RNA-binding proteins in immune regulation: a focus on CCCH zinc finger proteins

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Abstract

Nearly 60 CCCH zinc finger proteins have been identified in humans and mice. These proteins are involved in the regulation of multiple steps of RNA metabolism, including mRNA splicing, polyadenylation, transportation, translation and decay. Several CCCH zinc finger proteins, such as tristetraprolin (TTP), roquin 1 and MCPIP1 (also known as regnase 1), are crucial for many aspects of immune regulation by targeting mRNAs for degradation and modulation of signalling pathways. In this Review, we focus on the emerging roles of CCCH zinc finger proteins in the regulation of immune responses through their effects on cytokine production, immune cell activation and immune homeostasis.

Graphical Abstract
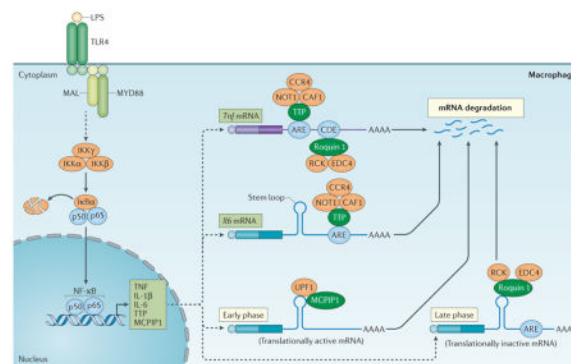
Immune responses combating infectious microorganisms are under precise control, as unchecked or inappropriate responses can be detrimental to the host and lead to inflammatory or autoimmune diseases[1]. Although the transcriptional control of immune responses has been studied extensively[2], the importance of post-transcriptional regulation of these processes is less well-defined. Post-transcriptional control can occur at each step of RNA metabolism, including splicing, capping, polyadenylation, export, localization, translation and decay. Recent studies have emphasized the importance of RNA metabolism, particularly mRNA degradation and translation, in the regulation of immune responses[3].

Zinc finger proteins are generally thought of as DNA-binding transcription factors. However, certain classes of zinc finger proteins, such as CCCH zinc finger proteins, often function as RNA-binding proteins and regulate RNA metabolism[4]. CCCH zinc finger proteins are characterized by one or more CCCH zinc finger domains containing three cysteines and a histidine. Nearly 60 CCCH zinc finger proteins have been identified in humans and mice. Although the functions of most CCCH zinc finger proteins remain obscure, emerging evidence suggests that some CCCH zinc finger proteins are involved in a broad range of biological processes that are associated with immune responses, including cytokine production, immune cell activation, immune homeostasis and antiviral innate immunity, as well as in regulation of cell differentiation and cancer cell growth (TABLE 1; see Supplementary information S1 (table)).

In this Review, we discuss the recent explosion of information on the role of CCCH zinc finger proteins in the regulation of immune responses. We focus on three protein families — tristetraprolin (TTP; encoded by *ZFP36*), roquin 1 and roquin 2 (encoded by *RC3H1* and *RC3H2*, respectively), and monocyte chemotactic protein-induced protein 1 (MCPIP1; also known as regnase 1 and encoded by *ZC3H12A*) — which provide an important layer of regulation of both innate and adaptive immune responses by targeting mRNA for degradation and through the modulation of signalling pathways.

CCCH zinc finger proteins

We previously identified 58 CCCH zinc finger proteins in mice and 56 CCCH zinc finger proteins in humans, through genome-wide surveys[5]. Based on updated information in GenBank, we identify here three new CCCH zinc finger proteins: CNOT4, HELZ2 and PAN3. In addition, two previously identified cDNAs that potentially encoded CCCH zinc finger proteins (BC003883 and BC019429) have now been deleted in the database. Thus, there are currently 59 and 57 CCCH zinc finger proteins in mice and humans, respectively (TABLE 1). *Zfp36l3* is a rodent-specific gene[6] and does not exist in humans. Human *ZC3H21* is a pseudogene and not listed in TABLE 1. Furthermore, a recent genomic analysis revealed 68 and 67 CCCH zinc finger proteins in *Arabidopsis thaliana* and rice, respectively[7], suggesting that CCCH zinc finger proteins are evolutionarily conserved.

Most CCCH zinc finger proteins with known functions act as regulators of RNA metabolism, including mRNA splicing, polyadenylation, export, translation and decay, and a number of CCCH zinc finger proteins also act as transcriptional repressors or signalling modulators (Supplementary information S2 (figure)). The molecular functions of 18 human

CCCH zinc finger proteins have not yet been determined, and the biological functions of many CCCH zinc finger proteins remain unknown. However, most of the characterized CCCH zinc finger proteins have been shown to be crucial regulators of immune responses, through the regulation of cytokine production, immune cell activation, immune homeostasis and antiviral responses.

Emerging evidence suggests that several CCCH zinc finger proteins, such as TTP, roquin 1 and MCPIP1, constitute a novel regulatory network that promotes the resolution of inflammation, controls the magnitude and duration of adaptive immune responses, and maintains immune homeostasis by targeting mRNA decay and modulation of signalling pathways[8–11]. TTP is the best-studied member of a small family of three proteins in humans that is characterized by a specific tandem CCCH zinc finger domain (FIG. 1). TTP was initially discovered as a gene that could be rapidly and transiently induced by the stimulation of fibroblasts with growth factors and mitogens[12]. It is now known that this protein can bind to AU-rich elements (AREs) in mRNA, leading to the removal of the poly(A) tail from the mRNA and increased rates of mRNA decay[13]. In addition to the tandem CCCH zinc finger domains, TTP contains three proline-rich domains and a conserved carboxy-terminal sequence that can bind the NOT1 scaffolding protein. A nuclear export sequence (NES) is located at the amino terminus of TTP (FIG. 1). The other two human family members, encoded by *ZFP36L1* and *ZFP36L2*, share very similar tandem CCCH zinc finger domains, as well as the putative C-terminal NOT1 binding sequences (FIG. 1).

Roquin 1 was identified through an *N*-ethyl-N-nitrosourea (ENU)-induced mutagenesis screen in mice[9]. In addition to a single CCCH zinc finger domain, roquin 1 contains a RING finger domain, a ROQ domain and a proline-rich domain (FIG. 1). Roquin 1 recognizes stem–loop motifs in the 3′ untranslated region (UTR) of its target mRNAs, through its ROQ domain and adjacent CCCH zinc finger domain, and promotes mRNA decay by recruiting the helicase RCK (also known as DDX6) and enhancer of mRNA-decapping protein 4 (EDC4)[14]. A second family member, roquin 2, shares a similar structure and has some overlapping functions with roquin 1 (FIG. 1).

MCPIP1 was identified as a novel protein containing a CCCH zinc finger domain and a PIN domain-like RNase domain; it also contains an ubiquitin-associated domain at its N terminus and a serine-rich domain at its C terminus (FIG. 1). MCPIP1 targets mRNA degradation via its intrinsic RNase activity[11]. Three other related proteins (termed MCPIP2, MCPIP3 and MCPIP4) share similar domain structures with MCPIP1 (REF. 10) (FIG. 1). Interestingly, phylogenetic analysis[5] showed that TTP, roquin 1 and MCPIP1 are evolutionarily closely related.

CCCH zinc finger proteins share many common features: the first is that each protein contains one or more CCCH zinc fingers, which defines this superfamily. Second, although other types of zinc fingers are well known as DNA-binding motifs, CCCH zinc fingers more commonly bind RNA by recognizing specific sequences or secondary structures in their mRNA targets[4]. For example, the tandem CCCH zinc finger motifs of the TTP protein family recognize specific ARE elements such as UUAUUUAUU in the 3′ UTR of their target mRNA[13], which is further confirmed by structural analysis[15]. Of note, a single CCCH

zinc finger can bind only weakly (albeit specifically) to AU-rich RNA[16], suggesting that proteins that contain a single CCCH zinc finger domain may bind RNA as dimers. Biochemical analysis indicates that both the ROQ domain and the adjacent CCCH zinc finger of roquin 1 contribute to the recognition of RNA[17]. However, structural analysis suggests that the ROQ domain of roquin 1 is sufficient for binding to the conserved decay element (CDE) in the 3′ UTR of tumour necrosis factor (*Tnf*) mRNA[18,19]. Although the CCCH zinc finger domain of MCPIP1 has been shown to play a part in the control of inter-leukin-6 (*Il6*) mRNA decay[11] (see later), the functional contributions of the CCCH zinc fingers in roquin 1 and MCPIP1 are not fully understood. Why CCCH zinc finger domains preferentially bind to RNA over DNA is also not well understood.

A third common feature of CCCH zinc finger proteins is that they commonly shuttle between different cellular compartments, such as from the nucleus to the cytoplasm, and between different RNA compartments, such as polysomes, stress granules and P-bodies, where they are thought to perform their various roles in RNA metabolism (BOX 1). A fourth common feature of this protein superfamily is that its members commonly interact with microRNA (miRNA) processing and effector pathways (BOX 2). Last, in addition to CCCH zinc finger domains, these family members contain other important functional domains. For example, MCPIP1 contains an RNase domain, and the roquin family proteins contain RING-type E3 ubiquitin ligase domains (FIG. 1). These domains greatly contribute to the functional variety within this protein superfamily.

