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Interleukin-2 Receptor Signaling in Regulatory T Cell Development and Homeostasis

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Abstract

Interleukin-2 (IL2) was initially identified from supernatants of activated lymphocytes over 30 years ago [1]. In the ensuing 15 years, the cDNAs for both *IL2* and the three chains of the interleukin-2 receptor (IL2R) were cloned [2–7]. Subsequently, many of the downstream biochemical pathways activated by the IL2 receptor complex were identified [8] and the structure of IL2 bound to this tripartite receptor complex was solved [9]. Thus, we now have a very good understanding of how each chain contributes to high affinity IL2 binding and signal transduction [10,11]. In contrast, over the past 30 years the role that IL2 plays in regulating lymphocyte function has involved many surprising twists and turns. For example, IL2 has been shown, paradoxically, to regulate both lymphocyte proliferation and lymphocyte death. In this review, we briefly outline the original findings suggesting a role for IL2 as a T cell growth factor, as well as subsequent studies pointing to its function as an initiator of Activation-induced Cell Death, but then focus on the newly appreciated role for IL2 and IL2R signaling in the development and homeostasis of regulatory T cells.

Interleukin-2 plays an important role in multiple aspects of T cell biology. Binding of IL2 to its receptor was initially demonstrated to be critical for inducing the proliferation of T cells in vitro [12]. Subsequent studies demonstrated that IL2R signaling leads to the activation of many genes associated with cell proliferation such as *c-myc* and *fos* [13]. These findings led to the idea that signaling via the IL2R complex would be critical for mounting effective immune responses in vivo and established IL2 as a T cell growth factor. However, the concept of IL2 as a critical factor required for T cell proliferation was essentially overturned in the early 1990s when mice lacking the *IL2* gene were first developed. The initial characterization of these mice demonstrated that *in vitro* proliferation of ConA-stimulated *IL2*^{−/−} T cells was impaired, but much more modestly than one would have predicted, suggesting the presence of alternative T cell growth factors [14]. It also was readily apparent that *in vivo*, T cell proliferation was clearly not impaired; *IL2*^{−/−} mice exhibited massive lympho-proliferation resulting in splenomegaly and lymphadenopathy. In addition, *IL2*^{−/−} mice rapidly developed signs of autoimmunity including, most prominently, a disease resembling ulcerative colitis [15]. These autoimmune manifestations ultimately resulted in death of 100% of *IL2*^{−/−} mice between 12–25 weeks of age. The reasons for this surprising onset of autoimmunity were at first unclear, but one potential explanation was provided by Michael Leonardo who demonstrated that IL2 plays an important role in sensitizing T cells for Activation-induced cell death [16]. When mice lacking the IL2R α or IL2R β chain were generated, they too exhibited multi-organ autoimmune disease

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resulting in early death [17,18]. Moreover, examination of *IL2R α ^{-/-}* mice demonstrated a significant defect in activation-induced cell death [17] suggesting that the primary mechanism that accounted for deregulated T cell homeostasis, and the onset of autoimmunity, was a failure of IL2-induced T cell death.

