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SWAP-70-like adapter of T cells: a novel Lck-regulated guanine nucleotide exchange factor coordinating actin cytoskeleton reorganization and Ca²⁺ signaling in T cells

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Summary

SWAP-70-like adapter of T cells (SLAT) is a recently identified guanine nucleotide exchange factor (GEF) for Cdc42 and Rac1, which is highly expressed in both thymocytes and peripheral T cells. Here, we present and discuss findings resulting from biochemical and genetic analyses aimed at unveiling the role of SLAT in CD4⁺ T-cell development, activation, and T-helper (Th) cell differentiation. *Slat*^{−/−} mice display a developmental defect at one of the earliest stages of thymocyte differentiation, the double negative 1 (DN1) stage, leading to decreased peripheral T-cell numbers. *Slat*^{−/−} peripheral CD4⁺ T cells demonstrate impaired T-cell receptor/CD28-induced proliferation and IL-2 production. Moreover, SLAT positively regulates the development of Th1 and Th2 inflammatory responses by controlling Ca²⁺/NFAT signaling. SLAT is also a positive regulator of the recently emerging Th subset, i.e., Th17 cells, as evidenced by its critical role in Th17 cell-mediated central nervous system inflammation. Furthermore, TCR engagement induces SLAT translocation to the immunological synapse, a process mediated by its Lck-dependent phosphorylation, which thereafter facilitates the triggering of SLAT GEF activity towards Cdc42 and Rac1, leading to NFAT activation and Th1/Th2 differentiation. Future work will aim to dissect the interacting partners of SLAT and may thus shed light on the poorly understood events that coordinate and link actin cytoskeleton reorganization to Ca²⁺ signaling and gene transcription in T cells.

Keywords

SWAP-70-like adapter of T cells; guanine nucleotide exchange factor; T-cell receptor; nuclear factor for activated T cells; calcium; T-helper cell differentiation

Introduction

Signals from the T-cell receptor (TCR) play a critical role in T-cell development, activation, differentiation, survival, and death. During T-cell development in the thymus, signals from the pre-TCR and the TCR control developmental progression (1,2). From the earliest thymic stage, the double negative (DN) stage, signals from the pre-TCR, resulting from the assembly of a TCRβ chain with a surrogate α chain, pTα, allow the survival of DN thymocytes, their proliferation, and differentiation into CD4⁺ CD8⁺ double positive (DP) thymocytes as well as participating in allelic exclusion completing the so-called 'β selection'. At this stage, successful

rearrangement of TCR $V\alpha$ and $J\alpha$ genes results in the synthesis of TCR α chains and assembly of the mature $\alpha\beta$ TCR on the cell surface. Furthermore, TCR signals are critically involved in both positive and negative selection in the thymus giving rise to $CD4^+$ or $CD8^+$ single positive (SP) cells and eliminating potentially autoreactive T cells from the repertoire. TCR signals also control the fate of naive T cells that migrate to the periphery, i.e. their survival and the maintenance of their numbers (homeostasis) (3,4). TCR engagement by specific antigen-major histocompatibility complex (MHC) molecules presented by antigen-presenting cells (APCs) is central to the effective induction of an antigen-specific T-cell response. Antigen exposure thus results in proliferation and subsequent differentiation into $CD4^+$ or $CD8^+$ effector T cells. On one hand, $CD4^+$ T-helper (Th) cells differentiate into three subsets of effector cells, Th1, Th2, and Th17, based on their distinct cytokine expression profiles and their subsequent immune regulatory functions (5–8). Th1 cells mainly secrete interleukin-2 (IL-2) and interferon- γ (IFN- γ) and mediate defense against infection by intracellular pathogens; Th2 cells secrete IL-4, IL-5, IL-6, IL-13, and IL-10 and mediate predominantly humoral immunity and allergic responses (9). The balance between Th1 and Th2 subsets determines susceptibility to disease states: development of excess Th2 cells can lead to allergy and asthma, while an overactive Th1 response can lead to autoimmunity (9). Lastly, Th17 cells produce IL-17, IL-17F, and IL-22, all of which regulate tissue inflammatory responses, and are critical for enhancing host protection against extracellular bacteria and fungi (6–8), which are not efficiently cleared by Th1 and Th2 responses. Th17 cells have been shown to have critical functions in the pathogenesis of a variety of organ-specific autoimmune inflammatory diseases, e.g. collagen-induced arthritis (CIA) (10), experimental autoimmune encephalomyelitis (EAE) (11,12), or psoriasis (13). On the other hand, $CD8^+$ T cells become cytotoxic cells and can efficiently kill target cells presenting the agonist peptide. Antigen-specific signals may also result in T-cell differentiation into long-lived memory cells.

T-cell activation is not a simple bimolecular event (i.e. a single receptor binding its ligand) but rather involves multiple interactions at the cell surface, including costimulatory molecules (e.g. CD28), adhesion molecules (e.g. integrins), and signaling from cytokine or chemokine receptors. The sum of these events, along with the qualitative difference in TCR signals and the nature of the APC, dictates the outcome of the antigen-specific T-cell response.

At a cellular level, for activation to occur, the formation of a stable conjugate between an antigen-specific T cell and a cognate peptide-bearing APC and the reorganization of molecules at the cell surface are required. The induction of T-cell activation is accompanied by large-scale rearrangements of the T-cell cytoskeleton, reorientation of the T-cell microtubule organizing center (MTOC), and the segregation of surface receptors, adapter proteins, and signaling molecules at the T cell/APC interface, also called the immunological synapse (IS) (14–17). Protein segregation within the IS was initially described as forming a supramolecular activation cluster (SMAC) comprised of a central region (c-SMAC) containing TCR and associated signaling proteins and a peripheral ring (p-SMAC) containing integrins [such as leukocyte-function-associated antigen-1 (LFA-1)] and adhesion molecules (17). However, several parameters including the nature of the T cell and the APC, costimulatory interactions, and the *in vivo* environment in which the interaction takes place, can dramatically influence IS architecture. The precise function of the IS in T-cell activation is still a matter of controversy, as the IS is thought to play a role both in triggering, sustaining, and terminating TCR signaling (16–19).

