nature immunology

REVIEW ARTICLE

https://doi.org/10.1038/s41590-022-01194-2

TCF-1: a maverick in T cell development and function

Fotini Gounari [1,2] ✉ and Khashayarsha Khazaie [2]

The T cell-specific DNA-binding protein TCF-1 is a central regulator of T cell development and function along multiple stages and lineages. Because it interacts with β-catenin, TCF-1 has been classically viewed as a downstream effector of canonical Wnt signaling, although there is strong evidence for β-catenin-independent TCF-1 functions. TCF-1 co-binds accessible regulatory regions containing or lacking its conserved motif and cooperates with other nuclear factors to establish context-dependent epigenetic and transcription programs that are essential for T cell development and for regulating immune responses to infection, autoimmunity and cancer. Although it has mostly been associated with positive regulation of chromatin accessibility and gene expression, TCF-1 has the potential to reduce chromatin accessibility and thereby suppress gene expression. In addition, the binding of TCF-1 bends the DNA and affects the chromatin conformation genome wide. This Review discusses the current understanding of the multiple roles of TCF-1 in T cell development and function and their mechanistic underpinnings.

T lymphocyte development is a highly ordered stepwise process that depends on the cooperative and highly orchestrated action of multiple transcription and epigenetic regulators[1]. These regulators organize cooperating complexes to establish the specific chromatin landscape and transcription profile required in each T cell lineage and developmental stage. The DNA-binding protein TCF-1, encoded by the *Tcf7* gene, has emerged as a central player in these processes[2–4]. A recent review by Xue and colleagues elegantly discusses findings on TCF-1 across the fields of T cell immunity, reflecting on the potential application of this knowledge to therapeutic intervention in viral infections and antitumor immunity[5]. This Review focuses on the current understanding of the molecular mechanisms through which TCF-1 leverages its diverse functions to shape T cell immunity.

TCF-1 is a member of the TCF/LEF family of high-mobility group (HMG) domain-containing proteins that have been conventionally viewed as effectors of the canonical Wnt signaling pathway (for reviews, see refs. [6–8]). The Wnt cascade is activated in response to signals induced by the binding of extracellular Wnt ligands to Frizzled and LRP5/6 receptors on the cell surface. This results in the stabilization of β-catenin, which is then transported to the nucleus, where it binds to TCF/LEF factors and promotes chromatin accessibility and gene expression. In the absence of Wnt signals, DNA-bound TCF/LEF factors interact with repressors of the Grg/TLE family that reduce chromatin accessibility and suppress transcription. This simplified view does not consider that in addition to the full-length TCF-1 protein, which can interact with β-catenin, TCF-1 is also expressed as isoforms that lack the β-catenin-interacting domain[9]. These short isoforms, originally presumed to have dominant-negative regulatory functions[10], are sufficient to support thymocyte maturation[11,12] and the generation of memory CD8$^+$ T cells in response to acute infection[13]. The long isoform, however, supports thymocyte survival and is needed for the optimal maturation of central memory CD8$^+$ T cells[13]. It is currently unclear whether the specific functions of the long isoform involve canonical Wnt signaling, since ablating β-catenin or γ-catenin, or manipulating the expression and function of Wnt pathway components, does not impair normal T cell development and function[14–18]. Together, these findings suggest that the functions of TCF-1 in T cell development are largely independent of classical Wnt signaling.

By contrast, in leukemia, autoimmunity and cancer, uncontrolled pathological activation of Wnt signaling in T cells engages TCF-1 to promote aberrant developmental progression and transformation of thymocytes, as well as immune imbalance[19–25]. Stabilizing mutations in β-catenin and activation of the Wnt signaling pathway have been reported in human T cell malignancies, including precursor, peripheral, cutaneous and adult (ATL) T cell leukemia[26–30]. Similarly, conditional stabilization of β-catenin in mouse CD4$^+$CD8$^+$ double-positive (DP) thymocytes induced leukemias with recurrent chromosomal translocations like the ones seen in human precursor T cell leukemia, providing mechanistic validation of the findings in human leukemias[20,25,31]. Importantly, we found that TCF-1 is responsible for the transformation of DP thymocytes with stabilized β-catenin, because conditional ablation of TCF-1 in these cells abolishes leukemogenesis (S. Arnovitz, P.S. Mathur, S.B. Morin, M. Tracy and F.G., unpublished data). Furthermore, in chronic inflammatory conditions, including inflammatory bowel disease and multiple sclerosis, as well as in colon cancer, regulatory T (T_{reg}) cells have been found to express high levels of β-catenin and to acquire proinflammatory properties[22–24,32]. Conditional stabilization of β-catenin in mouse T_{reg} cells induced an IPEX-like syndrome and experimental autoimmune encephalomyelitis, which is a model of multiple sclerosis. Mechanistically, the pathologies were linked to β-catenin/TCF-1-mediated changes in chromatin accessibility and gene expression[23,24].