The data suggesting that IL2 prevents autoimmunity via an effect on activation-induced cell death was compelling. However, new discoveries regarding a novel subset of CD4⁺ T cells began to shed doubt on whether the failure of IL2-induced T cell death was sufficient to account for the defects seen in IL2 deficient mice. Specifically, in the mid 1990s Sakaguchi and colleagues identified a population of CD4⁺ T cells that expressed high levels of CD25 and potently suppressed the proliferation of activated T cells in vitro. Moreover, they found that injection of CD4⁺CD25⁻ T cells into T-cell depleted host mice rapidly induced autoimmune disease; importantly, disease could be prevented or even reversed by the transfer of CD4⁺CD25⁺ T cells [19]. These studies established CD4⁺CD25⁺ T cells as the elusive suppressor T cells, more commonly referred to now as regulatory T cells (or Tregs), that were initially proposed over 35 years ago [20,21]. The discovery of this novel T cell subset characterized by high level IL2R expression, in conjunction with the observation that *IL2^{-/-}* and *IL2R β ^{-/-}* mice lacked CD4⁺CD25⁺ T cells, suggested that IL2-dependent signals were required for either Treg development, homeostasis, or function. Subsequent studies aimed at identifying genes involved in autoimmune disease have found distinct alleles of *IL2* or *IL2R α* associated with autoimmunity and defects in Tregs. For example, the insulin-dependent diabetes susceptibility locus 3 (*idd3*) in the NOD mouse has been shown to map to the *IL2* gene; importantly, Tregs from susceptible mice were found to have decreased regulatory T cell function [22]. Likewise, the *idd4* locus in NOD mice has been suggested to map to alterations in the downstream IL2 effector *STAT5* [23]. Finally, studies of human diabetes, multiple sclerosis and Graves' Disease have been mapped to distinct alleles for *IL2R α* [24–26]. These observations led to an explosive interest in regulatory T cells resulting in significant insights to all aspects of Treg biology. In the ensuing few pages we will specifically focus on the role IL2 plays in the differentiation and homeostasis of so-called natural Tregs that develop in the thymus.

Role of IL2 in the Development of CD4⁺CD25⁺Foxp3⁺Tregs

Seminal studies from three separate labs eventually established that the critical defect in IL2- or IL2R-deficient mice resulted from the absence of functional Tregs [27–30]. Compellingly, Malek and colleagues demonstrated that transfer of limited numbers of CD4⁺CD25⁺ T cells into *IL2R β ^{-/-}* mice prevented autoimmune disease. These findings clearly demonstrated that the defect in *IL2R β ^{-/-}* mice was not cell autonomous, but rather due to the absence of a functional Treg population [28]. These studies pointed to a critical role for the IL2R in Treg biology. However, whether the effect of the IL2R was on Treg development, homeostasis, or function remained unclear and has been quite controversial.

Malek and colleagues initially proposed that the IL2R was uniquely required for Treg development. Specifically, they developed a transgenic mouse model in which transcription of the *IL2R β* -chain was driven by the *lck*-proximal promoter, resulting in a thymic-restricted expression pattern. Crossing this transgene onto the *IL2R β ^{-/-}* background restored a population of CD4⁺CD25⁺ T cells in the thymus and periphery that suppressed lymphoproliferation and the lethal autoimmune disease normally observed in *IL2R β ^{-/-}* mice [31]. These findings suggested that IL2R β -dependent signals were required for Treg development in the thymus, but were dispensable for their homeostasis and function in the periphery. Subsequent studies in which neutralizing antibodies to IL2 were injected into neonatal mice demonstrated a reduction in numbers of CD4⁺CD25⁺ Tregs in the thymus [32], further supporting a role for IL2 in Treg development. In contrast, work by LaFaille and colleagues demonstrated that both splenocytes and purified CD4⁺CD8⁻ thymocytes from *IL2*

–/– mice could suppress the induction of disease in a mouse model of EAE [33]. This effect was not observed when CD4⁺ T cells were obtained from *IL2Rα*–/– mice. These studies indicated that there is a population of CD4⁺ T cells in both the thymus and spleen of *IL2*–/– mice that can exert regulatory activity when IL2 is restored. The initial explanation for these findings was that IL2 was required for Treg function. However, an alternative possibility was that IL2 was required for Treg homeostasis and that potentially reduced numbers of Tregs found in *IL2*–/– mice were rapidly expanded following transfer into IL2-competent host mice. Thus, early studies on the role of IL2 and the IL2R in Treg development led to apparently contradictory findings.

