STRUCTURE-FUNCTION STUDIES ON RHOA REVEAL MECHANISMS OF EFFECTOR ACTIVATION AND CELLULAR TRANSFORMATION

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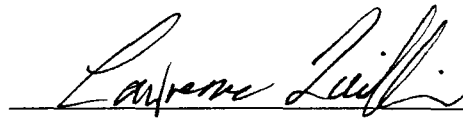

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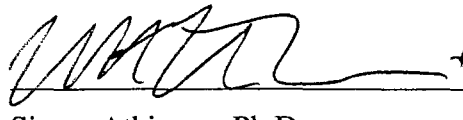

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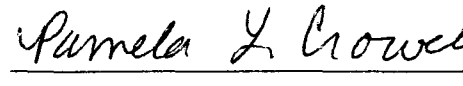

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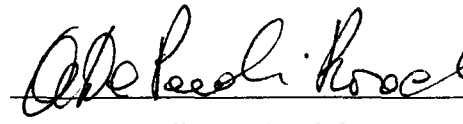

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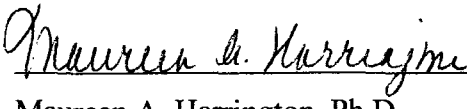

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ABSTRACT

The cells of all living organisms constantly sense and respond to environmental changes. This is achieved via signal transduction pathways that relay extracellular messages to the cellular response machinery. RhoA, a signaling intermediate in several pathways, regulates cytoskeletal organization and gene expression. When aberrantly expressed, RhoA also contributes to malignant cellular transformation, making it a potential cancer drug target. However, because the activity of RhoA is essential for normal cell survival, it is critical to delineate which of its many effector proteins/pathways are specifically involved in cellular transformation. We addressed these issues by studying the effector binding specificity of RhoA, the mechanism of effector activation, and by identifying the effector protein(s) that contributes to RhoA-induced transformation. Using mutagenesis and chimeric protein strategies, multiple effector binding sites were identified in RhoA. While Rho effectors can utilize the switch I domain to sense the GTP-bound state, unique residues within loop 6 are essential for determining both effector binding specificity and cellular functions. RhoA also contains a unique α-helix termed the insert region whose function is unknown. A deletion mutant, RhoΔRas that lacked the insert region was able to bind to effector proteins but failed to activate them. This suggests that effector interaction and activation are separable events, and that the insert region is specifically involved in the latter process. RhoΔRas was also defective in cellular transformation, while one of the RhoA downstream effector proteins, Rho-kinase/ROCK, was found to cooperate with RhoΔRas to transform cells. Y-27632, a Rho-kinase specific inhibitor, blocked RhoA-induced transformation. These data demonstrate that Rho-kinase is an effector of Rho that is required for its transforming activity.

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