response [15

insults. In fact, it is also described for natural killer (NK) cells, NKT cells, innate lymphoid cells (ILCs), mast cells, basophils, and eosinophils [29–31]. However, the constitutive expression of effector mRNAs also poses a health risk. Effector molecules are highly toxic, and aberrant protein production could lead to chronic activation, inflammation, tissue damage, and autoimmune diseases [3–5]. It is therefore imperative that protein production from preformed mRNAs should occur primarily upon pathogenic or aberrant cellular insults.

To preserve their silent state in the absence of infection, memory T cells tightly regulate the turnover of preformed cytokine mRNA and the initiation of its translation into protein (Figure 1A) [21]. In resting murine $CD8^+$ T cells, the mRNA of effector molecules is generally unstable, and this limits its accumulation [15]. However, rapid mRNA turnover is not sufficient to avoid aberrant protein production. It was recently shown that the translation of preformed mRNA in murine $CD44^{hi}$-memory-like $CD8^+$ and $CD4^+$ T cells was actively blocked by the RBP ZFP36L2 [21]. This process depended on the presence of AU-rich elements (AREs) within the 3′ untranslated region (3′UTR) of Ifng and Tnfa mRNA, and ZFP36L2 directly bound to these AREs in human and mouse T cells [21]. The interaction between AREs and ZFP36L2 hampered the recruitment of preformed mRNA to ribosomes, blocked its translation into protein, and prevented aberrant cytokine production in non-activated $CD44^{hi}$-memory-like $CD8^+$ and $CD4^+$ T cells in mice [21]. Moreover, germ-line deletion of the Ifng ARE region [32] has also resulted in chronic IFN-γ production in resting murine NK cells and NKT cells [33], indicating that the ARE-dependent translational block of cytokine production from preformed mRNA also occurs in other immune cells. Of note, a recent study in mice showed that skin-resident Staphylococcus epidermidis-specific $CD4^+$ and $CD8^+$ T cells constitutively expressed a type 2 immunity transcriptome; however, protein production of type 2 cytokines (i.e., IL-5 and IL-13) only occurred upon intradermal injection of chitin or exposure to insect bites [34]. Given that most transcripts encoding inflammatory mediators, including Il5 and Il13, contain AREs in their 3′UTR [35], it is conceivable that the translational block mediated by ZFP36L2 (or by another ARE-BP) is more broadly applied to block the undesirable release of effector molecules under homeostatic conditions.

AREs are not only present in inflammatory genes. They are found in ~16% of the human transcriptome [36]. New techniques that map RNA–protein interactions genome-wide to a single nucleotide of resolution (Box 2) have revealed that one RBP can interact with a subset of mRNAs sharing specific cis-regulatory elements, such as AREs [37]. In fact, ZFP36L2 does not only bind cytokine mRNAs [21]; together with its paralog ZFP36L1, it can also regulate the expression of transcription factors, kinases, and cell cycle genes in mice [37–40]. For example, CD2-Cre recombinase-mediated conditional deletion of ZFP36L1 and ZFP36L2 in mice resulted in increased expression of the oncogenic transcription factor Notch-1 in differentiating lymphocytes, which contributed to the development of T cell leukemia, relative to wild-type (WT) control mice [38,39]. Crosslinking immunoprecipitation (iCLIP) experiments in LPS-stimulated murine B cells revealed that ZFP36L1 targeted mRNAs encoding PIM-family kinases and cell cycle genes to enforce quiescence on late pre-B cells [37]. ZFP36L1 can also bind mRNAs encoding the

unresolved. We speculate that co-regulation through different RBPs and/or other trans-factors, such as non-coding RNA, defines a dynamic and flexible regulation. We anticipate that these regulatory mechanisms and the activity of trans-acting factors are cell type and transcript specific, depend on the subcellular localization of the target mRNA, and are instructed by context and signal strength.

