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Review Article

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RNA Metabolism in T Lymphocytes

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Conflict of Interest
The authors declare no potential conflicts of interest.

ABSTRACT

RNA metabolism plays a central role in regulating of T cell-mediated immunity. RNA processing, modifications, and regulations of RNA decay influence the tight and rapid regulation of gene expression during T cell phase transition. Thymic selection, quiescence maintenance, activation, differentiation, and effector functions of T cells are dependent on selective RNA modulations. Recent technical improvements have unveiled the complex crosstalk between RNAs and T cells. Moreover, resting T cells contain large amounts of untranslated mRNAs, implying that the regulation of RNA metabolism might be a key step in controlling gene expression. Considering the immunological significance of T cells for disease treatment, an understanding of RNA metabolism in T cells could provide new directions in harnessing T cells for therapeutic implications.

Keywords: T-lymphocytes; RNA; RNA metabolism; Immunity; Cellular

INTRODUCTION

RNA metabolism refers to the processes that encompass the life cycle of RNA, including RNA synthesis, RNA splicing, RNA modification, RNA decay, and overall quality control. mRNA is transcribed from DNA as a single-stranded RNA template, and is processed simultaneously for 5'-capping, 3'-polyadenylation and alternative splicing (1-3). In addition, various modifications, such as RNA methylation, transform RNA structure to increase the accessibility of RNA-binding proteins or to promote degradation by recruiting the CNOT complex (4,5). In the cell, quality control of gene expression largely relies on RNA, therefore, RNA metabolism is elaborately controlled, particularly when drastic, temporal, or poised gene expression changes are involved. For instance, RNA metabolism is extremely important in T cells. During their life cycle, T cells undergo multiple selection processes and dramatic changes of cellular state. In thymocytes, β-chain rearrangement of TCRs occurs through V(D)J recombination to pair with pre-TCRα at the double-negative (DN, $CD4^-CD8^-$) stage, which is called β-selection (6). Subsequent double-positive (DP, $CD4^+CD8^+$) thymocytes are positively selected depending on the binding ability of TCR to MHC-peptide complexes (6). Then, late DP and single-positive (SP, $CD4^+$ or $CD8^+$) thymocytes that show strong affinity to self-peptide:MHC complexes are removed by negative selection to prevent autoimmunity (6)

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Abbreviations

ADAR1, adenosine deaminase acting on RNA1; ALKBH5, AlkB homolog 5 RNA demethylase; ARE, AU-rich element; A-to-I, adenosine-to-inosine; BTG, B-cell translocation gene ; CELF, CUGBP Elav-like family member; DN, double-negative; DP, double-positive; EAE, experimental autoimmune encephalomyelitis; GC, germinal center; ICOS, inducible T cell co-stimulator; IL-7R, IL-7 receptor; Irf-7, interferon regulatory factor 7; LCMV, lymphocytic choriomeningitis virus; LEF-1, lymphoid enhancer-binding factor 1; LINE, long interspersed nuclear element; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; MKK, MAPK kinase; MS, multiple sclerosis; NSUN, NOP2/Sun RNA methyltransferase; poly(A), poly-A; PTBP, polypyrimidine-tract binding protein; RNA Pol II, RNA polymerase II; SLE, systemic lupus erythematosus; Socs, suppressor of cytokine signaling; SRSF, serine/arginine-

(**Fig. 1**). Matured naïve T cells maintain a quiescent state characterized by cell-cycle arrest and low metabolic activity, while they are poised for immediate response to antigen stimulation (7,8). Upon stimulation of both TCR and co-stimulatory molecules, naïve T cells become activated and increase in size with a duplication rate of 6-12 h, and reprogram their metabolic status (9). Together with TCR activation, various combinations of cytokines instruct activated T cells to differentiate into effector T cell subsets (**Fig. 1**). At the end of their effector function, the majority of T cells enter activation-induced programmed cell death, whereas some surviving effector T cells remain to comprise the memory pool of the adaptive immune system (10). Such a vast shift in the cellular status of T cells demands rapid and timely control of gene expression upon external cues. To this end, intrinsic regulation of gene expression by RNA metabolism is an effective strategy to achieve immediate 'switch on' or 'switch off' of target genes. Thus, understanding RNA metabolism in T cells may provide new insight into T cell biology.

Over the decades, a body of research has revealed how the complex processes of RNA metabolism are involved in a variety of physiological contexts. This review summarizes recent advances in RNA metabolism, with a particular focus on T cell-mediated immunity. Herein, we describe the molecular details of RNA metabolism in the T cell life cycle. In addition, we also briefly mention the pathological aspects of RNA dysregulation attributed to T cell-related disorders. The role of microRNA in T cells is excluded, as it has been previously reviewed elsewhere (11,12).

