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Calcium signaling in lymphocytes

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Abstract

In cells of the immune system, calcium signals are essential for diverse cellular functions including differentiation, effector function and gene transcription. After engagement of immunoreceptors such as T-cell and B-cell antigen receptors and the Fc receptors on mast cells and NK cells, the intracellular concentration of calcium ions is increased through the sequential operation of two interdependent processes: depletion of endoplasmic reticulum Ca^{2+} stores as a result of binding of inositol trisphosphate (IP_3) to IP_3 receptors, followed by “store-operated” Ca^{2+} entry through plasma membrane Ca^{2+} channels. In lymphocytes, mast cells and other immune cell types, store-operated Ca^{2+} entry through specialised Ca^{2+} release-activated calcium (CRAC) channels constitutes the major pathway of intracellular Ca^{2+} increase. A recent breakthrough in our understanding of CRAC channel function is the identification of STIM and ORAI, two essential regulators of CRAC channel function. This review focuses on the signaling pathways upstream and downstream of Ca^{2+} influx (the STIM/ ORAI and calcineurin/ NFAT pathways respectively).

Introduction

Calcium (Ca^{2+}) is a universal second messenger with a pivotal role in almost all cell types [1,••2,••3]. In cells of the immune system, including T cells, B cells, mast cells and many other cell types, Ca^{2+} signals control proliferation, differentiation, apoptosis and a variety of transcriptional programmes[4,••5,••6]. The consequences of Ca^{2+} signals can be distinguished by whether short- or long-term functions are affected. Short-term functions are generally influenced within minutes and are independent of new gene expression. They include the regulation of lymphocyte motility and the degranulation of allergen-sensitized mast cells or cytolytic CD8^+ T cells[7–10]. The interaction of T cells with antigen-presenting cells (APC) bearing antigenic peptides induces a quick increase of cytoplasmic Ca^{2+} concentration, which stops the movement of T cells and allows them to form stable immunological synapses, a process that is crucial for long-term function. Under conditions where high-affinity antigenic peptides and costimulatory signals are absent, T cells make only brief engagements with APC and display weak and infrequent Ca^{2+} spikes[11]. The long-term functions downstream of Ca^{2+} signalling include lymphocyte proliferation, expression of activation-associated genes, effector functions such as 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

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Conflict of interest. A.R. is a scientific advisor to CalciMedica, a company whose research on immune therapies includes a focus on inhibitors of STIM-ORAI pathway.

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of costimulation -- of an antigen-unresponsive state known as anergy[4]. These events all need sustained Ca^{2+} influx to keep cytoplasmic Ca^{2+} concentrations at higher than basal levels for several hours.

Integration and crosstalk between Ca^{2+} and other signalling pathways in lymphocytes

In lymphocytes and mast cells, the main mechanism for entry of extracellular Ca^{2+} across the plasma membrane is store-operated Ca^{2+} entry (SOCE) through Ca^{2+} release-activated calcium (CRAC) channels[1]. Opening of CRAC channels leads directly to the sustained increase of intracellular Ca^{2+} concentrations and the long-term functional consequences described above. The importance of Ca^{2+} influx through store-operated CRAC channels is highlighted by the existence of at least three families of patients with severe combined immunodeficiency (SCID) associated with severe defects in store-operated Ca^{2+} entry and CRAC channel function, resulting in severely compromised cytokine expression and lymphocyte function[12–14]. Besides CRAC channels, other channels found in lymphocytes include L-type voltage-gated Ca^{2+} channel subunits, the P2X receptor, TRPC channels, TRPV channels and some TRPM channels[••2,••3], but their physiological importance in lymphocyte function remains unclear.

CRAC channels open in response to a lengthy signaling cascade, initiated when immunoreceptors, such as T and B cell antigen receptors (TCR, BCR) as well as mast cell and NK cell Fc receptors, are bound by antigenic complexes (MHC-peptide and antigen-Ig, respectively). Immunoreceptor activation leads to recruitment and activation of protein tyrosine kinases and formation of large protein 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,••3,9]. 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 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, including the phosphatase calcineurin and its target 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). Simultaneously, DAG production activates the Ras-mitogen activated protein kinase (MAPK) and protein kinase C (PKC) pathways, which in turn lead to activation of the transcription factors AP-1 (a transcriptional complex formed by c-Jun and c-Fos) and NF κ B[15,16]. Thus Ca^{2+} signaling is integrated with other signaling pathways and the integration occurs at the level of the binding of transcription factors to DNA response elements, resulting in cell proliferation and cytokine gene expression. A notable example of such integration involves the formation of a cooperative NFAT:Fox:Jun complex on DNA, which drives the transcription of a large number of activation-associated genes[4,17].

