EXPERIMENTAL CELL RESEARCH 326 (2014) 280–294

Available online at www.sciencedirect.com

ScienceDirect

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

Research Article

Traction force microscopy in rapidly moving cells reveals separate roles for ROCK and MLCK in the mechanics of retraction

*Timothy R. Morin Jr., Sean A. Ghassem-Zadeh, Juliet Lee**

Department of Molecular and Cell Biology, University of Connecticut, 91 North Eagleville Road, Storrs, CT 06269, USA

ARTICLE INFORMATION

Article Chronology:
Received 29 January 2014
Received in revised form
15 April 2014
Accepted 18 April 2014
Available online 29 April 2014

Keywords:
Adhesion
Cell motility
Contractility
Mechanics
Retraction
Traction stress

ABSTRACT

Retraction is a major rate-limiting step in cell motility, particularly in slow moving cell types that form large stable adhesions. Myosin II dependent contractile forces are thought to facilitate detachment by physically pulling up the rear edge. However, retraction can occur in the absence of myosin II activity in cell types that form small labile adhesions. To investigate the role of contractile force generation in retraction, we performed traction force microscopy during the movement of fish epithelial keratocytes. By correlating changes in local traction stress at the rear with the area retracted, we identified four distinct modes of retraction. “Recoil” retractions are preceded by a rise in local traction stress, while rear edge is temporarily stuck, followed by a sharp drop in traction stress upon detachment. This retraction type was most common in cells generating high average traction stress. In “pull” type retractions local traction stress and area retracted increase concomitantly. This was the predominant type of retraction in keratocytes and was observed mostly in cells generating low average traction stress. “Continuous” type retractions occur without any detectable change in traction stress, and are seen in cells generating low average traction stress. In contrast, to many other cell types, “release” type retractions occur in keratocytes following a decrease in local traction stress. Our identification of distinct modes of retraction suggests that contractile forces may play different roles in detachment that are related to rear adhesion strength. To determine how the regulation of contractility via MLCK or Rho kinase contributes to the mechanics of detachment, inhibitors were used to block or augment these pathways. Modulation of MLCK activity led to the most rapid change in local traction stress suggesting its importance in regulating attachment strength. Surprisingly, Rho kinase was not required for detachment, but was essential for localizing retraction to the rear. We suggest that in keratocytes MLCK and Rho kinase play distinct, complementary roles in the respective temporal and spatial control of rear detachment that is essential for maintaining rapid motility.

*Corresponding author.
E-mail address: juliet.lee@uconn.edu (J. Lee).

http://dx.doi.org/10.1016/j.yexcr.2014.04.015
0014-4827/

Introduction

Continuous cell movement consists of repeated cycles of protrusion at the leading edge, with retraction at the rear. This is dependent on the organization of cytoskeletal function such that adhesion formation and protrusive force generation is maximized at the front, while increased contractility and de-adhesion facilitate retraction at the rear [42]. Although the biochemical basis of these processes has been studied for some time, it is still not clear how they are coordinated with one another. An increasing number of studies now show that myosin II dependent contractile force is central to the integration of cytoskeletal function in moving cells [8,49]. An essential part of this process is the coordination of protrusion with retraction, because this will influence both cell speed and mode of movement. Since, protrusion is generally considered to be the first, essential "step" in motility a larger number of studies have focused on this process than on retraction. However, learning more about the regulation of rear detachment is equally important for understanding how cytoskeletal functions are coordinated between the front and rear.

Early studies of motile fibroblasts were the first to demonstrate that retraction can be a major limiting step in cell motility [7,6,14]. The formation of large, stable, focal adhesions at the rear of fibroblasts provides greater resistance to forward movement than weaker more labile ones. Consequently, protrusion and retraction occur as separate phases, resulting in the slow, discontinuous movement that is typical of fibroblasts. In contrast, keratocytes and other fast moving cells types such as leukocytes or *Dicytostelium* amoebae form few or no focal adhesions, which impose less resistance to retraction [28]. This increases the degree of synchrony between protruding and retracting edges, which underlies the rapid, continuous motion of keratocytes. Thus the regulation of rear detachment is a key determinant of cell speed and mode of movement.

