Chemotaxis requires localized F-actin polymerization at the site of the plasma membrane closest to the chemoattractant source, a process controlled by Rac/Cdc42 GTPases. We identify Dictyostelium RacB as an essential mediator of this process. RacB is activated upon chemoattractant stimulation, exhibiting biphasic kinetics paralleling F-actin polymerization. racB null cells have strong chemotaxis and morphogenesis defects and a severely reduced chemoattractant-mediated F-actin polymerization and PAKc activation. RacB activation is partly controlled by the PI3K pathway. pi3k1/2 null cells and wild-type cells treated with LY294002 exhibit a significantly reduced second peak of RacB activation, which is linked to pseudopod extension, whereas a PTEN hypomorph exhibits elevated RacB activation. We identify a RacGEF, RacGEF1, which has specificity for RacB in vitro. racgef1 null cells exhibit reduced RacB activation and cells expressing mutant RacGEF1 proteins display chemotaxis and morphogenesis defects. RacGEF1 localizes to sites of F-actin polymerization. Inhibition of this localization reduces RacB activation, suggesting a feedback loop from RacB via F-actin polymerization to RacGEF1. Our findings provide a critical linkage between chemoattractant stimulation, F-actin polymerization, and chemotaxis in Dictyostelium.