NONCODING RNA

Turning the table on miRNAs

Transcripts with highly complementary sequences can target microRNAs (miRNAs) for degradation, but the physiological relevance of target-directed miRNA degradation (TDMD) has remained unclear. Bitetti et al. now identify a conserved vertebrate RNA that induces TDMD in the cerebellum of zebrafish and mouse to promote wild-type animal behaviors.

Manuel de la Mata and Helge Großhans

It is well known that miRNAs regulate target mRNAs by binding to partially complementary sequences. However, more recently, it has emerged that specific target RNAs can reverse the outcome (Fig. 1). In TDMD, binding of miRNAs to RNAs with extensive complementarity results in miRNA 3′-end tailing (addition of non-templated nucleotides, usually Us or As), trimming (shortening from the 3′ end), and miRNA loss. This process functions in the test tube with fly lysates[1], but also in cells in which viral RNAs can direct host miRNAs for degradation[2–4]. Given that viruses are great 'plagiarizers', it seemed reasonable to assume that cells would employ TDMD in physiological situations. However, no endogenous targets have been forthcoming.

Enter Bitetti et al.[5], who now describe how endogenous transcripts regulate miR-29b, one of three members of the miR-29 family, in the cerebellum of zebrafish and mouse[5]. They reveal that loss of miR-29b degradation causes aberrant explorative and anxiety-like behaviors in fish and mice. In a further twist to this exciting finding, the transcripts that direct degradation of miR-29b in fish and mouse appear evolutionarily related (Fig. 2a), but differ in one key aspect: the zebrafish libra transcript is a long noncoding RNA (lncRNA), previously known as linc-epb4.114 and renamed 'lncRNA involved in behavioral alterations' (libra). By contrast, its mouse counterpart is the *Nrep* mRNA, encoding a small, 8-kDa protein.

Strikingly, the *Nrep* mRNA shares large blocks of sequence identity with libra, including 20 nucleotides complementary to miR-29b in its noncoding 3′ untranslated region (UTR) (Fig. 2a). Previously, *Nrep* gene knockout (KO) had been shown to cause behavioral alterations[6]. Now, through elegant experiments, Bitetti et al.[5] show that scrambling of only the miR-29b complementary site partially recapitulates *Nrep*-KO phenotypes without affecting levels of *Nrep* mRNA and, presumably, protein (Fig. 2b). Future side-by-side experiments on the two mutant mouse strains, and ideally specific knockout of the protein, for example, through manipulation of the translation start codon, will be needed to delineate which functions of the *Nrep* gene are executed by the encoded protein versus 3′-UTR-mediated TDMD. Nonetheless, the findings of Shkumatava and colleagues provide strong evidence that TDMD accounts for at least some of the behavioral functions of the *Nrep* gene.

