Cell Mechanosensing*

Response of Living Cells to Their Mechanical Environment

Rumi De

Mechanical forces are known to play important roles in determining cellular functions and behaviours such as growth, migration, wound healing and tissue regeneration, to name a few. It is quite intriguing how cells sense the mechanical forces and properties of the surrounding matrix in which the cells float or rest. The cells are known to build numerous 'adhesion contacts' at the cell-matrix interface to probe the surroundings. These adhesion contacts, known as 'focal adhesions' are highly dynamic and strongly force sensitive. In this article, we discuss about the focal adhesions which act as mechanosensors and, in turn, regulate cellular activity.

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1. Motivation

Cells are the building blocks of life. As basic units of life, cells perform myriads of specialized functions such as encoding and decoding genetic information, synthesis and transport of molecules, and maintenance of their own internal structure. It has long been known that biochemical pathways play an important role in determining cell behaviours and functions. However, discoveries in recent years indicate that mechanical forces in the environment also significantly influence cellular activities [1]. In living systems, cells are often exposed to various forces induced by different interactions, such as those due to the adjacent cells, muscle tension, etc. Cells also experience periodic stretches, for e.g. cardiac muscle cells stretch during periodic heart beating and blood vessel cells stretch during oscillating blood pressure. These mechanical forces vastly affect how cells grow, migrate from one

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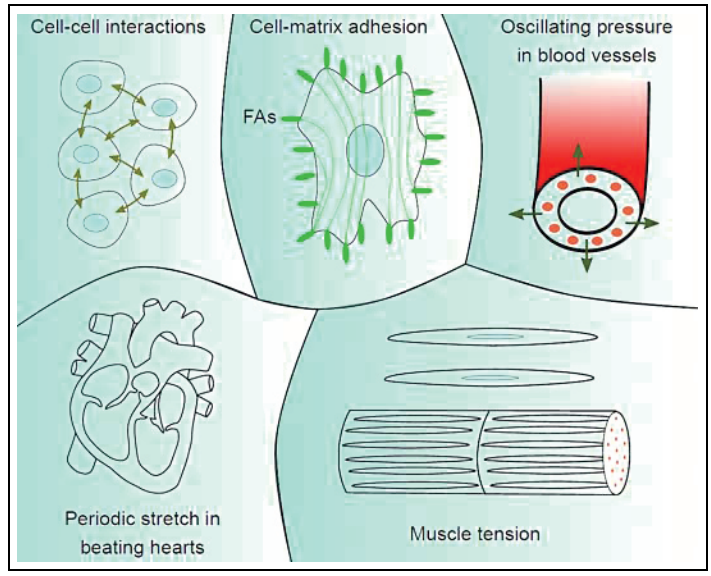

Figure 1. Schematic diagram of various mechanical interactions of cells in tissues.

place to other, or how cellular shape and morphology change or induce cell death [1– 4].

Recent experiments further reveal that differentiation of the cell into various cell types – muscle cells, bone cells, or brain cells – can be altered simply by tuning the elasticity of the extracellular matrix[1] (ECM) [5]. Also, a change in substrate stiffness can trigger a transformation from non-cancerous to cancerous cells.

Mechanical forces provide an important route for communication among cells and their microenvironment. We are yet to fully understand the underlying mechanisms of 'cell mechanosensing'. Some of the key questions which are being actively researched include: How do cells sense the mechanical forces in the surrounding matrix? How are cellular activities altered in the presence of an applied force?

[1] Extracellular matrix (ECM) is a network of proteins and other molecules in which the cells in tissues anchor themselves.

2. How Cells Feel the Mechanical Cues

Figure 1 shows a pictorial description of various mechanical interactions of cells with the surroundings. Cells constantly probe

their surroundings and respond to the variety of signals impinging on them by regulating different cellular processes. For several years, researchers have carried out investigations to understand how cells communicate with the extracellular matrix. These studies led to the discovery of 'focal adhesions' through which cells connect to the ECM, in 1970. Cells build up a large number of adhesion contacts along its periphery at the cell-matrix interface. These contact points are known as focal adhesions (FAs) [2]. FAs are multi-molecular protein assemblies linked to the ECM on one side and to the actin stress fibres in the cell cytoskeleton on the other. The cytoskeleton is like a pillar of a building. It provides structure and shape to the cell. The cytoskeleton is composed of a network of interlinking filaments – actin filaments, microtubule, and intermediate filaments. Actin stress fibres are bundles composed of 10–30 cross-linked actin filaments. Actin stress fibres are connected to myosin motors. These motor proteins convert chemical energy into mechanical work, cause the actin filaments to slide past each other, and generate contractile forces. Focal adhesions transmit these forces to ECM and act as mechanosensors. Cells exert contractile forces on the surrounding matrix and pull the matrix in order to probe its rigidity and presence of external forces, and then respond to them by regulating the internal activities.

