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The regulation of immune tolerance by FOXP3

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Abstract

The proper restraint of the destructive potential of the immune system is essential for maintaining health. Regulatory T (T_{reg}) cells ensure immune homeostasis through their defining ability to suppress the activation and function of other leukocytes. The expression of the transcription factor forkhead box protein P3 (FOXP3) is a well-recognized characteristic of T_{reg} cells, and FOXP3 is centrally involved in the establishment and maintenance of the T_{reg} cell phenotype. In this Review, we summarize how the expression and activity of FOXP3 are regulated across multiple layers by diverse factors. The therapeutic implications of these topics for cancer and autoimmunity are also discussed.

The transcription factor forkhead box protein P3 (FOXP3) belongs to the forkhead–winged-helix family of transcription factors. Its role as a broad regulator of gene expression is central to the identity and function of the most widely recognized and well-studied subset of immunoregulatory T cells: namely, the $CD4^+$ regulatory T (T_{reg}) cells. These T_{reg} cells are defined by the constitutive expression of FOXP3, although FOXP3 expression can also be transiently induced in non-T_{reg} cells upon activation[1,2]. Another defining characteristic of T_{reg} cells is their ability to suppress the activation and function of other leukocytes. This ability is central to their role in maintaining immune homeostasis. T_{reg} cells are also marked by their constitutively high expression of CD25 (also known as IL-2Rα, which is the high-affinity chain of the interleukin-2 (IL-2) receptor); this enables them to scavenge IL-2 from other cellular sources — a crucial trait, as T_{reg} cells do not produce their own supply of this survival-promoting and expansion-promoting cytokine[3].

Considerable heterogeneity exists among $FOXP3^+$ T_{reg} cells, and subsets arise in distinct tissues and display unique functional capabilities (BOX 1). In general, $FOXP3^+$ T_{reg} cells

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exert suppressive functions through a number of well-established mechanisms (as reviewed in REF. 4). For example, they secrete anti-inflammatory cytokines, express co-inhibitory molecules (such as cytotoxic T lymphocyte antigen 4 (CTLA4) and lymphocyte activation gene 3 protein (LAG3)) and can modulate the activity of antigen-presenting cells (APCs). T_{reg} cells can also deplete crucial growth factors from the microenvironment, thus sequestering these from effector cells and potentially 'starving' them into anergy or apoptosis[5]. They are also known to take up and consume scarce amino acids, and through expression of the ectoenzymes CD39 and CD73 they drive the accumulation of adenosine nucleosides, which disrupt effector cell metabolism, leading to anergy[6]. In addition, T_{reg} cells are reportedly equipped with cytotoxic potential, and they may suppress effector cells by simply killing them[7].

Box 1

Types of forkhead box protein P3-expressing regulatory T cells

Most circulating regulatory T (T_{reg}) cells arise in the thymus from self-reactive precursors. The expression of forkhead box protein P3 (FOXP3) is induced during the generation of these so-called thymus-derived T_{reg} cells (or tT_{reg} cells, formerly known as 'natural T_{reg} cells') in response to T cell receptor (TCR) engagement[33]. In addition, during tT_{reg} cell development an extensive pattern of epigenetically modified loci (including those within the *FOXP3* gene) emerges that predicts stable transcriptional commitment to a T_{reg} cell phenotype[14]. tT_{reg} cells are thought to mainly be responsible for preventing autoimmune diseases[49]. By contrast, extrathymic T_{reg} cells, known as peripherally derived T_{reg} (pT_{reg}) cells, arise from naive $FOXP3^{-}CD4^{+}$ T cells that are exposed to factors such as transforming growth factor-β and interleukin-2 in peripheral tissues. These pT_{reg} cells accumulate mostly at barrier sites (such as the gut) where they maintain immune homeostasis. *In vitro*-induced T_{reg} (iT_{reg}) cells can express considerable levels of FOXP3 but typically lack much of the epigenetic programming of their tT_{reg} cell counterparts, making their commitment to continuous FOXP3 expression and their suppressive phenotype less stable[64].

It is now becoming apparent that in addition to the above populations of $FOXP3^{+}$ T_{reg} cells, additional subpopulations can be defined. For example, activated (or 'effector') T_{reg} cells display a gene expression profile that is unique from that of 'resting' (or 'central') T_{reg} cells[31]. In addition, $FOXP3^{+}$ T_{reg} cells residing in certain peripheral tissues have been shown to display unique functions and gene products relative to their lymphoid tissue-dwelling counterparts[111,112]. Populations of human T_{reg} cells with different levels of CD25 expression and varying capacities for suppression and FOXP3 expression have also been reported[58].

The execution of these suppressive functions requires the proper regulation of genes within T_{reg} cells, and FOXP3 expression is crucial in the establishment and maintenance of the T_{reg} cell gene expression landscape. T_{reg} cell subsets are also capable of mediating several extra-immune functions, including angiogenesis (mediated by vascular endothelial growth factor

A expression), tissue repair and metabolic regulation at both the organismal level and the T cell level (BOX 2).

Box 2

Forkhead box protein P3 and metabolism

Naive $CD4^+$ T cells meet the modest metabolic demands of their largely quiescent lifestyle through the oxidation of pyruvate and fatty acids via the tricarboxylic acid (TCA) cycle. T cell activation, however, imposes considerable energetic and biosynthetic demands that are met through T cell receptor (TCR)-triggered and co-stimulatory molecule-triggered metabolic reprogramming events[113]. The activation of phosphoinositide 3-kinase (PI3K), AKT and mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) leads to the upregulation of genes that encode proteins crucial for the uptake and breakdown of glucose and other molecules (for example, amino acids). This enables the production of the energy and biosynthetic raw materials that are needed to accommodate effector T cell function and proliferation. Studies of T cell differentiation clearly show that T helper (T_H) cell lineage commitment can be affected by metabolic variables. In general, effector T cell lineages (such as the T_H1 and T_H17 cell lineages) require the robust induction of glycolysis; by contrast, an *in vitro*-induced regulatory T (iT_{reg}) cell fate is favoured when oxidative phosphorylation (OXPHOS) and fatty acid oxidation pathways are preferentially engaged[114].

Inhibiting glycolysis directs differentiating $CD4^+$ T cells towards an anergic fate and can result in the upregulation of forkhead box protein P3 (FOXP3)[115]. Chemically or genetic ablating mTOR, as well as knocking out other key facilitators of glycolysis-dominated metabolism, similarly favours the generation of iT_{reg} cells over the generation of effector cell lineages[102,115,116]. Similarly, forced activation of the lipid-metabolism regulator AMP-activated protein kinase during *in vitro* T cell differentiation drives FOXP3 upregulation and iT_{reg} cell differentiation, and can increase *in vivo* T_{reg} cell numbers in a mouse model of asthma[114]. By contrast, preventing fatty acid oxidation by using the carnitine palmitoyltransferase 1A inhibitor etomoxir reduces iT_{reg} cell differentiation[114]. Thus, the induction of FOXP3 expression by iT_{reg} cells is highly sensitive to metabolic factors.

Established T_{reg} cells similarly display a reliance on mitochondrial oxidative metabolism for their suppressive function[117]. Mutations that lead to the inappropriate dominance of a glycolytic, effector T cell-like metabolism destabilize the phenotype of T_{reg} cells[118] and induce loss of FOXP3 expression under certain conditions and an inability to suppress spontaneous inflammation. Nevertheless, T_{reg} cells probably still require some activity of glycolysis-favouring pathways for optimal fitness and expansion *in vivo*[119], as well as for other aspects of T_{reg} cell biology. In human T_{reg} cells, which can express multiple splice variants of FOXP3, glycolysis seems to have an important role in directing the splicing of *FOXP3* transcripts. Recently, De Rosa *et al.*[120] showed that inhibiting glycolysis during human iT_{reg} cell differentiation results in higher levels of bulk FOXP3 protein, but *FOXP3* transcript splicing was altered in such a manner that isoforms derived from transcripts containing the crucial exon 2 were lacking in the resulting T_{reg} cells. These

findings suggest that metabolic factors affect T_{reg} cells through means other than T cell lineage fate decisions.

Recently, the importance of FOXP3 as a regulator of metabolism in T_{reg} cells was also revealed. Gerriets *et al.*[121] found that triggering Toll-like receptor (TLR) pathways (particularly those induced by TLR1 and TLR2) activated signalling through the PI3K–AKT–mTORC1 axis in T_{reg} cells, and this signalling induced the expression of the glucose transporter GLUT1, supporting glycolysis. Although this promoted an increase in the number of T_{reg} cells, it impaired their suppressor function. By contrast, forced expression of FOXP3 in non-T_{reg} $CD4^+$ T cells suppressed PI3K–AKT–mTORC1 signalling and reduced the expression of glycolysis-associated genes while increasing the expression of those involved in oxidative metabolism. Interestingly, thymus-derived T_{reg} (tT_{reg}) cells and iT_{reg} cells expressing constitutively active AKT or GLUT1 were found to be more plentiful and more likely to express activation markers, but were unstable in terms of their FOXP3 expression and less suppressive than were wild-type control cells[121], thus being reminiscent of T_{reg} cells from mice that are unable to restrain PI3K and mTOR activity[122,123].

Although it is not completely clear how FOXP3 regulates the metabolic preferences of T_{reg} cells, Gerriets *et al.*[121] noted that FOXP3 did localize to the genes that encode a PI3K subunit and the pyruvate dehydrogenase kinase PDK3 with apparent repressive results[121]. Thus, FOXP3 expression in T_{reg} cells seems to be both in control of, and under the influence of, metabolic inputs.

The consequences of *FOXP3* mutation in mice and humans clearly demonstrate the importance of this transcription factor in immune homeostasis. Scurfy mice — which carry a nonsense mutation in *Foxp3* that results from a 2 bp insertion in the gene — express a truncated gene product. The T_{reg} cells in these mice lack suppressive function, and are unable to restrain hyperactivated T cells and their production of pro-inflammatory cytokines[8,9]. In humans, mutation of the *FOXP3* gene leads to the typically fatal immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome. Patients with this genetic disorder develop a number of immunopathologies within the first few months of life, including dermatitis, enteropathy, diabetes, thyroid disorders (owing to endocrine gland dysfunction) and anaemia[8,10].

As these phenotypes are well documented, it is not surprising that FOXP3 has for some time been regarded as key for the suppressive function of T_{reg} cells. Accordingly, many investigative efforts have focused on elucidating the factors and pathways that are responsible for influencing the expression and function of FOXP3 in T_{reg} cells. Although FOXP3 expression has been reported in other cell types (BOX 3), the relevance of this for immune homeostasis is less clear. In this Review, we discuss the diverse mechanisms that are responsible for controlling the induction and maintenance of FOXP3 expression in $CD4^+$ T_{reg} cells. We also present a number of recent breakthroughs that have added to our understanding of how the expression levels and activities of this crucial transcription factor are influenced. Lastly, the potential therapeutic implications of FOXP3-modulating pathways are discussed.

Box 3

Forkhead box protein P3 expression by non-regulatory T cells

Activated CD4$^+$ T cells

The expression of forkhead box protein P3 (FOXP3)-encoding transcripts, and even some FOXP3 protein, has been reported in non-regulatory T (non-T_{reg}) cells, particularly after activation[2]. In addition, the expression of FOXP3-encoding transcripts and FOXP3 protein can be seen transiently early on in the bifurcating pathways of T helper 17 (T_H17) cell and *in vitro*-induced T_{reg} (iT_{reg}) cell differentiation[73,102]. Whether or not FOXP3 has a role in recently activated T cells (moderating the extent of activation, for example) remains speculative. Regardless, it is likely that the extent and/or duration of FOXP3 expression in non-T_{reg} cells is limited by less-than-permissive epigenetic states in the *FOXP3* locus (for example, methylated conserved non-coding sequence 2) and by mechanisms that regulate both the nuclear exclusion and nuclear removal of the FOXP3 protein.

CD8$^+$ T_{reg} cells

A number of CD8$^+$ T cell populations with suppressive functions have been identified[124]. Although some are capable of attenuating both autoimmunity and antiviral responses without apparent FOXP3 expression[125], FOXP3$^+$CD8$^+$ T cells have also been reported in a number of settings, including human tumours[126]. However, the regulation and importance of FOXP3 in suppressive CD8$^+$ T cells remain to be fully demonstrated. A common observation in CD8$^+$ T_{reg} cells is that the levels of FOXP3, when its expression is detected at all, tend to be much lower than the levels found in CD4$^+$ T_{reg} cells, and some studies have reported that mouse FOXP3$^+$CD8$^+$ T cells are either mildly suppressive[127] or lack a suppressive function[128], suggesting that FOXP3 expression may be induced in activated effector CD8$^+$ T cells in certain microenvironments. Whether FOXP3 has an important role in directing the suppressive functions of CD8$^+$ T cells remains to be fully elucidated.

