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The calcium/NFAT pathway: role in development and function of regulatory T cells

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Abstract

Calcium signals are essential for diverse cellular functions in the immune system. Sustained Ca^{2+} entry is necessary for complete and long-lasting activation of calcineurin/ NFAT pathways. A growing number of studies have emphasized that Ca^{2+} / calcineurin/ NFAT pathway is crucial for both development and function of regulatory T cells.

Keywords

calcium; Foxp3; Stim1

1. Introduction

Calcium (Ca^{2+}) is a universal second messenger in all eukaryotic cells[1-3]. In immunocytes, including T cells, B cells, mast cells and many other cell types, Ca^{2+} signals control proliferation, differentiation and effector function as well as a variety of transcriptional programmes[4-6]. In lymphocytes, store-operated Ca^{2+} entry through calcium-release-activated calcium (CRAC) channels is the main mechanism to increase intracellular Ca^{2+} concentrations, which results in the activation of downstream signalling proteins, such as the calmodulin-dependent phosphatase calcineurin and calmodulin-dependent kinases, as well as downstream transcription factors including NFAT (nuclear factor of activated T cells). The consequences of Ca^{2+} signalling can be observed both in the short and long term. Short-term consequences, which occur in minutes, are independent of new gene expression. In contrast, the long-term consequences associated with gene expression are apparent only after several hours, including lymphocyte proliferation, the production of cytokines and chemokines, the differentiation of naïve T cells into various effector or memory T cells, and the establishment – in the absence of costimulation -- of an antigen-unresponsive state known as anergy[4,7].

The transcription factor NFAT has a crucial role in long-term Ca^{2+} signaling. NFAT can induce gene expression by itself but can also cooperate with other transcription factors. Recent evidence indicates that NFAT is part of a positive feedback pathway in which it cooperates with the forkhead transcription factor Foxp3 to induce the development and maintain the

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function of regulatory T cells. Here we review recent advances in our knowledge of the relationship between Ca^{2+} signaling, NFAT activation and regulatory T cells.

2. Mechanism of Ca^{2+} entry and Ca^{2+} signaling in lymphocytes

The main pathway to increase of intracellular Ca^{2+} is store-operated Ca^{2+} entry (SOCE) through Ca^{2+} release-activated calcium (CRAC) channels in lymphocytes[1]. Activation of immunoreceptors -- the T cell antigen receptor (TCR), the B cell antigen receptor (BCR) and Fc receptors (FcR) -- by antigen or antigen-antibody complexes leads to the recruitment and activation of protein tyrosine kinases and the formation of large signalling complexes scaffolded by adapter proteins, ultimately resulting in tyrosine phosphorylation and activation of phospholipase C (PLC)- γ (PLC- γ 1 in T cells and mast cells and PLC- γ 2 in B cells)[2,3,8]. PLC- γ hydrolyzes phosphatidylinositol-3,4-bisphosphate (PIP_2) to the two second messengers inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to IP_3 receptors in the endoplasmic reticulum (ER) membrane and causes the release of ER Ca^{2+} stores; in turn, ER store depletion opens store-operated CRAC channels, which permit sustained Ca^{2+} influx into the cell (Figure 1). As a result, several Ca^{2+} -dependent signaling proteins and their target transcription factors are activated.

Although the existence of store-operated Ca^{2+} entry channels was proposed more than twenty years ago, molecular identity and precise activation mechanism of these channels remained unclear until very recently. In just the past three years, major breakthroughs have revealed two key regulators of store-operated Ca^{2+} entry, STIM (an ER Ca^{2+} sensor)[9,10] and ORAI (a pore subunit of the CRAC channel)[11-13]. Both proteins were identified by large-scale RNA interference (RNAi) screens.

Stim (stromal cell interaction molecule[14]) was identified in two limited RNAi screens, performed in *Drosophila* and HeLa cells respectively[9,10]. Flies and worms express only one STIM protein[9], whereas mammals express two STIM proteins, STIM1 and STIM2, with 47% amino acid identity[15]. STIM1 and STIM2 are both single-pass transmembrane proteins with paired N-terminal EF-hands located in the ER lumen and protein interaction domains located both in the ER lumen and in the cytoplasm[16]. Both STIM1 and STIM2 are functional ER Ca^{2+} sensors that can trigger store-operated Ca^{2+} entry through CRAC channels in activated (store-depleted) cells. However, STIM1 is a prime activator of store-operated Ca^{2+} entry during the early phase of the response when ER Ca^{2+} stores are strongly depleted. On the other hand, STIM2 operates both in resting cells with Ca^{2+} -replete stores to control basal Ca^{2+} influx, but also during the late stages of a response when ER stores are becoming refilled. At these late times, STIM1 is inactivated by rebinding of Ca^{2+} whereas STIM2 remains active presumably because the affinity of STIM2 EF-hands for Ca^{2+} is two-fold lower than that of STIM1 EF-hands[17,18].

