

JB Review

Post-transcriptional regulation of inflammation by RNA-binding proteins via *cis*-elements of mRNAs

Received July 3, 2019; accepted August 26, 2019; published online September 12, 2019

Yutaro Uchida, Tomoki Chiba,
Ryota Kurimoto and Hiroshi Asahara*

Department of Systems BioMedicine, Tokyo Medical and Dental University, Tokyo, Japan

*Hiroshi Asahara, Department of Systems BioMedicine, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan. Tel.: +81-03-5803-5015, Fax: +81-03-5803-5810, email: asahara.syst@tmd.ac.jp

In human genome, there are approximately 1,500 RNA-binding proteins (RBPs). They can regulate mRNA stability or translational efficiency via ribosomes and these processes are known as ‘post-transcriptional regulation’. Accumulating evidences indicate that post-transcriptional regulation is the determinant of the accurate levels of cytokines mRNAs. While transcriptional regulation of cytokines mRNAs has been well studied and found to be important for the rapid induction of mRNA and regulation of the acute phase of inflammation, post-transcriptional regulation by RBPs is essential for resolving inflammation in the later phase, and their dysfunction may lead to severe autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus. For post-transcriptional regulation, RBPs recognize and directly bind to *cis*-regulatory elements in 3′ untranslated region of mRNAs such as AU-rich or constitutive decay elements and play various roles. In this review, we summarize the recent findings regarding the role of RBPs in the regulation of inflammation.

Keywords: chronic inflammation; *cis*-regulatory element; post-transcriptional regulation; RNA-binding protein; translation.

Abbreviations: AUF1, AU-rich element polyU binding degradation factor1; Arid5a, AT-rich interactive domain-containing protein 5a; ARE, AU-rich element; ARE-BP, AU-rich element-binding protein; BRF, butyrate response factor; CCCH zinc finger, Cys-Cys-Cys-His zinc finger; CDE, constitutive decay element; CELF, CUG-BP Elav-like family; CLIP, crosslinking and immunoprecipitation; CSF2, colony stimulating factor2; CUGBP1, CUG triplet repeat RNA-binding protein1; EAE, experimental autoimmune encephalomyelitis; ELAVL1, ELAV-like protein 1; GRE, GU rich element; HSC70, heat shock cognate 71 kDa protein; HSP70, heat shock protein 70; ICOS, inducible T-cell co-stimulator; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCP1, monocyte chemotactic protein induced protein; miRNA, micro RNA; NF- κ B, nuclear factor kappa B; PAR-CLIP, photoactivatable ribonucleotide enhanced crosslinking and

immunoprecipitation; PARN, poly(A) specific ribonuclease; PIN, PiT N-terminus; RA, rheumatoid arthritis; RBP, RNA-binding protein; RING finger domain, really interesting new gene finger domain; RRM, RNA recognition motif; SLE, systemic lupus erythematosus; STAT3, signal transducer and activator of transcription 3; T-ALL, T cell acute lymphoblastic leukemia; TNF α , tumor necrosis factor alpha; TNFRSF1B, TNF receptor superfamily 1B; TTP, tristetraproline; UTR, untranslated region; ZFP, zinc finger protein.

Aberrant expression of inflammatory cytokines causes severe autoimmune diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). The transcriptional regulation mechanisms of inflammatory cytokines underlying these inflammatory diseases have been extensively studied for decades. Transcriptional regulation via the nuclear factor kappa B (NF- κ B) and signal transducer and activator of transcription 3 (STAT3) pathways have been reported to be essential for the regulation of the immune system (1, 2).

However, recent studies have shown that the post-transcriptional regulation of inflammatory cytokines is also essential for their expression. In the acute phase of inflammation, the total amount of mRNA rapidly increases; while during the later phase of inflammation, post-transcriptional regulation determines the stability and translational efficiency of mRNA, thereby regulating the mRNA levels of cytokines (Fig. 1) (3–11). The role of RNA-binding proteins (RBPs) participate in post-transcriptional regulation is widely known and they perform multiple functions, including translational control and stabilization or degradation of mRNA. RBPs can recognize *cis*-elements or specific structures in the 5′ or 3′ untranslated regions (UTRs) of target mRNAs and determine mRNA fate by directly binding to these targets. There are approximately 1,500 RBPs in humans and some of them play important roles in the regulation of chronic inflammation (12). In this review, we summarize the post-transcriptional regulation of cytokines by RBPs that recognize *cis*-elements or specific structures of cytokines mRNAs.

Important *cis*-Elements and Structures of mRNA

Most of RBPs recognize *cis*-elements or specific structures of cytokine mRNAs and elicit multiple functions,

such as translational control, and destabilization of cytokine mRNAs. As shown in Fig. 2, these elements or specific structures are usually found in 3' UTR of mRNA. In this section, we have introduced several vital *cis*-elements of mRNA (Fig. 2) (13–16).

Adenine and uridine-rich element (ARE) is one of the most important *cis*-elements of mRNA usually composed of AUUUA pentamers and are known to regulate the stability of inflammatory mRNAs (13). ARE was initially identified as a conserved AU sequence in the 3' UTR, which mediated the selective degradation of colony stimulation factor 2 (CSF2) mRNA (17). AREs were also found in the 3' UTRs of many inflammatory cytokines, such as IL-3, IL-6, IL-8, and tumour necrosis factor α (TNF α) (18–20). Furthermore, mice lacking AREs in the 3' UTR of TNF α 's showed severe chronic inflammatory arthritis and Crohn's like inflammatory bowel disease, suggesting an important physiological role of AREs (19). Approximately, 4,000 genes with ARE-mRNA exist and are classified into three classes (20–22). Class I

(e.g. *c-myc* and *c-fos*) is characterized by having non-overlapping AUUUA pentamers, Class II (e.g. TNF alpha and IL-3) is characterized as having overlapping AUUUA pentamers and Class III AREs (e.g. *c-jun*) do not contain any AUUUA pentamer (21, 22). Though the mechanism of the regulation of ARE-containing mRNAs is still unknown, AREs are recognized and bound by many ARE-binding proteins (ARE-BPs). There are more than 20 ARE-BPs and they have multiple functions, such as degradation of mRNA and translational repression. The role of each ARE-BP is reviewed in the next section.