In addition, Ca^{2+} signaling directly regulates the Ras-MAPK signalling pathway. Ras is activated by Ras guanine nucleotide exchange factor (RasGEF) and inactivated by Ca^{2+} -activated RasGAPs, including CAPRI and GAPI[18]. Major RasGEFs in T cells are Sos (son of sevenless) and RasGRP1 (Ras guanine nucleotide releasing protein 1)[19,20]. RasGRP1 has a DAG-binding C1 domain and a pair of EF hand motifs that bind to Ca^{2+} directly[21]. RasGRP1 localizes in the cytoplasm of resting T cells and translocates to the Golgi apparatus in response to weak stimulation, such as with soluble anti-CD3 antibodies or with low-affinity peptides that induce positive selection of thymocytes[22,••23]. The RasGEF activity of RasGRP1 in the Golgi depends both on DAG and on increased intracellular Ca^{2+} concentration

[22]. RasGRP1 is barely expressed in thymocytes at the double-negative ($CD4^- CD8^-$, DN) to double-positive ($CD4^+ CD8^+$, DP) stages[24]; instead, the calcineurin/ NFAT pathway was shown to modulate Ras activation in $CD4^+ CD8^+ CD69^-$ DP thymocytes that have not yet undergone positive selection, altering the threshold for ERK activity and allowing weak TCR signals to induce positive selection[25]. B-Raf, MEK and ERK activation are all impaired in DP thymocytes from mice lacking the calcineurin regulatory subunit CnB1, suggesting that calcineurin/ NFAT-dependent transcription regulates the Raf/ Ras/ MAP kinase pathway at several levels, both upstream and downstream of Raf.

In T cells, a major pathway downstream of Ca^{2+} influx is the calcineurin-NFAT pathway, which is well characterized in its molecular aspects. Calcineurin is a calmodulin-dependent serine/threonine phosphatase, consists of the catalytic subunit calcineurin A and a regulatory subunit, calcineurin B. In mammals, there are three known isoforms of calcineurin A, CnA α , CnA β and CnA γ , and two isoforms of calcineurin B, CnB1 and CnB2. NFAT consists of a family of four transcription factors (NFAT1-4, also known as NFATc1-c4)[4,17]. All NFATs except NFAT3 are expressed in peripheral lymphocytes, however, NFAT1 is preferentially expressed in SP thymocytes, NFAT2 in DN thymocytes and B cells, NFAT4 in DP thymocytes [26,27]. An increase in $[Ca^{2+}]_i$ results in binding of Ca^{2+} -calmodulin to calcineurin and activation of its phosphatase activity. Dephosphorylation of cytoplasmic NFAT proteins by calcineurin unmasks the nuclear localization sequence and then NFAT proteins translocate into nucleus[4]. NFAT-driven gene expression is highly dependent on sustained Ca^{2+} influx and calcineurin activity, because a decrease in intracellular Ca^{2+} levels or treatment with the calcineurin inhibitor cyclosporin A results in the immediate export of NFAT from nucleus by NFAT kinases, which include GSK3 (glycogen synthase kinase 3), CK1 (casein kinase 1) and DYRK1A (dual-specificity tyrosine-phosphorylation regulated kinase 1A)[28–30].

The physiological roles of the calcineurin-NFAT pathway have been extensively studied using gene-disrupted mice. The most severe phenotypes are observed in *Cnb1*-deficient mice, in which conventional TCR $\alpha\beta$ T cell development is blocked at the double-positive (DP) to single-positive (SP) transition and thymocyte positive selection is impaired[31]. CnB1 is also crucial for B cell responses in vivo: mice lacking *Cnb1* specifically in B cells show reduced plasma cell differentiation and decreased antigen-specific antibody production during T cell-dependent immune responses [32]. In contrast, the impact on conventional T cell development observed in mice singly disrupted for individual *Nfat* genes or combinations of two *Nfat* genes is less than what is observed in *Cnb1*-deficient mice[33]. Probably, residual *Nfat* gene products can compensate for the loss of other isoforms.

Crosstalk between Ca^{2+} and Notch signaling appears to be important in both keratinocytes and T cells. In keratinocytes, Notch signaling increases calcineurin/NFAT activity by downregulating calcipressin, an endogenous calcineurin inhibitor[34]. In turn, calcineurin/ NFAT signaling affects the expression of Notch-responsive genes. Notch signaling is crucial for early T cell development[35]. Thus Notch signaling potentially cooperates with the calcineurin/NFAT pathway in T cells as well as in keratinocytes.