Natural killer T cells

Natural killer T cells (NKT cells) are lymphocytes that are capable of recognizing lipid antigens presented by CD1d. As well as producing effector-type cytokines, NKT cells are known to contribute to immune tolerance[129]. Invariant NKT (iNKT) cells in the lymph nodes of α-galactosylceramide-treated mice have been shown to upregulate their expression of FOXP3 in response to transforming growth factor-β (TGFβ). Furthermore, human blood-derived iNKT cells have been shown to express FOXP3 following *in vitro* treatment with TGFβ and neutralizing antibodies against effector cytokines[130]. It will be of interest to determine whether *ex vivo*-expanded, FOXP3$^+$ iNKT cells can be used therapeutically to correct dysregulated immune responses.

Macrophages

The degree of physiological FOXP3 expression by immune cell types other than T_{reg} cells remains, to many, a debated point. Results showing FOXP3 expression by a population of CD11b$^+$F4/80$^+$CD68$^+$ macrophages that have immunosuppressive, tumour-

promoting functions[131] have been dispelled by subsequent analyses of both naive and activated macrophages[132,133], and have been retracted. Recently, however, FOXP3 expression in macrophages was reassessed, and although FOXP3 expression was not observed in normal macrophages it was detected by western blot and reverse transcription PCR in macrophages that had infiltrated mouse renal cancer tumours[134]. These results suggest a potential role for FOXP3 specifically in tumour-infiltrating macrophages and highlight the possibility of therapeutically targeting these known tumour-abetting cells.

B cells

B cells with a suppressive function have been characterized. These 'regulatory' B cell populations are generally characterized by the production of anti-inflammatory cytokines (interleukin-10 (IL-10) or IL-35) upon activation[135], and they also express co-inhibitory surface molecules that limit inflammation in a number of autoimmune settings[136]. Regulatory B cells also contribute to the enforcement of tumour-induced immune tolerance, and to both the progression and spread of cancer in mice[137]. In humans, some regulatory B cells have been reported to also express FOXP3 (REF. 138). Such cells have been observed in patients with multiple sclerosis and patients with systemic lupus erythematosus, where their increased abundance correlates with increased disease activity[139,140], suggesting that the expansion of these populations may be a reactionary measure to limit the extent of inflammatory damage. The precise contributions of FOXP3 to regulatory B cells in these patients and the importance of FOXP3 as a physiological contributor to regulatory B cell function remain uncertain, as some regulatory B cells reduce inflammation without apparent FOXP3 expression.

Cancer cells

FOXP3 expression has been demonstrated in several malignant cell types, including breast, prostate, pancreatic, thyroid, gastric and ovarian cancer cells. Although *FOXP3* has been proposed to be a tumour suppressor gene[141,142], its biological function and the role it has in tumour cells remain to be fully defined. Additionally, beyond its direct effects on malignant cells, FOXP3 can have important and complex implications on the crosstalk that occurs between cancer cells and the tumour microenvironment. Thus, the factors that control the intracellular location and function of FOXP3 within cancer cells are likely to provide distinct biological activities and prognostic uses in different tumour cells.

Gene regulation by FOXP3

Target genes that are regulated by FOXP3

FOXP3 is capable of binding to more than 2,800 genomic sites, which corresponds to approximately 700–1,400 genes in developing and established T_{reg} cells[11–13]. By regulating these target loci, FOXP3 functionally cooperates with, or possibly reinforces, the gene expression patterns that arise from epigenetic programming initiated by T cell receptor (TCR) stimulation during T_{reg} cell development[14]. Despite the importance of FOXP3 for T_{reg} cell-mediated immunosuppression, the precise mechanisms involved are only now becoming clear.

Interestingly, the number of genes bound by FOXP3 constitutes only a small proportion (approximately 6–10%) of those known to be under its control[11]. Thus, it is thought that FOXP3 can positively or negatively control the transcriptional activity of many target genes indirectly by interacting with a number of cofactors. FOXP3 and its binding partners form a large protein complex that is 400–800 kDa in size (or perhaps even larger) involving more than 360 different factors, some of which are other transcription factors or chromatin-modifying factors[15,16]. The transcription factors nuclear factor of activated T cells (NFAT) and Runt-related transcription factor 1 (RUNX1; also known as AML1) bind to the promoter regions of FOXP3-regulated genes. The interaction between these factors and FOXP3 has been documented, and disrupting these interactions results in reduced T_{reg} cell function[17,18]. In addition, interferon (IFN) regulatory factor 4 (IRF4) has been shown to be another molecule that has an important collaboration with FOXP3. IRF4 interacts with FOXP3 and endows it with the ability to selectively regulate a proportion (approximately 20%) of the T_{reg} cell gene expression signature. These genes evidently encode factors that are crucial for the control of T helper 2 (T_H2) cell responses, as mice with IRF4-deficient T_{reg} cells are selectively defective in their ability to suppress the production of the T_H2-type cytokines IL-4 and IL-5 (REF. 19). In addition, increased interaction between IRF4 and FOXP3 is linked to superior suppression of T_H17 cell-mediated inflammation in a mouse arthritis model[20]. FOXP3 is also known to promote the expression of T_{reg} cell-specific genes by outcompeting FOS–JUN complexes for binding to NFAT[17]. Additionally, FOXP3 also interacts with the transcription factors GATA3, REL and RORγt[15,21,22], and most likely interacts with other transcription factors as well.

Epigenetic regulation and recruitment of cofactors by FOXP3

The selective activation or repression of genes by FOXP3 is dependent on its ability to facilitate epigenetic remodelling at its target loci. For example, the association of FOXP3 with the genes that encode IL-2 and IFNγ results in the deacetylation of histone H3, which is a modification that silences gene expression. By contrast, FOXP3 promotes the expression of glucocorticoid-induced tumour necrosis factor (TNF) receptor-related protein (GITR; also known as TNFRSF18), CD25 and CTLA4 by inducing histone acetylation near their gene promoters[23]. Central to this role as an epigenetic shaper of the T_{reg} cell genetic landscape is the now widely recognized ability of FOXP3 to associate with molecules that mediate epigenetic modifications. These interaction partners alter the methylation state of target loci with consequences for transcription factor binding. They also execute histone modifications. Such is the case with the FOXP3-associating histone acetyltransferases 60 kDa Tat-interactive protein (TIP60; also known as KAT5) and p300, and histone deacetylase 7 (HDAC7)[24,25].

The Ikaros family member EOS is another example of a cofactor that is important for the recruitment of epigenetic modifiers to FOXP3-regulated loci. This cofactor is highly expressed by T_{reg} cells and is essential for the repression of genes such as *Il2* by FOXP3 (REF. 26). EOS recruits carboxy-terminal binding protein 1 (CTBP1) and factors such as histone-lysine *N*-methyltransferase EHMT2 (also known as EuHMT2) to the *Il2* locus[26]. This prevents histone trimethylation (of histone H3K4) and acetylation (of histones H3 and H4), while promoting the methylation of histone H3K9 and the methylation of CpG

dinucleotides at the *Il2* promoter[26]. Through its role in the formation of this repressor complex, EOS can facilitate the epigenetic silencing of target genes by FOXP3 (REF. 26).

A group of FOXP3 cofactors (EOS, IRF4, SATB1, lymphoid enhancer-binding factor 1 (LEF1) and GATA1) help FOXP3 to enforce the T_{reg} cell gene expression signature. These cofactors seem to have redundant roles in facilitating the regulatory action of FOXP3, as the absence of one cofactor can be offset by the activity of another[27]. Such functional overlap could make the capacity of FOXP3 to regulate gene expression in T_{reg} cells quite resilient. However, precisely how redundant this network is in established T_{reg} cells remains to be determined. It is also possible that certain co-regulators may be necessary for particular T_{reg} cell functions in specific circumstances.

The consequences of altered interaction between FOXP3 and its co-regulators are nicely demonstrated in reporter mice that express an amino-terminal enhanced green fluorescent protein (GFP)–FOXP3 fusion protein (*Foxp3*^{tm2Ayr} mice). As the N-terminal domain is crucial for the interaction of FOXP3 with many cofactors, the presence of the GFP tag disrupts the interaction between FOXP3 and several of its many co-regulators, including TIP60, p300 (REFS 28–30) and EOS[20,26,28]. Although *Foxp3*^{tm2Ayr} mice do not experience overt autoimmunity, their T_{reg} cells show pathology-specific alterations in function. For example, non-obese diabetic (NOD) mice on this background develop diabetes faster than do wild-type NOD mice, as their T_{reg} cells express FOXP3 molecules that have reduced functionality[28]. Interestingly, however, T_{reg} cells expressing this GFP–FOXP3 fusion protein are more potent suppressors of antibody-mediated arthritis due to a preferential interaction between the fusion protein and IRF4 (REF. 20). These findings not only demonstrate how certain cofactors can be crucial for T_{reg} cell-mediated immune control, but they also suggest that T_{reg} cells may be optimized to suppress specific types of inflammation depending on the constituents of the FOXP3-containing functional complex.

Another FOXP3 interaction partner and chromatin modifier is the histone methyltransferase enhancer of zeste homologue 2 (EZH2), which is a component of Polycomb repressive complex 2 (REF. 31). EZH2 can be upregulated in T_{reg} cells in response to CD28 signalling[31]. FOXP3 and EZH2 preferentially form complexes in activated T_{reg} cells, and these complexes stabilize T_{reg} cell gene expression under conditions of inflammation and during activation[31]. Activated T_{reg} cells that lack EZH2 display unstable levels of FOXP3 expression and derepression of many genes[32]. In all, these results likely reflect the possibly distinct stage and function-specific roles of some FOXP3 interaction partners throughout the lifespan of a T_{reg} cell and across the heterogeneous subsets of FOXP3$^+$ T_{reg} cells.

T_{reg} cells, and the pathways and factors that are responsible for controlling FOXP3 expression and function in these important cells, have been under scrutiny for some time. In recent years, our understanding of these mechanisms has greatly improved. In the following sections, we aim to summarize the well-established tenets of FOXP3 biology and present several recent breakthroughs that have provided exciting new insights into the biology of FOXP3.

Regulation of the *FOXP3* gene

Structure of the *FOXP3* gene

The human *FOXP3* gene maps to the p-arm of the X chromosome, and this is clearly reinforced by the inheritance pattern of IPEX syndrome. The gene includes 11 exons, and a high degree of conservation is seen between the human and mouse genes, especially at the exon–intron interfaces[9,10]. In response to signalling via the TCR and co-stimulation pathways, the *FOXP3* promoter is bound and activated by transcription factors such as NFAT and AP-1 (REF. 33). In addition, the forkhead box O (FOXO) proteins FOXO1 and FOXO3 have been reported to bind to the *Foxp3* promoter as well as to other regulatory elements of the *Foxp3* gene[34] (discussed below), and cAMP-responsive element-binding protein (CREB)–activating transcription factor 1 (ATF1) complexes also drive the activation of the *FOXP3* promoter[35] (FIG. 1). Importantly, the promoter of the *FOXP3* gene has been characterized as having low levels of *trans*-activating potential. Instead, transcription at this important locus relies heavily on the contribution of conserved enhancer regions[33].

Both the initiation and maintenance of *FOXP3* transcription are highly dependent on key conserved non-coding sequences (CNSs), which serve as binding sites for a number of transcription factors (FIG. 1). CNS3 is found between exon 1 and exon 2 of *Foxp3* (REF. 36), and this region promotes the accumulation of permissive histone modifications at the *Foxp3* promoter, leaving it in an epigenetically poised state in both established $FOXP3^+$ cells and $FOXP3^-$ T_{reg} cell precursors. Thus, CNS3 has a crucial role as an epigenetic switch that controls *Foxp3* transcription in these cells in response to binding by REL, which is a crucial regulator of T_{reg} cell development in the thymus[37,38]. However, although it is indispensable for initiating *Foxp3* expression, CNS3 does not seem to be necessary for its maintenance.

By contrast, CNS2 (also known as T_{reg} cell-specific demethylated region) is important for maintaining the expression of FOXP3 in thymus-derived T_{reg} (tT_{reg}) cells after thymic egress. The CNS2 region is located within the first intron of *Foxp3*, and CpG elements within CNS2 become extensively hypomethylated during tT_{reg} cell development[39]. Demethylated CpG motifs in CNS2 and other regulatory elements in the *FOXP3* gene enable the binding of several transcription factors, including REL, CREB–ATF1, RUNX1–core-binding factor subunit-β (CBFβ), ETS1 and signal transducer and activator of transcription 5 (STAT5), as well as FOXP3 itself[33,36]. The maintenance of this epigenetic state enables the stable expression of FOXP3 by tT_{reg} cells under a variety of conditions, including tissue inflammation. *In vitro*-induced T_{reg} (iT_{reg}) cells, which are less stable than tT_{reg} cells in terms of their FOXP3 expression and suppressive activity, possess a methylated or partially demethylated CNS2 region[40,41]. By contrast, peripherally derived (pT_{reg}) cells generally have epigenetic profiles that resemble those of tT_{reg} cells[40], and they are thought to be functionally stable.