A major problem with the initial studies on the role of IL2 in Treg development was the use of CD25 as a surrogate marker for this cell population. Although relatively selective, CD25 is also upregulated on activated effector T cells [34]. Moreover, it was not clear whether all Tregs would be found within the CD25⁺ subset of CD4⁺ T cells. To more accurately dissect the role of IL2 in Treg biology, a unique marker was needed that could be utilized to track developing regulatory T cells in the thymus and mature Tregs in the periphery. Recent pioneering work by several groups identified a member of the forkhead-box family of transcription factors called Foxp3 which fulfills such a role. Specifically, the naturally occurring *scurfy* mouse mutant, which develops lethal multi-organ autoimmune disease, was found to have mutations in the *foxp3* gene that were predicted to disrupt Foxp3 function; restoration of wild type Foxp3 in these mice prevented disease [35]. Likewise, the genetic defect in human patients suffering from the autoimmune disease IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) were found to stem from mutations in the human *foxp3* gene [36,37]. Subsequent studies by the Ramsdell, Rudensky and Sakaguchi labs independently demonstrated that Foxp3 was both necessary and sufficient for Treg development [38–40]. Importantly, these studies also established Foxp3 as a unique marker for Tregs in the mouse. More recently, the Rudensky and Flavell labs generated mouse models in which cells that express Foxp3 can be directly identified using the fluorescent proteins GFP or DsRed respectively [41,42]. Using these mouse models and recent advances in intracellular flow cytometry for Foxp3, the controversial role of IL2 and IL2R signaling in Treg development and homeostasis is beginning to be clarified.

Using a Foxp3-GFP fusion knock-in mouse model Fontenot et al observed that CD4⁺Foxp3⁺ cells were clearly present in *IL2*–/– mice, although they failed to express CD25. Identical results were obtained in *IL2Rα*–/– mice. In both *IL2*–/– and *IL2Rα*–/– mice, numbers of thymic Tregs in young animals were slightly reduced (~2-fold) while peripheral Tregs were present in relatively normal numbers [43]. Similar conclusions were simultaneously reached by D’Cruz and Klein using a transgenic TCR system and intracellular staining for Foxp3 protein. In these studies, they found that thymic selection into the Treg lineage was not impaired in *IL2*–/– mice, but the peripheral maintenance of these cells was dramatically affected [44]. Although the studies by Fontenot et al and D’Cruz and Klein appeared to contradict the original findings of Malek and colleagues regarding the role of IL2Rβ in Treg development, more recent work examining Tregs in *IL2Rβ*–/– mice appears to have resolved this discrepancy. Specifically, using intracellular staining for Foxp3 we too have found that young *IL2*–/– and *IL2Rα*–/– mice have relatively normal numbers of thymic and peripheral Tregs. In contrast, mice in which IL2Rβ signaling is disrupted have a more dramatic defect in the population of regulatory T cells in the thymus and secondary lymphoid organs [45]. Similar results using *IL2Rβ*–/– mice have recently been obtained by Ziegler and colleagues [46]. To examine this in more detail, we generated *IL2*–/– *x* *IL15*–/– mice and observed that they had a comparable decrease in regulatory T cell numbers to that seen in *IL2Rβ*–/– mice [45]. These results demonstrate that IL2 and IL15 act as redundant factors, which are both capable of regulating Treg development via interactions involving the IL2Rβ chain. Importantly, these results provide an explanation for the earlier apparently discrepant findings of Malek and Lafaille. Namely, IL2 by itself is not required for Treg development due to the redundant role of IL15. However, the IL2Rβ

chain is required for efficient Treg development as it is a critical component of both the IL2 and IL15 receptors. Finally, it is important to point out that other γc -dependent cytokines, such as IL7, may allow for inefficient Treg development. This is based on the observation that while *IL2R β* ^{-/-} mice show a clear reduction in Tregs in both the thymus and peripheral lymphoid organs, a residual population of these cells can still be seen. In contrast, γc ^{-/-} mice are devoid of Tregs [43,45]. Taken together, these studies illustrate a predominant role for the IL2R β chain in driving the development of Tregs.

