

INSIGHTS

FOXP3 snatches transcription factors depending on the context

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FOXP3 hijacks DNA-binding proteins to regulate gene expression. In this issue of *JEM*, He et al. (<https://doi.org/10.1084/jem.20232068>) propose a dynamic model in which FOXP3 associates with DNA-binding proteins to regulate Treg cell function in response to environmental cues.

Regulatory T (Treg) cells are a subset of CD4⁺ T cells with immunosuppressive functions critically required for immune homeostasis and self-tolerance (Sakaguchi et al., 2020). Treg cell identity and function depend on the expression of the transcription factor (TF) forkhead box protein P3 (FOXP3) as mutations in the *FOXP3* gene result in a fatal multiorgan autoimmune inflammatory disease, both in human (IPEX syndrome [immune dysregulation, polyendocrinopathy, enteropathy, and X-linked]) and mice (scurfy) (Brunkow et al., 2001; Wildin et al., 2001). Subsequent studies identified the genes that are dependent on FOXP3 (Hill et al., 2007), which include both the induction of Treg-hallmark genes with immunoregulatory function, such as *Tnfrsf9* (4-1BB), as well as the suppression of genes associated with inflammation, such as *Ptpn22* (Marson et al., 2007; Zheng et al., 2007).

Mechanistically, FOXP3 has been shown to form a large protein complex with factors such as NFAT (Wu et al., 2006) or GATA-3, Runx1, and Foxp1, which directly regulate target gene expression in Treg cells (Rudra et al., 2012). Further studies have shown that FOXP3 induces changes in chromatin accessibility also at sites not directly bound by FOXP3, proving that FOXP3 acts not only in cis but also in trans to regulate gene expression (van der Veeke et al., 2020). Nevertheless, even >20 years after the

identification of FOXP3 as the key TF for Treg cells, the exact mode of action of FOXP3 and the associated DNA-binding proteins, specifically under varying immunological conditions, such as inflammation, remains poorly understood.

To analyze the effect these varying conditions have on FOXP3 and its binding partners, He et al. (2024) compared how FOXP3–chromatin binding differed between activated and resting Treg cells. While FOXP3 protein levels remained unchanged after activation, the authors could identify three distinct FOXP3–chromatin binding modes: constant, decreased, and increased in activated Treg cells compared to resting Treg cells isolated from lymphoid organs. As described in previous studies (Zheng et al., 2007), the authors could observe a correlation between the FOXP3 binding mode and gene expression, suggesting that FOXP3 binding at least partially controls gene expression.

To better understand how FOXP3 binding influences gene expression, the authors first analyzed genes which were not influenced by Treg cell activation. An analysis of TF binding motifs showed an increase in ETS family members near regions of constant FOXP3 binding. ETS1, a member of the ETS family, was found to co-precipitate with FOXP3, and a CRISPR-mediated knockdown of ETS1 in Treg cells caused a decrease in FOXP3 binding, thus leading the authors to



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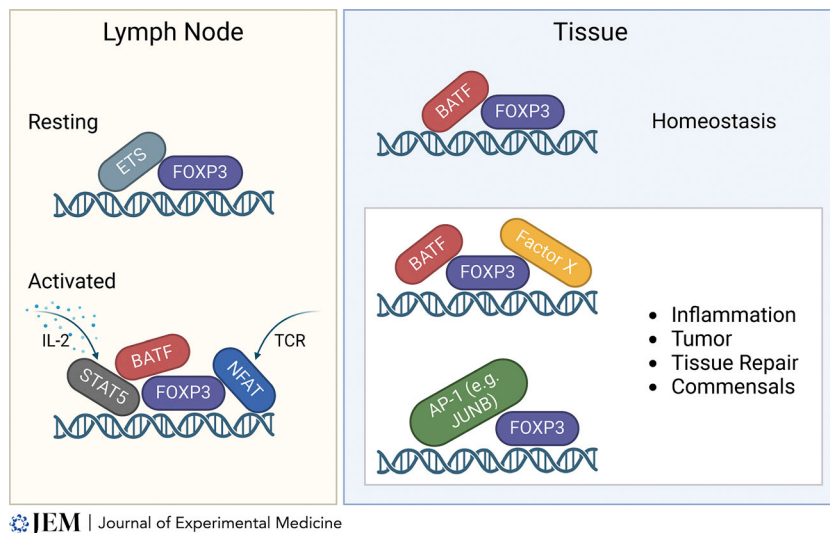
the conclusion that constitutive FOXP3 binding is supported by ETS proteins. Indeed, previous studies support this notion by demonstrating that ETS1 is important for the development of Treg cells by influencing either the stability or expression of FOXP3 (Mouly et al., 2010).