Store-operated Ca^{2+} entry through CRAC channels: STIM and ORAI

As a result of recent technological advances that have made it possible to perform large-scale RNA interference (RNAi) screens, two key regulators of store-operated Ca^{2+} entry, STIM (an ER Ca^{2+} sensor)[36,37] and ORAI (a pore subunit of the CRAC channel)[••38,•39,•40], have been identified by several groups. These proteins have been extensively investigated over the last two years, and their analysis has greatly advanced our understanding of the mechanisms of SOCE. *Stim* (stromal cell interaction molecule[41]) was identified in two limited RNAi screens, performed in *Drosophila* and HeLa cells respectively[36,37]. Flies and worms express

only one STIM protein[36], whereas mammals express two STIM proteins, STIM1 and STIM2, with 47% amino acid identity[42]. 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. The first EF-hand of each pair binds Ca^{2+} with low affinity matched to the high concentration of Ca^{2+} in the ER (~100–700 μM) [••6,36,37].

STIM1 is an acknowledged activator of store-operated Ca^{2+} entry and CRAC channel function as discussed below, but the role of STIM2 was initially controversial, with conflicting results reported by different groups[36,37,43]. An important clue as to the possible distinction between the two proteins came when STIM2 showed up as a major hit in an RNAi screen designed to find regulators of basal Ca^{2+} concentration, which is kept within very narrow limits in cells [••44]. Both STIM1 and STIM2 were able to sense depletion of ER Ca^{2+} stores and trigger store-operated Ca^{2+} entry via ORAI1, but RNAi-mediated knockdown of STIM2 selectively decreased basal cytosolic and ER Ca^{2+} levels in several cell lines. These findings are supported by studies of STIM-deficient cells. T cells and fibroblasts from mice with a conditional deletion of STIM1 showed a pronounced decrease in store-operated Ca^{2+} entry and CRAC channel function, which could be rescued efficiently by STIM1 and less efficiently by STIM2[••45]. T cells lacking STIM1 showed only a transient blip of NFAT nuclear localisation due to ER store depletion, whereas T cells lacking STIM2 showed a normal initial phase of NFAT nuclear localisation which however was not sustained[••45]. Together, these results confirm that 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 activated primarily during the early phase of the response when ER Ca^{2+} stores are strongly depleted, whereas STIM2 operates both in resting cells with Ca^{2+} -replete stores where it is already partially active and controls basal Ca^{2+} influx, and also during the late stages of a response when ER stores are becoming refilled. At these late time, STIM1 is inactivated by rebinding of Ca^{2+} whereas STIM2 remains active[••44,••45].

Drosophila Orai (*olf186-F*) was discovered as an essential regulator of store-operated Ca^{2+} influx in genome-wide *Drosophila* RNAi screens performed by three different groups [••38,•39,•40]. *Drosophila* Orai and its three human homologues, ORAI1-3, are small proteins with four transmembrane domains, whose N and C-termini are both located in the cytoplasm [••38,46,•47]. In humans, a single missense mutation in the *Orai1* gene caused severe combined immunodeficiency disease characterized by the absence of SOCE and I_{CRAC} [••38]. Mutational and electrophysiological analyses have identified *Drosophila* Orai and human ORAI1 as pore subunits of the CRAC channel[•47,•48,•49]. Consistent with this hypothesis, ORAI1 exists in the plasma membrane as at least a dimer, and its active form is likely to be a homotetramer [46,50]. Moreover, combined overexpression of ORAI1 and STIM1 leads to marked increases in CRAC current in cells[•49,51,52]. ORAI2 and ORAI3 have similar but not identical electrophysiological characteristics to ORAI1[53,54].

Gating of CRAC channels by local aggregation of STIM and ORAI

Following store depletion, STIM1 aggregates into small clusters (“puncta”) in the ER membrane[37,55]; STIM1 with an EF-hand mutation that impairs Ca^{2+} binding is constitutively present in puncta even in resting cells with replete Ca^{2+} stores[37]. The propensity of STIM1 to aggregate when its EF-hands are not bound by Ca^{2+} can be demonstrated using just a short recombinant N-terminal fragment of STIM1, containing only the EF-hands and the adjacent SAM (sterile α -motif) domain[56,57]. Once formed, the STIM1 clusters preferentially localize to sites of ER-plasma membrane apposition and colocalize partially with clusters of ORAI1[55,58,••59]. The regions of STIM1 clustering coincide with sites of Ca^{2+} entry[••60].

