/4$ and the molecule has no neighbors.

Another important factor, strain-dependent binding, is described using Kramers's theory:

$$k_{on} = k_{on}^0 \exp\left(-\frac{kz^2}{2k_BT}\right)\left(1 + 2\frac{wL}{z^2}\left(1 - \sqrt{1+\frac{z^2}{L^2}}\right)\right) \tag{17}$$

where k_{on} and k_{on}^0 (s^{-1}) are the effective and baseline binding rates of the circle state, w (nm) is the width of binding sites, and L (nm) is the offset of binding sites. This

equation indicates that varying the ECM rigidity will change the probability of forming molecular-ECM bonds. More compliant ECM undergoes larger deformation and results in greater equilibrium distances between adhesion molecules and ECM, which downregulates bond formation; stiffer ECM undergoes smaller deformation and results in shorter equilibrium distances, which upregulates bond formation. Thus, matrix rigidity could upregulate the adhesion growth by strain-dependent bond binding and stress-dependent bond unbinding.

6.5.3 Linear elastic chain adhesion (LECA) model

Although TDMM model can explain the relationship between the focal adhesion size or number and matrix rigidity well, anisotropic growth and shrinkage of focal adhesions in the direction of cell contraction still cannot be explained by the TDMM model or molecular clutch model [153, 154]. The one-dimensional linear elastic chain adhesion (LECA) model proposed by Nicolas and Safran identifies the physics underlying this behavior [155]. In their model, local contraction force originating from stress fibers will deform the adhesion layer leading to compression at the leading edge and expansion at the trailing edge. Note that adhesions are modeled as thin films whose stress-induce deformation is similar to an infinite, thin plate that can be solved by continuum elasticity theory. The different strain in the single focal adhesion, in turn, leads to different local biochemical signaling that will influence the local adhesion protein density. The variant adhesion protein density leads to structural changes that produce the directional, anisotropic growth of adhesions. Adhesion proteins (thin films), for simplicity, which are modeled as the chains of particles connected by linear elastic springs, can sense the elastic properties of the ECM through an interaction represented by a sinusoidal potential:

$$\mathrm{V(x)} = k_m \frac{a^2}{2\pi^2 \cos\left(2\pi x/a\right)} \tag{18}$$

where k_m (pN/nm) is the stiffness of the substrate; a (nm) is the equilibrium distance between the particles, and x (nm) is the current distance between the particles. The particle-spring system finally reaches mechanical equilibrium pulled by the

contraction force. The LECA model not only accounts for the anisotropic growth and shrinkage of focal adhesions in the direction of force, but also concludes that adhesions only grow within a range of force. Interestingly, in LECA model, adhesions are pulled by a shear force which is ignored in the TDMM model. Therefore, the direction of traction force should play a key role in adhesion-mediated mechanosensing. Recently, some studies show that talin orients at ~15° relative to the plasma membrane [78]. This conclusion may constitute the theoretical basis for future mathematical models. Later, an improved two-layer adhesion model also proposed by the Safran group concludes that adhesions will finally reach a finite size which is proportional to the matrix rigidity [156, 157].

6.5.4 Stochastic-elasticity (StoE) model

Recently, a stochastic-elasticity (StoE) model is proposed by Gao and colleagues to describe the dynamics of adhesion clusters between substrate and cell (both as the elastic media) which are subjected to a perpendicular/inclined tensile load [158, 159]. In StoE model, stochastic dynamic simulation of molecular bonds and continuum elastic theory of traction distribution on the surface are integrated into a single modeling framework. A scaling law of traction distribution within the adhesion domains calculated from classical elastic equations in contact mechanics shows that stress at the adhesion edge would increases with the increasing adhesion size, bond density and bond stiffness but deceases with the increasing substrate rigidity and cell rigidity (*e.g.*, cytoskeleton stiffening). According to this scaling law, bonds near the adhesion edge are subjected to a larger force, resulting in a larger dissociation rate as described by Bell model [160]. Also, bonds rebinding after rupture are less near the adhesion edge, according to

$$k_{on} = k_{on}^{0} \frac{l_{bind}}{Z} exp\left(-\frac{k_{LR}\delta^2}{2k_B T}\right) \tag{19}$$

where $k_B T$ (~4.1 pN·nm at physiological temperature) is the thermal energy; k_{on} (s^{-1}) is intrinsical on-rate when the receptor-ligand pairs are within a binding radius l_{bind} (nm), Z is the partition function for a receptor confined in a harmonic potential

between zero and δ. Therefore, the rupture of adhesion clusters is mainly caused by increasing stress near the adhesion edge with increasing adhesion size. Most importantly, increasing matrix rigidity can alleviate the stress concentration near the adhesion edge, thus leading to the stable adhesion domains. According to the above analysis, the StoE model successfully explains why the size of stable adhesion lies from a few hundred nanometers to a few microns.

6.5.5 Adhesion clustering model

As described in section 6.1, integrin clusters play an important role in cellular mechanosensing. Peng *et al.* developed a matrix-rigidity-dependent adhesion clustering (nucleation) model based on Monte Carlo simulation, in which three chemical reactions (*e.g.*, activation/deactivation of integrin, integrin-substrate binding and cross-linking between integrin) are analyzed in the model [161]. Interestingly, the integrin is activated by thermal undulations of local plasma membrane but not by other chemical factors (*e.g.*, Mn^{2+}, talin or ECM). The thermal undulations of local plasma membrane could reduce the matrix-rigidity-dependent activation energy barrier of integrin, which help cells to sense the matrix rigidity (just like the mechanism that cells test the substrate rigidity by pulling the integrin-ECM links using the intracellular traction). The matrix-rigidity-dependent mechanical energy barrier can be denoted as:

$$E_m = \frac{f_b^2}{2}\left(\frac{l_e - l_b}{f_b} + \frac{1 - v^2}{2Ea}\right) \tag{20}$$

where f_b (pN) is the thermal fluctuation force to activate the integrin; l_e (nm) and l_b (nm) are the length of bent/extended integrin, respectively; E (kPa) and v are the Young's modulus and Poisson's ratio; a (nm) is the radius of a single integrin. The model suggests that increasing matrix rigidity and integrin density both can increase the integrin cluster size. In addition to thermal undulations of plasma membrane, mechanical resistance caused by the glycocalyx between membrane and matrix also is an important factor to induce the integrin clustering, which has been confirmed by experimental observations and spatial-temporal lattice spring (STLS) model [162,

163]. Recently, a diffusion-dependent stochastic-elastic (StoE) model of molecular bond clustering between two elastic media is proposed by He and colleagues, which also suggests that matrix rigidity can promote the formation of stable bond clusters [164]. A matrix-adhesion-actomyosin-nucleus (MAAN) model is recently proposed by Shenoy and colleagues, which suggests that a stiffer substrate or nucleus could also promote the adhesion clustering [165].

6.5.6 Multi-scale stress fiber model

A mechanical model based on adhesion complexes, myosin II, actin filaments and substrate was proposed by Sun and colleagues [72]. The rigidity-sensing mechanism in their model is determined by the interaction of three mechanical elements, *i.e.*, adhesion complexes, myosin and actin filaments. Adhesion complexes provide a matrix surface rigidity-dependent viscous drag which increases with increasing matrix rigidity, suggesting that adhesion complexes will move faster and bear less force on soft than on stiff matrix. Then, the drag force can be written as:

$$F_{ac} = N_{ac} k \frac{2\pi RE}{2\pi RE + 3k} \frac{k_a}{k_d^0} \left(\frac{1}{k_d^0 + k_a} \right) v \tag{21}$$

where N_{ac} is the number of sliding molecules; $k\frac{2\pi RE}{2\pi RE+3k}$ (pN/nm) is the series protein stiffness of link between the matrix and actin cytoskeleton, R (nm^2) is the adhesion area; E (pN/nm) is the matrix rigidity; k (pN/nm) is the protein stiffness of adhesion and matrix surface; k_a (s^{-1}) is the overall attachment rate; k_d^0 (s^{-1}) is the off-rate at zero strain; v (nm/s) is the sliding rate. Myosin motors provide a steady-state force which obeys the Hill force-velocity relationship, suggesting that myosins generate smaller force when adhesion complexes slide fast. Hill model can be written as:

$$\frac{F_{myo}}{F_0} = \frac{v_0 - v}{v_0 + cv} \tag{22}$$

where F_{myo} (pN) is the myosin stall force; F_0 (pN) is the drag force experienced by myosin; v_0 (nm/s) and v (nm/s) are the sliding rates of actin-filament without and with drag force, respectively. Actin filaments in their model are modeled as rigid rods and are anchored (connected) by crosslink protein α-actinin (rigid nodes). As force is

applied, the actin-filament network is assumed to be in mechanical equilibrium. Single actin-filament experiences three different types of forces/torques and the equations as follows:

$$\sum_j F_{ij} + F_{ext}^i = 0 \tag{23}$$

$$\sum_j T_g^{ij} = 0 \tag{24}$$

where F_{ext}^i (pN) is the contraction force acted on actin-filament *i*; F_{ij} (pN) and T_g^{ij} (pN·nm) are the friction and torque about the center of mass applied on filament *i* by relative sliding of filament j, respectively. The model concludes that a random actin-filament network would bundle together and orient along force direction (contraction force of SFs) on stiff matrix, and such restructure of actin-filament network is an effective cellular mechanosensing mechanism. In addition to the orderly arranged SFs, adhesion complexes and myosin motors also display a mechanosensing behavior. For instance, adhesion complexes slide faster and myosin generates a smaller force on softer substrate. Thus they provide a simple, physically based model of cellular mechanosensing, which offers a different and novel view to traditional biochemical models. In addition to formation of SFs, the alignment of SFs on varying stiffness substrate (the polarization of SFs, *i.e.*, SFs prefer align to along the long axis of cells) is still unclear. Recently, Zemel *et al.* propose a force-dipoles (FD) model to solve this question, which suggests that such anisotropic alignment of SFs is dependent non-monotonically on matrix rigidity [166]. These findings provide a large amount of useful insight into the stress-fiber-based cellular mechanosensing to matrix rigidity.

6.5.7 Nucleus polarization and alignment model

Recently, a multiple-cells model was proposed to study the mechanism of the alignment and polarization of nucleus in response to matrix rigidity [167, 168]. The simulations based on this model show that matrix rigidity has significant impact on the magnitude and distribution of the in-plane stresses (cell-cell interaction). Structurally, nucleus envelope is mechanically integrated with the cytoskeleton

through lamin network, while the other end of cytoskeleton is linked to the ECM or neighboring cells through adhesion molecules. Hence, the nucleus is exposed to the forces transmitted from extracellular microenvironment via cytoskeleton. It has been demonstrated that cells in patterned cell monolayer reorient and polarize along the direction of the maximum principal stress, accompanied by the reorientation of actin cytoskeleton. The actin cytoskeleton is believed to provide structural support and geometric shape to cells, and it mainly sustains tensile stress, but hardly bears shear stress. The shear stress in cell will thus induce reorientation of cytoskeleton to align with the direction of principal stress where the shear stress is equal to zero. The mechanical stresses within the cytoskeleton will then be propagated to the nucleus, resulting in the reorientation and polarization of nucleus in the direction of the maximum principal stress. Thus the in-plane stresses are the driving force of the collective behaviors of cells and their subcellular structures, the change of matrix rigidity will finally influence the nucleus behaviors. Overall, this model offers a good platform to understand and predict various patterns of polarization and alignment of cell and nucleus for the process of tissue morphogenesis.

6.5.8 Gene circuit model

It has been shown that cells have more stress fibers formed on stiff matrix, which lead to an increasing tension in nuclear lamina. The high tension in the nuclear lamina could suppress the affinity of enzyme initiating phosphorylation and degradation of lamin-A filaments, leading to a high level of lamin-A and thus a stiffer nucleus. To investigate the effect of matrix rigidity on nuclear structural changes (*e.g.*, lamin-A concentration), a gene circuit model was proposed by Discher and colleagues [169] **(Fig. 6D)**. The gene circuit model contains a series of typical rate equations including synthesis (β_i) and turnover (α_i) rates of myosin II and filamin-A,

$$\frac{dm}{dt} = \beta_1 M - a_1 \left(\frac{m^{nx}}{K_m + m^{nx}} \right) \tag{25}$$

$$\frac{dl}{dt} = \beta_2 L - a_2 \left(\frac{l^{ny}}{K_l + l^{ny}} \right) \tag{26}$$

where m and M are levels of myosin and its mRNA (MYHN9) respectively; l and L

are levels of lamin-A and its mRNA (LMNA) respectively; β_1 (s^{-1}) and β_2 (s^{-1}) are first-order mRNA translational rates; a_1 (s^{-1}) and a_2 (s^{-1}) are maximal protein degradation rates. The novelty of this model is that cell and matrix mechanics are incorporated into reaction rate constant. For example, protein turnover rates of myosin II (K_m) and lamin-A (K_l) are coupled to matrix rigidity and myosin-generated stress, respectively, by log-linear functions usually obtained by fitting the experimental data. These phenomenological log-linear functions simulate the processes of increasing cytosolic tension on stiff matrix and then inhibiting lamin-A degradation under high cytosolic tension. The overall rate of degradation is generally represented by Michaelis-Menten kinetics. Two relationships between myosin II (M) and myosin structural gene (m) and between lamin-A (L) and lamin structural gene (l) could be obtained by solving the traditional rate equations. The model successfully explains the changes in collagen and cardiac myosin expression in a developing embryonic chick heart. The gene circuit model thus could predict tissue-level and long-time behaviors of biological mechanosensing behaviors **(Fig. 6B)**. However, whether tension could inhibit lamin-A degradation and exact rate constant are still unclear.

6.5.9 Signaling pathway model of cellular differentiation

Many mathematical models involving mechanochemical conversion have been developed, which help us to understand the interactions of various factors influencing actin cytoskeleton remodeling and transcription factor nucleocytoplasmic shuttling in a quantitative way. An integrated signaling pathway dynamic model has been proposed by Zaman and colleagues, which includes substrate rigidity, YAP/MAL dynamics, and actin cytoskeleton remodeling [170]. The model not only reproduces the existing experimental results (*e.g.*, regulation of location of YAP by tensioned cytosolic F-actin or SFs), but also provides a number of new predictions (*e.g.*, synergistic effect between chemo/mechanotransduction by multilevel crosstalk among YAP/TAZ and Hippo signaling pathway networks). The inputs of the model are the ECM rigidity and concentrations of LATS, while the output is the nuclear translocation of the relevant transcriptional molecule (YAP/TAZ). Most importantly,

the authors used a second-order Hill function to describe the relationship of FAK activity and ECM rigidity **(Fig. 5E)**. YAP/TAZ nuclear translocation has been shown to be influenced by SFs and RhoA as following,

$$\frac{d[YAP]}{dt} = \left(k_{cn} + k_{cy}[Fcyto][myo]\right)([YAP_{tot}] - [YAP]) - k_{nc}[YAP] \quad (27)$$

where $[YAP]$, $[YAP_{tot}]$, $[Fcyto]$ and $[myo]$ are concentrations of YAP in nucleus, YAP in nucleus and cytoplasmic, tensional F-actin and activity myosin, respectively; k_{cn} (s^{-1}) is the rate of YAP translocating from the cytoplasm to the nucleus with no active cytoplasmic F-actin and myosin; k_{cy} (s^{-1}) is the rate of YAP nuclear translocation rate due to the SF or the tensional cytoplasmic F-actin; k_{nc} (s^{-1}) is the rate of YAP translocation from nuclear to cytoplasm. It should be noted that the tensional cytoplasmic F-actin filaments are characterized by the product of the cytoplasmic F-actin concentration and active myosin concentration, *i.e.*, $[Fcyto]\times[myo]$. Although there is no apparent SF structure in 3D culture [171], more F-actin can still be found in the cell in rigid ECM and thus tensional cytoplasmic F-actin filament is a well equivalent component of SF structure [172]. MAL in the cytoplasm has been shown to bind with G-actin, which inhibits its ability to go to the nucleus and is associated with SRF to form the active SRF/MAL complex,

$$\frac{d[MAL]}{dt} = \left(\frac{k_{cnm}}{1 + k_{mg}[Gactin]^2}([MAL_{tot}] - [MAL]) - k_{ncm}[MAL]\right) \quad (28)$$

where $[MAL]$, $[MAL_{tot}]$ and $[Gactin]$ are the concentrations of MAL in nucleus, total MAL in cell and G-actin, respectively; k_{cnm} (s^{-1}) is the rate of MAL translocation from the cytoskeleton to the nucleus; k_{mg} (s^{-1}) is the decreasing effect due to cytoplasm MAL binding with G-actin and retained in the cytoplasm (interestingly, this inhibition effect is described as a second-order hill function); k_{ncm} (s^{-1}) is the rate of MAL trans-locating from the nucleus to the cytoplasm. Overall, their model provides a platform to study cell-ECM and cell-cell interactions, including the conversion of mechanical cues to biochemical signals and crosstalk among various signaling pathway networks, such as mechanical-cues-dependent YAP/TAZ signals and chemical-cues-dependent LATS and Smad signals [142].