Next, the authors analyzed how dynamic FOXP3 binding influences gene expression and found that regions with increased FOXP3 binding in activated Treg cells were correlated with an enrichment of AP-1 motifs while regions with decreased FOXP3 binding showed an enrichment of ETS motifs in resting Treg cells. These data could be recapitulated in activated Treg cells isolated from the tumor microenvironment (TME). This suggests that FOXP3 binding is controlled by distinct TF families, dependent on activation and environmental cues. They further speculate that during Treg cell activation, chromatin remodeling and FOXP3 binding specificity are controlled by shared regulators, such as AP-1 family members.

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Environmental cues direct FOXP3 binding partners in Treg cells. In resting lymph nodes, FOXP3 forms a complex with ETS family members, while during activation, FOXP3 forms a complex with AP-1 family members such as BATF. IL-2 stimulation induces FOXP3 binding to STAT5 while TCR stimulation induces FOXP3–NFAT binding. We speculate that under homeostatic conditions in the tissue, FOXP3 interacts with BATF to bind to DNA. Under different conditions in the tissue, such as under inflammation or in the tumor environment, external cues may influence the FOXP3 complex to include other DNA-binding proteins such as other AP-1 family members (e.g., JUNB) or yet undefined transcription factors (Factor X).

Using proximity proteomics, the authors identified dynamic changes in proteins adjacent to FOXP3 in response to IL-2 or TCR stimulation, which may play a role in modulating Treg cell function under different conditions. Inhibiting NFAT, a member of the FOXP3 complex (Rudra et al., 2012; Wu et al., 2006), resulted in reduced FOXP3 binding. Another possible candidate is BATF, a member of the AP-1 family that has previously been described to play an important role in Treg cell tissue adaptation (Hayatsu et al., 2017; Delacher et al., 2017, 2020). An analysis of TF binding sites revealed an enrichment of BATF binding site accessibility in activated Treg cells, and immunoprecipitation experiments showed that BATF and FOXP3 co-occupied a large fraction of genes. To further validate the role of BATF, He et al. (2024) used CRISPR to partially delete BATF in Treg cells, causing a decrease in FOXP3 binding as well as a reduction in protein expression of target genes, indicating that BATF supports FOXP3 binding. These data are consistent with previous reports that classify BATF as a “pioneering factor” which aids in the reorganization of chromatin before recruiting other factors such as ETS1 (Pham et al., 2019). Indeed, when He et al. (2024)

overexpressed both BATF and FOXP3 in CD4 T cells, they could observe a slight increase in both FOXP3 binding and the expression of FOXP3-dependent proteins CD25 and CTLA-4 as compared to cells that only overexpressed FOXP3. Moreover, BATF target specificity was independent of FOXP3, again indicating that BATF may act as a pioneering factor. Another example where FOXP3 exploits a pioneering factor has been previously described during Treg cell differentiation in the thymus, where FOXP3 replaces Foxo1 in an opportunistic manner (Samstein et al., 2012).

He et al. (2024) suggest a model of FOXP3–chromatin binding in the presence of another DNA-binding partner that associates with FOXP3, where FOXP3–DNA binding plays a stabilizing role and target specificity is determined by the other partner factor. Although the authors did not address this on a mechanistic level, this dynamic model provides an angle to further investigate FOXP3-dependent gene regulation. Further studies are needed to clarify the precise role of stabilizing versus target specificity promoting TF binding motifs. In addition, primary Treg cells from different tissues and activation states should be analyzed in the future to identify how external

stimuli induce context-specific interactions of FOXP3 with different DNA-binding proteins. The authors mainly used gene expression as their readout; therefore, future studies should include functionally relevant assays to understand the biology of perturbed Treg cells.

In their study, He et al. (2024) focused on the effects of three TFs that play a role in FOXP3 binding (NFAT, ETS1, and BATF). However, their proximity proteomics revealed 1,493 proteins that were highly enriched in proximity to FOXP3. Of these, 157 proteins had previously been described to interact with FOXP3. This leaves a large number of other potentially interacting DNA-binding proteins that could be further analyzed to shed more light on the mode of action of FOXP3. These interactions may also change in a context-dependent manner. While the authors demonstrated that FOXP3 binding partners differed when Treg cells were stimulated with TCR stimulus or IL-2, one could further speculate that other external stimuli such as cytokines or metabolites from inflamed environments, the TME, the process of tissue repair, or commensal bacteria may induce different sets of TFs that interact with FOXP3, consequently changing the gene target spectrum of FOXP3 (see figure). Perhaps these flexibly formed tandem complexes of FOXP3 and inducible TFs represent another way how Treg cells adapt to their local environment. These different complexes may exist simultaneously, which would allow Treg cells to respond to context-specific cues, e.g., support of tissue regeneration in an inflamed environment.

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