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Competing interests
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REGULATORY RNA

CIRCling the wagons to protect intestinal stem cells

Innate lymphoid cell–derived cytokine IL-13 promotes the maintenance of intestinal stem cells through stabilization of β-catenin. The circular RNA circPan3 regulates mRNA encoding the cytokine receptor subunit IL-13Rα and downstream IL-13 signaling to stabilize the β-catenin pathway in intestinal stem cells.

Frank Soveg, Jakob von Moltke and Ram Savan

The epithelium of the small intestine is tasked with balancing its role of absorbing nutrients with providing a physical barrier to protect against commensal microbes as well as pathogenic microbes. The barrier function of this surface is supported by the interplay of intestinal stem cells (ISCs) and immune cells associated with the intestinal mucosa. Therefore, crosstalk between these cells is important for intestinal homeostasis and host defense. In this issue of *Nature Immunology*, Zhu et al. show that the circular RNA (circRNA) circPan3 regulates such epithelial cell–immune cell crosstalk by controlling expression of the gene encoding the cytokine receptor subunit IL-13Rα (*Il13Ra1*) in the ISCs[1] (Fig. 1). The authors identify a novel role for circPan3 in regulating signaling via the type 2 cytokine IL-13 that is critical for the maintenance of ISCs. This detailed study combines extensive biochemical analysis and mouse genetics to provide novel insights into how circRNA promotes the self-renewal of ISCs and thereby contributes to intestinal homeostasis, with potential broad applicability to other mucosal tissues.

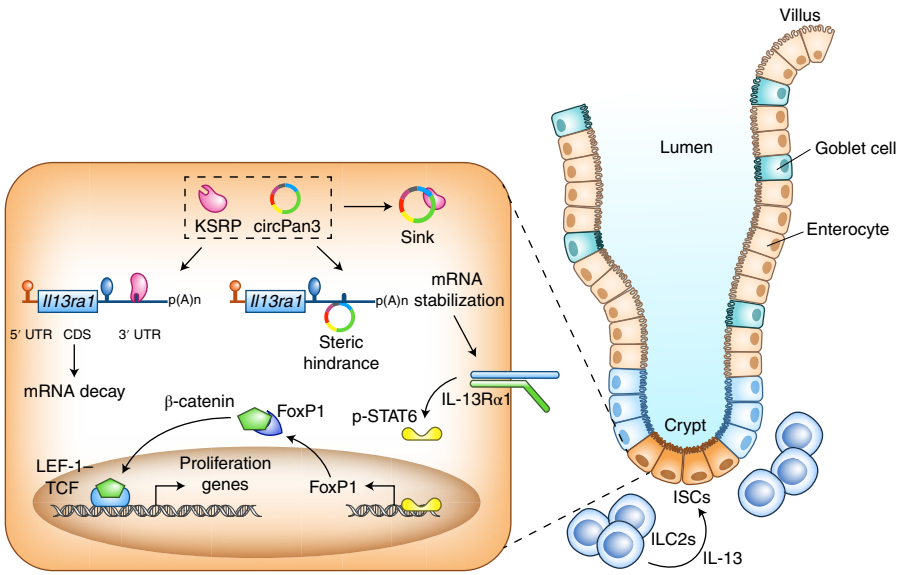

Fig. 1 | Role of circPan3 in ISC regulation. circPan3 is produced in ISCs, where it protects *Il13ra1* mRNA from degradation by KSRP. Signaling through the IL-13 receptor via phosphorylated (p-) STAT6 drives the expression of FoxP1, which binds to and stabilizes β-catenin. Translocation of stabilized β-catenin to the nucleus promotes the maintenance of ISCs. UTR, untranslated region; CDS, coding sequence; p(A)n, poly(A) tail.

