## Title

RNA circuits and RNA-binding proteins in T cells



## Journal

Trends in Immunology, 44(10)

## ISSN

1471-4906

## Authors

Zhu, Wandi S
Wheeler, Benjamin D
Ansel, K Mark

## Publication Date

2023-10-01

## DOI

10.1016/j.it.2023.07.006

Peer reviewed

HHS Public Access

Author manuscript

*Trends Immunol*. Author manuscript; available in PMC 2024 October 01.

Published in final edited form as:

*Trends Immunol*. 2023 October ; 44(10): 792–806. doi:10.1016/j.it.2023.07.006.

# RNA circuits and RNA binding proteins in T cells

**Wandi S. Zhu**[1,2], **Benjamin D. Wheeler**[1,2], **K. Mark Ansel**[1]

[1]Department of Microbiology & Immunology, Sandler Asthma Basic Research Center, University of California San Francisco, San Francisco, CA, USA, 94143

[2]Equal contribution

## Abstract

RNA is integral to the regulatory circuits that control cell identity and behavior. Cis-regulatory elements in mRNAs interact with RNA binding proteins (RBPs) that can alter RNA sequence, stability, and translation into protein. Similarly, long noncoding RNAs (lncRNAs) scaffold ribonucleoprotein complexes that mediate transcriptional and post-transcriptional regulation of gene expression. Indeed, cell programming is fundamental to multicellular life, and in this era of cellular therapies, it is of particular interest in T cells. Here, we review key concepts and recent advances in our understanding of the RNA circuits and RBPs that govern mammalian T cell differentiation and immune function.

## RNA Circuitry in T cells

Gene expression programs define cell identity and govern cell behavior. Layered regulatory circuits sculpt spatiotemporal patterns of gene activity to create impressive complexity and environmental responsiveness from a single genomic blueprint. RNA molecules are integral to almost all of these regulatory circuits. RNA is the synthetic product of transcription and the template for protein translation, but also act as substrates for post-transcriptional regulation and as active mediators of regulatory processes. In this review, we discuss RNA circuits that operate in T cells to regulate their development, differentiation, and function in mammalian immunity. We aim to illuminate RNA circuits through the lens of cis-regulatory logic, focusing on the RNA sequence and structural elements that function through interaction with RNA binding proteins (RBPs) and/or other RNA molecules. Our increasing understanding of RNA circuits sharpens our view of cell programming and may enable their use in genomic and cell therapies.

## RNA Binding Proteins

RNA binding proteins (RBPs) bind to linear and structural motifs in the coding region, introns, and untranslated regions (UTR) of transcripts to mediate alternative splicing, alternative polyadenylation usage (APA), RNA modifications, localization, stability, and

Address correspondence to: mark.ansel@ucsf.edu.

Declaration of interests

The authors declare no conflict of interest.

translation. Upon exposure to environmental stimuli or internal signaling, RBPs can relocalize and shuttle transcripts to different subcellular compartments to undergo different processes, including degradation and translation to generate an appropriate cellular response [1,2]. Regulatory circuits involving RBPs and their target transcript(s) modulate T cell differentiation and immune functions. Our expanding knowledge of the RBP repertoire and RNA binding sites in T cells provides an opportunity to deploy RNA-centric approaches for uncovering regulatory circuits that govern T cell function (Box 1).

## Experimental approaches for mapping RBP-RNA interactions

A variety of forward proteomic methods detect interactions between specific RBPs and their target transcripts. RNA immunoprecipitation (RNA IP) pairs immunoprecipitation of a specific RBP with quantitative RT-PCR (qPCR) to identify its associated transcripts (Fig. 1A). High throughput sequencing of crosslinking immunoprecipitation (HITS-CLIP) was developed to map the specific binding sites of an RBP (Fig 1B). This and many refined methods utilizing UV-crosslinking and RNAse digestion to produce small RNA libraries of bound sequences are widely used to produce transcriptome-wide RBP binding profiles at or near nucleotide resolution [3–7]. Conversely, RNA-centric reverse proteomics can be used to identify the RBP(s) that bind to known cis-regulatory regions (Fig. 1C). For example, **RNA aptamers** (See Glossary) have been instrumental in identifying RBPs that bind to AU rich elements (AREs) in the 3’UTR of mRNAs that encode the cytokines TNF-α and IFN-γ [8,9].

Recently developed methods profile the entire RBP repertoire and global RBP occupancy on transcripts. Both **RNA interactome capture** (RNA IC) [10–15] and the organic **phase separation methods OOPS and XRNAX** [16–18] systematically capture RBP:RNA complexes for downstream identification of proteins through mass spectrometry and binding sites through small RNA sequencing (Fig. 1D–E). Performed in the human Jurkat T cell line and in mouse and human primary T cells, these methods expanded the known repertoire of proteins that bind RNA [12,19] including non-canonical RBPs such as signal transducer and activator of transcription 1 (STAT1) and STAT4 [19]. Of the RBPs identified through RNA IC and OOPS, 439 were uniquely expressed in primary human T cells when compared to non-immune HEK293, U2OS and MCF10a cells [19]. These global RBP interactome data can guide the dissection of RBP-mediated post-transcriptional regulatory circuits modulating T cell function.

## RBP-mediated post-transcriptional processes

### Signal responsive alternative splicing and polyadenylation

Alternative splicing generates mRNA isoforms that can encode proteins with different localization, catalytic activity, or stability [20]. Splicing factors, including multifunctional RBPs, regulate splice site usage in a context-specific manner in T cells, forming signal-responsive RNA circuits that modulate T cell activation and immune function. To take a classic example, CD45, a transmembrane tyrosine phosphatase encoded by *Ptprc*, regulates cell signaling in T cells and other hematopoietic cells. Naïve T cells express long isoforms of the protein (e.g. CD45RA), but alternative splicing produces a shorter form (CD45RO)

in activated and memory T cells ($CD4^+$ and $CD8^+$). The interaction of Heterogeneous Nuclear Ribonucleoprotein L (HNRNPL) [21,22], HNRNPL-like (HNRNPLL) [23] and PTB-associated Splicing Factor (PSF) [24] with activation responsive sequences in the *Ptprc* mRNA regulates this process.

Antigen and costimulatory receptor signaling induce alternative splicing of many transcripts during T cell activation. For instance, activated human primary $CD4^+$ T cells generate alternatively spliced transcripts involved in apoptosis, including *CASP9*, *BIM,* and *BAX* [25]. The resulting shortened, inactive forms of CASP9, BIM, and BAX proteins inhibit apoptosis and instead promote cellular proliferation upon activation [25]. Binding motifs for CUGBP Elav-Like Family Member 2 (CELF2), Serine and Arginine Rich Splicing Factor 5 (SRSF5) and Polyprimidine Tract Binding Protein 1 (PTBP1) near the splice site in *CASP9* suggest a role for these RBPs [25]. In murine $CD8^+$ OT-I T cells, activation with antigen and costimulation via CD134 (OX40) and CD137 (41BB) induced TAR DNA Binding Protein (Tardbp)-mediated alternative splicing [26]. OT-I cells targeted with CRISPR to delete *Tardbp* generated a smaller pool of antigen-specific cells with less IFN-γ expression compared to non-targeted control OT-I cells [26]. Additionally, RBPs can regulate their own expression and function through splicing in response to TCR engagement. Downstream of TCR engagement, c-Jun N-terminal kinase (JNK) induces the expression of CELF2, which alternatively splices Map Kinase Kinase 7 (MKK-7) in Jurkat and primary human $CD4^+$ T cells [27,28]. The shorter isoform of MKK-7 phosphorylates JNK and reinforces CELF2 activity [28], generating a feedforward loop. RBP splicing proteins, such as SRSF1, can also regulate T cell homeostasis and development. [29,30]. Mice with T cell-specific ($Lck^{Cre}xSrsf1^{fl/fl}$) and regulatory $CD4^+$ T cell-specific (**Treg**) ($Foxp3^{YFP\text{-}Cre}xSrsf1^{fl/fl}$) depletion of SRSF1 develop systemic autoimmunity as well as express higher proportion of proinflammatory IL-$17a^+$, IL-$4^+$ and IFN-$\gamma^+$ $CD4^+$ effector T cells and Tregs [29,30]. In the thymus, $Lck^{Cre}xSrsf1^{fl/fl}$ mice showed reduction in mature single positive thymocytes as well as a reduction in peripheral T cells, suggesting a critical role of this RBP in late-stage thymocyte development [31]. Whether SRSF1 regulates T cell development and homeostasis through splicing mechanisms remains to be confirmed.

In addition to alternative splicing, transcripts can undergo alternative polyadenylation (APA). In most mRNA and many noncoding RNAs, the newly transcribed transcript is cleaved at the 3’ end by a multi-protein complex that recognizes the polyadenylation signal (PAS) composed of an AAUAAA motif and flanking U/G rich sequences [20], followed by non templated addition of adenosines. APA is regulated by RBPs that bind to regions in the 3’UTR to regulate PAS site usage. Activated mouse and human T cells engage APA to undergo global 3’UTR shortening, eliminating binding sites for trans factors including micro (mi)RNAs and RBPs [32,33]. Information on the regulatory circuits modulating APA in a cell-type or context specific manner remains limited. However, CELF2, a splicing protein [30] regulates APA upon T cell activation as shown by RNA-sequencing analysis and **3’RACE** in Jurkat T cells, and induces preferential usage of certain PAS sites in including in its own 3’UTR [34]. CELF2 competed with other RBPs in the polyadenylation complex for PAS sites in vitro [34]. However, further study is needed to elucidate this and other APA regulatory circuits in T cells.