GU-rich element (GRE) is also an essential *cis*-element that was identified through computational analysis and is composed of GUUUG pentamers. GRE can be seen in mRNAs of inflammatory proteins, such as *c-JUN*, *JUN-B* and TNF-receptor 1B. The transcripts containing GRE tend to be short lived. CUGBP1 was the first reported GRE-binding protein and it strongly destabilizes GRE-containing mRNAs (14).

The constitutive decay element (CDE) is another crucial element for determining the stability of cytokine mRNA. CDE was firstly reported as an important *cis*-element of TNF α mRNA for preventing excessive induction of TNF α , and its mechanism of decay is distinct from that of ARE (15). The later research revealed CDE in more than 50 vertebrate mRNAs, many of them encoding essential factors for development and inflammation. The stem loop structure in the CDE is the most crucial structure for its regulation (16). Regnase-1 (ZC3H12A), Roquin1/2 (RC3H1/2) and Arid5a recognize and regulate the stem loop in CDE and strongly regulate the expression of inflammatory cytokines, as reviewed in the latter section.

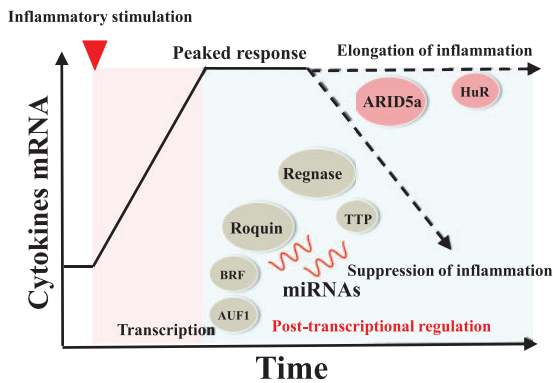


Fig. 1 The post-transcriptional regulation of inflammatory cytokines is important for the regulation of chronic inflammation. In the acute phase of inflammation, mRNA levels of cytokines rapidly increase and reach the peak. In the chronic phase of inflammation, post-transcriptional regulation by RBPs or miRNA determines the prolongation or termination of inflammation (3–11).

ARE-Binding Proteins in the Immune System

As reviewed in the previous section, ARE is regulated by several ARE-binding proteins (ARE-BPs). In this

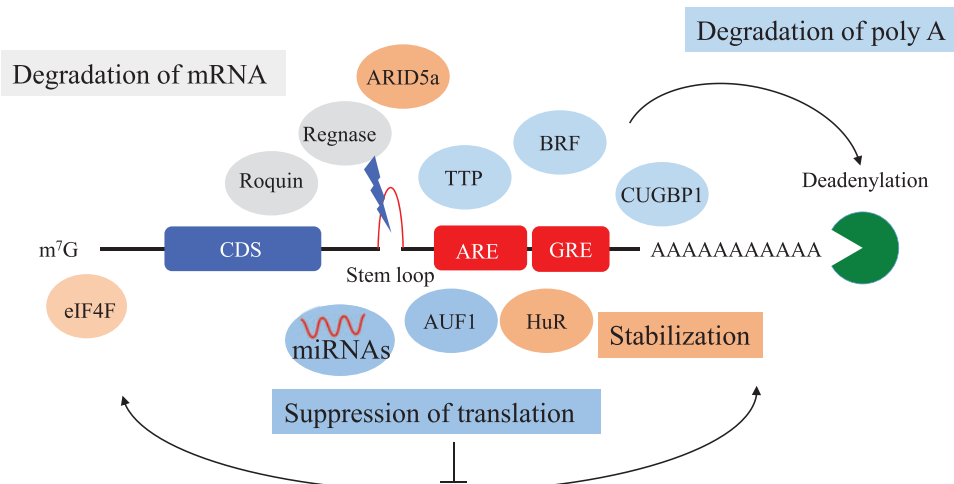


Fig. 2 Important RBPs and *cis*-elements for post-transcriptional regulation. Inflammatory cytokines contain several *cis*-elements, such as ARE. Many ARE-binding proteins bind to these *cis*-elements and regulate the levels of inflammatory cytokines (13–16).

section, we introduce several vital ARE-BPs involved in the regulation of inflammation.

TTP (ZFP36, Tristetraproline) is one of the well-known ARE-BPs. It destabilizes the mRNAs of inflammatory cytokines, such as IL-2, IL-6, IL1- β and TNF α by directly binding to the ARE in their mRNAs. As shown in Fig. 3, TTP harbours an RNA-binding domain, CCCH (Cys-Cys-Cys-His) zinc finger (11). By directly binding to AREs in mRNA, it shortens the poly (A) tail of mRNAs and promotes mRNA decay through the recruitment of exosome, XRN1 and decapping enzymes (23, 24). Its expression is rapidly and transiently induced by stimulation with mitogens and growth factors, which indicates its roles in the negative feedback loop of inflammation (11). The expression of TTP is suppressed by phosphorylation caused by the mitogen-activated protein kinase (MAPK)-MAPKAK2 (MK2) kinase cascade and this suppression leads to the stabilization of cytokine mRNAs (25, 26). Mice lacking TTP showed severe autoimmune-like phenotypes such as arthritis and cachexia, suggesting the important role of TTP in the negative feedback of inflammatory stimulation (27). Latest reports have revealed the importance of TTP with respect to viral infection and tumour immunity; and is now considered to possess variable roles as a suppressor of the immune system owing to its functions (28, 29).

BRF1 (ZFP36L1) and BRF2 (ZFP36L2) are members of the ZFP36 family, which share a highly conserved CCCH finger with TTP, and recognize AREs and accelerate mRNA decay (30). BRF1 KO mice were embryonic lethal because of chorioallantoic fusion, while mice lacking BRF2 survived only for a few days after birth possibly because of hematopoietic stem cell failure (31, 32). Both of BRF1 and BRF2 were expressed throughout the thymic development stage, and *Zfp36l1^{fl/fl} Zfp36l2^{fl/fl}* CD2-Cre dKO mice perturbed thymic development and T cell acute lymphoblastic leukemia (T-ALL) by aberrant expression of Notch1 (10). However, though their structures and regulatory mechanism seem to be similar, BRF1 and BRF2 play different roles in the immune system. BRF1 was reported to be essential for the maintenance of marginal-zone B cell compartment (33) and BRF2 was recently reported to prevent the chronic activation of memory T cells by blocking the recruitment (34).

