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REVIEW

Posttranscriptional regulation of T helper cell fate decisions

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T helper cell subsets orchestrate context- and pathogen-specific responses of the immune system. They mostly do so by secreting specific cytokines that attract or induce activation and differentiation of other immune or nonimmune cells. The differentiation of T helper 1 (Th1), Th2, T follicular helper, Th17, and induced regulatory T cell subsets from naive T cells depends on the activation of intracellular signal transduction cascades. These cascades originate from T cell receptor and costimulatory receptor engagement and also receive critical input from cytokine receptors that sample the cytokine milieu within secondary lymphoid organs. Signal transduction then leads to the expression of subset-specifying transcription factors that, in concert with other transcription factors, up-regulate downstream signature genes. Although regulation of transcription is important, recent research has shown that posttranscriptional and posttranslational regulation can critically shape or even determine the outcome of Th cell differentiation. In this review, we describe how specific microRNAs, long noncoding RNAs, RNA-binding proteins, and ubiquitin-modifying enzymes regulate their targets to skew cell fate decisions.

Introduction

Lymphocytes carry unique T cell receptors (TCRs) or B cell receptors that enable specific recognition of nearly all pathogen-derived antigens. Upon TCR binding to the cognate antigen, CD4 T helper cells become activated to coordinate responses of the immune system to diverse pathogens, including intracellular viruses, intra- or extracellular bacteria, fungi, or multicellular parasites. Among other functions (Table 1), CD4 T cells provide help to antibody-producing B cells and cytotoxic CD8 T cells, which are central effector cells of humoral and cellular responses of the adaptive immune system, respectively.

Mature CD4 T cells exit the thymus and home to secondary lymphoid organs, where they may recognize pathogen-derived peptides that are processed by antigen-presenting cells and presented to the TCR on major histocompatibility complexes (MHCs). In this initial antigen encounter in the periphery, the so-called priming step, several signals are integrated so that the naive CD4 T cell becomes productively activated and subsequently acquires specific effector functions (Table 1).

T helper cell activation and differentiation starts with antigen recognition. TCR binding to peptide/MHC class II activates tyrosine kinases, which enables the assembly of phosphorylation-dependent signaling complexes and ensuing activation of phospholipase C. Phospholipase C cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) into two second messengers, inositol-trisphosphate and DAG (Figs. 1, 2, 3, 4, and 5). Inositol-trisphosphate causes Ca^{2+}-store depletion of the ER, which triggers store-operated Ca^{2+} influx over the plasma membrane, thereby stimulating the phosphatase calcineurin to dephosphorylate and activate the transcription factor NFAT (Feske et al., 2006). DAG activates the Ras/MAPK cascade and PKC enzymes, which subsequently activate AP-1 and NF-κB transcription factors (Altman and Villalba, 2003). The combined induction of NFAT, AP1, and NF-κB is required for productive T cell activation, leading to the expression of IL-2, the hallmark cytokine of activated T cells. In addition to TCR signaling, B7 family members B7-1 (CD80) and B7-2 (CD86) on the cell surface of antigen-presenting cells induce costimulation through the CD28 receptor on T cells (Figs. 1, 2, 3, 4, and 5). The CD28 receptor directly interacts with the class I phosphatidylinositol 3-kinase (PI3K), thereby promoting the production of phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 signaling activates NF-κB and promotes cell survival. It also leads to cytoskeletal rearrangements, enhances signal transduction from the TCR, and activates mTOR to increase the metabolic activity of the cell (Acuto and Michel, 2003; Okkenhaug and Vanhaesebroeck, 2003; Gamper and Powell, 2012).

Our current understanding of T helper cell differentiation has been greatly influenced by in vitro cell culture systems that recapitulate T helper 1 (Th1), Th2, Th17, and induced regulatory T cell

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Table 1. **Differentiation and function of CD4 effector T cells**

CD4 subset	Figure	Differentiation and function
Th1	1	Stat4 activation upon IL-12 signaling promotes the expression of the master regulator T-bet and the signature cytokine IFNγ. Th1 cells are proinflammatory effector T cells involved in the activation of macrophages and cytotoxic T cells.
Th2	2	Stat6 activation upon IL-4 signaling induces the expression of the master regulator Gata3 and the signature cytokines IL-4, IL-5, and IL-13. Th2 cell effector functions drive immune responses directed against helminths and are central in allergic reactions.
Tfh	3	Tfh cell differentiation involves multiple cell–cell interactions that are spatially and temporally defined. Stat3 activation upon IL-6 signaling triggers the expression of the master regulator Bcl6. Tfh cells provide help to B cells through direct cell–cell interactions and release of different cytokines (e.g., IL-21 at first and subsequently IL-4, IL-17, or IFNγ). Their effector function is critical for controlling the development of humoral immunity and the generation of high-affinity antibodies.
Th17	4	Stat3 activation in the context of IL-6 and TGFβ signaling promotes the expression of the master regulator Rorγt and the signature cytokine IL-17. Th17 effector functions are proinflammatory and protect epithelial barriers of the gastrointestinal tract, the respiratory tract, and the skin from bacterial and fungal infections.
iTreg	5	Stat5 activation upon IL-2 signaling as well as TGFβ-dependent Smad signaling lead to the expression of the master regulator Foxp3. Treg cells suppress effector functions of other T cells through a variety of mechanisms, including IL-10 and TGFβ production, thereby limiting tissue damage and other inflammatory effects of acute or chronic immune responses.

Differentiation of CD4 T helper cell subsets can be described in a simplified scheme (Figs. 1, 2, 3, 4, and 5; O'Shea and Paul, 2010). Activation, proliferation, survival, and differentiation are induced by TCR and costimulatory receptor signaling. Additional engagement of cytokines with their receptors activates Stat proteins. Differential activation of Stats controls the expression of subset-specifying transcription factors, which in turn induce additional differentiation-associated genes, including those of signature cytokines.

(iTreg) differentiation. These reductionist approaches describe differentiation as a result of integrating TCR and costimulatory signals with coinciding signal transduction from cytokine receptors (Figs. 1, 2, 3, 4, and 5). The involved signaling cascades eventually promote transcription of subset-specific gene expression profiles. Once a gene expression program is established, it can be further stabilized and epigenetically fixed. The differential decisions are most strongly influenced by signals from cytokine receptors that are transmitted by Stat proteins (Table 1). Cytokine-induced phosphorylation of cytoplasmic Stats causes their homodimerization, nuclear translocation, promoter binding, activation of transcription, and expression of subset-specifying transcription factors (O'Shea et al., 2011). Subset-specifying transcription factors are not only necessary but also sufficient to drive differentiation into the respective T helper cell subset and hence have been at the center of many research activities (Ansel et al., 2006; Zhou and Littman, 2009; Shih et al., 2014).

Important cell fate decisions also occur posttranscriptionally by regulating the half-life or function of specific mRNAs or proteins, which challenges the long-held paradigm that only transcriptional networks control the differentiation of CD4 T cells. In this review, we summarize what is currently known about posttranscriptional and posttranslational regulation, focusing on the functions of noncoding RNAs (miRNAs and long noncoding RNAs [lncRNAs]), RNA-binding proteins (RBPs), and ubiquitin-modifying enzymes (E3 ligases/deubiquitinating enzymes [DUBs]) during differentiation processes of T helper cells.

Posttranscriptional and posttranslational mechanisms with an impact on CD4 T cell differentiation

Among the numerous mechanisms of posttranscriptional/posttranslational control of T cell differentiation, the best-understood examples (at this stage) involve miRNAs, lncRNAs, RBPs, and ubiquitin-modifying enzymes (see text box). Installing these layers of regulation likely provides more rigorous control over inappropriate stimulation of potent immune cells. Moreover, compared with transcriptional and epigenetic regulation, posttranscriptional/posttranslational processes are fast and therefore can instantly tune cell fate decisions in response to environmental cues. In the following, we discuss the mechanisms of posttranscriptional and posttranslational regulation for each of the five major CD4 T cell subsets.

Posttranscriptional regulation of Th1 cells

RBPs are involved in the differentiation of all CD4 T cell subsets, including Th1. The Roquin and Regnase family proteins have been found to interact with an overlapping set of target mRNAs and either induce mRNA decay through interaction with mRNA degrading enzymes or, as in the case of Regnase proteins, cleave target mRNAs endonucleolytically (Akira, 2013; Heissmeyer and Vogel, 2013; Jeltsch and Heissmeyer, 2016; Takeuchi, 2018). A homozygous single-point mutation (M199R) in the amino terminus of the RBP Roquin-1, the so-called *sanroque* mutation, weakened its posttranscriptional activity (discussed below in further detail) and caused an accumulation of Th1 cells (Fig. 1; Vinuesa et al., 2005; Yu et al., 2007; Lee et al., 2012). Moreover, the *sanroque* mutation or Regnase-1 deletion as well as the combination of both genotypes induced Th1 cells and led to lymphocyte infiltration and inflammation of multiple organs (Uehata et al., 2013; Cui et al., 2017). The increased IFNγ production in the *sanroque* mouse model was shown to drive a lupus erythematosus–like phenotype and T follicular helper (Tfh) cell accumulation. A potential direct or indirect regulation of *Ifng* mRNA by Roquin may explain these phenotypes (Vinuesa et al., 2005; Yu et al., 2007; Lee et al., 2012). Surprisingly, when the alleles encoding for Roquin-1 were inactivated in T cells in combination with those encoding for its paralog