On a molecular level, signaling from the TCR $\alpha\beta$ heterodimer depends critically on its non-covalently associated signal transduction components, the $CD3\gamma$, δ , and ϵ and ζ polypeptides (20,21). In particular, these proteins have cytoplasmic domains bearing immunoreceptor tyrosine-based activation motifs (ITAMs), which each contain tandem tyrosine residues spaced 8–12 amino acids apart. Engagement of the TCR results in rapid activation of Src-family

kinases Lck and Fyn, which phosphorylate the ITAM tyrosine residues. The phospho-ITAMs in turn recruit the tyrosine kinase ζ -associated protein of 70 kDa (ZAP-70) or the related Syk kinase via interactions with their tandem Src homology 2 (SH2) domains. This binding allows phosphorylation of ZAP-70 by Lck and, subsequently, its activation. Subsequently, many other proteins are also tyrosine phosphorylated, ultimately leading to the activation of a number of signaling pathways, including a rise in intracellular Ca^{2+} concentration (Ca^{2+} flux), activation of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK) cascades, and activation of multiple transcription factors, notably nuclear factor for activated T cells (NFAT), activated protein-1 (AP-1), and nuclear factor κB (NF- κB). Although NFAT, NF- κB , and AP-1 share a number of properties (i.e. DNA binding domains and nuclear translocation upon TCR stimulation) and must be coordinately activated to stimulate IL-2 gene transcription, these transcription factors are regulated by discrete signaling cascades and differ remarkably in their function, resulting in distinct functional out-comes (22–24). In this review, we focus specifically on what we have learned recently about the function of SLAT, a novel TCR-regulated protein linking the actin cytoskeleton to NFAT/ Ca^{2+} signaling, which is ultimately involved in the differentiation of naive CD4^{+} T cells into distinct Th subsets.

SLAT and SWAP-70: a new class of GEFs for Rho-family GTPases

SLAT isolation

Using a differential display approach to identify Th2-associated genes, we isolated a cDNA encoding a putative protein of 630 amino acids showing a significant (45%) amino acid homology with mouse SWAP-70 (see below) and, thus, termed it SWAP-70-like adapter of T cells (SLAT) (25). Concomitantly, a yeast two-hybrid interaction analysis of a human lymph node cDNA library aimed at identifying potential partners of the lymphoid-restricted transcription factor interferon regulatory factor-4 (IRF-4) led to the identification of a novel cDNA, hence termed IRF-4-binding protein (IBP) (26), encoding the human orthologue of Def-6, which has previously been shown to be expressed in myeloid progenitors but to be downregulated in differentiated macrophages, granulocytes, and erythrocytes (27). More recently, Def-6 has also been detected in skeletal muscle, where it influences myoblast differentiation by interacting with the skeletal muscle integrin chain $\alpha 7\text{A}$ (28). Furthermore, SLAT has been shown to be expressed in murine peritoneal and bone marrow-derived macrophages (BMMs), in which it modulates Fc γ receptor-mediated phagocytosis (29). It turned out that the amino acid sequence of human IBP also displays a significant sequence homology to SWAP-70 and was, in fact, the human homolog of SLAT, based on a comparison of the predicted amino acid sequence showing that human and murine IBP display 92% identity and 95% similarity over the entire sequence.

SWAP-70: structural features and function

SWAP-70 is predominantly expressed in activated mature B lymphocytes, although low levels can also be detected in mast cells and embryonic fibroblasts (30–33). SWAP-70 contains a putative EF-hand Ca^{2+} -binding domain, a coiled-coil region, three nuclear localization signals (NLSs), a nuclear export signal, a pleckstrin-homology (PH) domain, which is involved in membrane targeting through binding of phosphatidylinositol-3,4,5-triphosphate (PIP_3), and a domain weakly homologous to Dbl homology (DH) domains (30,31,33,34).

Although its sequence contains three potential NLSs, in small resting B cells, SWAP-70 is mainly found in the cytoplasm (33). Upon BCR stimulation, SWAP-70 translocates to the nucleus, where it was found to be a component of an enzyme complex called SWAP, also composed of B23 (nucleophosmin), C23 (nucleolin), and poly(ADP-ribose) polymerase (PARP), which promotes the recombination of immunoglobulin (Ig) switch regions in B cells

(33). SWAP-70, which is only expressed in B lymphocytes that had been induced to undergo Ig class switch and in switching B-cell lines (in contrast to B23, C23, and PARP, which are expressed in most cells), has a weak affinity for DNA, binds ATP, and is considered to be the specific recruiting element that assembles the switch recombinase from universal components. SWAP-70 is also critical for CD40-dependent switching to the IgE isotype (35). In activated, class-switching B-cell cultures, SWAP-70 is also found to be associated with membrane IgG but not IgM, an association mediated by its PH domain (33).

The DH domain is a hallmark of proteins that function as guanine nucleotide exchange factors (GEFs) for Rho-family guanosine triphosphatases (GTPases) (36). GEFs activate their target GTPases by catalyzing their conversion from the GDP-bound inactive state to the GTP-bound active state (36). Accordingly, SWAP-70 was found to be a Rac-GEF, which, independently of Ras, transduces signals from tyrosine kinase receptors to Rac, and, in turn, plays a role in specific processes during remodeling of the actin cytoskeleton, such as membrane ruffling [possibly via binding to non-muscle F-actin (37)] and lamellipodia formation (34,38,39). Recently, Pearce *et al.* (39) reported a role for SWAP-70 in B-cell adhesion and migration into secondary lymphoid organs. *Swap-70*^{-/-} B cells rolled and adhered normally yet accumulated in lymph node (LN) high endothelial venules, preventing their entry into LNs (39). This defect was due to an aberrant regulation of integrin-mediated adhesion during attachment, inducing defective polarization and leading to the lack of uropod formation or lamellipodia stabilization at a defined region. In addition to its role in B-cell activation, Ig class switching, migration, and homing, SWAP-70 also plays a role in T-cell-dependent immune response, particularly in the formation and function of germinal centers (GCs) (40). Indeed, SWAP-70 deficiency uncouples GC formation from T-dependent antibody and long-lived plasma cell production and causes extrafollicular generation of high-affinity plasma cells but does not adequately support the memory response (40).

In contrast to B cells, SWAP-70 is confined to the cytoplasm in mast cells, where it plays a role in development into mature mast cells and FcεRI-mediated degranulation (41). Furthermore, SWAP-70 regulates mast cell FcεRI-mediated signaling events such as LAT phosphorylation and Akt/PKB and p38 MAPK activation (42), which are important for the control of mast cell-dependent anaphylaxis. Finally, *Swap-70*^{-/-} bone marrow mast cells (BMMCs) also display impaired c-kit receptor signaling (38). *Swap-70*^{-/-} BMMCs are impaired in Ca²⁺ flux, in proper membrane translocation, and activity of Akt kinase, and in c-kit-induced translocation of Rac1 and Rac2, which are required for mast cell activation, cell-cell adhesion, and migration.

Expression and structural features of SLAT

Unlike SWAP-70, SLAT is abundant in thymocytes as well as in peripheral T cells (25,26, 43) and translocates from the cytoplasm to the plasma membrane (PM), specifically to the IS, upon antigen stimulation (25,44,45). However, the significant sequence homology between SWAP-70 and SLAT is further reflected at the level of the domain structure of the SLAT protein (Fig. 1).