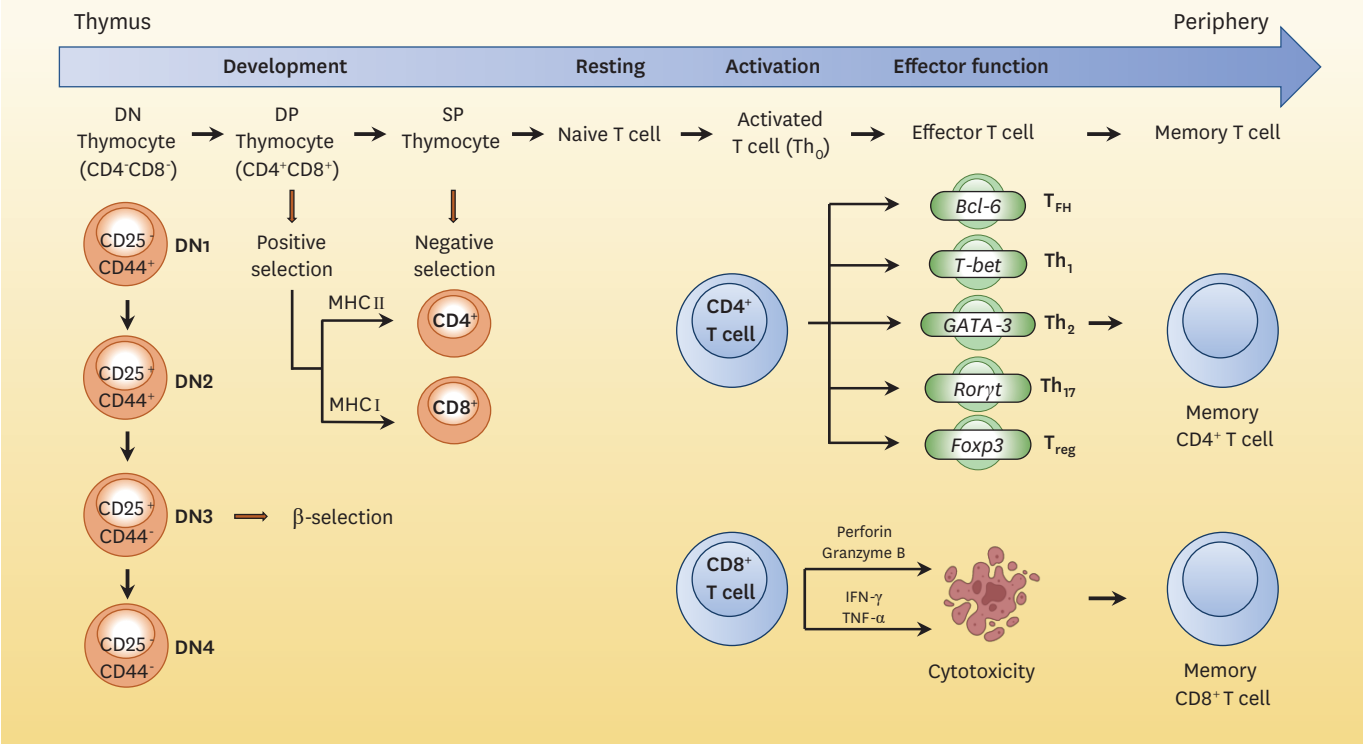

Figure 1. The life cycle of conventional T cells. T cells are produced in the bone marrow and mature into thymocytes in the thymus. In the early stage of T cell development, which is called the DN stage, they lack CD4 and CD8 coreceptors. This stage is divided into 4 sub-phases (DN1, DN2, DN3, and DN4) according to the surface expression of CD44 and CD25. During the DN3 phase, thymocytes undergo TCRβ rearrangement, known as 'β-selection.' As they express both CD4 and CD8, they become DP thymocytes. Positive selection occurs in the DP stage, which selectively removes thymocytes that are incapable of binding to MHC molecules. Thymocytes differentiate into CD4 or CD8 SP thymocytes depending on the recognition of peptides presented on MHC-I or MHC-II molecules. Subsequently, SP thymocytes undergo negative selection, leading to apoptosis upon strong interaction with self-antigens. Thymocytes that pass through these processes become naive T cells and circulate in the body. Upon cognate antigen stimulation and various cytokine signals, they are activated and differentiate into effector T cells. CD4 T cells differentiate into T_{FH}, Th1, Th2, Th17, and T_{reg} cells with expression of their master transcription factors, *Bcl-6*, *T-bet*, *GATA-3*, *Rorγt*, and *Foxp3*, respectively. Activated CD8 T cells have a cytotoxic effect and induce apoptosis in virus-infected or cancer cells. After playing an effector function, most effector T cells undergo apoptotic death, but a few survive and become memory T cells, responsible for the secondary immune response.

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rich splicing factor; SP, single-positive; TFH, follicular helper T cell; TUT, terminal uridyltransferase; UTR, untranslated region; U2AF, U2 small nuclear RNA auxiliary factor; YTHDF, YTH N6-methyladenosine RNA-binding protein

Author Contributions

Conceptualization: Hwang SS; Supervision: Hwang ss; Writing – original draft: Choi JO, Ham JH; Writing – review & editing: Choi JO, Ham JH, Hwang SS.

IMBALANCE OF TRANSCRIPTION AND TRANSLATIO IN T CELLS

The transition from naive to activated T cells triggers global upregulation of gene expression via rapid recruitment of RNA polymerase II (RNA Pol II) and simultaneously accelerates transcription rates (13). Mapping of *de novo* transcriptome and ribosome profiling with time-course tracking techniques has shown gene-specific discrepancies in approximately 95% of upregulated genes (13). The fastest response in transcription is for genes involved in basic cell physiology, such as ribosome biogenesis and translational machinery (13,14). Likewise, gene sets associated with production of cytokines and receptors exhibit a marked increase in transcriptional rates concomitant with translation (13). However, a large portion of genes show unmatched rates of transcription and translation, indicating high rates of protein synthesis relative to their *de novo* transcription rates (13). A study of the dynamics of mRNA translation using the pulsed SILAC technique has delineated an imbalance between transcription and translation rates in the early stage of T cell activation (15). Within the first 6 h after activation, the total mRNA copies showed only a mild (approximately 1.4-fold) increase (15). Notably, with negligible changes in ribosomal proteins and rRNA, the translation rate showed a 5-fold increase from 0.8/sec to 4.0/sec (15). This discrepancy can be explained by the presence of idling ribosomes, which are poised, and mRNAs are translationally repressed before activation (**Fig. 2**). Furthermore, similar evidence of idling translational machinery has been reported in the context of metabolic reprogramming during the naive-to-activation