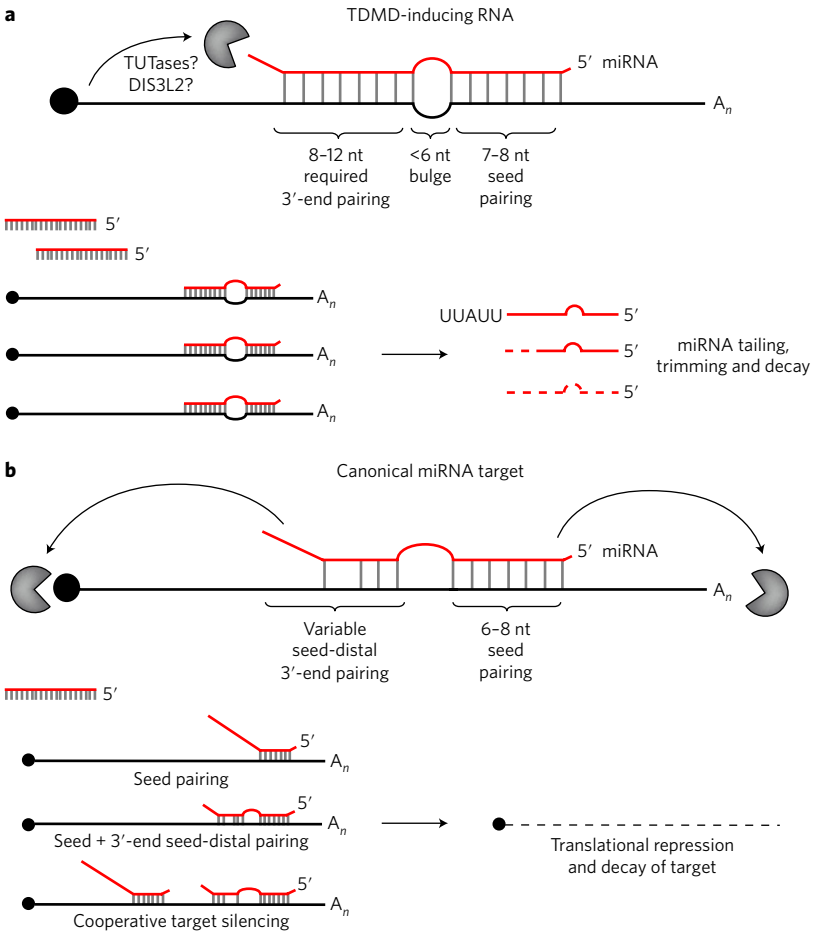

Fig. 1 | Target features determine distinct outcomes of miRNA binding. a, Schematic depiction of properties of TDMD-inducing RNAs, implicated enzymes, and mechanisms of TDMD. Substoichiometric target concentrations may suffice to trigger TDMD, and miRNAs may induce some extent of target degradation. **b**, Properties of canonical miRNA targets and outcomes of miRNA binding. Additional architectures such as 'centered' or 'seedless' interactions[16] have been omitted for clarity. Credit: Kim Caesar/Springer Nature

Conceptually, TDMD-mediated control of miRNA levels might be useful to diversify and rapidly alter miRNA expression patterns. By accelerating clearance of miRNA in specific contexts, TDMD would help to avoid inappropriate activity of this