## RNA methylation in T cells

RNA is subject to modification, such as methylation, that can affect its immunogenicity and stability within cells. The reversible methylation process is mediated by **"writer" complexes** comprised of the methyltransferases METTL3 and METTL14 and adaptor proteins, **"reader" proteins** including YTH N6-Methyladenosine RNA Binding Proteins (YTHDF1–3) and **"eraser proteins"** such as AlkB Homolog 5 (ALKBH5) that remove the methyl group [36]. Most research on RNA modification in T cells has focused on methylation of adenosine at the nitrogen-6 position (m6A) as it is one of the most abundant RNA modifications in eukaryotic cells [36]. However, recent work showed that 5-methylcytosine deposition on mRNA by NOP2/Sun RNA methyltransferase 2 (Nsun2) stabilizes *Il17a* mRNA in mouse **T helper 17** (Th17) cells, as evidenced by shorter *Il17a* mRNA half-life in Nsun2-depleted ($CD4^{Cre}xNsun2^{fl/fl}$) Th17 cells compared to WT Th17 cells [35], demonstrating a critical role for another RNA modification in regulating effector T cell function.

Studies that modulate m6A expression through removal of the writer or eraser RBPs demonstrated that this modification is crucial for regulating T cell function in different cellular and environmental contexts. After adoptive transfer of mouse naïve CD4+ T cells into immunodeficient $Rag2^{-/-}$ recipient mice in a transfer mouse model of colitis, donor $CD4^+$ mouse T cells deficient for METTL3 ($CD4^{Cre}xMettl3$; *Mettl3-cKO*) or METTL14 ($CD4^{Cre}xMettl14$; *Mettl14-cKO*) expressed lower levels of m6A as detected by high performance liquid chromatography, lower numbers of donor T cells, decreased proliferation as detected using CellTrace labeling, and remained in a $CD4^+$TGF-$\beta^+$CD45RB$^{hi}$ naïve state compared with donor WT T cells [37]. *In vitro* cultured *Mettl3*-cKO naïve $CD4^+$ T cells displayed longer transcript half-lives for *Socs1,* Socs3 and *Cish*, negative repressors of IL-7 mediated STAT5 signaling [37]. Mettl3-deficient Tregs from $Foxp3^{Cre}$x$Mettl3^{fl/fl}$ mice also expressed higher transcript levels of these negative repressors and decreased phosphoSTAT5 signaling after treatment with IL-2 *in vitro* [38]. Removing the eraser ALKBH5 also alters T cell function. For instance, conditional deletion of *Alkbh5* in $CD4^+$ T cells ($CD4^{Cre}$x$Alkbh5^{fl/fl}$) led to shorter IFN-$\gamma$ transcript half-life and decreased proinflammatory cytokine response in an experimental autoimmune encephalomyelitis (EAE) model [39].Together, these studies suggest that modulation of m6A methylation through "writer" and "eraser" RBPs is necessary for regulating T cell responses to environmental cues, specifically IL-7 and IL-2 mediated STAT5 signaling in mouse $CD4^+$ T cells [37,38], as well as production of proinflammatory cytokines [39].

Wilms Tumor-1 associated protein (Wtap) encodes an essential adaptor protein in m6A writer complexes. In contrast to *Mettl3*-cKO and *Mettl14*-cKO mice [37], mice with conditional deletion of *Wtap* in all T cells ($CD4^{Cre}xWtap^{fl/fl}$) or only in Tregs ($Foxp3^{YFP\text{-}Cre}xWtap^{fl/fl}$) developed spontaneous gut inflammation as evidenced by an increased colitis score and greater proportion of IFN-$\gamma^+$ and IL-17A$^+$ conventional $CD4^+$ T cells and Tregs, despite low or no expression of m6A [40]. While depletion of either the "writer" [37] or adaptor proteins [40] impaired proliferation, these studies differed on the effects on T cell receptor versus cytokine signaling. As described above, METTL3 Mettl3 deficiency affected IL-7 signaling [37], whereas Wtap-deficient T cells revealed that m6A destabilizes

transcripts that encode proteins involved in TCR signaling, such as *Orai1* and *Ripk1*, to inhibit TCR-induced cell death upon activation with anti-CD3 and anti-CD28, as shown by the increased half-life of these transcripts in Wtap1-deficient $CD4^+$ T cells ($CD4^{Cre\text{-}ERT2}xWtap^{fl/fl}$) compared to WT cells [40]. The discrepancy between the two models may be due to differences in the degree of reduced m6A deposition, although this remains to be further studies. Indeed, a comparison of the residual m6A and the transcripts that remained methylated could provide insight into this difference.

From another angle, m6A also contributes to regulating $CD4^+$ T cell differentiation through stabilization of transcription factor transcripts. For instance, in an acute Lymphocytic Choriomeningitis Virus (LCMV-Armstrong) mouse infection model, METTL3 was necessary for stabilization of *Tcf7* and other transcripts involved in **T follicular helper cell (Tfh)** differentiation, as evidenced by decreased half-life of *Tcf7* mRNA in Tfh cells from *Mettl3*-cKO mice [41]. In culture, METTL3-deficient naïve $CD4^+$ T cells skew towards **T helper 2** (Th2) over **T helper 1** (Th1) and Th17 cell fates, with greater proportion of IL-$4^+$ and IL-$13^+$ CD4 cells compared to IFN-γ and IL-17 producers [37]. Wtap-deficient and m6A-low thymocytes showed decreased CD8 expression on $CD8^+$ single-positive cells, suggesting a role for m6A in controlling the expression of key genes in T cell development [39]. m6A and the RBP machinery compose an important circuit for regulating transcript stability and various T cell functions. However, the balance between stabilizing and destabilizing certain transcripts in different cellular contexts in T cells is unknown. Targeting YTHDF2 with RNAi in HeLa cells caused accumulation of m6A-modified mRNA in the cell [42], while targeting members of the Insulin-like Growth Factor 2 Binding Protein (IGF2BP) family decreased m6A-modified mRNA expression transcriptome-wide in HeLa and HepG2 cells [43]. Taken together, these data suggest that YTHDF2 and IGF2BP2 may act antagonistically to destabilize or stabilize transcripts through m6A binding. This regulatory circuit may also modulate transcript stability in T cells, which express both YTHDF2 and IGF2BP family members.

## mRNA Stability and Translation

In the cytoplasm, RBPs bind cis-regulatory elements with specific RNA sequence and/or structural motifs to regulate transcript degradation by multiple mechanisms including endonuclease cleavage [44,45], decapping of the 5’ end [46] and deadenylation of the 3’ end [47,48] followed by exonuclease digestion. RBPs can also engage or inhibit translation to further fine-tune protein expression [9,44,49]. The following sections highlight these properties through discussion of select RBP families with prominent functions in T cell biology.

**AU Rich Elements (ARE) and ARE-BPs—**AU rich elements (ARE) and ARE binding proteins (ARE-BP) form complex circuits that modulate the duration and intensity of immune responses. AREs are typically characterized by the canonical pentamer AUUUA, though functional noncanonical sequence motifs also exist. AREs are common in 3’UTRs of cytokines, early activation genes and signal transduction genes such as *Nur77* and *IL-17* [50–53]. The loss of an individual ARE can lead to hyperinflammation and autoimmunity in mouse models, as was observed in TNF ΔARE mice, which lacked an ARE in the 3’UTR

of TNF, and developed chronic arthritis and inflammatory bowel disease marked by immune cell infiltration and tissue damage [54]. ARE-BP exert different regulatory mechanisms to modulate T cell effector function in a temporal and cell-type specific manner. For instance, Zinc finger protein 36 (ZFP36; also known as tristetraprolin, or TTP) is rapidly upregulated in activated mouse $CD4^+$ and $CD8^+$ T cells and maintained for several days [55]. During this time, ZFP36 and its family member ZFP36 like 1 (ZFP36L1) bind to AREs to regulate the stability and translation of mRNAs involved in T cell activation [55]; they can also functionally limit proliferation, effector cell function and inflammatory cytokine production [55,56]. In resting mouse $CD8^+$ memory T cells, ZFP36 like 2 (ZFP36L2) regulates IFN-γ production by repressing its translation in an ARE-dependent manner [9]. In contrast to the ZFP36 family, HuR (ELAVL1) binding to AREs can stabilize transcripts. Specifically, targeting HuR with RNAi increased the half-life of the ARE-containing mRNAs encoding GATA-3 and IL-13 in human in vitro cultured Th2 cells [57,58], and *Il17a* mRNA half life similarly increased in HuR-deficient mouse Th17 cells [59]. The mechanisms that determine ARE-BP specificity require further investigation. In T cell lines, incubation of ZFP36 and HuR with probes containing AREs from different genes showed competitive binding to a GM-CSF ARE probe, but also distinct binding specificity to IL-2 and c-Jun AREs. [60]. Additional ARE-BPs may also contribute to the regulation of ARE-containing mRNAs in T cells. Together, these studies demonstrate the complexity of ARE-directed RNA circuits that can regulate T cell responses.

**miRNA binding sites and Argonaute proteins—**RBPs of the Argonuate (Ago) family are programmed by miRNAs to recognize linear sequence motifs, and mediate translational repression and transcript instability of target transcripts. T cells exploit this flexibility in Ago target specificity by increasing Ago turnover and dynamically regulating their miRNA repertoire in response to activation [61]. HITS-CLIP revealed thousands of Ago2 binding sites in T cells [62,63]. Each miRNA can regulate tens to hundreds of mRNAs, sometimes through multiple binding sites in the same transcript. The myriad roles of individual miRNAs and families of miRNAs in T cell function has been reviewed elsewhere [64–66].

Ago and miRNAs participate in complex RNA circuitry with other RBPs. For example, the transcription factor BHLHE40 inhibits the expression of Regnase-4 and miR-146 in human memory $CD4^+$ T cells, both of which inhibit NF-kB signaling and cytokine expression by independently binding to an overlapping set of target mRNAs [67]. In primary mouse Tregs, *Mtor* is repressed by the cooperative activity of miR-150 and miR-99a, as elucidated using an *Mtor* 3'UTR reporter in mouse embryonic fibroblasts transfected with each miRNA alone, or both in combination [68]. In addition, RBPs and miRNAs can compete for binding sites. In primary mouse $CD4^+$ T cells, Roquin binds to *Pten* mRNA at a site that overlaps a miR-17 binding site [69]. Compared with wildtype cells, Roquin-deficient $CD4^+$ T cells exhibited increased Ago protein binding and decreased overall expression of *Pten,* consistent with a model wherein Roquin protects *Pten* from miR-17-mediated inhibition [69].

