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Insight into temperature-dependent microRNA function in mammalian hibernators

Perspectives on cold-influenced microRNA/target interaction

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Mammalian hibernation involves re-programming of metabolic functions, in part, facilitated by microRNA. Although much is known about microRNA function, we lack knowledge on low temperature microRNA target selection. It is possible that the thermodynamics of microRNA target selection could dictate unique temperature-dependent sets of microRNA targets for hibernators.

Mammalian hibernation is a natural winter survival phenotype that involves re-programming of metabolic functions in response to changes in an animal's surrounding environment. During hibernation, small mammals undergo an extreme depression of their metabolic rate (often to < 5% of euthermic values) in conjunction with a reduction in core body temperature (frequently decreasing from 37 °C to 0–5 °C).[1] To date, researchers have discovered and characterized a variety of molecular alterations that underlie this unique tolerance, including reversible post-translational protein phosphorylation, histone modification and post-transcriptional mRNA regulation by microRNA.[1-5] In this regard, we recently explored the regulation of microRNA in the brain tissue of hibernating little brown bats, identifying hibernation-responsive microRNA that are collectively involved in brain-associated cellular function.[3] Importantly, the necessity for microRNA to function at low temperature is unique to few mammals. This article presents an overview of microRNA biogenesis and function, and also expands on the current knowledge about temperature-modulated microRNAs. We also take the opportunity to introduce the possible existence of cold-influenced target interactions. It is possible that such interactions could dictate unique temperature-dependent sets of microRNA targets that exist only at the low body temperatures experienced by hibernating mammals.

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Hibernation research has now begun to highlight various adaptational roles for microRNA. These small non-coding RNA molecules are capable of inhibiting protein translation by binding to the 3′-untranslated region of target mRNAs. In particular, this research is exploring the possibility that microRNAs regulate the function of whole cellular processes.[3,4] A recent examples include the focused analyses of microRNAs involved in maintenance of brain function and muscle mass during hibernation in little brown bats (*Myotis lucifugus*).[3,4] In addition to pathway-focused analysis, studies have also begun to uncover unique structural alterations that exist in the precursor microRNA structures that allow efficient processing at low temperatures.[6]

MicroRNAs are short (~21 nucleotides in length) non-coding RNA transcripts involved in post-transcriptional regulation of gene expression in almost all eukaryotes. Currently, it is estimated that more than 60% of all protein-coding genes are regulated by microRNA. This likely results because of the ability of an individual microRNA species to target the transcripts of hundreds of genes, coupled with the fact that multiple microRNA species can target any given mRNA type, allowing for enormous regulatory potential and significant molecular crosstalk within the transcriptome.

Much work has been done in recent years to better understand the role of microRNA in mammalian hibernation. The initial study explored the expression of select microRNAs in tissues of hibernating 13 lined ground squirrels, *Ictidomys tridecemlineatus,* finding a torpor-responsive increase of miR-1 and miR-21 in kidney. Subsequently, miR-106b, a microRNA capable of regulating the hypoxia inducible transcription factor-1α (HIF-1α), was shown to decrease significantly in skeletal muscle of *I. tridecemlineatus* and liver of the little brown bat *M. lucifugus* during torpor.[2] Most recently, research on the brain of *M. lucifugus* identified the sequences of 344 mature microRNAs and proposed the involvement of 11 microRNAs in the regulation of brain-associated cellular function.[3] Analysis of microRNA potential targets (based at 37 °C) highlighted their potential involvement in focal adhesion and axon guidance during hibernation. Interestingly, these same processes were also independently shown to be regulated during hibernation in the brain of greater horseshoe bats (*Rhinolophus ferrumequinum*).[7]

Given the growing state of microRNA research, it is clear that these small RNA molecules have important roles to play in mammalian hibernation. However, of concern is the fact that the body of knowledge about hibernation-responsive microRNA is based mainly on expression profiling and microRNA target prediction