Together, these findings reveal the urgent need to better understand how TCF-1 leverages its Wnt-dependent versus Wnt-independent functions in T cells under physiological conditions and in the context of autoimmunity and cancer.

TCF-1 in T cell lineage specification and thymocyte differentiation

Notch activation enforces T cell specification on early thymic progenitors (ETPs) after their entry into the thymus[33,34] (Fig. 1). *Tcf7*

[1]Knapp Research Center, Section of Rheumatology, Department of Medicine, University of Chicago, Chicago, IL, USA. [2]Department of Immunology, Mayo Clinic, Scottsdale, AZ, USA. ✉e-mail: Gounari.Fotini@mayo.edu

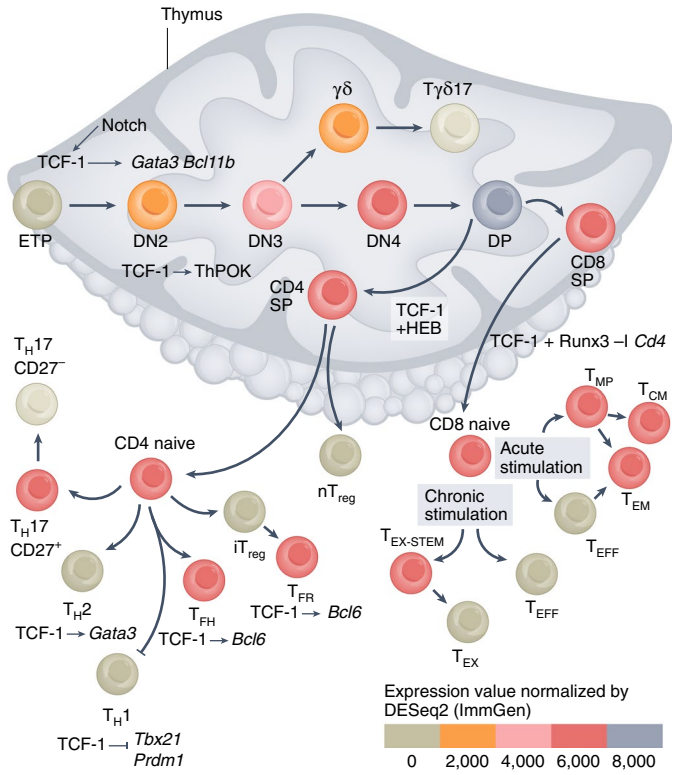

Fig. 1 | T cell development and the stages of TCF-1 implication. Notch signaling upregulates *Tcf7* expression in early ETPs[35–39]. TCF-1 induces the expression of genes encoding transcription factors critical for T cell specification, including *Gata3* and *Bcl11b*. The levels of TCF-1 increase progressively up to the CD4$^+$CD8$^+$ (DP) stage. In CD4$^+$CD8$^+$ (DP) thymocytes, TCF-1 cooperates with HEB to define their epigenetic landscape and transcription profile[44]. Following thymic selection, the DP cells become either CD4$^+$ or CD8$^+$ single-positive (SP) cells. TCF-1 fosters the CD4$^+$ T cell fate by promoting *Zbtb7b* (Th-POK) expression, and although TCF-1 is not required for commitment to the CD8$^+$ T cell lineage, it ensures CD8$^+$ T cell stability by cooperating with RUNX3 to suppress *Cd4* gene expression[48]. TCF-1 also suppresses *Rorc* (RORγT) and *Il17* expression in DP thymocytes, preventing their conversion to CD4$^+$ T_H17 and CD8$^+$ *Il17*-expressing cells[50,113]. After egress from the thymus, CD4$^+$ T cells differentiate into T_H subsets. TCF-1 promotes T_{FH} differentiation by inducing *Bcl6* and suppressing *Prdm1* (BLIMP-1) expression and limits T_H1 differentiation by suppressing *Tbx21* (T-bet) expression[63–65]. TCF-1 promotes T_H2 differentiation by directly upregulating *Gata3* (ref. [59]). TCF-1 is expressed by stem cell-like CD27$^+$ T_H17 cells, which persist in models of MS[54]. TCF-1 suppresses the generation of T_{reg} cells in the thymus (nT_{reg}) and the conversion of CD4$^+$ T cells to peripherally induced T_{reg} (iT_{reg}) cells It also enhances *Bcl6* expression to promote T_{FR} differentiation. CD8$^+$ T cells are critical for defense against acute and chronic viral infections and cancers. In acute viral infection, TCF-1 is important in driving the development of T_{MP} and T_{CM} cells. In both acute and chronic LCMV infection, downregulation of TCF-1 is needed for the differentiation of T_{EFF} and T_{EX} cells. In chronic viral infection and cancer, TCF-1 and BCL-6 support the differentiation and maintenance of $T_{EX\text{-}STEM}$ cells that express PD-1 and can respond to anti-PD-1 therapy.