## Secondary Structures and RBPs

Many RBPs interact with RNA structures, or a combination of structural and linear motifs. In mouse and human T cells, Roquin (both Roq1 and Roq2) and the RNA-binding

endonuclease Regnase-1 recognize **constitutive decay elements** (CDE), **alternative decay elements** (ADE) and other variations of a stem loop to exert regulatory function [47,70]. In steady state conditions, Roquin and Regnase bind to motifs in the 3'UTR to initiate transcript decay [44,71,72] or translational silencing [44,49]. Mutating multiple stem loops in the *Nfkbid* 3'UTR in a HeLa cell reporter system dramatically increased reporter mRNA half-life and enriched the transcript in translated polysomal fractions, suggesting that Roquin binding can induce translational repression in addition to mRNA decay for a target transcript. [49]. Upon TCR activation, both Roquin and Regnase are cleaved by paracaspase MALT1, releasing the regulatory circuits restraining the cells and promoting proinflammatory NFκB signaling and cytokine production, as was shown in mouse $CD4^+$ T cells and Jurkat cells [71,72].

Roquin proteins bind and repress the expression of *Icos*, *Ox40,* and transcripts in the NF-kB signaling pathway to maintain T cell quiescence [69,72,73]. Enhanced proportions of IL-17-producing Th17 cells [72] and $CXCR5^{hi}PD^{hi}$ Tfh cells were observed in mice with conditional deletion of Roquin in T cells ($CD4^{Cre}xRc3h1^{fl/fl}xRc3h2^{fl/fl}$). Limiting Roquin deficiency to Tregs (in $Foxp3^{YFP\text{-}Cre}xRc3h1f^{l/fl}xRc3h2^{fl/fl}$ mice) led to an expansion of $CXCR5^{hi}PD1^{hi}$ Tregs [69]. Together, these data suggest that Roquin restrains differentiation towards Th17, Tfh and **follicular regulatory T cells** (Tfr) [69,72]. In $CD4^{Cre}xRc3h1^{fl/fl}xRc3h2^{fl/fl}$ mice, absence of Roquin in $CD8^+$ T cells induces greater proportions of Granzyme B (GzB)+ and IFN-$\gamma^+$TNF-$\alpha^+$ cells, thereby restraining proinflammatory and cytotoxic function [74].

Regnase1 binds to similar target transcripts as Roquin and also restrains hyperinflammatory and autoimmune states, as observed in a genetic mouse model ($CD4^{Cre}$xRegnase-$1^{fl/fl}$) [71,74]. Specific examination of ICOS expression in *in vitro* polarized mouse Th1 cells revealed sustained ICOS expression throughout culture in Th1 cells lacking Roquin and Regnase-1 compared to deletion of either protein [74]. Additionally, co-immunoprecipitation assays in mouse $CD4^+$ T cells revealed that Roquin and Regnase-1 can interact with each other [74]. Mutagenesis of this interaction site in HeLa cells transfected with Roquin and Regnase plasmids decreased binding between the two RBPs [74]. Together, this suggests that while Roquin and Regnase can work independently, they may also interact and cooperatively regulate T cell function [74]. In contrast, AT rich interaction domain 5a (ARID5A) antagonizes Regnase function by competing for the same stem loops to stabilize target transcripts [75–78]. One such example is the *Stat3* 3'UTR which contains stem loops motifs for Regnase-1 and ARID5A. In HEK293 cells co-transfected with *Stat3* 3'UTR reporter and Regnase-1 plasmid, the addition of ARID5A into the system stabilized the reporter and enhanced reporter activity in a dose-dependent manner, demonstrating a competitive interaction between Regnase-1 and ARID5A. Additionally, ARID5A stabilizes *Ox40* and other target mRNAs, and increases Th17 differentiation, as evidenced by reduction of in vitro polarized Th17 cells from *Arid5a* deficient mice [77,78]. These findings add an additional layer of regulation beyond binary interactions between cis-elements and trans-acting RBPs.

From a clinical perspective, recent studies have leveraged RBPs, specifically Roquin and Regnase function, to generate new putative T cell therapies to treat cancer. For instance

deletion of these proteins in human chimeric antigen receptor **(CAR) T cells** or mouse antigen-specific $CD8^+$ T cells increases IFN-γ, TNF-α, IL-2 and GzB expression. Increased cell expansion and T cell persistence in tumors was also observed in models of pancreatic adenocarcinoma, OVA-expressing melanoma (B16-OVA) and OVA-expressing lymphoma (EL-4 OVA)[79–81]. Importantly, transfer of Regnase-1, Roquin, or double-deficient CAR T cells or $CD8^+$ OT-I cells slowed tumor growth in all the tumor models described [79–81]. Recipient mice given OT-I T cells with non-functional mutant Roquin-1 that also cannot interact with Regnase (Roquin-$1^{M199R/E212K}$) showed increased frequency of donor cells in the tumor and slower tumor growth in the B16-OVA model compared to recipients with WT OT-I donor cells [74]. Together, these studies have illuminated a potential avenue for targeting RBPs and their target mRNAs for potential therapeutic interventions in oncology.

## Long noncoding RNAs

Long non-coding RNA (lncRNA) are genomically encoded RNAs that do not contain an open reading frame and are not translated into proteins [82]. lncRNAs constitute a large portion of the human transcriptome with 96411 lncRNA genes annotated compared to 19890 coding genes [83,84]. Mouse annotations are similar with 87890 lncRNA genes annotated compared to 22186 coding genes [84,85]. lncRNAs have drawn significant research interest due to a high degree of cell type and tissue specificity in their expression [86]. Together, these observations suggest that lncRNAs perform important regulatory functions in a variety of cellular contexts.

## LncRNA discovery and annotation

The proliferation of annotated lncRNAs is the direct result of advances in long and short read sequencing technologies. Short read sequencing has been used to deeply and broadly survey the RNA landscape in many cells and tissue types, while long read sequencing has improved annotation of lncRNAs by capturing whole or nearly whole transcripts [87]. This is especially important for circular RNAs (circRNA), which are often the result of splicing events where the ends of the removed intron are ligated to create a circular topology. Long read sequencing greatly increases the likelihood that the ligated junction will be completely read through and correctly attributed to the circRNA rather than an un-spliced transcript [88]. There are other examples of post-transcriptional processing of lncRNAs, such as the **Malat1-associated small cytoplasmic RNA** (mascRNA), which is liberated from the parental Malat1 transcript by RNase P cleavage [89]. Attention to these details is important when annotating and detecting lncRNAs, especially for assays that rely on aligning short reads.

## LncRNA function in regulatory circuits

LncRNAs as a class do not have a specifically defined function but they contribute to a variety of regulatory circuits through their interaction with RBPs, other RNAs, and DNA [90]. Here, we focus on lncRNA-mediated regulatory mechanisms that have been described in T cells including transcriptional regulation, post-translational modification, and miRNA inhibition as a **competing endogenous RNA** (ceRNA) (Fig. 2).

## Transcriptional Regulatory Mechanisms

Many lncRNAs regulate gene transcription (Fig. 2A). Abundant lncRNAs can act at a distance on suites of target genes, while others act locally on genes in the same locus, as has been clearly defined for immunoglobulin locus lncRNAs in B cell **class switch recombination** [91]. Pediatric T cell acute lymphoblastic leukemia (T-ALL) cells highly express both *NOTCH1* and *NALT* (Notch associated lncRNA in T-ALL), which is located less than 100 bp away [92]. shRNA knockdown of *NALT* reduced *NOTCH1* expression in Jurkat T cells and slowed their growth when implanted into immunodeficient mice [92]. Importantly, a Gal4-λN/BoxB reporter system indicated that transcriptional activation is conferred by the lncRNA, as NALT tethering to the promoter induced the transcription of a reporter gene. Genetic deletion of the polyfunctional lncRNA Malat1 increased the expression of its neighboring genes, though no mechanism has so far been described [93]. The genomic locus of the lncRNA *Morrbid* in mice positively regulates the expression of *Bcl2l11* in cytotoxic $CD8^+$ T cells, but negatively regulates *Bcl2l11* expression in short-lived myeloid cells, suggesting that lncRNA loci can exhibit cell-type specific transcriptional regulation [94,95]. *Morrbid* overexpression did not alter *Bcl2l11* expression, but did reduce AKT signaling in cytotoxic T cells [95]. Thus, *Morrbid* is a functional lncRNA, but may not be responsible for the regulation of nearby genes. Further studies are needed to elucidate the genomic components and/or functional elements that confer *Bcl2l11* regulation in T cells and other immune cell types.

One common mechanism by which lncRNAs regulate transcription is by scaffolding transcription factors and chromatin modifiers (Fig. 2B), particularly proteins of the Polycomb repressive complexes (PRC). Malat1 associates with the PRC2 proteins EZH2 and SUZ12 and with nucleosomes bearing the repressive histone-3 trimethyl (H3K27me3) modification in HH cells, a human cutaneous T cell lymphoma cell line [96]. Another recent study showed that Malat1 interacts with Ezh2 in mouse $CD8^+$ T cells to maintain H3K27me3 marks on memory associated genes such as *Tcf7*, *Eomes*, *Zeb1*, *Lef1*, and *Bcl2* [97]. Moreover, in an adoptive transfer model, Malat1-deficiency in transgenic TCR P14 LCMV-specific $CD8^+$ T cells resulted in a greater proportion and number of memory cells [97]. Th17 cell differentiation and cytokine production is regulated by lncRNAs in a similar manner in both mice and humans. In mice, Malat1 is downregulated upon T cell activation and differentiation into the Th17 lineage [98,99]. Similar to $CD8^+$ T cells, Malat1 binds to Suz12 and enhances H3K27me3 deposition at the *Il17a-Il17f* locus, decreasing cytokine expression and colonic inflammation [98]. In contrast, lncRNA MIAT (Myocardial Infarction Associated Transcript) enhances Th17 differentiation and cytokine production in primary human T cells [100]. Of note, MIAT is highly expressed in T cells isolated from the synovium of rheumatoid arthritis (RA) patients, and targeting MIAT with **locked nucleic acid oligonucleotides** decreases chromatin accessibility at the *IL17A* locus [100]. Other lncRNAs have been shown to exhibit similar behaviors. For instance, T cell lymphoma-associated lncRNA1 (TCLlnc1) can modularly scaffold heterogeneous nuclear ribonucleoprotein D and Y-box binding protein 1 complexes to induce the expression of TGF-β in Jurkat T cells [101]. These examples demonstrate the modularity of lncRNA function in transcriptional regulation.