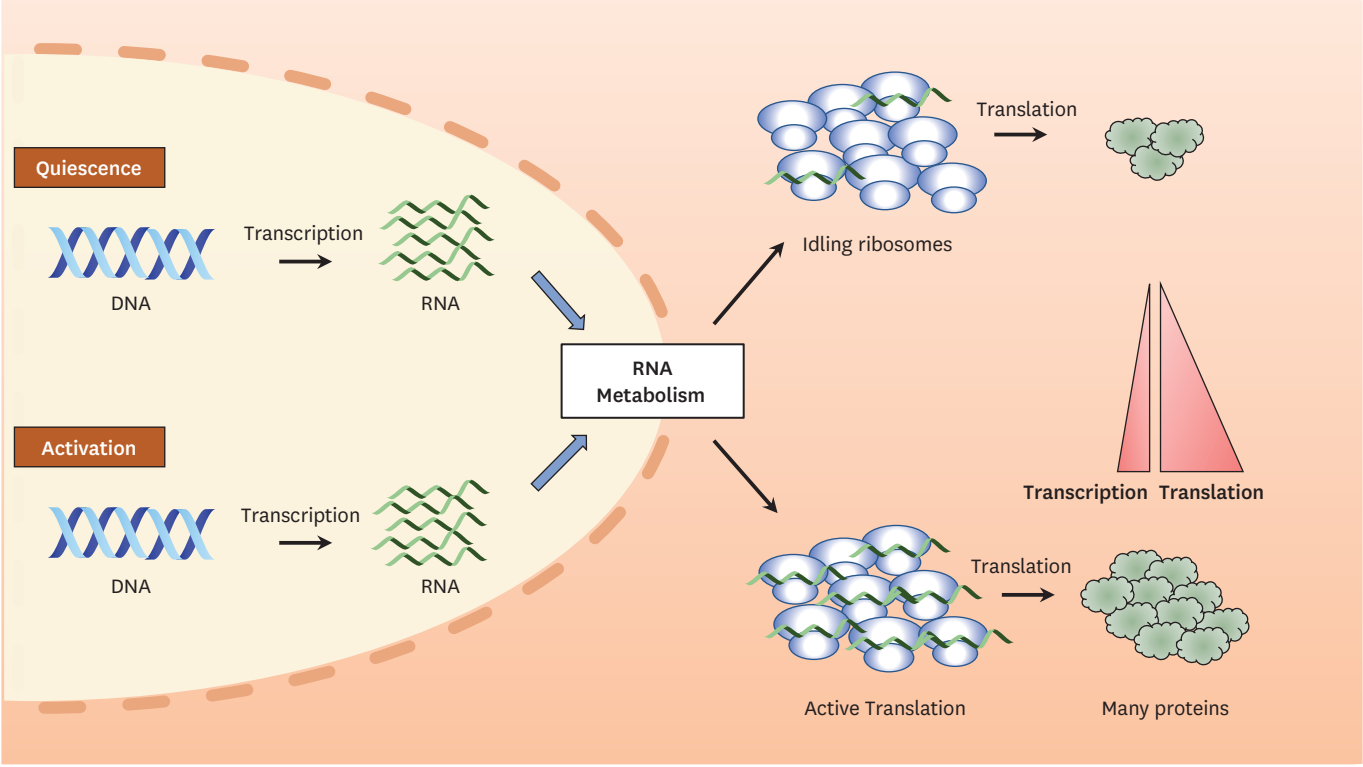

Figure 2. Imbalance between transcription and translation in quiescence and activation. During the transition between quiescence and activation, activated T cells become highly proliferative and increase in both size and internal mass. These processes require many proteins to support cell growth. During the early stage of T cell activation, the increase in transcription rates and corresponding RNA copy numbers are insufficient to explain such massive protein synthesis. The quantity of available ribosomal machinery is similar in quiescent and activated T cells, indicating translational preparedness in resting T cells in the presence of idling ribosomes. Imbalanced transcriptional and translational processes suggest that RNA level regulation might be a rate-limiting step for gene expression in the early stages of T cell activation, highlighting the significant role of RNA metabolism.

transition. Naive T cells accumulate mRNAs associated with glycolysis and fatty acid synthesis but stall translation until they are engaged by TCR stimulation (16). T cell activation leads to translation of the glucose transporter GLUT1 and acetyl-coenzyme A carboxylase 1, consequently completing metabolic reprogramming (16). Collectively, these studies indicate that a large portion of mRNAs remain untranslated in resting T cells, despite adequate translational machinery. This highlights the significance of RNA regulation combined with transcriptional and protein signaling regulation to control T cell homeostasis.

ROLE OF RNA SPLICING IN T CELL HOMEOSTASIS

Following transcription, primary transcripts, termed pre-mRNAs, undergo processing steps including 5'-capping, 3'-polyadenylation, and alternative splicing (1-3). RNA processing occurs concurrently with transcription because RNA pol II engages RNA processing machinery, including cap-binding proteins, polyadenylation complexes, and spliceosomes (3). 5'-capping and polyadenylation at the 3'-end influence mRNA stability, which is reviewed in the next section. Most pre-mRNAs in eukaryotic cells are alternatively spliced (17). 5' or 3'-splicing or exon skipping contributes to proteomic diversity by generating multiple isoforms from a limited number of genes (18). In addition, exon inclusion and intron retention determine the subsequent fates of mRNAs (19). As seen in other cell types, splicing events broadly regulate T cell homeostasis throughout their life cycle.

Global regulation of RNA splicing in T cells

A study using RNA-seq profiling of both human T cell lines and primary T cells found a comprehensive pattern of RNA splicing that characterized the isoform changes of 178 exons in 168 genes in response to stimulation (20). Transcription factors *Foxa1* and *Foxa2* (*Foxa1/2*) have recently been identified as global regulators of RNA splicing during the transition from DP to SP thymocytes (21). Double conditional knockout of *Foxa1/2* (*Foxa1**fl**/**fl**fl**/**fl**cre**Foxa2*) disrupted the development of SP population and caused aberrant RNA splicing (21). Combined analysis of human $CD4^+$ T cells using RNA-immunoprecipitation and mass spectrometry has also identified U2 small nuclear RNA auxiliary factor 2 (U2AF2) as a central regulator of RNA splicing by recruiting spliceosome to target mRNA, suggesting that U2AF plays an indispensable role in the optimal expression of many effector molecules, such as CD25 and CD62L, and subset-specific cytokines in T cells (22). Similarly, another study suggested that serine/arginine-rich splicing factor 1 (SRSF1) has an impact in T cell development (23). Additionally, this study also identified many binding partners of SRSF1 related to T cell differentiation factors, implying that SRSF1 has multiple effects on T cell biology (23). Furthermore, an abnormal decrease in SRSF1 was observed in patients with systemic lupus erythematosus (SLE) with a hyperresponsive T cell population (24). Consistent with these findings, mice lacking SRSF1 exhibit aberrantly elevated mTOR activity and reduced expression of its repressor, phosphatase and tensin homolog (24). A recent study demonstrated that different RNA splicing patterns affect the maintenance of T cell quiescence and it highlighted the negative effects of transcript variants containing long interspersed nuclear element 1 (LINE1). Owing to the unique splicing pattern in naive $CD4^+$ T cells, intronic LINE1 is inserted into the transcripts upregulated during T cell activation, and these variants act with nucleolin to repress gene expression in the chromatin in a cis-acting manner (25). The activation cue alters the splicing pattern, which reduces LINE1-containing transcripts via the splicing suppressor polypyrimidine-tract binding protein 1 (PTBP1), leading to the expression of the corresponding genes (25). Although PTBP1 regulates T cell

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activation and IL-2 production (26), its role in T cell biology remains unknown. Collectively, complicated patterns of RNA splicing for diverse gene sets globally orchestrate T cell homeostasis throughout the T cell life cycle.

