**REVIEW**

Roquin—a multifunctional regulator of immune homeostasis

JS Schaefer and JR Klein

Roquin-1 (Rc3h1) is an E3 ubiquitin ligase originally discovered in a mutational screen for genetic factors contributory to systemic lupus erythematosus-like symptoms in mice. A single base-pair mutation in the Rc3h1 gene resulted in the manifestation of autoantibody production and sustained immunological inflammation characterized by excessive T follicular helper cell activation and formation of germinal centers. Subsequent studies have uncovered a multifactorial process by which Roquin-1 contributes to the maintenance of immune homeostasis. Through its interactions with partner proteins, Roquin-1 targets mRNAs for decay with inducible costimulator being a primary target. In this review, we discuss newly discovered functions of Roquin-1 in the immune system and inflammation, and in disease manifestation, and discuss avenues of further research. A model is presented for the role of Roquin in health and disease.

*Genes and Immunity* (2016) 17, 79–84; doi:10.1038/gene.2015.58; published online 17 December 2015

ROQUIN-1 AS AN ATYPICAL E3 UBIQUITIN LIGASE

Structural analysis of Roquin-1 reveals the presence of a RING (really interesting new gene) finger domain, a CCCH-type (C3H1) zinc-finger domain, and a ROQ domain.1–2 The RING domain is a hallmark of the RING finger E3 ubiquitin ligase family, while the C3H1 and ROQ domains are emblematic of RNA-binding proteins. E3 ubiquitin ligases play a critical role in ubiquitination.3 Ubiquitination, or ubiquitylation, is a post-translational modification to regulate protein expression. It involves the concerted efforts of three distinct classes of enzymatic proteins to attach the ubiquitin molecule to a target protein (substrate). Via an ATP-dependent process, the E1 ubiquitin-activating enzyme transfers the activated ubiquitin from the E1 active site cysteine to the E2 active site cysteine. The E2 ubiquitin-conjugating enzyme transfers the activated ubiquitin from the E1 active site cysteine to the E2 active site cysteine. The E3 ubiquitin ligase binds both the E2 enzyme and the target substrate to catalyze the transfer of the activated ubiquitin to the target protein. The ubiquitin cascade may be repeated to poly-ubiquitinate the target or end with a single ubiquitin molecule (mono-ubiquitination). Dependent upon the number of ubiquitins, distinct signals and physiological outcomes are generated. Although poly-ubiquitination generally results in proteosomal-mediated decay of the target protein, both mono- and poly-ubiquitination can modulate other biological processes, including direct receptor signaling and trafficking from the cell surface, and affect protein–protein interactions and alter enzymatic activity.

The vast majority of over 600 identified E3 ligases are of the RING finger family. The homologous to E6-AP carboxyl terminus (HECT) E3 ubiquitin ligase and C-box E3 ligase comprise the other E3 ligase families.3–4 Whereas RING finger E3 ligases directly transfer the ubiquitin molecule from the E2 enzyme to the target substrate, the HECT E3 ligases act as intermediaries temporarily transferring the ubiquitin to the E3 active site before conjugating the ubiquitin to the target substrate. BRCA1, FANC and Mdm2 are three well-known E3 ligases.3–4 Mutations and altered expression of those proteins are associated with breast cancer, Fanconi anemia and colorectal cancer, respectively. The tumor suppressor p53 is a target of Mdm2; amplification of Mdm2 leads to excessive turnover of p53 resulting in cancer.3–4

Despite the presence of the RING finger domain, to date there have been no reports indicating that either the human or mouse Roquin-1 functions as an E3 ligase, nor has a function in the ubiquitination pathway been described yet. In contrast, the Roquin-2 paralog and the RLE-1 (regulation of longevity by E3) *Caenorhabditis elegans* homolog of Roquin-1 are the exceptions as both have been accorded roles and activities in the ubiquitination cascade. Studies in which *rle-1* was deleted in *C. elegans* resulted in an extended life span, reduced offspring and increased resistance to stressors including UV radiation, heat and certain pathogens. Protein levels but not the transcript levels of the DAF-16 transcription factor (TF) were elevated in mutants.5 Subsequent studies in wild-type *C. elegans* revealed that RLE-1 localized with DAF-16, while *in vitro* studies demonstrated that DAF-16 communoprecipitated with RLE-1.1–5 Further, those *in vitro* studies revealed poly-ubiquitination of DAF-16 with elevated RLE-1 expression.5

