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Uncovering mechanosensing mechanisms at the single protein level using magnetic tweezers

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ABSTRACT

Mechanosensing of the micro-environments has been shown to be essential for cell survival, growth, differentiation and migration. The mechanosensing pathways are mediated by a set of mechanosensitive proteins located at focal adhesion and cell–cell adherens junctions as well as in the cytoskeleton network. Here we review the applications of magnetic tweezers on elucidating the molecular mechanisms of the mechanosensing proteins. The scope of this review includes the principles of the magnetic tweezers technology, theoretical analysis of force-dependent stability and interaction of mechanosensing proteins, and recent findings obtained using magnetic tweezers.

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1. Introduction

In tissues, cells need to adapt to their microenvironment by sensing various chemical and physical cues. Recent development in cell biology has demonstrated mechanical force as one of the critical determinants involved in cell migration, cell differentiation, tissue development and maintenance. In tissues, cells adhere to extracellular matrix (ECM) through formation of integrin dependent focal adhesion and to the neighboring cells through cadherin dependent cell–cell adherens junctions. Forces are generated by actomyosin contraction, and propagated in the whole tissue [1,2]. Force sensing of cells is mediated by a set of mechanosensing proteins located at focal adhesion and cell–cell adherens junctions as well as in the cytoskeleton network (Fig. 1). It has been proposed that such mechanosensing proteins can change their conformations under force, resulting in switching their binding partners in a force-dependent manner. This way, they can process the mechanical cues into downstream biochemical reactions, resulting in mechanosensing signaling of cells [2].

In spite of the simplicity of the above hypothesis of the mechanosensing mechanism, it is technically challenging to directly test it in experiments. In order to do so, well-controlled forces in the physiological level (pN range) have to be applied to individual proteins, and the resulting dynamic conformational change of the proteins has to be probed at a nanometer resolution. In addition, the force-dependent interactions of the force-bearing mechanosensing proteins with their binding partners need to be investigated at a single-molecule level in real time. Thanks to the rapidly developing single-molecule manipulation technologies, such as atomic force spectroscopy (often referred as AFM, short for atomic force microscopy), optical tweezers, and magnetic tweezers [3], probing force-dependent conformational changes and interactions quantitatively at a single-molecule level has become possible. In addition to *in vitro* single-molecule manipulation experiments, Föster Resonance Energy Transfer (FRET) based force sensor has also been developed to measure force applied to mechanosensitive proteins *in vivo* [4,5].

However, applications of the single-molecule manipulation technologies to the studies of mechanosensing proteins are still challenging due to various technical limitations of the respective

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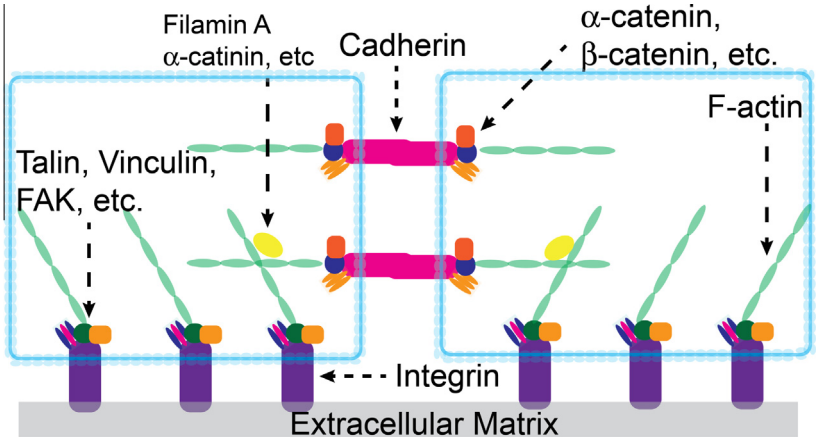

Fig. 1. Schematics of integrin mediated cell-matrix and cadherin mediated cell–cell adhesions. Actin cytoskeleton network and several known mechanosensitive proteins are highlighted.

technologies to apply low forces (pN range) directly to short tethers (<100 nm) over long duration of experiments (minutes to hours). One major difficulty is that the experimental time scale needed to study force-dependent conformational changes and interactions of single molecules often exceeds that can be provided by most of current single-molecule manipulation technologies due to rapid mechanical and thermal drifts. For example, the extensively studied 27th titin immunoglobulin (Ig) domain (I27) can be unfolded at a small force of ~5 pN, but with an ultraslow unfolding rate of $\sim 10^{-4}$ s^{-1} at this force range [6]. Therefore, studies of such proteins require a very long experimental time scale of several hours at <10 pN forces. Among the three main single-molecule manipulation technologies, magnetic tweezers excel in this aspect by providing the best long-time stability. In the subsequent sections, we review the basic principles of magnetic tweezers, thermodynamics of force sensing, and the recent applications of magnetic tweezers in studies of the molecular mechanisms of mechanosensing proteins.