Role of IL2 in the Homeostasis of CD4⁺Foxp3⁺ Tregs

The initial findings that thymic restricted expression of the IL2R β chain was sufficient to restore peripheral Treg numbers suggested that IL2R β -dependent signals might not be required for homeostasis of peripheral Tregs [31]. A potential caveat to these studies was the observation that the *IL2R β* transgene was weakly expressed in peripheral T cells. IL2R β expression levels were sufficient for the induction of very transient STAT5 activation but, importantly, did not induce clear biological responses [47]. Perhaps more tellingly, when regulatory T cells from these mice were transferred into *IL2R β* ^{-/-} mice (which lack Tregs but do produce IL2) they did not survive nor did they prevent autoimmunity [28]. Subsequent studies found that mature Tregs in mice expressing the thymic-restricted IL2R β chain proliferated poorly when compared to WT Tregs. Mixed bone marrow chimera experiments, in which the ability of cells expressing the thymic-restricted IL2R β chain versus WT cells were compared, were also carried out. In those studies, cells expressing the thymic restricted IL2R β chain contributed well to the thymic Treg compartment but were not effective at repopulating peripheral lymphoid organs when compared to WT Tregs [48]. Taken together, these studies suggested that IL2R β -dependent signals are required for homeostasis of Tregs as well.

A remaining question is whether IL2, as opposed to the IL2R (which can bind both IL2 and IL15), is also required for Treg homeostasis. As mentioned above, relatively normal numbers of Tregs are found in both *IL2*^{-/-} and *IL2R α* ^{-/-} mice indicating that IL2 may not be critical for Treg homeostasis [43–45]. In addition, purified Tregs injected into *rag2*^{-/-} mice underwent homeostatic proliferation that was not affected by IL2 neutralization, indicating that under lymphopenic conditions IL2 is not critical for Treg homeostasis [49]. However, it is important to note that Tregs do disappear in older *IL2*^{-/-} and *IL2R α* ^{-/-} mice (>8 weeks of age). Furthermore, gene microarray studies comparing Tregs from WT and *IL2*^{-/-} mice suggested that IL2 plays a predominant role in regulating metabolic activity and survival of Tregs [43]. Supporting these findings, Setoguchi et al carried out studies in which they injected Balb/c mice with IL2 neutralizing antibodies. In these studies they found a modest reduction of CD4⁺CD25⁺ T cells in the thymus, but a profound reduction in CD4⁺CD25⁺ T cells in secondary lymphoid organs such as the spleen and lymph nodes. Furthermore, this reduction of CD4⁺CD25⁺ T cells resulted in the spontaneous induction of autoimmune gastritis in BALB/c mice; similarly, IL2 neutralization in NOD mice led to an exacerbation of the diabetes phenotype that naturally develops in these animals [49]. We have obtained similar results when injecting neutralizing IL2 antibodies into C57Bl/6 mice; peripheral CD4⁺Foxp3⁺ Treg numbers were clearly reduced. Importantly, however, we found that this treatment only affected the CD4⁺CD25⁺ subset of Tregs, suggesting that other cytokines may play a role in maintaining homeostasis of CD4⁺CD25⁻Foxp3⁺ Tregs (MAB and MAF, unpublished observations). Taken together we believe the available data suggest a model (Fig. 1) in which IL2 plays a critical role in governing normal homeostasis of Tregs. This most likely reflects the downregulation of the IL7R α [50,51] and IL15R α chain (KBV and MAF, submitted) on mature Tregs, which renders them more dependent on IL2 for survival and expansion. Importantly, we have found that Tregs in *IL2*^{-/-} mice have upregulated expression of both the IL15R α and IL7R α chains (KBV and MAF, submitted); this most likely accounts for the ability of Tregs to survive (at least initially) in *IL2*^{-/-} mice. Likewise, under lymphopenic conditions,

IL15 and IL7 appear to be sufficient to maintain Treg homeostasis [49]. However, in wild type mice, in which Tregs express very low levels of the IL7R α and IL15R α chains and thus compete quite poorly with naïve and effector T cell subsets for endogenous IL7 and IL15, homeostasis of Tregs is predominately governed by IL2.