is among the first genes upregulated in ETPs in direct response to Notch signals[35–38]. We and others have established that the specification of ETPs to the T cell lineage requires TCF-1, and its loss results in an early block of T cell development[35,36]. TCF-1 positively regulates T cell lineage genes, including *Gata3*, *Il2ra* and *Bcl11b*, which leads to T cell commitment. In an elegant recent study, Rothenberg and colleagues validated these original findings by single-cell CRISPR perturbation of the genes encoding early acting transcription factors combined with single-cell RNA-sequencing[39]. These analyses established that TCF-1 was profoundly needed for progression through the earliest phase of the ETP stage, whereas GATA-3 becomes especially important after the initiating role of TCF-1 in T cell specification. TCF-1 was further found to have a critical role in the γδ versus αβ T cell lineage separation. Although the gradual upregulation of TCF-1 facilitates the αβ T cell fate after assembly of the pre-T cell antigen receptor (pre-TCR)[40,41], reduced TCF-1 expression favors the γδ T lineage fate[42,43]. In the γδ T cell lineage, TCF-1 also controls the γδ T cell effector fate, and essentially all TCF-1-deficient γδ cells convert into interleukin 17 (IL-17)-producing γδ effector cells[43]. More recent investigations using conditional CD4-Cre-mediated ablation of *Tcf7*, which targets CD4$^+$CD8$^+$ DP thymocytes, revealed functions of TCF-1 in these later stages of thymic development. Our own studies established that TCF-1 co-binds DNA regulatory sites together with other nuclear factors, including Ikaros, Runx1 and HEB, and in particular, it cooperates with HEB to establish the molecular profile of DP thymocytes[44]. Following positive selection, the DP thymocytes face a lineage choice of the CD4$^+$ or CD8$^+$ T cell lineage. This binary decision is guided by the transcription factors Th-POK or RUNX3, respectively[45–47]. Loss of TCF-1 impairs the CD4 lineage choice through insufficient Th-POK induction and elevated RUNX3 expression[48]. Thus, TCF-1 is a T cell-specification and fate-determining factor at multiple stages of thymocyte development.

TCF-1 in differentiation of peripheral CD4$^+$ helper T cell lineages. After thymic egress, circulating naive CD4$^+$ T cells have the potential to differentiate into several helper T (T_H) cell lineages in response to antigen stimulation. These include the T_H1, T_H2, T_H17 and follicular helper T (T_{FH}) cell lineages that act to control the source of the foreign antigen[49]. TCF-1 orchestrates the developsubment, equilibrium, and function of all these CD4$^+$ T cell lineages (for reviews, see refs. [5,50,51]). T_H17 cells have critical roles in host defense against bacteria and specific pathogens and drive pathogenic inflammation in autoimmunity and cancer[52]. Early studies suggested that germline deletion of *Tcf7* resulted in increased IL-17 gene expression both in the thymus and in peripheral T cells, which led to enhanced T_H17 differentiation[53]. However, these mice with germline TCF-1 deficiency also have abnormal T cell development owing to their constitutive lack of TCF-1. Moreover, in the context of experimental autoimmune encephalomyelitis, TCF-1 expression was shown to mark a CD27$^+$ T_H17 progenitor-like cell subset that after activation can give rise to a disease-promoting TCF-1^{lo}CD27$^-$ T_H17 cell subset[54]. T_H2 cells are critical for the immune response against extracellular parasites and are activated by allergens and toxins[55]. They can induce allergy and cooperate with T_H17 cells to mount tumor-promoting inflammation involving IL-33 and IL-10 (refs. [56–58]). TCF-1 promotes T_H2 cell polarization by transactivating GATA-3, the signature regulator of the T_H2 cell lineage[59]. T_H1 cells, differentiate in response to infection by intracellular pathogens, and have a shared transitional stage during their early differentiation steps with T_{FH} cells, which help B cells produce antibodies[60–62]. TCF-1 controls the bifurcation between the T_H1 and T_{FH} cell lineages in favor of T_{FH} by upregulating the BCL-6, which drives T_{FH} cell differentiation, and downregulating BLIMP-1, which normally suppresses BCL-6 (Fig. 1). BCL-6 limits T_H1 cell differentiation by directly suppressing the expression of critical T_H1 cell differentiation genes, including *Tbx21*, which encodes T-bet, the signature T_H1 lineage transcription factor[63–65]. Thus, starting from a common naive CD4$^+$ T cell, TCF-1 guides the maturation and the equilibrium of T_H cell lineages by selectively modulating the expression of key regulatory genes.

