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Review

Mechanical dynamics in live cells and fluorescence-based force/tension sensors

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ABSTRACT

Three signaling systems play the fundamental roles in modulating cell activities: chemical, electrical, and mechanical. While the former two are well studied, the mechanical signaling system is still elusive because of the lack of methods to measure structural forces in real time at cellular and subcellular levels. Indeed, almost all biological processes are responsive to modulation by mechanical forces that trigger dispersive downstream electrical and biochemical pathways. Communication among the three systems is essential to make cells and tissues receptive to environmental changes. Cells have evolved many sophisticated mechanisms for the generation, perception and transduction of mechanical forces, including motor proteins and mechanosensors. In this review, we introduce some background information about mechanical dynamics in live cells, including the ubiquitous mechanical activity, various types of mechanical stimuli exerted on cells and the different mechanosensors. We also summarize recent results obtained using genetically encoded FRET (fluorescence resonance energy transfer)-based force/tension sensors; a new technique used to measure mechanical forces in structural proteins. The sensors have been incorporated into many specific structural proteins and have measured the force gradients in real time within live cells, tissues, and animals.

1. Ubiquitous mechanical activity in non-muscle cells

Biomechanical activity was first recognized and studied in muscle cells. However, all cells, from osteocytes and endothelial cells to neurons and immune cells, are mechanically sensitive and can generate force. All biological processes, including differentiation, mitosis, meiosis, motility, apoptosis and homeostasis, involve the modulation and response to mechanical force [1]. Mechanical force obviously varies in different tissues and organs; whether it is periodical preload or postload in ventricles, fluid shear force on the vascular endothelium, strain in skin or compression, and bending of bones and cartilage. Along with chemical free energy and electrical free energy, mechanical force completes the sources of free energy available to cells, and all the free energy sources are coupled to downstream pathways that regulate physiological activity. For instance, stem cell phenotypy is affected by mechanical factors including substrate stiffness and surface topography. When mesenchymal stem cells are cultured on soft substrates that mimic the elasticity of brain tissue, the cells differentiate into neuronal precursors; matrices with intermediate stiffness that mimic muscle and induce myogenic commitment; while rigid matrices mimicking collagenous bone directly differentiate into osteogenic lineages [2]. The mechanical forces' connection to cell biology has been understudied, but is clearly an essential component of physiology and pathology.

2. Mechanical dynamics in live cells

Living cells generate and sense force; this interaction is used for homeostasis [3]. The cytoskeleton plays a fundamental role in conducting information and sensing mechanical force. The cytoskeleton is attached to the membrane through adhesion complexes involving integrins to facilitate mechanical communication between the cell and its surroundings. These differentiated couplers can translate mechanical force into electrical or biochemical signals through conformational changes [4,5]. The cytoskeleton also serves as tracks for motor proteins, including dynein, kinesin and myosin, which is the driving force behind most active transport of proteins and vesicles in the cytoplasm, as well as cell movement. For example, myosin is responsible for sliding actin filaments and cellular contractility; and the dynein motor is responsible for the wave like motion of the cilia, or the beat of the flagella. Furthermore, force generated by the movement of motor proteins is passed on along the cytoskeleton to mechanosensors, which can perceive and translate the force, activating various downstream signaling pathways that play important roles in the regulation of cell morphogenesis and polarity. The

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generation and transduction of cellular mechanical force all depend on the cytoskeleton, therefore, it is classified as a structural force.

Many factors, including extracellular mechanical stimuli, osmotic pressure, movement of motor proteins and mechanosensors, have real time effects on structural force. The system forms a network for the generation, perception, and transmission, as well as the translation of mechanical forces to maintain homeostasis in a mechanically changing environment. We are calling this the mechanical dynamics of living cells.

3. Mechanical stimuli and effects

Based on the origin of the force in and on cells, it can be classified into two groups: exogenous and endogenous. The former includes gravity, compression, stretch, strain, fluid shear, etc. Endogenous forces include osmotic pressure and the movement of motor proteins. As mentioned above, these mechanical factors play vital roles in the life of cells.

3.1. Exogenous mechanical stimuli

Investigations aimed at studying the effects of exogenous mechanical stimuli on cells *in situ* is difficult. Studying cell mechanics *in vitro* requires implementing a method to mimic the force that cells undergo in their physiological environment. There are a variety of such experimental methods and some are summarized in Table 1.

3.2. Endogenous mechanical stimuli

3.2.1. Movement of motor proteins

Motor proteins are a class of molecular motors, consisting of dynein, myosin and kinesin, that are able to move along the cytoskeleton. They play a major role in bidirectional transport in cytoplasm, which is essential for cell physiology, plasticity, morphogenesis, and survival [20]. They also link chemical catalysis to the production of directed force along protein filaments [21].

