ily portrait of the heterotrimeric G protein pathways is shown on the STKE Web site (3). Although the composite map appears quite complex, this is a first-level representation where the multiple isoforms of the different components are not shown. Since these maps are canonical representations, not all of these pathways and connections would be present in every cell type. As cell type–specific Connections Maps are constructed, it will be interesting to compare those with the canonical maps to determine which pathways occur in which cell type. The Gs pathway in Fig. 2 illustrates several general patterns that emerge from this complex picture. First, all G proteins engage multiple signaling pathways and consequently different cellular machines. This often helps produce effects with distinct rates of activation and duration of response. In neurons, cAMP can act through PKA to produce short-term effects on channel functions, and through Rap and MAPK to regulate gene expression and produce long-term effects through regulation of the transcriptional machinery. Second, it appears that all G proteins regulate the activity of GTPases such as Rap and Rho. Third, all G protein pathways either stimulate or inhibit one or more of the MAPK signaling pathways. All of these interconnections result in a complex and likely robust network in which signals from G protein–coupled receptors can be fully integrated with signals from other receptors.

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VIEWPOINT

Control of T Cell Function by Positive and Negative Regulators

Andrew L. Singer and Gary A. Koretzky*

T cells are an essential element of the body's immune system. Engagement of the T cell receptor is responsible for initiating the signaling events that can activate, inactivate, or eliminate T cells, depending on the magnitude and duration of the signal. Control of T cell signaling occurs through both positive and negative regulation, as well as through the actions of molecular scaffolds that contribute to the formation of signaling complexes. The T Cell Signal Transduction Pathway at the STKE Connections Maps highlights the molecular components that are responsible for T cell activation. Understanding the mechanisms that regulate T cell responsiveness will aid in the development of therapeutic agents to treat infection, cancer, and autoimmune disease and immune deficiency.

T cells play critical roles in the body's defense against pathogenic challenges and its ability to recognize and eliminate cells that have undergone malignant transformation. These abilities require the T cell to discriminate between "self" and "nonself," whether in the form of foreign antigens or inappropriate expression of endogenous proteins bycancerous tissues. The T cell antigen receptor (TCR) is responsible for making this distinction. One component of the TCR is a disulfide-linked dimer (α/β) whose proteins arise from gene segment rearrangements that provide the opportunity for virtually unlimited diversity. The α and β chains associate on the cell surface with the invariant CD3 complex, which transduces signals into the cell after α/β engagement by antigen (*1*). As T cells mature in the thymus, cells expressing TCRs that fail to interact with major histocompatibility proteins and thus cannot respond to foreign antigen, and cells expressing TCRs prone to interact with normal self antigens, are eliminated through a selection process that interprets the magnitude and duration of TCR signaling. Those cells that pass selection emigrate from the thymus, where they again rely on their TCRs to detect ligand engagement and to provoke an effector response. Studies over the past few years have provided a wealth of new information regarding the molecular events that occur after ligation of the TCR and ultimately result in biological effects.

As with all complex biological systems, each step of the signaling pathway initiated by TCR engagement (*2*) is subject to both positive and negative regulation (Fig. 1). For example, one of the first biochemical consequences of TCR binding is activation of Lck (*3*), a Src-family protein tyrosine kinase (PTK). Lck itself is regulated positively and negatively by other enzymes. Positive regulation is accomplished through the cell-surface CD45 protein tyrosine phosphatase, which is required to dephosphorylate a COOH-terminal tyrosine that negatively regulates Lck function (*4*).

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Lck enzymatic function is inhibited when COOH-terminal Src kinase (CSK), a cytosolic PTK, phosphorylates this tyrosine keeping Lck in a closed and, hence, inactive conformation (*5*). The balance between CD45 and CSK function on Lck is controlled in part by where the molecules are localized in the cells, which itself is a dynamic process modulated by other signals received by the cells.

Once Lck is activated by the engaged TCR, key substrates are phosphorylated, including specialized motifs of the CD3 chains (*6*). Phosphorylation of these immunoreceptor tyrosine-based activation motifs (ITAMs) creates docking sites for the tandem Src homology 2 (SH2) domains of the Syk PTK ZAP-70. As a result of ZAP-70 recruitment, the TCR is effectively endowed with PTK function, resulting in phosphorylation of numerous proteins and activation of a series of second-messenger cascades (*7*). These include, among others, phospholipase cγ-1, activation of which initiates the phosphatidylinositol pathway and leads to increases in calcium and diacylglycerol; Vav, an exchange factor for small guanosine-triphosphates (GTPases), which facilitate cytoskeletal reorganization; Itk and other members of the Tec PTK family; and components of lipid kinase signaling pathways [see the T Cell Signal Transduction Pathway (http://stke.sciencemag.org/cgi/cm/CMP_ 7019) for details (*2*)].