Methyl-CpG-binding domain protein 2 (MBD2) binds CNS2 and recruits the ten-eleven translocation (TET) demethylases that maintain the hypomethylation of CNS2 (FIG. 1). Ablating MBD2 function in mice results in decreased numbers of T_{reg} cells, and the remaining T_{reg} cells have a reduced suppressive activity[42]. These observations were linked

to a failure to maintain CNS2 hypomethylation in MBD2-deficient T_{reg} cells after thymic egress[42]. Conversely, DNA methyltransferase 1 (DNMT1) is known to promote methylation events at CNS2. This enzyme and similar factors may counteract the epigenetic programming that is responsible for tT_{reg} cell activity. Indeed, the pro-inflammatory cytokine IL-6 was recently shown to trigger the DNMT1-dependent methylation of CNS2 motifs in T_{reg} cells, whereas exposure to IL-2 or vitamin C mobilized TET enzymes to maintain hypomethylation at CNS2 (REFS 43,44) (FIGS 1,2). Exposure to IL-6 reduces the acetylation of histone H3 at the upstream promoter as well[45,46], adversely affecting *FOXP3* transcription. By contrast, transforming growth factor-β (TGFβ) signalling has been linked to the epigenetic stabilization of FOXP3 expression through the suppression of DNMT1 expression[47].

The CNS1 enhancer is particularly key for the induction of extrathymic FOXP3 expression in T cells. Similarly to CNS2, CNS1 is situated intronically. But it is uniquely indispensable for activating FOXP3 expression in response to TGFβ-induced SMAD signalling[48]. The binding of activated SMAD3 to CNS1 is a key event in the induction of FOXP3 expression during the differentiation of pT_{reg} cells but not that of tT_{reg} cells[36]. Accordingly, CNS1-deficent mice have a defective pT_{reg} cell compartment that fails to maintain immune tolerance at barrier sites such as the gut, but these mice do not succumb to autoimmunity[49]. Experiments showing that the appearance of CNS1 in the evolutionary record coincided with the appearance of placental mammals[50] strongly suggest that the ability to induce FOXP3 expression in non-T_{reg} cell precursors could be crucial for maintaining maternal tolerance to the semi-allogeneic fetus during pregnancy.

Transcription of the *FOXP3* gene

In tT_{reg} cell precursors, TCR stimulation triggers the activation of nuclear factor-κB (NF-κB) family members such as REL. These bind to the constitutively 'open' CNS3 enhancer of the *FOXP3* gene to initiate transcription. The importance of this signalling pathway in T_{reg} cell development in the thymus is clearly demonstrated by mice that lack its key mediators (for example, protein kinase Cθ (PKCθ), B cell lymphoma–leukaemia 10 (BCL-10), CARD-containing MAGUK protein 1 (CARMA1; also known as CARD11), TGFβ-activated kinase 1 (TAK1; also known as MAP3K7), IκB kinase 2 (IKK2; also known as IKKβ) and REL) as they display a markedly reduced tT_{reg} cell output[51]. Signalling via the TCR and the CD28-mediated co-stimulatory pathway is required for both the proper development and maintenance of T_{reg} cells[52,53], and this signalling also induces the activation of the RAS– RAF–mitogen-activated protein kinase (MAPK) signalling pathway, which is important for initiating *FOXP3* transcription[54,55]. These signalling cascades ultimately recruit numerous transcription factors to the promoter and regulatory elements of the *FOXP3* gene.

The role of cytokines

IL-2 receptor signalling triggers the activation of Janus kinases (JAKs) that in turn mediate the phosphorylation of STAT5. Activated STAT5 binds the *FOXP3* promoter and CNS2, driving the active transcription of this locus in T_{reg} cells. Mice that lack either STAT5 or JAK3 display substantially lower frequencies of T_{reg} cells than do wild-type controls[55]. By

contrast, constitutive STAT5 activity can rescue the T_{reg} cell pool from the negative effects of IL-2 deprivation[56]. IL-2 is also known to have an important role in stabilizing *Foxp3* expression[57]. Indeed, high expression of the IL-2 receptor subunit, CD25, has been shown to correspond to both highly stable *FOXP3* expression and potent suppressive functions in T_{reg} cells[58]. Indeed, while the pro-inflammatory cytokine IL-6 can trigger the methylation of CNS2 by recruiting DNMT1, thus reducing *Foxp3* transcription, IL-2 can prevent this by recruiting demethylating TET enzymes[44]. STAT3 activation triggered by IL-6 and other inflammatory cytokines (such as IL-21) can also inhibit *Foxp3* expression by obstructing the binding of IL-2-activated STAT5 to elements of the *Foxp3* gene[59]. Interestingly, TNF, a pro-inflammatory cytokine, has been shown to antagonize T_{reg} cell function under some circumstances, but it can also support the STAT5-mediated expansion of some T_{reg} cell populations[60]. Further study of the potential interplay between stabilizing and destabilizing signalling pathways in T_{reg} cells exposed to TNF is clearly necessary.

TGFβ is of particular importance in the development of extrathymically derived pT_{reg} cells. The TGFβ-triggered activation of SMADs leads to the binding of SMAD3–SMAD4 heterodimers to CNS1 in the *Foxp3* gene. This molecular event is essential for inducing *Foxp3* expression in naive $CD4^+$ T cells and thus promoting their acquisition of a T_{reg} cell phenotype in peripheral tissues and *in vitro*[48,49]. TGFβ may also have a role in tT_{reg} cell development[61]. Although some studies suggest that TGFβ is not required for tT_{reg} cell generation[62], others report that the cytokine can promote tT_{reg} cell survival during development[63] without directly influencing FOXP3 expression. Continuous exposure to TGFβ can prevent the loss of FOXP3 expression in iT_{reg} cells[64], but it is not clear if there is a similar stabilizing role for TGFβ in tT_{reg} cells.

The vitamin A metabolite all-*trans*-retinoic acid (ATRA) has been known for some time to augment the process of iT_{reg} cell differentiation from naive $CD4^+$ T cell precursors (FIG. 2). This may be due to the ability of ATRA to boost the TGFβ-driven phosphorylation of SMAD3 or to suppress inflammatory cytokine signalling that interferes with the upregulation of *Foxp3* (REFS 65,66). In addition, ATRA can influence the transcription of the *FOXP3* gene as it induces histone H4 acetylation at the *FOXP3* locus in T_{reg} cell precursors[67]. Epigenetic modifications of the CNS2 region and increased chromatin-binding by FOXP3 have been reported to improve the functional stability of ATRA-treated T_{reg} cells[45]. In addition, ATRA may support the stability of tT_{reg} cells *in vivo* by promoting resistance to the T_{reg} cell-destabilizing cytokine IL-6 (REF. 68).

Processing of *FOXP3* transcripts

In humans, *FOXP3* transcripts can be alternatively spliced into multiple variant isoforms, and this has important implications for the regulatory activity of the encoded transcription factor. The activation of T_{reg} cells via the TCR and co-stimulatory molecules can substantially alter their production of FOXP3 splice variants[69]. Isoforms of FOXP3 that have been cloned from peripheral blood mononuclear cell (PBMC)-derived cDNA include FOXP3Δ2, which lacks the region encoded by exon 2; FOXP3Δ7, which is missing exon 7 (and the leucine zipper domain); and FOXP3Δ2Δ7, which lacks both exon 2 and exon 7. Whereas full-length FOXP3 suppresses gene expression mediated by RORα, NF-κB and

NFAT, FOXP3Δ2 and FOXP3Δ2Δ7 display impaired inhibition of these inflammatory transcription factors[70]. Transfection studies have shown that forced expression of full-length FOXP3 in T cells limits their proliferation[70,71]. Even though variant constructs that lack exon 7 are consequentially defective at dimer formation, they yield FOXP3 molecules that are fully capable of suppressing T cell activation[70]. Indeed, compared with control T cells transfected with full-length FOXP3, FOXP3Δ2-transfected or FOXP3Δ2Δ7-transfected T cells show similar activation kinetics, and a similar upregulation of CD25 levels and reduction of CD127 levels[69,71]. Such findings suggest that these splice variants still induce some aspects of the T_{reg} cell programme. It has also been shown that the overexpression of FOXP3Δ2, like that of full-length FOXP3, could impart the ability to suppress the function of other T cells *in vitro*[72]. However, another study found that FOXP3Δ7 is prevalently expressed by PBMCs from patients with Crohn's disease, and showed that forced expression of FOXP3Δ2Δ7 can actually favour T_H17 cell differentiation in recently activated $FOXP3^+CD4^+$ T cells that are poised between the T_H17 and iT_{reg} cell fates[73].

Interestingly, the splicing events seen in Crohn's disease patients that could favour pro-inflammatory outcomes were linked to exposure to IL-1β, an implicated destabilizer of T_{reg} cell function[73]. It is not clear at present how the product of this IL-1β-induced splice variant of FOXP3 is functionally compromised relative to the full-length protein. However, this alteration of *FOXP3* transcript processing is associated with the suboptimal maintenance of immunological tolerance.

These findings suggest that different isoforms of FOXP3 may influence the differentiation or functionality of T_{reg} cells *in vivo*. It is not known, however, whether individual T_{reg} cells express one isoform at a time or more than one FOXP3 isoform simultaneously[69]. With the individual properties of each variant coming to light, it is possible that each possesses unique functions. In addition, the fact that splice variants exist in humans but not in mice should be recognized when applying mouse findings to human diseases.

Domains of FOXP3

The FOXP3 protein comprises three functionally important domains: namely, an N-terminal domain, a zinc finger and leucine zipper-containing region, and a C-terminal forkhead domain. All domains are apparently essential for the optimal function of $FOXP3^+$ T_{reg} cells, as mutations in any of the domains can lead to the scurfy or IPEX autoimmune phenotypes. The forkhead region is necessary for DNA binding, FOXP3–NFAT interactions and the homodimerization of FOXP3 molecules, and is responsible for the transcriptional repression of a number of immune-related target genes. Interestingly, the deletion of this domain results in the retention of some FOXP3 functions[74], an observation suggesting that enforcement of T_{reg} cell gene expression depends heavily on the interaction of FOXP3 with DNA-binding collaborator molecules. The N-terminal domain also confers the ability to repress the transcription of target genes[75] (hence its reputation as the 'repressor domain'), but it does not bind to DNA. A number of proteins interact with FOXP3 via the N-terminal domain, including EOS and hypoxia-inducible factor 1α (HIF1α). In addition, a chromatin-remodelling complex that includes HDAC7 or HDAC9 and TIP60, was shown to interact with this region of the protein, suggesting that it has a key role in facilitating the 'FOXP3

interactome': that is, the complex of FOXP3 and numerous co-regulatory molecules that can have collaborative and potentially redundant roles in promoting FOXP3-induced gene regulation[16]. Sequences encoded by exon 2 have also been shown to interact with RORγt and RORα[8]. These interactions were found to be important for inhibiting the activity of these transcription factors and promoting the generation of T_{reg} cells, rather than T_H17 cells, from naive $CD4^+$ T cells[8]. In addition, the leucine zippers and zinc finger domains are important for the homodimerization of FOXP3 (REFS 75,76), which is indispensable for its function[8,77]. Finally, distinct sites within the FOXP3 protein have been shown to determine the cellular distribution of the transcription factor, either ensuring or obstructing its typically constitutive nuclear localization in T_{reg} cells[75,78].

Post-translational modification of FOXP3

The expression and regulatory activity of FOXP3 are also regulated at the protein level. The pathways that lead to distinct post-translational modifications of FOXP3 — including acetylation, phosphorylation and ubiquitylation — have been recently shown to be important in this newly appreciated layer of control over T_{reg} cell function (FIG. 3).

Acetylation of FOXP3

Lysine acetyltransferases (KATs) catalyse the attachment of acetyl groups to diverse target proteins (including FOXP3) at specific lysine residues. Acetylated FOXP3 molecules have been shown to be more stable than those that are not acetylated, as they avoid ubiquitylation at targeted lysine residues and thus resist proteasomal degradation (discussed below). Acetylation improves the ability of FOXP3 to bind to chromatin and carry out its functions as a transcriptional regulator[79,80].

FOXP3 interacts with the KATs TIP60 and p300. These factors promote FOXP3 acetylation at residues K63, K263 and K268 (REF. 81), thus improving the stability of FOXP3 and its association with the promoters of its target genes[24,79,80]. The inhibition or deletion of p300 reduces the levels of both acetylated and total FOXP3 in T_{reg} cells, and negatively affects the fitness and function of these cells[81,82]. Importantly, p300 antagonism also undermines immune suppression in mouse models of cancer, thus enabling more robust antitumour immune responses that limit tumour growth[30]. The deletion of TIP60 also markedly decreases FOXP3 expression levels and leads to autoimmune pathology[81,82]. This T_{reg} cell dysfunction is accompanied by a loss of FOXP3 dimerization, and TIP60-deficient T_{reg} cells show reduced expression of FOXP3 and CTLA4, as well as inappropriate expression of the pro-inflammatory cytokines IL-6 and IL-17 (REF. 82). IL-6-induced STAT3 activity can downregulate the levels of the FOXP3 protein by inhibiting its acetylation[80,83]. By contrast, TGFβ augments the activity and/or stability of FOXP3 by driving its acetylation[80].

