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The molecular mechanisms of *Foxp3* gene regulation

Takashi Maruyama, Joanne E. Konkol, Brian F. Zamarron, and WanJun Chen

Mucosal Immunology Section, OIIB, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland, USA.

Abstract

Induction of *Foxp3* gene expression and acquisition of regulatory T cell fate is, understandably, a highly controlled process and one which many investigators want to illuminate. In studying the regulation of *Foxp3* gene expression, several conserved non-coding regions have been identified and the role of various transcription factors at these sites has been explored. What emerges, is that many factors, some positive, some negative, interact to collectively drive *Foxp3* gene expression and then maintain its expression in Foxp3⁺ regulatory T cells. TCR signaling is imperative for *Foxp3* gene expression and TGF- β is a key cytokine for initiating Foxp3 gene expression in naïve T cells. But other signaling pathways are also known to play a role in properly orchestrating *Foxp3* gene expression and regulatory T cell expansion. Here we review the recent progress in understanding the complex molecular events that drive *Foxp3* gene expression and allow functional regulatory T cells to develop.

Introduction

The vital role of CD4⁺CD25⁺Foxp3⁺ regulatory T cells (Tregs) in maintenance of a healthy immune system is starkly demonstrated in mice and humans with mutations in the *Foxp3* gene. The severe lympho-proliferative diseases, consequential morbidity and reduced life-span seen in scurfy mice and in Immune dysregulation, Polyendocrinopathy, enteropathy, X-linked syndrome (IPEX)-patients are due to Treg deficiencies resulting from *Foxp3* mutation [1–3]. Thus Tregs mediate dominant tolerance to self and have also been shown to be equally important in the control of autoimmune diseases, allergy, fetal-maternal tolerance, allograft tolerance and immunopathology. Tregs can develop intrathymically, from CD4⁺ single positive precursors [4, 5], which commit to the Treg lineage before exiting to the periphery. In addition Tregs can also be generated extrathymically from CD4⁺CD25⁻ naïve T cells in which Foxp3 expression is induced in a TGF- β -dependent manner [6].

As central as Tregs are to immune homeostasis, Foxp3 is central to Treg development and function. Indeed Foxp3 is considered the “master regulator” of Tregs. The current paradigm suggests that only CD4⁺CD25⁺ peripheral T cells and single positive (SP) thymocytes express Foxp3 [7]. Forced expression of Foxp3 in naïve (CD4⁺CD25⁻) T cells is sufficient to endow them with Treg properties; transduced cells not only express surface molecules associated with the Treg signature, such as CTLA-4, GITR and CD25, but are also able to suppress other cell types *in vitro* and *in vivo* [7, 8]. Generation of mixed bone-marrow chimeras consisting of wild-type and Foxp3^{-/-} cells demonstrated that CD4⁺CD25⁺ T cells capable of suppression could not be generated from Foxp3^{-/-} cells [8]. T cell specific

*Corresponding author. Tel.: +1 301 435 7168; Fax.: +1 301 402 1064; wchen@dir.nidcr.nih.gov (W Chen).

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deletion of *Foxp3* induced severe lympho-proliferative diseases similar to that observed in *Foxp3*^{-/-} mice [9]. Thus *Foxp3* is critical for and defines the CD4⁺CD25⁺ regulatory T cell population. Although the importance of *Foxp3* is well established the exact pathways by which its expression is induced, maintained and stabilized remain loosely defined, with the stimulus for *Foxp3* induction intrathymically being debated. Here we outline the current knowledge of the molecular mechanisms governing *Foxp3* expression, with a particular emphasis on the role played by TGF- β .

Foxp3 gene conserved regions

The *Foxp3* gene is found on the X chromosome. Critical for the initiation and regulation of *Foxp3* transcription is the *Foxp3* conserved promoter sequence, located up-stream of the transcriptional starting site (TSS) [10]. Detailed analysis of the *Foxp3* gene promoter in humans shows it is located in the region -511/+176 base pairs (bp) relative to the TSS. The promoter contains features common to most eukaryotic promoters; GC (138bp upstream of the TSS), TATA (44bp upstream of the TSS) and CAAT (218bp upstream of the TSS) boxes, mutation of which reduces core promoter activity [11]. Identification of proteins that bind this highly conserved region have shown that mutation of AP-1 and NFAT sites in this region decrease *Foxp3* promoter activity, highlighting a role for these transcription factors in the transactivation of the *Foxp3* promoter [11]. Other positive regulators that interact at the promoter to drive *Foxp3* expression may include Sp1 (an activator of transcription) and Sp3, which can bind the GC box.