Drosophila Orai (*olf186-F*) was identified as an essential regulator of store-operated Ca^{2+} influx by three different groups[11-13]. *Drosophila* expresses only one ORAI, whereas mammals express three homologues, ORAI, ORAI2 and ORAI3. ORAI proteins are small proteins with four transmembrane domains, whose N and C-termini are both located in the cytoplasm[11,19,20]. In humans, a single missense mutation in the *ORAI1* gene caused severe combined immunodeficiency disease characterized by the absence of SOCE and I_{CRAC} [11]. Mutational and electrophysiological analyses have identified *Drosophila* Orai and human ORAI1 as pore subunits of the CRAC channel[20-22].

The mechanism whereby STIM proteins regulate ORAI proteins has been extensively investigated over the last three years, and their analysis has greatly advanced our understanding of the mechanisms of SOCE. SOCE occurs in several steps. Following store depletion, STIM1 aggregates into small clusters ("puncta") in the ER membrane[10,23]. Next, the STIM1 clusters

preferentially localize to sites of preexisting and newly formed ER-plasma membrane (ER-PM) apposition and colocalize partially with clusters of ORAI1[23-25]. The oligomerization of STIM1 itself is critical for its accumulation at ER-PM sites, and regions that contain clusters of STIM proteins colocalize coincide with sites of Ca^{2+} entry[26]. Oligomerized STIM1 has been postulated to induce dimer-to-tetramer transition of ORAI1, which constitutes active and functional CRAC channels[27,28].

In T cells, STIM1 and ORAI1 behave differently after antigen stimulation. STIM1 and ORAI1 both translocate to the region of the immunological synapse that forms between T cells and antigen-presenting dendritic cells, colocalising with the T cell receptor (TCR) and co-stimulatory molecules[29]. This interface coincides with local Ca^{2+} influx from the extracellular space. Another group reported that STIM1 and ORAI1 formed cap-like structure at distal pole of the cell, which is opposite side of immunological synapse. This cap then moves from the distal pole to either an existing or a newly-forming immunological synapse[30]. Potentially, the cap may serve as a reservoir of preassembled Ca^{2+} channel components that can be delivered to newly-formed immunological synapses. Interestingly, tyrosine-phosphorylated $\text{IP}_3\text{R1}$ also colocalizes with the TCR after stimulation; this modification seems to increase the IP_3 sensitivity of the receptor while decreasing its inactivation by high concentrations of Ca^{2+} [31]. Presumably, co-localisation of these molecules at the immunological synapse is essential for augmentation and sustenance of local Ca^{2+} influx at the contact zone between T cells and antigen-presenting cells, thus facilitating long-term activation of antigen-stimulated T cells.

Besides store-operated Ca^{2+} entry, other Ca^{2+} influx pathways via other channels have been found in lymphocytes including L-type voltage-gated Ca^{2+} channel subunits and their scaffold protein, AHNAK1; ATP-responsive purinergic P2 receptors; TRPC channels; TRPV channels and some TRPM channels[2,3,32]. It was suggested that these channels regulate the concentration of intracellular Ca^{2+} in T cells and B cells, however, the magnitude of their contribution to Ca^{2+} influx is still controversial.