STIM1 and ORAI1 are the primary regulators of store-operated Ca^{2+} entry. Combined overexpression of STIM1 and ORAI1 leads to massive (up to 100-fold) increase in CRAC currents in many cell types, whereas expression of the individual proteins has less effect or may even be inhibitory[•49,51,52], implying that STIM1 is the major *limiting* component needed to open ORAI1-containing CRAC channels in response to store depletion. Whether there is a direct, physiologically relevant physical interaction between STIM1 and ORAI1 in cells remains controversial, however: no interaction, constitutive interaction and increased interaction after store depletion have all been reported [46,•47,•49]. Two reports suggest that overexpression of the cytoplasmic region of STIM1 in cells suffices to induce CRAC currents [61,62]. A coiled-coil domain in the cytoplasmic region of STIM1 is crucial for puncta formation and store-operated Ca^{2+} entry[63,64], and this region has been suggested to interact directly with a coiled-coil domain at the C-terminus of ORAI1, both in cell-free systems and by FRET after store depletion in cells[62]. Wu et al. estimated a minimum distance of 17 ± 10 nm between the ER and plasma membranes for ER-plasma membrane appositions at which ORAI1 and STIM1 come together[••59]; this distance could potentially be spanned by the cytoplasmic region of STIM1 in an extended conformation, but does not rule out the existence of a larger protein complex bridging STIM1 and ORAI1. Using chemical dimerisers to artificially bring together the ER and plasma membranes, Varnai et al. demonstrated that before store depletion, STIM1 was capable of diffusing freely within these regions even when the membranes were estimated to be only 4–6 nm apart, whereas ORAI1 was excluded unless the membranes were between 8 and 14 nm apart[•65]. After store depletion, an estimated distance of 12–14 nm between the ER and plasma membranes was required for STIM1 and ORAI1 to colocalise within the area of the apposed membranes instead of being confined to their periphery[•65]. This estimated distance is almost the same as the maximal distance for an efficient FRET signal (usually within 10 nm), suggesting that the possibility of indirect rather than direct interaction between STIM1 and ORAI1 still cannot be ruled out.

In T cells, 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[•66]. This interface coincides with local Ca^{2+} influx from the extracellular space. 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+} [67]. 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.

Physiological consequences of individual deficiency of STIM1, STIM2 or ORAI1 in mice

Stim1-deficient mice have been generated by our laboratory[••45] and by one other group [•68] *Stim1* deficiency results in perinatal lethality, leading us to investigate mice in which STIM1 was deleted only in peripheral T cells. *Stim1*-deficient T cells completely lack store-operated Ca^{2+} entry, I_{CRAC} and Ca^{2+} -dependent cytokine expression[••45]. In contrast, *Stim2*-deficient naïve T cells showed normal store-operated Ca^{2+} entry and cytokine production; this was not due to compensatory upregulation of STIM1, but rather reflects the fact that STIM2 constitutes only a very small proportion of total STIM protein in T cells. Mast cells from *Stim1*-deficient mice were defective in degranulation owing to a severe decrease of store-operated Ca^{2+} entry[•68]; this was also true of mast cells from mice with a gene-trap insertion in the *Orail* gene[•69]. Passive cutaneous anaphylaxis, a reaction which measures mast degranulation *in vivo*, was substantially impaired in the *Orail* gene-trap mutant mice, and in fact was decreased even in heterozygous (haploinsufficient) *Stim1*^{+/-} mice[•68,•69].

These data emphasise that mast cell function is strongly dependent on store-operated Ca^{2+} entry through STIM1 and ORAI1.

In contrast to NFAT, NF κ B is selectively activated by a large transient increase of Ca^{2+} concentration and activates target gene expression[1,4]. Activation of NFAT and NF κ B is also sensitive to the frequency of $[\text{Ca}^{2+}]_i$ oscillations[1,4]. NF κ B is preferentially activated by low-frequency Ca^{2+} oscillations, whereas both NFAT and NF κ B are activated by high-frequency oscillations. Consistent with these observations, nuclear translocation of p65 RelA is significantly decreased in STIM1-deficient mast cells and T cells[•68](M Oh-hora et al., unpublished).

T cell development was found to be normal in *Orai1* gene-trap mutant mice[•69], as well as in mice with a null mutation in the *Orai1* gene generated in our laboratory (Y Gwack, S Srikanth, M Oh-hora et al., unpublished). However, a major difference between these two mouse lines lies in the degree of impairment of T cell function. T cells from the ORAI1 gene-trap mice showed decreased cytokine expression in response to stimulation but no obvious impairment in store-operated Ca^{2+} entry or CRAC channel function[•69], suggesting that these mice are hypomorphs rather than true nulls. In contrast, T cells from ORAI1-null mice showed a clear impairment in all three functions, that was especially pronounced in previously activated and differentiated cells (Y Gwack, S Srikanth, M Oh-hora et al., unpublished). This result probably reflects compensation from ORAI2 or ORAI3; in particular, ORAI2 is expressed at relatively high levels in naïve T cells but is downregulated upon activation. Surprisingly, however, acute expression of ORAI2 in *Orai1*-null T cells did not reconstitute function, implying that other factors are needed.

