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Posttranscriptional T cell gene regulation to limit Tfh cells and autoimmunity

Simon H Jiang[1,2,3], Nan Shen[3,4] and Carola G Vinuesa[1,3]

T follicular helper (Tfh) cells are crucial to induce protective extrafollicular and germinal center antibody responses against protein antigens. Over the last decade, control of Tfh cell numbers has emerged as an important regulatory checkpoint which, when perturbed, may lead to production of autoantibodies. Recent progress in understanding how Tfh cells are kept limiting has revealed an important role for posttranscriptional control of gene expression mediated by microRNAs such as miR-17∼92, miR-155 and miR-146a, and the RNA-binding proteins Roquin and Regnase. Additionally, T cell microRNAs dysregulated in patients with systemic lupus erythematosus have been shown to influence processes such as DNA hypomethylation, IL-2 and CCL5 secretion, and Treg function, which contribute to autoantibody formation and tissue damage.

Addresses
[1] Department of Immunology and Infectious Disease, John Curtin School of Medical Research and Centre for Personalised Immunology, Australian National University, Canberra, Australia
[2] Department of Renal Medicine, The Canberra Hospital, Canberra, Australia
[3] China Australia Centre for Personalised Immunology, Shanghai Renji Hospital, China and Australian National University, Australia
[4] Joint Molecular Rheumatology Laboratory of the Institute of Health Sciences and Shanghai Renji Hospital, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai Jiaotong University School of Medicine, Shanghai, China

Corresponding authors: Jiang, Simon H (simon.jiang@anu.edu.au) and Vinuesa, Carola G (carola.vinuesa@anu.edu.au)

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Gene expression is initiated by transcription factors and then regulated by proteins and nucleic acids that control mRNA stability and translation. As genes are being transcribed, RNA-binding proteins (RBPs) bind the mRNAs, which undergo splicing and are exported from the nucleus into the cytoplasm [1]. RBPs and microRNAs (miRNAs) modulate the stability and translation of mRNA transcripts, and the outcome is in part determined by the recruitment of transcripts into subcellular compartments such as ribosomes, stress granules or processing bodies, respectively specialized in translation, storage or decay. Over the last 20 years, we have learnt in great detail the transcription factor networks that control T cell differentiation and function, but understanding regulation at the RNA level has been more challenging. It is only in the last few years that we have started to fully appreciate the impact of posttranscriptional gene expression on T cell homeostasis and its ability to control autoimmunity.

It has become apparent that over 50% of significant changes occurring upon T cell activation appear to be a result of mRNA stability regulation [2]. This may not be surprising since many T cell mRNAs encode for cytokines and regulatory proteins that are very unstable and their production rates need to change rapidly according to environmental cues (i.e. evolving cellular interactions, antigen recognition, stress). Furthermore, regulation of cytokine mRNA translation was shown to uncouple $CD4^+$ T helper cell differentiation from effector function, and this finding was triggered by the observation of stress granule formation upon T cell priming [3]. Stress granules are cytoplasmic compartments where RNAs are stored and remain translationally inactive. Naïve T cells receiving TCR signals produce and store cytokine mRNAs which are stored in stress granules and are only loaded onto ribosomes to be translated after subsequent TCR stimulation [3].

Posttranscriptional regulation of gene expression has since been shown to be particularly important for limiting the numbers of B follicular helper T (Tfh) cells and preventing autoimmunity. In this review, we summarise recent advances in understanding how RNA-binding proteins and microRNAs control Tfh cells and influence the development of systemic autoimmunity.

B follicular helper T cell development and function

T cell differentiation is first orchestrated by transcriptional regulators. One of the earliest events in Tfh cell differentiation — upregulation of CXCR5 — is dependent on expression of the transcription factor *Achaete-Scute Family BHLH Transcription Factor 2* (ASCL2) [4]. Thereafter, the Tfh differentiation program heavily relies on expression of *B-cell lymphoma 6* (BCL6) [5–7], which is induced upon DC-mediated activation of T cells with antigen and *inducible T-cell co-stimulator* (ICOS) [8,9], loss

of BCL6 selectively inhibits Tfh differentiation with little effect on other T helper subsets and BCL6 overexpression induces some features of Tfh differentiation [5–7]. BCL6 controls the expression of molecules essential to Tfh development, particularly those required for correct positioning at the T:B border and migration into follicles, including downregulation of CCR7, EBI-2, and S1PR1 [10]. BCL6 has also been shown to promote ICOS and PD-1 expression. Bcl-6 expression is positively regulated by the transcription factor BATF, which also induces expression of c-MAF, important for Tfh homeostasis and function through its ability to induce IL-21 [11]. The expression and/or actions of BCL6 are also opposed by a number of transcription factors including BLIMP1 [7], which is turned on in response to IL-2 signals; FOXP1 [12], FOXO1 [13], and KLF2 [14].