The specific roles of TCF-1 in T_{reg} cells

Among CD4$^+$ T cells, T_{reg} cells have indispensable roles in resolving tissue inflammation and preserving tolerance to self-antigens[66,67]. T_{reg}

cells are generated in the thymus or are converted in the periphery from naive CD4+ T cells. The function of TCF-1 in T_{reg} cells is complex. Earlier studies using a germline *Tcf7*-knockout model showed that heterozygous deletion of *Tcf7* increases the number of thymically generated T_{reg} cells[68], suggesting that TCF-1 negatively regulates T_{reg} cell development and TCR affinity. Consistent with that finding, the T_{reg} cell lineage- determining factor FOXP3 has been reported to suppress *Tcf7* gene expression[55]. More recent studies have demonstrated that TCF-1 interacts with Foxp3 and that chronic activation of Wnt signaling impairs Foxp3 functions through TCF-1 (ref. [69]). Subsequently, several studies have verified that in T_{reg} cells, TCF-1 shares a large number of DNA-binding sites with Foxp3 and regulates the expression of FOXP3 target genes to fine-tune T_{reg} cell properties[23,70,71]. In line with those findings, we have found that the targeted ablation of TCF-1 in T_{reg} cells enhanced their suppression of CD8+ T cell cytotoxicity but compromised their anti-inflammatory functions[72]. Interestingly, single-cell RNA-sequencing analysis has identified numerous T_{reg} cell subsets and has established that loss of TCF-1 has a greater impact in peripherally induced T_{reg} cells than in thymic T_{reg} cells[72]. T_{reg} cell-specific ablation of both TCF-1 and LEF-1 induced autoimmune pathologies[70], whereas loss of TCF-1 alone did not by itself render the mice unhealthy. However, in mice predisposed to polyposis due to heterozygous deletion of the adenomatous polyposis (*APC*) gene, the T_{reg} cell-specific loss of TCF-1 exacerbated pathogenic inflammation driving aggressive tumor growth[72]. Consistent with that, T_{reg} cells infiltrating human colorectal cancer tumors expressed lower levels of TCF-1 than did those in the healthy margins or peripheral blood[72]. These findings suggest that TCF-1 alters T_{reg} cell functions in response to environmental cues to produce appropriate or, in the case of autoimmunity and cancer, inappropriate and pathogenic immune responses[72]. A distinct sub-category of T_{reg} cells are the follicular regulatory T (T_{FR}) cells that act in the germinal centers to maintain immune homeostasis by suppressing excessive T_{FH} cell and B cell responses[73]. T_{reg} cell-specific loss of TCF-1 diminished the number of T_{FR} cells and impaired immune regulation within germinal centers[70]. This could contribute to the enhanced inflammation observed in mice with TCF-1-deficient T_{reg} cells[70,72]. Thus, TCF-1 expression in T_{reg} cells and T_{FH} cells is essential for selectively regulation of T_{reg} functions that control inflammation.

TCF-1 in CD8+ T cell survival and progenitor states

Expression of TCF-1 in CD8+ T cells distinguishes antigen-stimulated cells that have progenitor potential from their terminally differentiated counterparts. This distinction applies to T cells that are subjected to acute or chronic TCR stimulation.

TCF-1 is downregulated in mouse CD8+ T cells cells that respond to acute lymphocytic choriomeningitis virus (LCMV) infection as they progress from naive to terminally differentiated effector T (T_{EFF}) cells. However, expression of TCF-1 is maintained in a subset of T cell precursors with stem-cell-like features[74] that differentiate into long-lived central memory T (T_{CM}) cells[75–77]. Several subsets of antigen-experienced CD8+ T cells are defined on the basis of their longevity, progenitor properties, and effector functions, as well as expression of nuclear factors and cell surface markers (for review, see ref. [78]). Hybrid phenotypes have also been described on the basis of the expression of markers for migration and tissue residence (for review, see ref. [79]). TCF-1 deficiency alone diminishes, but does not ablate, CD8+ T_{CM} cells, whereas loss of both TCF-1 and LEF-1 almost completely eliminates the CD8+ T_{CM} precursor cells (T_{MP} cells), suggesting that TCF-1 together with LEF-1 contribute to the T_{MP} cell phenotype[80].

In mice with chronic LCMV infection, persistent TCR signaling drives the downstream activation of transcription factors that promote the expression of inhibitory receptors, such as PD-1, LAG-3 and TIGIT, and reduce expression of KLRG1 (ref. [81]), resulting in exhausted CD8+ T cells (T_{EX} cells). These transcription factors include the calcineurin-dependent factor NFAT (nuclear factor of activated T cells)[5], IRF4 (interferon regulatory factor-4), BATF (basic leucine zipper transcription factor, ATF-like), NR4A (nuclear receptor subfamily 4 group A) and TOX (thymocyte selection-associated HMG box). TOX is the master regulator of the exhausted epigenetic state. T_{EX} cells were initially thought to be dysfunctional; however, the loss of TOX also limits the persistence of functionally active antigen-specific CD8+ T cells, indicating that a subset of T_{EX} cells has progenitor properties[82–88]. This subset has elevated expression of TCF-1 and controls viral spread in chronically infected mice. These observations are consistent with TCF-1's modulating the epigenetic outcomes of TOX activity.

