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Exploring the molecular basis for mechanosensation, signal transduction, and cytoskeletal remodeling

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Abstract

Living cells respond to mechanical stimulation in a variety of ways that affect nearly every aspect of their function. Such responses can range from changes in cell morphology to activation of signaling cascades and changes in cell phenotype. Although the biochemical signaling pathways activated by mechanical stimulus have been extensively studied, little is known of the basic mechanisms by which mechanical force is transduced into a biochemical signal, or how the cell changes its behavior or properties in response to external or internal stresses. One hypothesis is that forces transmitted via individual proteins either at the site of cell adhesion to its surroundings or within the stress-bearing members of the cytoskeleton cause conformational changes that alter their binding affinity to other intracellular molecules. This altered equilibrium state can subsequently either initiate a biochemical signaling cascade or produce more immediate and local structural changes. To understand the phenomena related to mechanotransduction, the mechanics and chemistry of single molecules that form the signal transduction pathways must be examined. This paper presents a range of case studies that seek to explore the molecular basis of mechanical signal sensation and transduction, with particular attention to their macroscopic manifestation in the cell properties, e.g. in focal adhesion remodeling due to local application of force or changes in cytoskeletal rheology and remodeling due to cellular deformation.

Keywords: Mechanotransduction; Molecular mechanics and chemistry; Protein conformations; Cytoskeletal rheology and remodeling

1. Introduction

It is well known that living cells can sense mechanical stimuli. Forces applied to a cell or physical cues from the extracellular environment can elicit a wide range of biochemical responses that affect the cell's phenotype in health and disease (see for example reviews in Refs. [1–4]). Despite the wide relevance and central importance of mechanically-induced cellular response, the mechanisms for sensation and transduction of mechanical stimuli into biochemical signals, termed mechanotransduction, are still largely unknown. Various mechanisms have been proposed to explain this phenomenon and include: changes in membrane fluidity that act to increase receptor mobility and lead to enhanced receptor clustering and signal initiation [5,6]; stretch-activated ion channels [7]; mechanical disruption of microtubules [8]; and forced deformations within the nucleus [9]. Constrained autocrine signaling is yet another mechanism whereby the strength of autocrine signaling is regulated by changes in the volume of extracellular compartments into which the receptor ligands are shed [10]. Changing this volume by mechanical deformation of the tissues can increase the level of autocrine signaling. Finally,

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others have proposed conformational changes in intracellular proteins along the force transmission pathway, connecting the extracellular matrix with the cytoskeleton through focal adhesions, as the main mechanotransduction mechanism [6,11,12]. In particular, the hypothesis that links mechanotransduction phenomena to mechanically-induced alterations in the molecular conformation of proteins has been gaining increased support. For example, certain proteins that reside in 'closed' conformation can be mechanically triggered to reveal their cryptic binding clefts. Similarly, small conformational changes may also change binding affinity or enzyme activity, e.g. when protein binding occurs through hydrophobic site interactions, a conformational change could modify this function and potentially disrupt it totally. Still other examples exist now that lend evidence in support of force-induced changes in binding characteristics, such as seen by the reduced binding of RNA polymerase to DNA filaments stretched by optical tweezers [13] and the enhanced bundling of filaments following exposure of cryptic binding sites on fibronectin [14].

Force transmission from the extracellular matrix to the cell interior occurs through a chain of proteins, called focal adhesion sites, that are comprised of an integrin-extracellular matrix protein bond (primarily vitronectin and fibronectin), integrin-associated proteins on the intracellular side (paxillin, talin, vinculin, etc.), and proteins linking the focal adhesion complex to the cytoskeleton (see Fig. 1). Stresses transmitted through adhesion receptors and distributed throughout the cell could cause conformational changes in individual force transmitting proteins, any of which would be a candidate for force transduction into a biochemical signal. The process by which changes in protein conformation give rise to protein clustering at a focal adhesion or initiate intracellular signaling, however, remains largely unknown [15].

External stresses imposed on the cell are transmitted through the cytoskeleton to remote locations within the cell. To understand these stress distributions requires knowledge of cytoskeletal rheology, as governed by the structural proteins, actin filaments, microtubules, and intermediate filaments. For example, a simplified picture can be painted of the cytoskeletal rheology that is limited to actin filaments and actin cross-linking proteins living in a dynamic equilibrium. These cross-links constantly form and unbind at rates that are largely influenced by the forces borne by the individual molecules. Cytoskeletal rheology would then be determined at the molecular scale by the mechanics and binding kinetics of the actin cross-linking proteins as well as by the actin matrix itself [16].

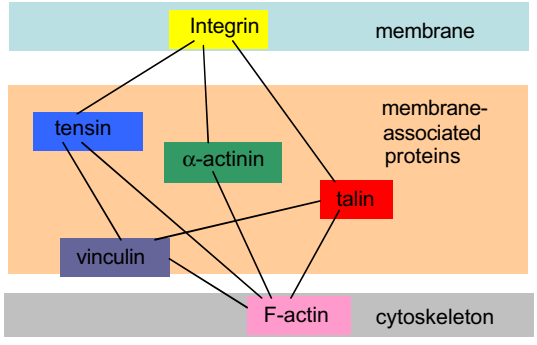

Fig. 1. A simplified view of the complex and interconnected pathways connecting extracellular domain to the cytoskeletal matrix within the confines of a focal adhesion.

To understand the phenomena related to mechanotransduction in living cells and their cytoskeletal rheology, the mechanics and chemistry of single molecules that form the biological signaling pathways that act in concert with the mechanics must be examined. This paper provides a range of case studies that seek to explore the molecular basis of mechanosensation, signal transduction, and cytoskeletal rheology and remodeling after deformation. Several examples are briefly presented that may help to introduce the reader to the different challenges that the field faces today, as well as approaches that may be used to attack these problems. Example 1, *Force-induced focal adhesion translocation: The spatial influence of force amplitude and frequency*, examines force magnitude and frequency thresholds for transducing local mechanical loads, applied via a magnetic trap, into biological signals through focal adhesion sites as marked by site translocation. Example 2, *The effect of cellular deformation on cytoskeletal rheology and remodeling*, provides evidence that mechanical force and deformation of scales comparable to those encountered by a neutrophil during transit through the microcirculation strongly impact the structure and function of the cell, as directly related to cytoskeletal rheology and post-deformation remodeling. In Example 3, *A coarse-grained model for force-induced protein deformation and kinetics*, a generic coarse-grained model is presented linking force applied to a single protein to its conformational change, expressed in terms of the mechanical properties of the protein conformational states. Finally, in Example 4, *Mechanical perturbation of the FAT–paxillin binding partnership*, molecular simulations are used to examine how force-induced changes in the molecular conformation of focal adhesion targeting domain (FAT) affect binding with its partner paxillin.

2. Case studies

2.1. Force-induced focal adhesion translocation: the spatial influence of force amplitude and frequency

2.1.1. Introduction

Focal adhesion site remodeling is used as a rapid and site-specific marker of mechanotransduction [11,17].