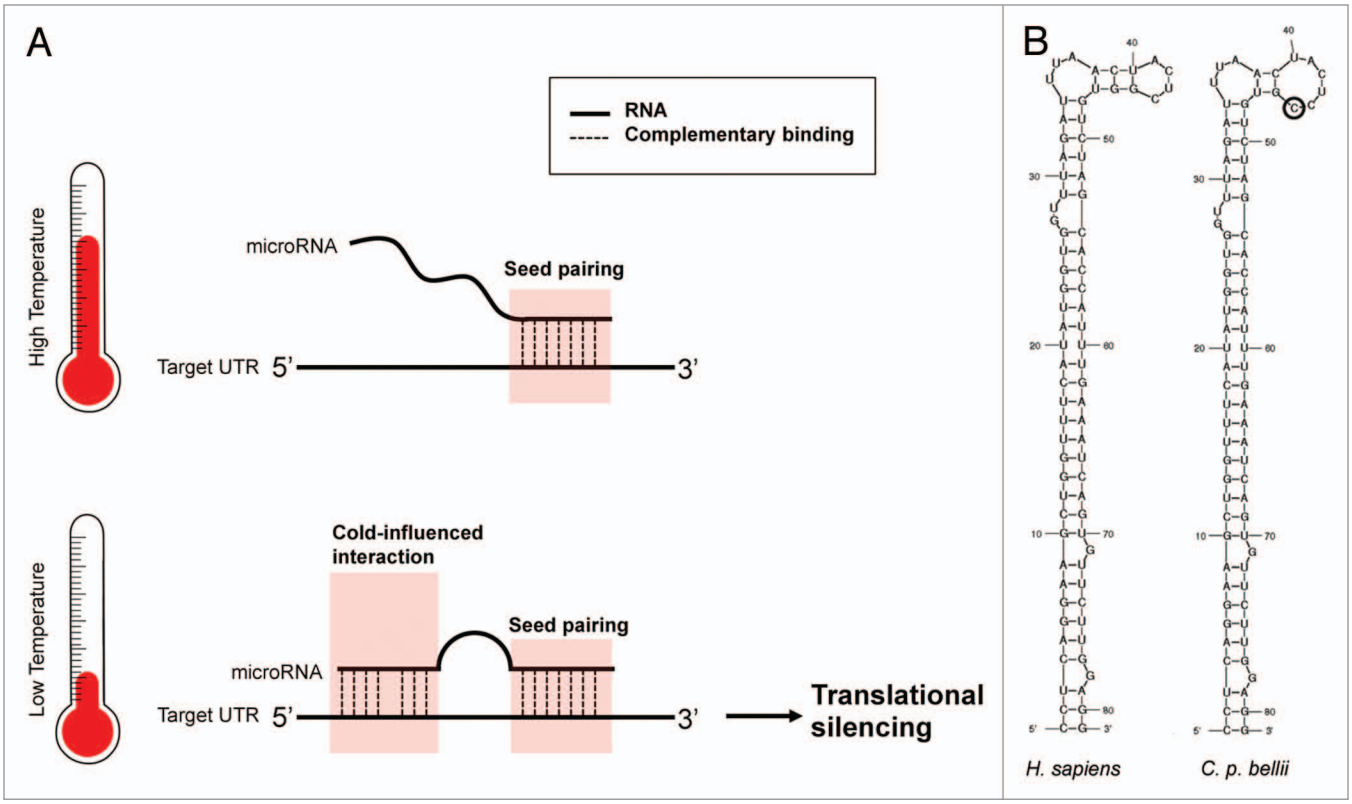

Figure 1. Mechanisms of possible temperature influence on microRNA expression and function. (**A**) Proposed mechanism of cold-influenced microRNA targeting. Low temperature, such as that experienced during mammalian hibernation, may act to stabilize microRNA/target interactions that are unstable at 37 °C, creating a unique subset of cold-influenced microRNA targets. (**B**) Nucleotide sequence and predicted secondary structure of pre-miR-29b transcripts from *H. sapiens* and *C. p. bellii* at 25 °C. The nucleotide substitution that leads to a terminal stem-loop formation that is unique to *C. p. bellii* is circled.

at euthermic body temperatures of 37 °C. Temperature-dependent influences that could contribute to microRNA interactions with target mRNAs have not yet been explored and will likely provide relevant insights into novel microRNA/target interactions that are unique to the hibernation phenotype.

MicroRNA target recognition relies on perfect Watson-Crick base pairing between nucleotides 2–8 of the microRNA (the crucial 'seed' region), with complementary binding from the 3' end of the microRNA acting to stabilize the interaction. Pairing normally occurs in the 3′-UTR of mRNAs and multiple microRNA recognition sites are often required for successful translational repression. Critically, the mean free energy threshold of -18 kcal/mol for microRNA/target is determined at 37 °C for almost all target prediction programs (including miRanda, targetscan and Diana microT). Although it is likely that a decrease in body temperature (such as that experienced by hibernators) will favorably stabilize the microRNA/target interactions that are predicted at 37 °C, it is also possible that select microRNA/target interactions that were once unfavorable at 37 °C, can reach the -18 kcal/mol threshold and become bonafide interactions at low temperature (such as 5 °C). This realization introduces the possibility that distinct cold-influenced microRNA/target interactions can exist, potentially playing unique hibernation-responsive roles and coordinating the stress response at low body temperatures (**Fig. 1A**). It is important to note that all other structural features of cold-influenced target interactions would still be required, including complementary seed pairing of the microRNA, whereas low temperature would influence the 3′-end complementary binding to stabilize the microRNA/target pair. Thus, the likelihood that microRNA could play an important role in helping various species to cope with temperature-related stress has garnered substantial interest, and future studies should start to consider the possible influence of temperature on microRNA targeting.

Apart from the possibility that temperature could play a critical role in the thermodynamics of microRNA targeting, studies have also documented distinct structural features in precursor microRNAs that can facilitate mature microRNA processing in cold-tolerant animals. Using the genome of the western painted turtle (*Chrysemys picta bellii*), a freeze tolerant species, researchers retrieved the precursor sequence of miR-29b, a microRNA that is overexpressed during freezing.[6] Based on its sequence, the secondary structure of western painted turtle pre-miR-29b was predicted to contain a single nucleotide mutation (nuc43) that resulted in a larger terminal stem-loop compared with the sequence from humans (**Fig. 1B**). Interestingly, secondary structures that

restrain the terminal loop region (as predicted for human) can decrease the efficiency of processing of precursor microRNA transcripts by the Dicer endonuclease in the range of 50%.[6] In addition to loop flexibility, slight alterations to loop structure and nucleotide sequence can also influence interactions between pre-microRNA and terminal loop binding proteins, impacting processing efficiency.

It is intriguing to hypothesize that microRNA expression and target selection may have unique low-temperature functions in mammals that endure low body temperatures. Future genome-wide analysis of microRNA structure and function are needed to determine such an outcome.

Conflicts of Interest Disclosure

The authors have no potential conflicts of interest to disclose

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