The tumor-induced T cell dysfunction occurs in early stages of carcinogenesis and is driven by the presence and persistence of tumor antigen[89]. Tumor-infiltrating CD8+ T cells become increasingly dysfunctional with time, expressing high levels of genes associated with reduced immune function, such as those encoding the transcriptional repressors Egr1, Batf and Blimp-1, and the inhibitory receptors PD-1, LAG-3, 2B4 and TIM3. Dysfunctional CD8+ T cells fail to produce effector cytokines in response to cognate antigen. By contrast, non–tumor-specific T cells that infiltrate the same tumor do not upregulate inhibitory receptors, and produce interferon-γ and tumor necrosis factor[89]. This finding suggests that chronic TCR stimulation by tumor antigens drives the dysfunctional CD8+ T cell phenotype. There is a growing consensus that elevated expression of TCF-1 identifies the tumor-antigen-specific CD8+ T cell subset that maintains long-term functional responses ($T_{EX\text{-}STEM}$) from the terminally differentiated T_{EX} subset[90–92] (for review, see ref. [5]). This concept may be oversimplified, as discussed below.

TOX and TCF-1 are both members of the family of transcription factors that contain the conserved HMG box region. TCF-1 partners with TOX to maintain an epigenetic state in CD8+ $T_{EX\text{-}STEM}$ cells that supports long-term survival and progenitor potential, but not necessarily functionality[83,85,93,94]. Expression of TCF-1 is under the control of TOX. In the absence of TOX, chronically stimulated CD8+ T cells maintain elevated levels of TCF-1 and fail to upregulate exhaustion markers. However, loss of TOX is not enough for the expression of interferon-γ and tumor necrosis factor by tumor-infiltrating CD8+ T cells. This finding has led to the suggestion that the expression of exhaustion markers is uncoupled from the loss of effector functions, but instead serves to prevent the overstimulation of T cells and activation-induced cell death[85]. This finding brings into question the immediate benefits of targeting TOX or TCF-1 for immunotherapy of cancer[95]. Further research is needed to establish the mechanisms responsible for functional inactivity of tumor-infiltrating cells, as well as the similarities and differences between dysfunctional CD8+ T cells in cancer and exhausted T cells in chronic viral infection[96].

Functions of TCF-1 in the Wnt cascade and the option of Wnt independence

It is intriguing that TCF-1 is crucial in all stages of T cell development and maturation, including various context-dependent T cell functions. The developmental-stage-specific and T cell-lineage-specific transcriptional profiles and biological outcomes attributed to the action of TCF-1 raise intriguing questions about its Wnt-dependent versus Wnt-independent functions, and the roles of molecular partners of TCF-1 that help to mediate diverse outcomes.

In the logic of the WNT cascade, DNA-bound TCF-1 enables directly interacting transcription and epigenetic factors to access specific genomic regions in order to regulate the chromatin landscape and transcription of the associated genes. In this scenario, β-catenin interacts with the full-length TCF-1 protein in complex with epigenetic and transcription regulators that enhance chromatin accessibility and gene transcription (Fig. 2a). In the absence of

β-catenin, repressors of the Grg/TLE family that bind TCF-1 at a region proximal to the HMG DNA-binding domain[6,97] reduce chromatin accessibility and suppress gene transcription (Fig. 2a)[98]. The extent to which β-catenin or repressors of the Grg/TLE family are involved in T cell development and function is uncertain and needs further study (for review, see ref. [5]). It is possible that other regulators that can bind TCF-1 outside the context of Wnt signaling also follow this scenario to modulate the chromatin landscape and transcription profile of cells, but the identities of such regulators remain to be elucidated.

Cooperation of TCF-1 with other transcription and epigenetic regulators

In addition to its direct interaction with regulators, TCF-1 also cooperates with other transcription factors to establish context- and developmental-stage-specific epigenetic and transcription profiles through co-binding to common DNA sites. Regulation through co-binding is supported by findings that accessible chromatin sites along thymocyte development are not only enriched for the TCF/LEF motif, but also motifs for ETS, RUNX and E2A families of transcription factors[44,99]. Comparing the genome-wide binding of TCF-1 to that of IKAROS, RUNX1 and HEB in DP thymocytes demonstrated an extensive overlap between TCF-1-occupied sites and sites bound by these transcription factors. Detailed studies focusing on the co-binding of TCF-1 and HEB showed that TCF-1 cooperates with HEB to establish and maintain the epigenetic and transcription profile of DP thymocytes (Fig. 2b)[44]. Furthermore, in T_{reg} cells, TCF-1 binding substantially overlaps the binding of Foxp3 (refs. [23,71]). We found that this overlapping binding limited the expression of genes involved in T_H17 inflammation, transforming growth factor-β (TGF-β) signaling, and T cell activation[72]. The genetic ablation of *Tcf7* led to the upregulation of these genes without compromising the core T_{reg} gene expression signature, and even upregulated *Foxp3* expression[72]. The interaction of TCF-1 with Foxp3 was further shown to involve Foxp3-mediated downregulation of *Tcf7* (ref. [71]). Thus, these new findings establish that TCF-1 engages transcription factors from several different families as its cooperating partners to shape the molecular profiles of T cells during development and function.