AUF1 (hnRNP D) is another ARE-BP that down-regulates ARE-containing mRNAs. There are four different alternatively spliced isoforms of AUF1 (p37, p40, p42 and p45) and all of them contain two RNA recognition motifs (35). The main role of AUF1 is the degradation of ARE-containing mRNAs and it plays an indispensable role in suppressing the immune system. Mice lacking AUF1 exhibited symptoms of severe endotoxin shock upon endotoxin challenge, due to the failure to degrade mRNAs of several critical inflammatory cytokines, such as IL1 β and TNF α (6). Though the functions of AUF1 seem to be similar to that of TTP, AUF1-dependent decay of mRNA is different from the TTP-dependent destabilization of mRNA. AUF1 forms a protein assembly with the cap-dependent translation initiation factor, eIF4G

and heat shock proteins such as, heat shock cognate 71 kDa protein (HSC70) and heat shock protein 70 (HSP70) (36, 37). AUF1-related mRNA decay is associated with displacement of eukaryotic initiation factor 4G (eIF4G) from AUF1, and subsequent ubiquitination and degradation of AUF1 by proteasomes (38). Photoactivatable ribonucleotide enhanced crosslinking and immunoprecipitation (PAR-CLIP) analysis and ribosomal profiling showed that AUF1 preferentially recognizes U-rich sequences, and at times promotes the translation of numerous mRNAs under several conditions, which suggests the multiple and complex roles of AUF1 (39).

Contrary to these mRNA degradation factors, embryonic lethal abnormal vision (ELAV/Hu) family proteins are the only RBPs that stabilize mRNAs by recognizing AREs (7, 40, 41). Though most of Hu protein family members (Hu-B, C and D) are expressed only in the central nervous system, HuR (ELAVL1) is the only widely expressed factors in humans. HuR has three RNA recognition motif (RRM) domains that are important for RNA binding (42). Most of HuR proteins are localized in nucleus; however, HuR accumulates in the cytoplasm by inflammatory stimulation and delays the onset of decay of ARE-containing mRNAs (7, 41, 43, 44). These findings suggested that HuR competes with mRNA destabilizing ARE-BPs, such as TTP, and protect target mRNAs from decay. HuR targets mRNAs of several inflammatory cytokines, such as IL-4, IL-13, TNF- α and IL-17, and mice lacking HuR were embryonically lethal because of defects in placental branching morphogenesis and embryonic development (45–52). Further studies using conditional-knockout mice revealed that HuR deletion in CD4⁺ T cells impaired Th17 differentiation; these mice were resistant to experimental autoimmune encephalomyelitis (EAE) (46). Moreover, elucidation of HuR targets and functions in all transcriptomes using PAR-CLIP analysis showed most of the binding sites of HuR to be located in 3' UTR of mRNAs with preferential bindings to the intronic sites or 3' UTR of mRNA. Binding motifs are U-rich sequences such as UUUUUUU, UUUAUUU and UUUGUUU as well as common AU-rich motifs such as UAUUUAU (53). Furthermore, another report showed that HuR stabilized transcripts with both intronic and 3' UTR binding sites rather than transcripts with only 3' UTR sites or intronic-binding sites. HuR alters splicing pattern and also protects mRNA from microRNA (miRNA)-induced mRNA decay, suggesting that HuR regulates expression outcomes at multiple stages of RNA processing (54).

GRE-Regulating Proteins in the Immune System

CUGBP1 is a member of the CELF family of RBPs and is the only well-studied RBP to be reported as a GRE-regulating protein (14). CUGBP1 has two RRM domains at the N terminus and one RRM domain at its C terminus, which are essential for binding to mRNA (55). CUGBP1 is well-known for binding to

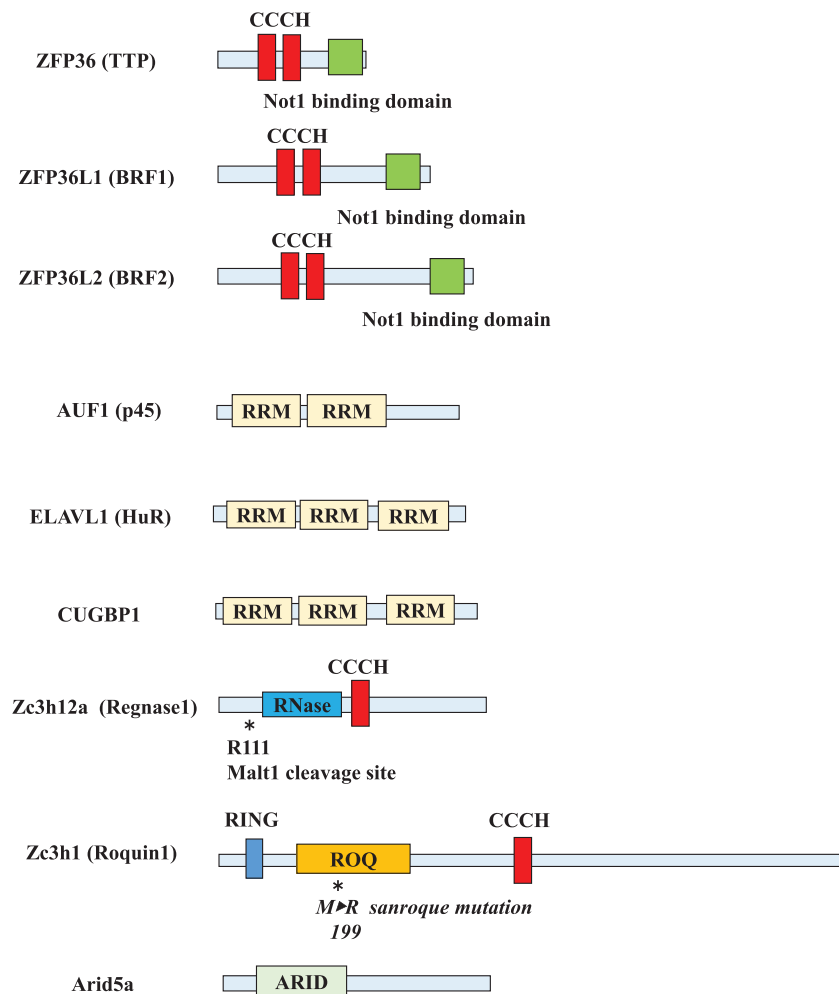


Fig. 3 The structure of RBPs. The structure of RBPs. CCCH domain and RRM domain are important for RNA binding and function (8, 9, 35, 42, 55, 61, 66). ARID, AT-rich interactive domain; CCCH zinc finger, Cys-Cys-Cys-His zinc finger; RRM, RNA recognition motif; RING finger domain, Really interesting new gene finger domain; ROQ, ROQ RNA-binding domain.

the abnormally extended CUG mRNA repeats in type I myotonic dystrophy (56). However, it was found that CUGBP1 also had the ability to destabilize GRE-containing transcripts by recruiting the poly(A)-specific ribonuclease (PARN) deadenylase (14, 57). As GRE are present in the mRNAs of important inflammatory factors, such as JUN, JUNB or TNFRSF1B, CUGBP1 is thought to play a suppressive role in the immune system.