At the T:B border, Tfh cells can prime B cells to differentiate into either extrafollicular plasma cells that induce rapid antibody production [15], as well as initiate germinal centers to produce long term high-affinity antibody responses [16,17]. Subsequent localisation into the B cell follicles is dependent on sustained expression of CXCR5 and ICOS and downregulation of EBI-2 [18–20]. This migration, associated with differentiation into 'GC-Tfh cells', is essential for selection of GC B cells and maintenance of GC reactions [21–23]. Within GCs, B cells undergo somatic hypermutation and affinity maturation [24,25] with subsequent emergence of B cell memory and long lived plasma cells. Tfh cells drive GC B cell growth and selection through production of IL-21 and expression of CD40 ligand [26–29]. A limiting number of Tfh cells favours competition of B cells for affinity-based selection by Tfh cells [30]. Limiting Tfh cell growth is a possible mechanism by which PD-1 expression by Tfh cells contributes to facilitate affinity maturation [31,32]. Although SHM occurs readily within germinal centers, it can also occur at lower rate in extrafollicular foci, particularly in the context of autoimmunity [33].

Mechanisms that act in T cells to limit autoantibody formation

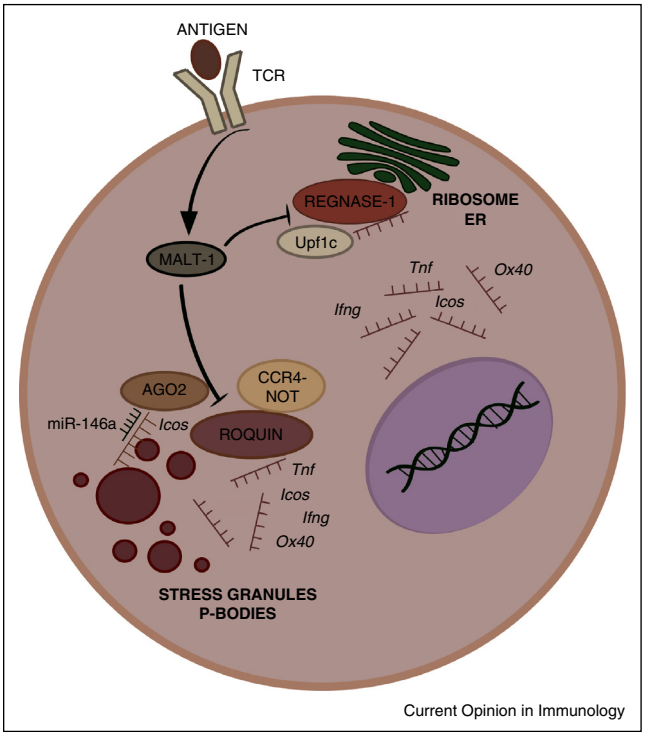
The result of the relatively stochastic SHM process is antibody of not only increased or decreased affinity, but also potential auto-reactivity [34,35]. The requirement for cognate T-cell help before GC formation and after SHM within GCs, provides regulatory checkpoints for non-autoreactive B cell selection as T cells have previously undergone positive and negative selection within the thymus and the latter appears to be more stringent than B cell selection in the bone marrow [36]. It therefore follows that dysregulation of this crucial checkpoint will significantly increase the risk of sustained autoantibody selection. Nevertheless, through the process of 'linked T cell help', self-reactive B cells that have bound self-antigens complexed to non-self peptides may receive help from perfectly tolerised T cells. Also, T cells are not normally tolerised against 'altered-self' — i.e. peptides that have been post-translationally modified through processes such as citrullination [37] or deamidation [38] — which are emerging as important triggers of autoimmunity. Thus, the control of self-reactivity cannot be exclusively delegated to central processes that ensure central T cell tolerance; additional mechanisms must operate to prevent autoantibody production.