A remarkable feature of the intestinal epithelium is its capacity for self-renewal, a function important for its normal turnover and response to pathogens[2]. ISCs reside in a stem cell niche at the crypt base and are responsible for the genesis of all intestinal epithelial cell lineages[3]. Under homeostatic conditions, the renewal and differentiation of ISCs maintains a constant turnover of the intestinal epithelium. Secreted factors, such as Wnt ligands from Paneth cells, are essential for maintaining the ISC pool[4]. The regulation of epithelial stem cells by immune cells and cytokines has been the subject of several high-profile studies. In the skin, resident regulatory T cells localize to the epithelial stem cell niche in the hair follicle, where they secrete factors such as Notch ligands to promote stem cell differentiation[5], while activation of the inflammasome endows epithelial cells with an epigenetic 'memory' that facilitates wound healing[6]. In the intestine, IL-13 produced during helminth infection both alters and increases ISC output[7,8], and the cytokine IFN-γ emanating from granulomas surrounding *Heligmosomoides polygyrus* larva induces a fetus-like reversion in the ISC niche[9]. Homeostatic signals from immune cells might also regulate the maintenance and differentiation of ISCs, although this area is underexplored.

Least understood are the post-transcriptional regulatory events important for the development and maintenance of ISCs. circRNAs are a recently discovered class of RNA formed by covalent ligation of the 5′ and 3′ ends[10]. Although they were once considered a rare and extraneous byproduct of splicing, RNA-sequencing

analysis has revealed that circRNAs are abundantly expressed and have biologically relevant roles. One example is the circRNA *CDR1as*, which acts as a sponge for the microRNA miR-7 in vivo; overexpression of *CDR1as* in zebrafish results in stunted midbrain growth[11]. Beyond acting as sponges for microRNA, circRNAs could regulate gene expression in a manner similar to that used by long non-coding RNAs, such as by binding to promoter regions, interacting with mRNA regulatory elements or sequestering RNA-binding proteins. In an added twist of complexity, some circRNAs have protein-coding potential. The many hundreds (or more!) of circRNAs with unknown function represent an inviting field ripe for discovery, especially in the context of immunoregulation.

To address whether circRNAs regulate the function of ISCs, Zhu et al. first profile the expression of circRNAs in Lgr5$^+$ ISCs[1]. They find over 2,000 potential circRNAs for which ISCs show enrichment and validate by RNA sequencing the ten circRNAs with the highest expression. They confirm the circularity of these RNAs by subjecting them to digestion with RNaseR, which selectively degrades linear RNAs. Knockdown of those ten circRNAs in enteric organoid cultures alone produces no difference in organoid growth. However, knockdown of circPan3, one of those ten circRNAs, in enteric organoids co-cultured with immunocytes results in a significant growth defect. These observations suggest that circPan3 might regulate cross-talk between intestinal immune cells and ISCs to provide important growth signals. Through the use of a *circPan3*^{RFP} reporter mouse, the authors visualize *circPan3* expression in the small intestine and colon, where *circPan3*^{RFP} expression is coincident with Lgr5$^+$ ISCs[1]. Accordingly, isolated *circPan3*^{RFP} cells are sufficient to generate fully differentiated crypt–villus organoids.