Lysine deacetylases (KDACs) and HDACs remove acetyl groups from target proteins. These enzymes can negatively affect FOXP3 expression and T_{reg} cell function, and KDAC inhibitors and HDAC inhibitors are well known for their ability to augment FOXP3 expression in T_{reg} cells and improve their suppressive performance[24,25,79,84]. Sirtuin 1 (SIRT1) is a class III KDAC, and its expression has been observed to be inversely proportional to that of FOXP3 during iT_{reg} cell differentiation[85]. Interestingly, whereas TCR

stimulation greatly upregulates SIRT1 levels in non-T_{reg} cells, TCR activation reduces SIRT1 expression in T_{reg} cells[86]. Forced SIRT1 expression in T_{reg} cells not only leads to the deacetylation of FOXP3 but also triggers the rapid polyubiquitylation of the transcription factor and its degradation by the proteasome[85]. Conversely, T_{reg} cell-specific deletion of SIRT1 causes increased FOXP3 expression, T_{reg} cell suppression and increased allograft tolerance[86]. These observations are associated with the elevated expression of T_{reg} cell-associated genes (for example, *Ctla4*) in the absence of SIRT1 (REF. 86). Chemical inhibition of this enzyme similarly increases FOXP3 levels and the *in vivo* suppressive potency of iT_{reg} cells[87].

The serine/threonine kinase and Hippo pathway participant mammalian sterile 20-like kinase 1 (MST1; also known as STK4) was recently shown to antagonize the FOXP3-deacetylating activity of SIRT1. In cell lines, MST1 interacts with FOXP3 and promotes its activity as a transcriptional suppressor by increasing its acetylation, and T_{reg} cells isolated from MST1-deficient mice have reduced levels of FOXP3 protein owing to its degradation[88]. Indeed, these findings are in line with the autoimmunity and poor T_{reg} cell differentiation seen in mice that lack MST1. It is noteworthy that MST1 is also capable of stabilizing FOXO proteins by limiting AKT activation[89]. Therefore, the positive effects of this kinase on FOXP3 levels may be multifaceted.

Finally, the acetylation of FOXP3 has been shown to be involved in the induction of pT_{reg} cells by commensal microorganisms in the gut. Certain species of gut bacteria produce short-chain fatty acids (SCFAs) that have a marked effect on host immunity[90]. In differentiating $CD4^+$ T cells, exposure to SCFAs such as butyrate was found to increase the induction of FOXP3 expression *in vitro* by promoting the deposition of activating histone modifications[91]. This ability of SCFAs to support T_{reg} cell induction seems to involve HDAC inhibition, and results in the reduced acetylation of the FOXP3 protein and of histones at the *Foxp3* locus[92].

Phosphorylation of FOXP3

FOXP3 is also subjected to phosphorylation. The C terminus of FOXP3 can be modified by an unknown kinase at S418. This reportedly augments the ability of FOXP3 to bind to DNA and dictates T_{reg} cell-associated gene regulation. In patients with arthritis, TNF activates protein phosphatase 1 (PP1), and this enzyme dephosphorylates FOXP3, ablating the function-augmenting modification[93]. This post-translational mechanism of FOXP3 regulation may explain why T_{reg} cells fail to curb inflammation in arthritic joints, even when they are abundant in number.

By contrast, the phosphorylation of FOXP3 at other sites can inhibit its ability to promote T_{reg} cell function. Cyclin-dependent kinase 2 (CDK2), which is activated by TCR signalling, is capable of phosphorylating four CDK motifs within the N-terminal domain of FOXP3 (REF. 94). These modifications might negatively affect FOXP3 expression and/or function, as CDK2-deficient T_{reg} cells are more suppressive than are wild-type controls[95]. The specific modification of S19 was observed *in vitro*, and mutation of this and other target residues in the repressor domain (S88, T114 and T175) was associated with an increased half-life of mouse FOXP3 and a heightened repression of target genes. Furthermore, the

ectopic expression of a mutant FOXP3 construct that is insensitive to S19 phosphorylation in $CD4^+CD25^-$ T cells resulted in a more pronounced suppressive function *in vitro* and *in vivo*[94]. Although it is uncertain how such modifications of FOXP3 interfere with its control of T_{reg} cell gene expression and phenotype, the N terminus of the protein is known to be important for the interaction of FOXP3 with numerous elements of the FOXP3 interactome[16,20,28,77]. It is possible that modifications to this region are disruptive to such complexes.

The kinase PIM1 was also recently shown to interact with and phosphorylate FOXP3. Li *et al.*[96] found that PIM1, which is highly expressed by human T_{reg} cells, can interact with the C-terminal domain of FOXP3 and target S422. The PIM1-induced phosphorylation of S422 interferes with FOXP3 activity and limits the expression of T_{reg} cell-associated genes, including those that encode CD25, CTLA4 and GITR. Although TCR signalling limits the induction of PIM1 expression, IL-6 can upregulate the expression of the kinase. Interestingly, inhibitory modification of S422 can be prevented by phosphorylation at S418 (REF. 96), which is a modification that is reported to augment T_{reg} cell function[93].

The related kinase PIM2 also executes an inhibitory phosphorylation of FOXP3. In human T_{reg} cells, PIM2 interacts with FOXP3 and targets multiple N-terminal sites (S33, S41 and a third unspecified site). Through an as yet unknown mechanism, PIM2 interferes with the expression of T_{reg} cell-associated genes, and the inhibition of PIM2 in mouse T_{reg} cells increases their suppressive capabilities[97]. Interestingly, PIM2 expression has been reported to be FOXP3 dependent and involved in the expansion of T_{reg} cells[98]. It is possible that the upregulation of this kinase may impair the suppressive function of T_{reg} cells in order to afford these cells some proliferative benefit. The lymphocyte-specific protein tyrosine kinase LCK phosphorylates FOXP3 in cancer cells at Y342 (REF. 99). This modification leads to the upregulation of FOXP3 in these cells[99], but it is unclear whether phosphorylation by LCK alters FOXP3 expression or function in T_{reg} cells. Taken together, these findings demonstrate that FOXP3 function can be upregulated or downregulated by site-specific, possibly cross-regulating, phosphorylation events.

Ubiquitylation of FOXP3

Ubiquitin chains interlinked at lysine residue 48 (K48 polyubiquitylation) are well known for facilitating the proteasomal degradation of proteins[100]. Several studies have shown that the FOXP3 protein can be modified in this manner and that this modification has major implications for T_{reg} cell function[101]. In differentiating $CD4^+$ T cells, HIF1 was found to physically interact with FOXP3 and trigger K48 polyubiquitylation. This leads to the proteasomal degradation of FOXP3, and accordingly, knocking down components of the HIF1 degradation machinery stabilized the FOXP3 protein pool[102].

The ubiquitin-dependent degradation of FOXP3 can also be precipitated in established T_{reg} cells by inflammatory stresses. The cellular levels of the FOXP3 protein are subject to constant turnover owing to K48-type polyubiquitylation-induced proteasomal degradation[103,104]. However, the half-life of FOXP3 can be markedly reduced by exposure to a range of inflammatory stresses *in vitro*, including lipopolysaccharide, pro-inflammatory cytokines and heat shock[103]. In addition, CC-chemokine ligand 3 (CCL3), which is

abundant in patients with psoriasis, has been reported to induce T_{reg} cell dysfunction and plasticity by triggering the K48-linked polyubiquitylation and degradation of FOXP3 (REF. 105).

Also among the ranks of FOXP3 interaction partners is the chaperone molecule heat shock 70 kDa protein (HSP70), which recruits the stress-activated, U-box domain type E3 ubiquitin ligase STUB1 (also known as CHIP). This ligase is capable of mediating the degradation of important transcription factors, including RUNX2, HIF1α[106,107] and FOXP3 (REF. 103). Although it is not ordinarily expressed at considerable levels by T_{reg} cells, STUB1 can be upregulated under *in vitro* conditions that can also bring about FOXP3 protein loss. This downregulation was shown to result from K48-type polyubiquitylation at residues K227, K250, K263 and K268 in human FOXP3 by STUB1 and from proteasome activity[103]. Ectopic STUB1-mediated FOXP3 loss disrupts the suppressive function of T_{reg} cells, reduces their expression of T_{reg} cell-associated genes and upregulates their expression of effector-type cytokines, such as IL-2 and IFNγ[103]. Conversely, knocking down STUB1 stabilizes FOXP3 expression and increases the suppressive potency of T_{reg} cells[103]. The situations and pathways that induce the activity of STUB1, and potentially other E3 ligases that substantially affect FOXP3 levels in T_{reg} cells, remain to be fully defined. In addition, it is unclear how the dramatic or incremental downregulation of FOXP3 at the protein level can affect the long-term identity and function of T_{reg} cells.

Deubiquitylation of FOXP3

As polyubiquitylation negatively affects the stability of the FOXP3 pool[79,85,102,103], it follows that deubiquitinases (DUBs) should preserve the cellular levels of FOXP3. Indeed, multiple DUBs seem to stabilize FOXP3 and the T_{reg} cell phenotype.

The DUB ubiquitin-specific peptidase 7 (USP7) is expressed by T_{reg} cells and is capable of interacting with FOXP3 to catalyse its deubiquitylation[104]. Knocking down USP7 reduces FOXP3 levels in T_{reg} cells and inhibits their suppressive functions[104]. The treatment of T_{reg} cells with a general DUB inhibitor has similar destabilizing effects[104]. By contrast, overexpressing USP7 in T_{reg} cells increases FOXP3 protein levels and augments T_{reg} cell activity[104]. A recent study has revealed that the conditional deletion of USP7 in mouse T_{reg} cells leads to a dramatic loss of immune regulation and to lethal autoimmunity within a month after birth[82]. This phenotype was associated with T_{reg} cell hyperproliferation, disrupted T_{reg} cell gene expression patterns, unstable FOXP3 protein levels and an ineffectual suppressive ability of T_{reg} cells. Yet, the T_{reg} cell-specific deficiency of USP7 did not alter the overall T_{reg} cell numbers or levels of FOXP3 expression in the thymus. These results clearly demonstrate the need to counteract the process of FOXP3 ubiquitylation in order to maintain a functionally stable pool of T_{reg} cells in the periphery. Excitingly, the authors of this study[82] also found that the *in vivo* administration of a specific USP7 antagonist inhibited FOXP3 expression and the suppressive activity of T_{reg} cells in tumour-bearing mice. Also, they showed that the antagonist suppressed the growth of tumours by augmenting antitumour immunity[82]. This suggests that targeting USP7 could have therapeutic potential in cancer.

Additional DUBs may also have the effect of stabilizing FOXP3. For example, mice that are deficient in USP44 display compromised FOXP3 expression in their T_{reg} cell compartment, and this has consequences for immune control (F.P. and J.B., unpublished observations). The DUB USP21 was also recently found to be expressed at relatively high levels in human[108] and mouse T_{reg} cells. The restricted deletion of USP21 in FOXP3$^+$ cells in mice resulted in the loss of immune homeostasis across multiple tissues (including the liver, lung and salivary glands) and was coincident with a substantial upregulation of inflammatory cytokines (predominantly IFNγ) by effector CD4$^+$ T cells[109]. Furthermore, compared with control T_{reg} cells, USP21-deficient T_{reg} cells produced substantial amounts of IFNγ, expressed less FOXP3, and they were less suppressive both *in vitro* and in a model of neuroinflammation[109].

Although several examples of ubiquitin-mediated mechanisms for FOXP3 downregulation exist, it is possible that — similarly to phosphorylation — unidentified enzymes (E3 ligases in this case) can also positively regulate aspects of T_{reg} cell biology by targeting distinct sites in FOXP3. Indeed, depending on the site and linkage type, protein ubiquitylation events can not only trigger degradation, but also alter the protein–protein interactions, signalling potential and cellular distribution of many proteins[110]. Thus, other types of ubiquitylation may have marked consequences on the functionality of the FOXP3 protein pool. It is also possible that degradative pathways that are dependent on ubiquitylation may compete with acetylating processes for target residues on FOXP3. Indeed, the degrees of FOXP3 ubiquitylation and acetylation can be inversely related[79,80,85], and some overlap exists between the lysine residues that have been identified as acetylation targets[87] and ubiquitylation targets[103,104].

The regulation of FOXP3 by protein modifications is a relatively new aspect of our understanding of these important elements of immune control. However, studies have already identified a number of enzymes and processes that could be exploited by future therapies that aim to fine-tune the suppressive functions of T_{reg} cells in disease settings (FIG. 2). With further study, we will no doubt discover additional targets that have such potential.