Box 1

CCCH zinc finger proteins in RNA compartments

Eukaryotic mRNAs are in dynamic equilibrium between different subcellular locations: actively translated mRNAs can be found in polysomes; mRNAs stalled in translation initiation can accumulate in stress granules; and mRNAs targeted for degradation or translational repression can accumulate in P-bodies[97]. During energy deprivation and oxidative stress, the CCCH zinc finger protein tristetraprolin (TTP) is recruited to stress granules[82] — a process that is inhibited by phosphorylation of TTP by the mitogen-activated protein (MAP) kinase p38. TTP also localizes to P-bodies. Overexpression of TTP can increase the contacts of P-bodies with stress granules, which may allow for the transit of stalled mRNAs into the decay compartment that is P-bodies. Moreover, TTP and ZFP36L1 can nucleate P-body formation by moving the mRNAs with AU-rich elements (AREs) into P-bodies[98,99].

Similar to TTP, roquin 1 can also localize in P-bodies. However, under stress conditions, roquin 1 can be completely re-localized to stress granules[14]. The ROQ domain of roquin 1 is necessary and sufficient for its localization to stress granules and for inducing the formation of these structures upon its overexpression, and is required to trigger *Icos* mRNA decay[17].

Overexpression of monocyte chemotactic protein-induced protein 1 (MCPIP1) in cells usually results in the formation of granule-like structures in the cytoplasm. Most MCPIP1-containing granules are adjacent to or in contact with P-bodies; however, only small portions of MCPIP1-containing granules actually overlap with the P-bodies[100]. The

identity of MCPIP1 containing granules is still controversial. We recently found that MCPIP1-containing granules overlapped with GW182 and argonaute 2 (AGO2), which are molecular markers of GW-bodies, suggesting that MCPIP1 may functionally interact with microRNA effector pathways[38]. However, a recent study indicated that MCPIP1 was localized on ribosomes on endoplasmic reticulum[32]. It is also possible that MCPIP1 protein is localized in both positions and exerts its specific roles. Nevertheless, further studies are required to define the identity of MCPIP1-containing granules, as this is important for understanding the molecular mechanisms of its action.

Interestingly, MCPIP1 expression completely blocks the formation of stress granules under different stress conditions[100], through which it may protect macrophages from stress-induced apoptosis. These studies also indicate that MCPIP1 functions differently to TTP and roquin 1 in the formation of stress granules. However, the physiological implications of this difference remain unclear. Other CCCH zinc finger proteins, such as ZAP, have also been shown to transiently localize to stress granules and P bodies[101]. Taken together, as RNA granules serve as key modulators of post-transcriptional and epigenetic gene expression, the dynamic localization and nucleation of these CCCH zinc finger proteins in RNA granules may have important roles in the regulation of immune responses.

Box 2

Interplay of CCCH zinc finger proteins with miRNAs

MicroRNAs (miRNAs) are a class of non-coding RNAs that modulate gene expression at the post transcriptional level and are involved in regulating many aspects of the immune response. It is well established that AU rich elements (AREs) in the 3′ untranslated region (UTR) of tumour necrosis factor *(Tnf)* mRNA dictates its degradation by tristetraprolin (TTP) binding. Interestingly, dicer 1, argonaute 1 (AGO1) and AGO2, which are molecules involved in miRNA processing and effector pathways, are required for the rapid decay of *Tnf* mRNA[102], suggesting the potential interaction of TTP with miRNA pathways. Interestingly, miR-16, a human miRNA containing a UAAAUAUU sequence that is complementary to the ARE sequence, is required for the decay of ARE containing RNA, a process that also depends on the ARE binding protein TTP. Moreover, TTP does not directly bind to miR-16 but interacts with argonaute family members to form a complex with miR-16 and assists in the ARE-mediated mRNA decay[102].

Roquin 1 was recently identified as a key factor to regulate miRNA homeostasis. Roquin 1 can decrease the half-life of mature miRNA by 50%, probably through increasing their mono-uridylation[103]. A loss of functional roquin 1 leads to accumulation of miR 146a in T cells, which also targets inducible T cell co stimulator (*Icos*) mRNA for degradation by binding to its complementary site that is different to roquin 1-binding site. These studies suggest that roquin 1 and miR-146a function in the same pathway. Loss of roquin 1 leads to increased levels of miR-146a, which may compensate for the role of roquin 1 in regulating *Icos* mRNA.

Moreover, roquin 1 interacts with AGO2, the core component of miRNA-induced silencing complex (miRISC), suggesting that roquin 1 may be involved in the miRNA effector pathway[103]. Similarly, monocyte chemotactic protein-induced protein 1 (MCPIP1) interacts with GW182, another core component of miRISC[38]. However, the functional significance of the interaction of these CCCH zinc finger proteins with miRISC is still an open question. One report also indicates that MCPIP1 counteracts dicer-mediated miRNA processing and suppresses miRNA biosynthesis via cleavage of the terminal loops of pre-miRNAs[104]. The implications of these studies in immune regulation need to be further explored.

Regulation of cytokine production

TNF

Cytokines are crucial mediators of inflammation and immune responses and their production is regulated at multiple levels. Although transcription is an essential first step, full regulation of cytokine production also involves many additional post-transcriptional checkpoints. These occur at the levels of mRNA splicing, polyadenylation, degradation and translation[20]. It is well established that many mRNAs that encode cytokines have short half-lives and are subject to ARE-mediated decay. For example, degradation of *Tnf* mRNA is crucial for restricting TNF production, and involves AREs in the 3′ UTR of *Tnf* mRNA. Indeed, specific deletion of AREs from endogenous *Tnf* transcripts results in more stable *Tnf* mRNA and hypersecretion of TNF[21]. The ARE-binding protein TTP is crucially involved in the regulation of TNF production by binding directly to AREs in the *Tnf* 3′ UTR and promoting TNF decay through recruitment of the CCR4–CAF1–NOT1 deadenylase complex and the 4EHP–GYF2 cap-binding complex[22,23] (FIG. 2). Interestingly, TTP expression is induced by TNF signalling and by many of the same agents that stimulate TNF production. Thus, TTP acts as one component of a negative feedback loop that controls TNF production by destabilizing its mRNA[8].

TTP-deficient mice develop a complex syndrome of inflammatory arthritis, dermatitis, cachexia, autoimmunity and myeloid hyperplasia[24]. These phenotypes are very similar to those seen in *Tnf*-transgenic mice[25] and mice lacking *Tnf* AREs[21]. In addition, treatment of young TTP-deficient mice with TNF blocking antibodies or crossing TTP-deficient mice with mice lacking TNF receptor 1 can prevent the development of most aspects of this syndrome[24,26]. Together, these studies suggest that TTP is essential for the normal control of TNF production.

Post-transcriptional control through AREs is often influenced by non-ARE sequences in the same transcripts. For example, the constitutive decay element (CDE) located downstream of the ARE in the 3′ UTR of *Tnf* mRNA has been proposed to prevent the pathological expression of TNF in conditions in which the ARE is inactive[27]. Indeed, roquin 1 has been shown to have a role in the regulation of *Tnf* mRNA decay via binding to the CDE[28]. Roquin 1 binds to the CDE through its unique ROQ domain; after doing so, it recruits the helicase RCK and decapping enzyme EDC4 for RNA degradation[28]. The coexistence of multiple regulatory RNA elements in a single mRNA ensures that several RNA-binding

proteins can work together to regulate the expression levels of important transcripts, such as those encoding TNF. Both ARE-mediated control by TTP and CDE-mediated control by roquin 1 are of crucial importance for controlling the production of TNF. This conclusion is further supported by *in vivo* evidence that roquin 1[san/san] mice developed TNF-driven inflammation and arthritis that was comparable to the disease that develops in TTP-deficient mice[29]. Together, these studies reveal a complex interplay of two CCCH zinc finger proteins in the regulation of TNF production (FIG. 2). Given that TNF is the most potent pro-inflammatory cytokine in mammals, and that increased levels of TNF often have detrimental consequences, including the development of septic shock and chronic inflammatory diseases such as rheumatoid arthritis, these pathways may be novel targets for anti-inflammatory treatments for rheumatoid arthritis and other inflammatory conditions.

IL-6

IL-6 is a multifunctional pro-inflammatory cytokine that has important roles in a variety of diseases[30], and its expression is tightly controlled at both the transcriptional and post-transcriptional levels. There are five ARE sites in the 3′ UTR of mouse *Il6* mRNA. It has been reported that TTP can promote *Il6* mRNA degradation by binding to ARE2, ARE3 and ARE4 (REF. 31) (FIG. 2). Moreover, IL-6 production was significantly elevated after injection of TTP-deficient mice with IL-1β, indicating that TTP directly regulated IL-6 production[31].