Alignment of the *Foxp3* locus from different species has highlighted the presence of several highly conserved regions. In addition to the promoter, these include three non-coding regions that could play a role in regulation of the *foxp3* gene [12, 13]. Here referred to a conserved non-coding sequence (CNS) 1, 2 and 3, although often referred to as enhancers, detailed analysis of these regions has been undertaken and a plethora of transcription factors have been shown to bind and influence *foxp3* gene induction. Highlighting well the multifactorial mechanism by which *Foxp3* expression is driven is the c-Rel enhanceosome model in which c-Rel promotes the formation of an enhanceosome consisting of p65, NFATc2, Smad3 and pCREB. These factors bind to the *foxp3* promoter and CNS1, 2 or 3, collectively promoting *Foxp3* expression [12–18]. Specifically, NF- κ B binding sites have been identified in the promoter region (at least four within 1kb upstream from TSS) and in a CpG island in CNS2 regions [14, 15]. NFAT binding sites have been located in the promoter region (three conserved sites within 500bp upstream from TSS) and the CNS1 region (+2145) [11, 16]. c-Rel binding sites are in the promoter (377bp upstream from TSS) and CNS3 region [12, 17]. Finally, a CpG island within the CNS2 region has one CREB binding site [18]. So from one model we have many transcription factors coordinately acting from different sites to drive *foxp3* expression. Importantly, this is a feat one transcription alone cannot achieve.

Examining the CNS regions in more detail, we will first look at CNS2, which is located 4kb down-stream of the *Foxp3* TSS. The chromatin structure of the CNS2 region in natural Tregs (*Foxp3*⁺ cells from the thymus; nTregs) has been shown to be in an “OPEN” chromatin state (defined as demethylated CpG motifs). In contrast, the CNS2 region of TGF- β induced Tregs (iTregs) has been observed to be in the “CLOSED” configuration [19, 20]. Pair this information with the demonstration that iTregs easily lose their regulatory phenotype when re-stimulated under culture conditions which induce Th1, Th2, or Th17 cell differentiation. nTregs on the other hand are relatively “stable” and don’t easily lose *Foxp3* expression when re-stimulated under these same conditions. However, caution should be taken in making the conclusion that “nTregs” are more stable than “iTregs”, as it is currently impossible to judge if the *in vitro* generated “iTregs” are at the same differentiation stage as the freshly

isolated “nTregs” from a naïve mouse. Nevertheless, these data begin to suggest CNS2 may play a role in maintaining the regulatory potential of the *Foxp3*⁺ cell. CNS2 deficient mice have been generated and these mice exhibit less *Foxp3*⁺ Tregs in peripheral lymph nodes. Importantly, these CNS2^{-/-} *Foxp3*⁺ Tregs lose *Foxp3* expression during proliferation in response to TCR stimulation [12]. Using the CNS2^{-/-} Zheng *et al* showed that the CNS2 region is required to stabilize *foxp3* expression in *Foxp3*⁺ Tregs [12].

Within the CNS2 region there are two Est-1 binding sites which are important for proper Treg development [21, 22]. Mutation of both Est-1 binding sites in CNS2 has been shown to significantly decrease *Foxp3* promoter activity [21, 22]. Est-1 KO mice were shown to have activated T cells and less *Foxp3*⁺ Tregs in the spleen. In addition, Est-1 KO T cells fail to generate *Foxp3*⁺ Tregs in response to TGF-β [22]. While Est-1 enrichment can be observed on the CNS2 element while de-methylated (or in an OPEN chromatin state) similar enrichment is not found when the CNS2 adopts the closed chromatin configuration [21, 22]. Some components (Azacytidine and Simvastatin amongst others) have been reported to de-methylate the CNS2 region and induce *Foxp3*⁺ Tregs, likely leading to the generation of a more stable Treg population [23, 24]. Binding of *Foxp3* to the CNS2 has been observed in nTregs, but not iTregs. De-methylation of a CpG island in the CNS2 region in nTregs allows *Foxp3* to bind it along with Cbf-β and RUNX-1 [12]. Cbf-β-deficient Tregs progressively lose *Foxp3* expression after transfer into lymphopenic mice [25]. Therefore, it has been suggested that the enrichment of these complexes at the CNS2 region may play an important role in the maintenance of *Foxp3* gene expression in nTreg cells [22, 26].