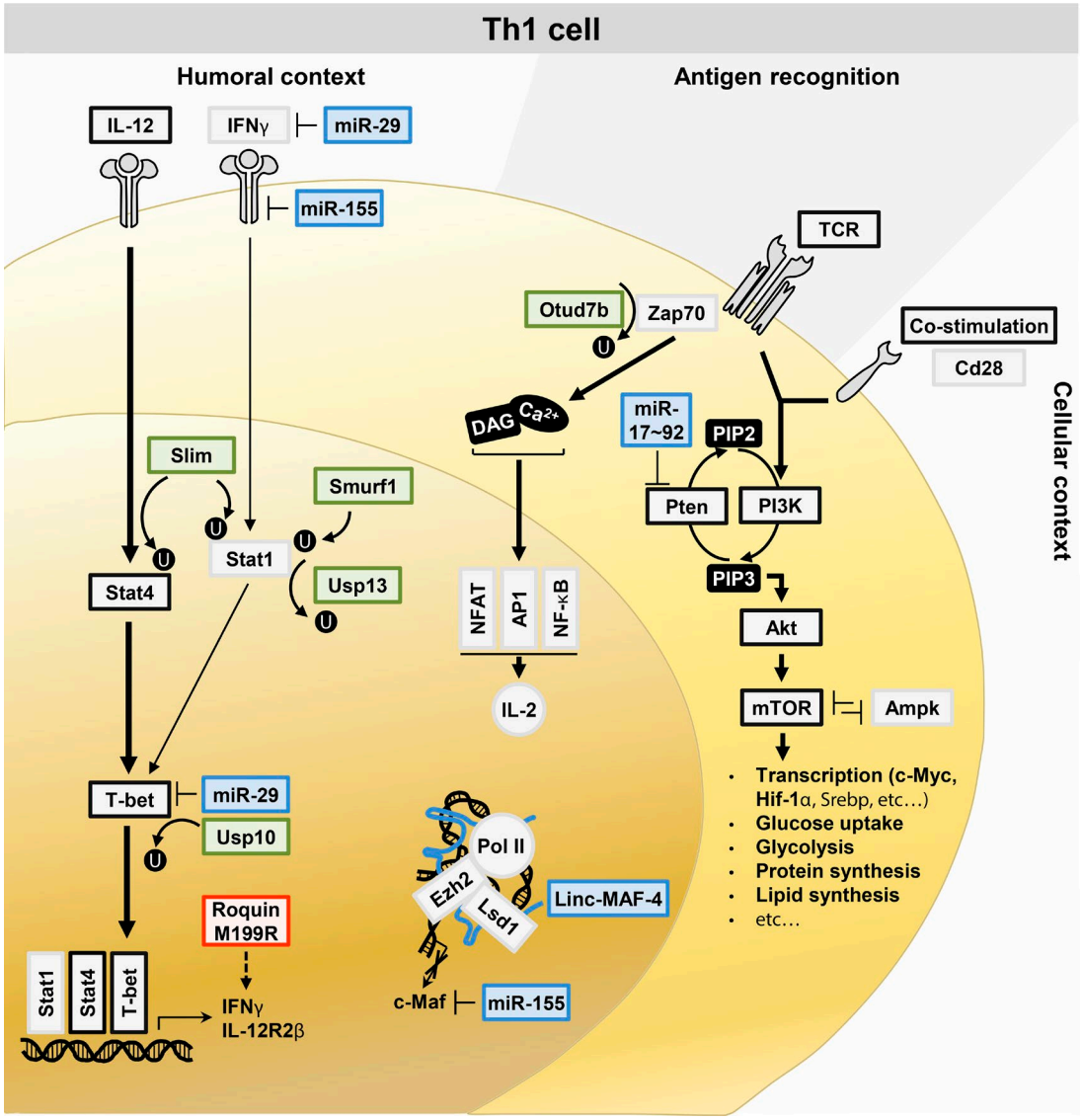

Figure 1. **Regulatory processes during Th1 differentiation.** Noncoding RNAs, RBPs, and E3 ligases/DUBs are shown in blue, red, and green, respectively. For clarity of depiction, these posttranscriptional regulators were not necessarily placed at the correct cellular site of activity. Dashed lines indicate an indirect effect. Molecular interactions are explained in the text.

Roquin-2, a stronger bias toward Tfh and Th17 than Th1 differentiation was observed (Vogel et al., 2013; Jeltsch et al., 2014).

Early miRNA profiling of primary hematopoietic cells and hematopoietic cell lines indicated that differential miRNA expression might contribute to lineage commitment (Monticelli et al., 2005). Subsequently, it was published that complete disruption of miRNA biogenesis resulted in the abnormal production of the Th1 cell signature cytokine IFNγ; however, the contributions of individual miRNAs remained unknown (Muljo et al., 2005; Cobb et al., 2006; Chong et al., 2008; Liston et al., 2008; Zhou et al., 2008). It is now clear that the miR-17~92 cluster as well as miR-155 promote, whereas miR-29 limits IFNγ expression (Fig. 1).

The miR-17~92 cluster of miRNAs has pleiotropic functions in hematopoietic cells. Germline deletion of miR-17~92 led to perinatal death, whereas deficiency of the paralogous clusters miR-106a~363 or miR-106b~25 did not result in obvious phenotypes (Ventura et al., 2008). During T cell activation, the transcription of miR-17~92 is strongly up-regulated in a CD28 costimulation–dependent manner (de Kouchkovsky et al., 2013), and posttranscriptional processing of the polycistronic pre-miRNA transcript results in six different mature miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a-1) that together promote CD4 T cell proliferation and survival (Jiang et al., 2011; Steiner et al., 2011). Several publications have described specific functions of the miR-17~92 cluster and individual miRNAs thereof in the differentiation of Th1, Th2, Tfh, Th17, and iTreg cells (Baumjohann, 2018), which will be discussed in the respective sections. Because of space restrictions, we do not depict individual miRNAs of the cluster in Figs. 1, 2, 3, 4, and 5.

IFNγ was up-regulated in CD4 T cells of transgenic mice that overexpressed miR-17~92 (Xiao et al., 2008), whereas T cell–specific deletion of this miRNA cluster decreased Th1 cell responses (Jiang et al., 2011). These findings could largely be attributed to miR-19b and the repression of its primary target

Factors involved in posttranscriptional and posttranslational gene regulation

RBPs

RBPs recognize RNA by binding to cis-acting elements, such as secondary structures or target sequences with regulatory potential, often found in the 5′ or 3′ UTRs of mRNAs. This interaction of the trans-acting factor with the cis-element typically initiates the recruitment of general regulators of mRNA half-life and/or translation efficiency. Many cytokine mRNAs that are expressed by T helper cells contain long 3′ UTRs. For instance, the 3′ UTR of the IL-17A mRNA makes up more than 70% of the entire transcript (Turner et al., 2014). Long 3′ UTRs can contain high numbers of encoded cis-elements that render them prone to posttranscriptional gene regulation, which can be mediated by trans-acting factors, such as miRNAs or RBPs, individually or in combination.

miRNAs

MicroRNAs are ∼22-nt-long, single-stranded, noncoding RNAs that, in complex with proteins of the miRNA-induced silencing complex, bind specific sequences in the 3′ UTRs of target mRNAs, which results in translational repression and mRNA degradation (Hoefig and Heissmeyer, 2008). Today, 2,588 mature miRNAs have been identified in humans (1,915 in mice), and the cellular network of miRNAs is believed to fine-tune tissue-specific gene expression. Typically, the impact of a single miRNA on the protein level of a target gene is rather small (Baek et al., 2008). However, the accumulation of silencing effects may be key to miRNA-mediated repression, because for each miRNA, the predicted target gene numbers range in the hundreds, whereas many 3′ UTRs offer more than one miRNA target site. This involves synergistic biological effects of several different miRNAs regulating the same mRNA or one miRNA regulating many different mRNA targets in the same pathway (Baumjohann and Ansel, 2013). Nevertheless, miRNA-dependent repression can determine cell fate choices, and often the deregulation of just one protein can account for much of the phenotype elicited by the genetic ablation of one specific miRNA (Xiao et al., 2007; Dorsett et al., 2008; Johnnidis et al., 2008; Teng et al., 2008).

lncRNAs

lncRNAs (typically >200 nt) are structurally and functionally different from miRNAs. In recent years, thousands of lncRNAs have been identified in the human and mouse genomes, many of which are of intergenic origin, have their own promoters, and are tissue-specifically transcribed. Such transcripts often possess a 5′ cap structure and are polyadenylated; nevertheless, they do not possess extended ORFs and hence have little coding potential. lncRNAs carry out diverse nuclear and cellular tasks, including nucleation of nuclear domains, bridging of proteins and chromatin, and acting as decoy or scaffold (Ulitsky and Bartel, 2013).

Ubiquitination

Although posttranscriptional regulators typically affect mRNA stability, the function and half-life of proteins are profoundly controlled by ubiquitination. This posttranslational modification involves three different types of proteins, termed E1, E2, and E3, which successively activate, transfer, and covalently link one or more 76-aa-long ubiquitins to target proteins. The highly conserved polypeptide chain of ubiquitin contains seven different lysines (K), all of which can be used to build side chains in polyubiquitination. Among many linkage possibilities, K48 polyubiquitination usually targets proteins for proteasomal degradation, whereas K63 polyubiquitination may confer activation of kinases or change the intracellular location of proteins. E3 ubiquitin ligases act in a target-specific manner, thereby explaining why hundreds of such gene products have evolved. Ubiquitination is a reversible process, and given its complexity, it is little surprising that ∼100 DUBs are predicted in the human proteome. In the last 10 years, the functions of a remarkable number of E3 ligases and DUBs have been elucidated. Their targets include key factors of T cell differentiation, such as the master transcription factors T-bet, Gata3, Roryt, Foxp3, and Bcl6 (Figs. 1, 2, 3, 4, and 5).

Pten (Fig. 1; Jiang et al., 2011). Recently, the role of miR-17∼92 in T cells was analyzed during lymphocytic choriomeningitis virus (LCMV) infection of mice (Baumjohann et al., 2013; Kang et al., 2013; Wu et al., 2015). In WT mice, the viral challenge would typically cause naive antigen-specific T cells to differentiate into Th1 or Tfh cells. These subsets control the infection by either producing IFNγ and TNFα or inducing germinal center (GC) reactions and humoral immune responses, respectively. Upon LCMV infection, mice with a T cell–specific ablation of the miR-17∼92 cluster predominantly exhibited a reduced clonal expansion of antigen-specific Th1, but also of Tfh cells (Wu et al., 2015). Consistently, opposing phenotypes were observed in transgenic mice overexpressing miR-17∼92 (Wu et al., 2015).