IL2R Signaling and Tregs

The IL2 receptor-signaling complex is composed of three distinct subunits, the α -chain (CD25), β -chain (CD122), and γ -chain (CD132) each of which have unique roles in facilitating IL2-dependent signal transduction. The IL2R α chain has a very short cytoplasmic domain and does not transmit intracellular signals [52]. Instead, IL2R α acts to confer high affinity binding of IL2 to the IL2R complex (K_d for IL2R β / γ c = 10^{-9} M versus 10^{-11} M for the IL2R α / β / γ c tripartite receptor)[10,34]. In contrast, the IL2R β and γ c chains play the predominate role in transducing intracellular signals. Neither of these chains has intrinsic enzymatic function; rather, they associate with the intracellular tyrosine kinases Jak1 and Jak3, respectively [53–56]. Ligand binding results in the activation of Jak1 and Jak3 which then phosphorylate multiple tyrosine residues found in the cytoplasmic tail of the IL2R β chain [57]. Therefore, the primary component of the IL2R complex that functions to convert extracellular ligand binding into intracellular signals is the IL2R β chain.

Taniguchi and colleagues first identified three distinct regions of the IL2R β -chain that were critical for IL2-dependent signal transduction using expression of mutant IL2 receptor constructs in the mouse pro-B cell line BAF-BO3 [58]. These regions include a serine rich region, an acidic region, and a carboxy-terminal region, referred to as the S, A, and H regions, respectively (Fig. 2). Numerous studies have shown that the primary role of the S-region in IL2R signaling is binding of JAK-1 to conserved motifs referred to as box1 and box 2 [59, 60]. The association of Jak-1 with the IL2R β chain, along with the association of JAK-3 with the γ c chain, leads to phosphorylation of tyrosine residues in the A and H-regions following receptor activation. Thus, the primary role of the A- and H-regions in IL2R signaling is to serve as docking sites for SH2-domain-containing adaptor or effector molecules. Three of these intracellular tyrosine residues, which are conserved across multiple species, appear to play particularly important roles. The first of these is the most membrane-proximal tyrosine residue (Tyr-338 in the human receptor; Tyr-341 in the murine receptor). Several groups demonstrated that Tyr-338 is critical for the recruitment of the adaptor protein Shc to the receptor complex [61–63]. This leads to recruitment of the adaptor protein Grb2 and activation of the Ras/Raf/Mek/Erk signaling cascade. In addition, the adaptor protein Gab2 is also recruited via SHC, and results in the recruitment and activation of PI3K [64]. These findings indicate that the A region is utilized to induce MAPK and PI3K signaling pathways. In contrast, the H-region, which contains two tyrosine residues, Tyr-392 and Tyr-510, appears to be responsible for activation of STAT5. Expression of IL2R mutants lacking either the A or H regions demonstrated that either receptor was capable of preventing autoimmunity when introduced into IL2R β ^{-/-} T cells in vivo [65,66]. This led to the suggestion that the Ras/PI3K and STAT5 pathways were redundant with regard to Treg development and homeostasis. However, subsequent work cast some doubt on this interpretation. Specifically, the role of the tyrosine residues in both the A- and the H-regions were further dissected using IL2R constructs containing various tyrosine to phenylalanine mutations. Using this strategy, Gaffen et al demonstrated that the transcription factor STAT5 could be activated by three tyrosine residues in the IL2R β -chain (Tyr-338 in the A-region, Tyr-392, and Tyr-510 in the H-region)[67]. Consistent with the initial studies examining IL2R signal transduction, STAT5 appears to bind most avidly to phosphorylated Tyr-510 and to a lesser extent to Tyr-392. Supporting these findings, IL2R β mutants containing just Tyr-392 or Tyr-510 were capable of activating STAT5. However, although direct binding of STAT5 to Tyr-338 based phospho-peptides has not yet been demonstrated, an IL2R β -mutant containing just Tyr-338 was capable of activating

STAT5. Thus, both the A- and H-regions are capable of activating STAT5 suggesting that perhaps STAT5 plays a key role in Treg development.