As a result of Ca^{2+} influx, several pathways downstream of Ca^{2+} influx are activated, including the serine/ threonine phosphatase calcineurin and its target transcription factor NFAT(nuclear factor of activated T cells); CaMK(Ca^{2+} - calmodulin-dependent kinase) and its target CREB (cyclic-AMP-responsive-element-binding protein); MEF2(myocyte enhancer factor 2) which is acted upon by both the calcineurin and CaMK pathways; and NF κ B(nuclear factor κ B). Among them, the calcineurin-NFAT pathway has a major role and is best characterized in terms of its downstream contributions to Ca^{2+} signalling and transcriptional effects. Calcineurin, the only calmodulin-dependent serine/threonine phosphatase, consists of the catalytic subunit calcineurin A (CnA α , CnA β and CnA γ) and a regulatory subunit, calcineurin B (CnB1 and CnB2). Increases in intracellular free Ca^{2+} ($[\text{Ca}^{2+}]_i$) result in activation of the phosphatase activity of calcineurin through binding of Ca^{2+} -calmodulin to the calcineurin regulatory and displacement of the calcineurin autoinhibitory domain from the enzyme active site.

NFAT consists of a family of four transcription factors (NFAT1-4, also known as NFATc1-c4)[4,7]. All NFATs except NFAT3 are expressed in peripheral lymphocytes, however, NFAT4 is preferentially expressed in thymocytes whereas NFAT1 and NFAT2 are prominent in peripheral T cells[33,34]. Dephosphorylation of cytoplasmic NFAT proteins by calcineurin unmasks the nuclear localization sequence following which NFAT proteins rapidly translocate into the nucleus[4]. NFAT-driven gene expression is highly dependent on sustained Ca^{2+} influx and calcineurin activity, because when $[\text{Ca}^{2+}]_i$ levels drop or cells are treated with the calcineurin inhibitor cyclosporin A (CsA), NFAT is immediately rephosphorylated and its

nuclear localization sequence is masked. This leads to rapid export of NFAT from the nucleus and cessation of the transcription of NFAT-dependent genes.

3. Regulatory T cells and Foxp3

Regulatory T cells, originally identified as CD25⁺CD4⁺ T cells, are a unique lineage of CD4⁺ T cells that exert active immune suppression and are essential for maintaining self-tolerance. Regulatory T cells can be mainly subdivided to two types that come from different origins. ‘Natural’ regulatory T (nTreg) cells develop from CD4⁺ T cells in the thymus. Another type of regulatory T cells, ‘induced’ regulatory T (iTreg) cells, are derived by differentiation of naïve T cells in peripheral lymphoid organs in the presence of transforming growth factor- β (TGF- β) and IL-2. nTreg and some iTreg cells characteristically express the forkhead transcription factor Foxp3 (forkhead box P3)[35,36], whose expression is regulated by several transcription factors, such as STAT5, Smad3 and NFAT(Figure 2). In addition to Foxp3, many genes are preferentially expressed in regulatory T cells, including CD25, CTLA-4, and GITR. Foxp3 is an acknowledged master regulator of thymic nTreg cell development and peripheral function, and is sufficient to induce regulatory T cell phenotype in conventional CD4⁺CD25⁻ T cells. Foxp3 can cooperate with other transcription factors, such as NFAT and Runx1 (also known as AML1), to regulate the expression or repression of characteristic genes in regulatory T cells[37]. Mutation of Foxp3 causes severe multiorgan autoimmune disease in both human (IPEX; immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) and mouse (*scurfy* mouse strain). The constant presence of nTreg cells is essential for maintaining peripheral tolerance because inducible deletion of Foxp3⁺ cells in adult mice results in an aggressive autoimmune disease similar to that observed in mice in which Foxp3 is deleted or mutated either in all tissues or in the CD4 lineage starting at the double-positive thymocyte stage[38].

4. Role of Ca²⁺ signaling in the development and function of regulatory T cells: ZAP70 and PLC γ

The first study showing importance of the Ca²⁺ signaling in regulatory T cell development was reported by Sakaguchi and colleagues two decades ago. They showed that when thymi from 1 week-old *nu/nu* mice that had been treated from birth with CsA were transplanted into recipient *nu/nu* mice, the recipient mice developed diverse organ-specific autoimmunity[39]. Autoimmunity could be prevented by inoculation of the recipients with normal thymocyte suspensions, leading the authors to suggest that “*CsA caused autoimmune disease by interfering selectively with the thymic production of certain suppressor T cells (which later became known as regulatory T cells) controlling self-reactive T cells*”. In human, treatment with CsA induces peripheral tolerance but also reduces the number and suppressive function of regulatory T cells in renal transplant patients[40]. These findings strongly suggest that Ca²⁺ signaling is essential for both development and function of regulatory T cells.