CDE-Regulating Proteins in the Immune System

The constitutive decay element is another essential structure for post-transcriptional regulation of the immune system. In this section, we focus on three characteristic CDE-regulating proteins, Roquin1/2, Regnase-1 and Arid5a.

Roquin1/2 (RC3H1 and RC3H2) are important factors for the destabilization of inflammatory cytokines mRNAs. Roquin has a CCCH zinc finger domain for RNA binding, a Really interesting new gene (RING) finger domain, a ROQ domain for recognizing stem loop structure and a proline-rich domain (16). The

relationship between Roquin and inflammatory cytokines was studied in *sanroque* mice, generated by screening a mutated mouse genome strain (9). The *sanroque* mice carried a T to G substitution that resulted in a M199R codon change (Fig. 3) (9). The *sanroque* mutation increased the number of follicular helper T cells, and showed severe autoimmune phenotypes. In these mice, the mutations prevented the downregulation of the mRNA of inducible T-cell Co-stimulator (ICOS), which was an essential co-stimulatory receptor for follicular T cells, and resulted in aberrant production of the cytokine, IL-21 (9). Furthermore, a conserved 47-base pair minimal region in the 3' UTR of ICOS mRNA was found to be essential for the regulation of ICOS by Roquin (58). However, though the mice lacking Roquin-1 exhibited perinatal lethality and conditional knockout of Roquin-1 in immune cells of mice that failed to maintain immune homeostasis, autoimmunity could be observed in these mice (59). Furthermore, even when Roquin-2 was ablated, a phenotype similar to that of Roquin-1-ablated mice was observed. T cells accumulated when both Roquin1/2 were ablated in CD4⁺ T cells, and these mice showed strong activation of

effector and follicular helper T cells, resulting in severe autoimmune phenotype. Both Roquin1/2 shared target genes, such as ICOS or Ox40, which are co-stimulatory receptors and redundantly regulate the polarization into follicular helper T cells (60). These targeted genes consisted of stem-loop structure in CDE, and Roquin1/2 recognized and bound to it via their ROQ domain, and subsequently recruited the Ccr4-Caf1-Not deadenylase complex and causing the degradation of target genes. More than 50 vertebrate mRNAs harbour CDE and many of them are regulated by Roquin1/2 (16).

Regnase-1 (ZC3H12A, MCPIP1) is another important RBP that down-regulates inflammatory cytokines. As shown in Fig. 3, Regnase-1 has a CCCH zinc finger domain, a PilT N-terminus (PIN)-domain like RNase domain. The CCCH zinc finger domain is essential for its binding to target mRNA and PIN structure is especially important for Mg^{2+} binding and enzymatic activity. Regnase-1 knockout mice survived only for a few weeks, and they exhibited splenomegaly, pulmonary inflammation and larger lymph nodes, suggesting the essential roles of Regnase-1 in mRNA decay. Regnase-1 recognizes the stem loop in CDE of cytokine mRNAs and causes the mRNA decay with its RNase activity (8). The target genes of Regnase-1 are important inflammatory cytokines or immune-related genes such as c-Rel, Ox40 and Il-2, and T cell receptor (TCR) stimulation led to cleavage of Regnase-1 at R111 by Malt1, thereby decreasing the levels of Regnase-1 and upregulating inflammation (Fig. 3) (61). The Regnase family consists of ZC3H12B and ZC3H12D, and both repress the expression of inflammatory cytokines. The mechanisms of action of ZC3H12D seems to be similar to that of Regnase-1, whereas ZC3H12B downregulates mRNAs via distinct mechanisms, which are not yet fully understood (62, 63). Further studies would be needed to fully comprehend the regulatory mechanisms by ZC3H12 family genes.

As we have already reviewed, Regnase-1 and Roquin share their roles in the regulation of the immune system and the regulatory mechanism itself seems to be similar. The paracaspase, MALT1, cleaves both Roquin and Regnase-1 and repressed important targets for promoting Th17 differentiation (64). However, Regnase-1 preferentially cleaves translationally active mRNAs and requires the helicase activity of UPF1 for its action, while Roquin regulates translationally inactive mRNAs in stress granules or P-bodies. These observations suggest that Regnase-1 is involved in the acute phase of inflammation, while Roquin has a role in the chronic phase of inflammation (65). Therefore, Regnase-1 and Roquin play distinct roles in inflammation and their cooperation is an essential factor for regulating the expression of inflammatory cytokines.

The AT-rich interactive domain-containing protein 5a (Arid5a) is a counter factor for Regnase-1 and Roquin1-2. Arid5a was initially thought to be a transcriptional factor like other Arid family proteins (66). However, it was found to have important roles in post-transcriptional regulation as an RBP. It recognizes the

stem loop structures in cytokines mRNAs and stabilizes them (5, 67). Arid5a can be induced by the stimulation of LPS, IL1- β and IL-6 (68). These inflammatory stimulations induce localization of Arid5a from the nucleus into the cytoplasm, thereby making it possible for Arid5a to upregulate cytokine genes (69). Arid5a KO mice showed resistance to endotoxin shock and EAE challenge, suggesting their important physiological roles in the immune system. Arid5a stabilizes mRNAs of Stat3, T-bet and Ox40, resulting in the upregulation of the polarization of each subset of helper T cells (67, 70, 71).