CNS3 is located 7 kb down-stream from the TSS and contains a c-Rel binding element [12]. In their examination of the role of the different CNS regions, Rudensky and colleagues, generated CNS3^{-/-} mice. CNS3^{-/-} mice exhibited reduced frequencies of *Foxp3*⁺CD4⁺ SP thymocytes, and CNS3^{-/-} naïve CD4⁺ T cells failed to develop into *Foxp3*⁺ cells following TCR stimulation in the presence of TGF-β [12]. As CNS3 contains a c-Rel binding sequence, the authors then examined *Foxp3* frequencies in chimeras generated from WT and c-Rel-deficient cells or WT and CNS3-deficient cells. In the resultant chimeras, the CNS3^{-/-} and c-Rel^{-/-} frequencies of *Foxp3*⁺ thymocytes were similar, leading to the suggestion that c-Rel binds the CNS3, which exists in an open chromatin configuration to facilitate *Foxp3* gene transcription and nTreg generation. Thus, unlike CNS2, it is suggested that factors bound at the CNS3 play an important role in the induction of *Foxp3* expression.

Finally, an examination of the role of CNS1 in *Foxp3* expression was also undertaken, however this will be discussed later. But even from this limited description of the factors interacting at the *Foxp3* promoter and CNS regions, one can see that many factors co-operate from different sites to co-ordinately drive *Foxp3* gene transcription. But a sequential series of events by which *Foxp3* is induced remains elusive.

TGF-β and *Foxp3* gene expression

How TGF-β promotes *Foxp3* expression remains an area of intense investigation, with a number of factors shown to bind the *Foxp3* locus in response to a TGF-β signal. However an exact, detailed pathway has not been determined. Here we outline the current data involving TGF-β and how it drives *Foxp3* expression.

We will begin by firstly discussing the remaining CNS region; CNS1 is located around 2kb downstream of the TSS and has been shown to contain NFAT and SMAD protein binding sites [16]. Smad proteins are prominent members of the canonical TGF-β signaling pathway. TGF-β-signaling induces phosphorylation of Smad2 and 3, this allows these proteins to interact with Smad4, which permits translocation of Smad2 and 3 to the nucleus and induction of Smad-mediated transcription [27, 28]. Despite the critical role of TGF-β in

induction of *Foxp3*, the Smad binding site in the CNS1 is the only Smad binding site located in either conserved and/or promoter regions of the *Foxp3* gene. Following TGF- β stimulation of T cells, Smad3, although not Smad2 or Smad4, has been shown to rapidly bind the CNS1 region suggesting a role for this transcription factor in *Foxp3* expression. However, a role for Smad2 and/or Smad4 is not precluded as in the absence of either protein induction of *Foxp3* in response to TGF- β is reduced (at least in cell culture) [29, 30].

