choice, active repression of critical targets and pathways plays a crucial, and perhaps pivotal, role in final commitment. The extent to which this holds for other tissue-restricted stem cells or for embryonic stem cells remains to be explored. For example, does the pluripotential state of embryonic stem cells depend on activation of stem cell genes or repression of genes that restrict developmental options? Considerable effort has been expended of late in gene expression profiling in a search for "stemness" genes, i.e., those which are specifically expressed in common in different types of stem cells (Ivanova et al., 2002; Ramalho-Santos et al., 2002). Whether this is a meaningful exercise is actively debated by stem cell investigators. The transcriptional promiscuity of HSCs, albeit restricted with respect to all potential developmental pathways, suggests that a focus on stemness genes per se may obscure the mechanisms operative in stem cells to control their transition from multipotentiality to single lineage differentiation.

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Amplifying Btk's Signal

The Tec kinase Btk is an important regulator of antigen receptor activation of phospholipase C-γ (PLC-γ). Data from Carpenter and colleagues (Saito et al., 2003, this issue of *Immunity*) now suggest that Btk also activates phosphatidylinositol-4-phosphate 5-kinase (PIP5K), thereby stimulating a positive feedback loop that generates PI(4,5)P_2, the substrate for both phosphoinositide 3-kinase (PI3K) and PLC-γ.

Phosphoinositides are critical intermediates that regulate many cellular processes including cell survival, growth and proliferation, Ca^{2+} mobilization, membrane trafficking, cytoskeletal rorganization, and migration (Takenawa and Itoh, 2001). PI3K, which generates phosphatidylinositol-3,4,5-trisphosphate (PIP_3), a phosphoinositide that binds to and activates proteins containing pleckstrin homology domains, is an important component of immunoreceptor signaling. Similarly, PLC-γ, which generates inositol trisphosphate (IP_3) and diacylglycerol (DAG), is a key activator of Ca^{2+} mobilization and PKC/Ras-mediated pathways. Both PI3K and PLC-γ share the common substrate, phosphoinositide 4,5 bisphosphate [PI(4,5)P_2], the product of phosphatidylinositol-4-phosphate 5-kinase (PIP5K). PI(4,5)P_2 is one of the most highly abundant phosphoinositides, yet, in other systems, receptor activation can significantly decrease PI(4,5)P_2 levels. Replenishing PI(4,5)P_2 levels should therefore be important for sustaining receptor responses. Although Rac has been implicated in activation of PIP5K via ARF6-mediated trafficking to the plasma membrane (Honda et al., 1999), relatively little is known about PIP5K activation in lymphocytes. New data from Carpenter and colleagues suggest that the Tec kinase Btk is a key player in this process (Figure 1) (Saito et al., 2003).

The Tec family of nonreceptor tyrosine kinases are notable for their amino-terminal pleckstrin homology domains, which bind to the products of PI3K, and PI3K activity is required for their function (reviewed in Schaeffer and Schwartzberg, 2000). Mutations affecting Btk cause the human immunodeficiency X-linked agammaglobulinemia (XLA) and the mouse mutant X-linked immunodeficiency, which are associated with impaired responses to B cell receptor engagement. Studies of Btk-deficient cells revealed that Btk is an important regulator of antigen-receptor-induced Ca^{2+} mobilization (Fluckiger et al., 1998; Takata and Kurosaki, 1996). Cells lacking Btk show defects in sustained Ca^{2+} influx, which are associated with decreased phosphorylation of PLC-γ and impaired IP_3 production. Tec kinases have also been implicated in similar roles downstream of the Fcε receptor in mast cells and the T cell receptor in T lymphocytes. As a consequence, lymphocytes deficient in Tec kinases show decreased activation of multiple downstream readouts dependent on IP_3 and DAG production, including activation of transcription factors, MAP kinases, and protein kinase C (Schaeffer and Schwartzberg, 2000).

Although Btk has been implicated in the direct phosphorylation of PLC-γ, tyrosine phosphorylation of PLC-γ can still be observed in lymphocytes deficient in Tec kinases, despite clear Ca^{2+} defects (Fluckiger et al., 1998; Schaeffer and Schwartzberg, 2000). Thus, it remained unclear whether phosphorylation was the only mechanism by which Tec kinases regulate PLC-γ activity. Moreover, overexpression of either wild-type or ki-

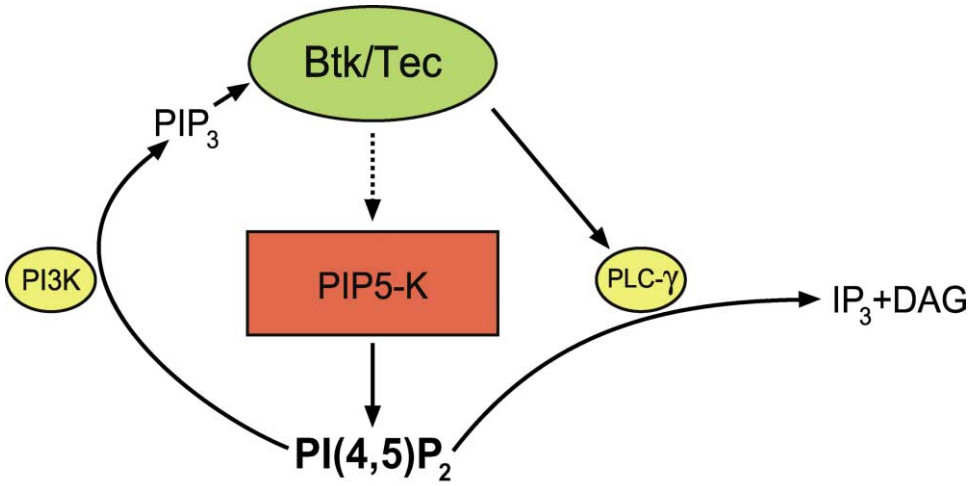

Figure 1. Btk Stimulates a Positive Feedback Loop in Antigen Receptor Signaling by Activating PIP5K to Increase Generation of $PI(4,5)P_2$, the Substrate for Both PI3K and PLC-γ