Exon inclusion and skipping

The RNA splicing factor CUGBP Elav-like family member 2 (CELF2) has been described for its multiple roles in the regulation of metabolism, alternative splicing, and translation (27). T cell activation increases the mRNA stability of CELF2, which is responsible for widespread alternative splicing in T cells (28). It controls alternative splicing of one-third of the genes associated with TCR signaling and T cell development via either exon skipping or inclusion (28). The best-known targets of CELF2-dependent RNA regulation in T cells are MAPK kinase 7 (MKK 7) and lymphoid enhancer-binding factor 1 (LEF1). Optimal production of effector cytokines by activated T cells requires JNK activity (29). MKK7 activates JNK, leading to activation of the transcription factor c-Jun (30). The complete form of MKK 7 is incapable of interacting with JNK, as exon 2 in the MKK 7 transcript is inserted within the JNK docking site and prevents binding (31). Enhanced CELF2 activity upon T cell activation causes exon skipping of introns flanking exon 2 in MKK 7, forming an intact docking site for JNK (31). Conversely, CELF2 directs exon inclusion, generating the full length of LEF-1, which contains exon 6 (32). LEF-1 coordinates the expression of TCRα in developing thymocytes; thus, it is critical for DN-to-DP transition during T cell development (32). Exon 6 in LEF-1 encodes a context-dependent regulatory domain that enables LEF-1 to interact with the co-activators Aly and Ets for TCRα expression (32,33). The splicing factor SRSF1 also plays a key role in exon inclusion. Phenotypically, the loss of SRSF1 in developing T cells (*Srsf1*$^{fl/fl}$ *Lck*^{cre}) resulted in impaired maturation in late stage T cell development (23). Genome-wide analysis of SRSF1 binding targets has shown that it binds to the pre-mRNA of *Il-27ra* and IFN regulatory factor 7 (*Irf-7*) in response to type I IFN signaling (23), which is critical for late T cell development and survival (34). In the absence of SRSF1, defective splicing of these genes, such as skipping of exon 3 in *Il-27ra* mRNA, or retention of intron 5 in *Irf-7* mRNA, introduced a premature termination codon that lead to mRNA decay, resulting in increased levels of pro-apoptotic factors, *Bax* and *Bcl2l11* (23) and compromised IL-27 signaling. Likewise, SRSF1 promoted the inclusion of exon 5 in the mRNA encoding the T cell surface glycoprotein CD6 (35). Downregulation of SRSF1 by T cell activation increases exon 5 skipping in CD6 mRNA (35). Although it has not been determined whether CD6 has a stimulatory or an inhibitory effect, skipping exon 5 in CD6 might limit its function because it encodes a ligand-binding domain (35). Splicing patterns of TCR-related molecules controlled by heterogeneous nuclear ribonucleoprotein (HNRNP) L and A1 determine the fate of CD4$^+$ T cells (36). Strong TCR signaling combined with cytokine signals induces the development of effector T cell subsets, whereas tonic TCR stimulation induces Treg differentiation (37). Weak TCR stimulation alters several substrates of Akt, including splicing factors that favor the activation of HNRNP L and HNRNP A1 (36). In conditions favoring the development of Tregs, distinct patterns in alternative splicing induce the expression of other variants of CD3ζ, CD3η and CD3, which lack the immunoreceptor tyrosine-based activation motif, and favor CD45RO instead of CD45RB by skipping exon 5, contributing to Treg generation (36). Similarly, the splicing pattern of mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) differs depending on whether T cells are resting or activated. In resting T cells, the splicing activity of HNRNP U generates the exon-excluded MALT1B isoform, whereas TCR stimulation relieves the suppressive effect of HNRNP U and allows the isoform, MALT1A, which contains exon 7, with enhanced binding ability to TRAF6 (38). From a disease perspective, distinct splicing events may drive pathological outcomes. For example, although the membrane-bound IL-7 receptor (IL-7R) plays an essential role in T cell

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survival and maintenance of homeostasis (39), aberrant skipping of exon 6 generates a secreted form of IL-7R, which is associated with an increased risk of multiple sclerosis (MS), as MS patients often possess a single nucleotide polymorphism in *Il-7r* exon 6 (40,41). On the contrary, RNA helicase DEAD box polypeptide 39 B is responsible for the formation of the complete form of IL-7R by preventing skipping of exon 6 (41).

Intron retention

The intron, a non-coding region of pre-mRNA, has a substantial impact on RNA regulation in T cells, as intron retention is one of the mechanisms regulating the number of transcripts linked to cell fate decisions (42). For example, selective induction of intron retention occurs in exons 4 to 6 of *Ptprc* mRNA (encoding CD45) by the RNA-binding protein HNRNPLL in T cells (43). Like exon splicing, global changes in intron retention are observed upon T cell activation, although the precise mechanism of intronic control of gene expression is still poorly understood. A study using strand-specific RNA-seq showed intronic sequences were favorably inserted in polyadenylated transcripts in resting $CD4^+$ T cells, displaying distinctive patterns of intron retention in T cells (44). Furthermore, intron retention was observed in 185 genes that were most frequently upregulated during T cell activation, and retained introns were dramatically decreased upon T cell stimulation (44). Collectively, this emphasizes that gene expression is dominantly controlled by splicing rather than transcriptional regulation.

EPIGENETIC MODIFICATION OF RNA IN T CELLS

In addition to RNA processing steps, internal modification of RNA is another important step to determine mRNA fate and gene expression. Chemical modification or addition of certain molecules to RNAs represents most of the internal modifications encompassing methylation (m^6A and m^5C), ribose modification (2'-O-methylation; 2'-OMe), adenosine (A)-to-inosine (I) modification, 5-hydroxymethylcytosine, and uridylation and isomerization of uridine (45). These modifications alter the characteristics of RNAs depending on the marked molecules, subsequently affecting pre-mRNA processing, stability, and mRNA translation. Advances in analytical chemistry and high-throughput sequencing methods have given rise to a new concept of "epitranscriptome," which has enabled the consideration of the molecular dynamics of chemically modified RNA. Correspondingly, many studies have actively been deciphering the role of RNA modification in T cell biology.