6.5.10 Signaling pathway model of cellular microstructure remodeling

Matrix rigidity not only influences cellular differentiation but also cell microstructure. A mathematical model of circular dorsal ruffles (CDRs) (*i.e.*, actin-rich ringlike structures that form on the dorsal surface of growth factor stimulated cells) was proposed by Chiam and colleagues, which suggests that the lifetime and size of CDRs depend on matrix rigidity via Rac-Rho antagonism [14] **(Fig. 5D)**. This model has two inputs, *i.e.*, matrix rigidity that is expressed by an increasing concentration of activated FAK (a log-linear function of matrix rigidity and FAK concentration) and PDGF stimulation that activates Arp2/3 through activation of Rac to form CDRs. These chemo/mechanotransduction signaling networks can be written in the form of mass action and Michaelis-Menten kinetics to form a set of coupled ordinary differential equation,

$$\frac{d[CDRactin]}{dt} = k_{ra}([Arp] + 1)[Gactin] - k_{dep}[CDRactin] \tag{29}$$

where [*CDRactin*] and [*Arp*] are the concentrations of CDR-actin and Arp2/3 complex; k_{ra} (s^{-1}) is the rate of CDR-actin formation without Arp2/3; k_{dep} (s^{-1}) is the rate of CDR-actin dissociation. The mathematical model also contains a set of coupled partial differential equations which represent the diffusion of various proteins between different simulation compartments (*e.g.*, extracellular, membrane and cytoplasm).

6.5.11 Signaling pathway model of fibrosis

It has been shown that the conversion of fibroblast into myofibroblast is also regulated by matrix rigidity **(Fig. 5B)**. An ODE-based model was developed to analyze the mechanisms of mechanical-regulation of αSMA production [73]. The model has two inputs, *i.e.*, growth factor signals (*e.g.*, TGF-β and FGF) that activate the downstream signals (*e.g.*, p38 and ERK) and matrix rigidity that is incorporated into the model with the level of intracellular kinases (*e.g.*, Src and FAK) via a scaling factor proportional to the log of matrix rigidity. The output is αSMA production,

$$\frac{d[\alpha SMA]}{dt} = k\frac{[pp38]}{[pERK]} \tag{30}$$

where [*αSMA*], [*pp38*] and [*pERK*] are the concentrations of αSMA, pp38 and pERK,

respectively; k (s^{-1}) is the rate of pp38 promotion of αSMA. This model suggests that αSMA production is enhanced by p38 and Src while inhibited by ERK. Modeling biological behaviors is challenging because of numerous interactions between different types of proteins and the difficulty to get quantitative data of intracellular chemical reaction kinetics. Computational models of mechanical-cue-related signaling networks have been used in many biological systems to clarify complex interactions, especially when intracellular protein activation states are difficult to quantify.

7. Cellular mechanosensing in response to various micropatterned geometries and microchannel

It has been shown that cell migration on 2D substrate is influenced by ligand density and integrin-fibronectin binding affinity (*e.g.*, a biphasic relationship between cell migration rate and fibronectin density, that is, cell migration rate will reach a maximum value at a particular fibronectin density) [173] **(Fig. 7A)**. Interestingly, there is also a biphasic relationship between cell migration rate and channel's sectional area in cell migration experiments through 3D microfluidic platform [174] **(Fig. 7B)**. Recently, cell spreading experiments on various micropatterned geometries (e.g., disk, pacman and crossbow shapes) show that strong traction force will appear at the corners of the patterns [175] **(Fig. 7C)**.

7.1 A three-dimension dynamic modeling of cell migration and spreading

Recently, a 3D integrated cell migration model is proposed by Asada and colleagues to study the various cellular mechanosensing behaviors (e.g., cell migration) in response to different micropatterned geometries and microchannel [176, 177] **(Fig. 7D)**. The cell plasma membrane and nuclear membrane are modeled as the two-layer elastic mesh structure, which can be connected by the SFs. Integrin clusters are placed at the nodes on the membrane mesh which can associate or disassociate with ligands on the substrate according to:

$$P_{on/off} = 1 - exp\,(-k_{on/off}\Delta t) \tag{31}$$

where $P_{on/off}$ is the binding/unbinding probabilities of integrin-FN bonds within a time interval Δt; $k_{on/off}$ (s^{-1}) is the association/dissociation rate. The nodes on the plasma membrane and nucleus membrane are described by Lagrangain equations:

$$m_i \frac{dv_i}{dt} = F_{D,i} + F_{FA,i} + F_{E,i} + F_{SF,i} + F_{L,i} \tag{32}$$

$$\frac{dx_i}{dt} = v_i \tag{33}$$

where v_i (nm/s) is the velocity vector of the i-th node; $F_{D,i}$, $F_{FA,i}$, $F_{E,i}$, $F_{SF,i}$ and $F_{L,i}$ (pN) are frictional dissipative force, adhesion force, elastic energy force, SF force and lamellipodium force, respectively; x_i (nm) is the coordinate of the i-th node. Above equations are solved numerically using a fourth order Rosenbrock method to determine the cell morphology and location. The relationships between cell migration rate and fibronectin density/channel's sectional area produced by the mathematical model are qualitatively consistent with experimental observations. This model provides a powerful platform to simulate dynamic process (e.g., cell migration, cell spreading and cancer cell metastasis) inside not only 2D but also 3D microenvironment, such as cylindrical lumens in the 3D ECM.

7.2 Cellular Potts Model (CPM)

A cellular Potts model based on simple tension-elasticity elements is proposed to study the dynamics of cell shape and traction on the various micro-patterned matrixes [178-180] **(Fig. 7D)**. Cells which are modeled as a collection of spins can be arbitrary shapes. Each spin configuration has an energy function which depends on the traction force in the cell (such traction force is balanced by the adhesion force). Evolution of cell shape and traction force can use Metropolis dynamics based on the energy minimization principle by assuming that cell spreading is a quasi-static process. During each time interval in the Metropolis algorithm, a lattice which sites at the edge of the cell is randomly chose and inverted. Then, the inversion is accepted with the probability $e^{\Delta H/k_B T}$, in which ΔH represents the energy difference between current state and inverted state,

$$\Delta H = H_{invert} - H_{current} \tag{34}$$

$$H = \sigma A + \gamma l + \sum_{arc\ i} \frac{EA}{2L_{0,i}} \left(L_i - L_{i,0}\right)^2 - \frac{E_0}{A_{ref} + A_{ad}} A_{ad} \tag{35}$$

where H_{invert} and $H_{current}$ are the energy functions after and before inversion, respectively; σA represents the surface tension (A represents the cell area); γl represents the line tension (l represents the cell perimeter); the third term represents the cell traction from concave actin fibers at cell edge; the last term represents the increasing energy by adding more adhesion sites. Thus, the evolution of cell shape with time can be obtained from the above methods. In a word, the CPM is a very useful platform to predict the cell shapes on the various micro-patterns.