Peripheral tolerance mechanisms that prevent formation of autoantibodies include the control of positive selection thresholds by limiting Tfh cell numbers [32,39–41], and the dominant regulation by regulatory T cells (Tregs), including the recently identified T follicular regulatory (Tfr) cells [42–44]. Indeed, accumulation of Tfh cells has been suggested to lower the thresholds for B cell selection, allowing escape of self-reactive or cross-reactive B cells. In a number of mouse models and humans with immune diseases, excessive numbers of Tfh cells have been shown to promote autoantibody formation and lupus-like disease in mice [32,39–41]. There are also accumulating reports of aberrant expansion of circulating Tfh cells correlating tightly with systemic autoimmunity in humans [45–49]. It is now evident that posttranscriptional regulation of gene expression is a powerful means to regulate Tfh cell function and numbers and control autoimmunity. This review focuses on such posttranscriptional mechanisms and describes recent findings on the roles of RNA-binding proteins such as Roquin and Regnase, and several microRNAs in these processes.

RNA-binding proteins regulating Tfh biology and autoimmunity

The importance of Tfh cells and posttranscriptional regulation to T cell tolerance was first revealed by discovery of the *sanroque* mouse model of SLE bearing a mutation in Roquin [39,50]. *Roquin/Rc3h1* and its paralog *Roquin2/Rc3h2* are both ubiquitously expressed genes and encode for the RNA-binding proteins ROQUIN and ROQUIN-2 [51••,52,53••] that regulate mRNA decay of multiple targets in T cells and myeloid cells (Figure 1). Roquin and Roquin-2 form HEPN-domain structures adjacent to a conserved N-terminal RING finger. The HEP-N domain contains a ROQ domain and a CCCH-type zinc finger [54••,55,56]. In the *sanroque* mouse model, a single ENU-induced point mutation within the ROQ domain of *Roquin-1* results in development of ANAs by 6–8 weeks, polyclonal hypergammaglobulinaemia, proliferative nephritis, hepatitis, and anaemia with thrombocytopaenia [39]. The secondary lymphoid organs of these mice develop spontaneous GC formation due to accumulation of Tfh cells — which is T cell autonomous — and reactive plasmacytosis [39,50]. Both CD4+ and CD8+ T cells had high surface expression of ICOS due to failed repression of *Icos* mRNA by Roquin [6,39,57].

Figure 1



Posttranscriptional regulation of gene transcripts exerted by Roquin and Regnase, to prevent autoimmunity. Roquin and Regnase cooperate to regulate an overlapping set of mRNAs in different intracellular compartments that are translationally inactive or active respectively. Regnase requires the helicase activity of UPF1, whereas Roquin associates with either the CCR4-Not decapping complex or AGO2/miR-146a, to influence decay of mRNA targets. Both Roquin and Regnase are cleaved by MALT-1 upon TCR signalling.

A causal link between Tfh cell accumulation and autoimmunity in *sanroque* was established through genetic elimination of Tfh cells (*sanroque.sh2d1a*$^{-/-}$ mice), which prevented autoantibody production and nephritis; and adoptive transfers of *sanroque* Tfh cells, which induced spontaneous germinal centers [50]. To date it is unclear if *sanroque* autoantibodies have an obligatory extrafollicular or follicular origin, since both routes require Tfh cells [15,58] and extrafollicular Tfh cells have long been shown to drive autoimmunity of extrafollicular origin in MRL.*lpr* mice [59]. Although GCs are likely to contribute, they are unlikely to be essential since complete ICOS deletion in *sanroque* still gave rise to some form of autoimmune disease and changed the antinuclear antibody pattern from a mixed cytoplasmic to a homogeneous nuclear one [60]. What is absolutely required for the autoimmune phenotype in *sanroque* mice is IFN-γ overexpression. All T cells in *sanroque* mice overexpress IFN-γ, from the early naïve stages to Tfh cells [60], which normally only express small amounts of this cytokine [61]. Absence of IFN-γR signalling or injections of mAb against IFN-γ for 3 weeks starting at 5 weeks of age completely prevented autoimmune pathology, myeloid cell expansion and Tfh/GC accumulation. Excessive IFN-γ was shown to increase Bcl-6 expression in T cells likely to promote Tfh cell accumulation. IFN-γ-mediated TFH cell accumulation, myeloid cell expansion and activation, and resistance to Treg suppression, are likely to explain the autoimmune syndrome in *sanroque*, with a possible additional contribution of excessive TNF, since Roquin can also repress TNF in macrophages cell autonomously to prevent autoantibody-driven autoimmunity [53••].

