

**P009** C-peptide activates Rac and Cdc42 and their downstream effectors PAK, LIMK and cofilin in CD4-positive cells  
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C-peptide, a cleavage product of proinsulin, induces CD4-positive cell chemotaxis. This process involves pertussis toxin sensitive G-proteins and PI 3-kinase  $\gamma$ . In chemokine-induced migration Rac and Cdc 42, members of the Rho family of GTP-ases, play an important role downstream of PI 3-kinase. We showed that Clostridium difficile toxin B, an inhibitor of Rho family of GTP-ases, inhibits C-peptide induced CD4-positive cell chemotaxis, suggesting involvement of Rho GTP-ases in this process. By affinity precipitation assays, using GST-PBD, we showed that 10 nM C-peptide induces both Rac and Cdc 42 activation with the maximum effect after 3 minute stimulation. When active, both GTP-ases bind to PAK, stimulating its phosphorylation and activation. Activated PAK phosphorylates and activates LIM Kinase, which in turn phosphorylates cofilin at Ser-3. Western blot analysis showed that 10 nM C-peptide induced Thr phosphorylation of PAK and LIMK, and Ser phosphorylation of cofilin, with the same maximal effect after 3 minutes. Phosphorylation inactivates cofilin, inhibiting its ability to depolymerize and sever F-actin, stabilizing actin filaments. These data suggest a chemotactic effect of C-peptide in CD4-positive cells and describe C-peptide signalling pathway downstream from PI 3-kinase leading to cofilin inactivation.