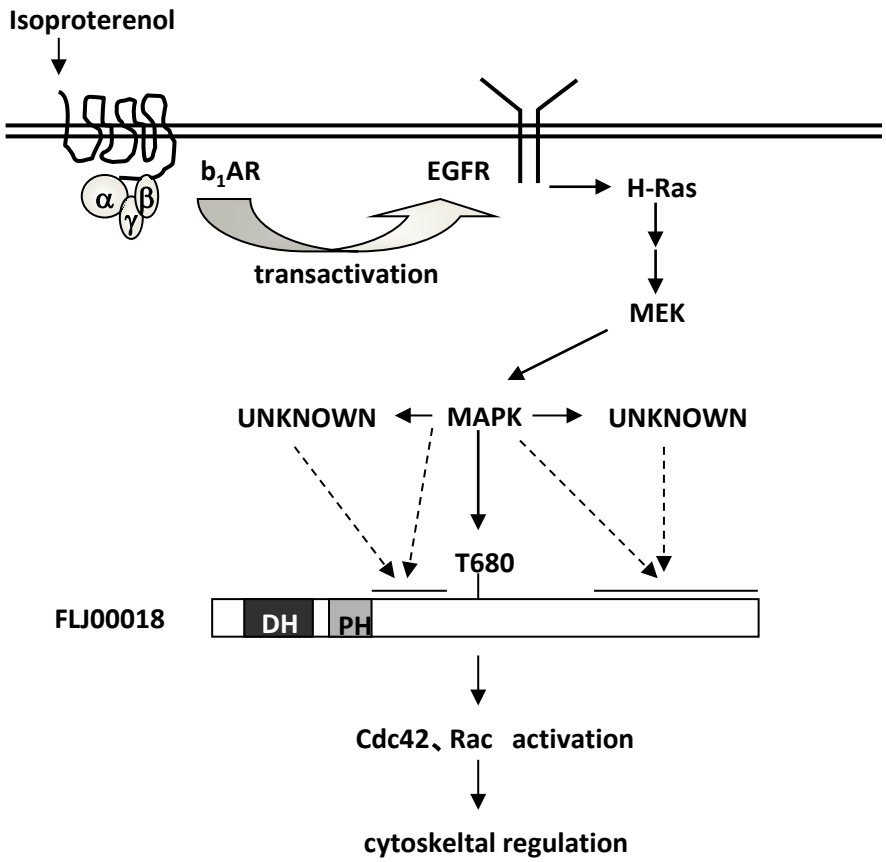
Threonine 680 phosphorylation of FLJ00018/PLEKHG2, a Rho family-specific guanine nucleotide exchange factor, by epidermal growth factor receptor signaling regulates cell morphology of Neuro-2a cells.



FLJ00018/PLEKHG2 is a guanine nucleotide exchange factor for the small GTPases Rac and Cdc42 and has been shown to mediate the signaling pathways leading to actin cytoskeleton reorganization. The function of FLJ00018 is regulated by the interaction of heterotrimeric GTP-binding protein Gβγ subunits or cytosolic actin. However, the details underlying the molecular mechanisms of FLJ00018 activation have yet to be elucidated. In the present study we show that FLJ00018 is phosphorylated and activated by β1-adrenergic receptor stimulation-induced EGF receptor (EGFR) transactivation in addition to Gβγ signaling. FLJ00018 is also phosphorylated and activated by direct EGFR stimulation. The phosphorylation of FLJ00018 by EGFR stimulation is mediated by the Ras/mitogen-activated protein kinase (MAPK) pathway. Through deletion and site-directed mutagenesis studies, we have identified Thr-680 as the major site of phosphorylation by EGFR stimulation. FLJ00018 T680A, in which the phosphorylation site is replaced by alanine, showed a limited response of the Neuro-2a cell morphology to EGF stimulation. Our results provide evidence that stimulation of the Ras/MAPK pathway by EGFR results in FLJ00018 phosphorylation at Thr-680, which in turn controls changes in cell shape.

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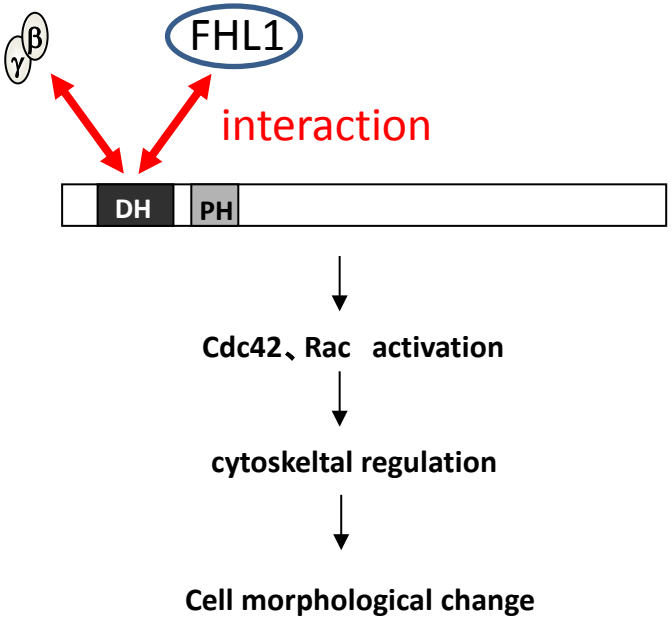
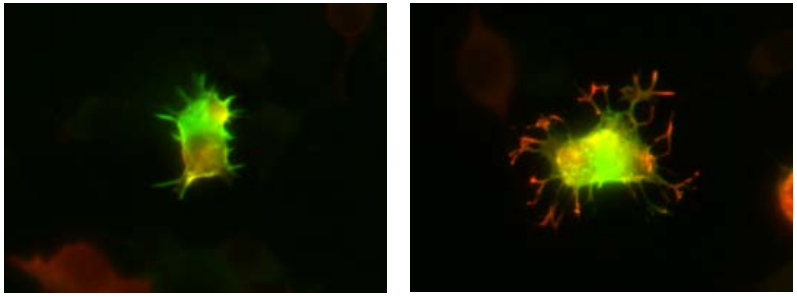
Four-and-a-half LIM domains 1 interacts with PLEKHG2/FLJ00018 and regulates cell morphogenesis





PLEKHG2/FLJ00018 is a Gβγ-dependent guanine nucleotide exchange factor for the small GTPases Rac and Cdc42 and has been shown to mediate the signaling pathways leading to actin cytoskeleton reorganization. Here we showed that the zinc finger domain-containing protein four-and-a-half LIM domains 1 (FHL1) acts as a novel interaction partner of PLEKHG2 by the yeast two-hybrid system. Among the isoforms of FHL1 (i.e., FHL1A, FHL1B and FHL1C), FHL1A and FHL1B interacted with PLEKHG2. We found that there was an FHL1-binding region at amino acids 58–150 of PLEKHG2. The overexpression of FHL1A but not FHL1B enhanced the PLEKHG2-induced serum response element (SRE)-dependent gene transcription. The co-expression of FHL1A and Gβγ synergistically enhanced the PLEKHG2-induced SRE-dependent gene transcription. Increased transcription activity was decreased by FHL1A knockout with the CRISPR/Cas9 system. Compared to PLEKHG2-expressing cells, the number and length of finger-like protrusions were increased in PLEKHG2-, Gβγ-, and FHL1A-expressing cells. Our results provide evidence that FHL1A interacts with PLEKHG2 and regulates cell morphological change through the activity of PLEKHG2.
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