SLAT harbors at its N-terminus a potential Ca²⁺ binding EF-hand domain, whose function remains elusive, followed by an imperfect ITAM-like sequence. This ITAM sequence contains negatively charged amino acids at conserved positions, the first YxxL/I motif, a spacer sequence of seven amino acids, and a second Tyr-containing sequence, YxxK, in which a lysine residue replaces the leucine/isoleucine typically found in ITAMs (Fig. 1). TCR/CD28 costimulation was found to lead to rapid Lck-dependent phosphorylation of Tyr-133 and -144 in this sequence in Jurkat T cells and in primary murine T cells (45). The relevance of this phosphorylation event for SLAT function in T cells is discussed later. In contrast, another

report identified instead Tyr-210 as a substrate for Lck phosphorylation by *in vitro* Lck kinase assays in non-T (293T) cells, i.e., not in the context of TCR stimulation (44).

SLAT next contains a PH domain, shown to be able to bind the phosphatidylinositol 3-kinase (PI3K) product PIP₃ (44,46). Gupta *et al.* (44) further described an Lck-dependent binding of PIP₃ to Arg-236 of the PH domain, whereas Oka *et al.* (46) identified several basic amino acids in the $\beta 1/\beta 2$ loop of the PH domain, namely Lys-225 and Arg-226/230/231, as being critical for PIP₃ binding but without any requirement for Lck activity. The PH domain is then flanked at its C-terminal side by a DH domain, exhibiting a TCR-regulated GEF activity towards Cdc42 and Rac1 (44,45,47), which are major regulators of TCR-mediated cytoskeleton dynamics. Of a particular note here is the arrangement of the PH–DH domains, which in both SLAT and SWAP-70 is the reverse of that observed in the best-characterized GEFs for Rho GTPases (known as Dbl family proteins), wherein the DH domain is invariably flanked by a C-terminal PH domain (36). Moreover, the DH domains of SLAT and SWAP-70 do not exhibit any statistically significant amino acid sequence homology to that of other known Dbl family members such as Vav1 and Tiam1. These structural and functional features thus define SLAT and SWAP-70 as members of a new subfamily of GEFs for Rho GTPases. In addition to its role as an upstream activator of Rho GTPases, SLAT interacts and cooperates with activated Rac1 to induce a change in cell shape, most likely independently of its GEF activity (46). The regions of SLAT interacting with activated Rac have been further mapped to two separate segments: one central part between amino acid residues 315 and 407, which did not show any homology to other Rac-interacting proteins, and an N-terminal region containing a stretch of residues weakly homologous to the Rac-binding domain conventionally found in Rac-interacting proteins, i.e. Leu-18, Leu-31, and Val-33. Finally, the predicted amino acid sequence contains a potential NLS, whose functionality and relevance have not been elucidated.

SLAT expression and its structural features suggest that it may play a novel and crucial role in TCR signaling by activating a subset of Rho-family GTPases, hence influencing T-cell responses in a unique manner. To address the role of SLAT *in vivo*, we and others used homologous recombination in embryonic stem cells to disrupt the *Slat* gene to generate SLAT-deficient (*Slat*^{−/−}) mice (43,45,48). The rest of this review focuses on what we have recently learned about the regulation and function of SLAT in T-cell physiology, i.e. T-cell development, activation/signaling, and Th differentiation.

SLAT and T-cell development

Slat^{−/−} mice are viable, fertile, and grossly normal (43,48). The overall thymic architecture is normal, with the DP thymocytes residing in the cortex while the CD4⁺ and CD8⁺ SP thymocytes are concentrated in the medulla. Analysis of thymic development showed that the overall thymus size and cellularity was reduced by about twofold in *Slat*^{−/−} mice by comparison to wildtype (WT) mice (43). Moreover, *Slat*^{−/−} and WT mice had similar proportions of DN, DP, and SP thymocytes, although the absolute number of each thymic subset was decreased by approximately 50%, suggesting a defect in differentiation and/or expansion of an early progenitor (43). This defect in thymic development was further traced to impaired expansion of the DN1 population, as evidenced by a specific reduction of bromodeoxyuridine (BrdU) incorporation into this subset (43), which contains the earliest intrathymic progenitors. The differentiation and proliferation of DN subsets are mainly governed by complex interrelationships between Notch, c-kit, and IL-7R signaling pathways (49,50), raising a question about the role of SLAT in this signaling network, apart from its TCR-dependent function in the periphery. Specifically, c-kit/stem cell factor interactions have been shown to be involved in the earliest stages of thymopoiesis by promoting the proliferation of these immature thymocytes (51), i.e. the stage affected by *Slat* deletion, suggesting that SLAT may

regulate c-kit signaling in the very immature thymocyte compartment. In this regard, it is interesting to note that SWAP-70, the homolog of SLAT, has been shown to regulate c-kit signaling in BMMCs (38).

SLAT and T-cell activation

In the periphery, we observed a mild CD4⁺ and CD8⁺ T-cell lymphopenia in both spleen and lymph nodes (LNs) of *Slat*^{-/-} mice (43), likely occurring as a direct consequence of the decreased thymic output. However, histological examination did not reveal any abnormality in splenic architecture in *Slat*^{-/-} mice, indicating that SLAT is not required to maintain a normal lymphoid tissue organization.

Regarding the impact of SLAT deficiency on T cell activation, we found that *Slat*^{-/-} peripheral CD4⁺ T cells displayed impaired TCR/CD28-induced proliferation and IL-2 production (43). However, phorbol myristate acetate plus ionomycin treatment bypassed this defect, demonstrating that SLAT is part of a signaling pathway downstream of the TCR but upstream of protein kinase C (PKC) and/or Ca²⁺ signaling that is necessary for full T-cell activation, in agreement with its recruitment to IS upon antigen stimulation. Interestingly, the absence of SLAT has no discernible impact on TCR-induced CD25 upregulation, and the proliferative defect of *Slat*^{-/-} CD4⁺ T cells could be overcome by adding exogenous IL-2, implying that this defect is due to altered TCR signaling and not IL-2R signaling.

SLAT, a key component of NFAT/Ca²⁺ signaling

Proximal TCR signaling events

Examination of TCR/CD28-mediated early signaling events revealed that SLAT is mostly dispensable for activation of early signaling events, with the exception of ERK1/2 and p38 MAPK pathways, whose TCR/CD28-mediated activation was reduced in the absence of SLAT (43,48). Indeed, after TCR/CD28 stimulation, *Slat*^{-/-} CD4⁺ T cells demonstrate intact activation of ZAP-70, linker for activation of T cells (LAT), phospholipase Cγ1 (PLCγ1), Akt, glycogen synthase 3β (GSK3β), and JNK, as well as normal NF-κB activation, while the magnitude and duration of ERK1/2 and p38 activation were modestly reduced by comparison with their WT counterparts. However, *Slat*^{-/-} CD4⁺ T cells failed to sustain intracellular Ca²⁺ influx upon TCR/CD28 ligation, leading consequently to defective translocation of NFAT to the nucleus (43). Another indication for the involvement of SLAT in NFAT activation was the demonstration that overexpression of SLAT in Jurkat T cells dramatically potentiated TCR-mediated transcriptional activation of NFAT but not of AP-1 or NF-κB (45).