RNA methylation in T cells

Methylation of RNA includes modifications such as *N*6-methyladenosin (m^6A), *N*1-mthyladenosine, and *N*5-methylcytosine (m^5C), which are modulated by several proteins referred to as "writers", "erasers", and "readers" (46,47). "Writers" transfer methyl residues on the RNA molecule, whereas "erasers" remove them by demethylase activity, and "readers" recognize methylated marks on RNAs. m^6A is one of the most abundant modifications and is enriched in 3'-untranslated region (UTR), long internal exons, and near the stop codons of RNAs (48). Methyltransferases such as METTL3 and METTL14 act as writers of m^6A modifications (49). An m^6A mark on RNAs has a multifunctional role by recruiting reader proteins that bind to the m^6A structure. Given that various RNA-binding proteins, such as RNA stabilizers or splicing factors, can recognize m^6A, changing its accessibility ultimately determines mRNA half-life and gene expression (50). For example, the binding of YTH N6-methyladenosine RNA-binding protein (YTHDF) 1, a reader protein, accelerates the translation of target mRNA by recruiting translation initiation factors (51). In addition, YTHDF2, another

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reader protein, recognizes m^{6}A-marked mRNA and induces decay by recruiting the CCR4-NOT complex (52). The ablation of YTHDF1 enhances anti-tumor immunity by facilitating cross-presentation of CD8$^+$ T cells from dendritic cells, suggesting that YTHDF1 has an indirect effect on T cells (4). The role of m^{6}A modification in T cell homeostasis has also been well described. Deletion of METTL3, which acts as an m^{6}A writer, disrupts IL-7-JAK/STAT5 signaling in T cell homeostasis and differentiation (53). *Mettl3*-deficient T cells (*Mettl3*$^{fl/fl}$*Cd4*^{cre}) adoptively transferred into RAG knockout mice failed to induce colitis due to their retained resting status (53). Upon IL-7 stimulation, METTL3-mediated m^{6}A marking selectively targeted suppressor of cytokine signaling-1 (*Socs1*), *Socs3*, and cytokine-inducible SH2-containing protein which are inhibitory proteins for STAT signaling, and induced their degradation, consequently activating IL-7-JAK/STAT5 signaling (53). In line with this, the same group has reported that Tregs lose their suppressive ability in the absence of METTL3 (*Mettl3*$^{fl/fl}$*Foxp3*^{cre}) due to the lack of m^{6}A modification of the inhibitory *Socs* gene family (5), as reported previously (53). However, the studies that describe the role of METTL3 in follicular helper T cells (T_{FH}) are controversial. Knockdown of METTL3 or METTL14 with shRNA promotes T_{FH} differentiation upon lymphocytic choriomeningitis virus (LCMV) infection (54). In contrast, another study showed that METTL3 deficiency disrupted T_{FH} differentiation due to the lack of stabilized transcription factor 7 via METTL3-mediated m^{6}A (55). Another contributing factor to the complexity of m^{6}A modifications in T cells is the demethylation of m^{6}A by the eraser protein such as AlkB homolog 5 RNA demethylase (ALKBH5) known to involve to CD4$^+$ T cell activation (56). TCR stimulation upregulated ALKBH5 expression, and the loss of ALKBH5 in T cells (*Alkbh5*$^{fl/fl}$*Cd4*^{cre}) resulted in enhanced m^{6}A modification of IFN-γ and CXCL2 mRNAs, increasing their vulnerability to decay and reducing their protein expression (56). Consistently, the adoptive transfer of ALKBH5-deficient T cells into RAG knockout mice failed to induce autoimmune colitis, and *Alkbh5*$^{fl/fl}$*Cd4*^{cre} mice exhibited resistance to experimental autoimmune encephalomyelitis (EAE) induction (56). Compared to m^{6}A modifications, the effects of m^{5}C and m^{1}A on T cells are unknown. The only known enzyme that catalyzes m^{5}C modification is NOP2/Sun RNA methyltransferase 2 (NSUN2). The CD4$^+$ T cells of patients with SLE exhibit a decreased pattern of m^{5}C and significantly reduced NSUN2 expression (57). Meanwhile, m^{1}A modification is mainly found in tRNAs (58). A recent study that screened for dynamic changes in the tRNA pool in T cells showed that the m^{1}A level in tRNA did not change during T cell proliferation and differentiation (59). However, the same study has identified 2 unique modifications, namely, wybutosine and ms2t6A, which occur in tRNAs bearing slippery codons, and prevents ribosomal frameshifting (59). These modifications were markedly reduced in activated T cells, and such modifications are likely to increase proteome-wide frameshifting (59).

Adenosine-to-inosine (A-to-I) modification during T cell development

Apart from mRNA modification, A-to-I modification of RNA is uniquely exploited during T cell development. Developing thymocytes migrate from the cortex into the thymic medulla, which contains abundant type I IFN, and IRF-7 is constitutively expressed in thymocytes (60). Dependency on IFN signaling in the absence of infection may cause unnecessary activation through increased expression of genes responsible for antiviral immunity, such as melanoma differentiation-associated protein 5, a sensor of ds RNA (61). The RNA-editing molecule adenosine deaminase acting on RNA1 (ADAR1) plays a compensatory role. ADAR1 catalyzes A-to-I modification upon recognition of endogenous dsRNA, preventing unwanted sensing of dsRNA by relieving the dsRNA structure (62). ADAR1 appears to have a similar role in developing thymocytes, as the expression of ADAR1 is upregulated during the late stage of thymic T cell maturation (63). Indeed, ADAR1-deficient thymocytes fail to fully mature and remain in the semi-mature stage, defined by a CD69$^-$MHC I$^+$ phenotype (63).

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Effects of RNA uridylation on T cell homeostasis

Although terminal uridylation of microRNA has been reported to be involved in its stability in $CD4^+$ T cells (64), little is known about the direct impact of RNA uridylation in T cells. In the RAW 264.7 murine macrophage cell line, terminal uridyltransferase 7 (TUT7) positively regulates the expression of IL-6 by controlling the mRNA stability of *Regnase-1* (also known as *Zc3h12a*). TUT7 expression requires p38 MAPK signaling and uridylated *Regnase*-1 mRNA by binding to the stem-loop in its 3'-UTR, ultimately facilitating its decay. Given the underlying mechanism of TUT7 shown in RAW264.7, it gives rise to the potential role of TUT7-mediated RNA uridylation in T cells indirectly because it shares similar signaling pathways upon TCR stimulation, and REGNASE-1 has a broad effect on T cell homeostasis by controlling mRNA decay. Thus, it may be an interesting area of research to explore in future studies.