To explore the role of circPan3 in vivo, Zhu et al. next generate circPan3-deficient mice[1]. Consistent with a role for circPan3 in enteric development, they find a significant reduction in the size of crypts and villi in unmanipulated circPan3-deficient mice. The rate of ISC proliferation and renewal after treatment with dextran sulfate sodium or radiation is also impaired in the circPan3-deficient mice. Importantly, the authors show that the effects on ISC development are specific to deletion of circPan3, as disruption of circPan3 does not alter the expression or activity of Pan3, and that Pan3-deficient mice do not have the ISC growth defects observed in circPan3-deficient mice. These observations are surprising, as Pan3 is one of the critical factors involved in mRNA decay, and one would expect widespread dysregulation of genes that could have biological consequences on the growth and proliferation of ISCs. Through transcriptomics analysis, the authors next identify *Il13ra1* as one of the many dysregulated genes in circPan3-deficient ISCs and find that, like deletion of circPan3, deletion of IL-13Rα1 in ISCs confers a growth defect onto ISCs co-cultured with immunocytes. Deletion of *Il13ra1* in vivo also results in a reduced number of ISCs. Through the use of RNA-immunoprecipitation approaches, the authors show that circPan3 binds to *Il13ra1* mRNA via three loop hairpins and that disruption of these hairpins abolishes the interaction of circPan3 with *Il13ra1*. They identify the RNA-binding protein KSRP as a negative regulator of *Il13ra1* mRNA by showing that KSRP binds to and promotes destabilization of *Il13ra1* through AU-rich element–mediated decay. Interestingly, KSRP and circPan3 compete for binding to *Il13ra1* mRNA, as the authors show that increasing the amount of circPan3 reduces the interaction of KSRP with *Il13ra1*. Deletion of *Il13ra1* in vivo also results in a reduced number of ISCs. There are several sources of IL-13 in vivo, including group 2 innate lymphoid cells (ILC2s) and lymphocytes of the T_H2 subset of helper T cells. Consistent with that, the authors observe a decrease in the number of ISCs in *Il2rg*$^{-/-}$ mice, which lack ILCs and natural killer cells, and are able to restore the number of ISCs by adoptive transfer or ILC2s and ILC3s, but not by adoptive transfer of natural killer cells or ILC1s[1]. Finally, they show signaling via the signal transducer STAT6 downstream of engagement of the IL-13 receptor in ISCs promotes stabilization of β-catenin. Proteomics analysis reveals that the transcription factor FoxP1 binds to β-catenin, and subsequent experiments confirm that signaling via STAT6 is required for this interaction. Published work has shown FoxP1 can augment Wnt–β-catenin signaling[12]. Zhu et al. show that FoxP1 expression depends on IL-13, IL-13Rα1 and STAT6, while the growth of organoids derived from FoxP1-deficient ISCs is impaired[1]. In contrast, overexpression of FoxP1 in FoxP1-deficient ISCs promotes organoid formation and results in stabilization of β-catenin and its translocation to the nucleus. Mechanistically, signaling via IL-13 promotes the survival and maintenance of ISCs through FoxP1-mediated stabilization of β-catenin. Interestingly, a published study has shown that IL-13 derived from T_H2 cells promotes ISC differentiation, while regulatory T cells promote ISC renewal, although these findings were reported in the context of helminth infection[13]. In all cases, it seems that signaling via IL-13 increases epithelial output from intestinal crypts, but the underlying mechanisms of this require further study.

An important, and perhaps surprising, finding of this work is the requirement for signaling via IL-13 on ISCs for their maintenance at steady state. As noted above, there are several possible sources of IL-13 in vivo, including ILC2s and T_H2 lymphocytes, but through the use of a series of genomic deletions and adoptive-transfer experiments, Zhu et al. conclude that ILC2s are the predominant source of IL-13, at least in the absence of infection[1]. How intestinal ILC2s are activated to produce IL-13 at steady state remains to be determined, and it is important to consider possible contributions from chronic colonization with protists such as *Tritrichomonas*[14].

Collectively, this work establishes circPan3 as a functional circRNA that acts as a sponge or through steric hindrance to prevent the decay of *Il13ra1* mRNA[1]. In the absence of circPan3, *Il13ra1* mRNA is susceptible to RNA-decay mechanisms involving the RNA-binding protein KSRP. Because the authors show that circPan3 and KSRP compete for *Il13ra1* mRNA[1], identifying the factors that regulate the amount of circPan3 and KSRP in ISCs is an interesting area of investigation. This will be informative for the understanding of how important the circPan3–KSRP balance is for the maintenance of ISCs. Another exciting avenue of inquiry will be determining if this post-transcriptional circuit is conserved in humans and if single-nucleotide polymorphisms in circPan3 or the *Il13ra1* 3′ untranslated region might correlate with intestinal dysfunction. We are tempted to speculate that circPan3 might broadly regulate IL-13 signaling in cells known to respond to IL-13, such as intestinal goblet cells, smooth muscle and functionally similar cells in other mucosal tissues such as the lungs. Importantly, this study highlights a critical role for circRNAs in regulating the cross-talk between intestinal epithelial cells and lymphoid cells. ❐

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Competing interests

The authors declare no competing interests.

CYTOKINE RNA STABILITY

Not immune to modification

The N^6-methyladenosine (m^{6}A) RNA-modification pathway substantially affects the outcome of viral infection. Studies now show that m^{6}A modification of transcripts encoding type I interferons limits the duration of anti-viral signaling.