Ca²⁺ signaling

TCR triggering induces a biphasic rise in [Ca²⁺]_i that consists of an early transient rise, resulting from the release of Ca²⁺ from the endoplasmic reticulum (ER), which serves as the primary trigger for a second phase of sustained Ca²⁺ mobilization, resulting from the opening of Ca²⁺ channels in the PM (52,53). The initial Ca²⁺ release is mediated by inositol-1,4,5-triphosphate (IP₃), a second messenger that binds and activates the IP₃ receptor (IP₃R), an ER Ca²⁺ channel. The tyrosine kinases Lck and ZAP-70 indirectly raise IP₃ levels by activating PLCγ1, which hydrolyzes PIP₂ into membrane-bound diacylglycerol and soluble IP₃. IP₃ binding is mandatory for channel activity, but other levels of regulation can increase channel sensitivity. In particular, another tyrosine kinase, Fyn, binds to the IP₃R, induces its tyrosine phosphorylation, and thereby further prolongs intracellular Ca²⁺ release (54,55). The early rise in [Ca²⁺]_i triggers the second Ca²⁺ wave by opening the Ca²⁺ release-activated Ca²⁺ (CRAC) channels (56), such as Orai/CRACM (57), thereby allowing Ca²⁺ influx from the extracellular space. Two RNA interference (RNAi)-mediated interference screens in *Drosophila* and HeLa cells revealed that stromal interaction molecule 1 (STIM1) plays a role in the activation of

CRAC channels (58,59). STIM1 is a single-pass transmembrane protein localized in the ER membrane, which contains a Ca^{2+} -binding EF-hand domain facing the ER lumen. STIM proteins were therefore postulated to act as sensors of Ca^{2+} concentration in the ER. The exact mechanism by which STIM proteins activate CRAC channels in the PM is not fully understood. After store depletion, however, STIM proteins form oligomers in the ER membrane and redistribute into discrete 'puncta', as a consequence of the dissociation of Ca^{2+} from the EF-hand domain (59), and then move to regions of ER-PM apposition that colocalize with clusters of Orai1-containing CRAC channels and sites of localized Ca^{2+} influx in the PM (60–62). Although STIM1 was reported to localize in the PM in addition to the ER (63), it is not clear whether this STIM fraction is involved in CRAC channel activation. Currently available data favor a model in which clusters of STIM1 in the ER act in *trans* on the CRAC channel localized in the PM. Ca^{2+} signaling then activates NFAT proteins that are heavily phosphorylated and reside in the cytoplasm in resting cells; when cells are stimulated, NFATs are dephosphorylated by calcineurin, a calmodulin-dependent serine/threonine phosphatase, and translocate to the nucleus, where they can form transcriptionally active complexes (64).

We analyzed the molecular basis of the defect in Ca^{2+} signaling in *Slat*^{-/-} mice in more detail (43). The failure of TCR/CD28 crosslinking and subsequent extracellular Ca^{2+} addition to induce a significant increase in $[\text{Ca}^{2+}]_i$ in *Slat*^{-/-} T cells when extracellular Ca^{2+} was chelated strongly suggests that the primary Ca^{2+} defect in *Slat*^{-/-} T cells resides in the receptor-induced release of Ca^{2+} from intracellular stores, which is required for subsequent Ca^{2+} influx.

However, as noted above, no defect in TCR-induced PLC γ 1 phosphorylation (and, hence, activation) or in IP_3 generation was observed in *Slat*^{-/-} T cells (43), suggesting that SLAT acts downstream of PLC γ 1-dependent IP_3 production to regulate Ca^{2+} release from the ER. A number of issues relating to the role of SLAT in controlling $[\text{Ca}^{2+}]_i$ remain unresolved. It is tempting to speculate that SLAT could regulate ER Ca^{2+} channels or sensors, e.g., the IP_3R (52) or STIM1 (63,65), or interact with other cytosolic proteins to allow efficient Ca^{2+} release (66).

SLAT, a regulator of actin cytoskeleton and IS formation

The SLAT DH domain possesses TCR-stimulated GEF activity towards Rac1 and Cdc42 (44,45,47). Consistent with the well-documented ability of Rho-family GTPases to control the dynamics of the actin cytoskeleton (67), SLAT has been reported to transduce TCR signals to and through the actin cytoskeleton. In fact, in the absence of SLAT, TCR-induced actin polymerization is reduced, a defect compensated by the expression of a constitutively active form of Rac2 in *Slat*^{-/-} T cells (48), thus linking the ability of SLAT to activate Rho GTPases to TCR-mediated cytoskeletal reorganization. Furthermore, in the absence of SLAT, antigen-specific IS formation and stabilization is also impaired (48), likely as a consequence of the TCR-mediated cytoskeletal remodeling defect, thus leading in turn to altered T-cell effector function.

SLAT, an actor in the interplay between NFAT/ Ca^{2+} signaling and actin dynamics

Actin reorganization, Ca^{2+} /NFAT, and gene expression in T cells

Despite the importance of Rho GTPase-mediated actin cytoskeleton reorganization in assembly of the IS and in controlling early events preceding gene transcription (such as migration, integrin activation, or T-APC conjugate formation) (68–70), the pathways linking actin rearrangement to Ca^{2+} /NFAT signaling and downstream changes in gene expression are far less clear.

There is growing evidence showing that the actin cytoskeleton plays a dual role in changes in T-cell function, first by enhancing T-cell activation by promoting conjugate formation and assembly of signaling complexes but also by downregulating activation by facilitating internalization of the TCR. Indeed, cytochalasin D (CytoD) treatment of T cells stimulated with superantigen-pulsed APCs results in diminished Ca^{2+} flux, whereas stimulation with anti-CD3 antibodies results in a prolonged Ca^{2+} response (71). Similarly, although one study showed that CytoD treatment impairs activation of the *IL-2* gene promoter (72), another study reported that at low doses of CytoD, Ca^{2+} signaling was prolonged and IL-2 production increased (73). Second, the finding that CytoD treatment impairs TCR-stimulated NFAT but not NF- κ B or AP-1 activation (74) suggests that the actin cytoskeleton does not globally regulate all TCR-stimulated signaling pathways. Lastly, antigen receptor-stimulated NFAT activation was previously found to require Rac1 activity in mast cells (75).

