

Regulatory mechanism of FilGAP activity by phosphorylation

生物科学専攻 細胞機能制御学

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FilGAP is a Rho GTPase-activating protein (GAP), which specifically regulates Rac. FilGAP is phosphorylated by ROCK, and this phosphorylation stimulates its RacGAP activity. However, it is unclear how phosphorylation regulates cellular functions and localization of FilGAP. We found that non-phosphorylatable FilGAP (ST/A) mutant is predominantly localized to cytoskeleton along actin filaments and partially co-localized with vinculin around cell periphery, whereas phosphomimetic FilGAP (ST/D) mutant is diffusely cytoplasmic. Moreover, phosphorylated FilGAP detected by Phos-tag is also mainly localized in the cytoplasm. Of the six potential phosphorylation sites in FilGAP tested, only mutation of serine 402 to alanine (S402) resulted in decreased cell spreading on fibronectin. FilGAP phosphorylated at S402 is localized to cytoplasm but not at the cytoskeleton. Although S402 is highly phosphorylated in serum-starved quiescent cells, dephosphorylation of S402 accompanied with the cell spreading on fibronectin. Treatment of the cells expressing wild-type FilGAP with Calyculin A, a Ser/Thr phosphatase inhibitor, suppressed cell spreading on fibronectin whereas cells transfected with FilGAP S402A mutant was not affected cell spreading by Calyculin A. Expression of constitutively activate Arf6 Q67L mutant stimulated membrane blebbing activity of both non-phosphorylatable (ST/A) and phosphomimetic (ST/D) FilGAP mutants. Conversely, depletion of endogenous Arf6 suppressed membrane blebbing induced by FilGAP (ST/A) and (ST/D) mutants. Our study suggests that Arf6 and phosphorylation of FilGAP may regulate FilGAP and phosphorylation of S402 may play a role in the regulation of cell spreading on fibronectin.