In last three years, increasing evidence has demonstrated the significance of Ca²⁺/ calcineurin/ NFAT pathway in regulatory T cells using gene-manipulated mice. (i) Mice with a mutation (Y136F) in the T cell transmembrane adapter LAT, which eliminates the docking site for PLC- γ 1 and results in lowered Ca²⁺ influx in response to TCR crosslinking, show a severe impairment of regulatory T cell development[41]. The autoimmune phenotype of LAT^{Y136F/Y136F} mutant mice has been attributed to impaired negative selection resulting in escape of autoreactive T cells into the periphery[42]. This autoimmune phenotype can be prevented by the transfer of CD4⁺CD25⁺ T cells, which suggests that suppressive function of residual regulatory T cells in periphery is also impaired in LAT^{Y136F/Y136F} mutant mice. (ii) In contrast to LAT^{Y136F/Y136F} mutant mice, SKG mice, which are prone to develop autoimmune arthritis in the Balb/c background because of a single missense mutation of

Zap70 gene[43], show a moderate defect of regulatory T cell function. SKG mice demonstrate a severe attenuation of signal transduction through ZAP70, and the self-reactive T cells in these mice have been postulated to arise through defects in thymic selection. Calcium mobilization is substantially impaired in SKG thymocytes, and SKG peripheral T cells show a severe defect of Ca^{2+} influx. Nevertheless, in SKG mice, the number of $\text{Foxp3}^+\text{CD25}^+\text{CD4}^+$ T cells and their in vitro suppressive function (assessed by their ability to diminish polyclonal TCR activation) are not significantly different from normal Balb/c mice. However, the suppressive ability of SKG $\text{CD25}^+\text{CD4}^+$ T cells is much less effective *in vivo*, as judged by co-transfer experiments in which SKG or Balb/c $\text{CD25}^+\text{CD4}^+$ T cells were transferred together with SKG $\text{CD25}^+\text{CD4}^+$ T cells to Balb/c nude mice[44]. In addition, the authors mention as unpublished data that the SKG mutation affects the repertoire of $\text{CD25}^+\text{CD4}^+$ regulatory T cells[45]. (iii) Another group generated mice with mutations in the *Zap70* gene, *murdock* (*mrd*) and *mrtless* (*mrt*), by *N*-ethyl-*N*-nitrosourea (ENU)-induced single nucleotide substitution[46]. They established three mutant mice, *mrd/mrd*, *mrd/mrt* and *mrt/mrt*. Among them, only *mrd/mrt* mice displayed a severe autoimmune phenotype and a significant decrease of thymic Foxp3^+ regulatory T cells. In the periphery, however, the number and function of these Treg cells were comparable with those of wild-type despite a severe decrease of Ca^{2+} entry in peripheral T cells. The phenotypes observed in these mice differ from those of mice with a null mutation in *Zap70* gene, which show a block at the DP to SP stage in T cell development. Presumably, in these mutant mice, Ca^{2+} influx is severely impaired but still induced to some degree, which allows these cells to maintain suppressive function in peripheral $\text{Foxp3}^+\text{CD25}^+\text{CD4}^+$ T cells.

5. Role of Ca^{2+} signaling in the development and function of regulatory T cells: STIM and ORAI

As mentioned above, the molecular identity of the essential components of store-operated Ca^{2+} entry – the ER Ca^{2+} sensors STIM1 and STIM2, and the pore subunits of the CRAC channel, ORAI1-3 -- is now known. This allows us to directly address the role of Ca^{2+} entry in regulatory T cells by using gene-disrupted mice rather than pharmacologic reagents. *Stim1*-deficient mice have been generated by our laboratory[18] and by other group[47-49]. Mice with an early *CD4Cre*-mediated deletion of *Stim1* shows normal thymic development, even though *Stim1*-deficient T cells completely lack store-operated Ca^{2+} entry, I_{CRAC} and Ca^{2+} -dependent cytokine expression[18]. *Stim2*-deficient naïve T cells showed normal store-operated Ca^{2+} entry and cytokine production, however, STIM2 was essential for maximal cytokine production and prolonged nuclear localization of NFAT in activated T cells. Despite the complete absence of store-operated Ca^{2+} entry in *Stim1*-deficient T cells, mice singly deficient for either the *Stim1* or *Stim2* genes possessed normal number of Foxp3^+ regulatory T cells in thymus and peripheral lymphoid organs. However, combined deletion of *Stim1* and *Stim2* resulted in a dramatic change of phenotype. Mice in which both *Stim1* and *Stim2* had been deleted by *CD4Cre* showed a striking decrease of regulatory T cell population in all immune organs and developed autoimmune symptoms[18]. In contrast to $\text{LAT}^{\text{Y136F/Y136F}}$ mutant mice, the effect was selective for the regulatory T cell lineage, since the mice showed normal thymic cellularity and normal development of thymocytes and peripheral T cells. There are a few but detectable residual $\text{Foxp3}^+\text{CD25}^+\text{CD4}^+$ regulatory T cells in *Stim1* and *Stim2* double knockout (DKO) mice. DKO $\text{CD25}^+\text{CD4}^+$ T cells demonstrate complete defect of store-operated Ca^{2+} influx and significant decrease of in vitro suppressive function. Furthermore, the lymphoproliferative phenotype is suppressed by the transfer of wild-type $\text{CD25}^+\text{CD4}^+$ T cells.