Focal adhesions provide a key mechanical link between the cell and the surroundings and act as mechanosensors.

3. Response of Focal Adhesions Under External Force

Biological focal adhesions are very different from physical adhesions. Physical adhesion is a passive process. On the other hand, cells actively reorganize and remodel FAs in the presence of mechanical forces. FAs adapt to the changes by tuning the growth, stability and contractile activities. The dynamics of FAs in response to time-varying forces is also very important as cells modulate in synchrony with the rhythmic cardiac cycles. It is observed that the orientation of FAs changes depending on the frequency of the time-varying stretch. Subjected to static or quasi-static stretch, FAs tend to orient along the stretch direction, whereas, under fast varying stretch, FAs opt to orient away from the stretch

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direction; for high-frequency cyclic stretch, FAs align nearly perpendicular to the applied stretch direction [4, 6, 7].

Understanding the dynamics of FAs is very challenging due to the large number of different components involved. Focal adhesions have a layered structure comprising a variety of proteins (such as integrin, vinculin, talin, paxillin, etc.) located at the interface of the cell plasma membrane and ECM. Experimental studies show that FAs are strongly force sensitive. The process of assembly and disassembly of FA proteins are affected by the rigidity of the ECM and the presence of external forces.

The growth, stability, and orientation of focal adhesions are strongly affected by time-dependent mechanical forces.

Forces applied to these cell-matrix bonds catalyse chemical processes or switch molecular interactions on or off. It alters the activation energy barriers along the kinetic pathways that lead to association and dissociation of FA bonds. Recent experiments show that FAs grow in the direction of the tensile force [2, 3]. The stability and lifetime of many focal adhesion molecules increase with the increase in tensile forces and reaches a maximal at a finite force after which stability decreases with higher force value. These bonds are called the 'catch bonds' [8]. Why and how mechanical force strengthens these catch bonds still remain elusive. Mechanical forces induce some conformational changes that activate the binding domains and strengthens the bond. An intuitive understanding of such bonds can be gained by understanding how two hooks connect with each other. A pair of interlocked hooks can unbind easily when they are loosely attached. On the other hand, if the interlocked hooks are pulled by a tensile force, they firmly bind and do not detach until a great force breaks them apart.

4. Theoretical Model of Focal Adhesions: An Example

Apart from experiments, considerable theoretical efforts are also underway to understand the dynamics of focal adhesions [3]. There is more than one approach to model FAs, here, we discuss one of the frameworks of modelling FAs.

Focal adhesions are generally modelled as a cluster of ligand-

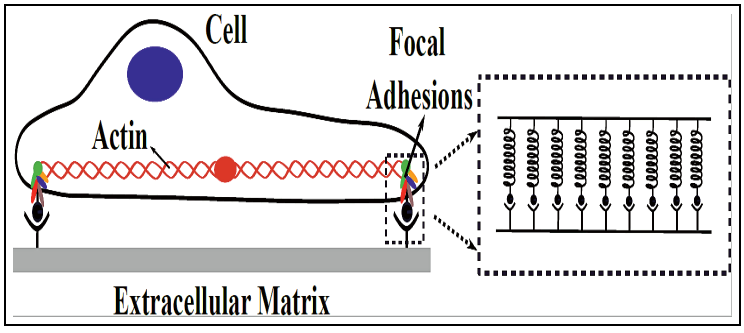

Figure 2. An illustration of a cell adhered to the extracellular matrix via two focal adhesions (FAs). The inset (dotted box) depicts FAs as a cluster of ligand-receptor molecular bonds.

receptor molecular bonds between the cell and ECM as illustrated in *Figure* 2. These bonds are considered as Hookean elastic spring. In a seminal work by Bell, the stability of the adhesion cluster was first addressed using the kinetic theory of chemical reactions [9]. The receptors (R) on the cell surface bind with the ligands (L) on the ECM to form the ligand-rector complexes (RL). The chemical kinetics is described as:

$$R + L \underset{K_{\text{off}}}{\overset{K_{\text{on}}}{\rightleftharpoons}} RL, \tag{1}$$

where K_{on} and K_{off} are the rate constants. K_{on} denotes the binding or association rate of the ligand-receptor bond and K_{off} is the breaking or dissociation rate. These rates are found to be force dependent.

Slip bonds weaken under force, whereas, catch bonds strengthen under force.

