

Force-induced accumulation of adhesion-related proteins around anchored actin filaments in HeLa cell extract

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Molecular assemblies at actin stress fibers (SFs), focal adhesions (FAs) and adherens junctions (AJs) are affected by mechanical forces, but the underlying molecular mechanism remains to be solved. Actin cytoskeleton including SF is anchored to extracellular substances through clusters of adhesion receptors (integrins for FAs and cadherins for AJs), enabling force transmission between intra- and extracellular environments. To examine the role of anchored actin filaments in the force-induced assembly of SF, FA and AJ proteins, we have developed an *in vitro* experimental model: small N-ethylmaleimide (NEM)-myosin-coated glass beads were linked to a NEM-myosin-coated glass coverslip via actin filaments and actin binding proteins. Mechanical forces were applied by centrifugation to the beads in the HeLa cell extract prepared by removing organelles and insoluble structures. In response to the applied forces, SF-, FA- and AJ-related proteins, zyxin,  $\alpha$ -actinin, vinculin, talin,  $\beta$ -catenin and cadherin, were accumulated between the beads and the coverslip. The accumulation was inhibited by cytochalasin D. These results suggest that force on anchored actin filaments and actin binding proteins causes the accumulation of SF-, FA- and AJ-related proteins without aid of subcellular organelle.