Dynein superfamily proteins are mechanoenzymes that move along microtubules, and they are comprised of two major groups: cytoplasmic dyneins and axonemal dyneins (also called ciliary or flagellar dyneins) [22]. Dyneins operate as complexes built around force-generating subunits called heavy chains, which contain the motor domain. The tail specifies oligomerization properties and serves as a platform for the binding of several types of associated subunits, which in turn mediate interactions with cargo either via direct binding or through the recruitment of adaptor proteins. Dynein also has an important associated protein complex called dynactin, which regulates dynein activity and the binding capacity of dynein for its cargos [23]. Cytoplasmic dynein performs a variety of cellular functions including: (1) Cytoplasmic dynein powers the transport of membrane bound vesicles and tubules, together with their resident molecules toward microtubule minus ends [24]. (2) Dyneins tethered to the cell cortex can apply a pulling force on the microtubule network by either walking toward the minus end of a microtubule or coupling to a disassembling plus end. This force is essential to cell division [25–27]. (3) At the outer nuclear envelope, dynein has been reported to contribute to nuclear rotation [28] and positioning [29], centrosome separation [30], and the breakdown of the nuclear envelope for open mitosis [31]. (4) At cell division, cytoplasmic dynein assists in assembling microtubules into the chromosome-segregating device known as the spindle [32,33]. (5) Cytoplasmic dynein localizes to the kinetochore; this dynein has an important role in the molecular surveillance mechanism that aids faithful chromosome segregation [34]. Dysfunctions of cytoplasmic dynein and dynactin contribute to many neurodegenerative and neurodevelopmental diseases, including short-rib polydactyly syndrome [35,36], motor neuron disease, ALS [37–39], lissencephaly [40,41], Alzheimer's disease [42], etc.

The kinesin superfamily proteins (KIFs) comprise three major groups based on the position of the motor domain: N-terminal motor domain KIFs (N-KIFs), middle motor domain KIFs (M-KIFs), and C-terminal motor domain KIFs (C-KIFs) [43]. N-KIFs and C-KIFs are composed of a motor domain, a stalk domain, and a tail region. The motor domain consists of ATP- and microtubule-binding sites which enable it to bind to microtubules and to move them along by hydrolyzing ATP. In general, the tail regions, and less frequently the stalk regions, recognize and bind to the cargo(s) [20,43,44]. Kinesins play a major role in intracellular transport and they can be classified into many groups based on the cargos transported and the location of the transport activity [43]: (1) Anterograde axonal transport, such as synaptic vesicle precursor and mitochondrial transport along the axon. (2) Dendritic transport in neurons, like the transport of NMDA and AMPA receptors and mRNA. (3) Conventional transport, including transport between the endoplasmic reticulum and Golgi apparatus, lysosomal transport, transport from the trans-Golgi network to the plasma membrane, and endosomal recycling. KIFs are also closely involved in various diseases, such as kinesin-1 in spastic paraplagia [45,46], amyotrophic lateral sclerosis (ALS) [47,48] and Alzheimer disease [49–51]; kinesin-3 in Charcot–Marie–Tooth disease [52,53] and multiple sclerosis [54,55]; kinesin-4 in congenital fibrosis of the extraocular muscles type 1 (CFEOM1) [56], etc.

Myosins are the only known actin-based motor proteins [57] and are classified into eighteen classes [58]. Most myosins form a dimer, consisting of a conserved catalytic motor domain (head) with actin- and ATP-binding sites, a neck region and a tail region that binds to cargos [57,59]. Myosins have various functions: (1) They are mechanoenzymes and their conformational changes associated with nucleotide binding, hydrolysis, and product release are crucial for the productive motility of myosin enzymes [60]. (2) Myosins, particularly the myosinII, have long coiled-coil domains that allow multimerization to take place. Thus, interactions between charged residues within these dimers mediate the formation of bipolar thick filaments that are responsible for contraction of muscle and cytokinesis [61,62]. (3) Myosins participate in many trafficking and anchoring events, such as directed migration of pigment containing vesicles involved with myosinVa in melanocytes [63] and correct targeting of the megalin receptors involved with myosinVI [64]. (4) Myosins play a role in actin-based projections including stereocilia and microvilli, which are essential for the normal function of hair cells in the inner ear and epithelial cells in the intestine and kidneys [65]. As for pathology, abnormalities of myosins are key factors in the development of diverse diseases, including autosomal dominant deafness (Myosin I) [66,67], May-Hegglin anomaly, Fechtner syndrome and Sebastian syndrome (Non-muscle Myosin II) [68,69], autosomal recessive deafness (Myosin III) [70], Griscelli syndrome and microvillus inclusion disease (Myosin V) [71,72], Usher syndrome type IB and unsyndromic deafness (Myosin VII) [73,74], loss of heterozygosity in lung cancer (Myosin XVIII) [75], etc.

The mechanism of force production by motor proteins is thought to involve structural changes in a deformable element of the motor that undergoes changes in structure under load, which creates strain followed by a strain-relieving structural change that causes the element to recoil back into its original conformation, producing force. In this process, motor proteins couple a chemical cycle of ATP hydrolysis to a mechanical cycle of motor interactions with its filament by the motor (Fig. 1) [21,76]. Take dynein for example; ATP induces dissociation of the motor–microtubule complex [77]. After detaching from the microtubule, the motor rearranges, becoming primed for a subsequent structural change (termed the powerstroke) that is thought to generate the force. Following a diffusive search, rebinding of the motor to a new site on the microtubule stimulates the release of ATP hydrolysis products, thus triggering the powerstroke [78]. Mechanical force produced by this mechanism is not only responsible for the cytoplasmic transport, but also essential for the generation of mechanical effects on the cytoskeleton; which can change its structure and eventually the shape of