Methods xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Methods

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

New approaches for understanding the nuclear force balance in living, adherent cells

Srujana Neelam [b], Richard B. Dickinson [a], Tanmay P. Lele [a,*]

[a] Department of Chemical Engineering, University of Florida, Gainesville FL 32611, United States
[b] Department of Biomedical Engineering, University of Florida, Gainesville FL 32611, United States

ARTICLE INFO

Article history:
Received 12 April 2015
Received in revised form 26 May 2015
Accepted 15 June 2015
Available online xxxx

Keywords:
Nuclear position
Nuclear shape
Cytoskeleton
LINC complex
Nuclear forces

ABSTRACT

Cytoskeletal forces are transmitted to the nucleus to position and shape it. Linkages mediated by the LINC (linker of nucleoskeleton and cytoskeleton) complex transfer these forces to the nuclear envelope. Nuclear position and shape can be thought to be determined by a balance of cytoskeletal forces generated by microtubule motors that shear the nuclear surface, actomyosin forces that can pull, push and shear the nucleus, and intermediate filaments that may passively resist nuclear decentering and deformation. Parsing contributions of these different forces to nuclear mechanics is a very challenging task. Here we review new approaches that can be used in living cells to probe and understand the nuclear force balance.

1. Introduction

Nuclear shape and structural abnormalities have been associated with a host of pathologies such as cancer, laminopathies and aging [1–4]. Nuclear positioning is also an important cellular function that contributes to cell polarity in critical functions such as wound healing [5]. Therefore, there is much recent interest in understanding how the nucleus is positioned and shaped in the cell. Given the large nuclear size, positioning it and shaping it in the cell requires generation of dynamic mechanical forces on it during cell migration. Cytoskeletal forces can be transferred to the nuclear surface through linkages between the cytoskeleton (and/or cytoskeletal motors) and nuclear envelope proteins [6–8].

Understanding nuclear mechanics is complicated because there are multiple, potentially competing mechanisms for generating nuclear forces. This includes myosin-mediated contractile forces [8–10], microtubule motors like dynein and kinesin [11–13] and passive resistance due to intermediate filaments like vimentin or keratin [14–17]. Parsing contributions of these different forces is a challenging task. Complicating matters further, a given cytoskeletal element may pull [18,19], push or shear [20–22], and the magnitude of these forces may vary depending on the context and cell type.

To enable design and reliable interpretation of experiments to understand nuclear forces, we have taken the view that nuclear position and shape are a result of a balance of competing forces. For example, in a migrating cell, forces generated in between the nucleus and the leading edge will act to generate a net force on the nucleus. This net force must be equal and opposite to a net force generated in the trailing edge. If this view is correct, then it gives rise to interesting questions. Is the net force from one side of the nucleus of a pushing or a pulling type? Of the various types of force generators, is there a dominant source of nuclear force? What is the magnitude of forces that are required to move and shape the nucleus? What are plausible physical explanations for nuclear motions such as nuclear rotations?

Studies in the field of nuclear mechanics have relied on a number of different methods including micropipette aspiration of isolated nuclei [23,24] and of trypsinized, whole cells [25], AFM measurements of nuclei [26], nuclear response to mechanical strain applied to adherent cells [27] and pulling on the cytoplasm [28]. Such approaches have been well-described in recent reviews [29,30]. Here we focus on multiple approaches developed in our laboratories designed to perturb and understand the nuclear force balance in living, adherent cells.

2. Modulating nuclear forces in migrating cells

To test the presence of a 'dominant' force generator and whether the net force acting on one side of the nucleus is tensile

* Corresponding author at: Department of Chemical Engineering, Bldg. 723, University of Florida, Gainesville, FL 32611, United States.
E-mail address: tlele@che.ufl.edu (T.P. Lele).

http://dx.doi.org/10.1016/j.ymeth.2015.06.014
1046-2023/

or compressive, an approach is required to selectively perturb forces only in the trailing or only in the leading edge of a migrating cell. Selectively inhibiting cytoskeletal forces by administering local doses through (for example) a micropipette to portions of the cell is challenging, considering that cytoskeletal inhibitors can diffuse throughout the small length of the cell much faster than kinetics for drug action. We approached this problem by engineering new lamellipodia in serum-starved non-migrating cells. Originally developed by Klaus Hahn's group [31], this method relies on photoactivation of Rac1 to engineer new lamellipodia [32,33]. The photoactivable Rac1 has a LOV2-Jα sequence fused to the N-terminus of constitutively active Rac1. The LOV2 domain when bound to the Jα helix blocks binding of effectors to Rac1, but when photoactivated, conformation changes cause dissociation of the Jα helix and exposes Rac1 to its effectors. To activate photoactivatable Rac1, an energy pulse from an Argon laser (488 nm) is focused on to a region of interest in cells expressing photoactivable Rac1 at regular intervals (time between intervals can be roughly 10 s). This can be easily accomplished on a conventional laser scanning confocal microscope. Photoactivation causes the formation of lamellipodia in serum-starved cells [10].

Upon engineering a lamellipodium, and then tracking the nucleus, we found that it 'drifts' toward the new lamellipodium (Fig. 1A and B) [10]. Importantly, triggering a new lamellipodium in a cell did not cause significant changes in cell morphology elsewhere over the time-scale of observation. Quantifying nuclear motion revealed that the nucleus undergoes a persistent motion toward the photoactivated spot, with smaller random positional fluctuations (Fig. 1C). This observation suggests that the forces on the nuclear surface may be locally increased due to the formation of a lamellipodium leading to a re-positioning of the nucleus.