Despite a role for Smad(s) in mediating TGF- β signaling and the known role of TGF- β signaling in the induction of *Foxp3* expression, there is a grey area between activation of Smad proteins and induction of *Foxp3* expression. Firstly, a time lag exists between the immediate Smad2/3 phosphorylation (which occurs within minutes) and the detection of *Foxp3* mRNA (which takes at least 10–12 hours) after TGF- β treatment. Secondly, as already stated, there is a lack of Smad binding sequences in the control elements of the *Foxp3* gene. These data suggest that there must be some intermediate transcription factor(s) linking TGF- β -signaling and Smad activation to *Foxp3* promoter activation. In line with this hypothesis, we have recently demonstrated that *id3* (inhibitor of DNA binding protein 3) could serve as one such transcription factor in the control of *Foxp3* gene expression [31]. Id3 belongs to a family of helix-loop-helix transcription factors [32]. Id3 lacks a DNA binding domain; instead Id3 complexes with basic Helix-loop-Helix proteins (E-protein) and inhibits their transcriptional activity [32, 33]. Id3 deficient mice have been shown to exhibit a Sjogren's syndrome-like autoimmune disease, with T cells playing an essential role in disease progression [34, 35]. While the mechanisms for disease development in these mice are still unclear, deletion of the Id3 gene in mice results in less *Foxp3*⁺ Tregs within the thymus, spleen and peripheral lymph nodes [31], which was primarily due to a defect in *Foxp3*⁺ Treg generation. In cell cultures, Id3^{-/-} CD4⁺ T cells fail to convert *Foxp3*⁺ Tregs in response to TGF- β [31]. As Id3 does not directly bind to the DNA, we sought to understand the mechanisms by which Id3 affects *Foxp3* promoter activity. We identified two indispensable, yet complementary, processes that are involved in TGF- β -mediated induction of *Foxp3* gene transcription. First, the E-protein E2A, was enriched on the *Foxp3* promoter in response to TGF- β [31]; the *Foxp3* promoter has multiple E-protein binding sites (CANNTG). Enrichment of E-proteins at the *Foxp3* promoter encouraged TATA binding protein enrichment and *Foxp3* mRNA production, suggesting that E2A proteins serve as positive regulators of *Foxp3* expression. Indeed, by a series of deletion and over-expression experiments, we have provided evidence that the enrichment of E2A binding at the *Foxp3* promoter promotes *Foxp3* gene transcription. Secondly, we showed that binding of GATA-3 is inhibitory to *Foxp3* expression, and it must be removed from the *Foxp3* promoter in order for *Foxp3* expression to occur. These two processes; enrichment of E2A and removal of GATA-3, were defective in Id3^{-/-} CD4⁺ T cells. Although many exciting questions remain to be answered, these findings emphasize that proper gene transcription requires not only the enrichment of positive “enhancer”(s) elements, but also (and at the same time) removal of negative “silencer” elements.

Other transcription factors have also been suggested to regulate positive *Foxp3* gene activation in naïve CD4⁺ T cells in cross-talk with TGF- β -signaling. Foxo proteins are reported to be involved in *Foxp3* gene expression [36–38], and are negatively regulated by the PI3K-Akt pathway, a pathway also inhibitory to the generation of Tregs [36–38]. In the absence of Foxo 1 and/or 3a decreases in thymic Tregs are seen. In addition, *in vitro* conversion of naïve CD4⁺ T cells to *Foxp3*⁺ cells by TGF- β is inhibited in Foxo deficient T cells. In fact, the response of Foxo-deficient T cells to TGF- β is misdirected and instead of becoming *Foxp3*⁺ cells, treated T cells secrete IFN- γ [38]. In non-lymphoid cells a direct interaction between Foxo proteins and TGF- β signaling components in the induction of TGF- β target gene expression was already demonstrated [39, 40], thus Foxo proteins could be a factor in the integration of signals mediated from both the TCR (via PI3-K) and TGF- β

receptor. Another factor downstream of TGF- β and involved in *Foxp3* induction is TGF- β inducible early gene 1 product (TIEG1), a transcription factor rapidly induced upon TGF- β stimulation. Ubiquitination of TIEG1 by the E3 ubiquitin ligase Itch promotes transcriptional activation of the *foxp3* promoter and *foxp3* expression [41]. Finally, RUNX3, which was shown to be induced by TGF- β signaling in CD4⁺ T cells, has been reported to positively regulate Foxp3 expression [42, 43]. RUNX family proteins form complexes with CBF- β which directly binds to the DNA [44]. In humans, RUNX1 and 3 are important for Foxp3 gene expression [42]. It has been reported that the proximal Foxp3 promoter region has three putative RUNX binding site (-53, -287 and -333 from TSS). RUNX-CBF β enrichment at the Foxp3 promoter activates *Foxp3* gene expression. In addition, RUNX1 can complex with Foxp3 and might be responsible for the suppression of IL-2 and IFN- γ production as well as the up-regulation of Treg associated molecules in response to TGF- β signaling [26].

