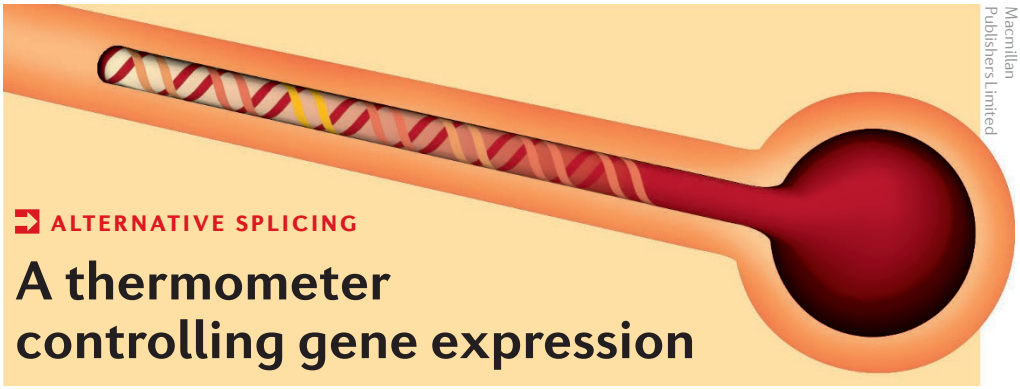
Nature Reviews Genetics | Published online 24 Jul 2017; doi:10.1038/nrg.2017.61



ALTERNATIVE SPLICING

A thermometer controlling gene expression

> alternative splicing acts like a thermometer to sense body temperature changes

To ensure a rapid response to environmental stress, the circadian clock — the endogenous oscillator that controls physiological and behavioural responses to the cycle of day and night — can be modulated by post-transcriptional mechanisms such as alternative splicing, instead of relying only on *de novo* transcriptional changes. A new study in *Molecular Cell* now reveals some of the molecular components and signalling cascades that lead to a switch in alternative splicing in response to minor physiological changes, such as the daily oscillations in body temperature in mice.

Preußner *et al.* first tested the effect of temperature changes on the rhythmic alternative splicing of the *U2af26* transcript in mouse neuronal N2A cells. The *U2af26* pre-mRNA was previously shown to be alternatively spliced in a circadian and light-inducible manner through the skipping of exons 6 and 7. Cold shock (32 °C) increased levels of U2af26Δ67, the isoform that is light-induced. By contrast, Δ67 isoform levels were significantly reduced after a heat shock (42 °C), with nearly exclusive formation of the full-length isoform, typical of the circadian night. The authors next tested a physiologically relevant temperature range (35–40 °C); alternative splicing of *U2af26* still changed fivefold and exhibited a near-perfect linear correlation with temperature.

The authors then compared mice kept under a 12-h light–dark cycle at Zeitgeber time (ZT; a standard of time based on the period of an environmental synchronizer, such as the 24-h cycle of light and darkness) 7 (when body temperature is low) and ZT14 (when body temperature is increased). The temperature measured in the brain correlated inversely with *U2af26* exon skipping in the cerebellum and the liver, that is, skipping of *U2af26* exons 6 and 7 also correlated with temperature *in vivo*. In young (12-day-old) mice, which are unable to regulate their own body temperature and thus do not oscillate in body temperature at ZT0 (the time of 'lights on') and ZT8, no increase was found in the U2af26Δ67 isoform in the cerebellum or the liver at ZT8. Decreasing the environmental temperature, which reduces the body temperature of young mice but not of older mice, revealed an increase in *U2af26* exon 6 and 7 skipping in the cerebellum and the liver only in young mice. This finding suggests that body temperature changes are sufficient for the regulation of alternative splicing *in vivo*.

A small interfering RNA (siRNA) screen targeting SR proteins, which are known to be involved in the heat shock response, implicated serine/arginine-rich splicing factor 2 (SRSF2) and SRSF7. Knocking down either protein increased exon 6 and 7 skipping, whereas overexpression led to a reduction. Combining protein knockdown with changes in temperature or temperature rhythms revealed that the response to temperature changes was either reduced or abolished. *In vivo* binding of SRSF2 and SRSF7 to the pre-mRNA was confirmed using individual-nucleotide resolution crosslinking and immunoprecipitation (iCLIP) data. Furthermore, the manipulation of SR-protein phosphorylation showed that hyperphosphorylation of SR proteins inhibits *U2af26*-variable exon inclusion, whereas dephosphorylation of SR proteins promotes exon inclusion.

To determine the extent of temperature-induced alternative splicing, the authors analysed RNA sequencing (RNA-seq) data from mouse embryonic fibroblasts kept at cold temperatures for 12 h. They identified 202 altered splicing events, including *U2af26* exon 6 and 7 skipping. According to a gene ontology term analysis, cold-responsive genes were predominantly involved in RNA binding, gene expression regulation or the regulation of metabolic processes. For all investigated targets, alternative splicing correlated with changes in temperature in the physiological range and in a linear manner. Taken together, the findings suggest that alternative splicing acts like a thermometer to sense body temperature changes, translating these into molecular consequences.

Linda Koch

ORIGINAL ARTICLE Preußner, M. *et al.* Body temperature cycles control rhythmic alternative splicing in mammals. *Mol. Cell* http://dx.doi.org/10.1016/j.molcel.2017.06.006 (2017)
FURTHER READING Takahashi, J. S. *et al.* Transcriptional architecture of the mammalian circadian clock. *Nat. Rev. Genet.* **18**, 164–179 (2017)