Conclusions and implications

A robust and functional T_{reg} cell compartment is requisite for managing a highly destructive immune system. In recent years, numerous breakthroughs have improved our understanding of how these cells function. As discussed in this Review, many of these breakthroughs have come from studies that have explored the mechanisms that control the expression of FOXP3, the transcriptional anchor of the major population of T_{reg} cells responsible for enforcing immune homeostasis. We have discussed the key role of this transcription factor in maintaining the gene expression patterns that underlie the characteristically suppressive phenotype of T_{reg} cells, and we have summarized a number of factors and pathways that are known to affect FOXP3 and the broader functions of T_{reg} cells. It is becoming clear that in addition to the transcriptional control of *FOXP3* expression, other mechanisms of regulation contribute to the overall abundance and activity of FOXP3 (for example, post-translational

modifications). Exploiting these newly appreciated aspects of FOXP3 biology may lead to an unprecedented level of therapeutic control over immune tolerance (BOX 4).

Box 4

Therapeutic targeting of forkhead box protein P3

Autoimmune disease

Regulatory T (T_{reg}) cell deficiency or dysfunction is thought to be central to the pathogenesis of diverse autoimmune diseases (as reviewed in REF. 143). Interestingly, T_{reg} cell numbers are not uniformly reported to be reduced in patients with autoimmune disease, and indeed T_{reg} cells can even be found in very high numbers in some afflicted tissues. For example, an abundance of T_{reg} cells has been reported in the synovial fluid of patients with rheumatoid arthritis (even though these cells are still very much outnumbered by effector T cells)[144]. It is therefore likely that functional or phenotypic abnormalities, the relative balance of T_{reg} cells and effector T cells, or elements in the inflammatory microenvironment are to blame for the failure of T_{reg} cells to enforce tolerance in these scenarios.

Inflammatory cytokines are likely to restrict T_{reg} cell function in patients with autoimmune diseases. As discussed in this Review, interleukin-1β (IL-1β), IL-6 and tumour necrosis factor (TNF) can antagonize forkhead box protein P3 (FOXP3) expression or function. By contrast, T_{reg} cell dysfunction may arise due to a paucity of stabilizing cues such as IL-2 (FIG. 2). In support of this, the concentrations of plasma transforming growth factor-β and the activity of signal transducer and activator of transcription 5 have been reported to be decreased in patients with systemic lupus erythematosus and patients with type 1 diabetes, respectively[145,146].

Augmenting the T_{reg} cell pool by administering additional, functionally suppressive T_{reg} cells as a cellular therapy represents an exciting strategy for treating autoimmune diseases by increasing both T_{reg} cell numbers and their frequency relative to potentially pathological effector leukocytes. The infusion of *ex vivo*-expanded T_{reg} cell populations is currently being explored as a means to alleviate organ transplant rejection and induce tolerance in graft recipients, and has shown some success[147,148]. Similar approaches may prove useful in the treatment of some autoimmune diseases. Preclinical studies using mouse models of autoimmunity and graft-versus-host disease have shown that the adoptive transfer of T_{reg} cells can either prevent or ameliorate severe inflammatory pathologies[149,150]. Recent clinical trials in adult and juvenile diabetes and in patients with Crohn's disease are also yielding promising results[151–153].

The optimization of T_{reg} cell adoptive transfer therapy could lead to its widespread use to combat autoimmune and inflammatory diseases. In this regard, the development of pretreatment and expansion regimens that improve the fitness, retention and function of T_{reg} cells post-transfer may advance therapies. Inhibitors of FOXP3 deacetylation or ubiquitylation could yield more-potent suppressors. In addition, neutralizing environmental factors that limit T_{reg} cell activity (for example, IL-6) or supplementation

with factors, such as IL-2, that boost T_{reg} cell function could be used to improve the efficacy of T_{reg} cell adoptive transfer therapy.

Cancer

T_{reg} cells can impede the effectiveness of antitumour immunity by suppressing the activity of cytotoxic immune cells; they also reduce the efficacy of immunotherapies and tumour vaccines[154]. In patients with cancer, T_{reg} cells are increased in number both within tumours and systemically, and an abundance of T_{reg} cells in the tumour microenvironment is linked to inadequate immunity and poor patient survival in many cancer types[155]. Strategies for depleting T_{reg} cells or inhibiting their suppressive functions are being pursued to improve both natural and therapeutically induced antitumour immune responses (as reviewed in REF. 155). The therapeutic anti-CD25 antibody daclizumab, for example, has been found to elicit a downregulation of FOXP3 that coincides with reduced T_{reg} cell-mediated suppression and increased immune priming in patients with breast cancer[156]. Alternatives to general T_{reg} cell depletion may also be effective, and include transient T_{reg} cell ablation and the selective targeting of tumour-associated T_{reg} cell subsets by taking advantage of specific surface markers that are expressed by these cells (for example, CC-chemokine receptor 4)[157].

One issue with these strategies is that certain molecular targets that are used for T_{reg} cell depletion are also expressed by activated effector immune cells. For these reasons, immunotherapies that target newly appreciated post-transcriptional mechanisms of FOXP3 regulation should be explored. Modulating T_{reg} cell function by depleting the pool of functional FOXP3 protein could transiently and specifically interrupt T_{reg} cell-mediated immune suppression in the cancer setting while preserving a suppressor cell population that has the transcriptional ‘blueprint’ for enforcing immune homeostasis. Preclinical studies of ubiquitin-specific peptidase 7 inhibition[82] and p300 inhibition[30], for example, suggest that such an approach has promise[82].

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References

1. Wang J, Ioan-Facsinay A, van der Voort EI, Huizinga TW, Toes RE. Transient expression of FOXP3 in human activated nonregulatory $CD4^+$ T cells. Eur J Immunol. 2007; 37:129–138. [PubMed: 17154262]
2. Gavin MA, et al. Single-cell analysis of normal and FOXP3-mutant human T cells: FOXP3 expression without regulatory T cell development. Proc Natl Acad Sci USA. 2006; 103:6659–6664. [PubMed: 16617117]

3. Sakaguchi S, Wing K, Miyara M. Regulatory T cells — a brief history and perspective. Eur J Immunol. 2007; 37(Suppl 1):S116–S123. [PubMed: 17972355]
4. Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol. 2008; 8:523–532. [PubMed: 18566595]
5. Shevach EM. Mechanisms of Foxp3$^+$ T regulatory cell-mediated suppression. Immunity. 2009; 30:636–645. [PubMed: 19464986]
6. Deaglio S, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med. 2007; 204:1257–1265. [PubMed: 17502665]
7. Cao X, et al. Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. Immunity. 2007; 27:635–646. [PubMed: 17919943]
8. Ramsdell F, Ziegler SF. FOXP3 and scurfy: how it all began. Nat Rev Immunol. 2014; 14:343–349. [PubMed: 24722479]
9. Brunkow ME, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet. 2001; 27:68–73. [PubMed: 11138001]
10. Bennett CL, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet. 2001; 27:20–21. References 9 and 10 describe the extremely negative consequences of *FOXP3* mutation on immune regulation in mice and humans. [PubMed: 11137993]
11. Zheng Y, et al. Genome-wide analysis of Foxp3 target genes in developing and mature regulatory T cells. Nature. 2007; 445:936–940. [PubMed: 17237761]
12. Samstein RM, et al. Foxp3 exploits a pre-existent enhancer landscape for regulatory T cell lineage specification. Cell. 2012; 151:153–166. [PubMed: 23021222]
13. Marson A, et al. Foxp3 occupancy and regulation of key target genes during T-cell stimulation. Nature. 2007; 445:931–935. [PubMed: 17237765]
14. Morikawa H, Sakaguchi S. Genetic and epigenetic basis of T_{reg} cell development and function: from a FoxP3-centered view to an epigenome-defined view of natural T_{reg} cells. Immunol Rev. 2014; 259:192–205. [PubMed: 24712467]
15. Rudra D, et al. Transcription factor Foxp3 and its protein partners form a complex regulatory network. Nat Immunol. 2012; 13:1010–1019. This study reveals the extensive interactions of FOXP3 with other transcription factors and epigenetic-modifying enzymes. [PubMed: 22922362]
16. Hori S. The Foxp3 interactome: a network perspective of T_{reg} cells. Nat Immunol. 2012; 13:943–945. [PubMed: 22990900]
17. Wu Y, et al. FOXP3 controls regulatory T cell function through cooperation with NFAT. Cell. 2006; 126:375–387. [PubMed: 16873067]
18. Ono M, et al. Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. Nature. 2007; 446:685–689. [PubMed: 17377532]
19. Zheng Y, et al. Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T_H2 responses. Nature. 2009; 458:351–356. [PubMed: 19182775]
20. Darce J, et al. An N-terminal mutation of the Foxp3 transcription factor alleviates arthritis but exacerbates diabetes. Immunity. 2012; 36:731–741. [PubMed: 22579475]
21. Zhang F, Meng G, Strober W. Interactions among the transcription factors Runx1, RORγt and Foxp3 regulate the differentiation of interleukin 17-producing T cells. Nat Immunol. 2008; 9:1297–1306. [PubMed: 18849990]
22. Loizou L, Andersen KG, Betz AG. Foxp3 interacts with c-Rel to mediate NF-κB repression. PLoS ONE. 2011; 6:e18670. [PubMed: 21490927]
23. Chen C, Rowell EA, Thomas RM, Hancock WW, Wells AD. Transcriptional regulation by Foxp3 is associated with direct promoter occupancy and modulation of histone acetylation. J Biol Chem. 2006; 281:36828–36834. [PubMed: 17028180]
24. Li B, et al. FOXP3 interactions with histone acetyltransferase and class II histone deacetylases are required for repression. Proc Natl Acad Sci USA. 2007; 104:4571–4576. [PubMed: 17360565]
25. Tao R, et al. Deacetylase inhibition promotes the generation and function of regulatory T cells. Nat Med. 2007; 13:1299–1307. References 24 and 25 are landmark studies demonstrating that FOXP3 is post-translationally modified by lysine acetylation (reference 24), and that preserving this

modification through HDAC inhibition can augment T_{reg} cell function in models of colitis and allograft transplantation. [PubMed: 17922010]

26. Pan F, et al. Eos mediates Foxp3-dependent gene silencing in $CD4^+$ regulatory T cells. Science. 2009; 325:1142–1146. [PubMed: 19696312]
27. Fu W, et al. A multiply redundant genetic switch 'locks in' the transcriptional signature of regulatory T cells. Nat Immunol. 2012; 13:972–980. This landmark study demonstrates that FOXP3 functions together with other cofactors to activate the expression of most of the T_{reg} cell signature. [PubMed: 22961053]
28. Bettini ML, et al. Loss of epigenetic modification driven by the Foxp3 transcription factor leads to regulatory T cell insufficiency. Immunity. 2012; 36:717–730. [PubMed: 22579476]
29. Xiao Y, et al. Histone acetyltransferase mediated regulation of FOXP3 acetylation and T_{reg} function. Curr Opin Immunol. 2010; 22:583–591. [PubMed: 20869864]
30. Liu Y, et al. Inhibition of p300 impairs $Foxp3^+$ T regulatory cell function and promotes antitumor immunity. Nat Med. 2013; 19:1173–1177. [PubMed: 23955711]
31. Arvey A, et al. Inflammation-induced repression of chromatin bound by the transcription factor Foxp3 in regulatory T cells. Nat Immunol. 2014; 15:580–587. [PubMed: 24728351]
32. DuPage M, et al. The chromatin-modifying enzyme Ezh2 is critical for the maintenance of regulatory T cell identity after activation. Immunity. 2015; 42:227–238. [PubMed: 25680271]
33. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol. 2012; 30:531–564. [PubMed: 22224781]
34. Ouyang W, et al. Foxo proteins cooperatively control the differentiation of $Foxp3^+$ regulatory T cells. Nat Immunol. 2010; 11:618–627. [PubMed: 20467422]
35. Kim HP, Leonard WJ. CREB/ATF-dependent T cell receptor-induced *Foxp3* gene expression: a role for DNA methylation. J Exp Med. 2007; 204:1543–1551. [PubMed: 17591856]
36. Zheng Y, et al. Role of conserved non-coding DNA elements in the *Foxp3* gene in regulatory T-cell fate. Nature. 2010; 463:808–812. This study demonstrates that the composition, size and maintenance of the T_{reg} cell population are controlled by *Foxp3* CNS elements that are engaged in response to distinct cell-extrinsic or cell-intrinsic cues. [PubMed: 20072126]
37. Ruan Q, et al. Development of $Foxp3^+$ regulatory T cells is driven by the c-Rel enhanceosome. Immunity. 2009; 31:932–940. [PubMed: 20064450]
38. Feng Y, et al. A mechanism for expansion of regulatory T-cell repertoire and its role in self-tolerance. Nature. 2015; 528:132–136. [PubMed: 26605529]
39. Toker A, et al. Active demethylation of the *Foxp3* locus leads to the generation of stable regulatory T cells within the thymus. J Immunol. 2013; 190:3180–3188. [PubMed: 23420886]
40. Ohkura N, et al. T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for T_{reg} cell development. Immunity. 2012; 37:785–799. [PubMed: 23123060]
41. Polansky JK, et al. DNA methylation controls *Foxp3* gene expression. Eur J Immunol. 2008; 38:1654–1663. [PubMed: 18493985]
42. Wang L, et al. Mbd2 promotes *Foxp3* demethylation and T-regulatory-cell function. Mol Cell Biol. 2013; 33:4106–4115. [PubMed: 23979593]
43. Yue X, et al. Control of Foxp3 stability through modulation of TET activity. J Exp Med. 2016; 213:377–397. [PubMed: 26903244]
44. Nair VS, Song MH, Ko M, Oh KI. DNA demethylation of the *Foxp3* enhancer is maintained through modulation of ten-eleven-translocation and DNA methyltransferases. Mol Cells. 2016; 39:888–897. References 43 and 44 provide insights into the mechanisms that are involved in regulating the methylation state of the CNS2 region of the *Foxp3* gene. [PubMed: 27989104]
45. Lu L, et al. All-*trans* retinoic acid promotes TGF-β-induced T_{reg}s via histone modification but not DNA demethylation on *Foxp3* gene locus. PLoS ONE. 2011; 6:e24590. [PubMed: 21931768]
46. Lal G, et al. Epigenetic regulation of *Foxp3* expression in regulatory T cells by DNA methylation. J Immunol. 2009; 182:259–273. [PubMed: 19109157]
47. Li C, Ebert PJ, Li QJ. T cell receptor (TCR) and transforming growth factor β (TGF-β) signaling converge on DNA (cytosine-5)-methyltransferase to control forkhead box protein 3 (*Foxp3*) locus

methylation and inducible regulatory T cell differentiation. J Biol Chem. 2013; 288:19127–19139. [PubMed: 23687305]