At the molecular level, Roquin-1/2 control Tfh cells and autoimmunity through their ability to bind target RNAs, such as *Icos*, *Ox40*, *Il6* and *Tnf* and induce their decay [39,51••,52,53••,55,56,62]. Stem-loop structures termed the constitutive decay element (CDE) in the 3′UTR of several mRNA targets including *Icos* and *Tnf* are recognised by at least two RNA-binding surfaces in the HEPN domain of Roquin [54••,55,56,63••,64]. IFN-γ mRNA was shown to accumulate and decay more slowly in *sanroque* T cells, suggesting this is another target of Roquin, although direct interaction between Roquin and *Ifng* mRNA has not been shown to date [60]. The precise mechanism through which Roquin-1, and presumably Roquin-2, mediate control of mRNA after binding is still not fully elucidated but is likely to involve several RNA regulation pathways including recruitment of the CCR4-CAF1-NOT deadenylation complex [63••], CDE-induced mRNA decay [63••]; and miRNA-mediated decay through recruitment of AGO2 [54••]. Although regulation through miRNA-mediated decay has been controversial and shown not to occur in Dicer-deficient MEFs and the Ago1–Ago4 deficient E7 cells [57]; it is possible that this operates in T cells in the case of certain miRNAs such as miR-146a [54••]. It is now known that the Roquin-1 paralogue Roquin-2 exerts overlapping but not completely redundant functions with Roquin-1 [53••,65].

Roquin-1 and Roquin-2 are regulated through TCR-induced MALT-1 cleavage. MALT-1 is an arginine specific protease, and target sites were identified in both Roquin-1 and Roquin-2 in addition to the known target Regnase-1 [66]. Regnase-1 and Roquin-1/2 co-operatively repress targets, through Regnase-1 nuclease activity and Roquin RNA binding (Figure 1). Varying levels of regulation of Regnase-1 and Roquin may fine tune target repression [67••]. Indeed, although Regnase-1 and Roquin target similar mRNAs, Regnase-1 operates in the ribosomes and endoplasmic reticulum together with the helicase UPF1 to degrade translationally active mRNAs during the early phase of inflammation, whereas Roquin functions in P-bodies and stress granules to degrade translationally-inactive mRNAs [68••] (Figure 1). It is therefore unsurprising that *Regnase-1*$^{-/-}$ mice have a similar phenotype to

the *sanroque* mouse: splenomegaly, lymphadenopathy, activated T cells with strong ANAs and multi-organ plasma cell infiltration [69]. *Regnase-1*$^{-/-}$ mice were also observed to have many mRNA transcripts also elevated in *sanroque* mice, including *Icos* and *Ifng*.

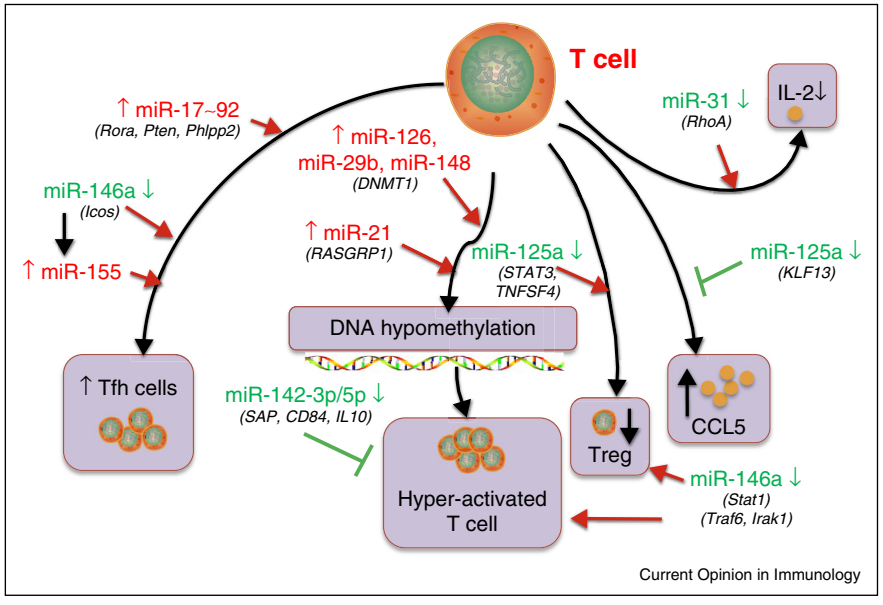
miRNAs in the regulation of Tfh cells and autoimmunity

miRNAs are a class of endogenous, non-coding small RNA which can regulate gene expression at the post-transcriptional level by targeting specific mRNAs for degradation or suppressing mRNA translation [70]. Accumulating evidence shows that miRNAs can govern leucocyte development and regulate innate and adaptive responses in physiological and pathological circumstances. Abnormal miRNA expression occurs in many autoimmune diseases including systemic lupus erythematosus (SLE) [71], and Tfh cells have been recently shown to be tightly regulated by several miRNAs (Figure 2).