Recent studies revealed that MCPIP1 is also a crucial regulator of IL-6 production by macrophages[11]. MCPIP1 binds to and cleaves a conserved stem–loop element in the 3′ UTR of *Il6* mRNA via its endonuclease activity and the helicase activity of UPF1 (REFS 11,32). As a result, MCPIP1-deficent mice have significantly higher levels of IL-6 than normal mice, and *Il6* mRNA is more stable in MCPIP1-deficient macrophages compared with wild-type macrophages[11]. Interestingly, a recent study showed that roquin 1 and MCPIP1 regulate an overlapping set of mRNAs, including *Il6* mRNA, via their recognition of a common stem–loop structure[32]. However, MCPIP1 and roquin 1 promote the decay of inflammatory mRNAs at different phases of the inflammatory response: MCPIP1 controls the early phase of inflammation via the cleavage and degradation of translationally active mRNAs and requires the helicase activity of UPF1 (FIG. 2). By contrast, roquin 1 controls the later phase of inflammation by removing translationally inactive mRNAs via a mechanism that is independent of UPF1 and involving RCK and EDC4 (REF. 32) (FIG. 2). Taken together, these results suggest that three CCCH zinc finger proteins — MCPIP1, roquin 1 and TTP — act together to control IL-6 production through different elements in the 3′ UTR of its mRNA. Further studies are needed to understand how these various mechanisms are integrated and regulated to determine the level of cytokine production by innate immune cells.

IL-10 and other cytokines

The range of TTP-targeted mRNAs includes not only those encoding pro- inflammatory cytokines, but also the anti-inflammatory cytokine IL-10 (REF. 33), a cytokine important for the resolution of inflammation. IL-10 inhibits acute and chronic inflammation by suppressing pro-inflammatory cytokine production by activated macrophages. *Il10* mRNA

was identified as a target of TTP through genome-wide analysis, and TTP enhances *Il10* mRNA degradation through binding to AREs in its 3′ UTR[33]. Furthermore, IL-10 was found to induce TTP expression in macrophages by activating signal transducer and activator of transcription 3 (STAT3), suggesting that IL-10-mediated TTP induction is part of a negative feedback loop that controls the production of this cytokine[34]. The discovery that TTP promotes the decay of *Il10* mRNA suggests that post-transcriptional control mechanisms can regulate both the initiation and the resolution of inflammatory responses.

Intensive studies have demonstrated that the feedback control mediated by these CCCH zinc finger proteins can also be applied to other cytokines, chemokines and pro-inflammatory molecules, such as IL-16, IL-8, IL-22, IL-23, CCL3, IFNγ, CXCL1, GM-CSF, iNOS and VEGF[35]. Tiedje *et al.* recently identified numerous mRNA targets of TTP, such as *Ler3, Dusp1* and *Tnfaip3*, which are feedback inhibitors of nuclear factor-κB (NF-κB) activation, suggesting a new role for TTP in the regulation of the NF-κB signalling pathway[36]. In addition, Sedlyarov *et al.*[37] have recently shown an increased influence of TTP-dependent mRNA decay on the expression profile of inflammatory cytokines at the transition to the resolution phase of the inflammatory response, which suggests that TTP-mediated mRNA decay directly controls the switch from the inflammatory to the resolution phase of the immune response in macrophages[37].

In addition to the three CCCH zinc finger proteins discussed above, other CCCH zinc finger proteins participate in the regulation of cytokine expression. For example, MCPIP4 (also known as TFL and p34 and encoded by *ZC3H12D*) is involved in the regulation of *Il6*, *Il1b*, *Tnf* and *Il2* mRNA decay through the targeting of their 3′ UTR[38–40]. The consequences of this regulation could be seen in MCPIP4-deficient mice, which produced elevated levels of IL-2, IL-6, TNF and IL-17A[40]. In studies of experimental autoimmune encephalitis, *Mcpip4*$^{-/-}$ mice contained a higher proportion of T helper 17 (T$_H$17) cells than did wild-type mice during the resolution phase. These results suggest that MCPIP4 may have an important role in attenuating local inflammation by suppressing the infiltration of T$_H$17 cells and may contribute to recovery from T cell-mediated autoimmune diseases[40].

Taken together, these studies suggest that mRNA stability is determined by the integration of multiple regulatory processes that have competing, additive or synergistic effects. These regulatory pathways are used to coordinate the expression of pro-inflammatory and anti-inflammatory cytokines to tune immune and inflammatory responses in a timely and efficient manner.

Regulation of immune cell activation

In addition to regulating immune responses through the control of cytokine production, CCCH zinc finger proteins can regulate immune cell activation via intrinsic pathways.

Macrophage activation

Macrophages are centrally involved in both innate and adaptive immunity. They express pattern recognition receptors (PRRs) that sense components of bacteria, viruses and parasites, as well as endogenous components. For example, lipopolyaccharide (LPS), a

component of the outer membrane of Gram-negative bacteria, is recognized by Toll-like receptor 4 (TLR4) expressed on the surface of macrophages. Binding of LPS to TLR4 triggers signalling cascades leading to the activation of the transcription factors NF-κB and AP-1, which results in the expression of genes encoding cytokines and other molecules[41] (FIG. 3).

During macrophage activation, MCPIP1 mRNA is induced by TLR ligands and pro-inflammatory cytokines such as TNF, IL-1β and CCL2 (REFS 10,11,42). Expression of MCPIP1, in turn, negatively regulates macrophage activation by multiple mechanisms (FIG. 3). As discussed above, MCPIP1 acts as an RNase to promote the degradation of a subset of mRNAs encoding pro-inflammatory cytokines, including IL-1β, IL-6 and IL-12p40, by directly targeting their 3′ UTRs[11,43]. In addition, several studies suggest that MCPIP1 may act as an adaptor molecule that recruits other proteins, such as TANK and USP10, to form a complex that inhibits NF-βB and the mitogen-activated protein kinase JNK by promoting the deubiquitylation of TNF-receptor-associated factor 3 (TRAF3) and TRAF6 (REFS 44– 46).

There are some controversial reports on the regulation of NF-κB signalling by CCCH zinc finger proteins. An early study suggested that MCPIP1 did not regulate NF-κB signalling, as macrophages isolated from *Mcpip1*^{−/−} mice did not show hyperactivation of NF-κB in response to LPS stimulation[11]. One possible explanation for this discrepancy is that some other CCCH zinc finger proteins may compensate for the effect of MCPIP1 deficiency on NF-κB activation. TTP was also reported to negatively regulate NF-κB signalling by acting as a co-repressor or by interfering with the nuclear translocation of p65 (REFS 47–49) (FIG. 3). Taken together, these studies suggest that these CCCH zinc finger proteins are multifunctional proteins. In controlling macrophage activation, they primarily act at a post-transcriptional level to promote mRNA decay of the inflammatory cytokines, but they may also attenuate the transcription of the inflammatory cytokines by inhibiting the signal transduction pathways that induce their expression (FIG. 3). The significance of these regulatory mechanisms can be seen from the phenotypes of the deficient mice. For example, mice specifically lacking TTP in myeloid cells are extremely sensitive to LPS-induced septic shock owing to a heightened production of TNF[50]. Mice lacking MCPIP1 spontaneously developed a systemic inflammatory syndrome with massive infiltration of inflammatory cells into many organs, especially the lungs and liver[51]. Furthermore, MCPIP1-deficient mice were extremely sensitive to LPS-induced septic shock due to overproduction of TNF[52]. It is noteworthy that TANK-deficient mice and MCPIP1-deficient mice share similar phenotypes, including splenomegaly, heightened production of inflammatory cytokines and early death[53], which further supports the hypothesis that these factors may be mediating their effects through the same pathway.

Although TTP and MCPIP1 act as negative regulators of NF-κB signalling, a recent study suggests that roquin 1 promotes the activity of the inhibitor of NF-κB kinase (IKK)–NF-κB pathway by promoting mRNA degradation of A20 (REF. 54). A20 is an ubiquitin-editing enzyme that inhibits the NF-κB signalling pathway[55]. Roquin 1 uses its ROQ and CCCH zinc finger domains to contact a non-CDE-type stem–loop structure that is preceded by an ARE in the 3′ UTR of *A20* mRNA and promotes its degradation[54]. These studies highlight

the importance of post-transcriptional regulation of gene expression to control crucial cellular signal transduction pathways (FIG. 3).

In addition, the CCCH zinc finger proteins ZC3H13 and ZC3H18 were reported to positively regulate the NF-κB pathway. ZC3H13 was found to have an important role in the transcription of NF-κB-induced latent membrane protein 1 (LMP1), TNF and IL-1β, but not for IKK activation, whereas ZC3H18 was crucial for IKK activation[56]. However, the mechanisms and physiological significance of ZC3H13 and ZC3H18 in the regulation of NF-κB-mediating signalling pathways need to be further investigated.

T cell activation

The failure of mechanisms that control T cell activation and function results in autoimmune diseases, which are characterized by the generation of autoantibodies and systemic inflammatory injury. As MCPIP1, TTP and roquin 1 are highly expressed by T cells, their function in T cell activation is of interest. MCPIP1-deficient mice showed severe systemic inflammation characterized by T cell and B cell hyper-activation, hyperimmunoglobulinaemia and production of autoantibodies[11].