2. Principles of magnetic tweezers

2.1. Magnetic tweezers apparatus

The basic idea of magnetic tweezers is to use an external magnetic field to apply forces to a paramagnetic bead typical with a diameter of 1–3 μm. When the bead is tethered to an end of a molecule with the other end of the molecule attached to a fixed surface, the molecule is then subject to an external force. In 1998, a highly efficient design of magnetic tweezers that is suitable for high resolution single-molecule studies was published by Strick et al. [7]. In this design, a basic magnetic tweezers apparatus consists of a reaction channel mounted on a microscope stage in which single-molecule tethers are formed, an optical microscope to image the tethered bead, a camera to record the bead images, permanent or electric magnets to generate a force perpendicular to the focal plane (x–y plane), and a computer to control the tweezers and analyze the bead fluctuation to obtain essential information of force and extension change of molecule (schematics in Fig. 2A). In this vertical design, the extension change is based on analyzing the diffraction patterns of the bead at different heights from the surface, which has been adopted by many other labs. Besides the perpendicular design, a transverse design was also developed, which applies forces in the focal plane. In the transverse design, the extension is determined by the centroid of bead [8].

The two designs have their respective strengths. In the vertical design, the tethers are formed on large coverslip surface, suitable for high-throughput multiplexing experiments [9,10]. The length of tethers can be shorter than 200 nm, ideal for high signal-to-noise measurements [11–14]. The tweezers can be built on a total internal reflection fluorescence microscope [15], allowing combination with single-molecule spectroscopy technologies such as single-molecule FRET (smFRET) [16,17]. In the transverse design, tethers can be as long as the dimension of the whole view area, ideal for studies of large DNA condensation by proteins [18–20]. The tethers are stretched in the focal plane, allowing direct observation of fluorescence labeled proteins on DNA [20]. The position of the bead can be determined with nanometer accuracy with long working distance non-contact objective, making it possible to control the temperature of the sample independently from the microscope, convenient for temperature dependent studies [21–23]. In the subsequent sections, we focus on the vertical design of the magnetic tweezers because of its strength in directly stretching short tethers formed by mechanosensing proteins.

2.2. Force generation

Force generation. As illustrated in Fig. 2B, a pair of magnets is placed above the sample stage, with its geometric center aligned along the optical axis. It produces a magnetic field, $\vec{B}$, along $\hat{x}$-direction (Fig. 1A and B) and a gradient perpendicular towards the magnets ($\hat{z}$-direction). The resulting force, $\vec{F} = \bigtriangledown(\vec{M}\cdot\vec{B}) = \hat{z}\frac{d}{dz}(\vec{M}\cdot\vec{B})$, where $\vec{M}$ is the induced magnet moment of the bead, is therefore along the $\hat{z}$-direction. In typical magnetic tweezers setup, the magnetization of the bead is saturated, resulting in a constant magnitude of M_{max}. Hence, the bead experiences a force, $\vec{F} = \hat{z}M_{max}B'(z)$. The logarithm of the magnitude of force, $\ln F = \ln M_{max} + \ln B'(z)$, consists of two independent contributions from the properties of bead and the magnets, respectively. For a given magnet-bead distance (d), forces applied to two different beads differ only by a constant $\Delta \ln F_{1,2}(d) = \Delta \ln M_{max,\,1,2} = \delta_{1,2}$. The slope $F'(d)$ is $< 10^{-2}$ pN/μm for typical magnetic tweezers settings [3,11,6]. Therefore, force is insensitive to drift in d and can remain stable over long time scale (hours). Furthermore, the spatial drift of the 3D position of the tethered bead can be effectively eliminated when a stuck bead is used as a reference [11,6]. The magnitude of the force can be tuned by controlling d. Various force controls, such as constant force (constant d) and loading-rate control with linearly increasing force (by programmed $d(t)$), can be easily achieved by moving the magnets using a computer-controlled motorized manipulator.

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