Another well-characterized, immune cell-expressed miRNA is miR-155. Similar to the miR-17∼92 cluster, miR-155 promotes T cell–dependent immunity in a variety of contexts. miR-155 maps to the *bic* locus, whose role in the development of B cell malignancies had been known before the discovery of miRNAs. miR-155 is strongly up-regulated during T cell activation and has important functions during humoral immune responses mediated by Tfh cells (Hu et al., 2014). However, miR-155 also drives T cell–dependent tissue inflammation by enhancing Th17 and Th1 differentiation. A Th1-promoting effect of miR-155 was demonstrated through retroviral overexpression of either a miR-155–encoding sequence or an antagomir of miR-155 in activated T cells, causing the increased or decreased production of the Th1 cytokine IFNγ, respectively. Although counterintuitive, one target of miR-155 is the mRNA encoding for the IFNγRα, which may contribute to the observed down-regulation of this receptor in Th1 cells (Banerjee et al., 2010; Fig. 1). Genetic ablation of miR-155 resulted in a bias toward Th2, and this effect was at least in part attributed to a direct, miR-155-dependent repression of the transcription factor c-MAF (Fig. 2), a potent transactivator of the Th2 cytokine IL-4 (Rodriguez et al., 2007; Thai et al., 2007). c-MAF expression is controlled both at the posttranscriptional level and epigenetically through Th1 subset–specific expression of the lncRNA linc-MAF-4 (Fig. 1). During T cell activation under either Th1- or Th2-polarizing conditions, linc-MAF-4 and c-MAF expression are inversely correlated, and linc-MAF-4 recruits the chromatin modifiers EZH2 and LSD1 to silence the c-MAF–encoding locus (Ranzani et al., 2015). The clinical relevance of these findings was recently suggested for multiple sclerosis (MS), as increased levels of linc-MAF-4 were found in peripheral blood mononuclear cells of MS patients (Zhang et al., 2017).

In contrast to miR-155 and miR-17∼92, miR-29 limits IFNγ expression in Th1 cells (Fig. 1). The miR-29 family consists of miR-29a, b, and c and is transcribed from two bicistronic clusters. miR-29s have pleiotropic effects in normal hematopoiesis, immune responses, and hematologic malignancies (Amodio et al., 2015). A broad repertoire of partly unique functions has been reported for miRNAs of this family. In a gain-of-function screen using miRNA-deficient CD4 T cells, the electroporation of miR-29a and miR-29b mimics reduced aberrantly high IFNγ levels (Steiner et al., 2011). Supporting these results, the activation of T cells from miR-29ab1 cluster-deficient mice under Th1-skewing conditions showed strong derepression of IFNγ and the master transcription factor T-bet (Smith et al., 2012). Both were confirmed as direct miR-29 targets in HEK-293 and T cells (Fig. 1; Ma et al., 2011; Steiner et al., 2011; Smith et al., 2012). Because IFNγ was identified as a driver of miR-29 expression (Smith et al., 2012), this negative feedback loop seems to control overshooting cytokine expression in Th1 cells.

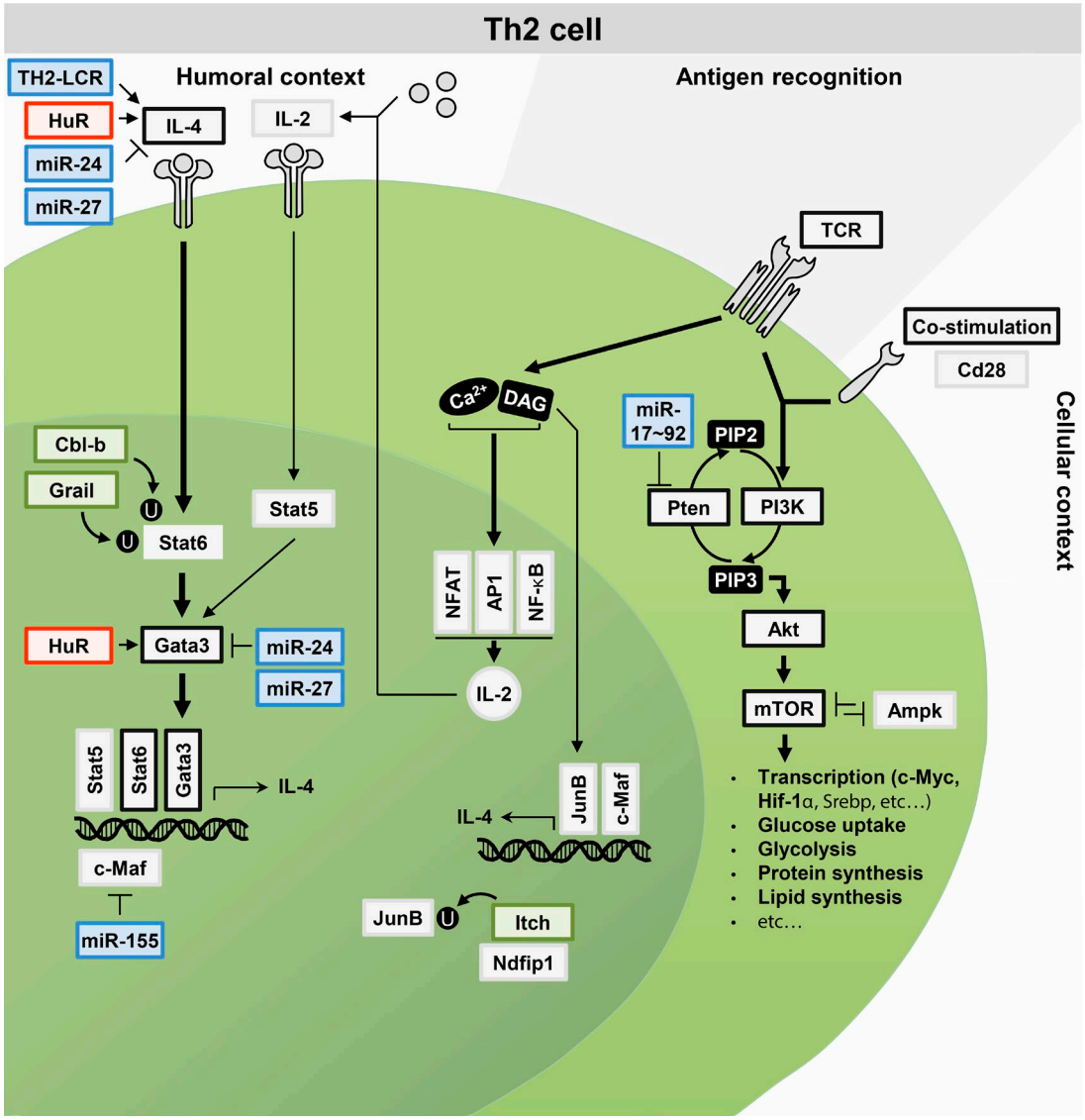

Figure 2. **Regulatory processes during Th2 differentiation.** Noncoding RNAs, RBPs, and E3 ligases/DUBs are shown in blue, red, and green, respectively. For clarity of depiction, these posttranscriptional regulators were not necessarily placed at the correct cellular site of activity. Molecular interactions are explained in the text.

Posttranslational regulation of Th1 cells

The strength of the TCR signal affects T cell activation and differentiation. Proximal pathway components are therefore tightly controlled and modulated by phosphorylation and/or ubiquitination. The deubiquitinase Otud7b has been identified as a positive regulator of Zap70 (Fig. 1), a tyrosine kinase and key upstream mediator of TCR signaling (Hu et al., 2016). Indeed, in vitro activated naive Otud7b$^{-/-}$ T cells produced less IL-2, and aged Otud7b-deficient mice had increased frequencies and numbers of naive CD4 T cells, whereas those of effector-memory phenotype were decreased. Additionally, in vitro differentiated Otud7b$^{-/-}$ Th1 cells produced less IFNγ, a result that was substantiated by in vivo experiments showing that Otud7b$^{-/-}$ mice generated fewer Th1 cells in response to bacterial infection or during myelin oligodendrocyte glycoprotein (MOG)–induced experimental autoimmune encephalomyelitis (EAE; Hu et al., 2016). Mechanistically, Otud7b deubiquitinated Zap70 at K544. Such deubiquitination attenuated its interaction with negative regulatory phosphatases (Carpino et al., 2009) and reinforced TCR signaling (Hu et al., 2016).

Differentiation of Th1 cells results from IL-12 and IFNγ signaling through Stat4 and Stat1, respectively (Fig. 1). In the context of TCR/Ca^{2+}-dependent NFAT activation, Stat4 induces expression of T-bet, which subsequently causes up-regulation of Th1 signature genes. In addition to the mentioned miRNAs, regulation of Th1 differentiation also occurs on the posttranslational level. The E3 ligase Slim was the first ubiquitinating enzyme found to target Stat proteins (Tanaka et al., 2005), which are rapidly activated and subsequently deactivated (Villarino et al., 2017). Slim-mediated ubiquitination of Stat1 and Stat4 triggered proteasome-dependent degradation and reduced IFNγ production (Fig. 1; Tanaka et al., 2005). Additionally, the E3 ligase Smurf1 was found to engage in the ubiquitination and destabilization of Stat1 (Fig. 1). In a series of biochemical experiments, Smurf1

