

P003 The proteolytic degradation of RhoB requires palmitoylation of its C-terminal end
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The small GTPase RhoB plays important roles in tumor suppression, susceptibility to apoptosis and regulation of endocytic traffic. Cellular levels of RhoB are subjected to a complex regulation through transcriptional and posttranscriptional mechanisms. RhoB is a short-lived protein whose turnover requires isoprenylation of a cysteine residue present in a C-terminal CAAX box motif (C193). RhoB posttranslational processing also involves the palmitoylation of two cysteine residues close to the isoprenylated cysteine (C189 and C192), a process important for RhoB localization and biological function. However, inhibition of RhoB isoprenylation precludes subsequent posttranslational processing. In order to assess the importance of palmitoylation for RhoB degradation we have used palmitoylation-deficient mutants, which retain the isoprenylation site. We have observed that mutation of C189 alters GFP-RhoB localization and reduces its degradation, whereas substitution of C192 elicits a diffuse cytosolic distribution and completely stabilizes the protein. Moreover, inhibition of protein palmitoylation by 2-bromopalmitate increases the protein levels of both endogenous and transfected RhoB, thus pointing to the involvement of posttranslational mechanisms in this effect. Taken together these observations suggest that the modifications of RhoB C-terminus, mainly C192 palmitoylation, play a key role in the localization and in the rapid turnover of the protein, both of which may be linked processes. Moreover, they identify a potential novel point of regulation of RhoB levels through the activity of RhoB palmitoyltransferases/esterases.