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Review

# The calcium feedback loop and T cell activation: How cytoskeleton networks control intracellular calcium flux ☆

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ABSTRACT

During T cell activation, the engagement of a T cell with an antigen-presenting cell (APC) results in rapid cytoskeletal rearrangements and a dramatic increase of intracellular calcium ($Ca^{2+}$) concentration, downstream to T cell antigen receptor (TCR) ligation. These events facilitate the organization of an immunological synapse (IS), which supports the redistribution of receptors, signaling molecules and organelles towards the T cell–APC interface to induce downstream signaling events, ultimately supporting T cell effector functions. Thus, $Ca^{2+}$ signaling and cytoskeleton rearrangements are essential for T cell activation and T cell-dependent immune response. Rapid release of $Ca^{2+}$ from intracellular stores, e.g. the endoplasmic reticulum (ER), triggers the opening of $Ca^{2+}$ release-activated $Ca^{2+}$ (CRAC) channels, residing in the plasma membrane. These channels facilitate a sustained influx of extracellular $Ca^{2+}$ across the plasma membrane in a process termed store-operated $Ca^{2+}$ entry (SOCE). Because CRAC channels are themselves inhibited by $Ca^{2+}$ ions, additional factors are suggested to enable the sustained $Ca^{2+}$ influx required for T cell function. Among these factors, we focus here on the contribution of the actin and microtubule cytoskeleton. The TCR-mediated increase in intracellular $Ca^{2+}$ evokes a rapid cytoskeleton-dependent polarization, which involves actin cytoskeleton rearrangements and microtubule-organizing center (MTOC) reorientation. Here, we review the molecular mechanisms of $Ca^{2+}$ flux and cytoskeletal rearrangements, and further describe the way by which the cytoskeletal networks feedback to $Ca^{2+}$ signaling by controlling the spatial and temporal distribution of $Ca^{2+}$ sources and sinks, modulating TCR-dependent $Ca^{2+}$ signals, which are required for an appropriate T cell response. This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters. Guest Editor: Jean Claude Hervé.



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## 1. Introduction

Host protection both from pathogens, such as viruses and bacteria, and from cancer is mediated by the immune system. As the first line of defense, cells of the innate arm of the immune system e.g. macrophages and dendritic cells, recognize and respond to pathogens in a non-specific manner. Cells at the site of infection then evoke an inflammatory response by releasing cytokines. Among other functions, these cytokines promote the recruitment and activation of both additional innate immune cells and cells of the acquired immune system, e.g. lymphocytes. A key step in the activation of the acquired immune response is the priming of naïve T lymphocytes by specialized antigen-presenting cells (APCs). The engagement between a peptide-specific T cell antigen receptor (TCR) and an APC bearing its cognate peptide subsequently results in cell cycle progression and proliferation. Antigen-primed T cells then survey the periphery for infected or transformed cells carrying their cognate antigen. Once a T cell specifically recognizes its target cell, it polarizes towards the T cell–target cell interface, the immunological synapse (IS), secreting cytolytic granules and/or cytokines that mediate the elimination of the malignant or infected target cell.

Hallmarks of T cell conjugation with an APC/target cell are rapid cytoskeletal rearrangements and a dramatic increase of intracellular calcium concentration. These events support the polarization of the T cell towards its target, forming an IS, which enables the redistribution of receptors, signaling molecules and organelles towards the T cell–APC contact surface and induces downstream signaling events, ultimately supporting T cell effecter functions.

TCR engagement with peptides conjugated to major histocompatibility complexes (pMHCs) presented on APCs, leads to activation of signal transduction pathways that promote a rapid release of $Ca^{2+}$ from the endoplasmic reticulum (ER) $Ca^{2+}$ stores [1–3]. $Ca^{2+}$ depletion induces the opening of $Ca^{2+}$ channels residing in the plasma membrane, known as $Ca^{2+}$ release-activated $Ca^{2+}$ (CRAC) channels [4,5]. These channels enable a sustained influx of extracellular $Ca^{2+}$ across the plasma membrane in a process termed store-operated $Ca^{2+}$ entry (SOCE) [6].

Prolonged elevation of intracellular $Ca^{2+}$ through CRAC channels is required for varied T cell functions, including proliferation, differentiation, maturation, gene transcription and cytokine production [7–9]. Interestingly, CRAC channels have been shown to be inhibited by $Ca^{2+}$ ions [4,5,10–13]. Thus, CRAC channels themselves cannot enable the sustained $Ca^{2+}$ influx required for T cell function.

Increased intracellular $Ca^{2+}$ levels are necessary for rapid cytoskeleton dependent polarization, which involves F-actin rearrangement and microtubule-organizing center (MTOC) reorientation [14,15]. Since $Ca^{2+}$ levels rise within seconds following TCR engagement, whereas actin rearrangements occur further downstream in the cascade, an intriguing question is whether cytoskeleton rearrangements induce feedback regulation of $Ca^{2+}$ signaling. Here, we will address this issue.