LncRNAs can also regulate post-translational modifications on transcription factors and other proteins (Fig. 2C). In mouse primary $CD4^+$ T cells, lncRNA-GM inhibits the dephosphorylation of FOXO1 by PP2A, which increases IL-23 Receptor expression [102]. This effect led to enhanced Th17 differentiation and increased pathology in EAE. The human orthologue of lncRNA-GM similarly enhanced Th17 transcriptional signature genes in $CD4^+$ T cells [102]. shRNA knockdown of NEAT1 (Nuclear paraspeckle assembly transcript 1) in human T cells enhanced ubiquitination of STAT3 [103]. The subsequent reduction in STAT3 abundance correlated with reduced IL-17 production [103]. Moreover, NEAT1 is upregulated in human RA peripheral blood mononuclear cells, and lentiviral delivery of NEAT1 shRNA into the joints relieved the degree of Type 2 collagen induction in the collagen-induced arthritis mouse model [103]. These findings suggest that NEAT1 may be of clinical relevance in human autoimmune diseases, although this remains to be robustly studied. Another notable example of lncRNA mediated modulation of protein abundance is the regulation of the vesicular trafficking system by the lncRNA Snhg1 (Small nucleolar RNA host gene 1) [104] (Fig. 2D). RNA immunoprecipitation showed that Snhg1 interacts with the vesicular sorting protein VPS13D and enhances its shuttling of CD127 to the cell surface of mouse and human $CD8^+$ T cells [104]. shRNA inhibition of either Snhg1 or VPS13D reduced cell surface CD127 expression and memory cell numbers in LCMV-Armstrong infected mice, as detected by flow cytometry [104]. Together, these findings further illuminate the great diversity of mechanisms by which lncRNAs mediate their cellular functions.

## Post-transcriptional Regulatory Mechanisms

LncRNAs can also regulate gene expression and cellular function post-transcriptionally by sequestering RBPs and miRNAs that regulate other mRNAs (Fig. 2E). For example, NORAD (Non-coding RNA Activated by DNA damage) binds to Pumilio proteins through multivalent interactions to induce their subcellular compartmentalization via liquid-liquid phase separation in the HCT116 human cancer cell line [105]. LncRNAs can also inhibit miRNA function through stoichiometric sequestration (as a decoy or “sponge”), miRNA degradation, or other yet to be defined mechanisms (Box 2). This phenomenon has gained substantial interest outside of T cell biology, and has been demonstrated in T cells as well.

In $CD8^+$ T cells, Malat1 inhibits miR-15/16 family miRNAs to enhance memory cell formation [63,106]. Precise disruption of the miR-15/16 binding site in Malat1 decreased expression of miR-15/16 targets including CD28 and Bcl-2 in mouse T cells, with reduced costimulation-associated gene expression and IL-2 production [106]. The murine circular RNA circRNA-1806 sponges miR-126, which targets adrenomedullin, which in turn induces the phosphorylation of c-Jun and JNK to regulate cell cycle progression in human glioblastoma cells [107] [108]. Targeting circRNA-1806 with RNA interference in mice reduced the clearance of the fungus *Cryptococcus neoformans* [107]. Lnc-AIFM2–1 sponges miR-330–3p in human $CD8^+$ T cells, and may thereby promote hepatitis B virus immune escape [109]. CD244 was identified as a key miR-330–3p target that exhibited reduced expression in Jurkat cells transfected with siRNA against Lnc-AIFM2–1. CD244 expression was associated with increased T cell apoptosis, and miR-330–3p mimics decreased HBV control in an *in vitro* co-culture model [109]. In $CD4^+$ T cells, Th17 differentiation

can be regulated by the lncRNA:miRNA circuit composed of LncITSN1–2 (LncRNA intersectin 1–2) and miR-125a [110]. IL-23R is a key target of miR-125a and Lnc-ITSN1–2 overexpression increased IL-17 and RORC mRNA expression in *in vitro* differentiated Th17 cells [110]. Together, these and additional emerging studies demonstrate that lncRNA:miRNA circuits modulate T cell activation, apoptosis, and differentiation, although the exact mechanisms and biological impact often remain to be further investigated.

## Experimental Approaches to Studying lncRNA Function

The tool kit to study lncRNA function is expanding. Previous genetic approaches have relied on the excision of large portions of the genetic locus of a given lncRNA [93,95] or the insertion of an early poly-A signal [111]. These interventions can cause significant disruption to the locus and regulatory regions such as enhancers for nearby or distant genes, confounding interpretation of observed phenotypes. To address this problem, many studies have focused solely on the transcript instead and employed RNAi to post-transcriptionally degrade a given lncRNA [97,103,107,109]. This approach has been best applied in mouse models where TCR transgenics are available and virally delivered shRNAs can be stably expressed in transduced T cells [97]. The limitations of RNAi include incomplete knockdown that may make observations of subtle phenotypes difficult, and the inability to attribute function to specific sequences within lncRNAs. LncRNA fragments can also be transcribed to probe protein binding through methods such as RNA-IP [101]. However, the advent of a multitude of CRISPR based technologies now allows more directed approaches to dissecting lncRNA function and mechanism of action (Box 3).

## Concluding Remarks

RNA circuits are a ubiquitous feature of genome regulation, and they play specific, essential roles in T cell proliferation, differentiation, and effector function – properties that are critical for effective immunity against pathogens and cancer, and for the prevention of allergy and autoimmunity. Cis-regulatory approaches that leverage high resolution RNA interactome mapping facilitate rigorous interrogation of RBP and lncRNA biological functions and mechanisms of action. Moreover, Recent findings and improved technologies for detecting and manipulating RNA circuits have accelerated progress in immunology and biomedicine, and are propelling the clinical translation of these discoveries. However, functional dissection of all the RBPs and lncRNA circuity, especially in primary T cells, remains limited and requires further investigation to fully leverage these mechanisms for T cell therapies (see Outstanding questions). Nevertheless, modulating T cell function through post-transcriptional processes represents a fruitful and exciting area of investigation.

## Acknowledgments

This work was supported by the US National Institute of Health (P01HL107202, R01HL109102, and T32AI007334), the Hooper Foundation, and the Sandler Asthma Basic Research Center.

## References


1. Decker CJ and Parker R (2012) P-bodies and stress granules: possible roles in the control of translation and mRNA degradation. Cold Spring Harb. Perspect. Biol 4, a012286 [PubMed: 22763747]
2. Ma W and Mayr C (2018) A Membraneless Organelle Associated with the Endoplasmic Reticulum Enables 3'UTR-Mediated Protein-Protein Interactions. Cell 175, 1492–1506.e19 [PubMed: 30449617]
3. Ule J et al. (2005) CLIP: a method for identifying protein-RNA interaction sites in living cells. Methods 37, 376–386 [PubMed: 16314267]
4. Hafner M et al. (2010) Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. Cell 141, 129–141 [PubMed: 20371350]
5. König J et al. (2010) iCLIP reveals the function of hnRNP particles in splicing at individual nucleotide resolution. Nat. Struct. Mol. Biol 17, 909–915 [PubMed: 20601959]
6. Van Nostrand EL et al. (2016) Robust transcriptome-wide discovery of RNA-binding protein binding sites with enhanced CLIP (eCLIP). Nat. Methods 13, 508–514 [PubMed: 27018577]
7. Hafner M et al. (2021) CLIP and complementary methods. Nature Reviews Methods Primers 1, 1–23
8. Leppek K and Stoecklin G (2014) An optimized streptavidin-binding RNA aptamer for purification of ribonucleoprotein complexes identifies novel ARE-binding proteins. Nucleic Acids Res. 42, e13 [PubMed: 24157833]
9. Salerno F et al. (2018) Translational repression of pre-formed cytokine-encoding mRNA prevents chronic activation of memory T cells. Nat. Immunol 19, 828–837 [PubMed: 29988089]
10. Baltz AG et al. (2012) The mRNA-bound proteome and its global occupancy profile on protein-coding transcripts. Mol. Cell 46, 674–690 [PubMed: 22681889]
11. Castello A et al. (2012) Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. Cell 149, 1393–1406 [PubMed: 22658674]
12. Perez-Perri JI et al. (2018) Discovery of RNA-binding proteins and characterization of their dynamic responses by enhanced RNA interactome capture. Nat. Commun 9, 4408 [PubMed: 30352994]
13. Garcia-Moreno M et al. (2019) System-wide Profiling of RNA-Binding Proteins Uncovers Key Regulators of Virus Infection. Mol. Cell 74, 196–211.e11 [PubMed: 30799147]
14. Huang R et al. (2018) Transcriptome-wide discovery of coding and noncoding RNA-binding proteins. Proc. Natl. Acad. Sci. U. S. A 115, E3879–E3887 [PubMed: 29636419]
15. Bao X et al. (2018) Capturing the interactome of newly transcribed RNA. Nat. Methods 15, 213–220 [PubMed: 29431736]
16. Trendel J et al. (2019) The Human RNA-Binding Proteome and Its Dynamics during Translational Arrest. Cell 176, 391–403.e19 [PubMed: 30528433]
17. Queiroz RML et al. (2019) Comprehensive identification of RNA-protein interactions in any organism using orthogonal organic phase separation (OOPS). Nat. Biotechnol 37, 169–178 [PubMed: 30607034]
18. Urdaneta EC et al. (2019) Purification of cross-linked RNA-protein complexes by phenol-toluol extraction. Nat. Commun 10, 990 [PubMed: 30824702]
19. Hoefig KP et al. (2021) Defining the RBPome of primary T helper cells to elucidate higher-order Roquin-mediated mRNA regulation. Nat. Commun 12, 5208 [PubMed: 34471108]
20. Blake D and Lynch KW (2021) The three as: Alternative splicing, alternative polyadenylation and their impact on apoptosis in immune function. Immunol. Rev 304, 30–50 [PubMed: 34368964]
21. Rothrock CR et al. (2005) HnRNP L represses exon splicing via a regulated exonic splicing silencer. EMBO J 24, 2792–2802 [PubMed: 16001081]
22. Shankarling G et al. (2014) Transcriptome-wide RNA interaction profiling reveals physical and functional targets of hnRNP L in human T cells. Mol. Cell. Biol 34, 71–83 [PubMed: 24164894]
23. Oberdoerffer S et al. (2008) Regulation of CD45 alternative splicing by heterogeneous ribonucleoprotein, hnRNPLL. Science 321, 686–691 [PubMed: 18669861]