In Bell's model, the dissociation rate of ligand-receptor bonds was proposed to increase exponentially with the mechanical force. Such bonds are called 'slip bonds' that weaken under force. The force-dependent dissociation rate of slip bonds is given as:

$$k_{\text{off}} = k_0 e^{f_{\text{b}}/f_0}, \tag{2}$$

where k_0 is the spontaneous dissociation rate in the absence of external force, f_{b} denotes the bond force, and f_0 is a molecular force scale typically of the order of piconewtons (pN) for FAs. On the other hand, the dissociation rate for catch bonds which get strengthened under force has been proposed as [7, 10],

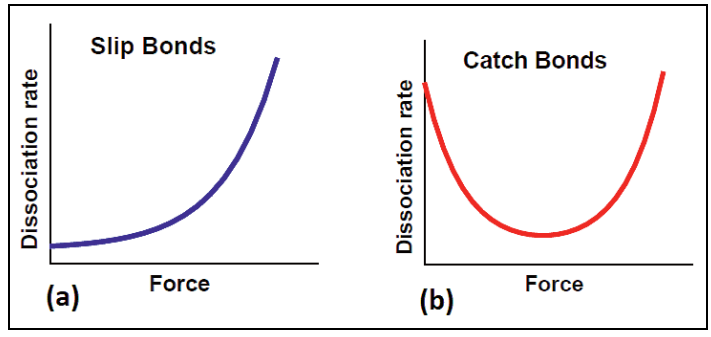

Figure 3. Schematic of bond dissociation rate as a function of applied force. For a slip bond, breaking rate increases with force. Howvever, for a catch bond, breaking rate decreases with increase in force up to an optimal value and then eventually increases with higher force value.

$$k_{\text{off}} = k_{\text{slip}} e^{f_b/f_0} + k_{\text{catch}} e^{-f_b/f_0} \ , \tag{3}$$

Focal adhesions undergo stochastic breaking or rebinding due to fluctuations in the surrounding microenvironment. Thus, one can write the master equation to describe the stochastic dynamics of FA bonds.

where k_{slip} and k_{catch} denote the rate constants for dissociation of the ligand-receptor pair via a slip pathway promoted by the force and a catch pathway opposed by the force respectively [10]; these rate constants depend on the type of adhesion molecules. *Figure* 3 depicts the force-dependent dissociation rate for the slip bonds and the catch bonds.

Focal adhesions undergo stochastic breaking or rebinding due to fluctuations in the surrounding microenvironment. Thus, one can write the master equation to describe the stochastic dynamics of FA bonds. Master equation is a gain-loss equation for the probability of each state of the system with regard to the time variable [11]. Thus, the master equation for a cluster of bonds can be written as:

$$\frac{dP_n}{dt} = K_{\text{on}} P_{n-1} + K_{\text{off}} P_{n+1} - (K_{\text{on}} + K_{\text{off}}) P_n, \tag{4}$$

where $P_n(t)$ is the probability that n bonds are formed at time t. The first two terms in the right-hand side represent the gain term, i.e., the tendency of the number of bonds in state n to increase due to the formation of new bonds in state $(n-1)$, and the dissociation of bonds in state $(n+1)$, respectively. The last term represents the loss of bonds in state n. Master equation is usually numerically solved by the Monte-Carlo method based on Gillespie's al-

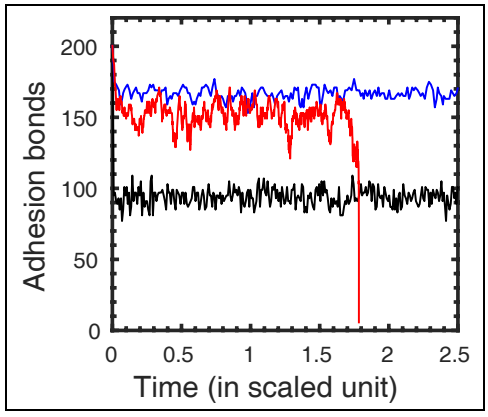

Figure 4. Time evolution of number of closed bonds in an adhesion cluster under applied force. The stochastic trajectories are simulated from the master equation using representative force values and the corresponsding reaction rates. Here, the black and the blue trajectories represent stable adhesion clusters and the red shows unstable cluster where all bonds disassemble after sometime.

gorithm [12] to study the time evolution of bond clusters[2]. One such typical simulation trajectories of instantaneous number of closed bonds in an adhesion cluster is shown in *Figure* 4. This kind of stochastic model framework along with other variations of such stochastic bond dynamics could predict the time evolution, growth, stability, and the orientation of adhesion clusters under external forces [7]. Other theoretical studies have also provided many insights on how focal adhesions respond to mechanical forces and regulate cell activities. (For more details refer [3].)

[2]For a detailed discussion on simulation algorithm and Gillespie's method, see the article by A B R Kumar and R Ramaswamy, Chemistry at the Nanoscale, *Resonance*, Vol.23, No.1, pp.23–40, 2018.

5. Summary and Outlook

In recent times, mechanobiology has become an increasingly exciting area of research. Of particular interest are the mechanical behaviours of cells and tissues with direct consequences in regulating biological functions and cellular organizations. How cells actively respond to the physical forces or how the mechanical signals are coupled with the biochemical signalling pathways that alter cellular activities or gene expressions still remains unclear. A better understanding of these aspects will not only help transform the field of cell biology but will also have wide implications in tissue engineering, regenerative medicine, cancer research and many biomedical applications.

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