7.3 Bio-chemo-mechanical model

In addition to CPM, Deshpande *et al*. proposed a bio-chemo-mechanical model to explain the cell contraction on the 2D micropatterned matrices [181-183]. Several phenomenological equations are incorporated into their continuum model to describe the following biochemical processes: 1) the formation of SFs is triggered by activation signals C (such as the concentration of Ca^{2+}),

$$C(t_i) = exp\left(\frac{-t_i}{\theta}\right) \tag{36}$$

where t_i is the time since the initially activation of Ca^{2+} signals and θ is the decay constant of the signal; 2) signal-dependent assembly and tension-dependent disassembly of SFs are followed by the first-order kinetic equation,

$$\frac{d\eta(\varphi)}{dt} = \left[(1 - \eta(\varphi))\frac{C(t)\bar{k}_f}{\theta}\right] - \left[(1 - \frac{\sigma(\varphi)}{\sigma_0(\varphi)})\eta(\varphi)\frac{\bar{k}_b}{\theta}\right] \tag{37}$$

where $\bar{k}_f$ and $\bar{k}_b$ are assembly and disassembly rate of SFs, respectively; $\sigma(\varphi)$ is the tension in SFs along the direction of φ; 3) contraction in SFs is generated between the actin and myosin through the cross-bridge dynamics (Hill equation describing the dynamics of muscle contraction). The bio-chemo-mechanical model can successfully predict that: 1) cell contraction increases with increasing matrix rigidity; 2) the boundary and cell shapes have an important influence on the anisotropic development of cell structure; 3) the concentration of SFs is high at the

focal adhesion. This model for the first time provides an integrated mechanochemical description of the cell spreading and contraction on 2D various micropatterned matrices.

7.4 The free-energy-based chemo-mechanical coupling model

Recently, Shenoy *et al.* also proposed a free-energy-based chemo-mechanical coupling model to explain why cells prefer to migrate towards stiffer matrix (*i.e.*, durotaxis) and display a rigidity-dependent cell contraction from the perspective of thermodynamics [184]. In this model, the second law of thermodynamics is used to describe the decreasing total free energy of cell:

$$dU_{mech} + dU_{chem} + dU_{moto} \leq 0 \tag{38}$$

where dU_{mech} and dU_{chem} are the changes of mechanical energy and chemical energy, respectively; dU_{moto} is the mechanical work done by myosin motors. When the cell is subjected to the external tension, several tension-dependent signaling pathways are activated (*e.g.*, Rho-cascade and Ca-cascade), which will increase the binding rate of myosin motors. Finally, more engaged myosin motors lead to an increase in cell contraction (*i.e.*, cytoskeleton tension), in turn, increasing the external tension. Most importantly, U_{chem} is related to the external tension and engaged myosin density, that is, an increasing external tension will cause a decrease of chemical free energy. Thus, the chemical and mechanical processes are integrated into a unified framework. According to this model, the strain and contraction distribution calculated from the model are consistent with a series of experiments, such as cells on 2D microposts or substrate and 3D matrices. Interestingly, the model also suggests that the chemical (mechanical) free energy of the cell decreases (increases) with increasing stiffness substrate, but the total free energy will decrease with increasing substrate stiffness. This provides an explanation of cell durotaxis from the perspective of thermodynamics.

Very recently, a mathematical model to describe the cellular nuclear morphology and stresses during cell transendothelial migration in microfluidic channel was also

proposed by Shenoy *et al.* [185]. Interestingly, this model can predict the effect of large and small constriction during cell transmigration on the nuclear envelope and chromatin deformation, which provides a very useful platform to understand the cell behaviors in 3D ECM, particularly cell differentiation influenced by the nuclear and chromatin shape. Although many cell behaviors (*e.g.*, migration, spreading) can be predicted by the above mechanical models, some more complex behaviors (*e.g.*, differentiation) cannot be captured by the mechanical model. Therefore, a more completed model involving interaction of mechanical cues and chemical cues is still needed to understand the mechanisms of interaction between cell and its complex mechanical environment.

8. Outlook and conclusions

Cells are complex living systems with behaviors integrated from the molecular (*e.g.*, bond dynamics in adhesions) to the subcellular (*e.g.*, arrangements of SFs) to the cellular level (*e.g.*, differentiation). Therefore, cellular mechanosensing is a multiscale process across wide temporal and spatial scales. Understanding of this is full of challenges and opportunities. For typical cellular mechanosensing modes (*e.g.*, cell-adhesions, myosin-actin filaments, and nuclear envelope), there exist various kinds of interactions between numerous proteins. In this article, we reviewed existing cellular mechanosensing models from the cell-ECM interactions to mechanotransduction in the cytoplasm and finally to nuclear responses. Many mathematical models have been established for each length scale, and successfully explained existing experimental observations. Although models have been established and used widely, we identified the following key issues that need to be addressed in this research field:

1) How to build a generic mathematical model to explain the phenomena that occur in different cells? Different cell types (also normal *vs*. diseased cells) or the same cell type in different microenvironments (*e.g.*, 2D *vs.* 3D) can display significantly different cellular mechanosensing behaviors [140, 186-191]. In view of the

complexity of both mechanical and biochemical signals controlling cellular mechanosensing, it is difficult to address this question through experiment alone. Manipulation of one particular component of one mechanosensing process may change the behavior of that process alone, but it may also affect several other related processes, leading to different behaviors. As a result of this complexity, more and more experimental studies have produced apparently contradicting conclusions [10, 146]. For example, the relationships between matrix rigidity and actin flow are distinct in different cell types, *e.g.*, biphasic relationship in neuron and monotonic relationship in fibroblast [125]. Recently, it has been shown that normal fibroblasts align to specific angles in response to 3D compression while cancer-associated fibroblasts show a random distribution [113]. Another studies show that fibroblasts align themselves along the stretch direction in 3D, while perpendicular to the stretch direction in 2D [85, 192]. So, is there a universal mathematical model to explain these distinct observations (such as, a universal molecular clutch model to explain the relationship between actin flow and matrix rigidity)?

2) How to build an integrated mathematical model to explain the multi-scale cellular mechanosensing? The events involved in cellular mechanosensing often occur at disparate timescales. Mechanosensitive ion channels can be activated on timescales of 0.1 s [193]; the tension-dependent association and dissociation of adhesion proteins usually happen within seconds [132, 194-197]; the turnover of mature adhesion happens within minutes [198]; viscoelastic relaxation of the ECM can last minutes to hours [199-201]; gene regulation and protein translation in cellular differentiation can last for few hours [202] or days [203]. Molecular and subcellular models like the molecular clutch model explain cell-adhesions dynamics in mechanosensing over seconds to hours. Cell and tissue models (*e.g.*, signaling network models) explain macroscopic cellular mechanosensing (*e.g.*, cell differentiation) over several days to weeks. Thus, it is important to provide a multi-scale mathematical model to quantitatively and qualitatively integrate the contributions to cellular mechanosensing across scales. A long term goal is to relate signaling network model inputs (*e.g.*,

functional relationships between signals such as FAK or Rho) and matrix rigidity to molecular models.

3) How are mechanical cues converted into chemical signals at cell-ECM interactions? Mechanical force and matrix rigidity can directly influence nuclear shape and gene location, but whether they can affect cell behaviors remains elusive [38, 204, 205]. Another pathway is conversion of mechanical cues into biochemical signals that sequentially activate intracellular signaling pathways to influence cell behaviors. Another interesting and important question to be solved is how a mechanical force or matrix rigidity translates into such signals. Cells sense force and matrix rigidity via specific sensors, likely amongst the hundreds of known adhesion proteins [206]. Adhesion proteins including Src, FAK and Rho could activate downstream signal pathways in a way that depends upon matrix rigidity. A hypothesis proposed by the Sheetz's group is that integrin or cadherin clusters are micro-platforms for biochemical reactions [30]. Dependence of cluster lifetime upon external mechanical cues offers a possible pathway for mechanochemical signal conversion [30].

A complete and precise mathematical model could help unravel these intrinsic and internal relationships among cell mechanosensing mechanisms. As explorations of the 3D cell microenvironment progress [6, 207], modeling will continue to play a central role in explaining experimental findings in terms of fundamental governing principles.