Given that IL2 induces multiple signal transduction pathways, it is important to know which of these pathways are actually active in Tregs. A key study by Bensinger et al demonstrated that activated CD4+CD25+ effector T cells induced the PI3K, Ras and STAT5 signaling pathways in response to IL2 stimulation. In contrast, freshly purified CD4+CD25+ Tregs exclusively activated STAT5 when stimulated with exogenous IL2 [68]. Supporting a role for STAT5 in Treg development and homeostasis, Burchill et al reported that transgenic mice expressing a constitutively active form of STAT5 in lymphocytes exhibit a significant increase in Tregs in both the thymus and secondary lymphoid organs [69]. In contrast, Treg numbers are reduced in mice expressing a STAT5 hypomorphic allele [69–71]. These studies pointed to a key role for STAT5 in promoting Treg homeostasis but did not address whether STAT5 regulated Treg development.

The generation of mice in which both STAT5a and STAT5b were completely deleted was required to examine the role of STAT5 in Treg development. Mice in which the STAT5a and STAT5b genes were flanked by loxP sites were ultimately generated by Hennighausen and colleagues [72]. Deletion of STAT5 in all tissues led to multiple defects, including impaired erythropoiesis, and perinatal lethality [72]. To examine the role of STAT5 in T cells, we crossed *STAT5a/b* floxed mice to *CD4-Cre* transgenic animals thereby allowing for selective ablation of STAT5 in T cells. We found that T cells lacking STAT5 were incapable of differentiating into Tregs, strongly supporting a role for STAT5 in Treg development [45]. Similar findings were reported by O'Shea and colleagues [73]. Thus, STAT5 is necessary for Treg development.

To determine whether IL2R β -dependent STAT5 signaling was sufficient to drive Treg development and survival, Yang and colleagues generated a series of IL2R β mutants which selectively activated just the STAT5 or Ras/PI3K pathways [45]. Hematopoietic stem cells from *IL2R β ^{-/-}* mice were transduced with retroviruses expressing these mutants and then used to generate bone marrow chimeras to examine the role of these two pathways in Treg development. These studies demonstrated that activation of STAT5 alone was sufficient to restore Treg development while activation of the Ras/PI3K pathway was not ([45] and JY and MAF, unpublished results). Supporting these studies, we found that expression of a constitutively active STAT5 transgene restored Treg development in both *IL2R β ^{-/-}* and γ c^{-/-} mice [45]. Taken together, these findings illustrate that IL2R β -dependent activation of STAT5 is both necessary and sufficient for the development and homeostasis of Tregs.

Although mouse models in which levels of STAT5 activation are manipulated indicate a critical role for STAT5 in Treg development, the exact means by which STAT5 directs this process has yet to be fully elucidated. One possible mechanism by which STAT5 signaling could influence Treg biology is through the induction or maintenance of the Treg lineage specification factor Foxp3. Recently Zorn et al identified a consensus STAT5 binding motif in the first intron of the human *foxp3* gene locus that is conserved throughout evolution. Introduction of luciferase constructs containing this motif into fibroblasts, in the presence of a constitutively active form of STAT5 or STAT3, resulted in the induction of luciferase activity [74]. These findings demonstrated that this motif was potentially functional, but did not demonstrate whether STAT5 or STAT3 actually bound these sites in vivo. We independently identified the same consensus STAT5 binding motif in the first intron of the *foxp3* gene, as well as several closely spaced evolutionarily conserved STAT5 binding motifs in the 5' region of the murine *foxp3* promoter (Fig. 3). Using chromatin immunoprecipitation assays, we detected binding of STAT5 to the promoter site, but not the intronic sites in sorted CD4+CD25+ T cells [45]. A recent report by O'Shea and colleagues confirmed our chromatin immunoprecipitation studies with regard to STAT5 binding to the *foxp3* promoter site.