In a small interfering RNA (siRNA) screen for regulators of the reactive oxygen species response, and the apoptosis signal-regulating kinase 1 (ASK1 or MAP3K5), Roquin-2 was identified as a candidate.5 In response to *H₂O₂* treatment, Roquin-2 coimmunoprecipitated with ASK1 in HeLa-S3 cells.6 Roquin-2-specific siRNA treatment reduced the Roquin-2-ASK1 interaction, prolonged the half-life of ASK1 protein and reduced the ubiquitination of ASK1.5 Interestingly, overexpression of Roquin-2 had the opposite effects, specifically, promoting the ubiquitination and turnover of ASK1.5

In combination, these studies clearly illustrate that both RLE-1 and Roquin-2 have E3 ligase activity. Whether or not the mouse and human Roquin-1 demonstrate a similar activity remains to be seen. And, it raises the question as to why this function has not been conserved evolutionarily.
ROQUIN-1 RNA TARGETS

There is ample evidence that Roquin-1 functions as an RNA-binding protein to regulate gene expression post-translationally. In the seminal papers, Roquin-1 was shown to limit the expression of inducible costimulator (ICOS). Subsequent analyses confirmed that Roquin-1 specifically recognized and bound to the ICOS 3'-UTR to regulate its expression. Although this was initially thought to involve miR-101 and the RNA-induced silencing complex (RISC) as ancillary control factors, later experiments with Dicer-deficient mouse embryonic fibroblasts ruled out the microRNA (miRNA) involvement.

Recent studies have focused on identifying additional RNA targets of Roquin-1 regulation as well as the mechanism by which Roquin-1 recognizes its targets. By using Icos as the standard, a conserved cis-regulatory element (CRE) was identified in the 3'-UTR as the recognition element for Roquin-1. This RNA secondary structure, alternatively identified as a constitutive decay element (CDE) or a stem-loop structure (SL), is AU-enriched with the consensus sequence being 5’-NNNNNUUCYRYGAANNNNN-3’. Although RNA immunoprecipitations (IPs), including HITS-CLIP (high-throughput sequencing of RNA isolated by crosslinking IP) and PAR-CLIP (photocleavable-ribonucleoside-enhanced cross-linking and IP) have identified ~3800 target mRNAs for Roquin-1, only A20, TNFA, OX40, NFKBID and NFKBIZ have subsequently been validated as Roquin-1 targets (Table 1).

Deletion studies identified the ROQ domain as both the structure critical for RNA binding and localization to stress granules. Crystallography of the ROQ domain in contact with the tumor necrosis factor-α (TNFa) CRE revealed three subdomains within the ROQ domain. Two of these subdomains were confirmed to be RNA-binding sites for stem-loop RNA and dsRNA, respectively, as determined by mutagenesis analysis. The ROQ domain is the site of the point mutation in sanroque mice. Crystallography analysis revealed the presence of a novel RNA interaction domain and the HEPN (higher eukaryotes and prokaryotes nucleotide-binding) domain, and further defined the structural consequences of the M199R mutation in which a previously buried hydrophobic residue (F234) is turned out and exposed.

Interestingly, regnase-1 (Reg1), a ribonuclease with a C3H zinc-finger domain, has recently been proposed to work in conjunction with Roquin-1 and Roquin-2 to regulate the inflammatory response. Similar to Roquin-1, Reg1 recognizes a common SL structure in the 3'-UTR of mRNAs to promote RNA turnover. Whereas Roquin-1 localizes to stress granules and processing (P) bodies, Reg1 localizes to ribosomes and the endoplasmic reticulum, thereby providing a spatiotemporally distinct manner of operation for Reg1. However, RNA-IP sequencing analysis identified seven target mRNAs that overlapped Roquin-1 and Reg1, including ICOS, OX40 and TNFA. The consensus recognition sequence for Reg1, 5'-UUGGAAGYRCUULUCCAA-3', is similar to that for Roquin-1, which may account for the overlap in mRNA targets. This again illustrates how Roquin-1 interacts with multiple players to adjust the expression of genes in an inflammatory response.

| Table 1. Targets of Roquin-1 |
|-----------------------------|
| **Gene** | **Citation** |
| Icos | Yu et al. |
| TNFA | Lepek et al. |
| OX40 | Vogel et al. and Schaefer et al. |
| A20 | Murakawa et al. |
| NFKBID | Lepek et al. |
| NFKBIZ | Lepek et al. |

ROQUIN-1 AND miRNAs

miRNAs are small non-coding RNAs that post-transcriptionally regulate gene expression. Given their regulatory roles, it is not surprising that studies aimed at understanding the relationship between Roquin-1 and miRNAs have been conducted. A recent study examined the effects of the sanroque mutation on miRNA expression. miR-146a-5p (24.9-fold) and miR-21 (13.9-fold) showed the greatest elevation in expression among 15 miRNAs that had more than twofold increases in expression in Roquin+/+ T cells compared to Roquin−/− T cells. Interestingly, no miRNAs were found to have decreased expression. RNA stability assays demonstrated that Roquin-1 controlled miR-146a stability. miR-146a had a longer half-life in Roquin+/+ T cells in comparison to Roquin−/− T cells. Further, Roquin-1 bound both miR-146a and the RISC subunit Ago2 (argonaute RISC catalytic component 2). The binding to Ago2 suggests that this may be a common mechanism used to regulate other miRNAs.