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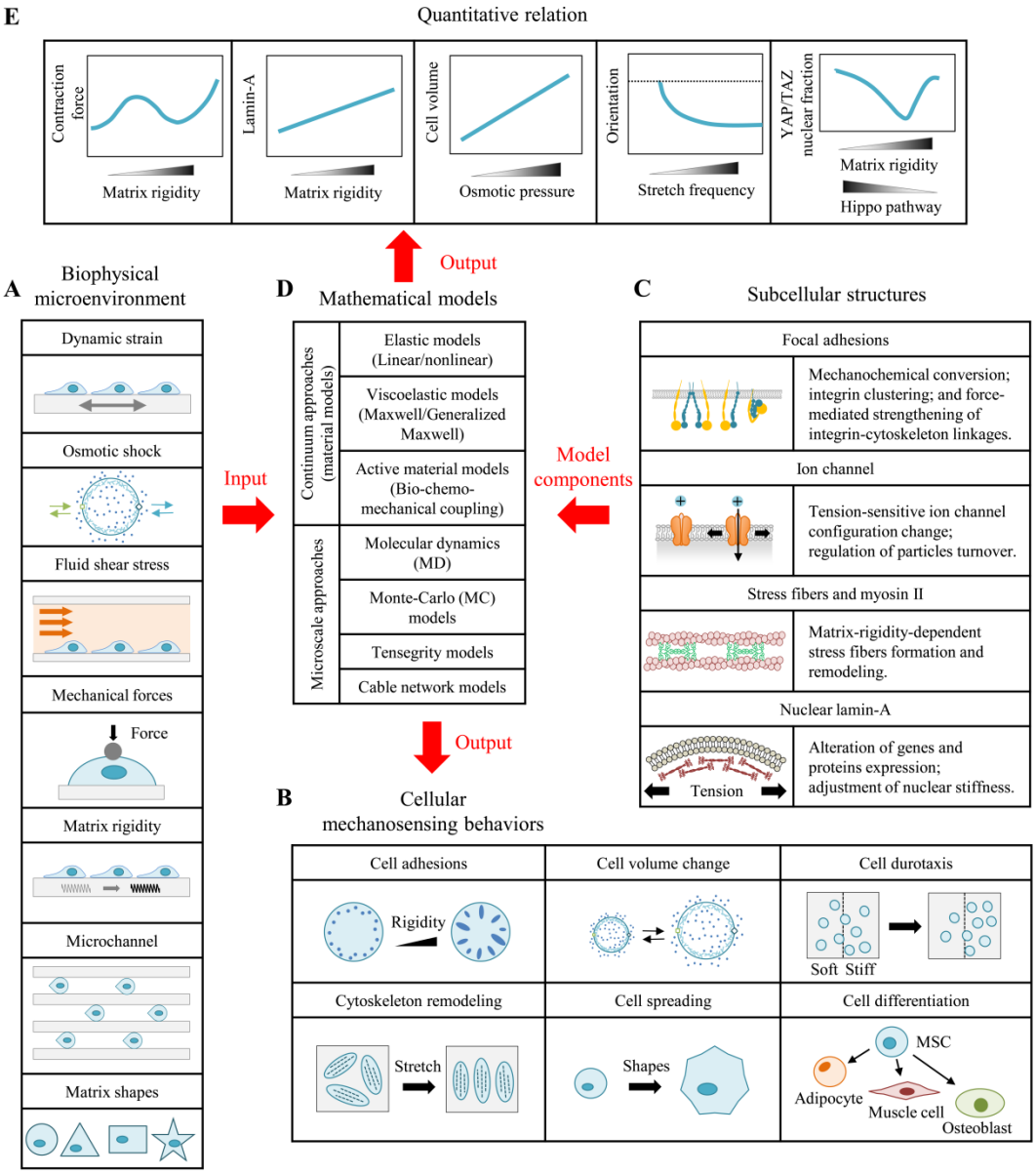

Figure 1. Application of cellular mechanosensing models in the mechanobiology research. More and more experiments have proved that cells can sense and respond biophysical cues (*e.g.*, dynamic strain, osmotic shock, shear flow, external forces, matrix rigidity, microchannel and various matrix shapes). For example, cells have bigger focal adhesions on stiffer matrix; cells can actively regulate their volume in response to osmotic shock; cells would reorient perpendicular to the strain direction on a cyclically stretched substrate; cellular behaviors also are regulated by interactions of mechanical cues and chemical signals. Many quantitative relationships between mechanical cues and cell behaviors can be obtained from various mathematical models based on different cellular mechanosensing components, and

then applied to the interpretation and prediction of experimental observations.

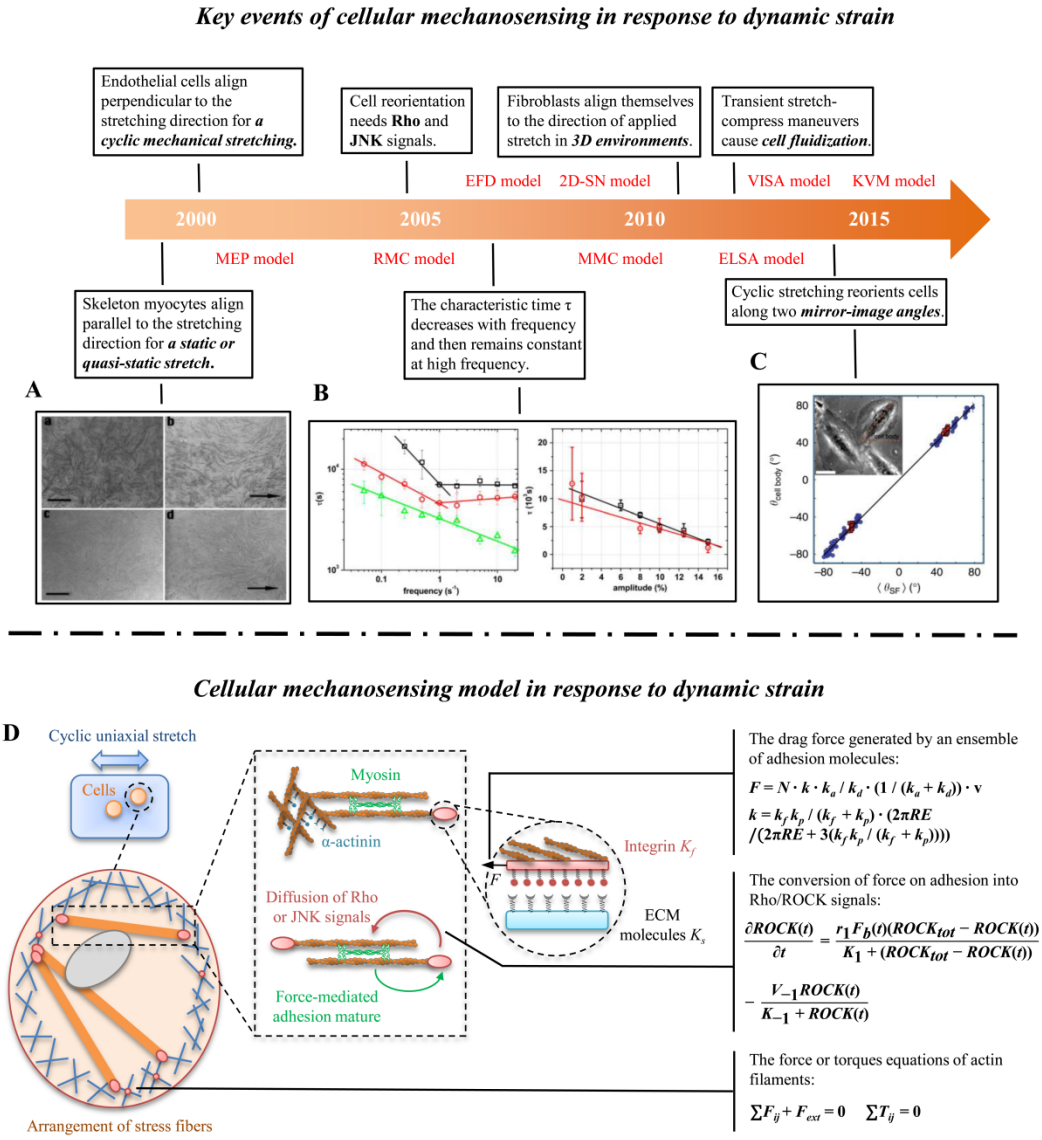

Figure 2. Cellular mechanosensing in response to dynamic strain. (A) Under conditions simulating mammalian long bone growth (*e.g.*, a static or quasi-static stretch), cultured myocytes respond to mechanical forces by lengthening and orienting along the direction of stretch [82]. (B) Many tissue cells (*e.g.*, fibroblasts and endothelial cells) prefer to align perpendicular to the direction of applied cyclic strain, especially at high frequency and larger stretching magnitude [83]. (C) Very recently, it has been show that cell reorientation to a uniform angle in response to cyclic stretching of the underlying substrate [84]. (D) This is the scheme of the mechanical SF model, showing adhesion complexes, myosin motors and the actin-filaments. Myosin motors generates force between antiparallel actin filament bundles, one of

which is anchored to matrix by adhesion complexes. Proteins in adhesion and actin-filaments system are drawn as masses on springs in order to indicate how they function in the model.

