The Kennedy pathway is vital for the synthesis of phosphatidylethanolamine (PE) that is incorporated into cell membranes and lipid-based signaling molecules. The three steps of the Kennedy pathway are catalyzed by ethanolamine kinase, CTP-phosphoethanolamine Cytidyltransferase, and selenoprotein I (SELENOI). Our data suggest that these enzymes are upregulated during T cell activation as a mechanism for increasing PE that is used to support increased membrane synthesis and lipidated signaling proteins. Moreover, we have analyzed micro-RNAs (miRs) and found that miR-16-1 and mR-15a, which are linked in the genome and co-expressed, have sequences predicted to target all three enzymes comprising the Kennedy pathway. Thus, we hypothesized that levels of miR-16-1/15a decrease upon T cell activation to allow increased expression of one or more of these three enzymes and thereby increased synthesis of PE. Using real-time PCR and western blot analyses, we found that both miR-16-1 and miR-15a decreased, while levels of the three enzymes increased, in mouse primary T cells upon T cell receptor (TCR) activation. This coincided with increased levels of different PE species as measured by LC-MS. Overall, these data support the model of miR-16-1/15a regulation of the Kennedy pathway in which miR-16-1/15a levels are higher in naïve T cells as a means to keep PE synthesis low. Upon TCR engagement, these miRs are reduced and all three enzymes are upregulated, thereby allowing increased PE synthesis required by activated T cells.