Shown to synergise with TGF- β for the generation of Foxp3⁺ cells in the gut, Retanoic acid (RA), a metabolic product of Vitamin A, promotes Foxp3 gene expression. RA has been shown to augment phosphorylation of Smad3 allowing translocation of Smad3 to the nucleus [45], although this was debated [46]. It has been reported that RA, functions to maintain homeostasis within the gut through the generation of Foxp3⁺ Tregs [46, 47]. Moreover, RA controls *Foxp3* expression directly through Retinoic acid Receptor (RAR) and Retinoid X Receptor (RXR), which bind the CNS1 region directly and positively regulate *Foxp3* expression [48, 49]. Although these reports demonstrate a role for a number of transcription factors in TGF- β -mediated *Foxp3* gene transcription, a systematic analysis of their interaction and regulators is needed to identify their precise effect on and at the *Foxp3* promoter and work to place these transcription factors within a network which mediates *Foxp3* induction.

There are also other cytokines and signaling stimuli that have been reported to affect Foxp3 and, ultimately, Treg development. Downstream of the TCR signal, Activator Protein 1 (AP-1) and Nuclear Factor of Activated T cells (NF-ATc2) are positive regulators of *Foxp3* gene expression [11]. Indeed, at least three NF-AT binding sites have been observed in the proximal *Foxp3* promoter region, around 500bp upstream from the TSS which positively regulate *Foxp3* expression [11]. During Foxp3⁺ Treg development in the thymus, TGF- β has been shown to be necessary for the initial generation of Tregs but another cytokine, IL-2, is important for their expansion [50]. IL-2 is required for the activation of Foxp3 gene expression through Stat5, a downstream signaling molecule of the IL-2 receptor, which is able to bind onto the Foxp3 promoter and upregulate *foxp3* expression [51, 52].

A chemical component has also been found to regulate Foxp3 expression; 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) can activate the Aryl hydrocarbon receptor (AHR) in T cells and induce Foxp3⁺ Tregs. So far three non-evolutionarily conserved AHR-binding (NCAB) sites in the Foxp3 promoter region and one conserved AHR-binding (CAB) site in exon 11 have been identified. AHR is enriched on the CAB and NCAB sites in the Foxp3 promoter in response to TCDD, and serves to positively regulate *foxp3* gene expression [53]. In addition, AHR-null T cells have less Foxp3⁺ Treg induction in response to TGF- β and, TGF- β induces AHR gene expression [54].

Repressors of Foxp3 gene transcription

Many cytokines and factors negatively regulate *Foxp3* gene transcription. Production of IL-4 following TCR stimulation up-regulates GATA-3 expression (the master Th2 transcription factor), which can bind to the *Foxp3* promoter region and suppress gene expression [55, 56]. TGF- β signaling prevents IL-4 production, thereby inhibiting Gata3

expression and consequent binding to the *Foxp3* promoter. In addition, the downstream IL-4 signaling protein Stat6 can bind to the *Foxp3* gene locus (around 2.2 kb downstream from the TSS) and directly prevent *Foxp3* gene activation [57]. Combined, these events negatively regulate Foxp3 expression and, as such, must be inhibited to allow for proper *Foxp3* gene transcription.

As well as IL-4, the Th1 cytokine IFN- γ activates Stat1 that also prevents *Foxp3* gene expression in murine T cells [58]. However, in human T cells, IFN- γ and IL-27 induced Stat1 can actually amplify TGF- β -mediated *Foxp3* gene expression. In addition, the human *Foxp3* gene has a Stat1 binding element within proximal region of the promoter [59]. Thus, more thorough studies are required to clarify these results and define the effects of Th1-cytokines on *Foxp3* gene transcription.

The inflammatory cytokine IL-6 is well known to inhibit Foxp3 expression by promoting acquisition of the Th17 cell fate, in naïve T cells [60]. Downstream of IL-6 signaling, the Stat3 transcription factor can bind directly onto the IL-17 promoter region and induce IL-17 expression. However, Stat3 has the opposite effect on Foxp3 expression. By binding onto the CNS2 region Stat3 inhibits gene transcription [61]. In fact, the balance of Stat3 and, the IL-2 activated, Stat5 has been shown to influence fate decisions of naïve T cells, in determining whether they differentiate into Tregs or Th17 cells [62]. Interestingly however, Stat3 expression in Tregs is reported to play a crucial role in keeping the regulatory function of Tregs in vivo [63], which seems to be attributable largely to Treg-derived IL-10 [64].