24. Heyd F and Lynch KW (2010) Phosphorylation-dependent regulation of PSF by GSK3 controls CD45 alternative splicing. Mol. Cell 40, 126–137 [PubMed: 20932480]
25. Blake D et al. (2022) Alternative splicing of apoptosis genes promotes human T cell survival. Elife 11
26. Karginov TA et al. (2022) Optimal CD8+ T cell effector function requires costimulation-induced RNA-binding proteins that reprogram the transcript isoform landscape. Nat. Commun 13, 3540 [PubMed: 35725727]
27. Mallory MJ et al. (2015) Induced transcription and stability of CELF2 mRNA drives widespread alternative splicing during T-cell signaling. Proc. Natl. Acad. Sci. U. S. A 112, E2139–48 [PubMed: 25870297]
28. Martinez NM et al. (2015) Widespread JNK-dependent alternative splicing induces a positive feedback loop through CELF2-mediated regulation of MKK7 during T-cell activation. Genes Dev. 29, 2054–2066 [PubMed: 26443849]
29. Katsuyama T and Moulton VR (2021) Splicing factor SRSF1 is indispensable for regulatory T cell homeostasis and function. Cell Rep. 36, 109339 [PubMed: 34233194]
30. Katsuyama T et al. (2019) Splicing factor SRSF1 controls T cell hyperactivity and systemic autoimmunity. J. Clin. Invest 129, 5411–5423 [PubMed: 31487268]
31. Qi Z et al. (2021) SRSF1 serves as a critical posttranscriptional regulator at the late stage of thymocyte development.
32. Sandberg R et al. (2008) Proliferating cells express mRNAs with shortened 3' untranslated regions and fewer microRNA target sites. Science 320, 1643–1647 [PubMed: 18566288]
33. Gruber AR et al. (2014) Global 3' UTR shortening has a limited effect on protein abundance in proliferating T cells. Nat. Commun 5, 5465 [PubMed: 25413384]
34. Chatrikhi R et al. (2019) RNA Binding Protein CELF2 Regulates Signal-Induced Alternative Polyadenylation by Competing with Enhancers of the Polyadenylation Machinery. Cell Rep 28, 2795–2806.e3 [PubMed: 31509743]
35. Yang W-L et al. (2023) Nsun2 coupling with RoRγt shapes the fate of Th17 cells and promotes colitis. Nat. Commun 14, 863 [PubMed: 36792629]
36. Shulman Z and Stern-Ginossar N (2020) The RNA modification N6-methyladenosine as a novel regulator of the immune system. Nat. Immunol 21, 501–512 [PubMed: 32284591]
37. Li H-B et al. (2017) m6A mRNA methylation controls T cell homeostasis by targeting the IL-7/STAT5/SOCS pathways. Nature 548, 338–342 [PubMed: 28792938]
38. Tong J et al. (2018) m6A mRNA methylation sustains Treg suppressive functions. Cell Res. 28, 253–256 [PubMed: 29303144]
39. Zhou J et al. (2021) $m^6A$ demethylase ALKBH5 controls $CD4^+$ T cell pathogenicity and promotes autoimmunity. Sci Adv 7
40. Ito-Kureha T et al. (2022) The function of Wtap in N6-adenosine methylation of mRNAs controls T cell receptor signaling and survival of T cells. Nat. Immunol 23, 1208–1221 [PubMed: 35879451]
41. Yao Y et al. (2021) METTL3-dependent m6A modification programs T follicular helper cell differentiation. Nat. Commun 12, 1333 [PubMed: 33637761]
42. Wang X et al. (2014) N6-methyladenosine-dependent regulation of messenger RNA stability. Nature 505, 117–120 [PubMed: 24284625]
43. Huang H et al. (2018) Recognition of RNA N6-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. Nat. Cell Biol 20, 285–295 [PubMed: 29476152]
44. Mino T et al. (2015) Regnase-1 and Roquin Regulate a Common Element in Inflammatory mRNAs by Spatiotemporally Distinct Mechanisms. Cell 161, 1058–1073 [PubMed: 26000482]
45. Mino T et al. (2019) Translation-dependent unwinding of stem-loops by UPF1 licenses Regnase-1 to degrade inflammatory mRNAs. Nucleic Acids Res. 47, 8838–8859 [PubMed: 31329944]
46. Tavernier SJ et al. (2019) A human immune dysregulation syndrome characterized by severe hyperinflammation with a homozygous nonsense Roquin-1 mutation. Nat. Commun 10, 4779 [PubMed: 31636267]

47. Leppek K et al. (2013) Roquin promotes constitutive mRNA decay via a conserved class of stem-loop recognition motifs. Cell 153, 869–881 [PubMed: 23663784]
48. Fabian MR et al. (2013) Structural basis for the recruitment of the human CCR4-NOT deadenylase complex by tristetraprolin. Nat. Struct. Mol. Biol 20, 735–739 [PubMed: 23644599]
49. Essig K et al. (2018) Roquin targets mRNAs in a 3'-UTR-specific manner by different modes of regulation. Nat. Commun 9, 3810 [PubMed: 30232334]
50. Winzen R et al. (2007) Functional analysis of KSRP interaction with the AU-rich element of interleukin-8 and identification of inflammatory mRNA targets. Mol. Cell. Biol 27, 8388–8400 [PubMed: 17908789]
51. Chen CY and Shyu AB (1994) Selective degradation of early-response-gene mRNAs: functional analyses of sequence features of the AU-rich elements. Mol. Cell. Biol 14, 8471–8482 [PubMed: 7969180]
52. Lee HH et al. (2012) Tristetraprolin down-regulates IL-17 through mRNA destabilization. FEBS Lett. 586, 41–46 [PubMed: 22138182]
53. Nicolet BP et al. (2021) Sequence determinants as key regulators in gene expression of T cells. Immunol. Rev 304, 10–29 [PubMed: 34486113]
54. Kontoyiannis D et al. (1999) Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies. Immunity 10, 387–398 [PubMed: 10204494]
55. Moore MJ et al. (2018) ZFP36 RNA-binding proteins restrain T cell activation and anti-viral immunity. Elife 7
56. Petkau G et al. (2022) The timing of differentiation and potency of CD8 effector function is set by RNA binding proteins. Nat. Commun 13, 2274 [PubMed: 35477960]
57. Stellato C et al. (2011) Coordinate regulation of GATA-3 and Th2 cytokine gene expression by the RNA-binding protein HuR. J. Immunol 187, 441–449 [PubMed: 21613615]
58. Casolaro V et al. (2008) Posttranscriptional regulation of IL-13 in T cells: role of the RNA-binding protein HuR. J. Allergy Clin. Immunol 121, 853–9.e4 [PubMed: 18279945]
59. Chen J et al. (2013) Posttranscriptional gene regulation of IL-17 by the RNA-binding protein HuR is required for initiation of experimental autoimmune encephalomyelitis. J. Immunol 191, 5441–5450 [PubMed: 24166976]
60. Raghavan A et al. (2001) HuA and Tristetraprolin Are Induced following T Cell Activation and Display Distinct but Overlapping RNA Binding Specificities *. J. Biol. Chem 276, 47958–47965 [PubMed: 11602610]
61. Bronevetsky Y et al. (2013) T cell activation induces proteasomal degradation of Argonaute and rapid remodeling of the microRNA repertoire. J. Exp. Med 210, 417–432 [PubMed: 23382546]
62. Loeb GB et al. (2012) Transcriptome-wide miR-155 binding map reveals widespread noncanonical microRNA targeting. Mol. Cell 48, 760–770 [PubMed: 23142080]
63. Gagnon JD et al. (08/2019) miR-15/16 Restrain Memory T Cell Differentiation, Cell Cycle, and Survival. Cell Rep. 28, 2169–2181.e4 [PubMed: 31433990]
64. Cho S et al. (2021) Cell-intrinsic and -extrinsic roles of miRNAs in regulating T cell immunity. Immunol. Rev 304, 126–140 [PubMed: 34549446]
65. Baumjohann D and Ansel KM (2013) MicroRNA-mediated regulation of T helper cell differentiation and plasticity. Nat. Rev. Immunol 13, 666–678 [PubMed: 23907446]
66. Naqvi RA et al. (2022) Regulatory roles of MicroRNA in shaping T cell function, differentiation and polarization. Semin. Cell Dev. Biol 124, 34–47 [PubMed: 34446356]
67. Emming S et al. (2020) A molecular network regulating the proinflammatory phenotype of human memory T lymphocytes. Nat. Immunol 21, 388–399 [PubMed: 32205878]
68. Warth SC et al. (2015) Induced miR-99a expression represses Mtor cooperatively with miR-150 to promote regulatory T-cell differentiation. EMBO J 34, 1195–1213 [PubMed: 25712478]
69. Essig K et al. (2017) Roquin Suppresses the PI3K-mTOR Signaling Pathway to Inhibit T Helper Cell Differentiation and Conversion of Treg to Tfr Cells. Immunity 47, 1067–1082.e12 [PubMed: 29246441]