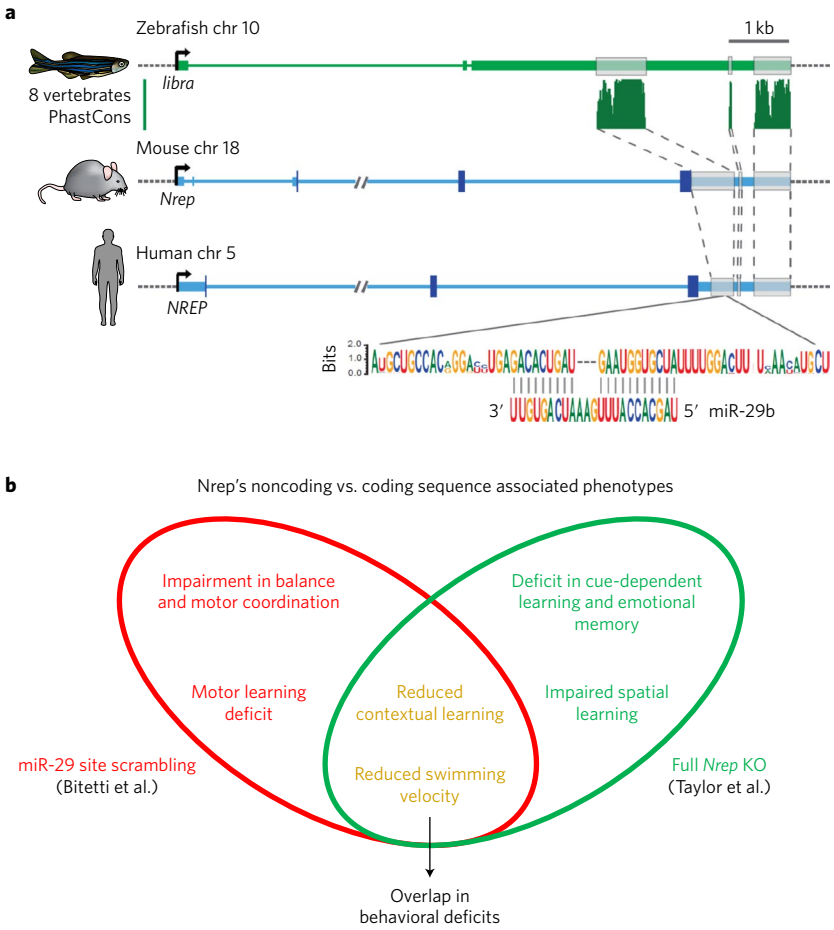

Fig. 2 | miR-29b TDMD as a conserved function of libra lincRNA and *Nrep* mRNA that modulates behaviors. a, Regions of sequence conservation between the *libra* lncRNA locus in zebrafish and the *NREP* loci in mouse and human. Boxed gray areas and PhastCons plots based on the eight-genome alignment indicate the location of deeply conserved sequences. Dark blue boxes in the mammalian loci represent the NREP-coding ORF. Light blue boxes represent 5′ and 3′ UTRs. The consensus sequence logo shows conservation of the miR-29 site, with vertical lines indicating Watson–Crick or wobble base-pairing. Panel **a** image and description were reproduced with permission from ref. [5], Nature America Inc. **b**, Mice with a scrambled miR-29-binding site partially recapitulate behavioral phenotypes of *Nrep*-KO mice that were tested in an independent study by Taylor et al.[6]. Note that the reduced swimming velocity phenotype of *Nrep*-KO mice was not stable over time. Credit: Kim Caesar/Springer Nature

miRNA. This appears particularly relevant because miRNAs tend to have long half-lives, preventing rapid downregulation of miRNA levels through repression of their transcription[7]. Because miRNAs are thought to compete for limiting amounts of cellular Argonaute (Ago) protein, depletion of one miRNA by TDMD might additionally facilitate loading of other miRNAs[7] (Fig. 3a).

In the case of *Nrep*, another aspect of TDMD may be important, namely its ability to 'uncouple' miRNAs that are cotranscribed from one genomic locus in a single primary transcript (Fig. 3b). Although mouse miR-29b derives from two genomic loci, either as an miR-29a/b-1 or an miR-29b-2/c primary transcript, only miR-29a and miR-29c accumulate in cerebellum granule cells, because TDMD depletes miR-29b. However, the adaptive value of establishing such specific expression domains for different miR-29 family members is not immediately clear. After all, miR-29a and miR-29c share a seed sequence with miR-29b, suggesting that the three family members regulate an overlapping target set[8] (Fig. 1b). Thus, one possibility is that the relevant biological outcome of *Nrep* activity is a sufficient reduction of the global abundance of miR-29 family miRNAs rather than depletion of miR-29b specifically. Alternatively, seed-distal miRNA pairing (Fig. 1b) may direct the different miR-29 family members to distinct, only partially overlapping sets of targets[9,10]. Identification of phenotypically relevant target(s) will ultimately solve this issue. In the meantime, examination of mice in which the *Nrep* 3′ UTR has been altered to target miR-29a or miR-29c, instead of miR-29b, might reveal how specific the mutant phenotypes are to miR-29b overexpression.

With libra and *Nrep* now established as the first endogenous TDMD inducers, it seems a safe bet to predict that more cases of TDMD remain to be discovered. Because prediction of conventional miRNA targets focuses mostly on seed matches[8] (Fig. 1b), it is not well suited to the identification of TDMD inducers. However, the features of the predicted libra/*Nrep*–miR-29 duplexes agree with those previously elaborated for artificial TDMD inducers with respect to overall pairing capacity, pairing to the miRNA 3′ end, and central loop size[3,11] (Fig. 1a), suggesting that parameters for a computational identification of TDMD inducers are emerging. The example of libra further suggests that searches for TDMD inducers might do well to include noncoding RNAs. Nonetheless, we caution against premature generalizations on lncRNA functions in TDMD. This is because most lncRNAs tend to be low in abundance and often localized to the nucleus. By contrast, functional, cytoplasmic miRNAs are thought to accumulate at hundreds of molecules per cell. Hence, we predict robust miRNA depletion to require substantial TDMD-inducer levels, even if we accept TDMD as a multiple turnover activity that can operate at substoichiometric concentration of inducer[11]. Hence, although cells typically contain a diverse set of lncRNAs, it remains quite possible, unless proven otherwise, that most may not execute specific cellular functions[12].