TCF-1 preserves chromatin accessibility

It has been suggested that in T cell development, TCF-1 promotes or preserves chromatin accessibility[44,71,99,100]. Vahedi and colleagues found that the upregulation of TCF-1 in early thymocytes undergoing T cell specification coincides with increased accessibility of chromatin sites that are enriched for the conserved TCF-1 DNA-binding motif[99]. Also during hematopoietic development, from hematopoietic stem cells to $CD4^+$ or $CD8^+$ single-positive thymocytes, the TCF-1 binding motif becomes progressively more enriched at accessible chromatin sites. The increase in chromatin accessibility parallels the progressive upregulation of *Tcf7* gene expression in developing thymocytes[99]. These findings have led to suggestions that TCF-1 may act as a pioneer-like factor[101] that initiates the T cell-specific chromatin landscape and establishes the T cell lineage identity (Fig. 2c). A detailed molecular analysis of TCF-1-sufficient and TCF-1-deficient lymphoid precursors cultured for a short time in T cell differentiation conditions (as described in ref. [39]) could help clarify the precise role of TCF-1 in T cell specification and may provide direct support for the role of TCF-1 as a pioneer factor. Other studies have shown that loss of TCF-1 in DP thymocytes[44], or in $CD8^+$ T cells[100], resulted in overall reduced chromatin accessibility at sites previously bound by TCF-1. At least in DP thymocytes, these accessibility changes were more evident in TCF-1-bound enhancer sites that contained its conserved motif, and loss of TCF-1 correlated with an overall reduction of target gene expression[44]. Along this line, Schietinger and colleagues have shown that the transition of $CD8^+$ $T_{EX\text{-}STEM}$ tumor-infiltrating lymphocytes (TILs), which express TCF-1, into terminal $CD8^+$ T_{EX} TILs, which do not, is associated with extensive changes in chromatin accessibility. Sites that progressively lose accessibility during this process are highly enriched for the TCF/LEF binding motifs[93], suggesting that TCF-1 sustains the open chromatin state in $T_{EX\text{-}STEM}$ TILs (Fig. 2d) and can serve as a 'placeholder' for other transcription factors (Fig. 2e). Similarly, following $CD4^+$ and $CD8^+$ T cell activation, TCF-1 is downregulated, and its canonical binding sites become inaccessible. Exceptions are TCF-1 sites that are co-bound by Ets1, or by activation-induced transcription factors, indicating that TCF-1 may act as a placeholder in these sites[102].

Collectively, these findings suggest that TCF-1 predominantly maintains and potentially also promotes chromatin accessibility in T cell development and support the notion that this function underlies its ability to maintain some differentiation potential or 'stemness' in T cells. These functions are probably independent of Wnt signals, as, in contrast to the critical need for TCF-1, β-catenin has not been shown to have a role in normal T cell development.

TCF-1 directly modifies the chromatin through its intrinsic HDAC activity

Deciphering how TCF-1 functions in T lymphocytes has become more challenging by the finding of Xue and colleagues that in addition to shaping the chromatin landscape through its interacting partners, TCF-1 has an intrinsic histone deacetylase (HDAC) activity. This activity, which was mapped to a region between the amino-terminal β-catenin-binding domain and the central HMG DNA-binding domain directly upstream of the Grg/TLE binding domain, can directly reduce chromatin accessibility (Fig. 2f). It has been suggested that the TCF-1 HDAC activity is essential for establishing the $CD8^+$ T cell identity[98] and for sustaining the ability of T_{FH} cells to provide B cell help[103]. Therefore, in order to determine the context-dependent functions of TCF-1 on chromatin accessibility, it is important to understand how its intrinsic HDAC activity orchestrates with the activities of regulators that directly interact with TCF-1, particularly those that promote chromatin accessibility.

TCF-1 shapes the 3D chromatin conformation

The HMG domain family of proteins, including TCF-1, bind to the minor groove of the DNA helix and have the ability to bend the DNA (reviewed in ref. [104]). Early studies by Grosschedl and colleagues established that LEF-1, a relative of TCF-1 that is also expressed in T cells, induces a substantial DNA bend to its binding site[105,106]. In particular, LEF-1, probably through its ability to bend the DNA after binding, facilitates interactions between proteins bound at nonadjacent sites and coordinates the assembly of a complex at the enhancer of the gene encoding TCRα. On the basis of the similarity of their HMG domains, it is expected that TCF-1 also bends DNA after binding, and this could have considerable implications for the ability of TCF-1 to regulate the three-dimensional (3D) chromatin conformation (Fig. 2). Newly developed technologies have made it possible to assess chromatin conformation changes genome wide and to associate them with lineage commitment stages. In this context, a comprehensive study combining high-throughput chromatin conformation capture (Hi-C) with chromatin accessibility and gene expression analyses has established that T cell commitment at the DN2-to-DN3 thymocyte stage is associated with major chromatin conformation changes[107]. Although that study did not implicate TCF-1, more recently, Xue and colleagues integrated Hi-C with epigenetic and transcription data to show that TCF-1 and LEF-1 promote the formation of extensively interconnected hubs by enforcing chromatin interaction and accessibility in $CD8^+$ T cells[100]. These findings open the way for further studies to independently assess the role of TCF-1 versus LEF-1 on chromatin conformation and to compare their differential impact in the various T cell lineages.