Unexpected requirement for STIM proteins in development and function of regulatory T cells

Unexpectedly, mice in which both *Stim1* and *Stim2* had been deleted in peripheral T cells by expression of Cre under control of the CD4 promoter showed a striking decrease of regulatory T cell (Treg) population in all immune organs and developed autoimmune symptoms[•45]. The effect was selective for the Treg lineage, since the mice showed normal thymic cellularity and normal development of thymocytes and peripheral T cells. Thus, Treg development appears to be especially sensitive to the absence of STIM-mediated Ca^{2+} influx (Figure 2). These data are consistent with other studies showing involvement of the calcineurin-NFAT pathway in Treg development and function[70–72].

How does the calcineurin-NFAT pathway impinge on Treg development and function? In “natural” (i.e. thymic-derived) regulatory T cells and “induced” regulatory T cells (i.e. induced by TGF β treatment in culture), NFAT regulates *Foxp3* expression in combination with Smad3 [•73]. 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[•73,74]. *Foxp3* can also physically interact with NFAT, and this complex regulates the suppressive function of *Foxp3*-expressing T cells in a mouse model of autoimmune diabetes[•72]. Together, these data show that STIM proteins, NFAT and *Foxp3* are all involved in a positive feedback pathway that enhances Treg development and function.

In contrast to the clear effect of combined *Stim1*, *Stim2* deletion, the role of the downstream proteins calcineurin and NFAT in Treg development and function is not yet fully resolved. Mice with a double deletion of *Nfat1* and *Nfat4* also show lymphoproliferative autoimmune phenotypes[75]. In these mice, Treg development and function appeared normal, and

hyperproliferation was attributed to the fact that their T cells were not effectively inhibited by wild-type Tregs[76]. Furthermore, *Cnbl*-deficient mice have not so far been reported to show any dysfunction or impaired development of Tregs[31]. Further studies are needed to elucidate the complex effects of mutations in the Ca^{2+} / calcineurin/ NFAT signalling pathway on T cell and Treg development.

Conclusions

The field of Ca^{2+} signalling has been reinvigorated in the last two years by the discovery of two key players in store-operated Ca^{2+} entry, STIM and ORAI. It is now possible to investigate the function of this pathway in molecular detail. Outstanding questions remain: What is the molecular composition of CRAC channels? How are CRAC channels gated through co-aggregation of STIM and ORAI? What are the unique biological functions of STIM and ORAI proteins and other components of the Ca^{2+} signalling pathway, including downstream effectors such as NFAT proteins, in vivo? The availability of conditional mouse genetic models will greatly facilitate the study of these questions in a physiological context in different cell types. Both store-operated Ca^{2+} entry and the Ca^{2+} -calcineurin-NFAT pathway are validated targets for the development of immunosuppressive drugs, the first because of the existence of a SCID patient with an Orai1 R186W mutation who shows only mild extra-immunological symptoms [12,••38] and the second because of the well-established clinical efficacy of the immunosuppressive drugs cyclosporin A and FK506[4,17]. A further understanding of these pathways will not only provide further insights into lymphocyte and mast cells activation, 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 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 $\text{PLC-}\gamma 1$. $\text{PLC-}\gamma 1$ hydrolyzes the membrane phospholipid PIP_2 to IP_3 and DAG. IP_3 opens IP_3 receptors (IP_3R), 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 concentration and activates the calcineurin-NFAT pathway as well as regulates the Ras-MAPK pathway.

Figure 2.

T cell development in thymus. $\text{CD4}^+ \text{CD8}^+$ double positive (DP) thymocytes differentiate into conventional CD4^+ or CD8^+ single positive (SP) $\alpha\beta\text{TCR}^+$ thymocytes, or $\text{CD4}^+ \text{CD25}^+$ Foxp3 $^+$ regulatory T cells (T_{reg} cells). STIM-dependent store-operated Ca^{2+} entry appears to be crucial for the development of T_{reg} cells but dispensable for the development of conventional $\alpha\beta\text{TCR}^+$ thymocytes[••45]. We speculate that other Ca^{2+} entry pathways might compensate for loss of STIM-dependent store-operated Ca^{2+} entry in the differentiation of DP thymocytes into conventional $\alpha\beta\text{TCR}^+$ SP thymocytes.