ROR γ t, is the key transcription factor of Th17 cells and is induced by TGF- β and IL-6 signaling [65]. Both Foxp3 and ROR γ t are induced by TGF- β , but which one dominates following TGF- β signaling depends on the presence of other cytokines (and their activated Stat proteins as indicated above), the strength of TCR engagement and the local concentration of TGF- β . Once Foxp3 is expressed in response to TGF- β , Foxp3 can complex with the ROR γ t protein and prevent ROR γ t transcriptional activity [66]. On the other hand, combined TGF- β and IL-6 signaling strongly induces ROR γ t which can bind to the *Foxp3* promoter directly and prevent Foxp3 expression [67]. In addition, low-dose exposure of TGF- β induces maximal expression of IL-23R and, together with inflammatory cytokines IL-6/IL-21/IL-23, inhibits Foxp3 expression and polarizes the naïve cell towards Th17 cell differentiation. High dose treatment of TGF- β inhibits expression of IL-23R, IL-22 and IL-17, thereby promoting Foxp3⁺ Treg differentiation [66]. Additionally, RA enhances TGF- β signaling and inhibits the expression of IL-6R α IL-23R and IRF-4, serving to prevent Th17 cell generation [68].

Recently, a new population has been identified which develops in response to TGF- β and IL-4, IL-9 producing T cells (Th9). Th9 express PU.1, but not Foxp3, ROR γ t, T-bet or GATA-3 [69–71]. PU.1 is a transcription factor of the Est-family, and is able to bind the IL-9 promoter region and positively regulates *Il-9* expression in response to TGF- β and IL-4. Importantly, PU.1 inhibits Foxp3⁺ Treg differentiation despite TGF- β signaling [72]. In addition, IRF-4 is also suggested to be important, not only for Th17, but also Th9 cell differentiation [73–75]. IRF-4 directly binds onto the IL-17A and IL-9 promoters, and upregulates IL-17 and IL-9 production. As such, IRF-4 deficient T cells fail to generate Th17 or Th9 cells in response to TGF- β and IL-6 or IL-4 co-signaling. Furthermore, IRF-4 KO T cells can be induced to Foxp3⁺ Tregs in response to TGF- β , and the combination of TGF- β and IL-6 signaling does not decrease Foxp3⁺ Treg induction. Interestingly, however, IRF-4KO Tregs lack suppressive functionality [76]. Although these studies expand the network of transcription factors that regulate the induction of Foxp3 and how this is balanced with the differentiation to other lineages, detailed cross-talk and interactions between the various lineage transcription factors, remains to be elucidated.

Away from cytokine-mediated effects, S1P1-signaling induces mTOR phosphorylation, and prevents the phosphorylation of Smad3. Thus S1P1-mediated phosphorylation of mTOR, serves to prevent TGF- β induced Foxp3 expression [77, 78]. In contrast, Rapamycin has been reported to dephosphorylate mTOR; and treatment with both Rapamycin and TGF- β enhances phosphorylation of Smad3 and, consequently, Foxp3 gene expression. Another inhibitory transcriptional factor for Foxp3 expression, Hes1, can be induced by Notch signaling. Hes1 can bind on the *Foxp3* promoter region (3bp up-stream from TSS, and 66bp/128bp down stream from TSS) and negatively regulate Foxp3 gene expression [79]. Interestingly, Hes proteins have been shown to be suppressors of bHLH molecules, inhibiting many bHLH family transcriptional factors including E47 (E47 and E12 are E2A encoded proteins) [80]. It will be interesting to determine whether Hes mediated suppression of *Foxp3* gene activation is mediated by down-regulation of E2A binding at the Foxp3 promoter.

So, TGF- β -mediated *Foxp3* gene expression requires not only positive regulatory factors working in cooperation, but also requires the repression of negative transcriptional activity to properly coordinate Foxp3 protein production.

Concluding remarks

In this paper, we have discussed the transcription factor network at play in the controlled expression of Foxp3 and the generation of regulatory T cells. We have emphasized a mutual regulation between the “*enhancer*” and the “*silencer*”, i.e. the activation of positive transcription factors and the suppression of negative transcription factors which collectively allow for the coordinated expression of Foxp3 and the generation of regulatory T cells. This balance of transcription factors mediates the subtle changes required for TGF- β -signaling to not only mediate the generation of Tregs, but may well also effect differentiation of Th17 and Th9 cells.

