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MOLECULAR BIOLOGY

Circles reshape the RNA world

The versatility of RNA seems limitless. The latest surprise comes from circular RNAs, which are found to counteract the function of another class of regulatory RNA — the microRNAs.

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The protein-coding function of messenger RNAs can be suppressed by the binding of short microRNA sequences. But how microRNA-induced suppression is itself inhibited is poorly understood. In two papers published on *Nature*'s website today, Memczak *et al.*[1] and Hansen *et al.*[2] describe highly stable, circular RNAs that bind several copies of a microRNA to terminate suppression of mRNA targets.

The circular RNA (circRNA) reported, called CDR1as by Memczak *et al.* and ciRS-7 by Hansen *et al.*, contains roughly 70 evolutionarily conserved binding sites for microRNA-7 (miR-7) and forms a complex with AGO proteins. The latter are part of the RNA-induced silencing complex, which allows miRNAs to recognize their target mRNAs. When Memczak and colleagues expressed human CDR1as/ciRS-7 in zebrafish embryos, its effects were the same as those seen when miR-7 expression was reduced — impaired midbrain development. Moreover, the authors' bioinformatic predictions indicated that thousands of circRNAs reside in the genome, consistent with previous reports[3,4].

Target suppression by miRNAs is highly nuanced. On the one hand, these sequences can induce AGO-mediated endonucleolytic mRNA cleavage triggered by complementarity between the mRNA and the miRNA at nucleotides 10 and 11. The destruction of the target, which follows, frees the miRNA to bind to its next target in a catalytic manner. On the other hand, miRNAs can inhibit protein translation by binding more stably to a target mRNA in a stoichiometric manner. This makes the target a 'reservoir' that prevents the miRNA from inhibiting other mRNA targets. The latter mechanism is an indication of the way in which competing endogenous RNAs (ceRNAs) act. These are mRNAs that share miRNA-response elements (MREs) with other mRNAs and so compete for binding to those miRNAs with which they also share MREs[5].

Like ceRNAs, circRNAs serve as miRNA reservoirs. However, circRNAs have numerous binding sites for a specific miRNA and so are completely dedicated to their role of harbouring miRNAs. Binding of a miRNA to a ceRNA not only prevents that miRNA from binding to other MREs, but can also suppress translation from the coding portion of the ceRNA. Hence, compared with circRNAs, ceRNAs operate in a more complex weave of interacting molecules that constrains translation. Other reservoirs of target sites also reside on distinct molecules. These include target mimics such as the *IPS1* gene in the plant *Arabidopsis thaliana*[6], decoys within pseudogenes such as *PTENP1* (ref. 7) and possibly 3′-untranslated regions of mRNA that are expressed separately from their associated protein-coding sequences[8].

Circularizing RNA enhances its stability by obviating a role for RNA exonuclease enzymes, which act on free 3′ and 5′ ends of an RNA molecule to cleave it. Moreover, with several binding sites dedicated to antagonizing a single miRNA, a circRNA can capture miRNAs from numerous targets in one fell swoop. Likewise, circRNA destruction could release a shower of miRNAs that target multiple mRNAs with the shared MRE. In fact, Hansen *et al.* outline a circRNA-destruction mechanism in which miR-671 binds CDR1as/ciRS-7 with greater complementarity than miR-7 and induces AGO-mediated cleavage of this circRNA.

Snapshot approaches to profiling miRNAs reveal that the greatest changes in their expression levels occur at transition points in development, cell differentiation or carcinogenesis[9]. Clearance of the mRNA–miRNA duplexes at these points and their replacement with different miRNAs could operate through circRNAs. For instance, as a brute means of vacuuming up miRNAs, circRNAs could increase in expression as cell differentiation from stem cells proceeds, to capture the exceedingly high levels of miRNAs expressed in stem cells. They could also clean up the opposite strands of mature miRNAs, which can be present in surprisingly large numbers[10], or potentially function therapeutically to divert cancer-associated miRNAs