Collectively, the crosstalk between chemically modified RNAs and T cell biology is an important mechanism of RNA-mediated T cell regulation. Given the importance of RNA modification in T cell regulation, it is worthwhile to explore the potential role of these mechanisms in controlling T cell function.

REGULATION OF RNA DECAY IN T CELLS

Many processes of RNA metabolism described above often closely correlate to RNA decay. Because of their unstable nature, typical mRNAs are protected by a 5'-7-methylguanosine cap (5'-cap) and 3' poly-A (poly(A)) tail (65,66). Degradation of mRNA occurs through multiple pathways. Most mRNAs undergo exonuclease-dependent mRNA decay that relies on poly(A) tail degradation in the 3' region of mRNA by de-adenylation complexes such as CCR4-NOT, exposing mRNA to an unfavorable environment (67). Subsequent decapping by the decapping complex *Dcp1*/*Dcp2* removes the 5'-cap, increasing the vulnerability of mRNA (68,69). Lastly, exonucleases catalyze mRNA degradation. Endonuclease-dependent mRNA decay is achieved by endonucleases such as REGNASE-1 (70). Without chemical removal on each side of the mRNA, endonucleases cause exposed 5'- and 3'- ends to be susceptible to exonucleases (71). For rapid and tight regulation of gene expression in response to extracellular stimuli, T cells often harness the mechanisms that enhance or abrogate mRNA stability.

Exonuclease-dependent mRNA decay

The role of the CCR4-NOT de-adenylase complex in silencing gene expression has been extensively reported in previous descriptions of T cell biology. For example, the expression of CNOT3, a regulatory component required for the integrity of the CCR4-NOT complex, is upregulated in DP thymocytes (72), implying the involvement of mRNA de-adenylation during T cell development. T cell-specific deletion of *Cnot3* (*Cnot3*$^{fl/fl}$-$CD4^{cre}$) has been shown to result in the failure of mature $CD4^+$ and $CD8^+$ T cell production with upregulated pro-apoptotic genes, including *Dap2ip* and *Bbc3* mRNA, in the early and late stages of positive selection, respectively (72). The RNA-binding proteins ZFP36L1 and ZFP36L2 (ZFP36L1/2) also utilize the CCR4-NOT complex to guide target mRNA degradation across the T cell life cycle. Mechanistically, ZFP36L1/2 belong to the ZFP36 family, which contain a CCCH zinc finger domain that can bind to conserved sequences in the AU-rich element (ARE) of the 3'-UTR (73). The ZFP36L family, including ZFP36L1/2, can interact with CCR4, leading to the recruitment of NOT4 and the final assembly of the CCR4-NOT de-adenylase complex, which limits target gene expression by promoting mRNA degradation (74). In the early developmental stages, ZFP36L1/2 downregulates the expression of *Notch-1*, which is crucial for thymocyte proliferation. Despite

its role in cell growth (75), uncontrolled signaling from Notch-1 induces thymocyte proliferation without proper maturation steps; therefore, it should be tightly regulated depending on the developmental stage. One study showed that mice with selective deficiency of ZFP36L1/2 in early thymocytes (*Zfp36l1*$^{fl/fl}$*Zfp36l2*$^{fl/fl}$-*Cd2*^{cre}) showed T cell acute lymphoblastic leukemia with aberrant accumulation of T cells bypassing β-selection (76). ZFP36L1/2 interacted with the highly conserved ARE region of Notch-1 mRNA, although the mechanism of Notch-1 mRNA decay is not clear (76). In subsequent works, it was determined that ZFP36L1/2 was also associated with the genome-wide suppression of cell cycle genes activating the E2F pathway and DNA-damage responses (77,78). Overall, ZFP36L1/2 mediated mRNA decay via CCR4-NOT seems to secure β-selection of early thymocytes by delaying cell proliferation and inhibiting DNA repair genes for uninterrupted V(D)J recombination. RNA regulation by ZFP36 family proteins influences the effector functions of mature T cells. ZFP36L2 limits the translation of IFN-γ by binding to AREs in the 3'-UTR of *Ifng* mRNA (79). Consistently, ZFP36 deficient mice were more resistant to antiviral response against LCMV infection with not only a dramatic increase of IFN-γ-expressing T cells, but also enhanced expression of activation and proliferation markers (80). RNA-binding proteins belonging to the ROQUIN family, including ROQUIN-1 and ROQUIN-2 (ROQUIN1/2), share a CCR4-NOT-mediated mechanism to regulate target mRNA availability. Although ROQUIN1/2 have a CCCH-type zinc finger domain, the ROQ domain is majorly responsible for their binding specificity, and these domains act synergistically (81,82). Through this domain, ROQUIN1/2 bind to the 3'-UTR (100-200 bp downstream of the stop codon) of the inducible T cell co-stimulator (ICOS) and limit the abundance of *Icos* mRNA by recruiting the CCR4-NOT complex (81,83). Since ICOS is essential for the regulation of T cell immunity, particularly in T_{FH} and germinal center (GC) responses (84), disrupted activity of ROQUIN1/2 spontaneously causes severe autoimmunity resembling SLE characterized by splenomegaly and abnormally increased GC formation, as seen in Sanroque strain mice which contain the M199R mutation in *Rc3h1* (encoding ROQUIN1) (85,86). Consistently, similar phenotypes have been observed in the T cell-specific deletion of ROQUIN1/2 (*Rc3h1/2*$^{fl/fl}$*CD4*^{cre}) (82). Recent work has also determined a role for CCR4-NOT-mediated mRNA decay in the quiescence maintenance of naive T cells. A study has identified B-cell translocation gene 1 (BTG1) and BTG2 (BTG1/2) as novel regulators of T cell quiescence by inducing global mRNA deadenylation and degradation (87). Remarkably, BTG1/2 were mainly expressed in quiescent T cells and swiftly turned off upon activation cues. BTG1/2-deficient T cells (BTG1/2$^{fl/fl}$-CD4^{Cre}) exhibited a global increase in mRNA abundance, leading to a compromised naive status, accompanied by the aberrant accumulation of effector memory population in steady-state conditions (87). In quiescent T cells, BTG1/2 can physically bind with poly(A) tail binding protein and the CCR4-NOT complex, even though they do not bear the direct RNA cognition domain, thereby facilitating constant deadenylation by inducing global mRNA decay machinery (87). Such a seemingly inefficient process is advantageous for a rapid response upon stimulation from the retained availability of pre-made mRNAs. Although the regulation of RNA metabolism in T cell quiescence has received less attention thus far, there is extensive evidence to elucidate how RNA metabolism is involved in T cell quiescence.