SLAT-mediated, Cdc42/Rac1-dependent NFAT activation

Several lines of evidence strikingly support the notion that the GEF activity of SLAT is cardinal for SLAT-induced Ca^{2+} /NFAT activation (45). First, latrunculin B, an inhibitor of actin polymerization, abrogated TCR-dependent, SLAT-mediated NFAT activation (45). Second, a dominant-negative Cdc42, and to a lesser extent Rac1, mutant blocked SLAT-dependent NFAT activation and, conversely, transduction of *Slat*^{-/-} CD4⁺ T cells with constitutively active Cdc42 restored NFAT nuclear translocation and Ca^{2+} mobilization (45), thus making a strong case for a causal role of the GEF activity of SLAT in inducing its Ca^{2+} -promoting activities.

Several Cdc42/Rac1 effectors can selectively regulate Ca^{2+} /NFAT signaling. Numerous studies indicated that Wiskott–Aldrich syndrome protein (WASP), a specific interacting partner and downstream effector of Cdc42, plays a key role in actin cytoskeleton rearrangement as well as in NFAT activation via its association with WIP [WAVE (WASP-family verprolin homologous protein)-interacting protein], preventing in turn WASP degradation (76–78). Similarly, the WAVE2 complex regulates actin cytoskeleton reorganization but is also required for NFAT (but not NF- κ B or AP-1) activation, reflecting its essential role in CRAC-mediated Ca^{2+} entry (79). Finally, Pak1 (p21-activated kinase 1) kinase, a common Rac1 and Cdc42 effector, is involved in NFAT activation via its Lck-mediated phosphorylation and resulting association with the adapter Nck (80).

Accumulating evidence shows that Rho GTPases regulate gene transcription through diverse distal effector elements of the TCR pathway, including MAPKs p38 and ERK (80–84), whose activation is also decreased in *Slat*^{-/-} T cells. Notably, Yablonski *et al.* (80) demonstrated that Rac/Cdc42 could influence NFAT by activating ERK through its target Pak1 in T cells. Furthermore, *Rac2*^{-/-} T cells show many similarities to T cells from *Slat*^{-/-} mice, namely, altered p38 and ERK activation, defective Ca^{2+} mobilization, cytoskeleton reorganization, and cellular activation (83). Cdc42 has also been recently shown to specifically modulate ERK activation (85). In addition, other studies provide evidence for a functional coupling of NFAT transcriptional activity to Ras/MAPK–ERK kinase (MEK)/ERK activation (86–88). Thus, Ras activation through MEK1–ERK was associated with increased NFATc4 nuclear translocation and transcriptional activity in cardiac myocytes (87). This result is also consistent with an observation made in T lymphocytes, whereby Vav signaling was coupled to Ras–MEK–ERK activation, which, in turn, promoted NFAT activation (88). Likewise, dominant negative or constitutively active MEK1 mutants blocked and induced NFAT activation in T lymphocytes, respectively (86). In agreement with these reports, SLAT-mediated NFAT activation was totally abrogated by an ERK inhibitor (45). These findings prompt us to suggest that the defective activation of MAPKs exhibited by *Slat*^{-/-} T cells may result from impaired Rho GTPase function and that SLAT may thus fulfill its role in NFAT activation through a specific Rac1/Cdc42–ERK pathway. Altogether, the identification of downstream effectors involved

in Cdc42/Rac1-dependent, SLAT-mediated NFAT activation may shed some light on the unresolved mechanism that links actin polymerization to Ca^{2+} /NFAT signaling.

SLAT and T-helper cell differentiation

Given that SLAT is a key regulator of the NFAT pathway (43), presumably via its GEF activity (45), lessons learned from mice deficient for the two NFAT family members most highly expressed in lymphocytes, i.e. NFATc1 and NFATc2, or mice deficient for GEF-induced effectors, are of special interest to understanding the role of SLAT in immune functions *in vivo*. NFATc1 deficiency results in an impaired Th2 response by decreasing IL-4 production (89), whereas NFATc2 deficiency enhances Th2 development by prolonging IL-4 transcription (90,91). Interestingly, mice lacking both NFAT isoforms have a profound defect in the production of both Th1 and Th2 cytokines (92). Moreover, transgenic mice expressing a dominant-negative form of NFAT were shown to be resistant to Th2-mediated allergic pulmonary inflammation (93). Lastly, Rac2 activates Th1-specific signaling (94) and CD4⁺ T cells from Wiskott–Aldrich syndrome patients (mutated in WAS gene encoding WASP) display defective Th1 response due to impaired NFATc2 activation (95), establishing a link between activation of Rho family GTPases, NFAT activation, and Th differentiation. In addition, the proximal promoter of the human *Il17A* gene contains two NFAT-binding sites, which appear to be important for IL-17 expression (96). Lastly, NR2F6, a nuclear zinc-finger orphan receptor, has been shown to interfere with the DNA binding of NFAT:AP-1 (but not NF- κ B) and, subsequently, transcriptional activity of the NFAT-dependent *Il-17A* cytokine promoter (97). Together, these findings show the emerging role of NFAT in Th17 differentiation and function.

SLAT and Th2 responses

Th functions were tested *in vivo* in *Slat*^{-/-} C57BL/6 mice to determine whether biochemical defects observed in *Slat*^{-/-} T cells, i.e. alteration of Ca^{2+} /NFAT signaling, translate into impaired T-helper responses *in vivo* (43). As expected, *Slat*^{-/-} mice were grossly impaired in their ability to mount a Th2-mediated lung inflammatory response (43). This phenotype was evidenced by reduced airway eosinophilia and levels of Th2 specific cytokines (IL-4/5) in the bronchoalveolar lavage (BAL) fluid from primed *Slat*^{-/-} mice as well as a reduction in pulmonary inflammation characterized by a drastically diminished perivascular and peribronchial infiltration of eosinophils and mononuclear cells (43). Additionally, immunized *Slat*^{-/-} mice displayed a drastic reduction in the hyperplasia of goblet cells and mucus production relative to that observed in primed WT mice (43).

In our earlier study, ectopic SLAT expression has been shown to have a moderate positive effect on IL-4 production (25). This study showed an inducible association of SLAT with ZAP-70, which may competitively reduce the association of ZAP-70 with TCR- ζ , consequently dampening the overall strength of TCR signaling. Based on studies showing that Th2 cell activation is associated with inefficient ZAP-70 activation (98–101), this study provided a potential mechanism for the ability of SLAT to positively regulate Th2 cell development and/or expansion, namely, via inhibition of ZAP-70-dependent signaling. Furthermore, another report using dominant-negative Ras transgenic mice, in which TCR-induced ERK activity and Th2 development were severely impaired, showed that the ERK pathway was necessary for Th2 development (102). Altogether, potential crosstalk or synergy between ZAP-70, Ca^{2+} /NFAT, and MAPK signaling pathways could underlie the defect in Th2 responses observed in *Slat*^{-/-} mice.