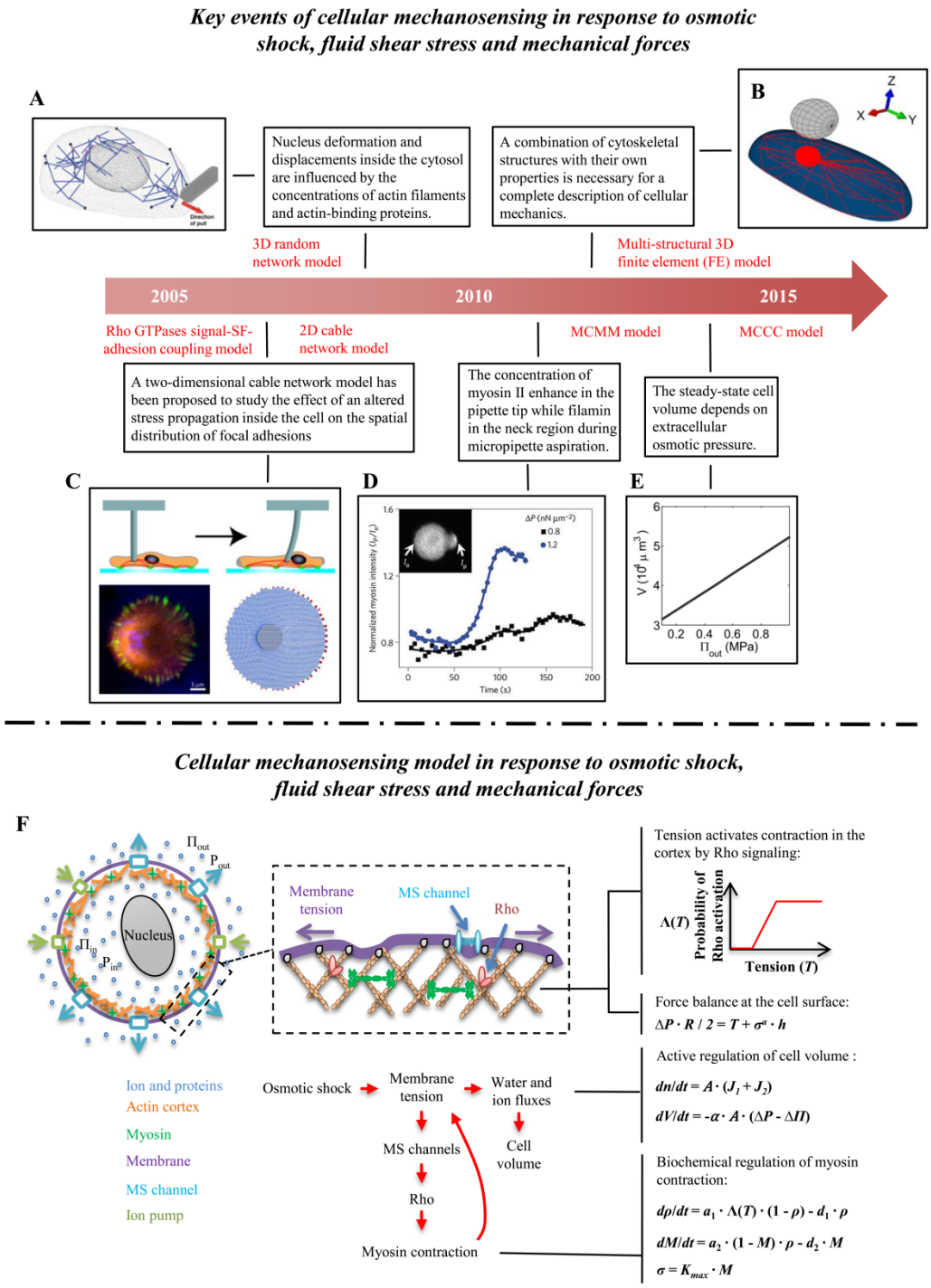

Figure 3. Cellular mechanosensing in response to osmotic shock, fluid shear stress and mechanical forces. (A) The 3D random network model of the actin cytoskeleton to study the nucleus deformation under micropipette pulling [123]. (B) The multi-structural 3D finite element (FE) model can be used to study the role of mechanical properties of the cytoskeleton in cell response under external compressive loads by AFM [120]. (C) A two-dimensional cable network model has been proposed

to study that how stress is transmitted through the actin cytoskeleton of adherent cells and consequentially distributed at the focal adhesions sites (FAs) [119]. (D) Myosin and α-actinin accumulation will increase in the pipette tip while filamin will increase in the neck region during micropipette aspiration [116]. (E) Steady cellular volume increases with increasing extracellular osmotic pressure [71]. (F) Schematic of the model calculation for a spherical cell during osmotic shock. The model includes the Rho signaling pathway that activates myosin assembly and active contraction in the cell cortex. At mechanical equilibrium, the membrane tension must be balance both osmotic pressure in the cell and active contraction in the cortex.

