RESEARCH HIGHLIGHT

RNA editing: new roles in feedback and feedforward control

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Biological systems use a variety of feedback and feedforward mechanisms to establish and maintain specific cellular programs. In a recent study published in *Cell Research*, Lin et al. add RNA editing to an S-nitrosylation-induced feedforward process, which, together with another recently reported paradigm of RNA editing-mediated feedback control, implicates a new role of RNA editing in fine-tuning biological outputs.

Feedback (both positive and negative) loops employ an output to enhance or suppress the input of a pathway, whereas feedforward controls leverage the output of a pathway to influence additional biological processes.[1] Both feedback and feedforward loops are key parts of regulatory biology, best exemplified by the highly intertwined regulatory networks in interferon signaling, where specific control mechanisms often involve the induction of activators/repressors of transcription or translation or post-translational modifications (PTMs) of various regulatory proteins.[2] RNA processing and regulatory RNAs (both long non-coding RNAs and microRNAs) have also been implicated in various feedback and feedforward controls.[3,4] However, there is no precedent for the involvement of RNA editing — adenosine-to-inosine (A-to-I) conversion within double-stranded RNA (dsRNA) catalyzed by Adenosine Deaminase Acting on RNA (ADAR) — in such regulation. This knowledge gap has now been filled by two recent studies.[5,6]

RNA editing represents a form of RNA metabolism that alters transcript sequences without changing DNA sequences, and ADAR-catalyzed A-to-I RNA editing is the most common RNA editing event in eukaryotic cells.[7] Different organisms often express a family of ADARs.[8] For example, the human genome encodes three ADARs: ADAR1, ADAR2 (also known as ADARB1), and ADAR3 (also known as ADARB2), with the former two functioning as active editing enzymes and ADAR3 acting as a regulatory protein. In *Caenorhabditis elegans*, on the other hand, ADR-1 is catalytically dead whereas ADR-2 is an active enzyme. ADAR-catalyzed conversion may alter the codon because of the recognition of inosine as guanine by the ribosome or modulate alternative splicing, RNA stability, and function of various regulatory RNAs, thus representing a key regulatory mechanism in diverse biological processes.[9]

In a recent study published in *Cell Research*,[6] Lin et al. revealed that S-nitrosylation of cathepsin B (CTSB) increased CTSB protein production through editing its own mRNA to increase RNA stability. S-nitrosylation is a form of PTM that modifies the thiol group in cysteine by nitric oxide, which has been implicated in numerous cellular signaling events.[10] CTSB, a cysteine protease in the lysosome, is a key target of S-nitrosylation; and in response to various stress signals, CTSB is released into the cytoplasm or even secreted extracellularly to induce proteolysis.[11] In the study by Lin et al., multiple stressors were shown to induce CTSB S-nitrosylation at C319 in endothelial cells, thereby inducing CTSB release into the cytoplasm to activate inflammation. Unexpectedly, this was accompanied with ~2-fold increase in CTSB protein to further drive the inflammatory response. This feedforward control was attributed to improved mRNA stability via stress-induced RNA editing in *CTSB* 3′UTR by ADAR1, which recruits the well-studied RNA stabilizer HuR.[12]

How does CTSB S-nitrosylation signal ADAR1-mediated editing in the nucleus? As illustrated in Fig. 1a, Lin et al. showed that stress signaling induced binding of S-nitrosylated CTSB to α-adducin (ADD1), an actin-binding protein that shuttles between the cytoplasm and the nucleus in a phosphorylation-dependent manner. S-nitrosylated CTSB triggered ADD1 dephosphorylation at S716 by the phosphatase PP6, thereby shifting ADD1 to the nucleus where it interacted with a nuclear matrix protein MATR3, which recruited ADAR1 to *CTSB* mRNA for editing. Because the S716A mutant that mimics dephosphorylated ADD1 could enter the nucleus to interact with MATR3, but failed to induce *CTSB* editing, this implies a co-factor(s) that may be independently induced by CTSB S-nitrosylation to synergize with the ADD1–MATR3 complex to guide ADAR1 to *CTSB* mRNA. It remains unclear whether CTSB S-nitrosylation may also induce other RNA editing events beyond those on its own mRNA.