Mice with a null mutation in the *Orail* gene have been generated in our laboratory[50], and another group has generated and analyzed *Orail* gene-trap mutant mice[51]. ORAI1-null mice showed a substantial decrease of store-operated Ca^{2+} entry in $\text{CD4}^+\text{CD8}^+$ double positive

thymocytes and obvious impairment in store-operated Ca^{2+} entry or CRAC channel function that was especially pronounced in previously activated and differentiated cells[50]. However, regulatory T cell development and function are normal in the mice, most likely because of residual store-operated Ca^{2+} entry that could reflect to possible compensation from other ORAI proteins.

These studies suggest that the development and function of regulatory T cells is remarkably sensitive to basal and induced levels of intracellular Ca^{2+} . A residual, even if very small, degree of store-operated Ca^{2+} influx allows some level of Treg development and function in *Stim1*^{-/-}, SKG and ZAP70 mutant *mrd/mrt* mice. In contrast *LAT*^{Y136F/Y136F} mutant mice with severely impaired IP3 production and therefore ER Ca^{2+} store depletion, and STIM1, STIM2 DKO mice, that show essentially no store-operated Ca^{2+} entry and likely have lower basal $[\text{Ca}^{2+}]_i$ levels[17], display decreased numbers and function of regulatory T cells and a striking phenotype of splenomegaly and lymphoproliferative disease.

6. Role of Ca^{2+} signaling in the development and function of regulatory T cells: calcineurin and NFAT

In contrast to the clear effect of absence of Ca^{2+} influx, the role of the downstream proteins calcineurin and NFAT in regulatory T cell development and function is not yet fully resolved. Mice with a double deletion of *Nfat1* and *Nfat4* also show lymphoproliferative autoimmune phenotypes[52]. In these mice, Foxp3 expression and in vitro suppressive ability are comparable with those of wild-type mice. Thus, regulatory T cell development and function appeared normal in NFAT1 and NFAT4 double deficient mice, and hyperproliferation was attributed to the fact that their CD25⁺CD4⁺ T cells were not effectively inhibited by NFAT1 and NFAT4 double deficient regulatory T cells[53]. Presumably, NFAT2 compensates for the loss of other isoforms in these mice since NFAT2 can bind to *Foxp3* enhancer region[54]. Furthermore, thymocyte development is blocked at the DP to SP stage in both CnA β -deficient mice and mice with an early *LckCre*-mediated deletion of *Cnbl* [55,56], however, autoimmune/hyperproliferative syndromes and dysregulation of regulatory T cells have not been reported.