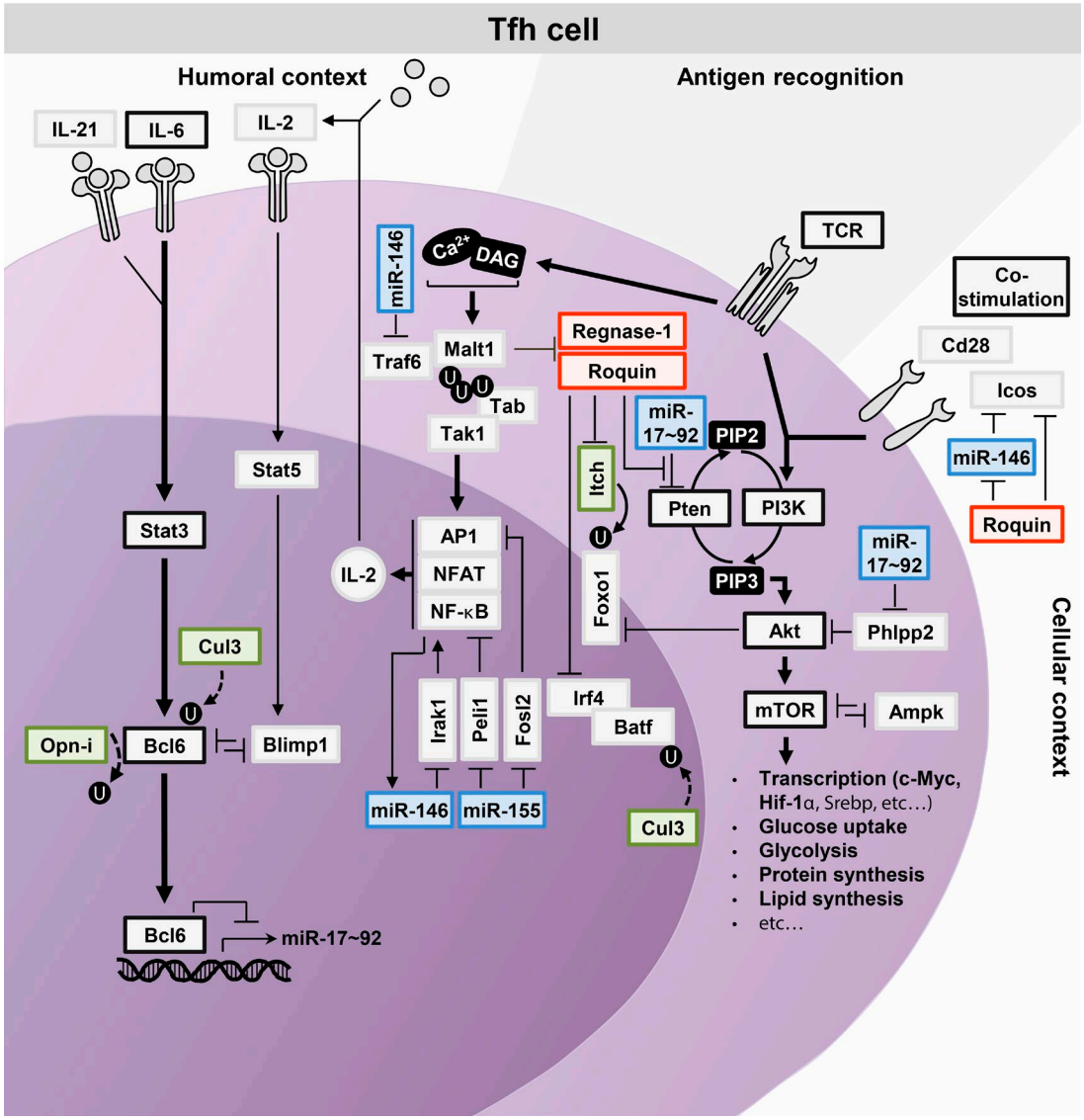

Figure 3. **Regulatory processes during Tfh differentiation.** Noncoding RNAs, RBPs, and E3 ligases/DUBs are shown in blue, red, and green, respectively. For clarity of depiction, these posttranscriptional regulators were not necessarily placed at the correct cellular site of activity. Dashed lines indicate indirect effects. Molecular interactions are explained in the text.

interacted with Stat1 and promoted its K48-linked polyubiquitination in a phosphorylation-independent manner, which negatively affected IFNγ signaling (Yuan et al., 2012). Interestingly, IFNγ also induced expression of Smurf1, thereby creating a negative feedback loop (Yuan et al., 2012).

Antagonizing the function of the E3 ligases Slim and Smurf1, the deubiquitinase Usp13 was identified in an RNAi screen searching for a positive regulator of IFNα-mediated antiviral effects (Yeh et al., 2013). Usp13 mainly interacted with the nonactivated form of Stat1 (Fig. 1). Affecting the same pathway further downstream, the deubiquitinase Usp10 was demonstrated to bind, deubiquitinate, and stabilize T-bet in the nucleus (Fig. 1; Pan et al., 2014).

Posttranscriptional regulation of Th2 cells

The majority of cytokine mRNAs contain adenine and uridine (AU)–rich elements (AREs) in their 3′ UTRs (Turner et al., 2014). These can be recognized by RBPs of the tristetraproline family and by the ARE/poly(U)-binding/degradation factor 1 (Auf1), which typically inhibit expression of the respective cytokine. AREs are also bound by the RBP Hu antigen R (HuR), which in contrast stabilizes these transcripts. So far, only HuR has been implicated in the differentiation of CD4 T cells, despite a likely function of other ARE-binding factors. Specifically, HuR was shown to promote Th2 cell differentiation, as it directly interacted with the 3′UTRs of the subset-specifying transcription factor Gata3, as well as with those of the signature cytokines IL-4 and IL-13 (Fig. 2; Stellato et al., 2011; Gubin et al., 2014).

Similar to some RBPs, miRNAs are involved in mRNA destabilization to influence Th2 cell differentiation. miRNA profiling of human CD4 T cells derived from asthma patients and healthy donors revealed elevated miR-19a expression in this Th2-driven disease (Simpson et al., 2014). Culturing miR-17~92–deficient mouse CD4 T cells under Th2-polarizing conditions

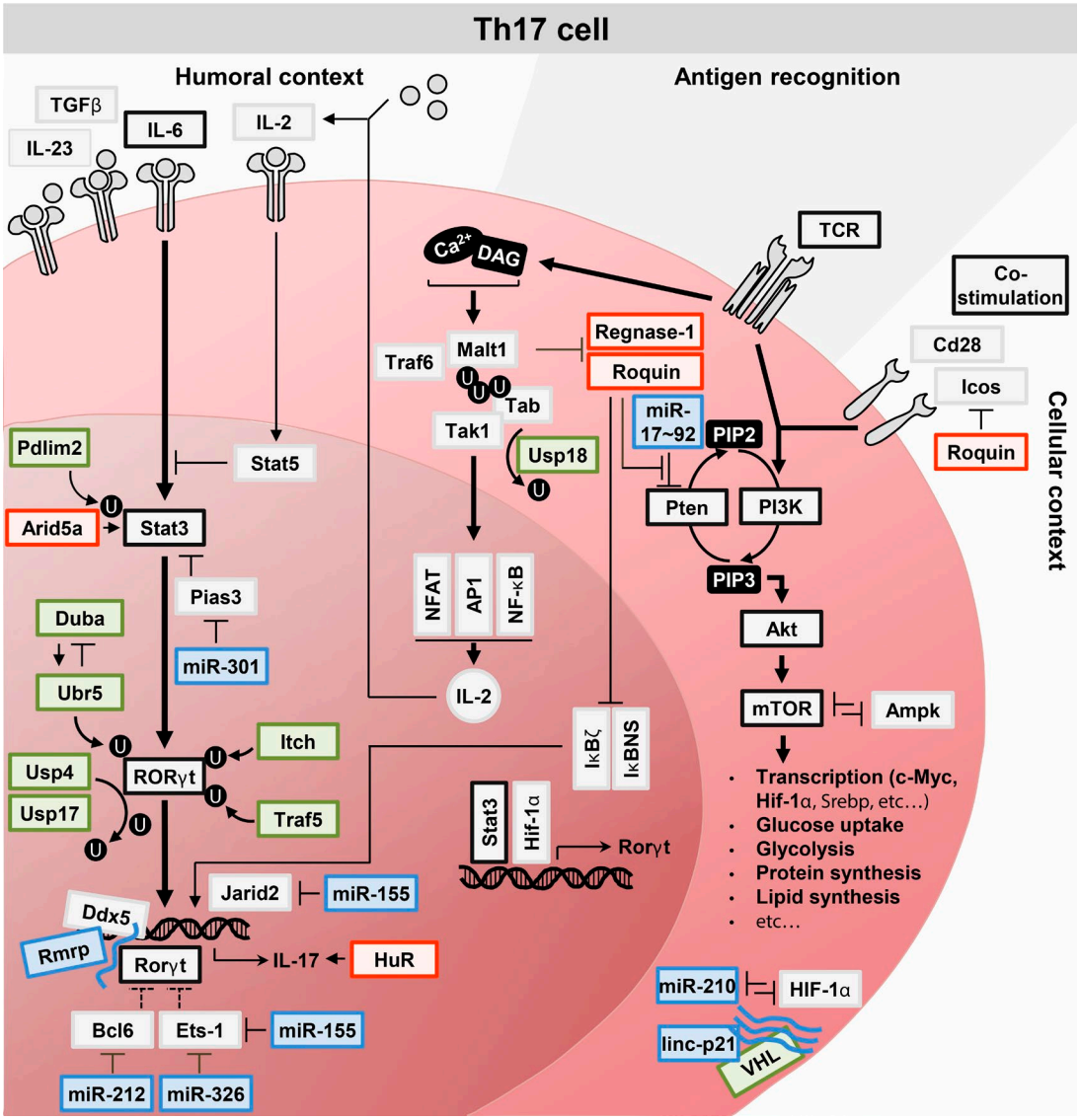

Figure 4. **Regulatory processes during Th17 differentiation.** Noncoding RNAs, RBPs, and E3 ligases/DUBs are shown in blue, red, and green, respectively. For clarity of depiction, these posttranscriptional regulators were not necessarily placed at the correct cellular site of activity. Dashed lines indicate a suspected effect. Molecular interactions are explained in the text.

