Temporal regulation of phosphotyrosine-modified Rac1 in response to epidermal growth factor stimulation

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Abstract

The role of protein tyrosine phosphatase (PTP) activity in the regulation of GDP (GTPase) exchange reactions has been investigated in a variety of systems. Tyrosine phosphorylation (pT) is known to influence PTP activity, providing cues for the regulation of protein signaling pathways. However, the mechanisms by which PTP activity may influence signaling are not well understood. In this study, we examined the role of PTP activity in the regulation of GTPase signaling pathways. We found that PTP activity is regulated by the activity of PTPs, and that PTP activity may influence signaling by modulating the activity of PTPs.

Results

1. APP3 is specific to pT modification

(a) Phosphorylation (pT, pS, and pY) was detected by IFA, and increasing amounts of phosphatase (DA) were added to the assay. (b) An increase in pT was observed in cells treated with DA. (c) Immunoprecipitation of pT was performed using antibodies specific to DA and pT. (d) The immunoprecipitated pT proteins were resolved by SDS-PAGE and transferred to a PVDF membrane. Immunoblotting was conducted using APY03 antisera. The pT modification was detected in cells treated with DA.

2. Detection and enrichment of pT proteins by APP3

(a) The amount of pT was measured using an ELISA. (b) The amount of pT was increased in cells treated with DA. (c) The amount of pT was increased in cells treated with DA, and the amount of pT was decreased in cells treated withDA.

3. Change of pT Racu and Rac1-GTP levels in HeLa treated with EGF

(a) Racu and Rac1-GTP levels were measured using an ELISA. (b) The amount of Racu was increased in cells treated with EGF. (c) The amount of Rac1-GTP was increased in cells treated with EGF. (d) The amount of Rac1-GTP was increased in cells treated with EGF, and the amount of Racu was decreased in cells treated with EGF.

Conclusions

1. APP3 and APP3 antisera are useful tools to detect endogenous tyrosine phosphorylated proteins.
2. Modifications such as tyrosine phosphorylation and/or Rac1 activation status are important factors in the regulation of protein signaling.
3. At steady state, activated Rac1 is essential for the tyrosine phosphorylation status of Rac1.

References


Images:

A) Immunofluorescence microscopy of a HeLa cell line. B) Immunoblotting of a HeLa cell line. C) Western blotting of a HeLa cell line. D) ELISA of a HeLa cell line.