How does the calcineurin-NFAT pathway impinge on Treg development and function? At the stage of nTreg and iTreg development, NFAT regulates *Foxp3* expression in combination with Smad3 in nTreg and iTreg cells[54]. Smad3 binds to a *Foxp3* distal enhancer early during TGF β treatment, whereas NFAT binding to the 5' enhancer occurs at later times, which suggests that NFAT is responsible for maintaining *Foxp3* expression. Indeed, *Foxp3* expression in activated, TGF β -treated T cells is blocked by treatment with either cyclosporin A or the Smad3 inhibitor SIS3[54,57]. There is structural, biochemical and functional evidence for cooperation between NFAT and Foxp3 (Figure 2). The crystal structure of NFAT-Foxp complexes on an NFAT-AP1 composite site in the *IL2* promoter has been solved. Foxp and AP-1 occupy the same DNA region and interact with overlapping, but not identical, residues of NFAT. The NFAT-Foxp3 complex represses, but the NFAT-AP1 complex activates, IL-2 expression. In contrast, Foxp3 upregulates expression of CTLA-4 and CD25 in cooperation with NFAT. These observations are confirmed by chromatin immunoprecipitation (ChIP) assays. Foxp3 proteins with mutations that disrupted NFAT:FOXP3 interaction were unable to regulate these genes properly[58]. Furthermore, NFAT-Foxp3 complex is essential for the suppressive function of Foxp3-expressing T cells in a mouse model of autoimmune diabetes since T cells expressing the mutant Foxp3 proteins (bearing mutations that disrupted the NFAT:FOXP3 interaction) were unable to suppress diabetes progression due to injection of diabetogenic T cells into neonatal mice[58]. Genome-wide ChIP-chip analysis revealed that the promoter regions bound by Foxp3 are also enriched for NFAT DNA-binding motifs[59].

7. Conclusions

The molecular aspects of regulatory T cells have been extensively studied during the last decade. Now we recognize that Ca^{2+} signalling is crucial for both the development and the function of regulatory T cells. The field of Ca^{2+} signalling also has been reinvigorated in the last three years by the discovery of two key players in store-operated Ca^{2+} entry, STIM and ORAI. It is now possible to validate the function of this pathway in regulatory T cells. Questions remain: Why is there a difference in the Ca^{2+} requirement for the development of conventional versus regulatory T cells, with Tregs dependent on store-operated Ca^{2+} entry involving STIM proteins whereas conventional T cells show no such requirement and can develop in the complete absence of STIM proteins? What are the biological functions of calcineurin and NFAT proteins in regulatory T cells? The availability of conditional mouse genetic models will greatly facilitate the study of these questions in a physiological context in different cell types.

Suppression of store-operated Ca^{2+} entry in conventional T cells results in immune deficiency with only mild extra-immunological symptoms, as shown by the phenotype of a surviving SCID patient with an *Orai1* R186W mutation[11,60]. Similarly, the Ca^{2+} -calcineurin-NFAT pathway is required for immune function, as shown by the well-established clinical efficacy of the immunosuppressive drugs cyclosporin A and FK506[4,7]. We have shown that the same pathways regulate the suppressive function of regulatory T cells, for instance at sites of pathogen infection or in progressively growing tumors. Thus drugs that inhibit store-operated Ca^{2+} entry or the Ca^{2+} -calcineurin-NFAT pathway might be expected to decrease both conventional immune responses and Treg-mediated suppression. A further understanding of the relationship of Ca^{2+} signaling to conventional and regulatory T cell function will not only provide further insights into molecular mechanism of regulatory T cells, but may also contribute to the development of new therapeutic modalities.

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Figure 1.

Store-operated Ca^{2+} entry in T cells. The binding of antigen/MHC complexes to the T cell receptor (TCR) triggers the activation of protein tyrosine kinases, such as LCK and ZAP70, which eventually results in tyrosine phosphorylation and activation of PLC- γ 1. PLC- γ 1 hydrolyzes the membrane phospholipid PIP₂ to IP₃ and DAG. IP₃ opens IP₃ receptors (IP₃R), which permits Ca^{2+} efflux from ER Ca^{2+} stores. The ER Ca^{2+} sensors STIM1 and STIM2 sense the resulting reduction of ER Ca^{2+} stores via their paired N-terminal EF-hands located in the ER lumen. After Ca^{2+} dissociates from the EF-hands, STIM proteins aggregate into small clusters ("puncta") in the ER membrane and trigger store-operated Ca^{2+} entry via the CRAC channel, ORAI1. Ca^{2+} influx elevates intracellular Ca^{2+} concentration and activates the calcineurin-NFAT pathway as well as regulating the CaMK-CREB pathway.

A **B** **Figure 2.**

Transcriptional regulation of the development and function of regulatory T cells. A). NFAT binds both promoter and enhancer regions of Foxp3 gene[54,57,61,62]. B). Crystal structure of NFAT - Foxp on IL-2 promoter. NFAT cooperates with Foxp3 to suppress IL-2 production [58]. Abbreviation: ARRE, antigen-receptor response element which also binds NFAT-Fos-Jun complexes in conventional activated T cells.