

An Assay for Activity of Rho

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Technology description

LPA induced transient Rho activation in serum-starved adherent cells, whereas in suspended cells Rho activation was sustained and had higher activity in the presence of serum. These conditions can be used to efficiently monitor Rho activation.

Soluble factors from serum such as lysophosphatidic acid (LPA) are thought to activate the small GTP-binding protein Rho based on their ability to induce actin stress fibers and focal adhesions in a Rho-dependent manner. Cell adhesion to extracellular matrices (ECM) has also been proposed to activate Rho, but this point has been controversial. An assay for GTP-bound cellular Rho was established. Plating Swiss 3T3 cells on fibronectin-coated dishes elicited a transient inhibition of Rho, followed by a phase of Rho activation. The activation phase was greatly enhanced by serum. In serum-starved adherent cells, LPA induced transient Rho activation, whereas in suspended cells Rho activation was sustained. Furthermore, suspended cells showed higher Rho activity than adherent cells in the presence of serum. These data indicate the existence of an adhesion-dependent negative-feedback loop. Both cytochalasin D and colchicine trigger Rho activation despite their opposite effects on stress fibers and focal adhesions.

Institution

[The Scripps Research Institute](#)

Inventors

[A Arvai](#)

联系我们



叶先生

电话 : 021-65679356

手机 : 13414935137

邮箱 : yeyingsheng@zf-ym.com