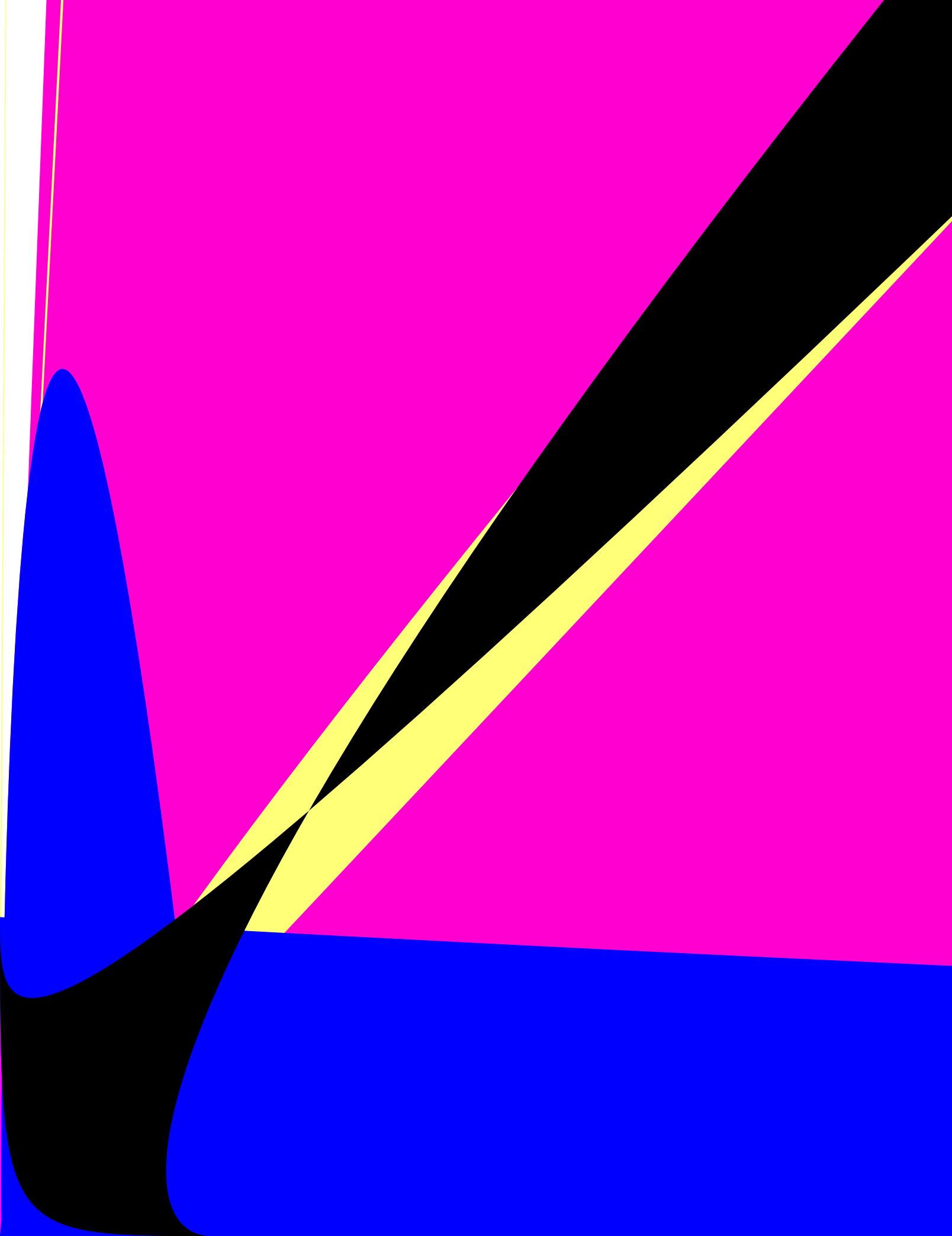
The transcript specificity of RBPs can be influenced by their affinity to target mRNAs. For example, the RBPs HuR and TIA-1 recognize generic U-rich elements [76,77

the same mRNA targets but, while Roquin acts in p-body and/or stress granules, Regnase-1 interacts with ribosomes within the endoplasmic reticulum [81]. Likewise, ZFP36L1 can interact with target mRNAs in HeLa cells in membrane-free structures associated with the endoplasmic reticulum [82]. Whether similar mechanisms also occur in T cells remains unknown and is challenging to study due to the small cell size and low cytoplasmic content of primary T cells.

RBPs can also be expressed, and thus be active, during different stages of T cell activation and differentiation. For example, whereas ZFP36L2 is already expressed by quiescent $CD8^+$ T cells, ZFP36 and ZFP36L1 expression increases upon activation [21,22]. Of note, ZFP36 can autoregulate its own abundance by binding to the AREs within the Zfp36 3′UTR in mouse RAW264.7 macrophage-like and human THP-1 myelomonocytic cells [83,84]. Given that Zfp36l1 and Zfp36l2 mRNAs also contain AREs, cross-regulation of these three family members is feasible. In conclusion, whether ARE-BPs cooperate in post-transcriptional regulatory events, or whether they act independently in a spatially and temporally compartmentalized manner is still under debate and requires evaluation at the level of individual transcripts.

Is Post-Transcriptional Regulation Involved in Antigen-Independent $CD8^+$ T Cell Responses?

Memory T cells can also be activated in an antigen-independent manner. They respond to cytokines, such as IL-12, IL-18, IL-15 and type I interferons (IFN- and IFN-), and to Toll-like receptors (TLR2 and TLR7) [85–88]. After receiving this so-called bystander stimulation, murine memory T cells undergo a robust program of activation that includes increased expression of the transcription factors T-bet and Eomesodermin, the activation markers CD69, CD25 and CD11a, and the cytotoxic molecules CD8



The differences in PKC-mediated post-transcriptional regulation may stem from different RBP-binding hubs present in the cytokine transcripts, which can comprise RNA sequences as well as secondary structures. For instance, only the 3′UTR of Tnfa contains a constitutive decay element (CDE) in addition to AREs [98]. Thus, it is conceivable that the CDE prevents Tnfa

to target genes independently from AP-1 and promotes the expression of inhibitory receptors, including PD-1 [111,112]. PD-1 also directly impairs CD28-mediated signaling [113], and blocks TCR- and CD28-mediated activation of PKC and PI3K/AKT, which results in loss of IL-2 production and T cell proliferation [108,114]. Given that CD28 co-stimulation supported the therapeutic benefit of anti-PD-L1 therapy in studies of CT26 colon carcinoma-bearing mice [115], it is possible that dysfunctional $CD8^+$ T cells use CD28-mediated signaling to restore effective im-

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