Mice with a T cell-specific deletion of *Mcpip1* have a similar phenotype to that of *Mcpip1*-global knockout mice, suggesting that MCPIP1 is crucial for preventing autoimmunity in a T cell-intrinsic manner[57]. The molecular mechanisms by which MCPIP1 negatively regulates T cell activation may involve the decay of a set of mRNAs encoding immunoregulatory molecules, such as inducible T cell co-stimulator (ICOS), REL, OX40, IL-2 and IL-2Rβ[57]. REL is a major component of the NF-κB pathway and has been shown to have an essential role in T_H1 cell activation associated with autoimmune disease[58]. The development of autoimmune disease in *Mcpip1*^{−/−} mice was partially inhibited by a loss of REL, suggesting that increased REL expression, due to defective mRNA degradation, contributes to the activation of T cells and subsequently B cells in *Mcpip1*^{−/−} mice[57].

As antigen recognition is required for T cell activation, the source (or sources) of antigens responsible for activating T cells in MCPIP1-deficient mice remain to be determined. The indigenous microbiota of mucosal surfaces, particularly the intestinal micro-flora, is thought to account for a substantial proportion of the antigenic stimuli for innate and adaptive immune cells[59]. Treatment of MCPIP1-deficient mice with broad-spectrum antibiotics significantly decreased the inflammatory phenotype and increased the lifespan of these mice, suggesting that microflora antigens contribute to T cell activation in the absence of MCPIP1 (REF. 51). In addition, adoptive transfer of activated T cells from MCPIP1-deficient mice into normal animals results in the generation of autoantibodies, suggesting that B cell activation in MCPIP1-deficient mice might be secondary to T cell activation[57]. Nevertheless, the intrinsic role of MCPIP1 in B cells warrants further investigation. Together, these studies suggest that MCPIP1 not only regulates the activation of macrophages but also controls T cell activation by targeting mRNA degradation.

In addition to mRNA decay, mRNA splicing is also crucial for suppressing aberrant T cell activation. For example, CD45 is a transmembrane tyrosine phosphatase that is expressed by all nucleated haematopoietic cells. CD45 is essential for T cell activation as it removes an

inhibitory phosphate group from kinases, such as SRC and LCK, and thereby allows T cell receptor (TCR) signalling[60]. However, expression of the spliced isoform of CD45 that lacks exon 4, 5 and 6 (CD45RO) has been shown to limit T cell activation[61]. During T cell activation, there is a shift in expression of CD45 isoforms from the full protein to the CD45RO isoform. This activation-induced alternative splicing may be part of a negative feedback regulatory loop to prevent aberrant TCR signalling. The CCCH zinc finger protein U2AF1L4 (also known as U2AF26) functions as a regulator of mRNA splicing and has been shown to cooperate with the zinc finger protein GFI1 in determining the splicing of CD45. U2AF1L4 expression facilitates the formation of the less-active CD45RO isoform, and by which it negatively regulates T cell activation[61].

In response to different antigens, activated T cells differentiate into different effector T cells such as T_H1, T_H2 and T_H17 cells. A recent study suggests that roquin 1 and MCPIP1 are crucial factors for controlling T_H17 cell differentiation by cooperatively repressing a set of common target genes encoding T^H17 cell-promoting factors such as IL-6, ICOS, REL, IRF4, IκBNS and IκBζ[62]. Mice with a combined deficiency of roquin 1 and roquin 2 specifically in T cells and their precursors developed severe T_H17 cell-mediated lung inflammation and gastritis[62]. Similar hyperinflammatory phenotypes were also observed in MCPIP1-deficient mice[11,51,63]. Furthermore, a recent study indicates that MCPIP1 negatively regulates IL-17 signalling and inflammation via suppressing the expression of IL-17 target genes and inducing decay of mRNA transcripts encoding IL-17 receptor subunits[64]. Although several studies reported that MCPIP1 was induced by IL-17 (REFS 65, 66), these studies suggest that these CCCH zinc finger proteins act as feedback inhibitors of IL-17 signalling.

In summary, these studies reveal that the regulation of mRNA metabolism by CCCH zinc finger proteins is crucial for suppressing aberrant activation of both innate and adaptive immune cells. The CCCH zinc finger proteins may control the immune cell activation by multiple mechanisms: inducing the decay of mRNA transcripts encoding immunoregulatory molecules, signalling transducers and inflammatory targets; recruiting other inhibitors to suppress crucial signalling pathways; and/or facilitating the formation of specific splicing isoforms that limit T cell activation. There are still many other questions waiting for answers. For example, the roles of these CCCH zinc finger proteins in the activation of other immune cells such as dendritic cells, natural killer cells and B cells remains to be determined. Further understanding of these post-transcriptional pathways may provide novel therapeutic targets for treatment of autoimmune diseases and inflammatory conditions.

Regulation of immune homeostasis

The regulation of costimulatory signalling in CD4+ T cells is crucial for maintaining peripheral T cell tolerance and immune homeostasis. Failure to control costimulatory signalling may break immunological tolerance and contribute to the development of autoimmune diseases such as systemic lupus erythematosus (SLE). The expression of ICOS, which is a member of the CD28 superfamily of costimulatory molecules, is upregulated by T cells upon activation. ICOS signalling is required for the development and proliferation of T follicular helper (T_{FH}) cells[67]. TFH cells promote the maturation of B cells into memory B

cells or antibody-secreting plasma cells in germinal centres[68]. Dysfunctional T_{FH} cells can lead to the aberrant selection of autoreactive germinal centre B cells and the production of autoantibodies that cause diseases such as SLE[69]. Roquin 1 is a crucial factor that controls *Icos* mRNA levels and maintains activation-induced expression of ICOS by CD4$^+$ T cells[70]. Recognition of the 3′ UTR of this mRNA by the RNA-binding domain of roquin 1 facilitates the degradation of the transcript through interactions with the decapping enzyme EDC4 and the helicase RCK[14].

Roquin 1$^{san/san}$ (also called *sanroque*), a single point mutation in the ROQ domain of the gene encoding roquin 1, leads to a lupus-like autoimmune phenotype in mice, which is marked by enhanced numbers of T_{FH} cells and spontaneous germinal centre formation[9]. These mice had high-level ICOS expression. Partial correction of ICOS overexpression in roquin 1$^{san/san}$*Icos*$^{+/-}$ mice was accompanied by a reduction in lymphadenopathy, splenomegaly, T_{FH} cell population expansion and germinal centre B cell numbers, suggesting that ICOS overexpression is an essential contributor to the lupus phenotype in roquin 1$^{san/san}$ mice[70]. Interestingly, unlike roquin 1$^{san/san}$ mice, the cell-specific ablation of roquin 1 in T cells or B cells did not cause autoimmunity[71], and similarly to roquin 1, T cell-specific deletion of roquin 2 does not affect immune cell homeostasis[72]. However, mice deficient in both roquin 1 and roquin 2 developed an autoimmune phenotype with increased T_{FH} cell numbers and germinal centre expansion, which phenotypically resembles roquin 1$^{san/san}$ mice[72]. These results suggest redundant functions of roquin 1 and roquin 2 in regulating ICOS expression in T_{FH} cells (FIG. 4).

The paradoxical observations between the autoimmune-prone roquin 1$^{san/san}$ mice and the healthy roquin 1-deficient mice are not fully understood. A recent elegant study showed that a RING-less roquin 1-mutant protein failed to localize to stress granules and resulted in a compensatory role for roquin 2 in the repression of ICOS and T_{FH} cells[29], thus roquin 1^{RINGless} mice did not show any phenotypes of autoimmunity[29]. By contrast, in roquin 1$^{san/san}$ mice, the ROQ-mutant roquin 1 still localized to mRNA-regulating stress granules and prevented a compensationary response by roquin 2; thus roquin 1$^{san/san}$ mice showed severe phenotypes of autoimmunity[29]. Nevertheless, more investigation into this paradoxical observation is required for a full understanding.

In view of the important role of RING domains in driving protein ubiquitylation, determining whether roquin 1 and roquin 2 contain E3 ubiquitin ligase activity is crucial for understanding their complicated roles. Roquin 2 was identified as an E3 ubiquitin ligase required for reactive oxygen species (ROS)-induced ubiquitylation and degradation of apoptosis signal- regulating kinase 1 (ASK1; also known as MAP3K5)[73]. As evident from research using *Caenorhabditis elegans*, mutation of the gene encoding the roquin 2 orthologue RLE1 results in the accumulation of the activated form of the ASK1 orthologue NSY1, which conferred resistance to *Pseudomonas aeruginosa* infection[73]. Moreover, Zhang *et al.* recently demonstrated that both roquin 1 and roquin 2 are functional E3 ubiquitin ligases that drive the assembly of polyubiquitin chains of different linkages[74]. Interestingly, a recent study showed that the RING domain of roquin 1 directly binds to the catalytic a1 subunit of adenosine monophosphate-activated kinase (AMPK) and represses its enzymatic activity[75]. As AMPK is a central regulator of cellular metabolism in response to cellular

stress and an inhibitor of mechanistic target of rapamycin (mTOR) signalling, roquin 1 may positively regulate T_{FH} cell formation by inhibition of AMPK activity (FIG. 4). Although this surprising observation[75] on the role of roquin 1 on T_{FH} cell formation needs more investigation, these results suggest that roquin 1 may fine-tune the regulation of T_{FH} cell differentiation through two different domains (RING domain and ROQ domain) and two different mechanisms (inhibition of AMPK and degradation of ICOS) (FIG. 4).