demonstrated reduced IL-4 and IL-13 expression. Transfection with miR-19a and miR-19b mimics restored WT cytokine levels (Fig. 2), whereas the remaining miRNAs of the cluster only marginally rescued the phenotype. Investigating 38 previously identified direct miR-19 targets, Simpson et al. (2014) found that knockdown of *Socs1* and *A20* specifically increased the production of Th2 cytokines, indicating a functional correlation between miR-19 and these two target genes. Further evidence came from a gain-of-function screen involving electroporation of miRNA mimics into miRNA and T-bet double-deficient T cells, which independently identified miR-19a as a positive regulator of IL-4 expression (Pua et al., 2016). In the same experiment, miR-24 and miR-27 were found to limit IL-4 production (Fig. 2). These miRNAs are encoded in two polycistronic clusters together with miR-23. In a mouse model of allergic airway inflammation, elimination of both clusters in T cells enhanced Th2 cytokine production and increased eosinophil infiltration of the lung (Pua et al., 2016). A network of overlapping miR-24 and miR-27 targets was identified that included the Th2 master transcription factor Gata3 (Fig. 2), but also factors not previously associated with Th2 cell biology, such as the deadenylation complex component Cnot6 (Pua et al., 2016). In a different study, transgenic mice were produced that overexpressed the whole miR-23~27~24 cluster or each of the three miRNAs individually in a T cell–specific manner (Cho et al., 2016). Surprisingly, cultivation of miR-23~27~24–expressing T cells under different polarizing conditions resulted in reduced differentiation of Th1, Th2, Th17, and iTreg cells, as opposed to the increased effector-memory phenotype and augmented cytokine production after ex vivo stimulation of CD4 T cells from this transgenic mouse. Analyzing the effects of individual miRNAs in the same experimental setting showed that miR-23 and miR-27 functions mirrored the results previously obtained for the whole cluster, whereas miR-24 promoted Th1, Th17, and iTreg differentiation. These findings show that under

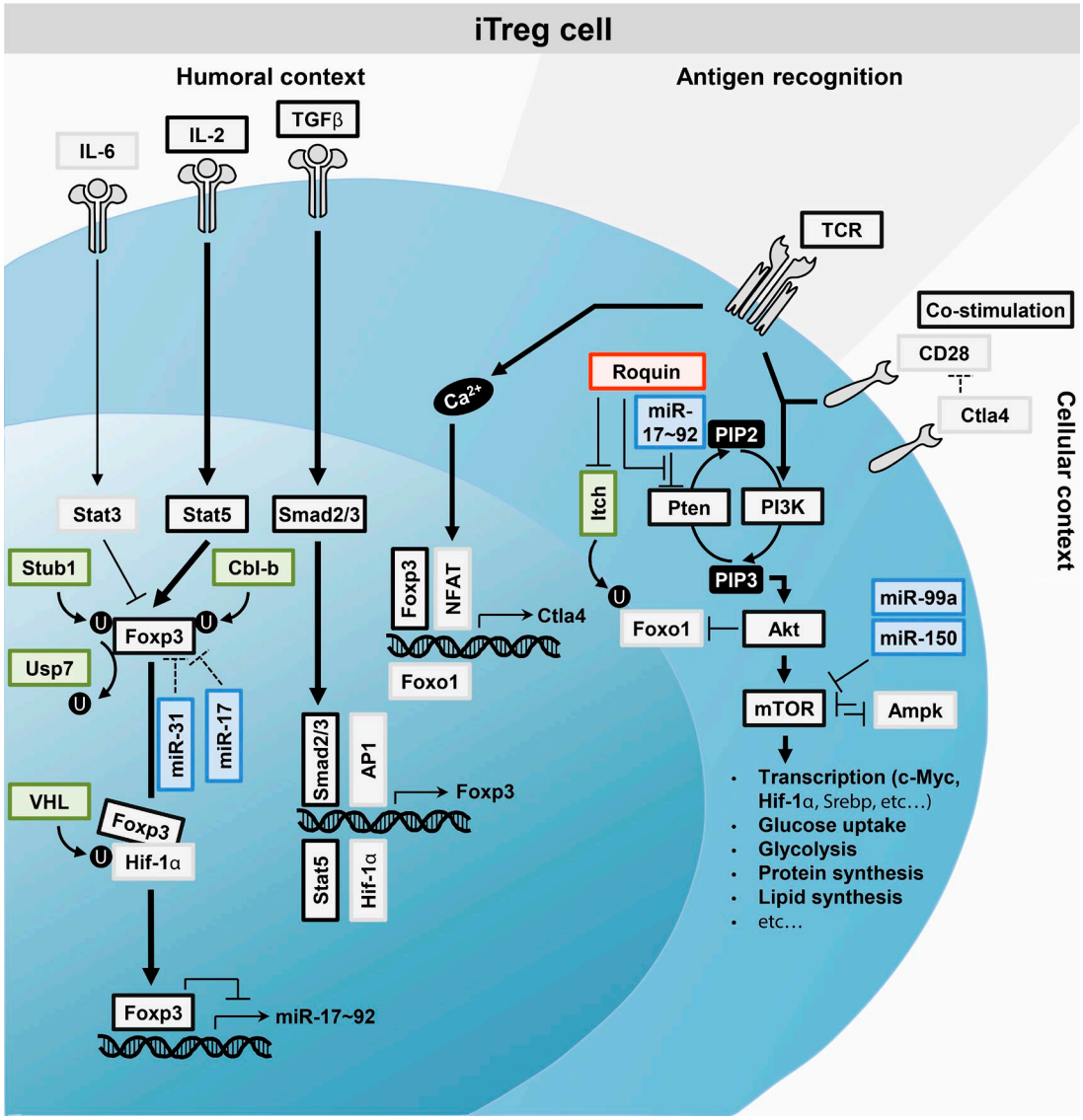

Figure 5. **Regulatory processes during iTreg cell differentiation.** Noncoding RNAs, RBPs, and E3 ligases/DUBs are shown in blue, red, and green, respectively. For clarity of depiction, these posttranscriptional regulators were not necessarily placed at the correct cellular site of activity. Dashed lines indicate an indirect effect. Molecular interactions are explained in the text.

different conditions, members of the same cluster can exhibit cooperative or antagonistic functions.

lincRNAs, which are lncRNAs expressed from intergenic transcripts, have also been found to have a supporting function for Th2 cell differentiation. A whole-genome sequencing approach identified 28, 17, or 29 lincRNAs to be selectively expressed in human Th1, Th2, or Th17 cells, respectively (Spurlock et al., 2015). Among these, the Th2-specific TH2-LCR promoted the expression of IL-4, IL-5, and IL-13, as demonstrated by a knockdown approach in developing human Th2 cells (Fig. 2). The Th2 locus control region (LCR) has previously been established by genomic deletion of sequences that showed Th2-specific demethylation after antigenic stimulation (Lee et al., 2005; Koh et al., 2010). The newly discovered expression and function of Th2-LCR now provide a molecular explanation why this genomic region has critical importance for Th2 responses in vitro and in vivo (Spurlock et al., 2015).

Posttranslational regulation of Th2 cells

The E3 ligase Itch has diverse yet specific functions in the differentiation of helper T cells, as it crucially influences Th2, Tfh, and Th17 cells (Figs. 2, 3, and 4). Itch-deficient (*itchy*) mice have activated, strongly proliferating T cells, increased IL-4 cytokine production, and elevated levels of IgG1 and IgE antibodies in addition to a severe immune dysregulation manifested in inflammation of the large intestine and skin (Perry et al., 1998; Fang et al., 2002). Molecularly, Itch ubiquitinates and induces proteasomal degradation of JunB (Fig. 2; Fang et al., 2002). JunB cooperates with c-MAF in the activation of IL-4 expression in Th2 cells (Li et al., 1999). The function of Itch likely depends on its association with and activation by Nedd4 family interacting protein 1 (Ndfip1), a transmembrane protein associated with intracellular vesicles and the Golgi apparatus. Ndfip1$^{-/-}$ mice phenocopied *itchy* mice, and the phenotypes were reminiscent of the human condition atopic dermatitis (Oliver et al., 2006).

Investigations of posttranslational regulation of IL-4 signaling indicate that the E3 ligases Grail and Cbl-b both target Stat6 and thereby control Th2 differentiation (Fig. 2). Grail expression was up-regulated by Stat6 and Gata3 during T cell activation in an IL-4–dependent manner, specifically in Th2 cells (Sahoo et al., 2014). As negative feedback, Th2 differentiation was strongly repressed by Grail. In vitro–differentiated naive Grail$^{-/-}$ T cells generated higher levels of the Th2 cytokines IL-4, IL-5, and IL-13 as well as increased expression of Gata3 and IL-4R. These results were confirmed in Grail-deficient mice, which not only produced more Th2 cells and cytokines after OVA immunization, but also were more susceptible to the induction of allergic asthma. Stat6 was identified as a direct target of the E3 ligase Grail, and its ubiquitination resulted in proteasomal degradation (Sahoo et al., 2014). Surprisingly, similar results were presented for Cbl-b, whose induced expression in Th2 cells appeared to be more dependent on TCR and costimulatory receptor signaling (Qiao et al., 2014). Whereas the exact site of Grail-mediated ubiquitination of Stat6 remains unknown, amino acids K108 and K398 of Stat6 were identified as being specifically ubiquitinated by Cbl-b. Consistently, reexpression of Stat6 with the corresponding mutations in Stat6$^{-/-}$ CD4 T cells increased the ability of reconstituted cells to produce IL-4 (Qiao et al., 2014).

Posttranscriptional regulation of Tfh cells

In Tfh cells, the subset-specifying transcription factor Bcl6 is up-regulated by Stat3 in response to the cytokines IL-6 and IL-21 (Fig. 3). Additionally, costimulatory signals transmitted through the Icos-PI3K-Akt axis are essential for Tfh cell differentiation (Rolf et al., 2010). Several miRNAs have been implicated in the regulation of Tfh cells, including miR-17∼92, miR-155, and miR-146 (Maul and Baumjohann, 2016).

