Imaging of Dynamic Changes of the Actin Cytoskeleton in Microextensions of Live NIH3T3 Cells with a GFP Fusion of the F-Actin Binding Domain of Moesin

ABSTRACT

Shape alone provided an inadequate criterion for distinguishing between retraction fibers and advancing, retracting or stable filopodia. Fluorescence imaging of C-moesin-GFP, however, paralleled the rapid and dynamic changes of the actin cytoskeleton in microextensions. Regional regulatory control is implicated because opposite changes occurred in close proximity and presumably independent of each other. This new and sensitive tool should be useful for investigating mechanisms of localized actin dynamics in the cell cortex.

INTRODUCTION

Background Lamellipodia, filopodia, retraction fibers and microspikes are dynamic and often transient membraneous structures on the surface of most cells. They can readily be observed in spreading, moving and dividing cultured cells, but also in migrating cells during development and inflammation, or in invading cancer cells in vivo. Recent evidence suggests that small GTPases of the rho family regulate this protrusive cell surface activity. Using a permeabilized Swiss3T3 cell system, Mackay et al. have shown recently that moesin, and possibly its relatives ezrin or radixin, are necessary for cellular responses induced by rho, namely the formation of lamellipodia, focal adhesion complexes and stress fibers in serum-starved fibroblasts. One or more members of this protein family is also required for the formation of filopodia in growth cones of neuronal cells, but how moesin interacts with the actin cytoskeleton during the dynamic restructuring of the cell cortex has not been entirely resolved. One suggestion has been that moesin needs to be in an "activated" form, a form capable to interact with actin filaments and to link filaments to sites in the plasma membrane. Recently, Nakamura et al. have deduced from in vitro co-sedimentation experiments with cellular forms of moesin that phosphatidylinositides and phosphorylation of a single threonine residue together are needed to convert moesin from an inactive protein to one that binds F-actin. Similarly, substitution of threonine 558 with aspartate simulated phosphorylation and activated F-actin binding of a recombinant form of moesin. An allosteric change has been proposed as the mechanism for this activation, since de-phosphorylation with a specific phosphatase obliterates binding. When expressed in cells, epitope-tagged versions of the C-terminal domain of ezrin co-distribute with stress fibers in fixed cells. This suggests that this peptide fused to GFP could serve as a probe for the imaging of dynamic changes of the actin cytoskeleton and, at the same time, of protrusive activity in live cells.

CONCLUSION

Conclusions Imaging of live NIH3T3 cells expressing the C-terminal F-actin binding domain of moesin fused to GFP before, during and after treatment with cytochalasin D, and retrospective analysis with fluorescent phalloidin are consistent with a pattern of actin microfilaments in different regions of the cells. The high sensitivity of this method allowed us to analyze dynamic and diverse changes that occur spontaneously in small areas of the cell surface and to distinguish microextensions according to their F-actin content, motility and life history. C-moesin-GFP may provide a sensitive new tool to study critical regulatory steps required to support the

highly dynamic interactions between different cytoskeleton and membrane components, and to unravel spatial and temporal relationships.