48. Schlenner SM, Weigmann B, Ruan Q, Chen Y, von Boehmer H. Smad3 binding to the *Foxp3* enhancer is dispensable for the development of regulatory T cells with the exception of the gut. J Exp Med. 2012; 209:1529–1535. [PubMed: 22908322]
49. Josefowicz SZ, et al. Extrathymically generated regulatory T cells control mucosal T_H2 inflammation. Nature. 2012; 482:395–399. This study demonstrates the importance of CNS1 for pT_{reg} cell generation and the maintenance of immune homeostasis at barrier sites. [PubMed: 22318520]
50. Samstein RM, Josefowicz SZ, Arvey A, Treuting PM, Rudensky AY. Extrathymic generation of regulatory T cells in placental mammals mitigates maternal–fetal conflict. Cell. 2012; 150:29–38. [PubMed: 22770213]
51. Benoist C, Mathis D. T_{reg} cells, life history, and diversity. Cold Spring Harb Perspect Biol. 2012; 4:a007021. [PubMed: 22952391]
52. Tai X, Cowan M, Feigenbaum L, Singer A. CD28 costimulation of developing thymocytes induces *Foxp3* expression and regulatory T cell differentiation independently of interleukin 2. Nat Immunol. 2005; 6:152–162. [PubMed: 15640801]
53. Salomon B, et al. B7/CD28 costimulation is essential for the homeostasis of the $CD4^+CD25^+$ immunoregulatory T cells that control autoimmune diabetes. Immunity. 2000; 12:431–440. [PubMed: 10795741]
54. Willoughby JE, et al. Raf signaling but not the ERK effector SAP-1 is required for regulatory T cell development. J Immunol. 2007; 179:6836–6844. [PubMed: 17982074]
55. Huehn J, Polansky JK, Hamann A. Epigenetic control of *FOXP3* expression: the key to a stable regulatory T-cell lineage? Nat Rev Immunol. 2009; 9:83–89. [PubMed: 19114986]
56. Thornton AM, Donovan EE, Piccirillo CA, Shevach EM. Cutting edge: IL-2 is critically required for the *in vitro* activation of $CD4^+CD25^+$ T cell suppressor function. J Immunol. 2004; 172:6519–6523. [PubMed: 15153463]
57. Chen Q, Kim YC, Laurence A, Punkosdy GA, Shevach EM. IL-2 controls the stability of Foxp3 expression in TGF-β-induced $Foxp3^+$ T cells *in vivo*. J Immunol. 2011; 186:6329–6337. [PubMed: 21525380]
58. Miyara M, et al. Functional delineation and differentiation dynamics of human $CD4^+$ T cells expressing the FoxP3 transcription factor. Immunity. 2009; 30:899–911. This paper describes the functional heterogeneity that is present within the human $FOXP3^+$ T_{reg} cell population, and the relationship between CD25 levels and suppressive potency. [PubMed: 19464196]
59. Laurence A, et al. STAT3 transcription factor promotes instability of nT_{reg} cells and limits generation of iT_{reg} cells during acute murine graft-versus-host disease. Immunity. 2012; 37:209–222. [PubMed: 22921119]
60. Nguyen DX, Ehrenstein MR. Anti-TNF drives regulatory T cell expansion by paradoxically promoting membrane TNF–TNF-RII binding in rheumatoid arthritis. J Exp Med. 2016; 213:1241–1253. [PubMed: 27270893]
61. Liu Y, et al. A critical function for TGF-β signaling in the development of natural $CD4^+CD25^+Foxp3^+$ regulatory T cells. Nat Immunol. 2008; 9:632–640. [PubMed: 18438410]
62. Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive $Foxp3^+$ regulatory T cells: more of the same or a division of labor? Immunity. 2009; 30:626–635. [PubMed: 19464985]
63. Ouyang W, Beckett O, Ma Q, Li MO. Transforming growth factor-β signaling curbs thymic negative selection promoting regulatory T cell development. Immunity. 2010; 32:642–653. [PubMed: 20471291]
64. Floess S, et al. Epigenetic control of the *Foxp3* locus in regulatory T cells. PLoS Biol. 2007; 5:e38. [PubMed: 17298177]
65. Hill JA, et al. Retinoic acid enhances Foxp3 induction indirectly by relieving inhibition from $CD4^+CD44^{hi}$ cells. Immunity. 2008; 29:758–770. [PubMed: 19006694]
66. Xiao S, et al. Retinoic acid increases $Foxp3^+$ regulatory T cells and inhibits development of Th17 cells by enhancing TGF-β-driven Smad3 signaling and inhibiting IL-6 and IL-23 receptor expression. J Immunol. 2008; 181:2277–2284. [PubMed: 18684916]

67. Kang SG, Lim HW, Andrisani OM, Broxmeyer HE, Kim CH. Vitamin A metabolites induce gut-homing FoxP3$^+$ regulatory T cells. J Immunol. 2007; 179:3724–3733. [PubMed: 17785809]
68. Zhou X, et al. Cutting edge: all-*trans* retinoic acid sustains the stability and function of natural regulatory T cells in an inflammatory milieu. J Immunol. 2010; 185:2675–2679. [PubMed: 20679534]
69. Kaur G, Goodall JC, Jarvis LB, Hill Gaston JS. Characterisation of *Foxp3* splice variants in human CD4$^+$ and CD8$^+$ T cells — identification of *Foxp3*Δ*7* in human regulatory T cells. Mol Immunol. 2010; 48:321–332. [PubMed: 20688398]
70. Ryder LR, et al. FoxP3 mRNA splice forms in synovial CD4$^+$ T cells in rheumatoid arthritis and psoriatic arthritis. APMIS. 2012; 120:387–396. [PubMed: 22515293]
71. Smith EL, Finney HM, Nesbitt AM, Ramsdell F, Robinson MK. Splice variants of human FOXP3 are functional inhibitors of human CD4$^+$ T-cell activation. Immunology. 2006; 119:203–211. [PubMed: 17005002]
72. Aarts-Riemens T, Emmelot ME, Verdonck LF, Mutis T. Forced overexpression of either of the two common human Foxp3 isoforms can induce regulatory T cells from CD4$^+$CD25$^-$ cells. Eur J Immunol. 2008; 38:1381–1390. [PubMed: 18412171]
73. Mailer RK, et al. IL-1β promotes Th17 differentiation by inducing alternative splicing of *FOXP3*. Sci Rep. 2015; 5:14674. [PubMed: 26441347]
74. Xie X, et al. The regulatory T cell lineage factor Foxp3 regulates gene expression through several distinct mechanisms mostly independent of direct DNA binding. PLoS Genet. 2015; 11:e1005251. [PubMed: 26107960]
75. Lopes JE, et al. Analysis of FOXP3 reveals multiple domains required for its function as a transcriptional repressor. J Immunol. 2006; 177:3133–3142. [PubMed: 16920951]
76. Li B, et al. FOXP3 is a homo-oligomer and a component of a supramolecular regulatory complex disabled in the human XLAAD/IPEX autoimmune disease. Int Immunol. 2007; 19:825–835. This study is noteworthy as it reveals that human FOXP3 forms a large molecular complex that is dysfunctional in T cells from patients with IPEX syndrome. [PubMed: 17586580]
77. Deng G, et al. Molecular and biological role of the FOXP3 N-terminal domain in immune regulation by T regulatory/suppressor cells. Exp Mol Pathol. 2012; 93:334–338. [PubMed: 23041265]
78. Hancock WW, Ozkaynak E. Three distinct domains contribute to nuclear transport of murine Foxp3. PLoS ONE. 2009; 4:e7890. [PubMed: 19924293]
79. van Loosdregt J, et al. Regulation of T_{reg} functionality by acetylation-mediated Foxp3 protein stabilization. Blood. 2010; 115:965–974. [PubMed: 19996091]
80. Samanta A, et al. TGF-β and IL-6 signals modulate chromatin binding and promoter occupancy by acetylated FOXP3. Proc Natl Acad Sci USA. 2008; 105:14023–14027. References 79 and 80 show that acetylation promotes the stability and DNA-binding ability of FOXP3. [PubMed: 18779564]
81. Xiao Y, et al. Dynamic interactions between TIP60 and p300 regulate FOXP3 function through a structural switch defined by a single lysine on TIP60. Cell Rep. 2014; 7:1471–1480. [PubMed: 24835996]
82. Wang L, et al. Ubiquitin-specific protease-7 inhibition impairs Tip60-dependent Foxp3$^+$ T-regulatory cell function and promotes antitumor immunity. EbioMedicine. 2016; 13:99–112. [PubMed: 27769803]
83. Gao Z, et al. Synergy between IL-6 and TGF-β signaling promotes FOXP3 degradation. Int J Clin Exp Pathol. 2012; 5:626–633. [PubMed: 22977658]
84. Wang L, de Zoeten EF, Greene MI, Hancock WW. Immunomodulatory effects of deacetylase inhibitors: therapeutic targeting of FOXP3$^+$ regulatory T cells. Nat Rev Drug Discov. 2009; 8:969–981. [PubMed: 19855427]
85. van Loosdregt J, et al. Rapid temporal control of Foxp3 protein degradation by sirtuin-1. PLoS ONE. 2011; 6:e19047. [PubMed: 21533107]
86. Beier UH, et al. Sirtuin-1 targeting promotes Foxp3$^+$ T-regulatory cell function and prolongs allograft survival. Mol Cell Biol. 2011; 31:1022–1029. [PubMed: 21199917]
87. Kwon HS, et al. Three novel acetylation sites in the Foxp3 transcription factor regulate the suppressive activity of regulatory T cells. J Immunol. 2012; 188:2712–2721. [PubMed: 22312127]

88. Li J, et al. Mammalian sterile 20-like kinase 1 (Mst1) enhances the stability of forkhead box P3 (Foxp3) and the function of regulatory T cells by modulating Foxp3 acetylation. J Biol Chem. 2015; 290:30762–30770. [PubMed: 26538561]

89. Du X, et al. Mst1/Mst2 regulate development and function of regulatory T cells through modulation of Foxo1/Foxo3 stability in autoimmune disease. J Immunol. 2014; 192:1525–1535. [PubMed: 24453252]

90. Sivan A, et al. Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science. 2015; 350:1084–1089. [PubMed: 26541606]

91. Furusawa Y, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013; 504:446–450. [PubMed: 24226770]

92. Arpaia N, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature. 2013; 504:451–455. References 91 and 92 demonstrate that the microbial production of SCFAs can positively influence FOXP3 upregulation and the generation of T_{reg} cells. [PubMed: 24226773]

93. Nie H, et al. Phosphorylation of FOXP3 controls regulatory T cell function and is inhibited by TNF-α in rheumatoid arthritis. Nat Med. 2013; 19:322–328. This study reveals a function-augmenting phosphorylation modification of FOXP3 that is adversely affected by TNF. [PubMed: 23396208]

94. Morawski PA, Mehra P, Chen C, Bhatti T, Wells AD. Foxp3 protein stability is regulated by cyclin-dependent kinase 2. J Biol Chem. 2013; 288:24494–24502. [PubMed: 23853094]

95. Chunder N, Wang L, Chen C, Hancock WW, Wells AD. Cyclin-dependent kinase 2 controls peripheral immune tolerance. J Immunol. 2012; 189:5659–5666. [PubMed: 23136201]

96. Li Z, et al. PIM1 kinase phosphorylates the human transcription factor FOXP3 at serine 422 to negatively regulate its activity under inflammation. J Biol Chem. 2014; 289:26872–26881. [PubMed: 25096571]

97. Deng G, et al. Pim-2 kinase influences regulatory T cell function and stability by mediating Foxp3 protein N-terminal phosphorylation. J Biol Chem. 2015; 290:20211–20220. References 94, 96 and 97 demonstrate how the phosphorylation of FOXP3 can limit its regulatory activity and T_{reg} cell function. [PubMed: 25987564]