As mentioned above, mice with a T cell–specific ablation of the miR-17∼92 cluster had defects not only in Th1 but also in Tfh cell differentiation (Baumjohann et al., 2013; Kang et al., 2013; Wu et al., 2015). A recent publication suggested a promoting contribution of miR-92a on human Tfh cell differentiation (Serr et al., 2016); however, the individual contributions of the remaining cluster miRNAs are not well understood. miRNAs of the miR-17∼92 cluster were found to directly target negative regulators of the PI3K-Akt-mTOR pathway. These include *Pten*, coding for a PIP3 phosphatase, and *Phlpp2*, an Akt-inhibiting phosphatase (Fig. 3; Xiao et al., 2008; Jiang et al., 2011; Baumjohann et al., 2013; Kang et al., 2013; Liu et al., 2014; Simpson et al., 2014). This is consistent with a positive, dose-dependent correlation of PI3K signaling and Tfh cell differentiation (Rolf et al., 2010). In addition to its Tfh-promoting function, miR-17∼92 inhibited subset-inappropriate gene expression through directly targeting the Th17-associated transcription factor *Rora* (Baumjohann et al., 2013).

An exciting and little-explored field of research is how RBPs and miRNAs engage in mutual regulation, cooperation, or antagonism. In addition to the Roquin-mediated inhibition of *Icos*, *Ox40*, and *Irf4* mRNA expression (Yu et al., 2007; Glasmacher et al., 2010; Vogel et al., 2013; Jeltsch et al., 2014; Schlundt et al., 2014; Janowski et al., 2016; Rehage et al., 2018), which are critical for Tfh differentiation, Roquin-1 can also interact with the Tfh cell–inhibiting miR-146a and Ago2 to regulate miRNA homeostasis (Srivastava et al., 2015). In another example, Regnase-1 down-regulates miRNAs by interacting with the terminal loop of pre-miRNA hairpins and interfering with Dicer-mediated miRNA biogenesis (Suzuki et al., 2011). Moreover, Roquin interacts with a putative stem-loop structure in the 3′ UTR of *Pten*, which overlaps with a miRNA binding site, thereby inhibiting the recognition of the complementary sequences by members of the miR-17∼92 cluster (Essig et al., 2017). As a result, Roquin-deficient T cells revealed increased PI3K-Akt-mTOR signaling. Hence, inhibition of mTOR signaling by rapamycin treatment in vivo prevented the spontaneous Tfh differentiation that is typically observed upon induced deletion of Roquin-encoding alleles (Essig et al., 2017).

Systemic deletion or transgenic overexpression of miR-155 reduced or enhanced GC responses, respectively (Fig. 3; Hu et al., 2014). In line with these findings, it has recently been shown in a Mir-146$^{-/-}$ mouse model of age-dependent inflammation that miR-155 was required for Tfh cell expansion, increased numbers of GC B cells, and generation of autoantibodies (Hu et al., 2014). 21 target genes, including Peli1 and Fosl2, were identified (Fig. 3). During Tfh cell differentiation, Peli1 and Fosl2 are involved in repressing the NF-κB and AP1 pathways, respectively (Hu et al., 2014). Moreover, miR-146a was found to be highly expressed in Tfh and GC B cells (Pratama et al., 2015). The mRNA of the important costimulatory receptor *Icos* was established as one of its direct targets in vitro (Pratama et al., 2015). In vivo experiments further highlighted the strong contribution of impaired Icos regulation in miR-146–deficient mice. Promoter analysis and subsequent experiments revealed that miR-146a expression was up-regulated by the NF-κB pathway and itself directly targeted Traf6 and Irak1 to create a negative feedback loop (Fig. 3; Taganov et al., 2006). miR-146a deficiency caused a spontaneous increase of Tfh and GC B cells, which was cell autonomous for T and B cells (Pratama et al., 2015). Therefore, miR-146a expression at later time points during Tfh cell differentiation opposes the activities of miR-155 and miR-17∼92, which are expressed early on. Adding to the regulatory complexity, a novel connection of miR-146a and an RBP has recently been elucidated, as Roquin was found to bind to mature miR-146a (Fig. 3) and induced its decay, possibly involving 3′-end mono-uridylation (Srivastava et al., 2015). These findings suggest cooperative regulation of mouse *Icos* mRNA by both factors (Fig. 3). However, Roquin-dependent regulation of the human ICOS 3′ UTR was unimpaired in cells with strongly reduced miRNA levels, including Dicer-deficient mouse embryonic fibroblast cells or Ago-deficient embryonic stem cells (Glasmacher et al., 2010).

Posttranslational regulation of Tfh cells

Adding to the pleiotropic functions of Itch in T helper cell development, it indirectly promotes Bcl6 expression (Fig. 3; Xiao et al., 2014). Analysis of T and B cell responses of *Itch*$^{fl/fl}$*; Cd4-Cre* mice after vaccinia virus infection revealed decreased Tfh and GC B cell numbers/frequencies as well as strongly reduced virus-specific antibody production (Xiao et al., 2014). Induced deletion of Foxo1 in *Itch*$^{fl/fl}$;*Foxo1*$^{fl/fl}$ T cells rescued the phenotype (Fig. 4). Mechanistically, Itch ubiquitinates Foxo1 for proteasomal degradation (Xiao et al., 2014). Interestingly, *Itch* mRNA is a target of the RBP Roquin, which down-regulated Itch expression to promote

Foxo1 abundance (Essig et al., 2017). Therefore, the E3 ligase Itch emerges as a potent positive regulator of Tfh differentiation while inhibiting Th2 and Th17 development. Conversely, Roquin proteins repress Tfh and Th17 cell differentiation, and their functions have been attributed to the ROQ domain, which is responsible for RNA binding and induced mRNA decay (Glasmacher et al., 2010; Jeltsch et al., 2014; Schlundt et al., 2014; Sakurai et al., 2015). However, the T cell–specific deletion of the RING domain of Roquin-1, but not combined deletion of the RING finger in Roquin-1 and Roquin-2, led to the paradoxical phenotype of decreased Tfh cell differentiation (Pratama et al., 2013; Ramiscal et al., 2015). Although induced activity of AMPK, a negative regulator of mTOR signaling, has been implicated in this phenotype, a direct target for the E3 ligase activity of Roquin is still elusive. Because complete deletion of Roquin-1– and Roquin-2–encoding alleles increased mTOR signaling (Essig et al., 2017), the RING finger may potentially be an intramolecular antagonist of Roquin function.

A mechanism to stabilize Bcl6 in response to Icos signaling involves the intracellular form of the phosphoprotein osteopontin (Opn-i; Fig. 3; Leavenworth et al., 2015). Opn-i is strongly expressed in Tfh cells, and sophisticated mouse models demonstrated that the intracellular form of Opn was essential for normal Tfh and GC B development. Intriguingly, costimulation via Icos induced binding of p85α, the regulatory subunit of PI3K, to Opn-i. This led to the translocation of Opn-i to the nucleus, where it directly or indirectly protected Bcl6 from proteasomal degradation (Leavenworth et al., 2015).

Bcl6 expression is also down-regulated by the histone-ubiquitinating E3 ligase cullin 3 (Cul3). Cul3 and Bcl6 are both highly and transiently expressed in $CD4^+CD8^+$ thymocytes and interact to generate Ub-histone marks that were maintained in single-positive $CD4^+$ thymocytes. Deletion of Cul3 in T cells resulted in the derepression of Batf and Bcl6 and increased Tfh cell frequencies (Fig. 3; Mathew et al., 2014). The transcription factor Batf is a critical determinant of Bcl6 induction and a global regulator of the Tfh program (Betz et al., 2010; Ise et al., 2011).

Posttranscriptional regulation of Th17 cells

Th17, Tfh, and Th1 cell differentiation are under control of the RBPs Roquin-1, Roquin-2, and Regnase-1 (Figs. 3 and 4). These proteins target a common set of mRNA transcripts through interactions with defined stem-loop structures in 3′UTRs (Matsushita et al., 2009; Leppek et al., 2013; Uehata et al., 2013; Schlundt et al., 2014; Schuetz et al., 2014; Tan et al., 2014; Sakurai et al., 2015; Janowski et al., 2016). Their targets include several immune-stimulatory genes: *Tnf*, *Icos* (Figs. 3 and 4), *Ox40*, *Nfkbiz* (coding for IκBζ; Fig. 4), *Nfkbid* (coding for IκBNS; Fig. 4), *cRel*, *Zc3h12a* (coding for Regnase-1; Figs. 3 and 4), *Irf4*, and *IL6* (Figs. 3 and 4; Matsushita et al., 2009; Suzuki et al., 2011; Leppek et al., 2013; Uehata et al., 2013; Vogel et al., 2013; Jeltsch et al., 2014; Mino et al., 2015). Indeed, *Nfkbiz*, *Nfkbid*, *cRel*, *Irf4*, and *Il6* have been described as key regulators of Th17 differentiation (Brüstle et al., 2007; Okamoto et al., 2010; Chen et al., 2011; Reinhard et al., 2011; Ruan et al., 2011; Kobayashi et al., 2014; Annemann et al., 2015). Regnase-1, Roquin-1, and Roquin-2 proteins all harbor conserved sequence motifs that are recognized and cleaved by the paracaspase Malt1 (Figs. 3 and 4), which inactivates these proteins downstream of the TCR (Uehata et al., 2013; Gewies et al., 2014; Jeltsch et al., 2014). Increased cleavage of Roquin proteins by Malt1 was correlated with high-affinity peptide/MHC recognition, and different targets of Roquin were recognized with very different affinity/avidity in cells (Schlundt et al., 2014). Thereby, differential gene regulation can be achieved in this system through altered TCR signal strength, which may then skew T helper cell differentiation (Jeltsch and Heissmeyer, 2016). The combined knockout of Roquin-1 and Roquin-2 elicited an accumulation of Th17 and Tfh cells in secondary lymphoid organs, as well as Th17 and neutrophils in the lung (Vogel et al., 2013; Jeltsch et al., 2014). A predisposition toward Th17, Tfh, and Th1 differentiation of Roquin-deficient T cells was demonstrated as a cell-intrinsic property in ex vivo–cultivated CD4 T cells (Vogel et al., 2013; Jeltsch et al., 2014; Essig et al., 2017).