Conclusion

Post-transcriptional regulation by various RBPs through *cis*-elements is essential for the accurate regulation of inflammation. Though ARE and CDE are well-known *cis*-elements in mRNAs, there might still be other important regulatory elements. Furthermore, we speculated that similar to Arid5a, other factors that play the roles of both transcriptional factors and RBPs might exist. For example, approximately 800 zinc finger proteins are encoded by the human genomes and most of them have not been classified into transcriptional factors or RBPs yet; and some of them are thought to play both roles. For understanding the mechanism of inflammation, especially chronic inflammation, further elucidation of post-transcriptional regulation by RBPs is required. Recently, several ways for analysing the roles and target of RBPs have been proposed. One of the most important innovative techniques is the CLIP, which makes it possible for us to fully understand the target molecules of RBPs with high sensitivity (72, 73). These techniques for analysing the role of RBPs could clarify the whole scenario of chronic inflammation in the next decade. Furthermore, since many RBPs share the same *cis*-elements and play multiple roles, there should be some regulatory systems for the expression and subcellular localization of each RBP. However, the entire mechanism of such regulations is still unknown. Therefore, the technological innovation for analysing these interactions are needed to understand the complex roles of RBPs in inflammation.

Acknowledgement

We are grateful to all members of the Asahara Lab for their helpful discussion. This review article was partly supported by AMED-CREST from AMED (JP15gm0410001, JP18gm0810008) and grants from the National Institutes of Health [AR050631, AR065379 to H.A.].

Conflict of Interest

None declared.

References

1. Medzhitov, R. and Horng, T. (2009) Transcriptional control of the inflammatory response. *Nat. Rev. Immunol.* **9**, 692–703

2. Yu, H., Pardoll, D., and Jove, R. (2009) STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat. Rev. Cancer* **9**, 798–809
3. Elkon, R., Zlotorynski, E., Zeller, K.I., and Agami, R. (2010) Major role for mRNA stability in shaping the kinetics of gene induction. *BMC Genomics* **11**, 259
4. Rabani, M., Levin, J.Z., Fan, L., Adiconis, X., Raychowdhury, R., Garber, M., Gnirke, A., Nusbaum, C., Hacohen, N., Friedman, N., Amit, I., and Regev, A. (2011) Metabolic labeling of RNA uncovers principles of RNA production and degradation dynamics in mammalian cells. *Nat. Biotechnol.* **29**, 436–442
5. Masuda, K., Ripley, B., Nishimura, R., Mino, T., Takeuchi, O., Shioi, G., Kiyonari, H., and Kishimoto, T. (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
6. Lu, J.Y., Sadri, N., and Schneider, R.J. (2006) Endotoxic shock in AUF1 knockout mice mediated by failure to degrade proinflammatory cytokine mRNAs. *Genes Dev.* **20**, 3174–3184
7. Fan, X.C. and Steitz, J.A. (1998) Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the in vivo stability of ARE-containing mRNAs. *EMBO J* **17**, 3448–3460
8. Matsushita, K., Takeuchi, O., Standley, D.M., Kumagai, Y., Kawagoe, T., Miyake, T., Satoh, T., Kato, H., Tsujimura, T., Nakamura, H., and Akira, S. (2009) Zc3h12a is an RNase essential for controlling immune responses by regulating mRNA decay. *Nature* **458**, 1185–1190
9. Vinuesa, C.G., Cook, M.C., Angelucci, C., Athanasopoulos, V., Rui, L., Hill, K.M., Yu, D., Domasch, H., Whittle, B., Lambe, T., Roberts, I.S., Copley, R.R., Bell, J.I., Cornall, R.J., and Goodnow, C.C. (2005) A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. *Nature* **435**, 452–458
10. Hodson, D.J., Janas, M.L., Galloway, A., Bell, S.E., Andrews, S., Li, C.M., Pannell, R., Siebel, C.W., MacDonald, H.R., De Keersmaecker, K., Ferrando, A.A., Grutz, G., and Turner, M. (2010) Deletion of the RNA-binding proteins ZFP36L1 and ZFP36L2 leads to perturbed thymic development and T lymphoblastic leukemia. *Nat. Immunol.* **11**, 717–724