Charles R. Hesser and Britt A. Glaunsinger

The function and fate of RNA can be heavily influenced by chemical alterations to its sequence; more than 150 such modifications exist. Classic examples of this include modifications to the wobble position of the tRNA anticodon loop, which contribute to expanded coding potential, and to the 5′ cap structure of mRNA[1]. Over the past several years, a remarkable array of gene-regulatory phenotypes have been ascribed to modification of cellular mRNA by m^{6}A, the most abundant internal modification on these transcripts[1]. Published studies have shown that the cellular m^{6}A machinery can also influence the outcome of viral infection, at least in part by affecting expression of m^{6}A-modified viral RNA[2,3]. Two new complementary studies, by Winkler and colleagues in this issue of *Nature Immunology*[4] and by Rubio and colleagues in *Genes & Development*[5], reveal that the influence of the m^{6}A pathway also extends to immunological regulators by demonstrating that transcripts encoding type I interferons (IFN-α and IFN-β (IFN-α/β)) — pleotropic mediators of anti-viral immunity — are also targets of m^{6}A modification. Thus, inhibiting m^{6}A modification can alter the outcome of infection by affecting the fate of both viral mRNAs and host mRNAs that drive anti-viral responses.

Published studies have indicated that m^{6}A is an important component of host–pathogen interactions. For example, it has a critical role in regulating cellular transition states and maintaining T cell homeostasis[6]. A role for m^{6}A in recognition by the innate immune system has also been proposed, as m^{6}A-modified RNAs show reduced activation of Toll-like receptor pathways[7]. For these reasons, interest in the role of m^{6}A in infectious contexts has burgeoned. While numerous studies of viral infection have found enrichment for m^{6}A in viral transcripts, precisely how the m^{6}A pathway regulates viral life cycles remains an active area of investigation. For hepatitis C virus, which has an entirely cytoplasmic life cycle, m^{6}A restricts viral assembly[2]. For influenza A virus and SV40 polyomavirus, which replicate in the nucleus, m^{6}A instead promotes viral gene expression[8,9]. However, for several viruses with complex life cycles (human immunodeficiency virus, hepatitis B virus and Kaposi's sarcoma–associated herpesvirus), a combination of pro-viral effects and anti-viral effects has been proposed[2,3,10]. Furthermore, the extent to which these phenotypes result from the binding of reader proteins in cis to methylated viral RNAs versus their influencing host pathways involved in the viral life cycle has remained unclear.

Viral infection substantially changes the host transcriptome, and as m^{6}A has been proposed to have a role in 'transcriptome-turnover' events[1], its presence in transcripts encoding anti-viral cytokines could affect the innate immune response by accelerating their degradation. Indeed, anti-viral cytokines are extensively regulated post-transcriptionally and are characterized by short transcript half-lives. For example, AU-rich elements are present in 3′ untranslated regions of many cytokine-encoding mRNAs and have a critical role in mRNA decay. Of particular interest in viral infection, IFN-α/β products induce resistance to viral infection but also cause a variety of autoimmune diseases when their production is dysregulated[11]. After being secreted, they exert autocrine and paracrine effects, binding to the IFN-α receptor (IFNAR) and triggering a JAK–STAT signaling cascade that leads to the production of molecules encoded by interferon-stimulated genes (ISGs), which restrain viral replication via multiple mechanisms[11]. Thus, even subtle changes to the stability of transcripts encoding IFN-α/β could substantially affect the abundance of IFN-α/β proteins and the production of molecules encoded by ISGs. If m^{6}A modification of transcripts encoding IFN-α/β contributed substantially to their post-transcriptional decay, could this contribute to the pro-viral effects ascribed to the m^{6}A pathway during various viral infections?

Winkler and colleagues address that issue by focusing on the role of m^{6}A in host transcripts during infection with several human and mouse viruses, including human cytomegalovirus (HCMV), a large dsDNA virus that replicates in the nucleus[4]. Once installed, m^{6}A acts as a molecular 'beacon' to recruit selective methyl-RNA-binding proteins known as 'readers'. Depletion of either the catalytic subunit responsible for the installation of m^{6}A (METTL3) or the reader protein YTHDF2 results in stabilization of the *IFNB* transcript and increased induction of downstream ISGs during infection with HCMV. This phenotype also develops during infection with each of three other human viruses and one mouse virus, suggestive of an evolutionarily conserved mechanism in which the m^{6}A pathway promotes the decay of transcripts encoding IFN-α/β. In support of this hypothesis, the authors observe that m^{6}A modification of transcripts encoding IFN-α/β is conserved across several human and mouse cell types and that viral gene expression is not required for the increased abundance of *IFNB* transcripts