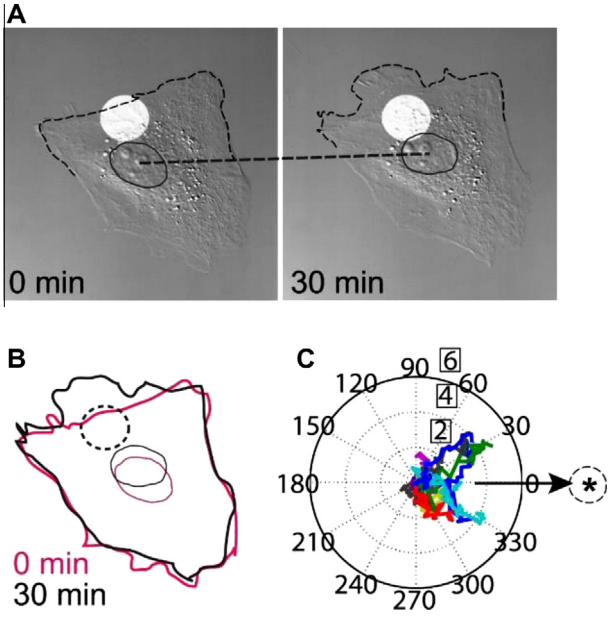

Fig. 1. Photoactivation of Rac1 to induce lamellopodium formation causes directional bias in nuclear translation. (A) DIC images of photoactivation experiment at 0 min and 30 min. Formation of new lamellopodium (black dashed line) at the photoactivation site (bright circular spot). Nucleus (outlined with black line) is observed to move towards the direction of new lamellopodium. (B) The overlap of nuclear and cell outlines at 0 min (red) and 30 min (black) show nuclear displacement. (C) Trajectories of the nucleus upon photoactivation ($n = 11$; angles are in degrees; *represents the photoactivation center, the nucleus-photoactivation center axis is oriented initially along the positive x axis); all trajectories start at the center. Boxed numbers are in microns. (Reprinted from The Biophysical society, volume 106, Actomyosin pulls to advance the nucleus in a migrating tissue cell, 1–9, Copyright (2014), with permission from Elsevier).

We were able to dissect the contributions of different cytoskeletal elements to the nuclear force balance using this approach. Because the assay relies on photoactivating Rac1 to induce lamellipodia, inhibiting myosin activity with blebbistatin, ROCK activity with Y27632, or disrupting microtubules (MT) with nocodazole did not prevent the formation of lamellipodia. This uniquely allowed us to observe nuclear motion in response to creation of a new lamellipodium in the presence and absence of key cytoskeletal force generators. Microtubule disruption did not interfere with nuclear motion toward the new lamellipodium, while myosin activity eliminated nuclear motion. In addition, the nuclear motion required an intact LINC complex as evident from a lack of nuclear motion in KASH4- expressing cells (KASH4 is a domain of nesprin-4 that binds to SUN1/2 proteins in the inner nuclear membrane; by over-expressing GFP-KASH4, endogenous KASH4 linkages with SUN1/2 are competitively inhibited [34]). These observations suggest that at least in the photoactivation assay, actomyosin forces may be increased on the nuclear surface. The net force is likely of a pulling type given that the direction of nuclear motion is toward the newly formed lamellipodium.

To manipulate forces in the trailing edge, we inserted a micropipette with a narrow tip (0.5 μm) under the trailing edge of fibroblasts. The method uses a standard Eppendorf Femtojet microinjection system to lower a micropipette close to the surface of the dish several hundred microns away from the cell. Next, the micropipette is lowered slowly to bend the main shaft as it contacts the surface of the dish, and translated toward the trailing edge. After translation under the trailing edge, the micropipette is raised through a distance of 3–5 μm, which causes detachment of the trailing edge.

Raising the micropipette to detach/rupture the trailing edge caused a rapid retraction of the trailing edge suggesting a dissipation of force from the trailing edge. Detaching the trailing edge caused nuclear motion toward the leading edge. If the net force from the leading edge is tensile as argued above, then the net force from the trailing edge also will be tensile and opposing, and forward nuclear motion in response to the detachment of the trailing edge is then a result of an imbalance of tensile forces between the front and back of the cell. In addition, such a model also predicts that the nucleus transmits tensile forces from the front to the back of the cell.

3. Traction force microscopy as a tool to probe the nuclear force balance

A limitation of the Rac1 photoactivation approach is that triggered lamellipodia may not fully capture the complex signaling events that occur during normal lamellipodial formation. We therefore developed an approach based on traction force microscopy (TFM, [35,36]) to infer the nuclear force balance in a migrating fibroblast [37]. The TFM method originally developed by Dembo and Wang [35] is a well-established technique in which cells are cultured on fibronectin-coated polyacrylamide gels. Fluorescent microspheres of 0.5 μm are suspended in the hydrogel prior to polymerization. Tracking position of the embedded microspheres under and in the vicinity of the cell and before and after complete removal of the cell from the gel allows calculation of traction stresses exerted by the cell. Traction stresses can be calculated from images of fluorescent microspheres using an algorithm developed by Dembo and Wang [35].

If the nucleus transmits internal tension from front to back of the cell, then it should coincide with where the tension is maximum. We therefore measured traction stresses generated by single migrating fibroblasts, and located the point of maximum tension (PMT) along the cell's contractile axis (Fig. 2). After calculating