98. Basu S, Golovina T, Mikheeva T, June CH, Riley JL. Cutting edge: Foxp3-mediated induction of Pim 2 allows human T regulatory cells to preferentially expand in rapamycin. J Immunol. 2008; 180:5794–5798. [PubMed: 18424697]

99. Nakahira K, Morita A, Kim NS, Yanagihara I. Phosphorylation of FOXP3 by LCK downregulates MMP9 expression and represses cell invasion. PLoS ONE. 2013; 8:e77099. [PubMed: 24155921]

100. Metzger MB, Hristova VA, Weissman AM. HECT and RING finger families of E3 ubiquitin ligases at a glance. J Cell Sci. 2012; 125:531–537. [PubMed: 22389392]

101. Ben-Neriah Y. Regulatory functions of ubiquitination in the immune system. Nat Immunol. 2002; 3:20–26. [PubMed: 11753406]

102. Dang EV, et al. Control of T_H17/T_{reg} balance by hypoxia-inducible factor 1. Cell. 2011; 146:772–784. This study highlights the importance of metabolic cues in T cell fate determination and shows that FOXP3 levels in developing T_{reg} cells are susceptible to ubiquitin-mediated degradation. [PubMed: 21871655]

103. Chen Z, et al. The ubiquitin ligase Stub1 negatively modulates regulatory T cell suppressive activity by promoting degradation of the transcription factor Foxp3. Immunity. 2013; 39:272–285. This paper demonstrates the crucial role of the stress-activated STUB1–HSP70 complex in promoting FOXP3 downregulation and T_{reg} cell inactivation. [PubMed: 23973223]

104. van Loosdregt J, et al. Stabilization of the transcription factor Foxp3 by the deubiquitinase USP7 increases T_{reg}-cell-suppressive capacity. Immunity. 2013; 39:259–271. This study reveals a molecular mechanism by which the rapid temporal control of FOXP3 expression in T_{reg} cells can be regulated by USP7, thereby modulating T_{reg} cell numbers and function. [PubMed: 23973222]

105. Chen L, Wu J, Pier E, Zhao Y, Shen Z. mTORC2–PKBα/Akt1 serine 473 phosphorylation axis is essential for regulation of FOXP3 stability by chemokine CCL3 in psoriasis. J Invest Dermatol. 2013; 133:418–428. [PubMed: 23223135]

106. Li X, et al. CHIP promotes Runx2 degradation and negatively regulates osteoblast differentiation. J Cell Biol. 2008; 181:959–972. [PubMed: 18541707]
107. Luo W, et al. Hsp70 and CHIP selectively mediate ubiquitination and degradation of hypoxia-inducible factor (HIF)-1α but not HIF-2α. J Biol Chem. 2010; 285:3651–3663. [PubMed: 19940151]
108. Zhang J, et al. Identification of the E3 deubiquitinase ubiquitin-specific peptidase 21 (USP21) as a positive regulator of the transcription factor GATA3. J Biol Chem. 2013; 288:9373–9382. [PubMed: 23395819]
109. Li Y, et al. USP21 prevents the generation of T-helper-1-like T_{reg} cells. Nat Commun. 2016; 7:13559. [PubMed: 27857073]
110. Kirisako T, et al. A ubiquitin ligase complex assembles linear polyubiquitin chains. EMBO J. 2006; 25:4877–4887. [PubMed: 17006537]
111. Feuerer M, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med. 2009; 15:930–939. [PubMed: 19633656]
112. Burzyn D, et al. A special population of regulatory T cells potentiates muscle repair. Cell. 2013; 155:1282–1295. [PubMed: 24315098]
113. Wang R, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. Immunity. 2011; 35:871–882. [PubMed: 22195744]
114. Michalek RD, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory $CD4^+$ T cell subsets. J Immunol. 2011; 186:3299–3303. This study shows that distinct metabolic pathways are preferentially utilized by effector T cells and T_{reg} cells, and that manipulating these pathways can alter T cell subset skewing and function. [PubMed: 21317389]
115. Shi LZ, et al. HIF1α-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of T_H17 and T_{reg} cells. J Exp Med. 2011; 208:1367–1376. [PubMed: 21708926]
116. Delgoffe GM, et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. Immunity. 2009; 30:832–844. [PubMed: 19538929]
117. Beier UH, et al. Essential role of mitochondrial energy metabolism in $Foxp3^+$ T-regulatory cell function and allograft survival. FASEB J. 2015; 29:2315–2326. [PubMed: 25681462]
118. Lee JH, Elly C, Park Y, Liu YC. E3 ubiquitin ligase VHL regulates hypoxia-inducible factor-1α to maintain regulatory T cell stability and suppressive capacity. Immunity. 2015; 42:1062–1074. This study shows that metabolic reprogramming in T_{reg} cells can adversely affect their expression of FOXP3 and their suppressive function in an IFNγ-dependent manner. [PubMed: 26084024]
119. Zeng H, et al. mTORC1 couples immune signals and metabolic programming to establish T_{reg}-cell function. Nature. 2013; 499:485–490. [PubMed: 23812589]
120. De Rosa V, et al. Glycolysis controls the induction of human regulatory T cells by modulating the expression of *FOXP3* exon 2 splicing variants. Nat Immunol. 2015; 16:1174–1184. This paper introduces a possible connection between the metabolism of differentiating iT_{reg} cells and the processes that are involved in the alternative splicing of the *FOXP3* transcript. [PubMed: 26414764]
121. Gerriets VA, et al. Foxp3 and Toll-like receptor signaling balance T_{reg} cell anabolic metabolism for suppression. Nat Immunol. 2016; 17:1459–1466. This study demonstrates that the activation of T_{reg} cells by TLR ligands can induce metabolic changes that disrupt their suppressive functions while promoting T_{reg} cell population expansion; by contrast, FOXP3 directly regulates metabolic activity in T_{reg} cells to restrict this TLR-dependent process. [PubMed: 27695003]
122. Shrestha S, et al. T_{reg} cells require the phosphatase PTEN to restrain T_H1 and T_{FH} cell responses. Nat Immunol. 2015; 16:178–187. [PubMed: 25559258]
123. Park Y, et al. TSC1 regulates the balance between effector and regulatory T cells. J Clin Invest. 2013; 123:5165–5178. [PubMed: 24270422]
124. Ware R, et al. Human $CD8^+$ T lymphocyte clones specific for T cell receptor Vβ families expressed on autologous $CD4^+$ T cells. Immunity. 1995; 2:177–184. [PubMed: 7895174]

125. Kim HJ, et al. CD8$^+$ T regulatory cells express the Ly49 Class I MHC receptor and are defective in autoimmune prone B6-Yaa mice. Proc Natl Acad Sci USA. 2011; 108:2010–2015. [PubMed: 21233417]

126. Kiniwa Y, et al. CD8$^+$ Foxp3$^+$ regulatory T cells mediate immunosuppression in prostate cancer. Clin Cancer Res. 2007; 13:6947–6958. [PubMed: 18056169]

127. Mayer CT, et al. CD8$^+$ Foxp3$^+$ T cells share developmental and phenotypic features with classical CD4$^+$ Foxp3$^+$ regulatory T cells but lack potent suppressive activity. Eur J Immunol. 2011; 41:716–725. [PubMed: 21312192]

128. Le DT, et al. CD8$^+$ Foxp3$^+$ tumor infiltrating lymphocytes accumulate in the context of an effective anti-tumor response. Int J Cancer. 2011; 129:636–647. References 127 and 128 suggest that FOXP3 expression is seen in some CD8$^+$ T cells that have a suppressive function. [PubMed: 20857491]

129. Bendelac A, Savage PB, Teyton L. The biology of NKT cells. Annu Rev Immunol. 2007; 25:297–336. [PubMed: 17150027]

130. Monteiro M, et al. Identification of regulatory Foxp3$^+$ invariant NKT cells induced by TGF-β. J Immunol. 2010; 185:2157–2163. [PubMed: 20639482]

131. Manrique SZ, et al. Foxp3-positive macrophages display immunosuppressive properties and promote tumor growth. J Exp Med. 2011; 208:1485–1499. [PubMed: 21670203]

132. Mayer CT, Kuhl AA, Loddenkemper C, Sparwasser T. Lack of Foxp3$^+$ macrophages in both untreated and B16 melanoma-bearing mice. Blood. 2012; 119:1314–1315. [PubMed: 22308282]

133. Put S, et al. Macrophages have no lineage history of Foxp3 expression. Blood. 2012; 119:1316–1318. References 132 and 133 show that, contrary to an initial study, FOXP3 is not expressed by several macrophage types. [PubMed: 22308283]

134. Devaud C, et al. Foxp3 expression in macrophages associated with RENCA tumors in mice. PLoS ONE. 2014; 9:e108670. [PubMed: 25264896]

135. Rosser EC, Mauri C. Regulatory B cells: origin, phenotype, and function. Immunity. 2015; 42:607–612. [PubMed: 25902480]

136. Yang M, Rui K, Wang S, Lu L. Regulatory B cells in autoimmune diseases. Cell Mol Immunol. 2013; 10:122–132. [PubMed: 23292280]

137. Olkhanud PB, et al. Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting CD4$^+$ T cells to T-regulatory cells. Cancer Res. 2011; 71:3505–3515. [PubMed: 21444674]

138. Noh J, Noh G, Kim HS, Kim AR, Choi WS. Allergen-specific responses of CD19$^+$CD5$^+$Foxp3$^+$ regulatory B cells (B_{reg}s) and CD4$^+$Foxp3$^+$ regulatory T cell (T_{reg}s) in immune tolerance of cow milk allergy of late eczematous reactions. Cell Immunol. 2012; 274:109–114. [PubMed: 22398308]

139. Vadasz Z, et al. The expansion of CD25^{high}IL-10^{high}FoxP3^{high} B regulatory cells is in association with SLE disease activity. J Immunol Res. 2015; 2015:254245. [PubMed: 26504851]

140. de Andres C, et al. New regulatory CD19$^+$CD25$^+$ B-cell subset in clinically isolated syndrome and multiple sclerosis relapse. Changes after glucocorticoids. J Neuroimmunol. 2014; 270:37–44. [PubMed: 24662004]

141. Zuo T, et al. FOXP3 is an X-linked breast cancer suppressor gene and an important repressor of the HER-2/ErbB2 oncogene. Cell. 2007; 129:1275–1286. [PubMed: 17570480]

142. Wang L, et al. Somatic single hits inactivate the X-linked tumor suppressor *FOXP3* in the prostate. Cancer Cell. 2009; 16:336–346. [PubMed: 19800578]

143. Buckner JH. Mechanisms of impaired regulation by CD4$^+$CD25$^+$FOXP3$^+$ regulatory T cells in human autoimmune diseases. Nat Rev Immunol. 2010; 10:849–859. [PubMed: 21107346]

144. Sarkar S, Fox DA. Regulatory T cell defects in rheumatoid arthritis. Arthritis Rheum. 2007; 56:710–713. [PubMed: 17328040]

145. Talaat RM, Mohamed SF, Bassyouni IH, Raouf AA. Th1/Th2/Th17/T_{reg} cytokine imbalance in systemic lupus erythematosus (SLE) patients: correlation with disease activity. Cytokine. 2015; 72:146–153. [PubMed: 25647269]

146. Long SA, et al. Defects in IL-2R signaling contribute to diminished maintenance of FOXP3 expression in $CD4^+CD25^+$ regulatory T-cells of type 1 diabetic subjects. Diabetes. 2010; 59:407–415. [PubMed: 19875613]

147. Di Ianni M, et al. T_{reg}s prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. Blood. 2011; 117:3921–3928. [PubMed: 21292771]

148. Martelli MF, et al. HLA-haploidentical transplantation with regulatory and conventional T-cell adoptive immunotherapy prevents acute leukemia relapse. Blood. 2014; 124:638–644. [PubMed: 24923299]

149. Masteller EL, et al. Expansion of functional endogenous antigen-specific $CD4^+CD25^+$ regulatory T cells from nonobese diabetic mice. J Immunol. 2005; 175:3053–3059. [PubMed: 16116193]

150. Roncarolo MG, Battaglia M. Regulatory T-cell immunotherapy for tolerance to self antigens and alloantigens in humans. Nat Rev Immunol. 2007; 7:585–598. [PubMed: 17653126]

151. Bluestone JA, et al. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. Sci Transl Med. 2015; 7:315ra189.

152. Marek-Trzonkowska N, et al. Therapy of type 1 diabetes with $CD4^+CD25^{high}CD127$-regulatory T cells prolongs survival of pancreatic islets — results of one year follow-up. Clin Immunol. 2014; 153:23–30. References 151 and 152 report encouraging results from trials using the adoptive transfer of T_{reg} cells as a therapy in patients with diabetes; the data suggest that *ex vivo*-expanded T_{reg} cells can be used to treat autoimmune diseases. [PubMed: 24704576]

153. Desreumaux P, et al. Safety and efficacy of antigen-specific regulatory T-cell therapy for patients with refractory Crohn's disease. Gastroenterology. 2012; 143:1207–1217. [PubMed: 22885333]

154. Nishikawa H, Sakaguchi S. Regulatory T cells in cancer immunotherapy. Curr Opin Immunol. 2014; 27:1–7. [PubMed: 24413387]

155. Liu C, Workman CJ, Vignali DA. Targeting regulatory T cells in tumors. FEBS J. 2016; 283:2731–2748. [PubMed: 26787424]

156. Rech AJ, et al. CD25 blockade depletes and selectively reprograms regulatory T cells in concert with immunotherapy in cancer patients. Sci Transl Med. 2012; 4:134ra162. This study shows the potential benefits of targeting T_{reg} cells in patients with cancer as a means to augment antitumour immunity.

