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Christopher S. Chen

University of Pennsylvania, chrischen@seas.upenn.edu

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Separate but not equal: Differential mechanical roles for myosin isoforms

Abstract

Cells undergo many structural-mechanical changes as an inextricable component of cellular motility, cytokinesis, and changes in cell shape. The mere act of receptor-mediated adhesion to extracellular matrix involves massive changes in cytoskeletal organization, spreading, and flattening of cells against the matrix, and the generation of traction forces through the contractile activity of cells. The primary force-generating proteins involved in these mechanical processes are thought to be the nonmuscle myosin II's (NMM-II). Of the three different nonmuscle myosin II isoforms that have been identified, two (NMM-IIA and NMM-IIB) are found almost ubiquitously in higher organisms. Yet, it has remained unclear whether these molecules play redundant, overlapping, or distinct roles in the varying mechanical functions of cells.

Comments

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Separate but not equal: Differential mechanical roles for myosin isoforms

Christopher S. Chen

Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104

Cells undergo many structural-mechanical changes as an inextricable component of cellular motility, cytokinesis, and changes in cell shape. The mere act of receptor-mediated adhesion to extracellular matrix involves massive changes in cytoskeletal organization, spreading and flattening of cells against the matrix, and the generation of traction forces through the contractile activity of cells. The primary force-generating proteins involved in these mechanical processes are thought to be the non-muscle myosin II's (NMM-II). Of the three different non-muscle myosin II isoforms have been identified, two (NMM-IIA and NMM-IIB) are found almost ubiquitously in higher organisms. Yet, it has remained unclear whether these molecules play redundant, overlapping, or distinct roles in the varying mechanical functions of cells.

Isolating the role of different myosin isoforms in various cellular functions has been difficult in part because knockout of these proteins in mice leads to embryonic lethality (1, 2). Because cells can be isolated from NMM-IIB null mice, which die late in gestation, some insights into the function of NMM-IIB have been made. In contrast, NMM-IIA knockout leads to early lethality, leaving the community at a loss to conduct comparative studies addressing the relative contributions of these two different myosin isoforms to various cellular processes.

Cai, Sheetz and colleagues (3) in the *Biophysical Journal* have now used RNAi-mediated knockdown of non-muscle myosin IIA to provide the first insights into its role relative to NMM-IIB in regulating cellular mechanics. The authors used retrovirus-mediated expression of shRNA targeted against NMM-IIA to generate clonal cell lines with the protein knocked down by as much as 80%. Using these cell lines, they first examined whether loss of NMM-IIA affected the magnitude of traction forces generated by cells against an underlying sensor that was made of an array of elastomeric posts. It was previously shown that the deflections of such posts could be used to report traction forces (4, 5). The authors showed that while knockout of NMM-IIB led to a loss of 30% of traction force-generating capacity in fibroblasts (confirming previous studies (6)), knocking down NMM-IIA resulted in a 60% loss in force. They further showed that knocking down NMM-IIA in the NMM-IIB null cells results in 85% loss in force, and was comparable to the traction force obtained when control cells were exposed to the general myosin II inhibitor, blebbistatin. Together, these findings showed that NMM-IIA in fact is the principal force-generating myosin in nonmuscle cells, and that NMM-IIA and B together account for nearly all of the force-generating capacity in cells. Interestingly, the authors further showed that NMM-IIA was uniquely important to actin retrograde flow and regulating cell spreading against extracellular matrix, and confirmed previous reports (7) of distinct spatiotemporal distribution for the two isoforms along the radial axis of cells.

Several natural questions are raised by this study. While the direct quantitative comparison between NMM-IIA and NMM-IIB needs to be taken with some caution, especially given that the knockdown is partial, one cannot deny that the two myosins appear to have important and distinct roles in regulating cell mechanics. More direct comparisons between knockdowns of both isoforms in sister cells will be an important next step in confirming the quantitative comparisons made in this study. The differential contribution of NMM-IIA and -IIB to traction forces, actin retrograde flow, and cell spreading together with the observation of distinct spatiotemporal distribution of the two isoforms compels one to speculate that these different processes are causally related, but additional studies will be required to map these relationships more definitively. If NMM-IIA and NMM-IIB indeed have distinct functions, then are they independently regulated by distinct molecular pathways, or are they coordinately regulated by the same upstream signals? Because several regulatory signals can impinge

on myosin activity, such as the calcium-independent Rho/ROCK and the calcium-dependent myosin light chain kinase (MLCK) signaling pathways, it remains unclear whether these differentially regulate the two isoforms in different settings. In addition to biochemical regulation, it has been suggested that mechanical stresses, whether applied externally or generated by myosins, can themselves feedback to affect the contractile activity of cells. Are these two isoforms equally predisposed to such mechanical feedback? And, does the mechanical activity of one isoform then affect the function of the other? Perhaps one of the most important avenues to examine is how these two isoforms confer their differential functions. These myosins appear to associate with different proteins, which may in part regulate their localization. More work will need to be done to examine if such interactions are solely responsible for these differential roles.

It may at first appear pedagogically simpler to have a control system in which all NMM-II isoforms performed essentially redundant functions (analogous to the case of a single NMM-II isoform in lower organisms), until one attempts to explain the myriad of complex processes that this one protein can perform. The study presented by Sheetz and colleagues (3) clearly illustrates, by recognizing two of the myosins for each of their unique contributions to cellular mechanics, that fighting the urge to oversimplify such complexities will ultimately lead us to a clearer understanding of how cells function.

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