Taken together, these studies revealed the importance of roquin 1- and roquin 2-mediated post-transcriptional networks in T_{FH} cell differentiation and the maintenance of immune homeostasis. Investigations into the mechanisms through which roquin proteins and MCPIP1 repress their target mRNA molecules have highlighted the complex and intricate feedback loops these RNA-binding proteins use to augment or control adaptive immune responses.

Regulation of the regulators

The expression and function of CCCH zinc finger proteins are precisely regulated by multiple mechanisms, which add another layer of control on the regulation of innate and adaptive immune responses (FIG. 5). For example, *Mcpip1* mRNA is induced by TLR ligands and inflammatory cytokines through the NF-κB signalling pathway[76]. However, during LPS- and IL-1β-induced macrophage activation, MCPIP1 protein is rapidly degraded by the ubiquitin–proteasome system, which is dependent on IKK-mediated phosphorylation[77] (FIG. 5). Consistent with this observation, MG132, which is a potent proteasome inhibitor, has been shown to significantly increase MCPIP1 protein levels in HepG2 or HeLa cells[78]. In addition, MCPIP1 protein can target the 3′ UTR of its own mRNA and promote its degradation[77]. In activated T cells, MCPIP1 is regulated through TCR-induced MALT1 (mucosa-associated lymphoid tissue lymphoma translocation 1)-mediated cleavage. MALT1 is an arginine-specific protease that cleaves MCPIP1 at arginine 111; the resulting fragments are rapidly degraded by other proteases[57]. Similarly to MCPIP1, both roquin 1 and roquin 2 are also cleaved by MALT1 upon T cell activation[62] (FIG. 5).

Importantly, the function of MCPIP1 on mRNA decay is regulated by other RNA-binding proteins. For instance, AT-rich interactive domain-containing protein 5α (ARID5α) can compete with MCPIP1 for binding to the 3′ UTR of *Il6* mRNA and protect it from MCPIP1-mediated degradation. Furthermore, ARID5a deficiency in mice attenuates acute inflammation and autoimmunity[79]. Together, these results suggest that both the expression and function of MCPIP1 are dynamically regulated during the course of an immune response: MCPIP1 expression is suppressed by TLR signalling during the early phase of an infection, allowing the immune system to be activated and fully respond to the microbial challenge, whereas, in the later phases of the response, MCPIP1 expression is restored by increased transcription[76] and helps to promote the resolution of inflammation by promoting the degradation of inflammatory cytokines and immune molecules.

Similarly to MCPIP1, the transcription of *Zfp36* is also regulated by NF-κB signalling in LPS-stimulated macrophages[80]. TTP can also promote the degradation of its own mRNA by

binding to the ARE on its 3′ UTR[81]. Phosphorylation of TTP by p38–MK2 has been reported to promote its sequestration by 14-3-3 proteins, resulting in diminished mRNA decay activity[82] (FIG. 5). Additionally, phosphorylated TTP may be impaired in its ability to recruit mRNA decay enzymes, resulting in decreased deadenylation of target transcripts[82–84]. In addition, the function of TTP in the regulation of cytokine production may be antagonized by another RNA-binding protein. For example, as an ARE-binding protein, HuR can compete with TTP for ARE-binding sites on the *Il6* 3′ UTR, thereby stabilizing *Il6* mRNAs[85]. TTP can also be ubiquitylated by the adaptor molecule TRAF2 in a phosphorylation-dependent manner[86,87]. Ubiquitylated TTP no longer affects NF-κB activity but promotes the activation of JNK[86]. A further study suggests that TTP degradation by proteasomes is ubiquitin independent but instead is dependent on intrinsically disordered N- and C-termini of the protein[88]. These studies suggest that the expression and function of TTP are regulated by multiple mechanisms.

Taken together, these studies suggest that the expression and function of TTP, roquin 1 and MCPIP1 can be governed by the same signalling pathways that control immune cell activation in response to TCR, TLR and cytokine receptor signalling. It is likely that some of CCCH zinc finger proteins may act as a point of convergence for the activity of numerous kinases, including IKK, ERK1, ERK2, JNK and p38 MAP kinase. For example, TTP can be modified by different kinases in response to different environmental cues, and the different modified states of TTP have different roles in the regulation of target decay or inhibition of signalling pathways. Precise regulation of these regulators provides further layers of control to fine tune immune responses (FIG. 5).

Associated diseases and therapeutic potential

Although emerging evidence from studies of genetically engineered animals indicates that TTP, roquin 1 and MCPIP1 are crucial regulators of inflammation and immune homeostasis, mutations of these genes in humans have not been clearly implicated in disease development. Suzuki *et al.*[89] reported that a single nucleotide polymorphism (SNP) within the human *ZFP36* promoter resulted in twofold greater promoter activity. These authors reported that patients with rheumatoid arthritis with the GG genotype might be prone to higher disease activity than those with the AG or AA genotypes. This suggests that this SNP mildly affects *ZFP36* promoter activity, and may thus influence the activity of rheumatoid arthritis and perhaps other inflammatory diseases[89]. Another polymorphism in the protein coding domain of *ZFP36* has also been shown to be significantly associated with rheumatoid arthritis in African–Americans[90]. Associations between human disease and mutations of *RC3H1* (encoding roquin 1) and *MCPIP1* have not been the subject of published investigations but certainly warrant future study.

Given their importance in immune regulation and RNA metabolism, CCCH zinc finger proteins are possible targets for the treatment of autoimmunity, viral infections and cancer, although the effects of targeting CCCH zinc finger proteins on an organismal level are currently unknown. Based on their complex function, both increasing and decreasing CCCH zinc finger protein expression and activity could have therapeutic benefits for specific indications. For instance, increasing MCPIP1 expression, by the selective suppression of

degradative enzymatic activity within the cells, such as that exhibited by MALT1 and proteasomes, could potentially improve outcomes in diseases in which excessive inflammatory responses are considered detrimental, such as septic shock, atherosclerosis and certain viral infections. MI-2, a specific inhibitor of MALT1, was recently reported to selectively enhance MCPIP1 expression in CD4^{+} T cells and consequently promote apoptosis of T cell lines that were latently infected with HIV-1 in the presence of cell stimuli *in vitro*[91]. Given that MI-2 is a highly specific and non-toxic small molecular inhibitor of MALT1 (REF. 92), it would be interesting to explore its therapeutic potential for inflammatory diseases and viral infections *in vivo*. By contrast, suppressing MCPIP1 and roquin 1 activity may be a novel strategy for enhancing cancer immunotherapy or responses to vaccines. Recent studies with a mouse model of regulated TTP overexpression demonstrated a protective effect against several models of immune and inflammatory disease, supporting the potential role of TTP as a therapeutic target[93,94].

Conclusions and perspectives

Regulation of RNA metabolism is an important component of gene expression that facilitates the fine-tuning of transcript levels during physiological conditions and during the rapid and profound switch in global gene expression associated with inflammation and immune responses. Long-term dysregulation of RNA metabolism can often result in disease states, including inflammatory and autoimmune diseases[95]. CCCH zinc finger proteins have emerged as important regulators of multiple facets of RNA metabolism and immune responses, with promising therapeutic potential. During the innate immune response, several CCCH zinc finger proteins, such as TTP, roquin 1 and MCPIP1, promote the resolution of inflammation by helping to eliminate the mRNAs of certain pro-inflammatory cytokines. During the activation of B cells and T cells, these CCCH zinc finger proteins intrinsically control the magnitude and duration of adaptive immune responses and maintain immune homeostasis via multiple mechanisms. Failure of these important regulatory controls (for example, in mice that are genetically deficient for TTP, roquin 1 or MCPIP1) often leads to the development of systemic inflammatory responses and autoimmune syndromes. In addition to the regulation of immune responses, other CCCH zinc finger proteins such as ZAP (encoded by *ZC3HAV1*), PARP12 (encoded by *ZC3HDC1*) and TOE1 are important regulators of viral replication (Supplementary information S3 (box)).