Elevated expression of both miR-146a-5p and miR-21 has been associated with inflammatory disease. miR-146a has been shown to be highly expressed in T follicular helper (Tfh) cells and regulate ICOS expression. Another miRNA of note that had elevated expression in these studies was miR-223 (3.4-fold), a validated regulator of Roquin-1 expression and has been demonstrated to have elevated expression in patients with inflammatory bowel disease and the interleukin-10 knockout (IL-10−/−) mouse model of chronic intestinal inflammation. Those studies indicate that, in addition to target mRNAs, Roquin-1 regulates miRNA stability, including several miRNAs that regulate its own expression.

REGULATION OF ROQUIN PROTEINS

A recent study investigating the paracaspase MALT1 in immune function has shed some light on the regulatory control of the Roquin-1 protein. It began with the initial observation that when CD4+ T cells were stimulated with phorbol myristate acetate and ionomycin, Roquin-1 and Roquin-2 protein levels decreased over the 3-h time course. Previous studies established Reg1 as a substrate for MALT1-mediated proteolytic cleavage to regulate gene expression. On the basis of those studies, Jeltsch et al. investigated the ability of MALT1 to cleave the Roquin proteins. When phorbol myristate acetate/ionomycin stimulation of CD4+ T cells was repeated in the presence of pharmacological inhibitors of MALT1 activity (z-VRPR-fmk, mepazine and thioridazine), cleavage of the Roquin proteins was reduced. A subsequent series of genetic and molecular studies confirmed that MALT1 was able to cleave Roquin-1 and Roquin-2 as well. Those studies further reinforce the importance of Roquin-1 in Th17-cell differentiation and function, and IL-17 expression, thus adding an additional post-transcriptional method for regulating Roquin-1 expression.

ROQUIN INVOLVEMENT IN INFLAMMATION AND AUTOIMMUNITY

The Roquin-1 gene mutation was identified in C57BL/6 mice treated with N-ethyl-N-nitrosourea, a mutagen that induces single base-pair alterations in spermatogonial cells at a rate of ~1 per 0.5 megabases. Animals were bred and screened for systemic lupus erythematosus based on the presence of antinuclear antibodies as a predictive biomarker. The mutation was linked to the Rc3h1 (Roquin-1) gene, a gene of previous unknown function. Animals homozygous for the mutation, referred to as sanroque or Roquin+/+, were found to have extensive immunological alterations that included antibodies to double-stranded DNA, IgG immune complexes, anemia, glomerulonephritis and necrotizing hepatitis, splenomegaly and lymphadenopathy and autoimmune
thrombocytopenia and plasmacytosis. CD44\(^{7,8}\), CD62L\(^{9}\), CD4\(^{+}\) and CD8\(^{+}\) T cells were present, and animals had high-level ICOS expression\(^{2}\) in both male and female animals that was evident as early as 6–8 weeks of age. Sanroque mice have dysregulated IL-5 and IFN-\(\gamma\) synthesis.\(^{2}\)

It is notable that despite the extreme immunological perturbations in sanroque mice, they show minimal if any changes in the Th1 or Th2 responses to foreign antigen compared to wild-type animals. T-cell tolerance is normal and deletion of intrathymic CD4\(^{+}\) T cells and self-reactive T cells is unaffected. Activation-induced cell death and the proportional numbers of CD4\(^{+}\) CD25\(^{+}\) Tregs remain unaltered in sanroque mice.\(^{2}\) Those changes are T-cell autonomous as seen from studies of mixed bone marrow chimeras, and in radiation chimaeras using bone marrow from Roquin-deficient mice.\(^{33}\)

A prominent immunological feature of the sanroque phenotype is lymph node germinal center disorganization resulting in large numbers of Th17 cells. In addition, sanroque mice have elevated levels of several molecules involved in immune activation or regulation. These include ICOS, regulated upon activation, normal T-cell expressed (RANTES, CCL5), CXCR5 and the programmed cell death 1 (PCD1) molecules, among others.\(^{2,3,4,5}\) Mesenteric lymph node (MLN) T cells in sanroque mice have increased ICOS and OX40 expression on CD4\(^{+}\) T cells.\(^{36}\) CD4\(^{+}\) CD62L\(^{−}\) cells have increased expression of KLRG1,\(^{36}\) a population of short-lived effector cells.\(^{37}\)

Both CD28 and ICOS can function as costimulatory signals during T-cell activation. CD28 is constitutively expressed on most T cells, whereas ICOS is upregulated during activation on effector and memory T cells,\(^{38,39}\) and is present on peripheral Tregs.\(^{39,40}\) The regulation of ICOS by the action of Roquin-1 occurs by binding to the ICOS promoter.\(^{41}\) Sanroque mice had increased ICOS levels in comparison to wild-type mice,\(^{11}\) thus demonstrating targeted action of Roquin-1 on ICOS.