However, they also reported significant binding of STAT5 to the conserved intronic STAT5 binding sites, as well as a site located several kb 5' of the *foxp3* transcription start site. These latter studies also demonstrated that STAT3 was completely dispensable for *foxp3* transcription and Treg development [73]. Taken together, these studies clearly demonstrate that STAT5 can bind to the *foxp3* gene. However, additional experiments are required to determine the biological significance of these STAT5 binding sites.

In summary, studies over the last few years have helped illuminate the key role that IL2 and IL2R β -dependent signals play in Treg development and homeostasis. However, many questions still remain. For example, although STAT5 has been shown to bind to distinct sites in the *foxp3* gene, the functional relevance of these binding sites remains to be determined. Likewise, transcription factors that potentially cooperate with STAT5 to promote *foxp3* transcription remain to be identified. Finally, how TCR-dependent and IL2R β -dependent signals interact to promote Treg development remains to be established. The answer to these questions will provide a more complete picture of how IL2R β -dependent signals entrain regulatory T cell development and homeostasis.

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Figure 1. Model of Cytokine-dependent Treg Development and Homeostasis

In the thymus, IL2 plays the predominant role in driving Treg development. In the absence of IL2, IL15 is sufficient for regulatory T cell development. Other γ c-dependent cytokines such as IL7 may play a minor role in this process and most likely account for the few Tregs found in $IL2R\beta^{-/-}$ mice as compared to $\gamma c^{-/-}$ mice in which Tregs are absent. In the spleen of wild type mice, IL2-dependent signals lead to the downregulation of both the IL7R α and IL15R α chains. This renders mature Tregs primarily dependent on IL2 for their survival and expansion. In contrast, in $IL2^{-/-}$ mice, IL15R α and IL7R α remain expressed at high levels and allow for survival of mature Tregs. Likewise, under lymphopenic conditions, such as occurs upon introduction of Tregs into $rag2^{-/-}$ mice, Tregs do survive and expand. This most likely occurs due the absence of other T cell subsets thereby leading to increased amounts of available IL15 and IL7 that can stimulate Treg survival despite lower expression of IL15R α and IL7R α receptors by those cells. In addition, the absence of IL2 signals may lead to re-expression of the IL15R α and IL7R α rendering these cells more sensitive to IL15 and IL7.

Figure 2. Signaling Components of the IL2 Receptor Complex

The IL2R is composed of three distinct subunits - IL2R α , IL2R β , and IL2R γ . The IL2R β -chain consists of three previously identified signaling domains, the A-, H-, and S-regions. The tyrosine-containing A and H regions are labeled, while the JAK1 binding domain or S-region is shaded in gray. Phosphorylation of Tyr-338 in the A region recruits the adaptor protein SHC; SHC then binds Grb2 and Gab2, leading to activation of the Ras-Raf-MAPK and PI3K/Akt signaling pathways, respectively. Phosphorylation of Tyr-392 and Tyr-510 in the H region recruits STAT5 and leads to its activation; in the absence of these two tyrosine residues Tyr-338 can also induce STAT5 activation.

Figure 3. STAT Binding Motifs in the Murine *foxp3* gene

The murine *foxp3* gene locus contains many motifs to which activated STAT molecules can bind. Black boxes represent exons while vertical lines represent consensus STAT binding motifs. The three closely linked sites (separated by 7 base pairs) in the promoter and the three sites labeled intronic are conserved across species.