11. Carballo, E., Lai, W.S., and Blakeshear, P.J. (1998) Feedback inhibition of macrophage tumor necrosis factor- α production by tristetraprolin. *Science* **281**, 1001–1005
12. Gerstberger, S., Hafner, M., and Tuschl, T. (2014) A census of human RNA-binding proteins. *Nat. Rev. Genet.* **15**, 829–845
13. Zubiaga, A.M., Belasco, J.G., and Greenberg, M.E. (1995) The nonamer UUAUUUAUU is the key AU-rich sequence motif that mediates mRNA degradation. *Mol. Cell. Biol.* **15**, 2219–2230
14. Vlasova, I.A., Tahoe, N.M., Fan, D., Larsson, O., Rattenbacher, B., Sternjohn, J.R., Vasdewani, J., Karypis, G., Reilly, C.S., Bitterman, P.B., and Bohjanen, P.R. (2008) Conserved GU-rich elements mediate mRNA decay by binding to CUG-binding protein 1. *Mol. Cell.* **29**, 263–270
15. Stoecklin, G., Lu, M., Rattenbacher, B., and Moroni, C. (2003) A constitutive decay element promotes tumor necrosis factor α mRNA degradation via an AU-rich element-independent pathway. *Mol. Cell Biol.* **23**, 3506–3515
16. Leppek, K., Schott, J., Reitter, S., Poetz, F., Hammond, M.C., and Stoecklin, G. (2013) Roquin promotes constitutive mRNA decay via a conserved class of stem-loop recognition motifs. *Cell* **153**, 869–881
17. Shaw, G. and Kamen, R. (1986) A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell* **46**, 659–667
18. Stoecklin, G., Hahn, S., and Moroni, C. (1994) Functional hierarchy of AUUUA motifs in mediating rapid interleukin-3 mRNA decay. *J. Biol. Chem.* **269**, 28591–28597
19. Kontoyiannis, D., Pasparakis, M., Pizarro, T.T., Cominelli, F., and Kollias, G. (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
20. Chen, C.Y. and Shyu, A.B. (1995) AU-rich elements: characterization and importance in mRNA degradation. *Trends Biochem. Sci.* **20**, 465–470
21. Chen, C.Y., Xu, N., and Shyu, A.B. (1995) mRNA decay mediated by two distinct AU-rich elements from c-fos and granulocyte-macrophage colony-stimulating factor transcripts: different deadenylation kinetics and uncoupling from translation. *Mol. Cell. Biol.* **15**, 5777–5788
22. Peng, S.S., Chen, C.Y., and Shyu, A.B. (1996) Functional characterization of a non-AUUUA AU-rich element from the c-jun proto-oncogene mRNA: evidence for a novel class of AU-rich elements. *Mol. Cell. Biol.* **16**, 1490–1499
23. Hau, H.H., Walsh, R.J., Ogilvie, R.L., Williams, D.A., Reilly, C.S., and Bohjanen, P.R. (2007) Tristetraprolin recruits functional mRNA decay complexes to ARE sequences. *J. Cell. Biochem.* **100**, 1477–1492
24. Lykke-Andersen, J. and Wagner, E. (2005) Recruitment and activation of mRNA decay enzymes by two ARE-mediated decay activation domains in the proteins TTP and BRF-1. *Genes Dev.* **19**, 351–361
25. Bourcier, C., Griseri, P., Grepin, R., Bertolotto, C., Mazure, N., and Pages, G. (2011) Constitutive ERK activity induces downregulation of tristetraprolin, a major protein controlling interleukin8/CXCL8 mRNA stability in melanoma cells. *Am. J. Physiol. Cell Physiol.* **301**, C609–618
26. Deleault, K.M., Skinner, S.J., and Brooks, S.A. (2008) Tristetraprolin regulates TNF TNF- α mRNA stability via a proteasome dependent mechanism involving the combined action of the ERK and p38 pathways. *Mol. Immunol.* **45**, 13–24
27. Taylor, G.A., Carballo, E., Lee, D.M., Lai, W.S., Thompson, M.J., Patel, D.D., Schenkman, D.I., Gilkeson, G.S., Broxmeyer, H.E., Haynes, B.F., and Blakeshear, P.J. (1996) A pathogenetic role for TNF α in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency. *Immunity* **4**, 445–454
28. Coelho, M.A., de Carne Trecesson, S., Rana, S., Zecchin, D., Moore, C., Molina-Arcas, M., East, P., Spencer-Dene, B., Nye, E., Barnouin, K., Snijders, A.P., Lai, W.S., Blakeshear, P.J., and Downward, J. (2017) Oncogenic RAS signaling promotes tumor immunoresistance by stabilizing PD-L1 mRNA. *Immunity* **47**, 1083–1099.e6
29. Moore, M.J., Blachere, N.E., Fak, J.J., Park, C.Y., Sawicka, K., Parveen, S., Zucker-Scharff, I., Moltedo, B., Rudensky, A.Y., and Darnell, R.B. (2018) ZFP36