The finding of small intestinal inflammation in sanroque mice\(^{36}\) is particularly interesting given that, whereas there are numerous models of inflammatory bowel disease for the large intestine inflammation,\(^{41–44}\) few models have been identified for small intestinal inflammatory bowel disease. One such model is the SAMPL1/YirFc mouse, in which most animals develop ileitis by 20–30 weeks of age. In sanroque mice, the T-cell profile in the mucosal epithelium and lamina propria differs from that of the MNLNs in that ICOS\(^{+}\) cells are present in the epithelium but not the lamina propria, and because OX40 is not appreciably expressed on T cells in either mucosal site whereas it is present on MLN T cells.\(^{36}\) Moreover, the extensive chemokine dysregulation consisting of elevated levels of CCL1, CCL24, CCL25, CCL20, CXCL1 and IL-13 would favor chronic inflammation in the small intestine of sanroque mice.\(^{36}\)

**ROQUIN AND IMMUNE-BASED DISORDERS**

Two divergent sets of findings have been reported with regard to the role of Roquin-1 in angioimmunoblastic T-cell lymphoma (AITL). Roquin\(^{\text{aun}}\)/Roquin\(^{\text{aun}}\) heterozygous mice were reported to develop AITL-like disease at ~4 months of age in ~50% of the animals.\(^{45}\) Animals had lymph node tumors (enlarged lymph nodes) with characteristic histopathologic and cellular features of AITL in humans.\(^{45}\) In a study of 12 human AITL lymph node biopsies, although expression of CXCL13, ICOS and PD1 was not determined by histocytotoxicity, Roquin-1 and mid101 gene expression remained unaltered compared to tissues from control patients.\(^{46}\) Thus, whereas Roquin\(^{\text{aun}}\)/Roquin\(^{\text{aun}}\) heterozygous mice display some of the phenotypic and pathologic features of AITL, this may reflect a concomitant rather than a direct effect of partial Roquin-1 impairment.

Although much information has been gleaned from studies using Roquin-1 mutant sanroque mice, findings using T-cell lines or transgenic (Tg) mice with Roquin-1 overexpression have been equally illuminating. Stimulation of EL4 T-cells via CD3/CD28 in cells that overexpressed Roquin-1 resulted in lower ICOS expression as expected, but elevated CD28 expression with increased IL-2 and TNFα synthesis.\(^{47}\) Elevated levels of IL-2 are consistent with the role of CD28 as a primary IL-2 signal through Grb2.\(^{48,49}\) Inhibition of AKT and JNK signaling suppressed CD3/CD28 increases caused by Roquin-1 overexpression.\(^{47}\) Consistent with those findings, Tg mice that overexpressed Roquin-1 had suppressed ICOS and elevated CD28 T-cell expression, and had more IL-2 and TNFα synthesis following CD3/CD28 stimulation.\(^{37}\)

In a model of collagen-induced arthritis, Roquin-1 Tg mice mounted stronger collagen-induced arthritis responses and had suppressed levels of ICOS, elevated CD28, and an imbalance in Th1/Th2 that favored Th1 cells.\(^{50}\) By day 45 post collagen-induced arthritis induction, levels of IFN-\(\gamma\), TNFα, IL-6 and IL-17 were elevated in Tg mice.\(^{50}\) Th1-cell numbers and germinal center B cells were unaffected in Roquin-1 Tg mice. Selective overexpression of Roquin-1 in T cells using a T-cell-specific promoter also led to more severe T-cell-mediated hepatitis, increased numbers of Th17 cells and production of proinflammatory cytokines.\(^{51}\) Taken together, those findings are at odds with the notion that the primary function of Roquin-1 is to limit and modulate the immune response. Rather, Roquin-1 may have a dual function as both an activator and suppressor of immunity, as discussed below.