SLAT, Th1/Th17 responses, and autoimmunity

The response of *Slat*^{-/-} mice in a Th1-mediated lung inflammatory model was also defective. Severely reduced numbers of lymphocytes and neutrophils were found in the BAL fluid from primed *Slat*^{-/-} C57BL/6 mice as well as decreased level of IFN γ and lack of cellular infiltration/inflammation in the airways (43).

In apparent contrast, Fanzo *et al.* (48) paradoxically found that aging (>5-month old) and preferentially female *Slat*^{-/-} mice on a mixed C57BL/6 $\times$ 129 background spontaneously developed a lupus-like autoimmune syndrome characterized by the accumulation of effector/memory T cells and IgG⁺ B cells, proteinuria, glomerulonephritis, profound hypergammaglobulinemia, and autoantibody production. Moreover, antigen-experienced T cells from *Slat*^{-/-} mice exhibited a significant resistance to apoptosis, most likely accounting for the accumulation of effector/memory T cells and, in return, for the development of the autoimmune disorder observed in aged *Slat*^{-/-} mice. The peripheral T-cell homeostasis defect observed in *Slat*^{-/-} mice (43) might be related to the spontaneous lupus-like syndrome reported in this study (48). Indeed, several studies showed that lymphopenia can predispose mice to several autoimmune diseases such as insulin-dependent diabetes mellitus, systemic lupus erythematosus, colitis, or rheumatoid arthritis (103,104). The compensatory homeostatic proliferation, normally a consequence of such lymphopenia, has been reported to drive the development of autoimmunity via the rapid expansion of autoreactive short-lived memory-like T cells within the depleted niche (105,106). However, the biologic relevance of the lupus-like disease observed in that study (48) is unclear, given the mixed background of the mice and the finding that epistatic interactions between the non-autoimmune-prone strains 129 and C57BL/6 can induce a lupus-like disease (107).

For over a decade, IFN γ -secreting Th1 cells were thought to be the population central to the pathogenesis of autoimmunity, considering the clear association of the Th1 effector phenotype to diseases like rheumatoid arthritis, multiple sclerosis, and type 1 diabetes. In the respective animal models, however, the loss of the major Th1 cytokines, IFN γ , IL-12, and IL-18, surprisingly did not hamper disease development (108–111). In fact, IFN γ and IL-12 deficiency led to an even more severe inflammation in CIA and EAE. As a result of these discoveries, the simplistic notion that Th1 cells and their respective cytokines are the sole regulators of autoimmunity had to be revised. Recently, it has been extensively shown that a third Th subset, i.e., Th17 cells, has critical functions in the pathogenesis of a variety of organ-specific autoimmune and inflammatory diseases (112,113), including EAE (11,12), CIA (10), and psoriasis (13). We showed that *Slat*^{-/-} mice on a C57BL/6 background were resistant to EAE induction, displaying both dramatically reduced onset and severity of the disease (114). This defective *in vivo* response was correlated with a lack of demyelination of the spinal cord's white matter and reduced encephalitogenic CD4⁺ T-cell infiltration into the central nervous system as well as impaired Th1 and Th17 responses in both the central nervous system and draining LNs. Consistent with a key role of SLAT in autoimmunity, naive *Slat*^{-/-} CD4⁺ T cells were unable to differentiate properly into Th17 cells *in vitro* under TGF- β - and IL-6-dependent polarizing conditions, indicating that SLAT acts as a positive regulator of Th17 differentiation (114). In contrast to our study, a recent publication (115) reported that *Slat*^{-/-} BALB/c mice crossed to DO11.10 transgenic mice expressing an MHC class II-restricted ovalbumin-specific TCR spontaneously and rapidly develop rheumatoid arthritis-like joint disease and large vessel vasculitis associated with enhanced IL-17 and IL-21 production. Nevertheless, these observations, made in the setting of a restricted TCR repertoire (in the DO11.10 TCR transgenic background), have not been confirmed in mice expressing an intact polyclonal TCR repertoire, as young *Slat*^{-/-} BALB/c mice do not develop obvious signs of arthritis (115), questioning the physiological and pathological relevance of findings based on the study of *Slat*^{-/-} mice on a TCR transgenic background.

SLAT-mediated NFAT activation and T-helper cell differentiation

To further dissect the role of SLAT in Th cell differentiation, a number of *in vitro* studies have examined the ability of *Slat*^{-/-} naive CD4⁺ T cells to differentiate under defined culture conditions. Bécart *et al.* (43) showed that SLAT was required for efficient Th1 and Th2 differentiation *in vitro*. Moreover, the addition of ionomycin could (i) restore a proper nuclear translocation of NFAT and (ii) rescue decreased production of both IL-4 and IFN γ in *Slat*^{-/-} T cells (43), indicating that SLAT regulates early TCR signaling (prior to Th1/Th2 commitment) and controls lung inflammation by promoting nuclear localization of both NFATc1 and NFATc2 induced by early TCR/CD28 instructive signals. In contrast, Fanzo *et al.* (48) failed to observe a defect in IL-4 production by unskewed or Th2-polarized *Slat*^{-/-} CD4⁺ T cells and observed that the defect in IFN γ production under neutral conditions was rescued by addition of IL-12 to the primary culture. Although the *Slat*^{-/-} mice used in these two studies were of the same origin, they differ notably by their genetic background; thus, Bécart *et al.* (43) used *Slat*^{-/-} mice on a homogeneous C57BL/6 background, whereas Fanzo *et al.* (48) studied *Slat*^{-/-} mice on a mixed 129 $\times$ C57BL/6 genetic background, providing a likely explanation for these discrepancies.

Regulation of SLAT-dependent NFAT-mediated Th differentiation: structure-function analysis

Although SLAT clearly plays a crucial role in Ca²⁺ /NFAT-mediated Th1/2 differentiation of CD4⁺ T cells (43), the underlying molecular mechanisms have remained elusive. In this regard, SLAT possesses several prominent TCR-inducible features, such as Lck-dependent tyrosine phosphorylation (44,45), activation-induced translocation to the IS/PM (25,44,45), and GEF activity towards Cdc42 and Rac1 (44,45,47), all of which may be relevant for NFAT activation. Based on previous reports emphasizing the role of other actin regulatory proteins in Ca²⁺ /NFAT signaling, the GEF activity of SLAT is likely to be important for these events. SLAT tyrosine phosphorylation may also control its GEF activity, as it is the case for other DH-containing proteins (e.g. Vav1). The translocation of SLAT to the membrane/IS may also be potentially regulated by its PH domain and play a crucial role in SLAT-mediated T-cell activation and, thus, possibly in downstream NFAT activation.

