Experimental Cell Research ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Review Article

Focal adhesions, stress fibers and mechanical tension

Keith Burridge [a,*], Christophe Guilluy [b]

[a] Department of Cell Biology and Physiology, and Lineberger Comprehensive Cancer Center, 12-016 Lineberger, CB#7295, University of North Carolina, Chapel Hill, NC, USA
[b] Inserm UMR_S1087, CNRS UMR_C6291, L'institut du Thorax, and Université de Nantes, Nantes, France

ARTICLE INFO

Article history:
Received 5 October 2015
Accepted 23 October 2015

Keywords:
Myosin II
RhoA
Integrins
Substratum rigidity

ABSTRACT

Stress fibers and focal adhesions are complex protein arrays that produce, transmit and sense mechanical tension. Evidence accumulated over many years led to the conclusion that mechanical tension generated within stress fibers contributes to the assembly of both stress fibers themselves and their associated focal adhesions. However, several lines of evidence have recently been presented against this model. Here we discuss the evidence for and against the role of mechanical tension in driving the assembly of these structures. We also consider how their assembly is influenced by the rigidity of the substratum to which cells are adhering. Finally, we discuss the recently identified connections between stress fibers and the nucleus, and the roles that these may play, both in cell migration and regulating nuclear function.

Contents

1. Introduction

Growing in culture many cell types, particularly those of mesenchymal origin, display prominent bundles of filamentous actin (F-actin) associated with myosin II, α-actinin and several other cytoskeletal proteins. These structures, known as stress fibers (SFs) occur in several distinct forms. Frequently, they are associated at one or both ends with adhesions to the underlying matrix, known most commonly as focal adhesions (FAs). For over 40 years there has been considerable interest in the functions of these structures, their role in cell migration, and how they assemble and disassemble. Much evidence has indicated that these structures are mechanosensitive [1–5] and it was concluded earlier that mechanical tension contributes to their assembly [1,6,7]. However, several recent studies have challenged this view and demonstrated a more complex situation [8–11]. Here, we consider the role of mechanical force in the assembly of SFs and FAs.

It is often forgotten, even by those who study FAs and SFs, that these structures are not needed for cell migration [1,12]. Many cells (e.g. leukocytes) do not develop FAs or SFs but migrate highly effectively. Indeed, the presence of FAs can hinder cell migration due to excessive adhesion. Nevertheless, many migratory cells do display FAs and SFs. In these cells there must be a dynamic coupling of adhesion strength and traction force for cells to move forward. It is important that both adhesion and traction at the front are stronger than at the rear, and so mechanisms must exist

* Corresponding author.
E-mail addresses: Keith_Burridge@med.unc.edu (K. Burridge), christophe.guilluy@univ-nantes.fr (C. Guilluy).

http://dx.doi.org/10.1016/j.yexcr.2015.10.029

Please cite this article as: K. Burridge, C. Guilluy, Focal adhesions, stress fibers and mechanical tension, Exp Cell Res (2015), http://dx.doi.org/10.1016/j.yexcr.2015.10.029

for modulating these. Force at the front of migrating cells derives from both retrograde actin flow and myosin-generated tension [4].

For many years, the term stress fiber was most commonly applied to the large bundles of F-actin that traverse much of the cell and that are anchored at both ends by FAs. However, it was readily apparent that a variety of structures were often referred to as stress fibers. In 1998, Small and colleagues distinguished 2 types of SF: ventral SFs, which are anchored at each end by a FA, and dorsal SFs, which are anchored only at one end by a FA close to the cell front. Dorsal SFs extend back toward the nucleus and upwards toward the dorsal cell surface. They also discussed "arcs", convex bundles of F-actin that form behind the leading edge of migrating or spreading cells and which move rearwards below the dorsal surface [13]. Subsequent studies have included arcs, often referred to as transverse arcs, as a form of SF [14]. Arcs contain many of the same proteins, but unlike other types of SF, they are not directly anchored by adhesions to the matrix. However, arcs can give rise to ventral SFs and will be considered here as a form of SF.

One complicating factor in the relationship between mechanical tension and the assembly of FAs and SF is that the three types of filament bundle collectively referred to as SFs differ in their genesis, behavior, and relationship to FAs. Additionally, different models have been used to study how SFs and FAs assemble.