**SANROQUE MICE VS ROQUIN-DEFICIENT MICE**

One of the most enigmatic findings involving Roquin comes from studies in which the Roquin-1 gene has been disrupted leading to complete Roquin-1 protein ablation. Roquin-1 knockout mice are born with a caudal spine defect and impaired lung development, resulting in poor perinatal survival,\(^{52}\) thus implying a role for Roquin-1 in embryonic development that extends beyond the immune system. R3h1\(^{−/−}\) mice had higher levels of ICOS expression on CD4\(^{+}\) and CD8\(^{+}\) T cells, and an increase in overall numbers of CD8\(^{+}\) T cells bearing an effector-like phenotype.\(^{52}\) Numbers of eosinophils and cells of the monocyte/macrophage lineages also were elevated. Notably, splenic germinal centers were otherwise unaffected in R3h1\(^{−/−}\) mice.\(^{52}\)

In mice in which Roquin-1 ablation targeted the entire hematopoietic system or B cells, immunological changes were similar to what was seen in T-cell-targeted mice. Animals did not develop autoantibodies and lacked autoimmunity in the kidney, liver and lung.\(^{52}\) Those findings indicate that Roquin-1 ablation in and of itself is not sufficient to cause a breach in self-tolerance or promote autoimmunity. R3h2\(^{−/−}\) mice displayed the same perinatal lethality as R3h1\(^{−/−}\) mice.\(^{52}\) ICOS expression was elevated in R3h2\(^{−/−}\) mice.\(^{52}\) Roquin-2 has been shown to regulate inflammatory signals mediated by ASK1.\(^{52}\)

Several interpretations can be drawn from those studies. Because the Roquin-1 protein in sanroque mice is present but mutated, consisting of a M119R substitution, whereas the protein is fully absent in R3h1\(^{−/−}\) mice, it may be inferred that the mutated version of the protein is more detrimental in the context of immunobiological changes than a complete lack of the protein.\(^{52}\) The basis for that remains unclear; however, it has been demonstrated that the mutated form of Roquin-1 binds more than threefold tighter to Icos mRNA than WT Roquin-1,\(^{13}\) thus perhaps causing long-lasting disturbances within the immune system. Additional studies will be needed to address this.

The possibility that the Roquin-1 paralog, R3h2, might compensate for the lack of autoimmunity in R3h1\(^{−/−}\) has been
addressed in mice carrying deletions in both Rc3h1 and Rc3h2. Ablation of Rc3h1 and Rc3h2 resulted in an immunophysiologic phenotype that mimicked that of sanroque mice and, unlike individual knockout animals, double knockouts had increased numbers of Tfh cells.\textsuperscript{1,3,6} At odds with those findings are studies in which Rc3h1\textsuperscript{−/−} mice were generated by insertion of a gene-trap into intron 1 (Rc3h1\textsuperscript{gt/gt} mice), which resulted in a phenotype more typical of that seen in sanroque animals.\textsuperscript{3,6} Although Rc3h1\textsuperscript{gt/gt} mice had caudal spine deformity and poor post-birth survival\textsuperscript{3,6} similar to that of Rc3h1\textsuperscript{−/−} and Rc3h2\textsuperscript{−/−} mice,\textsuperscript{1,3,6} Rc3h1\textsuperscript{gt/gt} mice developed extensive inflammation in the small intestine, though not in the colon, and had a destructive inflammatory response in the kidney, lungs' liver and spleen\textsuperscript{33} that was reminiscent of sanroque mice. That phenotype was similarly retained in chimeric mice made from the bone marrow of Rc3h1\textsuperscript{gt/gt} mice injected into irradiated syngeneic animals,\textsuperscript{33} confirming that hematopoietic and not systemic disruption of Roquin-1 expression was the primary factor responsible for the development of immunopathology. The basis for the differences in the immunological findings of Rc3h1\textsuperscript{−/−}, Rc3h2\textsuperscript{−/−} and Rc3h1\textsuperscript{gt/gt} mice is unclear; however, it could reflect variations in the methods used to render mice Roquin deficient. Yet, this seems unlikely given that in all the three animal systems, mice were devoid of Roquin gene and protein expression.