70. Janowski R et al. (2016) Roquin recognizes a non-canonical hexaloop structure in the 3'-UTR of Ox40. Nat. Commun 7, 11032 [PubMed: 27010430]
71. Uehata T et al. (2013) Malt1-induced cleavage of regnase-1 in CD4(+) helper T cells regulates immune activation. Cell 153, 1036–1049 [PubMed: 23706741]
72. Jeltsch KM et al. (2014) Cleavage of roquin and regnase-1 by the paracaspase MALT1 releases their cooperatively repressed targets to promote T(H)17 differentiation. Nat. Immunol 15, 1079–1089 [PubMed: 25282160]
73. Vogel KU et al. (2013) Roquin paralogs 1 and 2 redundantly repress the Icos and Ox40 costimulator mRNAs and control follicular helper T cell differentiation. Immunity 38, 655–668 [PubMed: 23583643]
74. Behrens G et al. (2021) Disrupting Roquin-1 interaction with Regnase-1 induces autoimmunity and enhances antitumor responses. Nat. Immunol 22, 1563–1576 [PubMed: 34811541]
75. Masuda K et al. (2013) Arid5a controls IL-6 mRNA stability, which contributes to elevation of IL-6 level in vivo. Proc. Natl. Acad. Sci. U. S. A 110, 9409–9414 [PubMed: 23676272]
76. Masuda K et al. (2016) Arid5a regulates naive CD4+ T cell fate through selective stabilization of Stat3 mRNA. J. Exp. Med 213, 605–619 [PubMed: 27022145]
77. Hanieh H et al. (2018) Arid5a stabilizes OX40 mRNA in murine CD4+ T cells by recognizing a stem-loop structure in its 3'UTR. Eur. J. Immunol 48, 593–604 [PubMed: 29244194]
78. Zaman MM-U et al. (2016) Arid5a exacerbates IFN-γ-mediated septic shock by stabilizing T-bet mRNA. Proc. Natl. Acad. Sci. U. S. A 113, 11543–11548 [PubMed: 27671645]
79. Wei J et al. (2019) Targeting REGNASE-1 programs long-lived effector T cells for cancer therapy. Nature 576, 471–476 [PubMed: 31827283]
80. Zhao H et al. (2021) Genome-wide fitness gene identification reveals Roquin as a potent suppressor of CD8 T cell expansion and anti-tumor immunity. Cell Rep 37, 110083 [PubMed: 34879274]
81. Mai D et al. (2023) Combined disruption of T cell inflammatory regulators Regnase-1 and Roquin-1 enhances antitumor activity of engineered human T cells. Proc. Natl. Acad. Sci. U. S. A 120, e2218632120
82. Carpenter S (2022) Long Noncoding RNA, Springer International Publishing
83. Nurk S et al. (2022) The complete sequence of a human genome. Science 376, 44–53 [PubMed: 35357919]
84. Zhao L et al. (2021) NONCODEV6: an updated database dedicated to long non-coding RNA annotation in both animals and plants. Nucleic Acids Res. 49, D165–D171 [PubMed: 33196801]
85. Mus musculus Annotation Report[Online]. Available: https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Mus_musculus/109/. [Accessed: 25-Mar-2023]
86. Gibb EA et al. (2011) Human cancer long non-coding RNA transcriptomes. PLoS One 6, e25915 [PubMed: 21991387]
87. Carbonell Sala S et al. (2021) Annotation of Full-Length Long Noncoding RNAs with Capture Long-Read Sequencing (CLS). Methods Mol. Biol 2254, 133–159 [PubMed: 33326074]
88. Ashwal-Fluss R et al. (2014) circRNA biogenesis competes with pre-mRNA splicing. Mol. Cell 56, 55–66 [PubMed: 25242144]
89. Wilusz JE et al. (2008) 3' end processing of a long nuclear-retained noncoding RNA yields a tRNA-like cytoplasmic RNA. Cell 135, 919–932 [PubMed: 19041754]
90. Wilusz JE et al. (2009) Long noncoding RNAs: functional surprises from the RNA world. Genes Dev. 23, 1494–1504 [PubMed: 19571179]
91. Zheng S et al. (2015) Non-coding RNA Generated following Lariat Debranching Mediates Targeting of AID to DNA. Cell 161, 762–773 [PubMed: 25957684]
92. Wang Y et al. (2015) LncRNA NALT interaction with NOTCH1 promoted cell proliferation in pediatric T cell acute lymphoblastic leukemia. Sci. Rep 5, 13749 [PubMed: 26330272]
93. Zhang Bin; Arun Gayatri; Mao YuntaonbspS.; Lazar Zsolt; Hung Gene; Bhattacharjee Gourab; Xiao Xiaokun; Booth CarmennbspJ.; Wu Jie; Zhang Chaolin; Spector DavidnbspL. et al. (2012) The lncRNA Malat1 Is Dispensable for Mouse Development but Its Transcription Plays a cis-Regulatory Role in the Adult. CellReports 2, 111–123

94. Kotzin JJ et al. (2016) The long non-coding RNA Morrbid regulates Bim and short-lived myeloid cell lifespan. Nature 537, 239–243 [PubMed: 27525555]
95. Kotzin JJ et al. (2019) The long noncoding RNA *Morrbid* regulates CD8 T cells in response to viral infection. Proc. Natl. Acad. Sci. U. S. A 116, 11916–11925 [PubMed: 31138702]
96. Kim SH et al. (2017) Association of the long non-coding RNA MALAT1 with the polycomb repressive complex pathway in T and NK cell lymphoma. Oncotarget 8, 31305–31317 [PubMed: 28412742]
97. Kanbar JN et al. (2022) The long noncoding RNA Malat1 regulates CD8+ T cell differentiation by mediating epigenetic repression. J. Exp. Med 219
98. Ma S et al. (2022) LncRNA Malat1 represses Th17 effector program by maintaining a critical bivalent super-enhancer and promotes intestinal inflammation bioRxiv, 2022.03.21.485192
99. Masoumi F et al. (2019) Malat1 long noncoding RNA regulates inflammation and leukocyte differentiation in experimental autoimmune encephalomyelitis. J. Neuroimmunol 328, 50–59 [PubMed: 30583215]
100. Khan MM et al. (2022) Long Intergenic Noncoding RNA MIAT as a Regulator of Human Th17 Cell Differentiation. Front. Immunol 13, 856762 [PubMed: 35784351]
101. Zhao P et al. (2021) A novel lncRNA TCLlnc1 promotes peripheral T cell lymphoma progression through acting as a modular scaffold of HNRNPD and YBX1 complexes. Cell Death Dis. 12, 321 [PubMed: 33767152]
102. Chen Y et al. (2022) lncRNA-GM targets Foxo1 to promote T cell-mediated autoimmunity. Sci Adv 8, eabn9181
103. Shui X et al. (2019) Knockdown of lncRNA NEAT1 inhibits Th17/CD4+ T cell differentiation through reducing the STAT3 protein level. J. Cell. Physiol 234, 22477–22484 [PubMed: 31119756]
104. Zhang Y et al. (2021) The lncRNA Snhg1-Vps13D vesicle trafficking system promotes memory CD8 T cell establishment via regulating the dual effects of IL-7 signaling. Signal Transduct Target Ther 6, 126 [PubMed: 33758164]
105. Elguindy MM and Mendell JT (2021) NORAD-induced Pumilio phase separation is required for genome stability. Nature 595, 303–308 [PubMed: 34108682]
106. Wheeler BD et al. (2023) The lncRNA Malat1 Inhibits miR-15/16 to Enhance Cytotoxic T Cell Activation and Memory Cell Formation bioRxiv, 2023.04.14.536843
107. Zhang L et al. (2020) CircRNA-1806 Decreases T Cell Apoptosis and Prolongs Survival of Mice After Cryptococcal Infection by Sponging miRNA-126. Front. Microbiol 11, 596440 [PubMed: 33281794]
108. Ouafik L 'houcine et al. (2009) Adrenomedullin promotes cell cycle transit and up-regulates cyclin D1 protein level in human glioblastoma cells through the activation of c-Jun/JNK/AP-1 signal transduction pathway. Cell. Signal 21, 597–608 [PubMed: 19166930]
109. Xie C et al. (2023) Lnc-AIFM2–1 promotes HBV immune escape by acting as a ceRNA for miR-330–3p to regulate CD244 expression. Front. Immunol 14, 1121795 [PubMed: 36845111]
110. Nie J and Zhao Q (2020) Lnc-ITSN1–2, Derived From RNA Sequencing, Correlates With Increased Disease Risk, Activity and Promotes CD4+ T Cell Activation, Proliferation and Th1/Th17 Cell Differentiation by Serving as a ceRNA for IL-23R via Sponging miR-125a in Inflammatory Bowel Disease. Front. Immunol 11, 852 [PubMed: 32547537]
111. Nakagawa S et al. (2012) Malat1 is not an essential component of nuclear speckles in mice. RNA 18, 1487–1499 [PubMed: 22718948]
112. Liu J and Cao X (2023) RBP-RNA interactions in the control of autoimmunity and autoinflammation. Cell Res 33, 97–115 [PubMed: 36599968]
113. McFarland AP et al. (2014) The favorable IFNL3 genotype escapes mRNA decay mediated by AU-rich elements and hepatitis C virus-induced microRNAs. Nat. Immunol 15, 72–79 [PubMed: 24241692]
114. Farh KK-H et al. (2015) Genetic and epigenetic fine mapping of causal autoimmune disease variants. Nature 518, 337–343 [PubMed: 25363779]
115. Taylor KE et al. (2021) PICS2: next-generation fine mapping via probabilistic identification of causal SNPs. Bioinformatics 37, 3004–3007 [PubMed: 33624747]