Further structure–function analysis and the study of the regulation of SLAT subcellular localization provided insights into the mechanisms by which SLAT coordinates NFAT activation and Th differentiation. This work has been carried out by examining the function and localization of SLAT mutants (deleted for specific domains or carrying defined point mutations) overexpressed in Jurkat T cells, or expressed in primary *Slat*^{-/-} CD4⁺ T cells using retroviral transduction to establish the physiological relevance of the findings (45). SLAT-transfected Jurkat T cells showed a dose-dependent increase in NFAT reporter activity, which was abrogated by cyclosporine A, an inhibitor of calcineurin, confirming the involvement of SLAT in modulating TCR-induced NFAT transcriptional activity (45). However, SLAT was dispensable for TCR-mediated NF κ B and AP-1 activation (45).

Tyrosine phosphorylation of SLAT

Consistent with findings that IS assembly and translocation of signaling molecules to the membrane/IS greatly influence T-cell activation (16), a strict correlation between the structural features required for SLAT membrane/IS recruitment and its ability to activate NFAT was observed; both events required the Lck-dependent phosphorylation of Tyr-133 and Tyr-144 (45). In contrast, Tyr-210, shown to be an Lck substrate in pervanadate-stimulated 293T cells (44), was not required for TCR-induced NFAT activation (45). Of note, phosphorylation of Tyr-133/144 was required but not sufficient to induce NFAT activation as a SLAT mutant

mimicking phosphorylation of these residues (Y133/144D) still needed TCR stimulation to activate NFAT and translocate to the cell membrane (45).

Independence of SLAT function on its PH domain

Strikingly, the combined PH–DH domains or the DH domain alone were not sufficient, and PI3K-dependent binding of the PH domain to PIP₃ was not even required, for SLAT translocation to the IS and, in return, for NFAT activation and proper Th1/2 differentiation (45). Thus, a point mutation of Arg-236 (R236C), shown to be critical for binding of PIP₃ to the SLAT PH domain (44), as well as pretreatment with the PI3K inhibitor LY294002 had no effect on antigen-induced SLAT translocation to the membrane or IS, NFAT activation, or Th1/2 differentiation of CD4⁺ T cells.

Relevance of SLAT recruitment to membrane for its function

The fact that the isolated catalytic domain of SLAT, the DH domain, which displays GEF activity in COS-7 cells by itself (45), failed to translocate to IS and activate NFAT raised the question whether the IS translocation of SLAT was a prerequisite for NFAT activation. In this regard, unlike the native DH domain alone, constitutive targeting of the DH domain to the PM, accomplished by fusing the N-terminal acylation sequence of Lck to the SLAT DH domain (MyrDH) and, thus, bypassing TCR-induced SLAT translocation to the IS/membrane, enhanced TCR-stimulated NFAT activation and rescued the Th differentiation defect of *Slat*^{-/-} CD4⁺ T cells (45). This finding demonstrates that mislocalization of the SLAT DH domain in the cytosol upon TCR engagement underlies its inability to activate NFAT and substantiates the conclusion that membrane/IS association of SLAT is a prerequisite for NFAT activation. Enforced membrane targeting of the isolated DH domain *per se* was not sufficient to activate NFAT but still required TCR stimulation, indicating that additional TCR-induced signals, beyond the mere membrane/IS translocation of SLAT, are required for its proper function.

Role of SLAT GEF activity in NFAT activation

We further found that the MyrDH-induced NFAT activation was dependent on functional Cdc42 activity (45), as indicated by the ability of a dominant-negative Cdc42 mutant to block SLAT-dependent NFAT activation and, conversely, the restoration of NFAT nuclear translocation, Ca²⁺ mobilization, and Th1 differentiation in *Slat*^{-/-} CD4⁺ T cells by a transduced, constitutively active Cdc42 protein. This causality between activation of Rho family GTPases and Th differentiation is consistent with reports showing that (i) Rac2 activates Th1-specific signaling (94) and (ii) CD4⁺ T cells from Wiskott–Aldrich syndrome patients display defective Th1 response because of impaired NFATc2 activation (95). Altogether, these findings establish a causal link between the GEF activity of SLAT and the resulting activation of Cdc42 on one hand and its critical function in promoting activation of the Ca²⁺/NFAT signaling pathway and, consequently, Th differentiation, on the other.

These data raised the question of the regulation of SLAT GEF activity specifically involved in NFAT activation. A recent study proposed a model for regulation of SLAT GEF activity, reminiscent of that for Vav GEF activity, based on a tyrosine phosphorylation-dependent relief of an intramolecular inhibitory interaction (44). In this model, the N-terminal region of SLAT would serve a regulatory function by maintaining SLAT in a closed conformation until a stimulus-induced phosphorylation relieves this autoinhibitory interaction. This would expose the PH domain allowing it to bind PIP₃ and leading to full activation of SLAT by enabling its DH domain to interact with target Rho GTPases. This model is based on findings showing that deletion of the N-terminal region of SLAT renders it constitutively active and capable of activating Rac1 and Cdc42 in COS-7 or 293T cells (44); however, the behavior of this mutant in T cells has not been studied. Nevertheless, as the SLAT DH domain alone failed to induce

TCR-regulated, Cdc42/Rac1-mediated NFAT activation (45), the regulatory mechanism accounting for SLAT GEF activity in the context of NFAT activation in T cells appears to be more complex. In particular, our recent findings (45) prompted us to speculate that the TCR-induced, Lck-dependent tyrosine phosphorylation of SLAT combined with its segregation to the membrane/IS are the mandatory key steps in stimulating its GEF activity, which is necessary, in turn, for proper NFAT activation.

Speculative model for SLAT-mediated NFAT activation and Th1/2 differentiation

Based on our findings, we can propose the following regulatory model to account for the TCR-induced localization and function of SLAT in the course of T-cell activation and differentiation (Fig. 2). First, Lck-mediated phosphorylation of SLAT creates a binding site(s) for an undefined critical TCR-regulated adapter protein (implicating a potential adapter function for SLAT), and/or induces a conformational change in SLAT. As a result of this modification, SLAT is recruited to the IS, where it can access and activate Rho GTPases, which thereafter induce reorganization of the actin cytoskeleton, and subsequently lead to Ca^{2+} /NFAT activation and Th1/2 differentiation.

Concluding remarks and perspectives

TCR signaling triggers interlinked pathways involving a plethora of proteins including tyrosine and serine/threonine kinases, adapter proteins, and cytoskeleton-regulating proteins, leading to highly regulated and fine-tuned responses. In this complex network, SLAT has been identified as a member of a new class of Rho GTPases specific for Cdc42 and Rac1 (44,45). Studies focusing on CD4^{+} T-cell responses attributed to SLAT a pivotal role in the regulation of Th1, Th2, and Th17 inflammatory responses by controlling Ca^{2+} /NFAT signaling through its role in actin cytoskeleton reorganization and Cdc42/Rac1 activation (25,43,45). Although recent work improved our understanding of the molecular basis of SLAT function in CD4^{+} T cells, important questions remain to be answered.