A long-standing view is that myosin II dependent contractile forces are directly involved in pulling up the rear edge, and is supported by studies which show that retraction is impaired when myosin II is inhibited [21,54,26]. In addition, myosin II activity has been shown to act in combination with a number of biochemical mechanisms to trigger retraction [9,40,25]. Furthermore, myosin II may indirectly weaken adhesions through force-induced changes in enzyme activity, kinetics [18,51,53] or structural changes in adhesion components [44]. There is evidence to suggest that the mechanism(s) of detachment may vary depending on both substratum adhesiveness and adhesion strength. For example, retraction in fibroblasts is dependent on calpain-induced adhesion disassembly when they are attached to surfaces of high adhesiveness but not when this is low [41]. Similarly, retraction in neutrophils and *Dictyostelium* amoebae is generally dependent on myosin II activity, except when cells are attached to weakly adhesive surfaces [21,15]. Studies of retraction have generally been performed with fibroblastic cells, and so may not accurately reflect the mechanisms used by fast moving cell types.

Changes in intracellular calcium concentration $[Ca^{2+}]_i$ play a key role in regulating retraction, because they can regulate both contractility via MLCK, and adhesion disassembly by activating enzymes such as calpain or calcineurin (Kirfel, 2004). In addition, calcium signals can specify the location and timing of retraction through increasing gradients of $[Ca^{2+}]_i$ toward the cell rear [4] or by transient increases in $[Ca^{2+}]_i$, respectively [33,36,27]. Furthermore, stretch-activated calcium channels (SACs) can trigger calcium transients that induce retraction in keratocytes and *Dictyostelium* amoebae, when the rear becomes temporarily stuck [27,32]. Thus SACs can maintain rapid motility by increasing the degree of synchrony between protruding and retracting edges. It is noteworthy that retraction in leukocytes is also dependent on calcium transients suggesting that they may be particularly important for regulating detachment in cell types whose function depends on rapid movement.

The use of traction force microscopy (TFM) in a variety of cell types has shown that the largest traction forces are located at the rear consistent with their role in retraction [29,35,48,47,20,34]. In ameboid cell types, increased contractility at the rear is thought to facilitate retraction by pulling up the rear or by squeezing the cell forward. In keratocytes, calcium dependent increases in traction stress were shown to facilitate retraction through a combination of increased contractility and adhesion disassembly [11,13]. However, it is still not clear how increased contractility at the rear contributes to retraction, because the relationship between traction stress magnitude and rate of detachment has not been measured directly.

In this study, we have used TFM to measure changes in traction stress at the rear during retraction at high temporal and spatial resolution. In addition, we examined the role of contractility in rear detachment by using inhibitors to modulate calcium dependent and independent regulation of contractility, through MLCK and ROCK, respectively. Comparison of the effects of these treatments on the mechanics of detachment revealed distinct functions for MLCK and ROCK in the respective temporal and spatial regulation of retraction. Our data suggest that modulation of MLCK activity is sufficient to regulate detachment, while Rho kinase localizes retraction to the rear. We propose that this represents a new paradigm for the regulation of detachment, and is of particular importance for fast moving cell types whose function depends on rapid motility.

Methods

Reagents

Stock solutions were made as follows: ML7 (EMD Millipore, MA) at 2 mM in 50% ethanol, Y-27632 (EMD Millipore, MA) at 5 mM in PBS, the active S-enantiomer of blebbistatin at 5 mM in DMSO, calcyculin A at 10 μM in 100% ethanol, and calcimycin (A-23187, free acid, Invitrogen Corporation, CA) at 50 mM in DMSO. Before use, all inhibitors were made up at twice their final working concentration so that this was halved when an equal volume was added to the cell chamber. Inhibitor solutions were made up in RPMI 1640 (Sigma-Aldrich Corporation, MO) supplemented with ~2% serum, and syringe-filtered before use.

Preparation of polyacrylamide substrata

Polyacrylamide (PA) gels were prepared as described in detail elsewhere [43] with some minor modifications. Briefly, 22 mm^2 square, zero thickness coverslips (Electron Microscopy Sciences, Hatfield, PA) were "activated" in order to facilitate binding of the polyacrylamide gel to the coverslip. Solutions of 40% polyacrylamide and 2% bis-acrylamide (Bio-Rad, Hercules, CA) were made into a