116. Schaid DJ et al. (2018) From genome-wide associations to candidate causal variants by statistical fine-mapping. Nat. Rev. Genet 19, 491–504 [PubMed: 29844615]
117. Ota M et al. (2021) Dynamic landscape of immune cell-specific gene regulation in immune-mediated diseases. Cell 184, 3006–3021.e17 [PubMed: 33930287]
118. Nathan A et al. (2022) Single-cell eQTL models reveal dynamic T cell state dependence of disease loci. Nature 606, 120–128 [PubMed: 35545678]
119. Simeonov DR et al. (2017) Discovery of stimulation-responsive immune enhancers with CRISPR activation. Nature 549, 111–115 [PubMed: 28854172]
120. Van Nostrand EL et al. (2020) A large-scale binding and functional map of human RNA-binding proteins. Nature 583, 711–719 [PubMed: 32728246]
121. Kleaveland B et al. (2018) A Network of Noncoding Regulatory RNAs Acts in the Mammalian Brain. Cell 174, 350–362.e17 [PubMed: 29887379]
122. Han J et al. (2020) A ubiquitin ligase mediates target-directed microRNA decay independently of tailing and trimming. Science 370
123. Shi CY et al. (2020) The ZSWIM8 ubiquitin ligase mediates target-directed microRNA degradation. Science 370
124. Hou Y et al. (2021) Circular RNA circRNA_0000094 sponges microRNA-223–3p and up-regulate F-box and WD repeat domain containing 7 to restrain T cell acute lymphoblastic leukemia progression. Hum. Cell 34, 977–989 [PubMed: 33677796]
125. Deng L et al. (2019) Circ-LAMP1 promotes T-cell lymphoblastic lymphoma progression via acting as a ceRNA for miR-615–5p to regulate DDR2 expression. Gene 701, 146–151 [PubMed: 30922709]
126. Roth TL et al. (2018) Reprogramming human T cell function and specificity with non-viral genome targeting. Nature 559, 405–409 [PubMed: 29995861]
127. Mandal PK et al. (2014) Efficient ablation of genes in human hematopoietic stem and effector cells using CRISPR/Cas9. Cell Stem Cell 15, 643–652 [PubMed: 25517468]
128. Schumann K et al. (2015) Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. Proc. Natl. Acad. Sci. U. S. A 112, 10437–10442 [PubMed: 26216948]
129. Hendel A et al. (2015) Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. Nat. Biotechnol 33, 985–989 [PubMed: 26121415]
130. Garcia-Doval C and Jinek M (2017) Molecular architectures and mechanisms of Class 2 CRISPR-associated nucleases. Curr. Opin. Struct. Biol 47, 157–166 [PubMed: 29107822]
131. Han J et al. (2014) Efficient in vivo deletion of a large imprinted lncRNA by CRISPR/Cas9. RNA Biol. 11, 829–835 [PubMed: 25137067]
132. Guo R et al. (2023) LINC00478-derived novel cytoplasmic lncRNA LacRNA stabilizes PHB2 and suppresses breast cancer metastasis via repressing MYC targets. J. Transl. Med 21, 120 [PubMed: 36782197]
133. Wu Y et al. (2013) Correction of a genetic disease in mouse via use of CRISPR-Cas9. Cell Stem Cell 13, 659–662 [PubMed: 24315440]
134. Iancu O et al. (2023) Multiplex HDR for disease and correction modeling of SCID by CRISPR genome editing in human HSPCs. Mol. Ther. Nucleic Acids 31, 105–121 [PubMed: 36618262]
135. McAuley GE et al. (2023) Human T cell generation is restored in CD3δ severe combined immunodeficiency through adenine base editing. Cell DOI: 10.1016/j.cell.2023.02.027
136. Abudayyeh OO et al. (2017) RNA targeting with CRISPR-Cas13. Nature 550, 280–284 [PubMed: 28976959]
137. Yang L-Z et al. (2019) Dynamic Imaging of RNA in Living Cells by CRISPR-Cas13 Systems. Mol. Cell 76, 981–997.e7 [PubMed: 31757757]

**Box 1.**

### Integrating genetic data to identify cis-regulatory elements

Mutations that alter RBPs and their regulatory activity can lead to hyperinflammation, autoimmunity and other immune disorders [46,112]. Polymorphisms that alter cis-regulatory regions bound by RBPs (as well as those that change RBP proteins themselves) alter cell states and affect immune responses. For example, GWAS studies showed that a common single nucleotide polymorphism (SNP) in the 3'UTR of *interferon lambda 3* (*IFNL3*) is strongly associated with hepatitis C virus (HCV) clearance (i.e. versus chronic infection). This SNP disrupts a cis-regulatory sequence through which RBPs and miRNAs induce *IFNL3* transcript instability in the Huh7 hepatocyte cell line, modulating expression of the antiviral protein it encodes and subsequent clearance of HCV [113].

Genome-wide association studies (GWAS) have identified numerous genetic variants associated with immune-mediated diseases, some of which were identified to be highly probable causal variants through fine-mapping [114–116]. For instance, **expression quantitative trait loci** (eQTL) analyses have been used to understand which disease-associated SNPs affect gene expression in immune cells, thereby identifying candidate DNA cis-regulatory elements [117,118]. However, using eQTL and GWAS alone to dissect individual functional cis-elements can be challenging, as individual variants may only work in a cell-specific and/or context-specific manner. Biochemical data that illuminate the activity of cis-regulatory sequences can aid the interpretation of genetic and gene expression data, as illustrated by the discovery of a stimulation-responsive *IL2RA* enhancer through integrating GWAS and gene expression data with biochemical maps of chromatin accessibility in human T cell subsets [119].

Similar approaches can be used to discover cis-regulatory elements involved in post-transcriptional regulation. With large RBP binding data repositories available from the encyclopedia of RNA elements (ENCORE; https://www.encodeproject.org/encore-matrix/?type=Experiment) [120] and other sources, future studies can combine biochemical analyses with genetic and gene expression data to identify regions containing functional RNA cis-regulatory elements that might impact human health and disease.

**Box 2.**

### Mechanisms of miRNA inhibition by lncRNAs

Despite numerous studies suggesting that large ceRNA networks regulate gene expression in a variety of cellular contexts, how exactly lncRNA:miRNA interactions lead to the inhibition and/or degradation of miRNAs remains generally uncharacterized. A notable study involving the lncRNA Cyrano demonstrated that extensive 5’ and 3’ base pairing between miR-7 and Cyrano led to the degradation of miR-7 in the mouse brain [121]. Subsequent mechanistic studies in mouse cell lines elaborated on these findings, reporting that extensive 3’ binding in addition to **seed sequence** pairing between a miRNA and its target can lead to target mediated miRNA degradation (TDMD) and Zswim8-mediated ubiquitination of Ago2 [122,123]. However, many of the lncRNA:miRNA interactions characterized in T cells do not involve extensive 3’ binding [109,124,125]. Furthermore, some lncRNAs can reduce miRNA activity without notable degradation of the target miRNA [106], indicating that abundant ceRNAs may be able to operate via competitive binding alone, perhaps by redirecting the subcellular localization of target miRNAs [106]. Resolving the mechanisms by which lncRNAs and other ceRNAs regulate miRNA-driven circuits is a key area for further research that may reveal new strategies for therapeutic application.

**Box 3.**

## Genomic and Transcriptomic Editing Approaches for Dissection of lncRNA and RBP Function

Genome editing technologies allow detailed interrogation of specific sequences and binding site-dependent lncRNA functions. The non-viral targeting of human T cells with CRISPR-Cas9 systems opened the door to efficient and rapid genetic editing in T cells [126–129]. These systems have most often been employed to induce insertions and deletions (indels) in an exon of a coding gene [130], which is often insufficient to change the function of a lncRNA or the global function of a 3' UTR. There are instances where traditional Cas9 induced indels may have functional outcomes for RBP and lncRNA function. For instance, the miR-15/16 binding site within Malat1 in humans T cells? can be directly targeted by Cas9, and indels at this site disrupt miRNA binding [106]. Where a single guide targeting approach is insufficient, paired single guide RNAs can be used to excise portions of the genome containing putative functional sequences [131,132]. These larger deletions may alter neighboring functional DNA or RNA regulatory elements, but they can be precisely targeted in somatic cells without the use of recombinase ,or the insertion of residual foreign sequences.

**Homology directed repair** (HDR) templates can be used to create more specific mutations, albeit with reduced efficiency [133]. This approach can be used to disrupt binding sites while maintaining nucleotide content and RNA length [104]. It can also be used to insert large novel sequences [134]. Genomic base editing can also be targeted with sgRNAs by fusing catalytically inactive Cas9 to an adenosine deaminase [135]. For instance, CD3δ was recently targeted in human hematopoietic stem cells to restore T lymphopoiesis in immunodeficient patients bearing a pathogenic mutation in this gene [135]. In this context, base editing produced higher desired mutation rates with lower indel byproducts compared to HDR strategies.

Cas13 systems target RNA transcripts directly. Cas13 exhibits equivalent or better depletion of transcripts compared to RNAi, can be provided transiently or stably, and does not alter genomic sequences [136]. In addition, catalytically inactive Cas13 paired with fluorescent labeling allows dynamic tracking of specific RNA transcripts in living cells [137]. This has been used to reveal the dynamics of NEAT1 interaction with **paraspeckles** in HeLa cells, and might be paired with orthogonal Cas13 or Cas9 probes to investigate RNA-RNA or RNA-DNA interactions in a sequence-specific fashion.

## Highlights

- Recent work has defined the RBPome of human and mouse T cells and generated transcriptome-wide maps of RBP occupancy.
- mRNAs encoding crucial T cell functional proteins are regulated by AU-rich elements and Roquin/Regnase-binding RNA structural elements.
- m6A modification of RNA is essential for proper differentiation, homeostasis, and function of multiple types of T cells.
- Technological advances have driven extensive annotation of lncRNAs expressed in T cells, and new methods for functional as well as the mechanistic interrogation of these regulatory RNAs.

### Outstanding Questions

- How are sequence and structural determinants, RNA modifications, and RBP cooperation/competition integrated to regulate RNA circuit outputs?
- By what mechanism(s) do lncRNA ceRNAs inhibit miRNAs, and do lncRNAs perform this function differently than protein-coding mRNAs?
- How can RNA circuits be manipulated in T cells to enhance immunity or treat autoimmunity and allergy?

## Significance

RNA circuits are key regulators of cytotoxic and helper T cell activation, survival, differentiation, and function in immunity. Moreover, Emerging technologies and increased interest in RNA circuits involving RBPs and lncRNAs has accelerated the pace of discovery in RNA immunology. This is relevant, as RNA circuits might be targeted directly, or used to enhance cellular and gene therapies in a variety of disorders.