157. Sugiyama D, et al. Anti-CCR4 mAb selectively depletes effector-type $FoxP3^+CD4^+$ regulatory T cells, evoking antitumor immune responses in humans. Proc Natl Acad Sci USA. 2013; 110:17945–17950. [PubMed: 24127572]

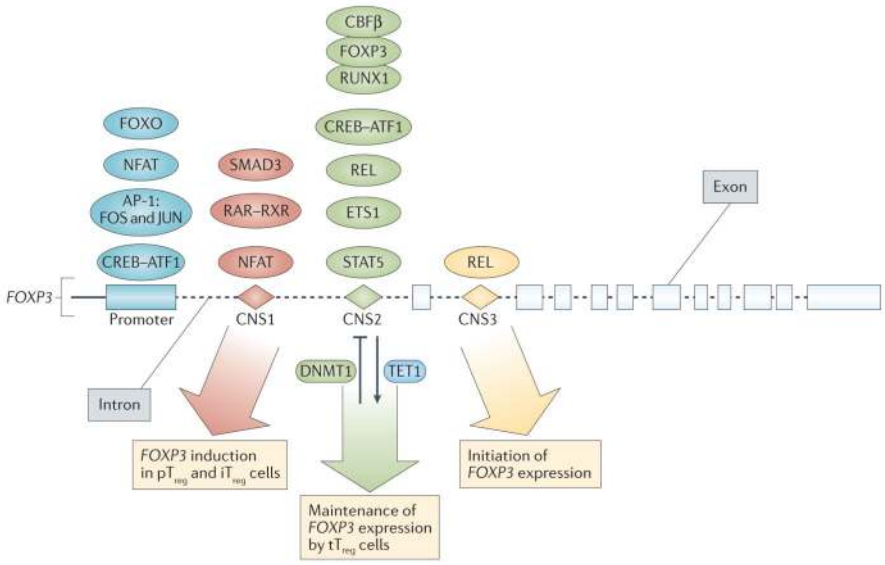


Figure 1. The control of forkhead box protein P3 expression by transcription factors and regulatory elements within the *FOXP3* gene locus

The figure depicts the characterized coding and non-coding elements of the gene that encodes forkhead box protein P3 (*FOXP3*) along with the transcription factors that are reported to activate the transcription of the gene and their sites of interaction. Transcription factors that bind to the promoter, conserved non-coding sequence 1 (CNS1), CNS2 and CNS3 regions of *FOXP3* are shown in blue, red, green and yellow, respectively. Also depicted are the CNS2-targeting methylating enzyme DNA methyltransferase 1 (DNMT1) and the demethylating enzyme ten-eleven translocation 1 (TET1), which influence the inactive and active transcriptional status of that region, respectively. ATF1, activating transcription factor 1; CBFβ, core-binding factor subunit-β; CREB, cAMP-responsive element-binding protein; FOXO, forkhead box protein O; iT_{reg}, *in vitro*-induced regulatory T; NFAT, nuclear factor of activated T cells; pT_{reg}, peripherally derived regulatory T; RAR, retinoic acid receptor; RUNX1, Runt-related transcription factor 1; RXR, retinoid X receptor; STAT5, signal transducer and activator of transcription 5; tT_{reg}, thymus-derived regulatory T.

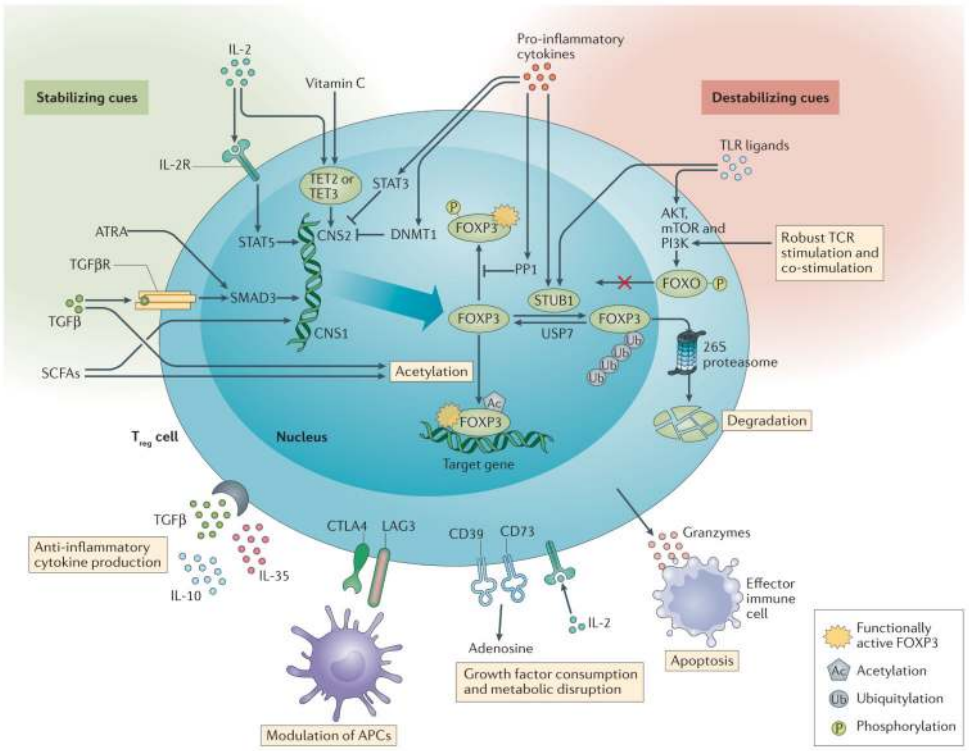
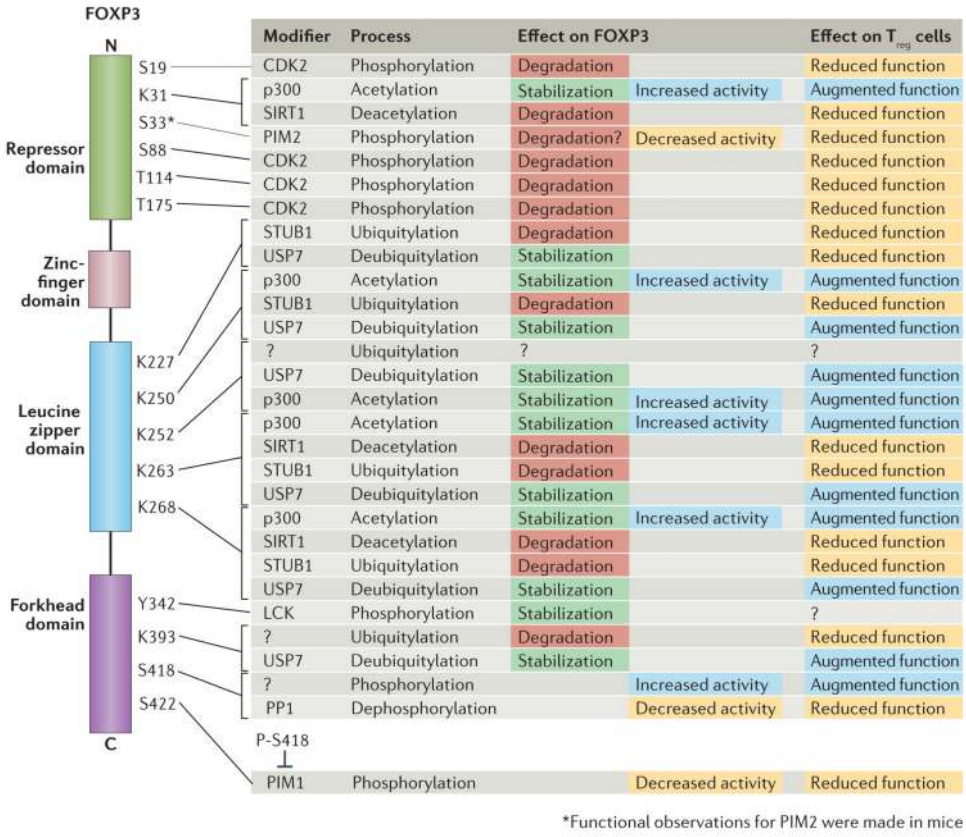


Figure 2. Environmental cues modulate the transcription, stability and function of forkhead box protein P3

The induction and maintenance of forkhead box protein P3 (*FOXP3*) transcription can be positively influenced by cytokines such as transforming growth factor-β (TGFβ) and interleukin-2 (IL-2), and by other factors in the tissue microenvironment, such as retinoic acid, vitamin C and short-chain fatty acids (SCFAs). By contrast, FOXP3 is negatively regulated by pro-inflammatory cytokines, such as tumour necrosis factor (TNF) and IL-6, and by other factors, such as Toll-like receptor (TLR) activation or robust T cell receptor (TCR) and co-stimulatory molecule signalling. These factors can alter the post-translational modifications that are made to the mature FOXP3 protein to either stabilize or deplete the cellular pools of FOXP3 and modulate its functional capacity. By affecting FOXP3 expression and function, these factors influence the many functions of regulatory T (T_{reg}) cells, such as their production of anti-inflammatory cytokines, their modulation of antigen-presenting cell (APC) function, their consumption of growth factors and their ability to induce apoptosis in effector immune cells. ATRA, all-*trans*-retinoic acid; CNS, conserved non-coding sequence; CTLA4, cytotoxic T lymphocyte antigen 4; DNMT1, DNA methyltransferase 1; FOXO, forkhead box protein O; IL-2R, IL-2 receptor; LAG3, lymphocyte activation gene 3 protein; mTOR, mechanistic target of rapamycin; PI3K, phosphoinositide 3-kinase; PP1, protein phosphatase 1; STAT, signal transducer and activator of transcription; TET, ten-eleven translocation; TGFβR, TGFβ receptor; USP7, ubiquitin-specific peptidase 7.



Modifier	Process	Effect on FOXP3		Effect on T_{reg} cells
CDK2	Phosphorylation	Degradation		Reduced function
p300	Acetylation	Stabilization	Increased activity	Augmented function
SIRT1	Deacetylation	Degradation		Reduced function
PIM2	Phosphorylation	Degradation?	Decreased activity	Reduced function
CDK2	Phosphorylation	Degradation		Reduced function
CDK2	Phosphorylation	Degradation		Reduced function
CDK2	Phosphorylation	Degradation		Reduced function
STUB1	Ubiquitylation	Degradation		Reduced function
USP7	Deubiquitylation	Stabilization		Reduced function
p300	Acetylation	Stabilization	Increased activity	Augmented function
STUB1	Ubiquitylation	Degradation		Reduced function
USP7	Deubiquitylation	Stabilization		Augmented function
?	Ubiquitylation	?		?
USP7	Deubiquitylation	Stabilization		Augmented function
p300	Acetylation	Stabilization	Increased activity	Augmented function
p300	Acetylation	Stabilization	Increased activity	Augmented function
SIRT1	Deacetylation	Degradation		Reduced function
STUB1	Ubiquitylation	Degradation		Reduced function
USP7	Deubiquitylation	Stabilization		Augmented function
p300	Acetylation	Stabilization	Increased activity	Augmented function
SIRT1	Deacetylation	Degradation		Reduced function
STUB1	Ubiquitylation	Degradation		Reduced function
USP7	Deubiquitylation	Stabilization		Augmented function
LCK	Phosphorylation	Stabilization		?
?	Ubiquitylation	Degradation		Reduced function
USP7	Deubiquitylation	Stabilization		Augmented function
?	Phosphorylation		Increased activity	Augmented function
PP1	Dephosphorylation		Decreased activity	Reduced function
PIM1	Phosphorylation		Decreased activity	Reduced function

*Functional observations for PIM2 were made in mice

Figure 3. Post-translational modifications of forkhead box protein P3 and their impact on regulatory T cell function

Depicted on the left is a schematic representation of the mature forkhead box protein P3 (FOXP3) molecule showing its functional domains and reported post-translational modification sites. The table on the right summarizes the types of modification that can occur in each region of FOXP3, the effects of these modifications on FOXP3 protein stability and function, and the subsequent impact on the suppressive function of regulatory T (T_{reg}) cells. CDK2, cyclin-dependent kinase 2; PP1, protein phosphatase 1; SIRT1, sirtuin 1; USP7, ubiquitin-specific peptidase 7.