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Actin deficiency induces Cofilin phosphorylation: Proteome analysis of HeLa cells after β -actin gene silencing

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Actin-binding proteins regulate the dynamic structure and function of actin filaments in the cell. Much is known about how manipulation of the actin-binding proteins affects the structure and function of actin filaments; however, little is known about how manipulation of actin in the cell affects actin-binding proteins. We addressed this question by utilizing two technologies: RNA interference and 2-dimensional gel electrophoresis. We knocked down β -actin expression in HeLa cells using short interfering RNA and applied 2-DGE to examine alterations in the HeLa cell proteome. We revealed a 2-5 fold increases of four protein spots on 2-D gels and identified these proteins by mass spectrometry. Three of the four proteins were actin-binding proteins, including cofilin, which promotes both disassembly and assembly of actin filaments but becomes inactivated when phosphorylated. Further examination revealed that the cofilin total protein level barely increased, but the phosphorylated cofilin level increased dramatically in HeLa cells after β -actin siRNA treatment. These results suggest that in response to siRNA-induced β -actin deficiency HeLa cells inactivate cofilin by phosphorylation rather than down-regulate its protein expression level. This study also demonstrates that the combination of RNA interference and 2-dimensional gel electrophoresis technologies provides a valuable method to study protein interactions in a specific cellular pathway.