Taken together, the study by Shkumatava and colleagues[5] is an important step toward understanding physiological functions of TDMD, which we expect to inspire further research into the prevalence and mechanisms of TDMD. For instance, previous work on artificial targets has suggested a particularly active TDMD pathway in neurons[11], a view consistent with earlier observations that miRNAs appear generally much less stable in neurons than in other cell types[13]. It is thus intriguing that libra and *Nrep* direct TDMD in vertebrate brains. However, a more comprehensive survey seems clearly warranted to understand whether and how cells or tissues may differ in their TDMD activity[14]. Mechanistically, the coincidence of reduced miRNA levels with accumulation of 3′-terminally tailed and trimmed miRNAs suggests that tailing and trimming are stages en route to full miRNA degradation. However, this hypothesis awaits rigorous

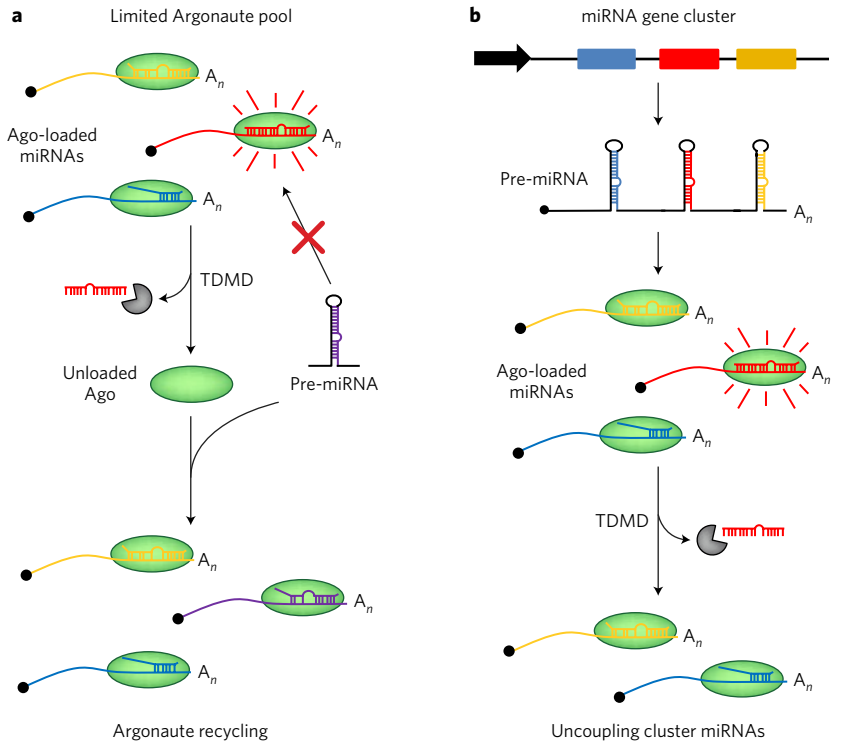

Fig. 3 | Control and diversification of miRNA expression patterns through TDMD. a, TDMD-inducing RNAs may promote recycling of Argonaute (Ago) proteins (green ovals) by enabling loading of newly expressed miRNAs. **b**, TDMD may also facilitate expression of specific miRNA family members from a single gene cluster, for example, in a tissue- or developmental-time-specific manner. Shine emphasis is shown on 'TDMD-primed' Ago–miRNA–target complexes. Credit: Kim Caesar/Springer Nature

testing. Identification of factors involved in the different steps followed by individual or combined manipulation would appear to offer a way forward. A previous study that used modified antisense oligonucleotides as TDMD inducers implicated terminal U transferases (TUTases) and DIS3L2, an exonuclease that prefers substrates with 3′-terminal oligo-U-tails, in TDMD[15] (Fig. 1a). Knowledge of libra/*Nrep* and potentially additional endogenous TDMD-inducers will offer an opportunity to test the roles of these enzymes in TDMD under physiological conditions and screen for other relevant factors. ❐

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Competing interests

The authors declare no competing interests.

CHROMATIN

Rewriting the past: de novo activity of PRC2 restores global H3K27 methylation patterns

Inheritance of Polycomb repressive complex 2 (PRC2)-mediated gene silencing involves self-propagation of histone H3 lysine 27 (H3K27) methylation from an initial nucleation site, but how the first H3K27 methylation marks are established is not fully understood. A recent study reveals that PRC2 can reconstitute H3K27 methylation de novo in cells that have lost the mark. This reconstitution is dependent on the PRC2 core component SUZ12, which provides a novel link between initiation and self-propagation of this critical epigenetic mark.

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PRC2 mediates mono-, di- and trimethylation of H3K27 (H3K27me1, H3K27me2 and H3K27me3, respectively) to repress developmental genes in different species, including flies, mammals and plants. In mammals, H3K27me3 is enriched in CpG islands (CGIs) and is required for transcriptional silencing[1]. Following replication, pre-existing