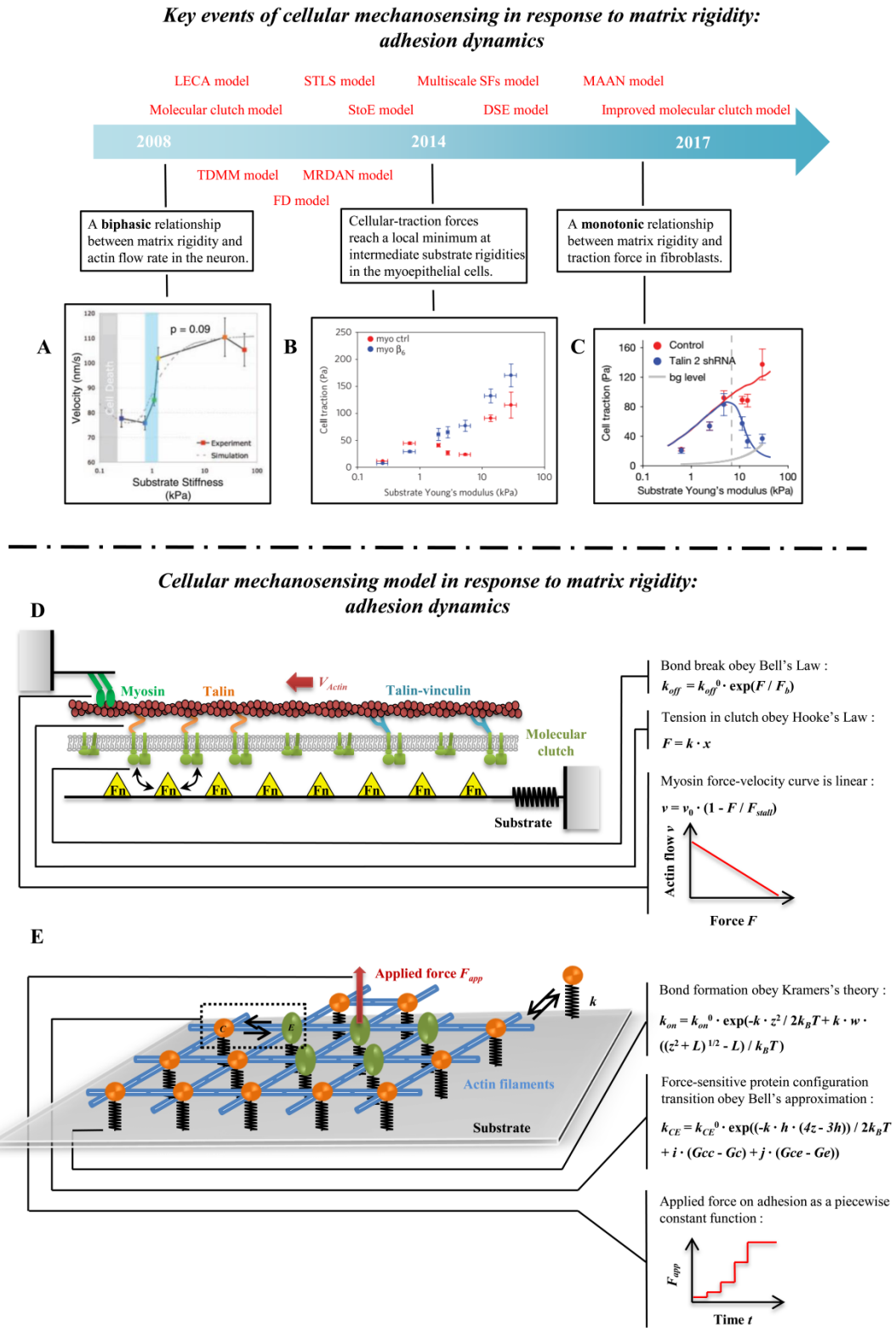

Figure 4. Cellular mechanosensing in response to matrix rigidity: adhesion dynamics. (A) Neurons have higher actin flow rate on stiffer substrate [146]. (B-C) The distinct actin flow/matrix rigidity relationships in breast myoepithelial and fibroblasts [10, 25]. (D) Schematic of the uniaxial molecular clutch model. Actin

polymerization and depolymerization at the tips of filopodia are coupled to the substrate through molecular clutches, and these molecular clutches resist the retrograde flow driven by myosin motors and membrane fluctuations. With increasing tension, the following transformation of molecular clutches could happen: talin unfolding and refolding, clutch reinforcement by vinculin binding, clutch configuration change for signaling activation, and weakest-link rupture. (E) Schematic of the two-dimensional molecular-mechanical adhesion model. Each molecular may bind to the substrate through a flexible spring, and may exist in two state, load-dependent transition of circle state and ellipse state.

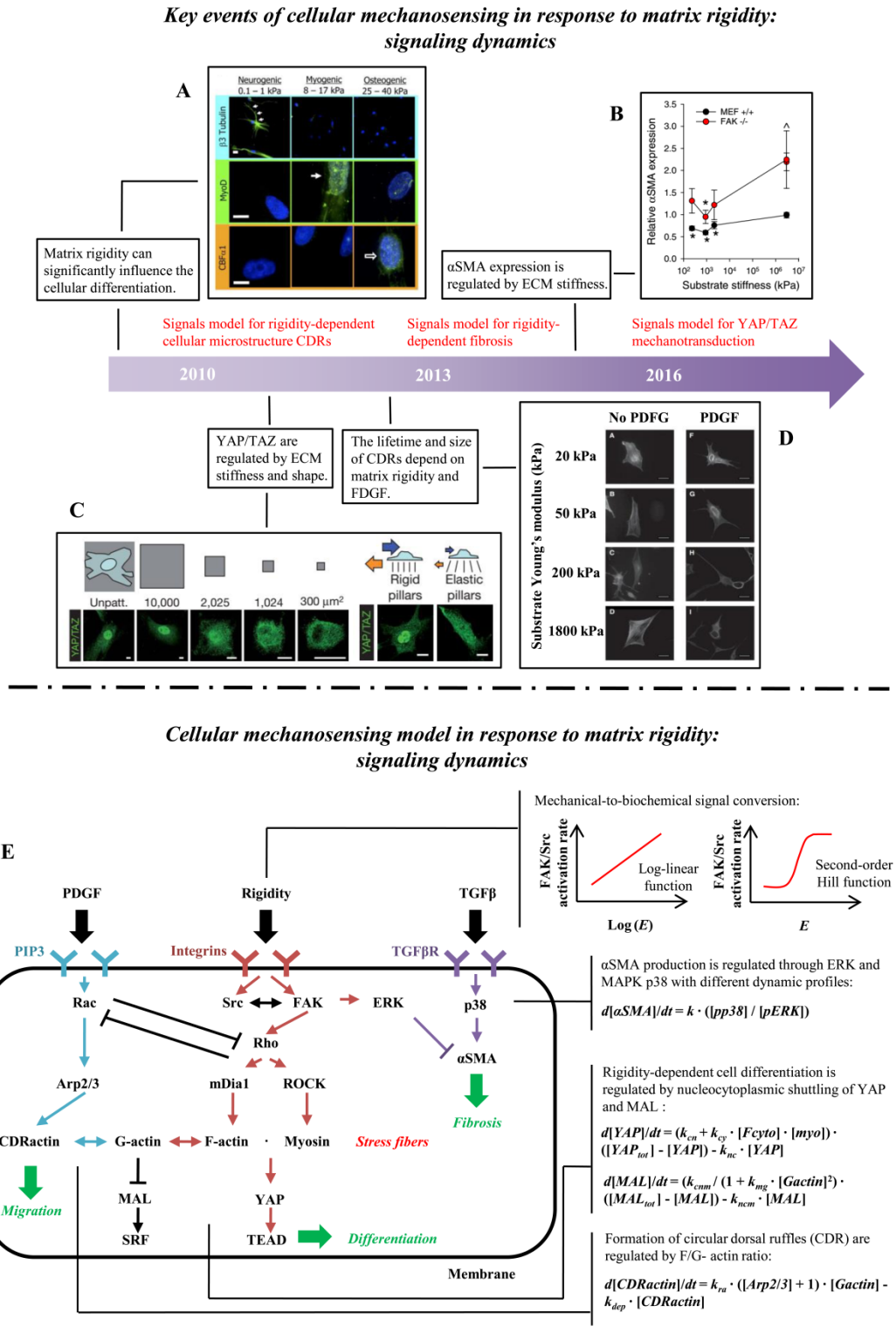

Figure 5. Cellular mechanosensing in response to matrix rigidity: signaling dynamics. (A) The lineage of mesenchymal stem cells (MSCs) is strongly affected by the modulus of the substratum upon which they are cultured [26]. (B) The conversion of fibroblast into myofibroblast is also regulated by external mechanical cues [73]. (C)

YAP/TAZ nuclear translocation has been shown to be influenced by ECM stiffness and shape [144]. (D) FDGF can significantly increase the lifetime and size of CDRs on stiff substrate [14]. (E) Signaling pathway dynamic model for cell shape, migration and differentiation. The matrix rigidity is transmitted to intracellular signals via adhesion molecules, such as FAK and Src. Adhesion-mediated mechanosensing signals are Rho/ROCK/myosin II, Rho/mDia1/F-actin, SF/YAP/TEAD and SF/MAL/SRF. Other chemical cues related signals are TGFβ/p38/αSMA and PDGF/Rac/Arp2/3. The synergistic effect between the mechanical sensing and the chemical signals is predicted due to the interaction of Rac/Rho and ERK/p38.