Mice with Regnase-1–deficient T cells exhibit autoantibody production and excessive IFNγ levels, which may contribute to the increased Tfh frequencies (Uehata et al., 2013). Ex vivo–stimulated CD4 T cells from spleens of these mice showed strongly increased IFNγ-producing Th1 cells and somewhat increased IL-17A–producing Th17 cells (Uehata et al., 2013; Cui et al., 2017). Indirect evidence that Regnase-1 impinges on Th17 cell differentiation also comes from studies on a posttranscriptional antagonist of Regnase-1 function, Arid5a, which competes with Regnase-1 for binding to stem-loop structures in 3′UTRs (Masuda et al., 2013, 2016). In gain- and loss-of-function experiments, it was shown that Arid5a or Regnase-1 stabilized or destabilized *IL-6* and *Stat3* mRNAs, respectively (Fig. 3; Masuda et al., 2013, 2016). Consistently, Arid5a-deficient T cells showed reduced differentiation into Th17 cells, and $Arid5a^{-/-}$ mice were less susceptible to EAE, a mouse model with similarities to the human disease MS (Masuda et al., 2013).

Apart from RBPs, many noncoding RNAs have been identified to skew Th17 cell differentiation (miR-17∼92, miR-155, miR-301, miR-132/212, miR-326, miR-210, Rmrp, and lincRNA-p21). T cell–specific deficiency of miR-17∼92 reduced Th17 cell differentiation and ameliorated symptoms of EAE after $MOG_{35–55}$ immunization (Liu et al., 2014). Th17 development could be partly compensated by retroviral reintroduction of miR-19b or miR-17 (Jiang et al., 2011). Paradoxically, miR-20b, a miRNA that shares an identical seed sequence with miR-17, repressed rather than promoted Th17 cell differentiation in vitro. Furthermore, miR-20b was down-regulated in CD4 T cells of EAE mice (Zhu et al., 2014) as well as in blood cells of MS patients (Keller et al., 2009). Consistently, the transcripts of Rorγt and Stat3 were identified as direct targets of miR-20b (Zhu et al., 2014). Along the same lines, miR-18a was demonstrated to be a potent inhibitor of Th17 differentiation in vitro and in vivo, repressing *Smad4*, *Hif1α*, and *Rora* (Montoya et al., 2017).

miR-155 controls the epigenetic regulator Jarid2, whose down-regulation is required for Th17 differentiation and expression of IL-17 (Escobar et al., 2014). miR-155–dependent differentiation of pathogenic Th17 cells was elucidated by analyzing EAE in $miR155^{-/-}$ mice after $MOG_{35–55}$ immunization (O'Connell et al., 2010). Compared with their WT counterparts, $miR155^{-/-}$ mice displayed fewer neurological symptoms at later time points and with reduced incidence (Fig. 4).

An early event during Th17 differentiation involves Stat3 phosphorylation in response to IL-6 stimulation. This process is promoted by miR-301a (Mycko et al., 2012), a miRNA that was identified in a profiling approach analyzing MOG_{35-55}–responsive CD4 T cells. In vitro, miR-301a was strongly expressed in Th17 cells, and transfection of a miR-301a antagomir inhibited IL-6–induced Stat3 phosphorylation. Pias3, a potent inhibitor of Stat3, was identified as a direct target of miR-301a (Fig. 4). Consistently, down-regulation of miR-301a or expression of Pias3 in the transferred MOG-specific T cells resulted in the expected ameliorated or exacerbated EAE disease, respectively (Mycko et al., 2012). Stat3 regulates the transcription of the lineage-specifying factor Rorγt, whose activity critically depends on posttranscriptional and posttranslational regulation (discussed below).

Enrichment of Rorγt-containing complexes and subsequent mass spectrometry identified the helicase Ddx5 (Fig. 4) as a potential cofactor (Huang et al., 2015). T cell–specific deletion of Ddx5 inhibited Th17 differentiation in vitro, and its function as a cofactor depended on its helicase activity. Immunoprecipitation of Ddx5 or Rorγt and sequencing of associated RNA identified the lncRNA *Rmrp*, which has a crucial role in the localization of the tripartite Rorγt/Ddx5/*Rmrp* complex on Rorγt-bound genes (Fig. 4; Huang et al., 2015). The importance of *Rmrp* sequence integrity for Rorγt activity was elegantly confirmed by CRISPR/Cas9-mediated single nucleotide exchange (270G>T) in the *Rmrp* locus of the mouse genome. This point mutation inactivates *Rmrp* function and is often found in human cartilage-hair hypoplasia syndrome, a rare disorder that includes symptoms such as defective immunity and predisposition to lymphoma (Mäkitie et al., 1998; Bonafé et al., 2005).

Optimal Rorγt-mediated transcription of the IL-17 signature cytokine genes partly depends on the aryl hydrocarbon receptor (Veldhoen et al., 2009), which induces miR-132/212 expression during Th17 differentiation (Nakahama et al., 2013). Mice deficient in the miR-132/212 cluster exhibited reduced EAE scores potentially because of inappropriate Tfh gene expression in Th17 cells in response to increased Bcl6 levels. Accordingly, Bcl6, the lineage-specifying factor of Tfh cells and negative regulator of Th17 differentiation, was identified as a miR-212 target (Fig. 4; Nakahama et al., 2013).

Th17 and Th1 cells have been recognized as key drivers of chronic inflammatory demyelination of the central nervous system in MS patients. miR-326 and *IL17a* were correlated in their expression, and miR-326 was strongly overexpressed in CD4 T cells of relapsing, but not remitting, MS patients (Du et al., 2009). This result could be recapitulated in an EAE mouse model, and here Ets-1, a known repressor of Th17 development (Moisan et al., 2007), was identified as a direct target of miR-326 (Du et al., 2009) as well as of miR-155 (Fig. 4; Na et al., 2016). The diagnostic value of miR-326 was independently confirmed by a study analyzing peripheral blood lymphocytes from relapsing and remitting MS patients (Honardoost et al., 2014).

As T cells become activated, metabolic reprogramming leads to the Warburg effect: increased glycolysis and decreased oxidative phosphorylation, even in the presence of oxygen. In Th17 cells, metabolic reprogramming strongly depends on the transcription factor hypoxia-inducible factor 1-α (Hif-1α; Shi et al., 2011), which promotes transcription of the lineage-specifying transcription factor Rorγt (Fig. 4; Dang et al., 2011). Hif-1α levels are rigorously controlled by hypoxia-responsive miR-210 and lincRNA-p21 at the posttranscriptional level, but also posttranslationally by an E3 ligase called von Hippel–Lindau protein (VHL; Fig. 4). In response to hypoxia and/or TCR signaling, the up-regulation of Hif-1α promotes a dramatic but delayed increase in miR-210 expression (Wang et al., 2014). In turn, miR-210 directly repressed Hif-1α, thereby creating a negative feedback loop, possibly preventing an overshooting inflammatory response (Fig. 4; Wang et al., 2014).

Under hypoxic conditions that often occur after activation of T cells, binding of the E3 ligase VHL to Hif-1α is impaired, resulting in reduced polyubiquitination and an increased half-life of Hif-1α (Jaakkola et al., 2001). Additionally, Hif-1α is stabilized in a positive feedback loop through Hif-1α–dependent lincRNA-p21 expression (Yang et al., 2014). Mechanistically, lincRNA-p21 impedes the Hif-1α-VHL interaction by binding to both proteins independently (Fig. 4), thereby blocking a region in VHL that recognizes Hif-1α. This function of lincRNA-p21 indirectly promotes glycolysis and Th17 differentiation.

Finally, the Th17 signature cytokine IL-17 is also subject to posttranscriptional gene regulation. Its mRNA transcript was demonstrated to be a direct target of HuR (Fig. 4), an RBP with a known role in Th2 differentiation (Fig. 2). HuR-deficient T cells were less able to differentiate into Th17 cells, had less proliferative capacity, and showed attenuated disease scores of EAE, compared with WT (Chen et al., 2013a).

Posttranslational regulation of Th17 cells

At least four DUBs have been identified to coordinate Th17 differentiation by controlling TCR and IL-17 proximal signaling (Usp18), as well as Rorγt stability (Usp4, Usp17, and Duba). Deficiency of Usp18 reduced Th17 cell differentiation in vitro and in vivo as a result of hyperactivated NF-κB/NFAT signaling and increased levels of IL-2 (Liu et al., 2013). To activate the canonical NF-κB pathway, TCR proximal signaling involves the Tak1-Tab1 complex, of which Tak1 is a K63 ubiquitin-activated kinase (Fig. 4; Wang et al., 2001). Interestingly, in transfection experiments, Usp18 bound to and deubiquitinated Tak1, thereby reducing its kinase activity and the associated downstream signaling (Liu et al., 2013). Adoptive transfer of $Usp18^{-/-}$ CD4 T cells into $Rag1^{-/-}$ mice followed by MOG-dependent EAE induction caused delayed onset and less severe symptoms of EAE, most likely because of the inhibition of Th17 differentiation by the observed increase in IL-2 production (Liu et al., 2013).

IL-6 signals through Stat3, which promotes Th17 and suppresses iTreg lineage commitment. Activated Stat3 has been proposed as a nuclear target of the E3 ligase Pdlim2 (Fig. 4; Tanaka et al., 2011; Qu et al., 2012). Injection of $Pdlim2^{-/-}$ mice with heat-killed *Propionibacterium acnes* caused significantly more granuloma formation in the liver as compared to injected WT mice. T cells from $Pdlim2^{-/-}$ mice showed increased Th17 cytokine production including IL-17A, IL-22, IL-21, but also IFNγ or IL-4 in response to stimulation with these bacteria or upon stimulation with agonistic anti-CD3/anti-CD28 (Tanaka et al., 2011). Consistently, $Pdlim2^{-/-}$ mice were more susceptible to EAE as compared to WT counterparts (Qu et al., 2012).