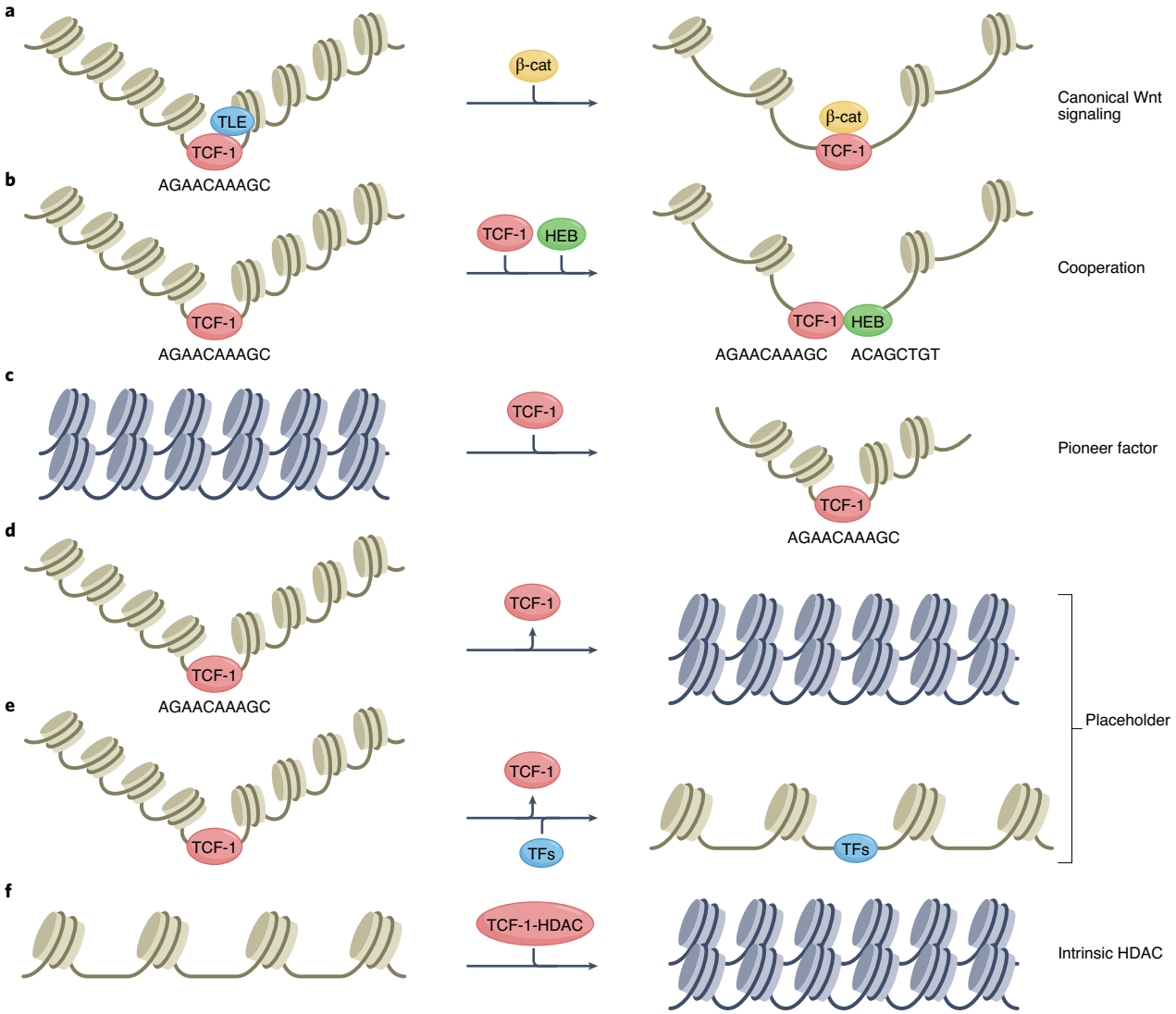

Fig. 2 | Understanding the fundamental molecular functions of TCF-1. TCF-1 has multiple molecular functions that are superimposed on its ability to bend the DNA after binding and to regulate the 3D chromatin conformation. **a**, In the absence of β-catenin (β-cat), TCF-1 can bind with the repressors of the Grg/TLE family at a region proximal to the HMG DNA-binding domain[6,97] to reduce chromatin accessibility and suppress gene transcription[98]. Extracellular Wnts stabilize β-catenin by disrupting its degradation complex, allowing its nuclear translocation and interaction with the full-length TCF-1 protein. This results in the recruitment of epigenetic and transcription regulators that enhance chromatin accessibility and gene transcription. **b**, TCF-1 cooperates with other transcription factors to establish context- and developmental-stage-specific epigenetic and transcription profiles through co-binding to common DNA sites. **c**, TCF-1 could function as a pioneer-like factor and initiate changes in the chromatin landscape that establish the T cell lineage identity[101]. **d**,**e**, TCF-1 may function as a 'placeholder,' to promote or maintain T cell-specific chromatin accessibility, independently of Wnt signals. **f**, TCF-1 has intrinsic HDAC activity and can directly reduce chromatin accessibility.