SLAT has been shown to translocate to the membrane/IS upon TCR/CD28 stimulation in a Lck-dependent but PH-independent manner (45). A major question emerging from this finding relates to the tyrosine phosphorylation-dependent mechanism that selectively recruits SLAT to the IS and the identification of proteins mediating this recruitment. Thus, further biochemical studies will aim to define potential SLAT-binding partners following TCR-mediated SLAT phosphorylation on Tyr-133 and Tyr-144 within its ITAM-like motif and accounting for its translocation to the IS. Phosphorylation of these tyrosine residues may generate binding sites for SH2 domain-containing proteins such as Syk or ZAP-70, initiating a cascade of signaling pathways resulting in the formation of an IS-localized signalosome. In this regard, SLAT and ZAP-70 have been shown to interact upon TCR stimulation (25). Furthermore, SLAT phosphorylation may also induce interaction with adapters, such as LAT or Src homology 2 domain-containing leukocyte phosphoprotein of 76 kDa (SLP-76), whose function is to link proximal and distal signaling events downstream of the TCR by nucleating a multi-molecular signaling complex that facilitates activation of intermediate signaling cascades such as those stimulated by Rho GTPases.

The SLAT DH domain clearly contains GEF activity towards Cdc42 and Rac1. However, the DH domain residue(s) essential for the GEF activity has not been identified, a task rendered difficult by the fact that the DH domain of SLAT lacks significant primary amino acid sequence homology with 'conventional' DH domains or even with the DH domain of its close relative, SWAP-70. Furthermore, as SLAT controls NFAT activation supposedly via its GEF-mediated Cdc42 activation and, hence, via cytoskeleton actin reorganization, future studies exploring the signaling events downstream of SLAT GEF activity may shed light on the poorly

understood events that coordinate and link actin cytoskeleton reorganization to Ca^{2+} signaling and gene transcription in T cells.

Recent reports focused on the role of SLAT in CD4^+ T-cell activation and Th1/Th2/Th17 differentiation. However, SLAT is broadly expressed in the peripheral T-cell compartment; thus, its role in other T-cell subsets or states of activation such as regulatory T cells, CD8^+ T cells, or memory T cells may be of major interest to broaden our understanding of SLAT function in T-cell physiology.

Beyond these fundamental questions, strategies designed to selectively block the function of SLAT in T cells (e.g. by inhibiting the translocation of SLAT to the IS or, more directly, its GEF activity) may provide an efficient means of blocking the Ca^{2+} /NFAT signaling machinery in T cells and hence be therapeutically useful for the treatment of NFAT-dependent autoimmune and inflammatory diseases, especially given the predominant and abundant expression of SLAT in T cells

(<http://biogps.gnf.org/?referer=symatlas#goto=gene-report&id=50619>). In that regard, SLAT may turn out to be an attractive drug target with potentially similar importance to that of the recently discovered T-cell CRAC channel, Orai1 (116–118) and the Ca^{2+} sensor, STIM1 (58,63). Lastly, additional work is required to define the precise role that SLAT plays in T-cell development through its selective action in DN1 thymocytes. Altogether, resolution of these outstanding questions is likely to firmly establish SLAT as a novel and clinically relevant player in the complex network of T-cell activation and function.

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Fig. 1. Domain structure of SWAP-70-like adapter of T cells (SLAT)

Sequence analysis predicts the existence of a number of domains, which are shown in the figure. Numbers above the scheme refer to amino acid residues. A comparison of the ITAM-like sequence of SLAT with the consensus ITAM is also shown. Gray boxes indicate conserved amino acids in the ITAM. The ITAM-like domain contains negatively charged amino acids at conserved positions, the first YxxL/I motif, a spacer sequence of seven amino acids, and a second Tyr-containing sequence, YxxK, in which a lysine residue replaces the leucine/isoleucine typically found in ITAMs. The two tyrosines (Y) of this ITAM-like sequence, Tyr-133 and Tyr-144, are phosphorylated in a Lck-dependent manner upon TCR stimulation. The PH domain has the ability to bind PIP₃ but is not required for TCR-induced translocation of SLAT to the membrane/IS. The DH domain has catalytic GEF activity towards Cdc42 and Rac1. The functionality and relevance of the putative EF hand domain and the nuclear localization signal (NLS) have yet to be determined.

Fig. 2. A hypothetical model of SWAP-70-like adapter of T cells (SLAT) function in Ca^{2+} /NFAT signaling and Th cell differentiation

TCR stimulation leads to the sequential activation of protein tyrosine kinases such as Lck and ZAP-70, which phosphorylate adapter proteins such as LAT. Consequently, phosphorylated LAT binds the SH2 domain of PLC γ 1, resulting in the phosphorylation/activation of PLC γ 1. PLC γ 1 catalyses the hydrolysis of the membrane phospholipid PIP $_2$ to IP $_3$ and DAG. IP $_3$ binding to its receptor (IP $_3$ R) in the ER triggers Ca^{2+} release from intracellular stores, which, in return, triggers the opening of plasma membrane CRAC channels. Ca^{2+} influx through CRAC channels and elevated intracellular Ca^{2+} concentration activate Ca^{2+} -dependent enzymes such as calcineurin, which dephosphorylates cytoplasmic NFATc1/2. Dephosphorylated NFATc1/2 translocate to the nucleus to induce gene transcription and influence Th cell responses; NFATc1 and NFATc2 positively regulate Th2 and Th1 responses, respectively. In this scenario, TCR engagement induces SLAT phosphorylation on Tyr-133 and Tyr-144 in its ITAM-like sequence (step 1), an event required for its translocation to the plasma membrane/IS (step 2). The exact mechanism mediating this relocalization remains elusive but does not involve the SLAT PH domain. Once at the membrane, additional TCR

signals stimulate SLAT GEF activity towards Cdc42 and Rac1 (step 3), leading to activation of actin-regulatory proteins, e.g., WASP, WAVE, or PAK1, which promote actin polymerization (step 4). SLAT-mediated Cdc42/Rac1-dependent actin reorganization thereafter promotes Ca^{2+} release from intracellular stores (step 6a) and, consequently, Ca^{2+} influx (steps 6b-c) and NFAT activation (steps 6d-e), by acting downstream of IP_3 production (step 5). The exact mechanism underlying this function is currently unknown. SLAT-mediated, Ca^{2+} -dependent NFAT activation (steps 6a-c) controls Th1/Th2 commitment, depending upon the NFAT isoform involved (steps 7a and 7b, respectively).