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Review

A biomechanical perspective on stress fiber structure and function ☆

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ABSTRACT

Stress fibers are actomyosin-based bundles whose structural and contractile properties underlie numerous cellular processes including adhesion, motility and mechanosensing. Recent advances in high-resolution live-cell imaging and single-cell force measurement have dramatically sharpened our understanding of the assembly, connectivity, and evolution of various specialized stress fiber subpopulations. This in turn has motivated interest in understanding how individual stress fibers generate tension and support cellular structure and force generation. In this review, we discuss approaches for measuring the mechanical properties of single stress fibers. We begin by discussing studies conducted in cell-free settings, including strategies based on isolation of intact stress fibers and reconstitution of stress fiber-like structures from purified components. We then discuss measurements obtained in living cells based both on inference of stress fiber properties from whole-cell mechanical measurements (e.g., atomic force microscopy) and on direct interrogation of single stress fibers (e.g., subcellular laser nanosurgery). We conclude by reviewing various mathematical models of stress fiber function that have been developed based on these experimental measurements. An important future challenge in this area will be the integration of these sophisticated biophysical measurements with the field's increasingly detailed molecular understanding of stress fiber assembly, dynamics, and signal transduction. This article is part of a Special Issue entitled: Mechanobiology.

The ability of a eukaryotic cell to adhere, spread, migrate and resist deformation depends on the ability of the cell to generate force against the surrounding extracellular matrix (ECM). These forces are not only essential for structural regulation in individual cells but can also control morphological changes in tissue during development [1]. Changes in the magnitude and direction of these forces at either the cell or tissue length scale can contribute to the development of diseases such as atherosclerosis, osteoporosis and cancer. The cytoskeleton, an interconnected network of filamentous proteins consisting of actin filaments (F-actin), microtubules, intermediate filaments, and their associated molecular motors and other accessory proteins, acts as a physical and biochemical link between the cell and the ECM [2]. The cytoskeleton senses, generates and mediates coordinated forces to maintain tensional homeostasis and control normal cell and tissue function [3,4]. It has been shown to contribute to cellular contractility and matrix reorganization in both highly simplified two-dimensional culture paradigms as well as more complex, three-dimensional microenvironments [5], reflecting the need to study how cytoskeletal components generate, transmit and withstand forces over time as a means of understanding how cells behave in vivo.

Stress fibers represent an important component of the cytoskeleton. Stress fibers are bundles of actin filaments with alternating polarity held together by various crosslinking proteins such as α-actinin and zyxin. Often, but not always, stress fibers also contain non-muscle myosin II (NMMII) bipolar filaments. Although stress fibers can resemble myofibrils in their composition, they exhibit a less organized structure; if sarcomeres are present, they are not as regular as myofibrils and actin filaments are not uniformly located along the fiber length [6]. Contraction of NMMII produces a force along the length of the fiber that is transmitted through cellular adhesions to the ECM allowing the fibers to be in isometric tension.

Stress fibers are physiologically important in processes that require cellular contraction such as wound healing and exocrine gland secretion [7]. For example, during wound healing, tension borne by specific stress fibers within fibroblasts can induce recruitment of α-smooth muscle actin to these fibers, which in turn permits even greater generation of tensile force [8,9]. This tension generation can activate latent Transforming Growth Factor β1 (TGF β1) within the matrix, promoting these fibroblasts to differentiate into myofibroblasts that drive tissue compaction [10]. This increased stress fiber-driven contractility therefore initiates a positive feedback loop of increased tension generation and myofibroblast differentiation that contributes to eventual wound closure [11,12]. Epithelial cells that line the wound site also develop actomyosin cables that contract to facilitate wound closure [13]. These principles are not limited to wound healing; epithelial cells around exocrine glands also form stress fibers whose contraction promotes

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secretion. In the presence of oxytocin, myosin is activated, leading to increased contraction around the mammary gland and eventual secretion of milk [14,15].

The growing appreciation of the physiological importance of stress fibers has spurred significant interest in quantifying the mechanical properties of stress fibers and the roles they may play in supporting cellular structure, motility and tissue processes. Thus, a rich variety of tools have recently emerged to study the mechanical and structural properties of stress fibers and determine new physical models of how stress fibers contract and contribute to the overall mechanics of the cell. In this review, we will discuss both in vitro (cell-free) and in vivo (live-cell) tools available to study the mechanical properties of stress fibers and how these tools have been used to advance our understanding of stress fiber biomechanics. Several excellent reviews have covered broad aspects of stress fiber structure and function [2,16,17]. Here, we will concentrate on current biomechanical models of stress fiber structure and function and end with a discussion of unanswered questions in the field.