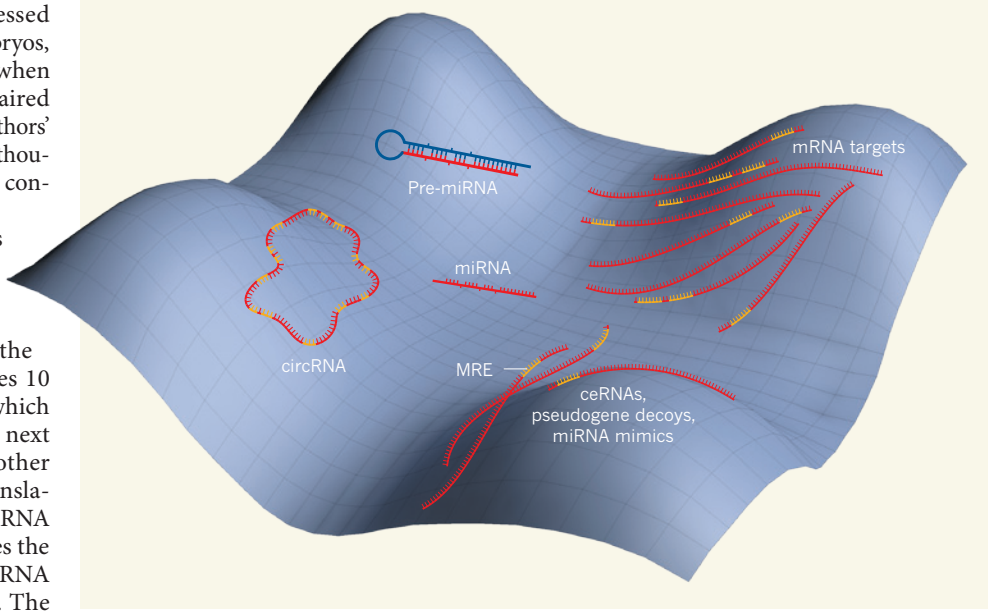

Figure 1 | Constraints on evolutionary change in microRNAs. MicroRNAs (miRNAs) lie in a fitness valley constrained by their numerous interactions, which include those with the hairpin structure of the precursor miRNA (pre-miRNA), the many target mRNAs and other RNAs that terminate or modulate miRNA binding to target sequences by competing against them. The latter category includes competing endogenous RNAs (ceRNAs), pseudogene decoys and miRNA mimics. Two studies[1,2] introduce circular RNAs (circRNAs) as another constraining factor. MRE, miRNA-response element.

from promoting an oncogenic pathway. In all these cases, however, the circRNAs sequester miRNAs, and so a knowledge gap remains regarding how miRNAs are destroyed.

To function optimally, the number of miRNA-binding sites on each circRNA is probably under selection pressure to attract nearly all of a specific miRNA population from all of its target sites. If so, the number of miRNA-binding sites on a circRNA multiplied by the number of copies of the circRNA in a single cell will inform us about the collective strength of all MREs for a particular miRNA. Many miRNAs operate at copy numbers of 10^3 per cell — a likely lower boundary for the number of circRNA sites required to mop them all up. However, for circRNAs to win out against mRNA targets in the competition for miRNA binding, they must have a greater affinity for the miRNAs. High affinity can be thermodynamically built into the circRNA sequence, but may also require an excess of circRNA-encoded miRNA-binding sites relative to the total number of other relevant MREs in the cell. Certainly, modellers will soon be romping through this territory.

The fitness 'landscape' that has contributed to maintaining each of the roughly 21-nucleotide miRNAs unchanged over major parts of evolution includes circRNAs (Fig. 1). The selection pressure on each miRNA nucleotide is undoubtedly high: an miRNA sequence must base-pair to itself to form the hairpin-shaped precursor miRNA; it must pair with a host of target mRNAs; and it must pair with binding sites that terminate or modulate target interaction. Despite the enormous number of possible miRNA sequences, the small amount of change in miRNAs implies that the remaining evolutionary space for innovation is limited; in other words, miRNAs have approached molecular perfection. Throughout animal evolution, nature has tinkered with the sequences of a relatively constant set of coding genes, whereas miRNA innovation, in general, is more reliant on the invention of completely novel sequences[11]. Perhaps the ease with which hairpin-shaped miRNA precursors can arise as potential regulatory elements — and fit 'digitally' into a wealth of genomic non-coding sequence, including circRNAs — could serve as a driver of evolution.

As a footnote, a better naming system for circRNAs is needed. 'ciRS-7' denotes binding to miR-7, and therefore assumes that other circRNAs in this category will also neatly align with a single miRNA. 'CDR1as' assumes that circRNAs will bear some relationship to a named gene — in this case, an antisense sequence to the cerebellar degeneration-related gene. With thousands of these circRNAs in the genome, they require their own numbering system. My suggestion is that this one is called circR-1. ■

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