- RNA-binding proteins restrain T cell activation and antiviral immunity. *Elife* **7**, e33057
30. Stoecklin, G., Colombi, M., Raineri, I., Leuenberger, S., Mallaun, M., Schmidlin, M., Gross, B., Lu, M., Kitamura, T., and Moroni, C. (2002) Functional cloning of BRF1, a regulator of ARE-dependent mRNA turnover. *EMBO J.* **21**, 4709–4718
 31. Stumpo, D.J., Byrd, N.A., Phillips, R.S., Ghosh, S., Maronpot, R.R., Castranio, T., Meyers, E.N., Mishina, Y., and Blackshear, P.J. (2004) Chorioallantoic fusion defects and embryonic lethality resulting from disruption of Zfp36L1, a gene encoding a CCCH tandem zinc finger protein of the Tristetraprolin family. *Mol. Cell Biol.* **24**, 6445–6455
 32. Stumpo, D.J., Broxmeyer, H.E., Ward, T., Cooper, S., Hangoc, G., Chung, Y.J., Shelley, W.C., Richfield, E.K., Ray, M.K., Yoder, M.C., Aplan, P.D., and Blackshear, P.J. (2009) Targeted disruption of Zfp36L2, encoding a CCCH tandem zinc finger RNA-binding protein, results in defective hematopoiesis. *Blood* **114**, 2401–2410
 33. Newman, R., Ahlfors, H., Saveliev, A., Galloway, A., Hodson, D.J., Williams, R., Besra, G.S., Cook, C.N., Cunningham, A.F., Bell, S.E., and Turner, M. (2017) Maintenance of the marginal-zone B cell compartment specifically requires the RNA-binding protein ZFP36L1. *Nat. Immunol.* **18**, 683–693
 34. Salerno, F., Engels, S., van den Biggelaar, M., van Alphen, F.P.J., Guislain, A., Zhao, W., Hodge, D.L., Bell, S.E., Medema, J.P., von Lindern, M., Turner, M., Young, H.A., and Wolkers, M.C. (2018) Translational repression of pre-formed cytokine-encoding mRNA prevents chronic activation of memory T cells. *Nat. Immunol.* **19**, 828–837
 35. Wagner, B.J., DeMaria, C.T., Sun, Y., Wilson, G.M., and Brewer, G. (1998) Structure and genomic organization of the human AUF1 gene: alternative pre-mRNA splicing generates four protein isoforms. *Genomics* **48**, 195–202
 36. Knapinska, A.M., Gratacos, F.M., Krause, C.D., Hernandez, K., Jensen, A.G., Bradley, J.J., Wu, X., Pestka, S., and Brewer, G. (2011) Chaperone Hsp27 modulates AUF1 proteolysis and AU-rich element-mediated mRNA degradation. *Mol. Cell Biol.* **31**, 1419–1431
 37. Sinsimer, K.S., Gratacos, F.M., Knapinska, A.M., Lu, J., Krause, C.D., Wierzbowski, A.V., Maher, L.R., Scudato, S., Rivera, Y.M., Gupta, S., Turrin, D.K., De La Cruz, M.P., Pestka, S., and Brewer, G. (2008) Chaperone Hsp27, a novel subunit of AUF1 protein complexes, functions in AU-rich element-mediated mRNA decay. *Mol. Cell Biol.* **28**, 5223–5237
 38. Laroia, G., Cuesta, R., Brewer, G.a., and Schneider, R.J. (1999) Control of mRNA decay by heat shock-ubiquitin-proteasome pathway. *Science* **284**, 499–502
 39. Yoon, J.H., De, S., Srikantan, S., Abdelmohsen, K., Grammatikakis, I., Kim, J., Kim, K.M., Noh, J.H., White, E.J., Martindale, J.L., Yang, X., Kang, M.J., Wood, W.H. 3rd, Noren Hooten, N., Evans, M.K., Becker, K.G., Tripathi, V., Prasanth, K.V., Wilson, G.M., Tuschl, T., Ingolia, N.T., Hafner, M., and Gorospe, M. (2014) PAR-CLIP analysis uncovers AUF1 impact on target RNA fate and genome integrity. *Nat. Commun.* **5**, 5248
 40. Hinman, M.N. and Lou, H. (2008) Diverse molecular functions of Hu proteins. *Cell. Mol. Life Sci.* **65**, 3168–3181
 41. Peng, S.S., Chen, C.Y., Xu, N., and Shyu, A.B. (1998) RNA stabilization by the AU-rich element binding protein, HuR, an ELAV protein. *EMBO J.* **17**, 3461–3470
 42. Ma, W.J., Cheng, S., Campbell, C., Wright, A., and Furneaux, H. (1996) Cloning and characterization of HuR, a ubiquitously expressed Elav-like protein. *J. Biol. Chem.* **271**, 8144–8151
 43. Lopez de Silanes, I., Zhan, M., Lal, A., Yang, X., and Gorospe, M. (2004) Identification of a target RNA motif for RNA-binding protein HuR. *Proc. Natl. Acad. Sci. U S A.* **101**, 2987–2992
 44. Tran, H., Maurer, F., and Nagamine, Y. (2003) Stabilization of urokinase and urokinase receptor mRNAs by HuR is linked to its cytoplasmic accumulation induced by activated mitogen-activated protein kinase-activated protein kinase 2. *Mol. Cell Biol.* **23**, 7177–7188
 45. Cok, S.J., Acton, S.J., and Morrison, A.R. (2003) The proximal region of the 3'-untranslated region of cyclooxygenase-2 is recognized by a multimeric protein complex containing HuR, TIA-1, TIAR, and the heterogeneous nuclear ribonucleoprotein U. *J. Biol. Chem.* **278**, 36157–36162
 46. Chen, J., Cascio, J., Magee, J.D., Techasintana, P., Gubin, M.M., Dahm, G.M., Calaluze, R., Yu, S., and Atasoy, U. (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
 47. Herjan, T., Yao, P., Qian, W., Li, X., Liu, C., Bulek, K., Sun, D., Yang, W.P., Zhu, J., He, A., Carman, J.A., Erzurum, S.C., Lipshitz, H.D., Fox, P.L., Hamilton, T.A., and Li, X. (2013) HuR is required for IL-17-induced Act1-mediated CXCL1 and CXCL5 mRNA stabilization. *J. Immunol.* **191**, 640–649
 48. Abdelmohsen, K. and Gorospe, M. (2010) Posttranscriptional regulation of cancer traits by HuR. *Wiley Interdiscip. Rev. RNA* **1**, 214–229
 49. Casolaro, V., Fang, X., Tancowny, B., Fan, J., Wu, F., Srikantan, S., Asaki, S.Y., De Fanis, U., Huang, S.K., Gorospe, M., Atasoy, U.X., and Stellato, C. (2008) Posttranscriptional regulation of IL-13 in T cells: role of the RNA-binding protein HuR. *J. Allergy Clin. Immunol.* **121**, 853–859.e4
 50. Stellato, C., Gubin, M.M., Magee, J.D., Fang, X., Fan, J., Tartar, D.M., Chen, J., Dahm, G.M., Calaluze, R., Mori, F., Jackson, G.A., Casolaro, V., Franklin, C.L., and Atasoy, U. (2011) Coordinate regulation of GATA-3 and Th2 cytokine gene expression by the RNA-binding protein HuR. *J. Immunol.* **187**, 441–449
 51. Atasoy, U., Curry, S.L., Lopez de Silanes, I., Shyu, A.B., Casolaro, V., Gorospe, M., and Stellato, C. (2003) Regulation of eotaxin gene expression by TNF-alpha and IL-4 through mRNA stabilization: involvement of the RNA-binding protein HuR. *J. Immunol.* **171**, 4369–4378
 52. Katsanou, V., Milatos, S., Yiakouvaki, A., Sgantzis, N., Kotsoni, A., Alexiou, M., Harokopos, V., Aidinis, V., Hemberger, M., and Kontoyiannis, D.L. (2009) The RNA-binding protein Elavl1/HuR is essential for placental branching morphogenesis and embryonic development. *Mol. Cell Biol.* **29**, 2762–2776
 53. Kishore, S., Jaskiewicz, L., Burger, L., Hausser, J., Khorshid, M., and Zavolan, M. (2011) A quantitative analysis of CLIP methods for identifying binding sites of RNA-binding proteins. *Nat. Methods* **8**, 559–564