TCF-1 leverages its functions through abundance

The expression levels of TCF-1 in developing T cells are highly regulated. ETP thymocytes express low levels of TCF-1. Expression of TCF-1 is progressively upregulated up to the DP stage, at which it reaches a level that is unusually high for a transcription factor. Past the DP stage, TCF-1 expression levels are reduced but remain relatively high in all peripheral T cell lineages with the exception of the T_{reg} cells, which express low levels of TCF-1. Polarization of CD4$^+$ T cells to the T_H lineages is invariably marked by downregulation of TCF-1. Similarly, progression of naive CD8$^+$ cells to the terminal effector or terminal exhaustion stages is associated with downregulation of TCF-1, whereas the progenitor exhausted CD8$^+$ T cells that are long lived and have progenitor properties express high levels of TCF-1.

Recent studies have highlighted the molecular impact of TCF-1 downregulation. In particular, the reduced expression of TCF-1 after CD4$^+$ and CD8$^+$ T cell activation was suggested to underlie the observed accessibility loss of sites uniquely bound by TCF-1 (ref. [102]). The increased generation of thymic T_{reg} cells after heterozygous TCF-1 deletion[68], and the downregulation of TCF-1 by FOXP3, which reduces chromatin accessibility and associated gene expression[71] in T_{reg} cells, further support the suggestion that the levels of TCF-1 determine its functions. TCF-1 is also downregulated in colon tumor-infiltrating T_{reg} cells, indicating that T_{reg} cells adapt to their environment by regulating the levels of TCF-1, which in this case contributed to their enhanced tumor-promoting properties[72]. Therefore, variations in TCF-1 levels can substantially impact the interplay between TCF-1 and other molecular partners that affect

key biological processes, including cell differentiation, cell fate decision and cellular function in health and disease.

Future perspectives

Deciphering how TCF-1 orchestrates its diverse functions during T cell development represents a challenging puzzle. This challenge is because TCF-1 has essential roles in multiple T cell lineages and developmental stages, and its activity is context dependent. Therefore, any effort to delineate the molecular functions of TCF-1, must take into consideration in each T cell lineage and developmental stage: (1) the epigenetic and transcription profile of the cells; (2) the cooperating and interacting partners; and (3) the physiological levels of TCF-1. An additional parameter to consider in each context is the redundant functions of the transcription factor LEF-1, the WNT-responsive close relative of TCF-1 that is expressed in all T cells and shares considerable structural homology with TCF-1. TCF-1 and LEF-1 share the ability to bind β-catenin and Grg/TLE factors, and they are both expressed as shorter isoforms that do not bind β-catenin. The genome-wide DNA binding of LEF-1 overlaps that of TCF-1 in cells in which it has been assessed, including DP thymocytes[44] and T_{reg} cells[70]. Simultaneous loss of both TCF-1 and LEF-1 in many T cell lineages and stages mainly strengthens the phenotypes observed after simple deletion of TCF-1 (refs. [48,63,70]). It is even puzzling why the loss of TCF-1 has a much stronger impact on T cell development than does the loss of LEF-1, and it is unclear whether this is due to the lower levels of LEF-1 expression, as in DP thymocytes, or to unknown functional differences. Although studies up to now have mainly identified functional similarities between TCF-1 and LEF-1 in T cell development, it would be interesting to uncover their differences and the molecular basis of their functions.

Future investigations can take advantage of new single-cell and genome-wide technologies. For example, single-cell technologies, including scCrisprCAS9 perturbations[39], scRNAseq, and scATACseq, have already provided a wealth of information on the roles of TCF-1 in T cell specification[99], on T_{reg} cell heterogeneity, and on tumor-infiltrating T cells[72,108–112]. Similarly, integration of genome-wide transcription, epigenetic and chromosome conformation data is beginning to more accurately predict molecular functions of TCF-1. Addressing the molecular functions of TCF-1 will require new model systems in which TCF-1 expression and/or protein levels can be temporally modulated in specific T cell developmental stages and lineages. Given that TCF-1 regulates multiple T cell properties, knowing how it functions in each situation will provide the knowledge base with which to design context-specific therapeutic interventions in autoimmunity and cancer.

Received: 29 November 2021; Accepted: 22 March 2022;
Published online: 29 April 2022

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Acknowledgements

This work was supported by US National Institutes of Health grants R01 AI 108682 (to F.G. and K.K.) and R01 AI 147652 (to F.G.).

Competing interests

The authors declare no competing interests.

Additional information

Correspondence should be addressed to Fotini Gounari.

Peer review information *Nature Immunology* thanks Christelle Harly and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling editor: Laurie A. Dempsey, in collaboration with the *Nature Immunology* team.