nase-inactive Btk had been shown to increase PIP_3 levels and to partially rescue Ca^{2+} mobilization in Btk-deficient DT-40 cells, suggesting that Btk may have kinase-independent functions in regulating phosphoinositide metabolism (Scharenberg et al., 1998; Tomlinson et al., 2001).

The work of Saito and colleagues in this issue of *Immunity* helps clarify this role of Btk (Saito et al., 2003). In this paper, the authors confirm that overexpression of Btk increases both Ca^{2+} mobilization and PIP_3 levels, independent of Btk kinase activity. Speculating that Btk may affect phosphoinositide regulation, the authors then show that PIP5K activity can be coimmunoprecipitated with Btk and that overexpression of Btk or the Btk-PH domain causes membrane localization of PIP5K, similar to that induced by ARF6. A direct interaction between the PH domain of Btk and PIP5K activity could be detected. While most of the work utilized overexpression studies, Btk-deficient DT-40 cells exhibited decreased $PI(4,5)P_2$ levels upon antigen stimulation, suggesting physiologically important roles for this process (Figure 1). Overexpression of both Btk and PIP5K prolonged BCR-induced Ca^{2+} mobilization and increased PIP_3 levels, arguing that, together, these molecules amplify signals in response to antigen receptor stimulation.

The interaction between Btk and PIP5K has important implications for establishing a positive regulatory loop in antigen receptor signaling. By activating PIP5K, Btk creates more substrate both for PI3K, which is required for Btk activation, as well as for PLC-γ, which is a downstream effector of Btk. This leads to a particularly efficient mode of signal amplification, by both increasing Btk activation and providing more substrate for its target. This amplification loop may account for the effect of Tec kinases on the prolonged phase of capacitive Ca^{2+} entry, which may be more dependent on continued signal propagation. Indeed, T cells deficient in Tec kinases have phenotypes resembling those resulting from decreased signal duration (Schaeffer and Schwartzberg, 2000). Decreased $PI(4,5)P_2$ levels and reduced PI3K activity may also account for decreased activation of Akt in Btk-deficient cells.

Such signal amplification could also contribute to other pathways downstream of antigen receptors. $PI(4,5)P_2$ itself plays important roles in actin cytoskeleton organization—$PI(4,5)P_2$ binds to and regulates a number of actin regulatory proteins including profilin, gelsolin, α-actinin, and WASP, the Wiskott-Aldrich Syndrome Protein, mutations of which disrupt the actin cytoskeleton in T cells and platelets (Takenawa and Itoh, 2001). Intriguingly, recent data implicate Tec kinases as regulators of actin cytoskeleton reorganization. Expression of activated BTK, like localized activation of PIP5K by ARF6, increases actin accumulation and membrane ruffling (Nore et al., 2000). Moreover, mutations affecting the Tec kinase Itk impair TCR-driven actin reorganization and cell polarization (Grasis et al., 2003; Labno et al., 2003). Data suggest that Itk may affect actin polymerization in a kinase-independent manner, reminiscent of the kinase-independent regulation of PIP5K by BTK (Grasis et al., 2003). Although it is not known whether Itk can regulate PIP5K, it is interesting to speculate that local recruitment of PIP5K to the site of antigen presentation via interactions with Itk could contribute to T cell polarization. Rac/ARF6 are also likely to play important roles in this process, emphasizing the many layers of positive-feedback that may occur in signaling pathways.

It is also intriguing to speculate that Tec kinases may participate in amplification loops in other pathways. In chemokine signaling, stimulation of G protein-coupled receptors causes the localized activation of PI3K and the rapid recruitment of proteins containing PIP_3 binding PH domains to the leading edge of the polarized cell, setting up a cellular compass for migration (Bourne and Weiner, 2002). This gradient of PIP_3 and PH containing proteins within the cell appears greater than the chemokine gradient outside the cell, suggesting an internal amplification loop. A positive-feedback loop that supplies more substrate for PI3K could provide a mechanism for such signal amplification. Indeed, evidence now suggests that Tec kinases participate in chemokine signaling (Gilbert et al., 2003).

Btk's roles in phosphoinositide signaling are likely to be more complex than this paper suggests. Mutations

affecting Tec kinases decrease activation of Rho family GTPases (Labno et al., 2003) and production of phosphatidic acid, both of which help activate PIP5K via ARF6 (Honda et al., 1999), suggesting multiple levels of PIP5K regulation. Nonetheless, given the functions of phosphoinositides in membrane trafficking, membrane-actin interactions and cell adhesion, the role of Tec kinases in regulating PIP5K raises many questions about how these kinases affect signaling pathways in lymphocytes and the nature of the defects in XLA. While the consequences of these interactions remain to be explored, this paper provides interesting insight into the interplay and feedback between components in signaling pathways.

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