1. Stress fiber composition

Stress fibers are generally defined as bundles of 10–30 thin filaments, composed of F-actin crosslinked by actin-binding proteins such as α-actinin, fascin and filamin. These thin filament bundles are frequently but not always interleaved with thick filaments composed primarily of NMMII motors, the key force-generating component in stress fibers (Fig. 1A). NMMII is a hexamer that consists of two essential light chains (ELCs), two regulatory light chains (RLCs) and two heavy chains. The heavy chains contain the head domain, which is a globular structure that can both directly engage F-actin and hydrolyze ATP to provide the free energy required to power the contractile sliding of the thick filaments against the thin filaments, thus creating tension within the stress fiber (Fig. 1B and C). Phosphorylation of the RLC facilitates this process by allowing myosin to uncoil and assemble into linear thick filaments, as well as enhancing its ATPase activity [18].

There are three NMMII isoforms in mammalian cells: NMMIIA, IIB and IIC, with the isoform specified by the heavy chain. NMMIIA and IIB are the predominant isoforms, and much effort has been devoted to understanding their differential biophysical properties and contributions to cell mechanics and motility. Much less is known about the in vivo function of NMMIIC, although it appears to play central roles in specific physiological contexts. For instance, NMMIIC is critical for the outgrowth of neuronal processes, can modulate neuronal cell adhesion [19] and is important in the cytokinesis and motility of cancer cells [20].

Within polarized cells, there is an overall differential distribution of NMMIIA and IIB with an observable NMMIIA-rich state in the front and an NMMIIB-rich state in the rear [21]. The differential localization of NMMII isoforms is thought to correspond to distinct mechanical functions: NMMIIB found at the cell rear promotes directional migration by preventing protrusion formation and properly positioning the Golgi apparatus, the nucleus and microtubules, whereas NMMIIA found at the

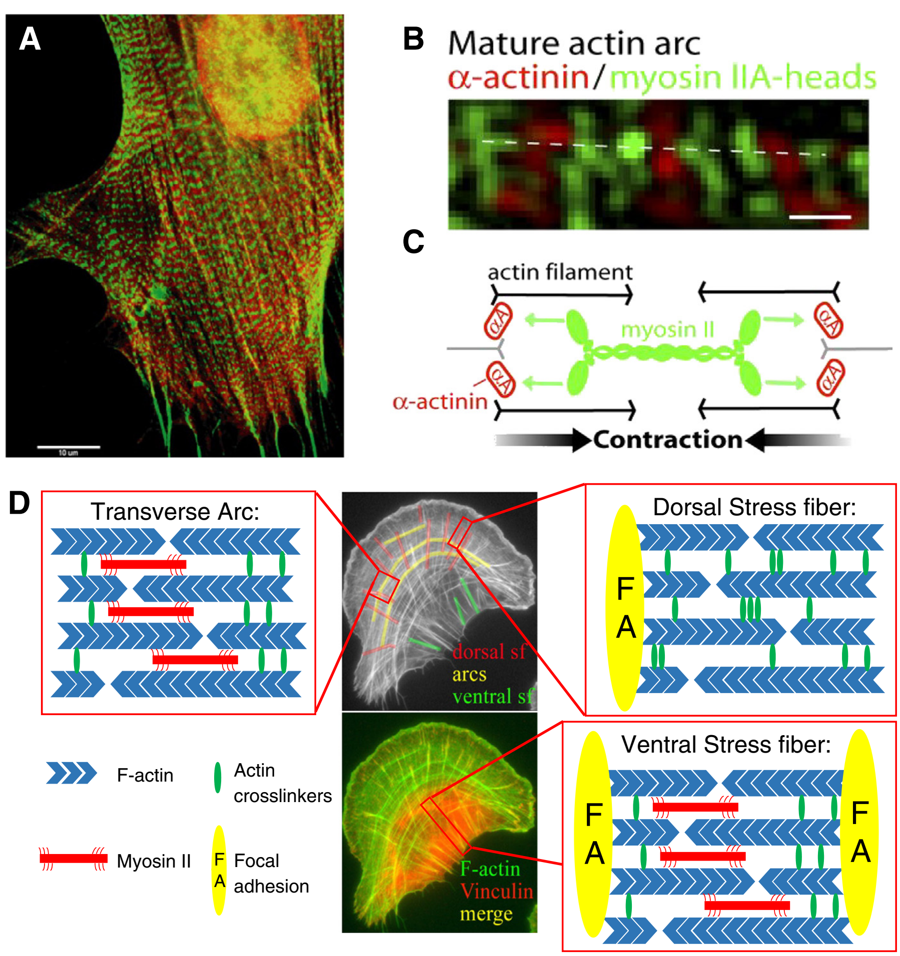

Fig. 1. Stress fiber structure and composition. (A) A gerbil fibroma cell is stained for myosin light chain (red) and α-actinin (green) to illustrate the periodic localization of proteins along the stress fiber [30]. (B) Structured illumination microscopy of the nanoscale organization of myosin IIA minifilaments in actin fibers using U2OS cells expressing α-actinin mApple (green) and myosin IIA-mEGFP [31]. (C) Schematic showing how the interaction of myosin minifilaments can lead to sarcomere contraction of stress fibers [31]. (D) Schematic of the three stress fiber subtypes indicated in a U2OS human osteosarcoma cell stained for F-actin (green) and the focal adhesion marker vinculin (red) (images from [40]). Boxed regions highlight the structural differences between the three stress fiber subtypes.

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