REGULATION OF ROQUIN-1 GENE EXPRESSION
The factors that regulate Roquin-1 gene expression are only now beginning to come to light. Colonic tissues of Il10\textsuperscript{−/−} mice, an animal model extensively used in studies of human inflammatory bowel disease, have decreased Roquin-1 gene expression and elevated ICOS and IL-17 gene expression.\textsuperscript{25} Those patterns were reversed, however, following in vitro treatment of colonic intraepithelial lymphocytes with exogenous IL-10, suggesting that IL-10 functions in some manner to regulate Roquin-1 expression in intraepithelial lymphocytes with exogenous IL-10, suggesting that IL-10 may directly modulate Roquin-1 expression. Of interest, treatment of EL4 T cells with IL-10 resulted in increased gene expression of all the negative regulatory elements during the generation of a homeostatic immune response, as well as during chronic inflammation or autoimmunity. By using MLN T cells, we observed that stimulation through the TCR/CD3 complex or following exposure to a Th17-inducing cocktail, Rc3h1 expression was suppressed compared to control Ig stimulation (Figure 1). In extending those findings, lower levels of Roquin-1 during a primary immune response would predictably lead to elevated ICOS and OX40 levels,\textsuperscript{1,2,3,6} thus promoting strong T-cell activation. The benefit to the host would be a robust immune response in dealing with infection and foreign antigen challenge. A potent Roquin-1 response at this stage, however, could have negative consequences by holding ICOS expression down, as seen in studies in Roquin-1 Tg mice, although that response could be partially compensated for by elevated IL-2, IFN-γ, TNFα, IL-6 and IL-17 synthesis.\textsuperscript{37,54} Interestingly, a unique population of CD4\textsuperscript{+} CD25\textsuperscript{+} FoxP3\textsuperscript{−} ICOS\textsuperscript{+} regulatory T cells has been identified,\textsuperscript{40} demonstrating that immunological significance of ICOS regulation by Roquin-1 may be more complex than previously appreciated.

The natural immune response to antigen under homeostatic conditions proceeds systematically through expansion and contraction phases. The patterns just described would largely define the role for Roquin-1 during the initial expansion phase. Although beneficial overall, failure to shut down the response would have significant deleterious consequences by preventing movement into the contraction phase, potentially leading to chronic inflammation or autoimmunity. Thus, upregulation of Roquin-1 expression would serve to suppress the expression of ICOS, RANTES, CXCR5 and CCL5 on peripheral T cells, thereby limiting effector cell activity.

Additional studies will be needed to understand how the cells of the immune system lose and gain Roquin expression. Clearly, Roquin-1 gene regulation through TFs such as STAT1, STAT3, GATA2, c-Rel and IKZF2, as discussed above, along with production of immune-modulating cytokines, would likely come into play. IL-10, in particular, may be a key regulatory factor in this pathway by independently curtailing the expression of TNFα, IFN-γ and IL-17.\textsuperscript{57} Second, however, IL-10 may directly modulate Roquin-1 gene expression by increasing the expression of Roquin-1 TFs.\textsuperscript{54} The net effect of IL-10-mediated Roquin-1 modulation would be to further limit ICOS and OX40 expression and influence Th17-cell regulation. The latter is supported by studies demonstrating that T cells deficient in IL-10 signaling are more likely to have increases in Tfh cells.\textsuperscript{58} A model of how Roquin-1 expression contributes to the immune response is shown in Figure 2.
In summary, Roquin poses to be an interesting molecular switch used in the regulation of the immune response. Although in some areas, differences exist in findings regarding the role of Roquin in immunity, these will likely be resolved as work continues into this exciting aspect of immunobiology. This work was supported by NIH grant DK035566 and a grant from the Crohn’s and Colitis Foundation of America.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES

1. Yu D, Tan AH, Hu X, Athanasopoulos V, Simpson N, Silva DG et al. Roquin represses autoreactivity by limiting inducible T-cell co-stimulator messenger RNA. Nature 2007; 450: 299–303.
2. Vinuesa CG, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM et al. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature 2005; 435: 452–458.
3. Metzger MB, Pruneda JN, Klevit RE, Weissman AM. RING-type E3 ligases: master regulators of the immune response. Although in some findings regarding the role of Roquin in immunity, these will likely be resolved as work continues into this exciting aspect of immunobiology. This work was supported by NIH grant DK035566 and a grant from the Crohn’s and Colitis Foundation of America.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES

1. Yu D, Tan AH, Hu X, Athanasopoulos V, Simpson N, Silva DG et al. Roquin represses autoreactivity by limiting inducible T-cell co-stimulator messenger RNA. Nature 2007; 450: 299–303.
2. Vinuesa CG, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM et al. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature 2005; 435: 452–458.
3. Metzger MB, Pruneda JN, Klevit RE, Weissman AM. RING-type E3 ligases: master regulators of the immune response. Although in some findings regarding the role of Roquin in immunity, these will likely be resolved as work continues into this exciting aspect of immunobiology. This work was supported by NIH grant DK035566 and a grant from the Crohn’s and Colitis Foundation of America.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES

1. Yu D, Tan AH, Hu X, Athanasopoulos V, Simpson N, Silva DG et al. Roquin represses autoreactivity by limiting inducible T-cell co-stimulator messenger RNA. Nature 2007; 450: 299–303.
2. Vinuesa CG, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM et al. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature 2005; 435: 452–458.
3. Metzger MB, Pruneda JN, Klevit RE, Weissman AM. RING-type E3 ligases: master regulators of the immune response. Although in some findings regarding the role of Roquin in immunity, these will likely be resolved as work continues into this exciting aspect of immunobiology. This work was supported by NIH grant DK035566 and a grant from the Crohn’s and Colitis Foundation of America.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES

1. Yu D, Tan AH, Hu X, Athanasopoulos V, Simpson N, Silva DG et al. Roquin represses autoreactivity by limiting inducible T-cell co-stimulator messenger RNA. Nature 2007; 450: 299–303.
2. Vinuesa CG, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM et al. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature 2005; 435: 452–458.
3. Metzger MB, Pruneda JN, Klevit RE, Weissman AM. RING-type E3 ligases: master regulators of the immune response. Although in some findings regarding the role of Roquin in immunity, these will likely be resolved as work continues into this exciting aspect of immunobiology. This work was supported by NIH grant DK035566 and a grant from the Crohn’s and Colitis Foundation of America.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES

1. Yu D, Tan AH, Hu X, Athanasopoulos V, Simpson N, Silva DG et al. Roquin represses autoreactivity by limiting inducible T-cell co-stimulator messenger RNA. Nature 2007; 450: 299–303.
2. Vinuesa CG, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM et al. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature 2005; 435: 452–458.
3. Metzger MB, Pruneda JN, Klevit RE, Weissman AM. RING-type E3 ligases: master regulators of the immune response. Although in some findings regarding the role of Roquin in immunity, these will likely be resolved as work continues into this exciting aspect of immunobiology. This work was supported by NIH grant DK035566 and a grant from the Crohn’s and Colitis Foundation of America.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES

1. Yu D, Tan AH, Hu X, Athanasopoulos V, Simpson N, Silva DG et al. Roquin represses autoreactivity by limiting inducible T-cell co-stimulator messenger RNA. Nature 2007; 450: 299–303.
2. Vinuesa CG, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM et al. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature 2005; 435: 452–458.
3. Metzger MB, Pruneda JN, Klevit RE, Weissman AM. RING-type E3 ligases: master regulators of the immune response. Although in some findings regarding the role of Roquin in immunity, these will likely be resolved as work continues into this exciting aspect of immunobiology. This work was supported by NIH grant DK035566 and a grant from the Crohn’s and Colitis Foundation of America.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES

1. Yu D, Tan AH, Hu X, Athanasopoulos V, Simpson N, Silva DG et al. Roquin represses autoreactivity by limiting inducible T-cell co-stimulator messenger RNA. Nature 2007; 450: 299–303.
2. Vinuesa CG, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM et al. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature 2005; 435: 452–458.
3. Metzger MB, Pruneda JN, Klevit RE, Weissman AM. RING-type E3 ligases: master regulators of the immune response. Although in some findings regarding the role of Roquin in immunity, these will likely be resolved as work continues into this exciting aspect of immunobiology. This work was supported by NIH grant DK035566 and a grant from the Crohn’s and Colitis Foundation of America.