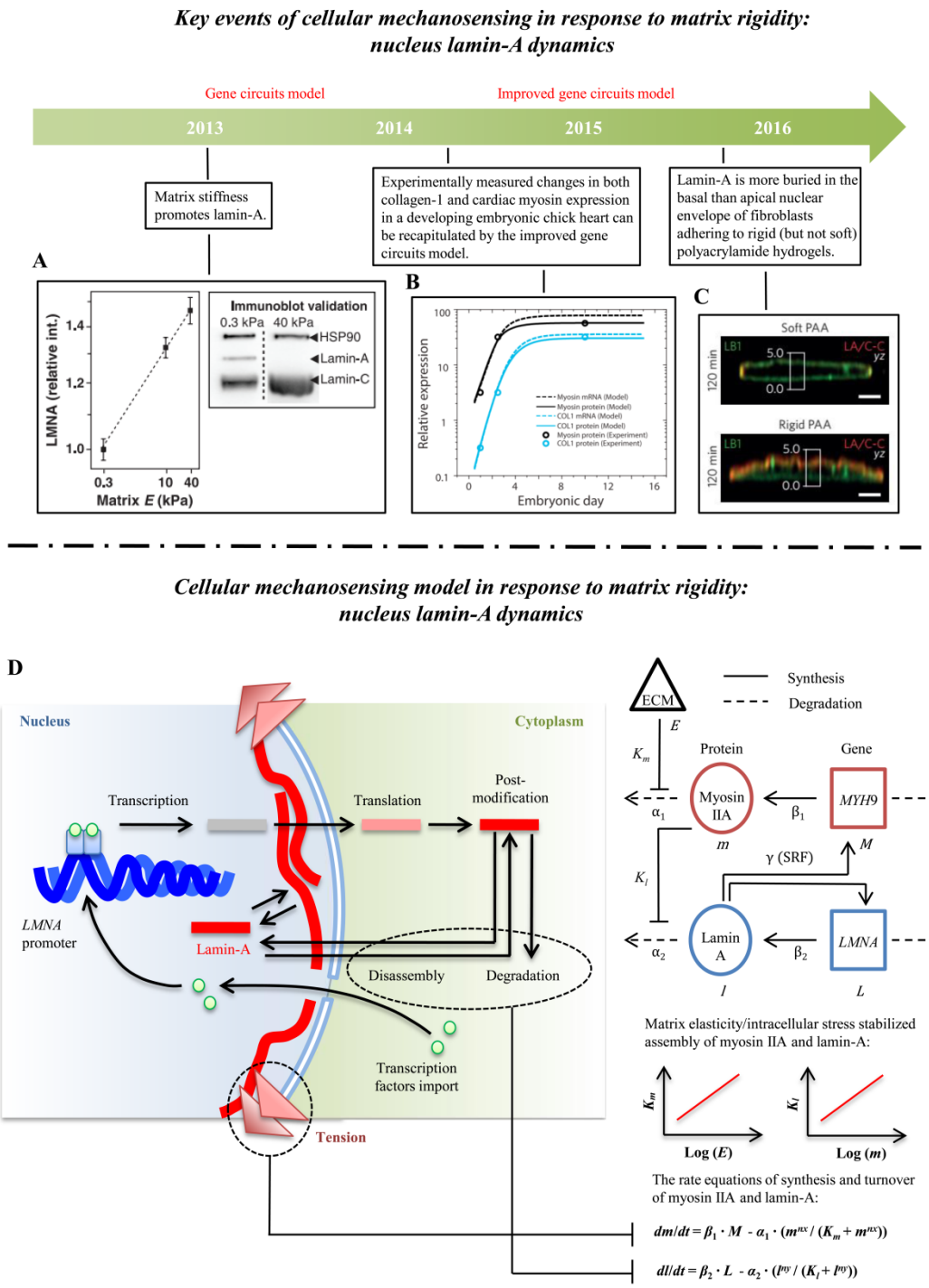

Figure 6. Cellular mechanosensing in response to matrix rigidity: nucleus lamin-A dynamics. (A) The level of lamin-A increases with increasing matrix stiffness [11]. (B) The levels of collagen-1 and cardiac myosin increase first and then reach a maximum value during the development of embryonic chick heart [169]. (C) Lamin-A is more buried in the basal than apical nuclear envelope of fibroblasts adhering to rigid (but not soft) polyacrylamide hydrogels [204]. (D) Schematic of the

gene circuit model concludes that matrix rigidity can regulate the levels of nuclear lamin-A. Stiff (soft) matrix can enhance (inhibit) the contraction force and then increase the nuclear tension in lamin layer, resulting in deceased lamin-A degradation rate. Tension acted on nuclear by stress fibers can also influence the transcription factors nucleoplasm shuttling, which would further regulate the lamin-A expression.

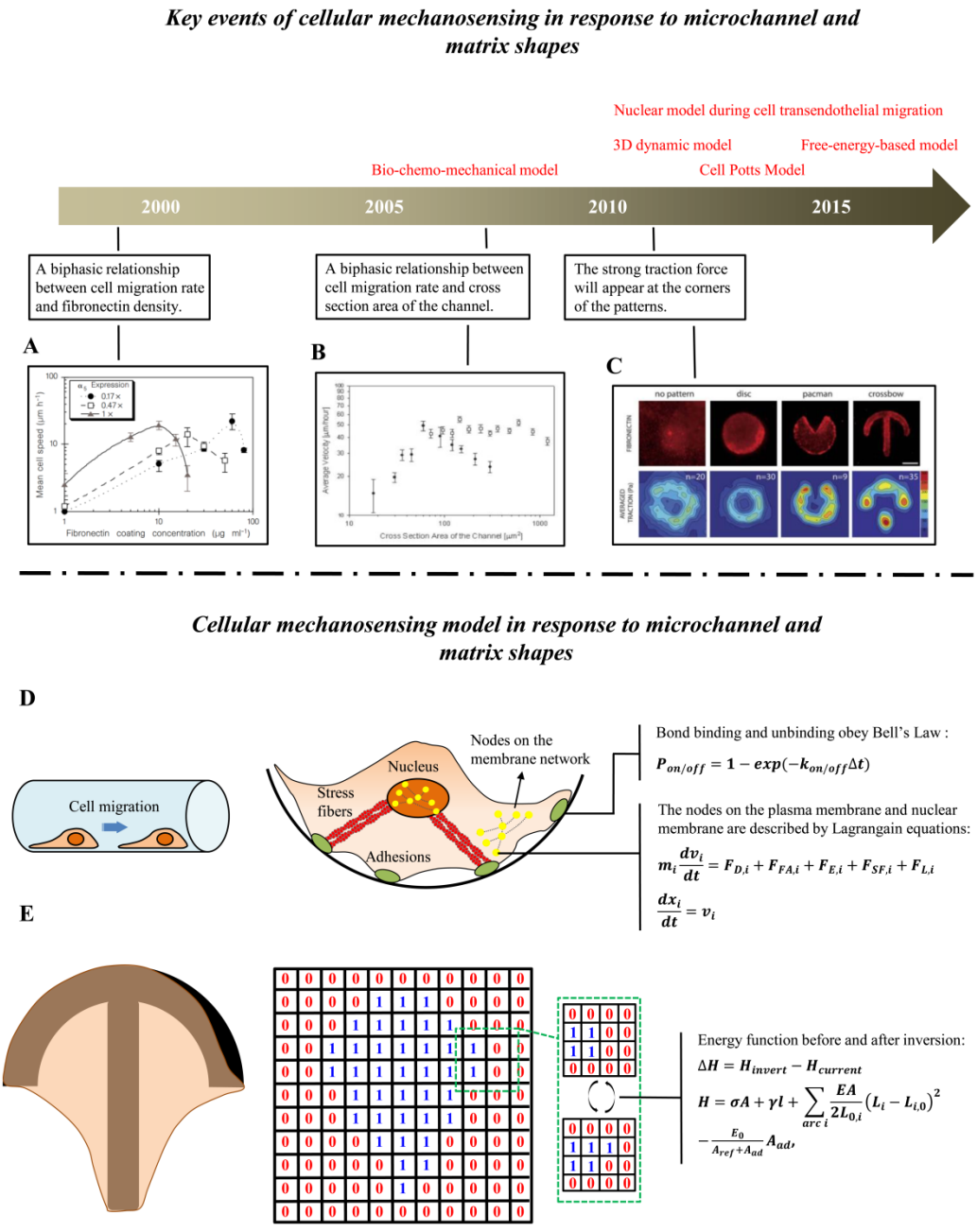

Figure 7. Cellular mechanosensing in response to microchannel and matrix shapes. (A) A biphasic relationship between cell migration rate and fibronectin density, that is, cell migration rate will reach a maximum value at a particular fibronectin density) [173]. (B) Interestingly, there is also a biphasic relationship between cell migration rate and channel's sectional area in cell migration experiments through 3D microfluidic platform [174]. (C) Recently, cell spreading experiments on various micropatterned geometries (e.g., disk, pacman and crossbow shapes) show that strong traction force will appear at the corners of the patterns [175]. (D) 3D

integrated dynamic model of cell migration on the curved substrate. The plasma membrane and nucleus membrane are modeled as two elastic mesh networks. The integrin-fibronectin is also incorporated into model through Monte Carlo simulation. (E) A cell Potts model based on simple tension-elasticity elements is proposed to study the dynamics of cell shape and traction on the various micro-patterned matrixes. Cells which are modeled as a collection of spins can be arbitrary shape because of a sufficiently plenty of spins. Each spin configuration has an energy function which based on the traction force in the cell (such traction force is balanced by the adhesion force). Evolution of cell shape and traction force can use Metropolis dynamics based on the energy minimization principle by assuming that cell spreading is a quasi-static process.