Despite the missing link, the study by Lin et al. established RNA editing as a key regulatory component in a feedforward circuitry. This finding joins another study published earlier by Li et al., showing RNA editing as part of a negative feedback loop to dampen hyperactivation of ciliary kinases in *C. elegans*.[5] In this biological system, the kinase DYF-5 needs to be tightly regulated, as deletion of the kinase leads to long cilia whereas overexpression of the kinase blocks ciliogenesis. DYF-5 is activated by phosphorylation at T164. Unexpectedly, the T164E mutant that mimics phosphorylated DYF-5 was constitutively activated in vitro, but the mutant kinase behaved like a recessive loss-of-function mutant in animals to develop elongated cilia. Genetic repressor screen revealed ADR-2 whose inactivation rescued the phenotype.

Mechanistically, the hyperactivated DYF-5 induced antisense transcription within its own gene, resulting in the formation of dsRNA that induced ADR-2-mediated RNA editing. The edited sequences impaired splicing to cause intron retention, which led to nonsense-mediated decay. Thus, the hyperactivation of the kinase limits the expression of its own gene by enlisting antisense transcription and RNA editing in a feedback control loop (Fig. 1b). Interestingly, hyperactivated DYF-5 also similarly impacted the

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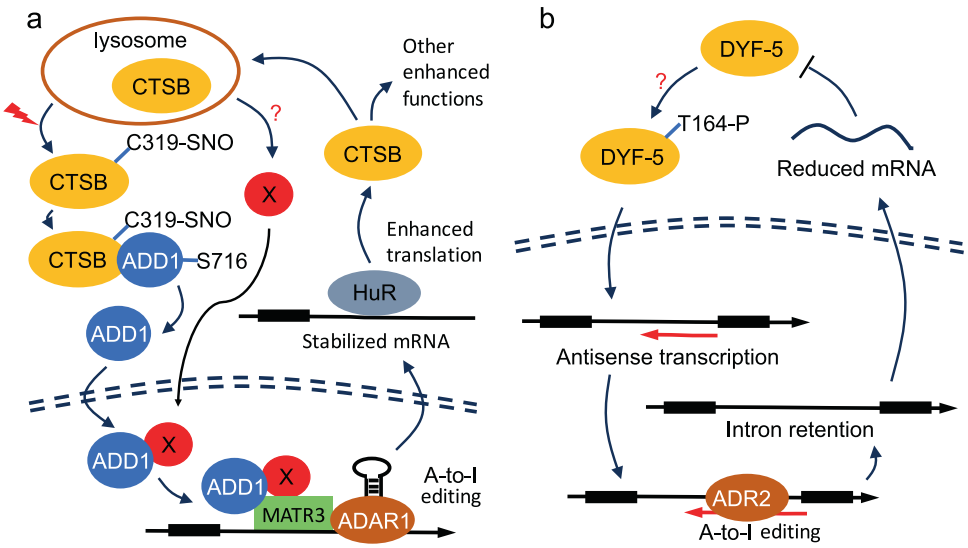

Fig. 1 RNA editing as part of regulatory loops. a RNA editing in feedforward control. In endothelial cells, stress induces CTSB S-nitrosylation, which induces ADD1 dephosphorylation and translocation to the nucleus. An uncharacterized, likely also stress-induced factor X, may join ADD1 to form a complex with MATR3, which in turn recruits ADAR1 and targets the complex to *CTSB* mRNA to catalyze RNA editing. The altered sequence binds HuR, leading to increased RNA stability to enhance CTSB translation. **b** RNA editing in feedback control to maintain ciliary homeostasis in worm. The ciliary kinase DYF-5 becomes activated by phosphorylation, and through an unknown mechanism, such activated kinase induces antisense transcription within the kinase gene, leading to the formation of dsRNA that recruits ADR-2 to edit its own pre-mRNA. Some critical splicing signals are impaired to cause intron retention, thereby triggering nonsense-mediated mRNA decay to limit DYF-5 translation. Interestingly, hyperactivated DYF-5 also induces antisense transcription to limit the expression of the other two ciliary kinases, indicating a coordinated feedback control program to prevent ciliopathies in worm.

other two ciliary kinases to achieve coordinated regulation. However, it remains unclear mechanistically how an hyperactivated ciliary kinase would prompt antisense transcription and whether such antisense transcription is widespread or limited to the loci that express these ciliary kinases.

In summary, despite many mechanistic details that remain to be worked out, these studies have firmly established RNA editing as a regulatory strategy in feedback or feedforward control, thus adding a new functional dimension to this conserved mode of RNA metabolism.

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ADDITIONAL INFORMATION

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