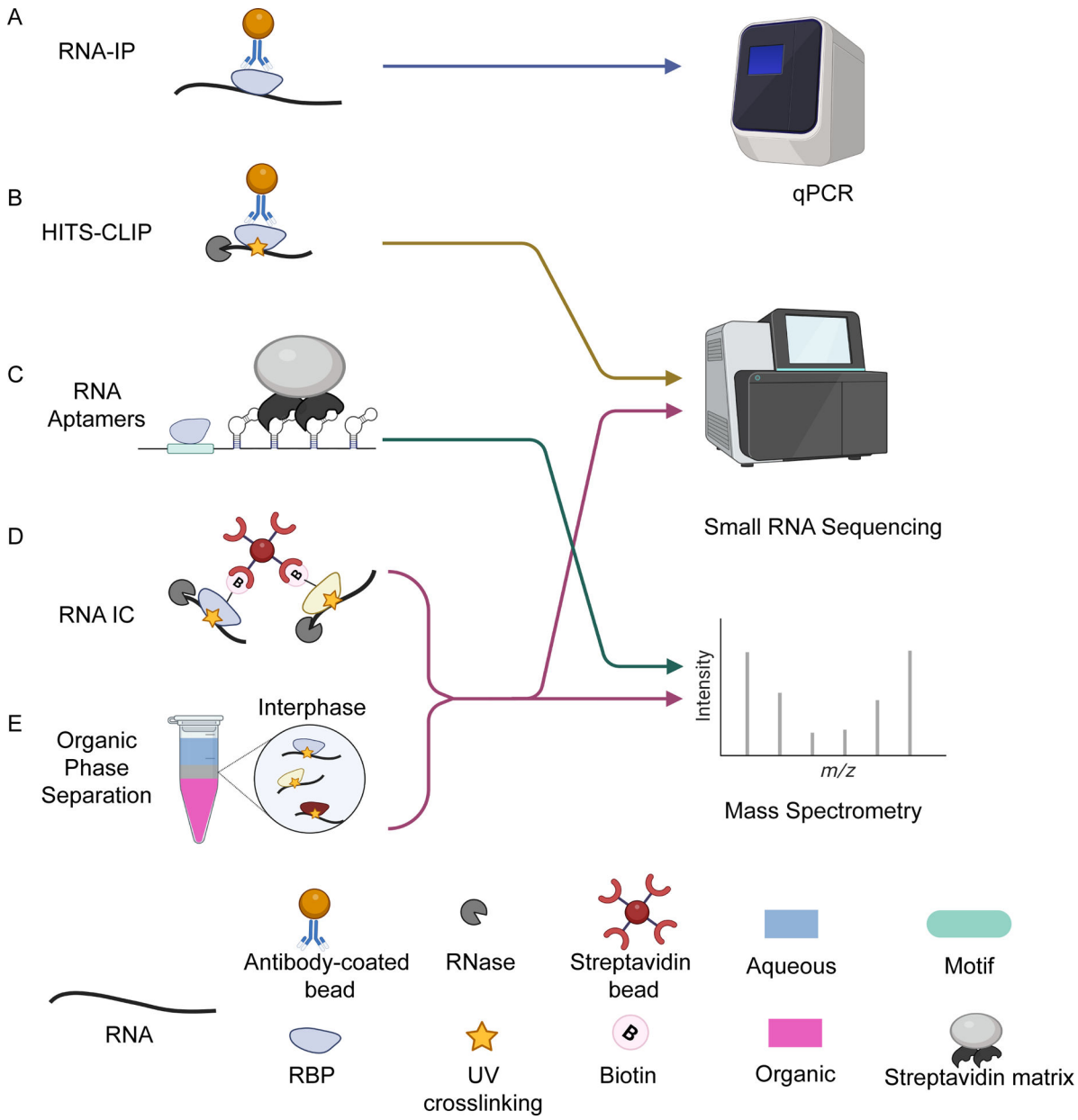


**Figure 1.**

Ribonucleoprotein capture methods performed in T cells (and other cell types). **(A)** RNA immunoprecipitation (RNA IP) captures RBP-RNA interactions using antibody-coated beads to pull down specific RBPs and their bound transcripts in the cell under native conditions. The bound transcript is processed for quantitative PCR to determine target transcripts by the specific RBP.

**(B)** Crosslinking immunoprecipitation with high throughput sequencing (HITS-CLIP) methods use UV radiation to covalently bind RBPs to their transcripts. The ribonucleoprotein complexes (RNPs) are captured using antibody coated beads and undergo RNase digestion to generate small RNAs containing the bound region. These fragments are then sequenced to determine the transcriptomic binding profile.

**(C)** RNA aptamers contain small, structured motifs that recognize small molecules and can be used to pull down and identify RBPs that bind to a sequence of interest. The illustration depicts an aptamer with modified streptavidin binding structures (S1m) and the sequence of interest. Streptavidin matrix is used to pull down the protein-bound aptamer and the proteins are processed for mass spectrometry.

**(D)** RNA interactome capture methods (RNA IC) biotinylate the proteins and use streptavidin beads to extract RBP-bound RNA. Captured RPBs are identified using mass spectrometry and RNA undergoes library preparation and sequencing to determine RBP binding sites.

**(E)** Organic phase separation can be used to systematically identify RBPs and RBP binding profiles of a cell. These methods use phenol phase separation which partitions proteins and RNA into the organic and aqueous phase respectively. RNPs that separate into the interphase are captured and processed for mass spectrometry and/or sequencing. Figure was created using Biorender.com.

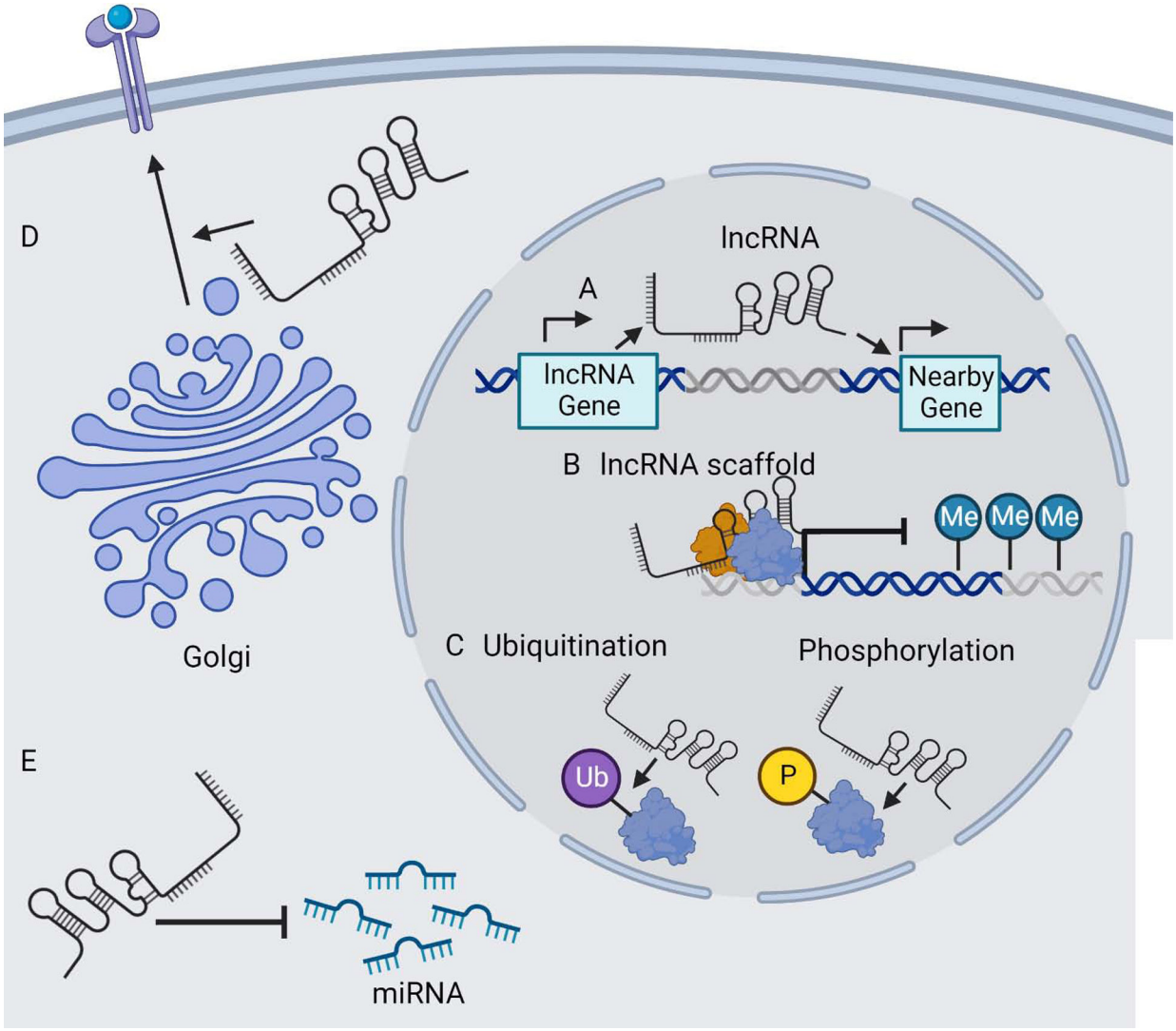


**Figure 2.**

lncRNA Functions Identified in T cells **(A)** Transcription of lncRNAs has significant impacts on the expression of other genes in the same locus. In T cells the mechanisms of these *cis*-regulatory effects are still not well defined.

**(B)** lncRNAs often regulate a host of other protein-coding genes via *trans*-regulatory mechanisms. This is often done via the scaffolding of various transcription or epigenetic factors and facilitating their binding to chromatin. This can influence the deposition of histone regulatory modifications such as H3K27me3.

**(C)** lncRNAs can regulate transcription factors in a post-translational fashion by influencing the addition of ubiquitin or phosphoryl groups resulting in degradation, inhibition, or activation of the transcription factor.

**(D)** lncRNAs can influence protein activity of the golgi vesicle trafficking network. In particular VPs13d activity is enhanced by the presence of a lncRNA and this is essential for the cell surface expression of important cytokine receptors such as CD127/IL7R.

**(E)** lncRNAs act as ceRNAs for miRNAs, which reduces the amount of miRNA induced inhibition of protein coding targets. lncRNA acting as a ceRNA often leads to the degradation of the miRNA but in some cases may inhibit the miRNA solely via stoichiometric competition.

Figure was created using Biorender.com.