54. Mukherjee, N., Corcoran, D.L., Nusbaum, J.D., Reid, D.W., Georgiev, S., Hafner, M., Ascano, M., Jr., Tuschl, T., Ohler, U., and Keene, J.D. (2011) Integrative regulatory mapping indicates that the RNA-binding protein HuR couples pre-mRNA processing and mRNA stability. *Mol. Cell* **43**, 327–339
55. Tsuda, K., Kuwasako, K., Takahashi, M., Someya, T., Inoue, M., Terada, T., Kobayashi, N., Shirouzu, M., Kigawa, T., Tanaka, A., Sugano, S., Guntert, P., Muto, Y., and Yokoyama, S. (2009) Structural basis for the sequence-specific RNA-recognition mechanism of human CUG-BP1 RRM3. *Nucleic Acids Res* **37**, 5151–5166
56. Timchenko, L.T., Miller, J.W., Timchenko, N.A., DeVore, D.R., Datar, K.V., Lin, L., Roberts, R., Caskey, C.T., and Swanson, M.S. (1996) Identification of a (CUG)_n triplet repeat RNA-binding protein and its expression in myotonic dystrophy. *Nucleic Acids Res.* **24**, 4407–4414
57. Moraes, K.C., Wilusz, C.J., and Wilusz, J. (2006) CUG-BP binds to RNA substrates and recruits PARN deadenylase. *RNA* **12**, 1084–1091
58. Yu, D., Tan, A.H., Hu, X., Athanasopoulos, V., Simpson, N., Silva, D.G., Hutloff, A., Giles, K.M., Leedman, P.J., Lam, K.P., Goodnow, C.C., and Vinuesa, C.G. (2007) Roquin represses autoimmunity by limiting inducible T-cell co-stimulator messenger RNA. *Nature*. **450**, 299–303
59. Bertossi, A., Aichinger, M., Sansonetti, P., Lech, M., Neff, F., Pal, M., Wunderlich, F.T., Anders, H.J., Klein, L., and Schmidt-Supprian, M. (2011) Loss of Roquin induces early death and immune deregulation but not autoimmunity. *J. Exp. Med.* **208**, 1749–1756
60. Pratama, A., Ramiscal, R.R., Silva, D.G., Das, S.K., Athanasopoulos, V., Fitch, J., Botelho, N.K., Chang, P.P., Hu, X., Hogan, J.J., Mana, P., Bernal, D., Korner, H., Yu, D., Goodnow, C.C., Cook, M.C., and Vinuesa, C.G. (2013) Roquin-2 shares functions with its paralog Roquin-1 in the repression of mRNAs controlling T follicular helper cells and systemic inflammation. *Immunity* **38**, 669–680
61. Uehata, T., Iwasaki, H., Vandenbon, A., Matsushita, K., Hernandez-Cuellar, E., Kuniyoshi, K., Satoh, T., Mino, T., Suzuki, Y., Standley, D.M., Tsujimura, T., Rakugi, H., Isaka, Y., Takeuchi, O., and Akira, S. (2013) Malt1-induced cleavage of regnase-1 in CD4(+) helper T cells regulates immune activation. *Cell* **153**, 1036–1049
62. Wawro, M., Kochan, J., Krzanik, S., Jura, J., and Kasza, A. (2017) Intact NYN/PIN-Like Domain is Crucial for the Degradation of Inflammation-Related Transcripts by ZC3H12D. *J. Cell. Biochem.* **118**, 487–498
63. Wawro, M., Wawro, K., Kochan, J., Solecka, A., Sowinska, W., Lichawska-Cieslar, A., Jura, J., and Kasza, A. (2019) ZC3H12B/MCPIP2, a new active member of the ZC3H12 family. *RNA* **25**, 840–856
64. Jeltsch, K.M., Hu, D., Brenner, S., Zoller, J., Heinz, G.A., Nagel, D., Vogel, K.U., Rehage, N., Warth, S.C., Edelman, S.L., Gloury, R., Martin, N., Lohs, C., Lech, M., Stehlein, J.E., Geerlof, A., Kremmer, E., Weber, A., Anders, H.J., Schmitz, I., Schmidt-Supprian, M., Fu, M., Holtmann, H., Krappmann, D., Ruland, J., Kallies, A., Heikenwalder, M., and Heissmeyer, V. (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
65. Mino, T., Murakawa, Y., Fukao, A., Vandenbon, A., Wessels, H.H., Ori, D., Uehata, T., Tartey, S., Akira, S., Suzuki, Y., Vinuesa, C.G., Ohler, U., Standley, D.M., Landthaler, M., Fujiwara, T., and Takeuchi, O. (2015) Regnase-1 and Roquin Regulate a Common Element in Inflammatory mRNAs by Spatiotemporally Distinct Mechanisms. *Cell* **161**, 1058–1073
66. Amano, K., Hata, K., Muramatsu, S., Wakabayashi, M., Takigawa, Y., Ono, K., Nakanishi, M., Takashima, R., Kogo, M., Matsuda, A., Nishimura, R., and Yoneda, T. (2011) Arid5a cooperates with Sox9 to stimulate chondrocyte-specific transcription. *Mol. Biol. Cell* **22**, 1300–1311
67. Hanieh, H., Masuda, K., Metwally, H., Chalise, J.P., Mohamed, M., Nyati, K.K., Standley, D.M., Li, S., Higa, M., Zaman, M.M., and Kishimoto, T. (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
68. Nyati, K.K., Masuda, K., Zaman, M.M., Dubey, P.K., Millrine, D., Chalise, J.P., Higa, M., Li, S., Standley, D.M., Saito, K., Hanieh, H., and Kishimoto, T. (2017) TLR4-induced NF-kappaB and MAPK signaling regulate the IL-6 mRNA stabilizing protein Arid5a. *Nucleic Acids Res.* **45**, 2687–2703
69. Higa, M., Oka, M., Fujihara, Y., Masuda, K., Yoneda, Y., and Kishimoto, T. (2018) Regulation of inflammatory responses by dynamic subcellular localization of RNA-binding protein Arid5a. *Proc. Natl. Acad. Sci. U S A.* **115**, E1214–E1220
70. Zaman, M.M., Masuda, K., Nyati, K.K., Dubey, P.K., Ripley, B., Wang, K., Chalise, J.P., Higa, M., Hanieh, H., and Kishimoto, T. (2016) Arid5a exacerbates IFN-gamma-mediated septic shock by stabilizing T-bet mRNA. *Proc. Natl. Acad. Sci. U S A.* **113**, 11543–11548
71. Masuda, K., Ripley, B., Nyati, K.K., Dubey, P.K., Zaman, M.M., Hanieh, H., Higa, M., Yamashita, K., Standley, D.M., Mashima, T., Katahira, M., Okamoto, T., Matsuura, Y., Takeuchi, O., and Kishimoto, T. (2016) Arid5a regulates naive CD4+ T cell fate through selective stabilization of Stat3 mRNA. *J. Exp. Med.* **213**, 605–619
72. Hafner, M., Landthaler, M., Burger, L., Khorshid, M., Haussler, J., Berninger, P., Rothballer, A., Ascano, M., Jr., Jungkamp, A.C., Munschauer, M., Ulrich, A., Wardle, G.S., Dewell, S., Zavolan, M., and Tuschl, T. (2010) Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. *Cell* **141**, 129–141
73. Licatalosi, D.D., Mele, A., Fak, J.J., Ule, J., Kayikci, M., Chi, S.W., Clark, T.A., Schweitzer, A.C., Blume, J.E., Wang, X., Darnell, J.C., and Darnell, R.B. (2008) HITS-CLIP yields genome-wide insights into brain alternative RNA processing. *Nature* **456**, 464–469