
Recent studies have demonstrated that PH domains specific for PI(3,4,5)P3 accumulate at the leading edge of a number of migrating cells and that PI3Ks and PTEN associate with the membrane at the front and back, respectively, of chemotaxing Dictyostelium discoideum cells. However, the dependence of chemoattractant induced changes in PI(3,4,5)P3 on PI3K and PTEN activities have not been defined. We find that bulk PI(3,4,5)P3 levels increase transiently upon chemoattractant stimulation, and the changes are greater and more prolonged in pten– cells. PI3K activation increases within 5 s of chemoattractant addition and then declines to a low level of activity identically in wild–type and pten– cells. Reconstitution of the PI3K activation profile can be achieved by mixing membranes from stimulated pi3k1−/pi3k2− cells with cytosolic PI3Ks from unstimulated cells. These studies show that significant control of chemotaxis occurs upstream of the PI3Ks and that regulation of the PI3Ks and PTEN cooperate to shape the temporal and spatial localization of PI(3,4,5)P3.