CONFLICT OF INTEREST
The authors declare no conflict of interest.
31 Uehata T, Iwasaki H, Vandenbon A, Matsushita K, Hernandez-Cuelar E, Kuniyoshi K et al. Malt1-induced cleavage of regnase-1 in CD4(+) helper T cells regulates immune activation. Cell 2013; 153: 1036–1049.
32 Vinuesa CG, Goodnow CC. Illuminating autoimmune regulators through controlled variation of the mouse genome sequence. Immunity 2004; 20: 669–679.
33 Montufar-Solis D, Vigneswaran N, Nakra N, Schaefer JS, Klein JR. Hematopoietic not systemic impairment of Roquin expression accounts for intestinal inflammation in Roquin-deficient mice. Sci Rep 2014; 4: 4920.
34 Linterman MA, Rigby RJ, Wong R, Silva D, Withers D, Anderson G et al. Inducible costimulator (ICOS) is a marker for highly suppressive antigen-specific T follicular helper cells and systemic inflammation. J Immunol 2013; 187: 3845–3855.
35 Hondowicz BD, Batheja AO, Metzgar MH, Caton AJ, Erikson J. ICOS expression by effector T cells influences the ability of regulatory T cells to inhibit anti-chromatin B cell responses in recipient mice. J Autoimmun 2010; 34: 460–468.
36 Schaefer JS, Montufar-Solis D, Nakra N, Vigneswaran N, Klein JR. Small intestine inflammation in Roquin-mutant and Roquin-deficient mice. PLoS One 2013; 8: e56436.
37 Joshi NS, Cui W, Chandel A, Lee HK, Urso DR, Hagman J et al. Induction, binding specificity and function of human ICOS. Eur J Immunol 2000; 30: 3707–3717.
38 Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A et al. B7/CD28 costimulation is essential for the homeostasis of the CD4(+)CD25(+) immunoregulatory T cells that control autoimmune diabetes. Immunity 2000; 12: 431–440.
39 Vocanson M, Rozieres A, Hennino A, Poyet G, Gaillard V, Renaudineau S et al. Inducible costimulator (ICOS) is a marker for highly suppressive antigen-specific T cells sharing features of TH17/TH1 and regulatory T cells. J Allergy Clin Immunol 2010; 126: 280–289.
40 Sadlack B, Merz H, Schorle H, Schimpl A, Feller AC, Horak I. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. Cell 1993; 75: 253–261.
41 Kuhn R, Kohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993; 75: 263–274.
42 Claesson MH, Rudolph A, Kofoed S, Poulsen SS, Reimann J. CD4(+) T lymphocytes injected into severe combined immunodeficient (SCID) mice lead to an inflammatory and lethal bowel disease. Clin Exp Immunol 1996; 104: 491–500.
43 Powrie F, Leach MW, Mausze S, Caddie LB, Coffman RL. Phenotypically distinct subsets of CD4(+) T cells induce or protect from chronic intestinal inflammation in B 17 scid mice. Int Immunol 1993; 5: 1461–1471.
44 Ellyard JL, Chia T, Rodriguez-Pinilla SM, Martin JL, Hu X, Navarro-Gonzalez M et al. Heterozygosity for Roquin2 leads to angioimmunoblastic T-cell lymphoma-like tumors in mice. Blood 2012; 120: 812–821.
45 Auguste T, Travers M, Tarte K, Arme-Thomas P, Arichtounin C, Martin-Garcia N et al. ROQUN/RCH1 alterations are not found in angioimmunoblastic T cell lymphoma. PLoS One 2013; 8: e64536.
46 Kim HJ, Ji YR, Kim MO, Yu DH, Shin MJ, Yuh HS et al. The role of Roquin overexpression in the modulation of signaling during in vitro and ex vivo T-cell activation. Biochim Biophys Acta 2012; 1824: 280–286.
47 Watanabe R, Harada Y, Takeda K, Takahashi J, Ohnuki K, Ogawa S et al. Grb2 and Gads exhibit different interactions with CD28 and play distinct roles in CD28-mediated costimulation. J Immunol 2006; 177:1085–1091.
48 Ji YR, Kim HJ, Yu DH, Bae KB, Park SJ, Yi JK et al. Enforced expression of roquin protein in T cells exacerbates the incidence and severity of experimental arthritis. J Biol Chem 2012; 287: 42269–42277.
49 Ji YR, Kim HJ, Yu DM, Bae KB, Park SJ. Over-expression of Roquin aggravates T cell mediated hepatitis in transgenic mice using T cell specific promoter. Biochem Biophys Acta 2014; 452: 822–827.
50 Bertossi A, Aichinger M, Sansonetti P, Lech M, Neff F, Pal M et al. Loss of Roquin induces early death and immune deregulation but not autoimmunity. J Exp Med 2011; 208: 1749–1756.
51 Pratama A, Ramiscal RR, Silva DG, Dias SK, Athanasopoulos V, Fitch J et al. Roquin-2 shares functions with its paralog Roquin-1 in the repression of mRNAs controlling T follicular helper cells and systemic inflammation. Immunity 2013; 38: 669–680.
52 Schaefer JS, Montufar-Solis D, Klein JR. A role for IL-10 in the transcriptional regulation of Roquin-1. Gene 2014; 549: 134–140.
53 Shen X, Hong F, Nguyen VA, Gao B. IL-10 attenuates IFN-alpha-activated STAT1 in monocytic cells. J Biol Chem 2012; 287: 14969–14977.
54 Kim HJ, Barnitz RA, Kreslavsky T, Brown FD, Moffett H, Lemieux ME et al. Stable inhibitory activity of regulatory T cells requires the transcription factor Helios. Science 2015; 350: 334–339.
55 Schaefer JS, Montufar-Solis D, Vigneswaran N, Klein JR. ICOS promotes IL-17 synthesis in colonic intraepithelial lymphocytes in IL-10/-/- mice. J Leukoc Biol 2010; 87: 301–308.
56 Cai G, Nie X, Zhang W, Wu B, Lin J, Wang H et al. A regulatory role for IL-10 receptor signaling in development and B cell help of T follicular helper cells in mice. J Immunol 2012; 189: 1294–1302.