In the future, searching for associations between mutations in the genes for CCCH zinc finger proteins and various inflammatory disorders and cancer should help to assess the biological roles of members of this protein superfamily in humans. Furthermore, elucidating how these CCCH zinc finger proteins are regulated and exploiting this knowledge for therapeutic application, by enhancing or repressing their expression and/or function, is a crucial next step in understanding these multifaceted proteins. At a more basic level, it would be extremely interesting to determine how cross-regulation of two different domains occurs in a single protein. For example, does the E3 ubiquitin ligase activity of roquin 1 RING domain influence the RNA decay process mediated by its ROQ domain and CCCH zinc finger? Alternatively, does ROQ and CCCH zinc finger-mediated RNA binding affect its RING domain- mediated ubiquitylation function? Based on structural studies, it seems that the RING domain of roquin 1 can interact with its CCCH zinc finger domain and affect

its RNA-binding activity[74]. A recent study also showed that the N-terminal domain of MCPIP1 was associated with its PIN domain and significantly enhanced its RNase activity[96]. Lastly, as most CCCH zinc finger proteins have not been well-characterized, further studies on the function and mechanisms of these less- characterized proteins will provide valuable information for understanding this fascinating group of proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Glossary

RNA metabolism	Refers to any events in the life cycle of RNA molecules, including their synthesis, folding and unfolding, modification, processing and degradation.
Zinc finger	A finger-shaped fold in a protein that permits it to interact with DNA and RNA. The fold is created by the binding of specific amino acids in the protein to a zinc atom.
AU-rich elements	(AREs). Found in the 3′ untranslated region (3′ UTR) of many mRNAs that encode proto-oncogenes, nuclear transcription factors and cytokines. AREs are one of the most common determinants of RNA stability in mammalian cells.
RING finger domain	RING (really interesting new gene) finger domain is a protein structural domain of zinc finger type that contains a C_3HC_4 amino acid motif and binds two zinc cations. Many proteins containing a RING finger domain have a key role in the ubiquitylation pathway.
Polysomes	Polysomes (or polyribosomes) are a cluster of ribosomes that are attached along the length of a single molecule of mRNA. Polysomes read this mRNA simultaneously, helping to synthesize the same protein at different spots on the mRNA.
Stress granules	Dense aggregations in the cytosol composed of proteins and RNA molecules that appear when the cell is under stress. The RNA molecules stored in these granules are stalled translation pre-initiation complexes.

P-bodies	Cytoplasmic domains that contain proteins involved in diverse post-transcriptional processes, such as mRNA degradation, nonsense- mediated mRNA decay, translational repression and RNA-mediated gene silencing.
MicroRNA	(miRNA). A small, RNA molecule that regulates the expression of genes by binding to the 3′ untranslated region of specific mRNAs.
Roquin 1^{san/san} mice	Mice with a single point mutation (M199R) in the ROQ domain of the gene encoding roquin 1. These mice develop a lupus-like autoimmune phenotype, marked by enhanced numbers of T follicular helper cells and spontaneous germinal centre formation.
MALT1	(Mucosa-associated lymphoid tissue lymphoma translocation protein 1). A protein of the paracaspase family that shows proteolytic activity. Since many of its substrates are involved in the regulation of inflammatory responses, the protease activity of MALT1 has emerged as an interesting therapeutic target.
14-3-3	A family of proteins that functions as adaptor molecules in protein interactions and can regulate protein localization and enzyme activity.

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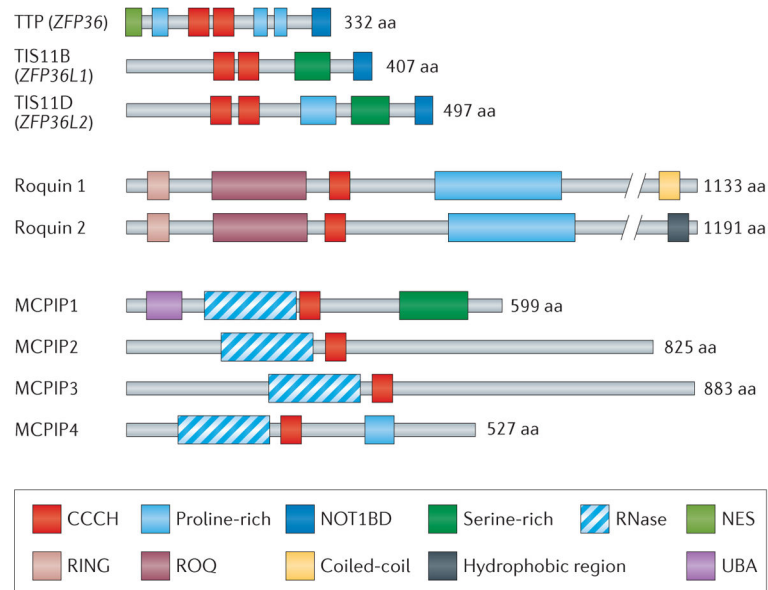
Figure 1. Schematic structures of human tristetraprolin (TTP), roquin and monocyte chemotactic protein-induced protein (MCPIP) protein families

The protein domains are presented as boxes as indicated; NOT1-binding domain (NOT1BD) has been identified by a recent study[105]. NES, nuclear export sequence; RNase, ribonuclease domain; UBA, ubiquitin-associated domain.
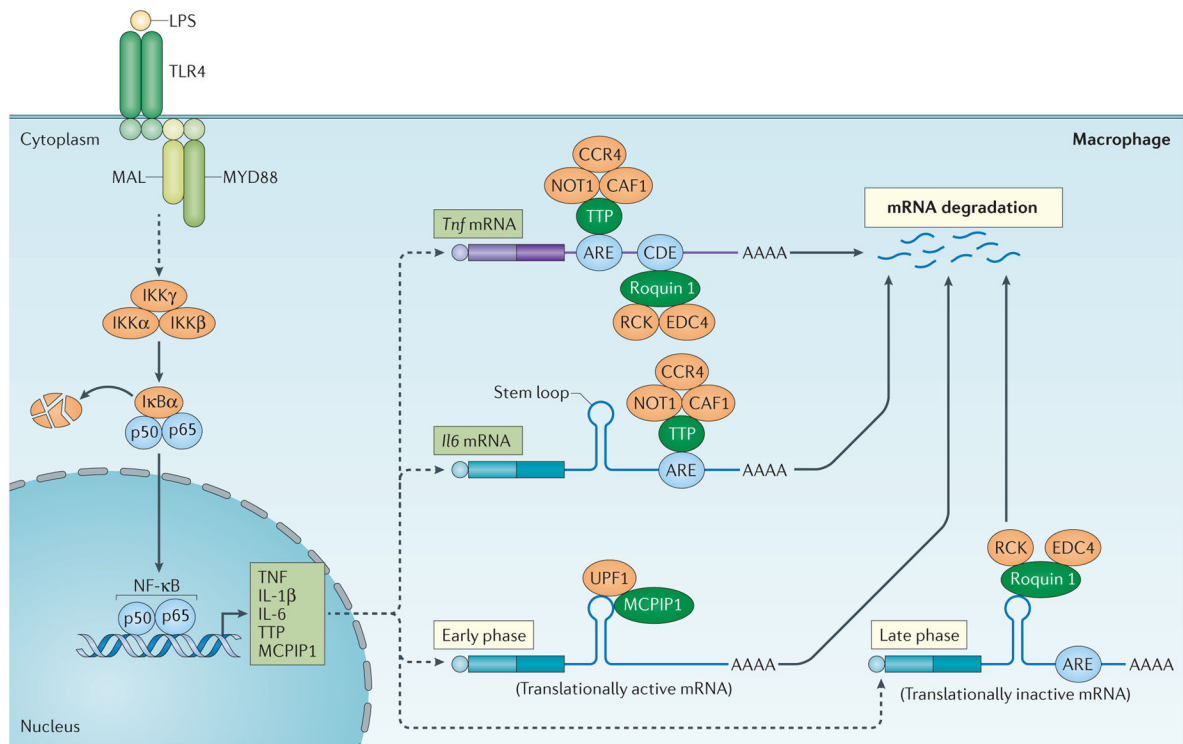
Figure 2. Regulation of cytokine production by CCCH zinc finger proteins