The miR-17∼92 cluster has been shown to target *Pten*, *Phlpp2* and *Rora* to promote Tfh cell differentiation and its function in the GC response [72•,73] (Figure 2). *Rora* degradation limits expression of non-Tfh genes [72•], whereas repression of PHLPP2, with some contribution from PTEN repression, enhance PI(3)K signalling — an important mediator of ICOS signals — thus promoting Tfh cell formation. Mice overexpressing miR-17∼92 in T cells formed large numbers of Tfh cells and developed lymphoproliferative disease and autoimmune SLE-like symptoms [73].

miR-146a has been associated with various autoimmune diseases and acts in various cell-types to repress multiple targets. It can suppress TRAF6 and IRAK1 in both myeloid cells and T cells, and its deficiency results in pro-inflammatory IL-6 and TNF production [74,75]. In Tregs, miR-146a represses STAT-1, which is important for repression of effector Th1 responses [76]. miR-146a directly targets *Icos* in all T cell subsets and maturation stages and ICOS overexpression caused by miR-146a deficiency leads to spontaneous and cell-autonomous Tfh cell accumulation [77••] (Figure 2). The spontaneous Tfh cell accumulation in *Mir146a*$^{-/-}$ mice has also been shown to be at least in part mediated miR-155, which acts in T cells to promote Tfh differentiation targeting multiple regulatory genes [78••] (Figure 2). miR-146a also acts in B cells to repress GC formation. In the absence of miR-146a, ICOSL is overexpressed in GC B cells and DCs, which is likely to compound the T cell-automomous effect leading to increased Tfh cell numbers [77••]. ICOSL has no obvious target sites for miR-146a but is known to be regulated by NF-KB, expression of which is dampened indirectly by miR-146a [79]. Intriguingly, Roquin-1 is capable of binding miR-146a and the core RISC component Argonaute 2 (AGO2) and limit miR-146a longevity [54••]. Given that Roquin-1 binds both miR146a and its target *Icos* mRNA and induces the decay of both RNA species, an open hypothesis is that Roquin-1 promotes miRNA-mediated ICOS repression. In humans, miR-146a is also thought to contribute to SLE through its ability to regulate type I interferon responses by targeting STAT1 and IRF5 [80]. A novel genetic variant in the promoter region

Figure 2



miRNAs that regulate Tfh cell numbers, T cell activation or Treg activity, known to influence systemic autoimmunity. Summary of miRNAs shown to regulate Tfh cells or T cell activation, Treg function, IL-2 or CCL5 production, and linked to autoimmunity (SLE) when dysregulated. Experimentally validated and physiologically-relevant mRNA targets are indicated below individual miRNAs.

of miRNA-146a is associated with SLE susceptibility and individuals carrying the risk-associated allele have significantly reduced expression of miR-146a [81].

A number of miRNAs, although not shown to influence Tfh cells directly, are also likely to regulate T cell help for self-reactive B cells and exacerbate autoimmunity (Figure 2). For example, miR-31, which is underexpressed in SLE T cells, impairs IL-2 expression by targeting RhoA, a negative regulator of NFAT [82] (Figure 2). IL-2 expression limits Tfh cell formation and has been proposed to maintain natural regulatory T cells; it has been reported to be lower in T cells from SLE patients. Furthermore, NFAT has been shown to be important for the development of Tfr cells [83], which limit the numbers of Tfh cells and have been proposed to prevent autoimmunity [42–44]. miR-125a, which is significantly down-regulated in PBMCs of SLE patients and selectively expressed in T cells, may contribute to excessive secretion of CCL5 by SLE T cells by targeting KLF13, promoting leucocyte infiltration and tissue damage [84] (Figure 2). Dysregulation of several miRNAs also contribute to the hypomethylated DNA status characteristic of CD4+ T cells from SLE patients: miR-21, miR-148a, miR-126 and miR-29b have all been found to be upregulated in lupus CD4+ T cells and to contribute to DNA hypomethylation by targeting DNMT1 [85,86] (Figure 2). miR-21 can also inhibit the RAS-MAPK-ERK signalling pathway upstream of DNMT1 in T cells [85].

Concluding remarks

The florid autoimmunity observed in the *sanroque* mouse demonstrated the capacity of abnormal Tfh responses to cause SLE-like autoimmunity and revealed important posttranscriptional networks of Tfh cell control. Investigation into the mechanisms through which Roquin-1/2, Regnase and miRNAs repress mRNAs has highlighted the complex and intricate feedback loops these RBPs and small RNAs use to augment or control adaptive immune responses. Further work will be important to delineate the upstream signals and cues that determine the activity of these RNA-regulating factors.

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