On the other hand, there are actin rearrangements that are partially triggered by the formation of $Ca^{2+}$-independent complexes that influence ongoing $Ca^{2+}$ flux [16–22]. Additionally, some evidence suggests that actin rearrangements may be part of the TCR triggering process itself and, therefore, precede $Ca^{2+}$ flux [20,23–25]. In agreement with these observations, the inhibition of actin polymerization by cytochalasin D has been shown to reduce T cell $Ca^{2+}$ mobilization and T cell activation, as indicated by IFNγ production [26]. These effects support the notion that the remodeling of the actin cytoskeleton is essential for $Ca^{2+}$ signaling. However, the mechanisms underlying the linkage between cytoskeleton rearrangements and $Ca^{2+}$ signaling are not entirely understood. Recent observations have provided additional insights into how cytoskeleton rearrangements control crucial activities, such as $Ca^{2+}$ signaling.

In this review, we briefly summarize the known principles regarding actin polymerization in cells, and focus on the less well understood role of cytoskeleton remodeling in maintaining $Ca^{2+}$ signaling required for full T cell activation.

## 2. TCR triggering leads to $Ca^{2+}$ influx

The intracellular $Ca^{2+}$ concentration in resting T cells is maintained at ~50–100 nM, whereas the extracellular $Ca^{2+}$ concentration is ~1 mM, resulting in a ~$10^4$-fold resting concentration gradient of $Ca^{2+}$ across the plasma membrane. Following the engagement of the TCR with a pMHC on an APC, the intracellular $Ca^{2+}$ concentration can increase to ~1 μM [1] through the sequential operation of two interdependent processes: (i) Release of phospholipase C gamma1 (PLCγ1)-dependent intracellular $Ca^{2+}$ stores, and (ii) extracellular $Ca^{2+}$ influx through store-operated plasma membrane $Ca^{2+}$ channels [1–3] (Fig. 1).

### 2.1. PLCγ1 signals to induce intracellular $Ca^{2+}$ store release

TCR–pMHC engagement leads to the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) on the cytosolic side of the TCR–CD3 complex by the Src-family kinases, Lck and Fyn [27,28]. These phosphorylation events enable the recruitment and activation of the tyrosine kinase ζ-chain associated protein of 70 kDa (ZAP-70), which in turn, enhances the phosphorylation of the ζ-chain, promoting the formation of small protein aggregates, known as microclusters (MCs) [23,29–37]. These MCs, which function as integrated signaling machines, consist of multiprotein complexes that are essential for intracellular signaling pathways downstream of the TCR engagement, such as $Ca^{2+}$-mediated signaling [38–41].

Of particular importance in $Ca^{2+}$ signaling is the recruitment and activation of the intracellular enzyme PLCγ. Two forms of this protein have been identified, PLCγ1 and PLCγ2, of which T cells express predominantly the PLCγ1 form [42]. Two tyrosines, 775 and 783, located between the carboxyl terminus Src homology (SH) 2 domain and the SH3 domain, are crucial for the enzymatic activation of PLCγ1 in vivo [43–46]. Following tyrosine 783 phosphorylation by interleukin-2 (IL-2)-inducible T-cell kinase (Itk), PLCγ1 undergoes a conformational change that involves an intramolecular interaction between the carboxyl terminus SH2 domain and the phosphorylated tyrosine 783 [47]. Furthermore, all three SH domains of PLCγ1 are essential for its efficient recruitment, phosphorylation and activation in T cells [48].

The recruitment and activation of PLCγ1 at T cell MCs depend on several signaling molecules, including linker for activation of T cells (LAT); SH2 domain-containing leukocyte protein of 76 kDa (SLP-76); Vav1, a guanine nucleotide exchange factor (GEF); Itk; and c-Cbl [36,49,50] (Fig. 1). Reduced PLCγ1 phosphorylation and impaired $Ca^{2+}$ mobilization have been described in T cell deficient or impaired in these molecules [16,39,40,48,51–55]. Indeed, both T cell development and signaling are abolished in the absence of either LAT or SLP-76, demonstrating their essential role in signal propagation [56–60].

LAT is a transmembrane adaptor protein that phosphorylation of its tyrosines provides docking sites for the recruitment of SH2 domain containing proteins, including PLCγ1, Grb2, and Grb2-related adaptor protein (GADS). Furthermore, these interactions constitute a platform for an indirect association between LAT and SH3 domain ligands of these proteins, such as SLP-76, which binds GADS SH3 domain through its proline rich domain (PRD), and c-Cbl, which interacts with the SH3 domain of Grb2 [57,61–63].

A study investigating the phosphorylation sequence and kinetics of the individual tyrosines on LAT revealed that the kinetics of LAT tyrosine 132 phosphorylation are much slower than that of tyrosine 191. This delayed phosphorylation of LAT tyrosine 132 is thought to ensure the tight control of PLCγ1 activity, and thus, is important for the regulation of signaling pathways downstream of this protein, including $Ca^{2+}$ signaling [56].