Upon activation of Toll-like receptor (TLR4) by lipopolysaccharide (LPS), signalling results in activation of the inhibitor of nuclear factor-κB (NF-κB) kinase (IKK) complex (that is, IKKγ, IKKα and IKKβ) and the phosphorylation and degradation of IκBα. Upon release, NF-κB translocates into the nucleus and activates the expression of genes encoding tumour necrosis factor (TNF), interleukin-1β (IL-1β) and IL-6, as well as tristetraprolin (TTP) and monocyte chemotactic protein-induced protein 1 (MCPIP1). TTP can bind to the AU-rich elements (AREs) on the 3′ untranslated region (UTR) of *Tnf* and *Il6* mRNAs and promote their decay, at least in part, by recruiting the CCR4–CAF1–NOT1 deadenylase complex. There is a second layer of regulation of *Tnf* mRNAs, involving the binding of roquin 1 to constitutive decay element (CDE), which promotes mRNA degradation through the recruitment of RCK and enhancer of mRNA-decapping protein 4 (EDC4). MCPIP1 and roquin 1 also bind to the stem–loop structures of the conserved elements in the 3′ UTR of *Il6* mRNA and respectively promote its degradation in the early phase of the inflammatory response through the recruitment of UPF1 and in the late phase through the recruitment RCK and EDC4. MAL, MYD88 adaptor-like; MYD88, myeloid differentiation primary response gene 88; Ub, ubiquitylation.
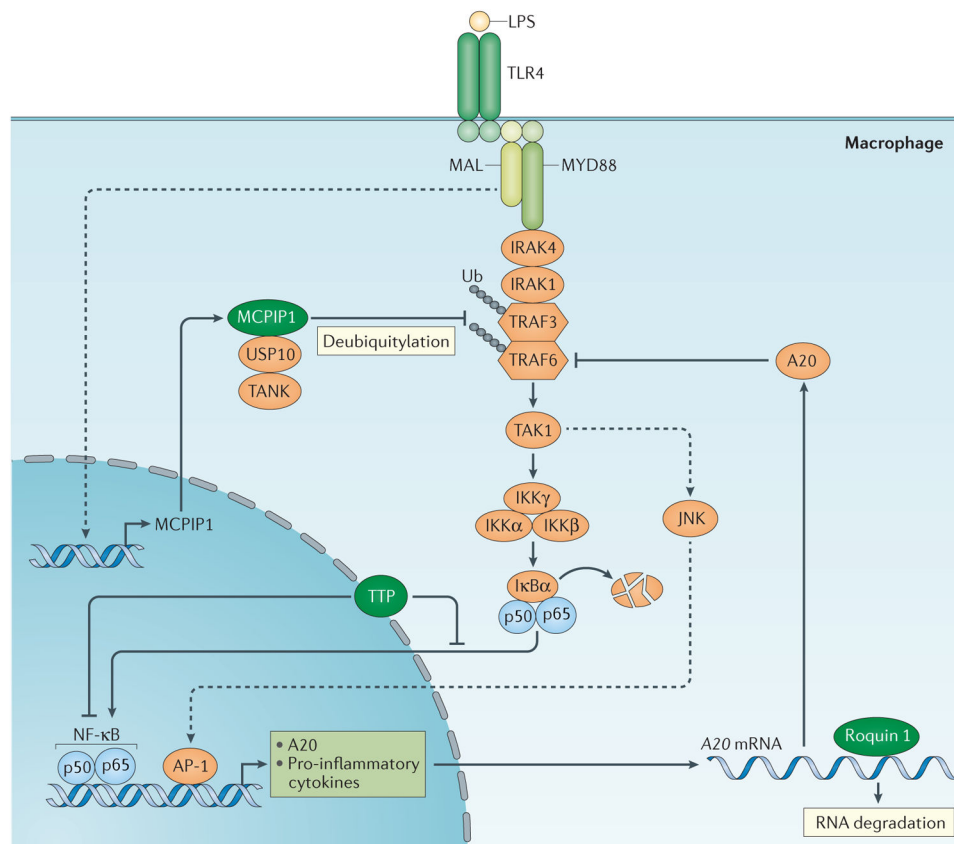
Figure 3. Regulation of macrophage activation by CCCH zinc finger proteins
Macrophages are activated in response to environmental cues, and the activated cell surface receptors (exemplified by Toll-like receptor 4 (TLR4) in the figure) will initiate signalling, which ultimately transduces into the nucleus and activates gene transcription. The newly synthesized mRNAs are exported from the nucleus to the cytoplasm, where they can be translated into proteins or be subject to degradation. Tristetraprolin (TTP) may suppress signal transduction or transcription of pro-inflammatory cytokines by acting as a co-repressor of nuclear factor-κB (NF-κB) or by blocking NF-κB translocation into the nucleus. Monocyte chemotactic protein-induced protein 1 (MCPIP1) can act as an adaptor molecule to recruit the deubiquitinase USP10, which targets TNF-receptor-associated factor 6 (TRAF6) via TANK and promotes the deubiquitylation of TRAF6 to inhibit signal transduction. By contrast, roquin 1 can promote *A20* mRNA degradation, which is a negative regulator of NF-κB signalling, thereby removing a negative feedback loop and facilitating macrophage activation. IKK, inhibitor of NF-κB kinase; IRAK, interleukin-1 receptor-associated kinase; LPS, lipopolysaccharide; MAL, MYD88 adaptor-like; MYD88, myeloid differentiation primary response gene 88.
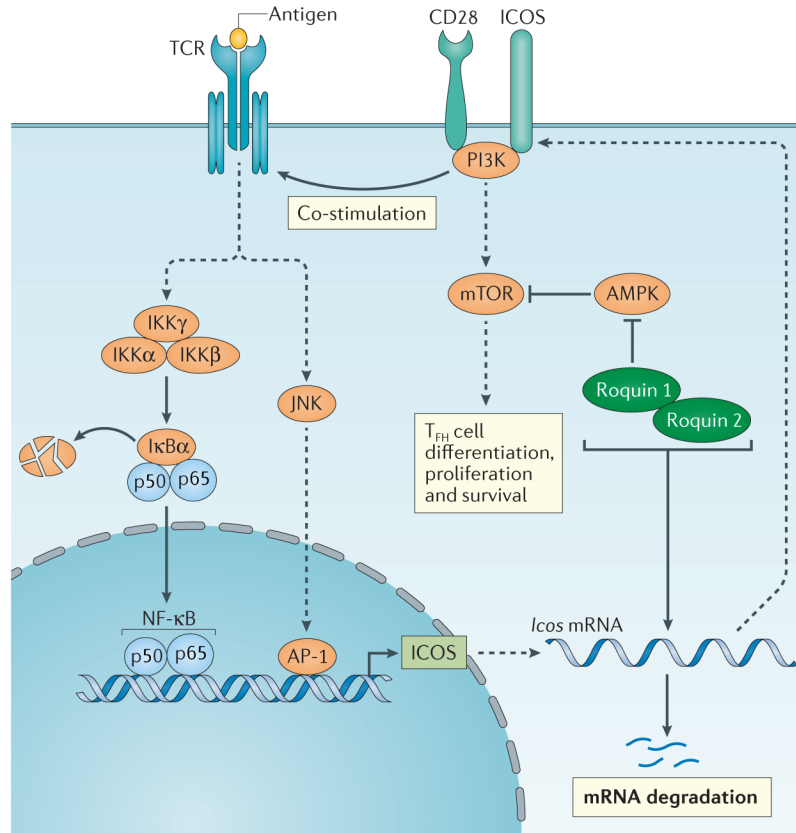
Figure 4. Roquin 1 and roquin 2 regulate T$_{FH}$ cell differentiation

Inducible T cell co stimulator (ICOS) is upregulated by T cells upon activation. ICOS signaling is required for T follicular helper (T$_{FH}$) cell differentiation and proliferation. Roquin 1 and roquin 2 redundantly promote *Icos* mRNA degradation by recruiting the RNA decay proteins enhancer of mRNA-decapping protein 4 (EDC4) and RCK through their ROQ domain and CCCH zinc finger domains (not shown), thereby negatively regulating T$_{FH}$ cell differentiation. However, the RING domain of roquin 1 protein promotes T$_{FH}$ cell differentiation by directly interacting with the catalytic unit of adenosine monophosphate-activated kinase (AMPK) and suppressing AMPK activity, by which it enhances mechanistic target of rapamycin (mTOR) signalling and increases T$_{FH}$ cell differentiation, proliferation and survival. IKK, inhibitor of NF-κB kinase; NF-κB, nuclear factor-κB; PI3K, phosphatidylinositol 3 kinase; TCR, T cell receptor.
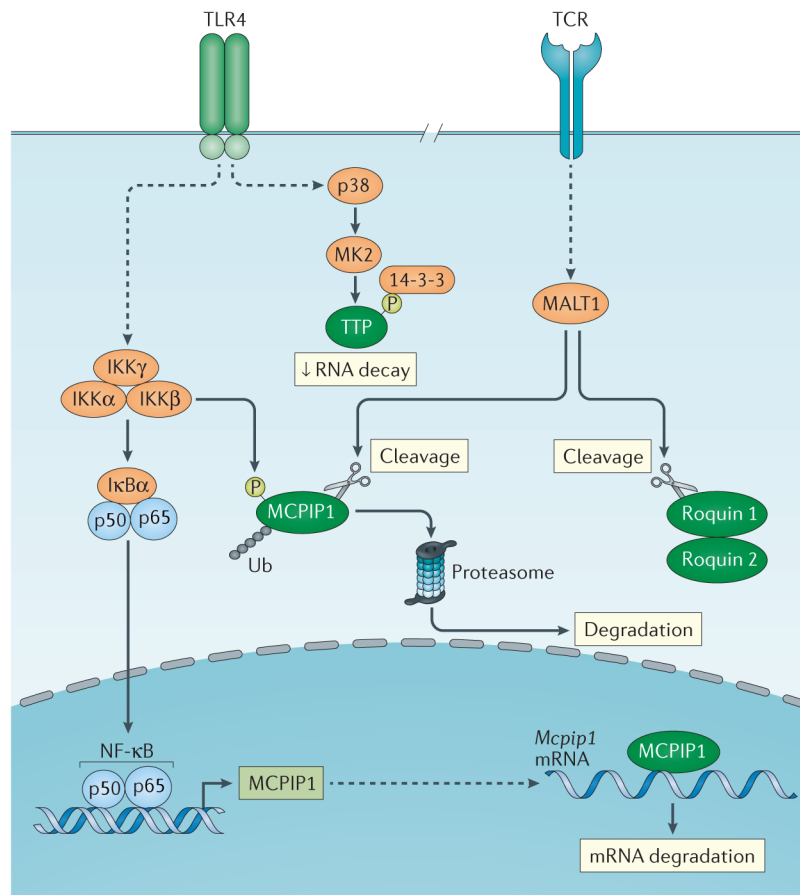
Figure 5. Expression and function of CCCH zinc finger proteins are regulated by multiple mechanisms

Toll-like receptor 4 (TLR4) signalling results in the activation of the inhibitor of nuclear factor-κB (NF-κB) kinase (IKK) complex and NF-κB. Then NF-κB translocates into the nucleus and activates the expression of monocyte chemotactic protein-induced protein 1 (*Mcpip1*) mRNA. MCPIP1 can bind its own mRNA and promote its own mRNA degradation. Moreover, the activated IKK complex can phosphorylate MCPIP1, which is followed by ubiquitylation and degradation by proteasomes. T cell receptor (TCR) activation can activate mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1), a paracaspase. MCPIP1, roquin 1 and roquin 2 can be cleaved by MALT1, and the fragments are rapidly degraded by the proteasome. Tristetraprolin (TTP) can be phosphorylated by p38–MK2 and be inactivated by the recruitment of 14-3-3 protein.