- Foxp3 gene conserved regions
- TGF- γ and Foxp3 gene expression
- Repressors of Foxp3 gene transcription

Abbreviations

TGF-β	transforming growth factor beta
Foxp3	forkhead box P3
TCR	T-cell receptor
Id3	inhibitor of DNA binding protein 3
E2A	basic Helix-Loop-Helix protein E2A
GATA3	Trans-acting T-cell-specific transcription factor GATA-3

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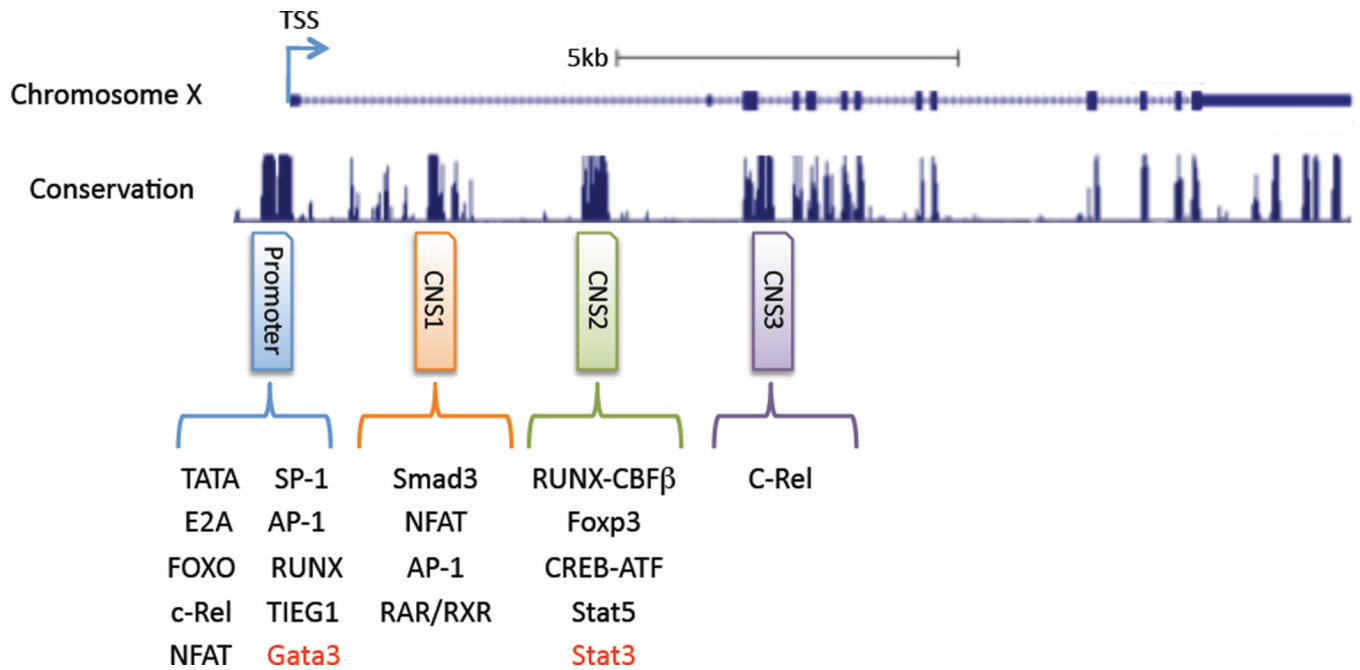


Figure 1. A map of *Foxp3* gene regulation

Foxp3 is located on chromosome X and it has been reported that several conserved regions exist on this gene (Promoter, CNS1, CNS2 or CNS3). So far, epigenetic studies have revealed the role of each of these conserved regions. The promoter region is necessary for directly controlling gene expression. CNS1 serves as a TGF- β sensor, so called enhancer regions. CNS2 is important for stabilizing *foxp3* expression. Transcription factors can bind on each of the conserved regions and are either Positive (Black) or Negative (Red) regulators for *Foxp3* protein expression.