2. Systems for analyzing SF and FA assembly

Most studies have examined the assembly and disassembly of these structures as cells spread and migrate on coverslips coated with extracellular matrix (ECM) (most commonly fibronectin) [4,14–17]. This system is well suited to analyzing adhesion dynamics and actin organization as cells migrate. The second experimental model for examining FA and SF assembly was pioneered by Ridley and Hall [18]. They exploited the observation that some cells lose their SFs and FAs when deprived of serum to become quiescent. Upon re-addition of serum or other factors that activate RhoA, FAs and SFs rapidly reassemble. It should be noted that these cells are usually in a non-migratory state and often confluent. This system was used to identify RhoA as a key regulatory protein controlling the assembly of these structures. It was also the system used to show that RhoA-induced assembly of FAs and SFs was blocked by a variety of inhibitors of myosin activity and contractility. This led to the conclusion that RhoA-stimulated myosin activity drives the assembly of SFs and FAs [7]. The bundling of F-actin to form a SF was attributed not only to the tension generated by myosin but also to myosin's crosslinking of F-actin [7,19]. Contractility inhibitors available at the time of these experiments were relatively non-specific. However, subsequent studies using blebbistatin and Y27632, which inhibit the activities of myosin II and ROCK respectively, have also shown that inhibiting myosin activity blocks the formation of most FAs and SFs [20,21]. Similarly, knockout or knockdown of myosin II expression prevented maturation of nascent adhesions into FAs [21,22]. In one study this was found for both myosin IIA and IIB [22], whereas in another this was dependent on knockout of myosin IIA but not IIB [21]

This second model system (stimulation of quiescent cells with serum or Rho-activating factors) was well suited to microinjection of constitutively active or dominant negative constructs. In addition, it has the advantage of allowing synchronous assembly of FAs and SFs to be studied in many cells. However, many cell types are resistant to serum-starvation; they either maintain FAs and SFs, or show only a slight decrease in these structures when deprived of serum. Another disadvantage is that this system does not recapitulate the events that occur as cells migrate and engage the ECM at new sites.

Cell migration involves a series of transitions that affect both the adhesions and organization of the actin cytoskeleton. As the lamellipodium extends, driven by Arp2/3 complex-mediated actin polymerization, initial adhesions form as integrin receptors engage the underlying matrix [4,23]. Such "nascent adhesions" are often transient and many rapidly disassemble. Maturation of adhesions that are not disassembled occurs at the transition between the lamellipodium and lamella, where retrograde actin flow changes from being driven by actin polymerization to myosin-based contraction. Whereas actin is organized in the lamellipodium as a branching dendritic network, in the lamella it is often bundled into the different SF types [24]. The maturing adhesions elongate in the direction of retrograde actin flow and retard the rate of rearward movement of actin [25]. They act as "molecular clutches" that couple the force of retrograde flow into forward extension of the lamellipodium [4]. Consistent with this clutch-like function, it was observed that in stationary cells FAs are often pulled toward the nucleus, whereas in migrating cells they are stationary [26]. The small maturing adhesions are often referred to as "focal complexes" [27]. Some continue enlarging to become classical "focal adhesions". However, the different types of adhesion are poorly defined and it is often difficult to distinguish one type from another. In general, FAs are dependent on RhoA activity, whereas focal complexes are dependent on active Rac1 or Cdc42 [27].

Hotulainen and Lappalainen used live cell imaging to analyze assembly of the different SF types in migrating osteosarcoma cells [14]. They observed dorsal SFs initiating at small adhesions forming behind the leading edge. As the cell front extended away from the adhesion, dorsal SFs elongated. This SF growth was inhibited by depleting cells of the formin mDia1. Alpha-actinin was incorporated into the growing SF. Recruitment of myosin II into the dorsal SF was a relatively late event. A subsequent study using higher resolution imaging concluded that little if any myosin II is incorporated into dorsal SFs [16]. Transverse arcs arose behind the lamellipodium from the combination of short myosin filaments plus actin filaments generated at the leading edge by the Arp2/3 complex. In this system, ventral SFs developed most commonly from the fusion of each end of an arc with a dorsal SF. The annealing of two dorsal SFs growing from opposite sides of a cell also gave rise to ventral SFs, again anchored at each end by FAs. A subtype of ventral SFs (discussed later) is the perinuclear "actin cap", where ventral SFs wrap over nuclei and anchor to elongated FAs [17]. The different SF types are illustrated in Fig. 1.

Burnette and colleagues used the same cells to explore the factors that maintain the lamella flat as cells migrate [16]. They determined that dorsal SFs, which they found contained little to no myosin II, acted as struts connecting the ventral adhesions with the dorsal contractile actin meshwork. Their analysis revealed that contraction of transverse arcs generated tension on dorsal SFs, and this caused the dorsal SFs to pivot, thereby flattening the lamella [16].

3. The role of myosin and tension in the development of stress fibers and focal adhesions

Besides the evidence that blocking Rho-mediated myosin activity inhibited ventral SF and FA assembly in quiescent cells [7], support for mechanical tension stimulating assembly comes from several observations. For example, shear stress at levels equivalent to that experienced in arteries induced endothelial cells in culture to develop SFs [28]. Direct evidence for mechanical tension stimulating growth of FAs came from Riveline and colleagues who applied force directly to individual cells with a glass rod. They observed growth of adhesions by IRM optics and incorporation of

Please cite this article as: K. Burridge, C. Guilluy, Focal adhesions, stress fibers and mechanical tension, Exp Cell Res (2015), http://dx.doi.org/10.1016/j.yexcr.2015.10.029