Table 1

Human CCCH zinc finger proteins

Gene name[*]	Other names	CCCH zinc fingers (number of repeats)	Molecular functions	Biological functions
CNOT4	CLONE243; NOT4	$CX_8CX_4CX_3H$ (1)	Deadenylation	Megakaryocyte differentiation
CPSF4	CPSF30; NAR; NEB1	$CX_{7-8}CX_{4-5}CX_3H$ (5)	mRNA splicing	Antivirus
CPSF4L	–	$CX_{7-8}CX_{4-5}CX_3H$ (4)	ND	ND
DHX57	DDX57	$CX_7CX_5CX_3H$ (1)	ND	ND
DUS3L	DUS3	$CX_{10}CX_5CX_3H$ (1)	ND	ND
HELZ	DHRC; HUMORF5	$CX_8CX_5CX_3H$ (1)	RNA helicase	ND
HELZ2	PDIP1; PR1C285	$CX_5CX_5CX_3H$ (1)	RNA helicase	Adipocyte differentiation
LENG9	–	$CX_7CX_5CX_3H$ (1)	ND	ND
MBNL1	EXP; MBNL	$CX_7CX_6CX_3H$ (2)	mRNA splicing	Myoblast differentiation
MBNL2	MBLL; MBLL39	$CX_7CX_{4-6}CX_3H$ (4)	mRNA splicing	Myoblast differentiation
MBNL3	CHCR; MBLX	$CX_7CX_6CX_3H$ (2)	mRNA splicing	Myoblast differentiation
MKRN1	RNF61	$CX_7CX_5CX_3H$ (3)	E3 ligase	Tumorigenesis
MKRN2	RNF62; HSPC070	$CX_{7-9}CX_5CX_3H$ (2)	Putative E3 ligase	Neurogenesis
MKRN3	CPPB2; RNF63; ZNF127	$CX_{7-9}CX_5CX_3H$ (3)	Putative E3 ligase	Central precocious puberty
NUPL2	CG1; NLP1	$CX_7CX_5CX_3H$ (1)	RNA export	ND
PAN3	–	CX_8CX_5CX3H (1)	Deadenylation	ND
PPP1R10	CAT53; FB19; PNUTS	$CX_8CX_5CX_3H$ (1)	Adaptor protein	Inhibition of HIV replication
PRR3	CAT56	$CX_8CX_5CX_3H$ (1)	ND	ND
RC3H1	ROQUIN; ROQUIN 1; RNF198	$CX_8CX_5CX_3H$ (1)	mRNA decay	Immune homeostasis
RC3H2	ROQUIN 2; RNF164; MNAB	$CX_8CX_5CX_3H$ (1)	mRNA decay	Immune homeostasis
RNF113A	CWC24; TTD5; ZNF183	$CX_8CX_5CX_3H$ (1)	ND	ND
RNF113B	RNF161; ZNF183L1	$CX_8CX_5CX_3H$ (1)	ND	ND
TIPARP	PARP7; ARTD14	$CX_7CX_5CX_3H$ (1)	mRNA splicing	Antivirus
TOE1	–	$CX_8CX_5CX_3H$ (1)	Deadenylation	Inhibition of HIV replication
TRMT1	TRM1	$CX_7CX_5CX_3H$ (1)	tRNA methyltransferase	ND
U2AF1	U2AF35; U2AFBP; FP793	$CX_8CX_5CX_3H$ (2)	mRNA splicing	Blood cell differentiation
U2AF1L4	U2AF26; U2AF-RS3	$CX_8CX_5CX_3H$ (2)	mRNA splicing	T cell activation
ZC3HAV1L	C7ORF39	$CX_{7-12}CX_{4-5}CX3H$ (2)	ND	ND
ZC3H1	PARP12; ARTD12; MST109	$CX_{7-12}CX_5CX_3H$ (3)	mRNA translation	Inflammation and antivirus
ZC3H2	ZC3HAV1; ZAP; PARP13	$CX_{7-11}CX_{4-5}CX_3H$ (3)	mRNA decay	Antivirus
ZC3H3	SMICL	$CX_{7-8}CX_{4-6}CX_3H$ (5)	Polyadenylation	ND
ZC3H4	C19ORF1	$CX_{7-8}CX_5CX_3H$ (3)	ND	ND
ZC3H5	UNK; UNKEMPT	$CX_{6-14}CX_{5-6}CX_3H$ (5)	mRNA translation	Neuronal differentiation

Gene name[*]	Other names	CCCH zinc fingers (number of repeats)	Molecular functions	Biological functions
ZC3H5L	UNKL	$CX_{6-14}CX_{5-6}CX_3H$ (5)	ND	ND
ZC3H6	ZC3HDC6	$CX_{7-8}CX_5CX_3H$ (3)	ND	ND
ZC3H7A	HSPC055	$CX_{7-8}CX_5CX_3H$ (3)	ND	ND
ZC3H7B	ROXAN	$CX_{7-8}CX_5CX_3H$ (3)	ND	ND
ZC3H8	FLIZ1	$CX_{7-8}CX_5CX_3H$ (3)	Transcription repressor	Thymocyte homeostasis
ZC3H9	ZGPAT; ZIP; GPATC6	$CX_7CX_5CX_3H$ (1)	Transcription repressor	Tumour suppressor
ZC3H10	ZC3HDC10	$CX_7CX_{4-5}CX_3H$ (3)	ND	Putative tumour suppressor
ZC3H11A	ZC3HDC11A	$CX_{7-8}CX_{4-5}CX_3H$ (3)	mRNA export	ND
ZC3H12A	MCPIP1; REGNASE 1	$CX_5CX_5CX_3H$ (1)	Ribonuclease	Inflammation and immunity
ZC3H12B	MCPIP2	$CX_5CX_5CX_3H$ (1)	Putative ribonuclease	ND
ZC3H12C	MCPIP3	$CX_5CX_5CX_3H$ (1)	Putative ribonuclease	Inflammation
ZC3H12D	MCPIP4; TFL; P34	$CX_5CX_5CX_3H$ (1)	Putative ribonuclease	Inflammation and immunity
ZC3H13	KIAA0853	$CX_8CX_5CX_3H$ (1)	mRNA splicing	Inflammation
ZC3H14	UKP68; MSUT2;SUT2	$CX_5CX_{4-5}CX_3H$ (5)	Polyadenylation	Neuronal differentiation
ZC3H15	LEREPO4; HTO10	$CX_7CX_5CX_3H$ (1)	ND	HIV replication
ZC3H16	RBM22; CWC2; FSAP47	$CX_7CX_5CX_3H$ (1)	mRNA splicing	ND
ZC3H17	RBM26; PPP1RB2; ARRS2	$CX_8CX_5CX_3H$ (1)	ND	ND
ZC3H18	NHN1	$CX_7CX_5CX_3H$ (1)	mRNA export	Inflammation
ZC3H19	ZMAT5; SNRNP20	$CX_8CX_5CX_3H$ (1)	ND	ND
ZC3H20	RBM27; ARSS1; PSC1	$CX_8CX_5CX_3H$ (1)	ND	ND
ZC3H22	ZRSR2; URP; U2AF1RS2	$CX_{7-8}CX_5CX_3H$ (2)	mRNA splicing	Blood cell differentiation
ZFP36	TTP; NUP475; TIS11; G0S24	$CX_8CX_5CX_3H$ (2)	mRNA decay	Inflammation and immunity
ZFP36L1	TIS11B; BRF1; BERG36	$CX_8CX_5CX_3H$ (2)	mRNA decay	Immune cell maturation
ZFP36L2	TIS11D; BRF2; ERF2	$CX_8CX_5CX_3H$ (2)	mRNA decay	Immune cell maturation

C, cysteine; H, histidine; ND, not determined; X, any amino acid.

[*] 57 human CCCH zinc finger proteins are identified based on updated information in GenBank.

Zc3h21 and Zfp36l3 are rodent-specific genes; therefore, the mouse genome encodes 59 CCCH zinc finger proteins. Additional information, such as the full description of gene names and references for biological function, is presented in Supplementary information S1 (table).