As it became clear that TCR engagement leads to multiple signaling pathways, experiments were designed to investigate how these various biochemical cascades are integrated. These efforts led to the discovery of a number of hematopoietic-specific adaptor proteins that also serve as substrates of the TCR-stimulated PTKs. Examples include linker of activated T cells (LAT) and SH2 domain-containing leukocyte phosphoprotein of 76 kilodaltons (SLP-76). Although their precise mechanism of action remains undetermined, both of these molecules are known to be absolutely essential for T cell function (*8*). Lack of either protein results in a complete block in T cell development at an early stage (presumably due to defects in signaling through the pre-TCR), and mutation of either adaptor in mature T cells results in severely dysregulated TCR function.

Appreciation of the critical role of adaptors in TCR signaling underscores the broader issue of the role of multimolecular signaling complexes in determining the appropriate cellular response after TCR engagement. Important studies pioneered by several groups have begun to sort out the dynamics of complex formation and the subcellular localization of the various components (*9*). Determining their precise physiological consequences will require application of even more sophisticated cell biology and imaging approaches to allow for real-time, high-resolution visualization in living cells.

Although recent years have taught us much about the initiation and propagation of TCR signaling, less is known about how T cell activation is terminated. It is clear, however, that this process occurs at many levels. For many years it has been known that TCR engagement under some circumstances leads to proliferation and enhanced effector function, whereas under other conditions, TCR ligation leads to unresponsiveness ("anergy"). Although it has long been clear that the effect depends, at least in part, on what other receptors are engaged on the T cells [for example, adhesion molecules or other coreceptors, such as CD28 or CTLA4 (*10*)], it also appears that the type or strength of signal delivered by the TCR itself may modulate how the cells respond. Additionally, TCR engagement activates proteins that have the potential to terminate T cell responses. These include phosphatases that counterbalance the effects of the PTKs that initiate T cell activation, as well as other proteins, such as the ubiquitin ligase Cbl, which is also a substrate of the TCR-stimulated PTKs. Although Cbl has been postulated to function in numerous pathways, and its precise mechanism remains unclear, compelling genetic and biochemical evidence indicates that this protein is an essential negative regulator of TCR signaling (*11*). TCR-derived second messenger pathways also play a critical role in the most effective termination signals—those leading to apoptosis of activated T cells. This "activation-induced cell death" is essential for T cell homeostasis, because patients defective in this process suffer from severe lymphoproliferation and susceptibility to developing autoimmunity and lymphoma (*12*). Finally, recent findings have restored confidence in earlier observations (*13*) that a non–cell autonomous mechanism exists that interferes with T cell activation events. Antigen-specific regulatory cells, thus far characterized best by surface phenotype, appear to play a key role in protection from inappropriate T cell activation that may lead to autoimmunity (*14*). Clearly, it will be only through a more complete understanding of the interplay among immune-system cells, and of activating versus inhibitory signals initiated by the TCR, that we will identify the best targets for, and devise the best approaches to, modulation of T cell function for therapeutic advantage.

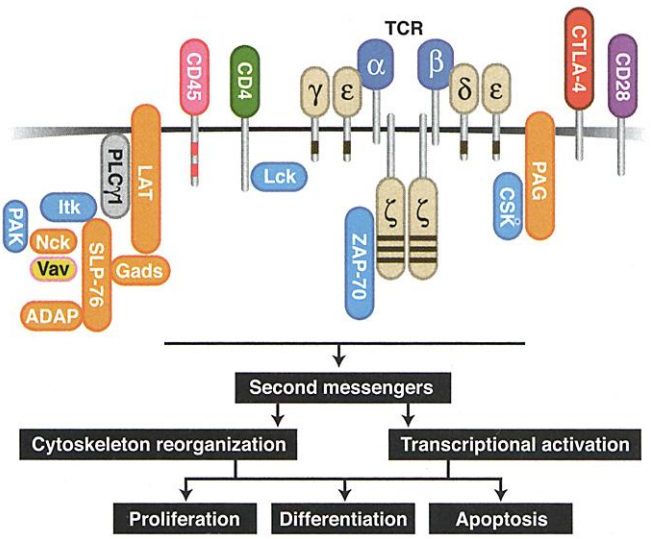

Fig. 1. Signaling initiated by engagement of the T cell receptor. The formation of a multimolecular complex, nucleated by the adapters SLP-76 and LAT, coordinates numerous second messenger cascades leading to cytoskeletal reorganization and transcriptional activation. The cellular response is also determined by the engagement of costimulatory receptors such as CD28. TCR signaling is regulated at multiple steps, and it is through the integration of these positive and negative regulators that T cells are driven toward proliferation, differentiation, or apoptosis. Adaptor proteins are shown in orange with kinases shown in blue. The immunoreceptor tyrosine-based activation motifs are shown as black lines on the TCR, and the two phosphatase domains of CD45 are shown as red lines. See the T Cell Signal Transduction Pathway for details.

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