Endonuclease-dependent mRNA decay

REGNASE-1 acts as an endonuclease that harbors a PIN-like RNase domain and specifically cleaves mRNA upon recognition of the stem-loop structure cooperatively with helicase upstream frameshift 1 (88). In the immune system, it is known to destabilize mRNAs encoding the inflammatory cytokines IL-6 and IL-12 (89). Mice with homozygous knockout of REGNASE-1 (*Zc3h12a*$^{-/-}$) succumbed within 12 weeks after birth due to severe anemia and showed significantly high effector/memory splenic T cells (89). Similarly, another study has shown that the loss of REGNASE-1 induced spontaneous autoimmune phenotype marked by the increased

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expression of T cell activation markers and inflammatory cytokines (90). In contrast, mice with a mutant form of REGNASE-1 with enhanced stability exhibited lymphopenia and were unresponsive to skin allografts due to disrupted thymic T cell development (91), indicating that REGNASE-1 controls T cell homeostasis in diverse aspects. In addition to its role in exonuclease-dependent mRNA decay, ROQUIN also cooperates with REGNASE-1 and induces target mRNA degradation as they share target mRNAs associated with inflammatory responses and Th17 differentiation (92). In contrast, upon TCR stimulation, REGNASE-1 is degraded in a MALT-1-dependent manner, relieving T cells from suppressive conditions (90).

Mechanisms protecting of mRNA from decay

As mentioned previously, ELAVL1 is a stabilizer of mRNA. Similar to other molecules contributing to mRNA decay, ELAVL1 interacts with introns and ARE regions in the 3'-UTR of mRNA, but it increases the mRNA lifespan by interfering with exonuclease access and destabilizing degrading complexes (93).

A study using Jurkat human T cell line has shown that long 3'-UTR is favorably targeted by ELAVL1 and is important for target protein localization (94). During T cell development, thymocytes deficient in ELAVL1 (*Elavl1*$^{fl/fl}$*Lck*^{cre}) exhibited defective β-selection and a failure of thymus egression concomitant with hyperproliferation of thymocytes (95). While the study did not clearly address the molecular mechanism of ELAVL1, it suggested that ELAVL1 is a stabilizer of *p53*, ultimately inducing cell cycle arrest of DN thymocytes (95). Recently, it was shown that ELAVL1 regulates the translation of *Ccnd3* mRNA (encoding cyclin D3) via direct interaction (96). However, further studies are required to understand the multifunctional role of ELAVL1 in cell-cycle genes during T cell development.

RNA stabilizing activity of ELAVL1 regulates broad aspects of T cell function. The mRNA encoding IL-2 receptor α chain (CD25) requires ELAVL1 interaction for optimal translation, as the loss of ELAVL1 in CD4$^+$ T cells (HuR$^{fl/fl}$*Lck*^{cre}) has shown defective IL-2 homeostasis (97). In effector T cell subsets, ELAVL1 stabilizes mRNA encoding IL-4, IL-13, and IL-17A. Deletion of ELAVL1 in activated T cells (HuR$^{fl/fl}$OX40^{cre}) abrogated Th17 responses as they failed to induce EAE, while Th2 responses were unchanged (98,99). Moreover, transcriptome-wide analysis revealed potential targets of ELAVL1 associated with T cell activation pathways, further suggesting a comprehensive role of ELAVL1 in T cell function.

CONCLUSION

To date, T cell studies have mainly focused on their functions and cellular interactions from a macroscopic view, while the intrinsic activities of T cells have been poorly understood. Although clinical techniques involving T cells currently has improved medical therapeutics, there are still many limitations with utilizing T cells as an effective drug target. Therefore, more research is needed to gain a fundamental understanding of T cell biology. Recent breakthroughs in technical and analytical tools have enabled us to scrutinize the RNA world across T cell life cycles. A large portion of mRNAs remain untranslated in resting T cells and diverse RNA metabolism is intrinsically involved in T cell functions (summarized in **Fig. 3**). Several studies have also implicated that dysregulation of RNA metabolism in T cells is associated with pathological outcomes in both human and animal models (**Table 1**). Given the central role of RNA and the underlying mechanisms of T cell regulation, reconsidering the significance of RNA metabolism in T cells may open new paradigms for future therapeutic strategies.