In Th17 cells, increased activation of Stat3 leads to increased expression of Rorγt, whose function is heavily influenced by posttranscriptional and posttranslation mechanisms, including miRNAs (miR-212 and miR-326), lncRNAs (Rmrp), E3 ligases (Itch, Ubr5, and Traf5), and DUBs (Usp4, Usp17, and Duba). Whereas Usp4 and Usp17 simply stabilized Rorγt during Th17 differentiation by means of K48-linked ubiquitin removal (Fig. 4; Han et al., 2014; Yang et al., 2015), regulation by Duba was found to be more complex. Duba stabilized the E3 ligase Ubr5, which not only ubiquitinated Rorγt for proteasomal degradation, but also destabilized Duba in a negative feedback loop (Rutz et al., 2015). Consistently, T cell–specific deletion of Duba in mice increased Th17 and moderately decreased Treg cell frequencies, but caused Rorγt expression in some of the Treg cells (Rutz et al., 2015). Furthermore, injection of anti-CD3 antibodies triggered exacerbated inflammation in the lamina propria of the small intestine of *Duba*$^{fl/fl}$;*Cd4-Cre* mice (Rutz et al., 2015).

E3 ligases involved in the regulation of Rorγt include Ubr5, Itch, and Traf5 (Fig. 4). Apart from the well-known "itchy" phenotype of the skin, mice with an ablation of Itch developed spontaneous colitis and an increased incidence of associated colorectal cancer (Kathania et al., 2016). Colonic mucosa cells of these animals had elevated Rorγt and IL-17 levels, and the cellular sources of IL-17 production were identified as Th17 cells, innate lymphoid cells, and γδ T cells (Kathania et al., 2016). Increased IL-17 expression was likely a consequence of reduced Rorγt ubiquitination in the absence of Itch (Kathania et al., 2016). Although Ubr5 and Itch destabilized Rorγt, the E3 ligase Traf5 catalyzed K63-linked ubiquitination via its RING domain, stabilized its target, and increased IL-17a expression (Fig. 4; Wang et al., 2015). Together, this large number of E3 ligases and DUBs involved in the regulation of Rorγt underscore its pivotal role in the differentiation as well as pathogenicity of Th17 cells. However, the extent to which these ubiquitin-modifying enzymes work in a redundant, unique, or cooperative manner is currently unclear.

Posttranscriptional regulation of Treg cells

Only a few publications have directly investigated the posttranscriptional regulation of Treg cells. The RBP Lin28b has an interesting function in the differentiation of fetal Treg cells. Lin28b specifically recognizes terminal loops of pre-miRNA from let-7 family members and induces their degradation (Heo et al., 2008, 2009). In human fetal naive CD4 T cells, Lin28b expression levels were far higher compared with adult cells, and Lin28b knockdown led to increased let-7 miRNA maturation and decreased TGFβ signaling through TGFβR1, TGFβR2, and SMAD2 (Bronevetsky et al., 2016). Because TGFβ signaling is crucial for Treg cell differentiation (Fig. 5), Lin28b promotion of TGFβ signaling via inhibition of let-7 processing, and indirect stabilization of let-7 targets in the TGFβ pathway, help to explain the observed increased frequency of these cells in secondary lymphoid organs of the fetus and the resulting tolerance to maternal antigens. In contrast, naive CD4 T cells deficient for the RBPs of the Roquin family differentiated less efficiently into iTregs (Jeltsch et al., 2014). This phenotype could be rescued by inhibitors of PI3K-Akt-mTOR signaling, which promote iTreg differentiation (Sauer et al., 2008). These findings are consistent with Roquin targeting *Icos*, *Pten*, and *Itch* mRNAs (Essig et al., 2017) and with the importance of Foxo1 (Kerdiles et al., 2010) and Pten for Treg cell differentiation and function (Huynh et al., 2015; Shrestha et al., 2015).

Thus far, the combined inactivation of the miR-17∼92 cluster revealed its function as a driver of Th1, Th2, Tfh, and Th17 differentiation; however, it also inhibited the generation of Treg cells in vitro and in vivo (Jiang et al., 2011; de Kouchkovsky et al., 2013). These effects were mainly caused by miR-19 and miR-17, the latter of which repressed two regulators of Treg development, *Tgfbr2* and *Creb1* (Jiang et al., 2011). It was recently shown that miR-17 expression increased upon IL-6–mediated up-regulation of the transcription factor Hif-1α (Yang et al., 2016). Elevated miR-17 levels directly repressed cofactors (e.g., *Eos*) of the subset-specifying factor Foxp3. Accordingly, overexpression of miR-17 in Treg cells exacerbated pathology in a murine colitis model (Yang et al., 2016).

miR-31 was also identified as a negative regulator of iTreg differentiation (Fig. 5). Its expression is triggered by TCR signaling and down-regulated through TGFβ1-induced Foxp3 expression (Zhang et al., 2015). T cell–specific deletion of miR-31 ameliorated the symptoms of EAE and skewed the CD4 T cell–mediated immune balance away from pathogenic Th17 cells in the central nervous system. The orphan G protein–coupled receptor Gprc5a is a direct target of miR-31, but its molecular function in T cell differentiation has not been investigated so far. Nevertheless, a combined knockout (*miR-31*$^{fl/fl}$;*Cd4-Cre*;*Gprc5a*$^{-/-}$) rescued the miR-31 knockout–associated effect on EAE (Zhang et al., 2015).

An in vitro forward screen of adenovirally overexpressed miRNAs in naive T cells identified a small set of miRNAs that reciprocally increased iTreg and decreased Th17 cell differentiation (Warth et al., 2015). Dissecting the cooperative functions of induced miR-99a and constitutively expressed miR-150 demonstrated that together they repressed the transcript of the Th17-promoting factor mTOR, thereby supporting iTreg cell differentiation (Fig. 5; Warth et al., 2015).

A few publications have also described effects of posttranscriptional gene regulation on Treg cell plasticity. For instance, miR-10a reduces the expression Bcl6 in iTreg cells, thereby preventing their plasticity toward the Tfh cell differentiation program (Jeker et al., 2012; Takahashi et al., 2012). In another example, miR-146a directly repressed Stat1 expression, which was required in Treg cells to efficiently suppress Th1 responses and IFNγ-driven pathology (Lu et al., 2010). Finally, the lack of Roquin-encoding alleles in Tregs imposed a Tfr gene expression program that enabled these cells to regulate GC B cell responses but impaired their ability to protect from T cell transfer–induced colitis (Essig et al., 2017).

Posttranslational regulation of Treg cells

The reciprocal differentiation of Th17 and iTreg cells partly depends on the opposing functions of Stat3 and Stat5 (Figs. 4 and 5). In response to IL-2 signaling, T cells activate Stat5, which subsequently leads to transcriptional up-regulation of Foxp3 and repression of the Th17 gene expression program including the cytokine IL-17. It has recently been demonstrated that Foxp3 is subject to posttranslational regulation, involving factors such as Stub1, Usp7, and Hif-1α (Fig. 5; Laurence et al., 2013). The E3 ligase Stub1 required the stress response protein Hsp70 for

binding and ubiquitinating Foxp3 to trigger its proteasomal degradation (Chen et al., 2013b). Conversely, Usp7 deubiquitinated and stabilized Foxp3 (Fig. 5), which was found to be ubiquitinated at five different lysine residues (van Loosdregt et al., 2013). Interestingly, Th17-promoting IL-6 signaling reduced Foxp3 levels in three ways, by down-regulation of Usp7 (Yang et al., 2012) and by up-regulation of Stub1 (Chen et al., 2013b) as well as Hif-1α (Dang et al., 2011). In fact, Hif-1α can bind Foxp3 directly and target it for proteasomal degradation in a VHL E3 ligase–dependent manner (Fig. 5; Dang et al., 2011). Treg cell–specific deficiency of VHL increased Hif-1α levels, which was followed by augmented glycolytic reprogramming, increased IFNγ expression, and conversion into Th1 cells. Treg cells isolated from mice that lacked VHL expression in Tregs no longer suppressed inflammation in an adoptive transfer model of colitis (Lee et al., 2015).

During the development of thymic regulatory T cells, Foxp3 was found to be ubiquitinated and destabilized by the combined activity of the E3 ligases Stub1 and Cbl-b in a TCR/CD28-dependent manner (Fig. 5; Zhao et al., 2015). Particularly, CD28 costimulation potentiated auto-ubiquitination and degradation of Cbl-b (Zhang et al., 2002). Consistently, in $CD28^{-/-}$ mice, the decreased numbers and frequencies of Treg cells were partially rescued after additional deletion of Cbl-b (Zhao et al., 2015).

Within the reciprocal iTreg and Th17 differentiation, the latter involves many more regulatory mechanisms, which might be necessitated because of the high detrimental potential of Th17 cells. However, it is also tempting to speculate that iTreg cell differentiation can operate as a default pathway that is pursued in the absence of Th17-inducing factors (Wang et al., 2010).

Concluding remarks

In recent years, research in the field of posttranscriptional and posttranslational regulation has advanced our understanding of subset-specific gene expression in CD4 T cells. Defining the basis of T cell differentiation as a concerted and long-lasting change in gene expression, inducing and shaping the output of de novo transcription, appears to be only one way to reach this goal. It is now becoming apparent that stability of mRNA, modulation of translation, and protein stability and function might play much larger roles than previously appreciated. Consistently, the differentiation into individual T cell subsets is placed under tight posttranscriptional control by RBPs and miRNAs, which typically target the mRNAs of key signal transducers, transcription factors, and cytokines or their receptors or costimulatory receptors (Figs. 1, 2, 3, 4, and 5). Most strikingly, in all the CD4 T cell subsets, cytokine-mediated signaling is regulated by a large number of E3 ligases or DUBs (Figs. 1, 2, 3, 4, and 5). Ubiquitination and deubiquitination are often directed toward lineage-specifying factors, but also target signal transducers, such as Stat4, Stat6, and Stat3. Because these posttranslational modifications occur at the protein level, they may serve to directly and quickly implement regulatory signals. In contrast, slower but longer-lasting epigenetic changes may result from the large number of lncRNAs with unknown functions, which are highly and specifically expressed in T cell subsets. A future challenge will be to integrate all the identified individual activities of E3 ligases/DUBs, noncoding RNAs, and RBPs and ascertain how all these factors influence each other and have concerted cooperative or antagonizing effects.

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