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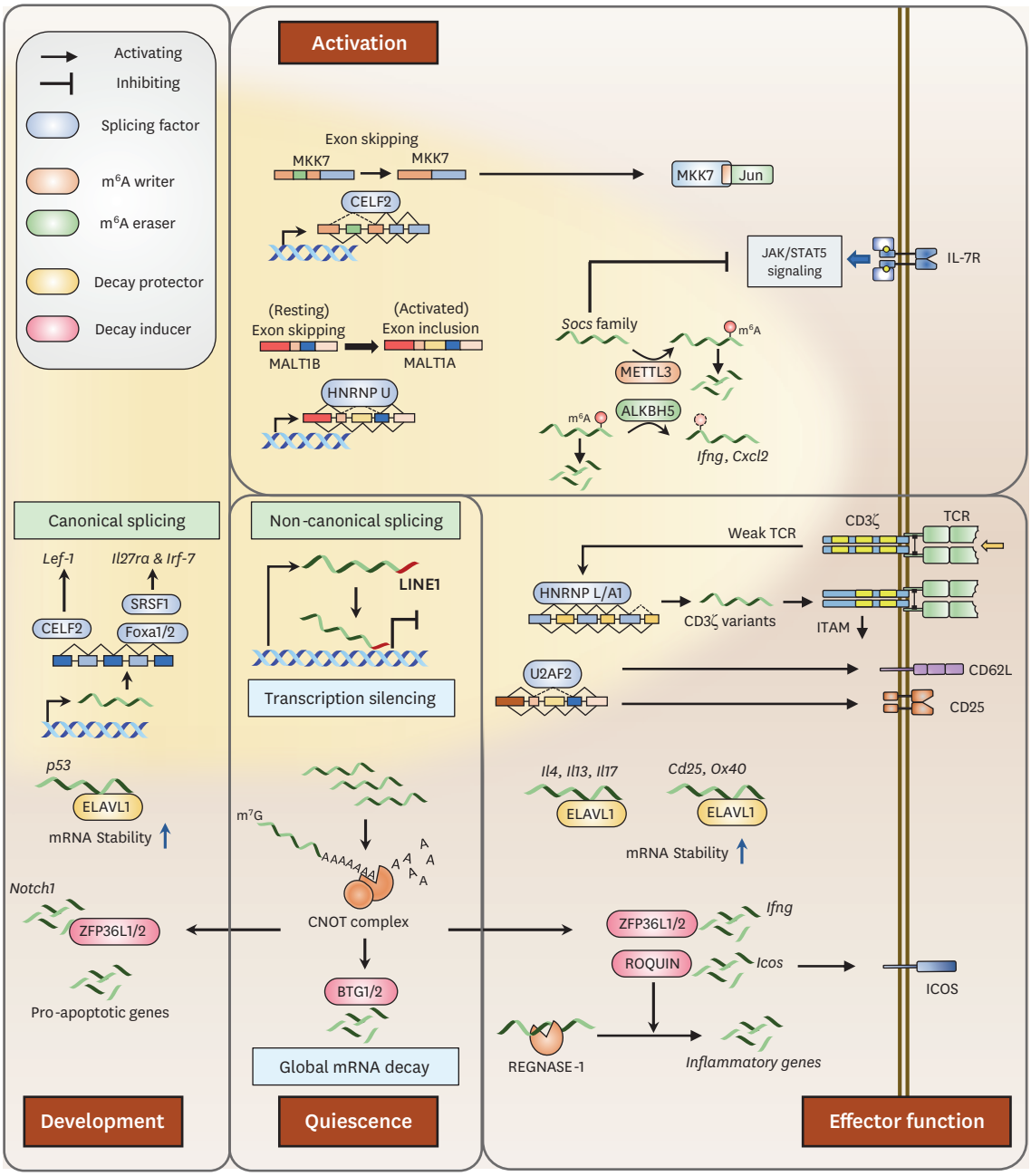

Figure 3. Dynamics of RNA metabolism in T cells. RNA metabolism affects T cell function throughout their life cycle. During the developmental phase, several genes, such as such as *Lef-1*, *Il27ra*, and *Irf7*, which play a critical role in thymocyte maturation, are processed by splicing factors, CELF2, SRSF1, and FOXA1/2. *p53* mRNA stabilized by ELAVL1 supports thymocyte proliferation, whereas ZFP36L1/2 mediated *Notch-1* mRNA decay secures thymocyte selection by limiting uncontrolled proliferation. The CNOT complex, which has de-adenylase activity, degrades mRNAs by shortening poly(A) tails at the 3'-end. BTG1 and BTG2 cooperate with the CNOT complex to maintain quiescence of resting (naive) T cells by degrading global mRNAs, leading to a poised status. Distinct splicing patterns in resting T cells, which contain LINE1 in mRNAs, suppress the corresponding gene expression. T cell activation affects the exon skipping or inclusion processes driven by CELF2 or HNRNP U, which generates binding sites containing MKK7 or full-length MALT1A. m^{6}A modification of inhibitory *Socs* family mRNAs by the m^{6}A writer, METTL3, facilitates mRNA decay by enhancing IL-7R/JAK/STAT5 signaling. In contrast, ALKBH5, an m^{6}A eraser, inhibits the degradation of *Ifng* and *Cxcl2* mRNAs by removing m^{6}A marks upon T cell activation. Splicing factor U2AF2 is involved in the optimal expression of CD62L and CD25. A low degree of TCR signaling stimulates the splicing factor HNRNP L/A1, forming a CD3ζ variant that lacks one of the ITAMs. ELAVL1 enhances the mRNA stability of various effector molecules such as IL-4, IL-13, IL-17, CD25, and OX40. Excessive effector functions are inhibited by selective mRNA decay. ZFP36L1/2 and ROQUIN recruit the CNOT complex and degrades *Ifng* and *Icos* mRNA, respectively. Endonuclease activity by REGNASE-1 also contributes to controlling the abundance of mRNAs from inflammatory genes.
ITAM, immunoreceptor tyrosine-based activation motif.

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Table 1. Effect of RNA metabolism on disease and pathological phenotypes

RNA metabolism	Associated factor	Impact on T cells	Species	Related phenotype/Diseases	References
RNA splicing	FOXA1/2	Positive selection DP thymocytes	Mouse	T cell developmental defects	(21)
	SRSF1	Exon inclusion of *Il-27ra* and *Irf-7*	Mouse	T cell developmental defects	(23)
		Enhanced mTOR signaling	Human	SLE	(24)
		Reduced PTEN activity			
	DDX39B	Prevention of IL-7R exon 6 skipping	Human	MS	(40,41)
RNA modification	METTL3	m^6A writer; promoting IL-7-JAK-STAT5 signaling pathway	Mouse	T cell activation and effector function	(53)
		m^6A writer; unknown	Mouse	T_{FH} differentiation upon LCMV infection	(54)
	ALKBH5	m^6A eraser; inducing degradation of *Ifng* and *Cxcl2* mRNA	Mouse	T cell activation and effector function	(56)
	NSUN2	m^5C modification; unknown	Human	SLE	(57)
RNA decay	ZFP36L1/2	β-selection of thymocytes	Mouse	T-ALL	(76,79,80)
		Limiting the abundance of *Ifng* mRNA		T cell activation and effector function	
	ROQUIN1/2	Inducing decay of *Icos* mRNA	Mouse	T_{FH} differentiation	(82,85,86)
				Spontaneous SLE-like autoimmunity	
	BTG1/2	Global mRNA decay in resting T cell	Mouse	T cell quiescence	(87)
	REGNASE-1	Inflammatory mRNAs decay	Mouse	Severe anemia	(89-91)
				Spontaneous autoimmunity	
				T cell developmental defects	

m^5C, N5-methylcytosine; m^6A, N6-methyladenosin; PTEN, phosphatase and tensin homolog deleted on Chromosome 10; T-ALL, T-cell acute lymphoblastic leukemia.

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