The heat's on: nuclear stress bodies signal intron retention

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The cellular response to heat shock requires massive adaptation of gene expression driven by the transcription factor HSF1, which assembles in nuclear stress bodies together with human satellite III RNA and numerous splicing factors. In this issue of *The EMBO Journal*, Ninomiya *et al* demonstrate that nuclear stress bodies serve as a platform for phosphorylation of the SR protein SRSF9 by the CLK1 kinase, which promotes retention of a large number of introns during the recovery phase from heat shock.

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See also: K Ninomiya *et al* (February 2020)

Thirty years ago, the laboratory of Jean-Paul Fuchs discovered that heat shock (HS) induces the assembly of ribonucleoprotein particles into electron-dense granules that are tightly associated with the nuclear matrix (Mähl *et al*, 1989). A few years later, Richard Morimoto's laboratory observed that HSF1, the major transcription factor driving expression of HS-induced genes, accumulates upon thermal stress in nuclear granules of human, but not mouse, cells (reviewed in Biamonti & Vourc'h, 2010). The group of Guiseppe Biamonti was then able to connect the two observations by showing that scaffold attachment factor B (SAFB), an RNA-binding and chromatin-associated protein that binds to hnRNP complexes and regulates the transcription of heat shock protein genes, co-localizes with HSF1 in subcellular structures we now call nuclear stress bodies (nSBs). These studies already revealed the dynamic nature of nSBs, as HSF1 relocates to nSBs within minutes upon HS, whereas SAFB

associates with nSBs later during the HS response and remains in nSBs during the recovery phase.

Another important piece to the puzzle was added by the laboratories of Claire Vourc'h and Guiseppe Biamonti through their discovery that nSBs assemble on heterochromatic DNA regions composed of a subclass of human satellite III (HSATIII) repeats located primarily on chromosome 9q12 and that HSF1 binds with high affinity and specificity to HSATIII DNA. Satellite III and closely related satellite II are primate-specific pericentromeric repeats located on most human chromosomes. They are composed of divergent 5-bp repeat units that vary in their arrangement and array size from several kbp to Mbp, overall accounting for approximately 1.5% of the human genome (Altemose *et al*, 2014). HS causes activation of HSF1, which accumulates at HSATIII DNA regions and promotes massive transcription of HSATIII RNA. Interestingly, this RNA remains close to its site of transcription and builds an architectural scaffold of nSBs. The accumulation of HSATIII RNA is further responsible for the sequestration of SR proteins (SRSFs), RNA-binding proteins that serve as essential regulators of splicing. SRSFs are characterized by a C-terminal intrinsically disordered region (IDR) containing stretches of serine-arginine (SR) and serine-proline (SP) dipeptides. In particular, SRSF1 (SF2/ASF), SRSF7 (9G8), and SRSF9 (SRp30) were found to accumulate in nSBs (Biamonti & Vourc'h, 2010). While nSBs were proposed to play a role in controlling pre-mRNA splicing already at the time of their first description (Mähl *et al*, 1989), a clear function could so far not be assigned to these nuclear condensates.

Now, the laboratory of Tetsuro Hirose provides experimental evidence that nSBs are essential to suppress splicing of a large number of pre-mRNAs by mediating intron retention during the recovery phase following HS (Ninomiya *et al*, 2019). To demonstrate this, the authors abolished nSBs by knockdown (KD) of HSATIII RNA expression in HeLa cells and conducted a transcriptome-wide splicing analysis during and after HS. Their main observation is that HSATIII KD cells fail to retain > 500 introns in > 400 different mRNAs. Lack of intron retention in KD cells occurs primarily during the recovery phase following HS and affects two classes of mRNAs: Class I show retention of one (sometimes two) intron(s) at normal temperature; their pre-mRNAs are fully spliced during HS; and intron retention (in wild-type cells) is re-established during the recovery phase. Class 2 mRNAs are fully spliced at both normal temperature and during HS, while intron retention sets in only during the recovery phase (Fig 1). Notably, the authors demonstrate that intron retention prevents export of the pre-mRNAs from the nucleus and leads to reduced levels of spliced mRNAs, indicating that intron retention is an effective means to suppress gene expression at the posttranscriptional level.

To pursue the mechanism by which HSATIII RNA promotes intron retention, Ninomiya *et al* purified HSATIII RNA-associated proteins by RNA-protein crosslinking inside cells followed by capture of HSATIII RNA using a biotinylated antisense probe. Mass spectrometry analysis revealed 141 proteins associated with HSATIII RNA, including known constituents of nSBs such as the SR proteins SRSR1, SRSF7, and SRSF9. Of particular interest was the

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Figure 1. Phosphorylation of SRSF9 promotes intron retention during recovery from heat shock.

Under normal conditions, SR proteins are hyperphosphorylated at their intrinsically disordered regions containing SR and SP dipeptides. Upon heat shock, SR proteins are rapidly dephosphorylated. Concomitantly, HSF1 accumulates at human satellite III (HSATIII) repeat DNA arrays, recruits the acetyltransferase CBP to acetylate histone tails, and drives transcription of HSATIII RNA by RNA polymerase II (POL2). HSATIII RNA forms a scaffold for the assembly of nuclear stress bodies (nSBs) and recruits certain hnRNP and SR proteins including SRSF9. Ninomiya *et al* (2019) discovered that during the recovery from heat shock, CLK1 kinase associates with nSBs where it re-phosphorylates SRSF9. Hyperphosphorylated SRSF9 in turn prevents splicing of > 500 introns, which leads to intron retention within > 400 pre-mRNAs and thereby inhibits expression of the corresponding mRNAs.

isolation of CDC2-like kinase 1 (CLK1), known to phosphorylate SR/SP repeats within the IDR of SR proteins. SR proteins are hyperphosphorylated under normal conditions, yet become dephosphorylated during HS (Shi & Manley, 2007). Interestingly, Ninomiya *et al* (2019) observe that CLK1 associates with HSATIII RNA and localizes in nSBs only during the recovery from HS, and that re-phosphorylation of SRSF9 during the recovery phase depends on CLK1 and HSATIII RNA. The study then comes full circle by the demonstration that intron retention during the recovery phase is dependent on SRSF9 and CLK1, and that a non-phosphorylatable SRSF9 mutant, in contrast to the wild-type protein, cannot restore intron retention of two mini-gene reporters in SRSF9 KD cells. Taken together, the authors' tour de force provides

compelling evidence that phosphorylation of SRSF9 by CLK1 occurs in nSBs and drives intron retention during the recovery phase from HS. Intron retention appears ideally suited to shape stress responses given that it represents a powerful mechanism to suppress gene expression in a reversible manner while keeping the pre-mRNA ready for instant activation. Such a mechanism might be of particular interest for long genes whose transcription can take more than an hour, whereas splicing of a single intron may be achieved within seconds.

The current study by Ninomiya *et al* (2019) points to an essential role for nSBs in preparing cells for recovery from HS. An interesting question is whether nSBs are also important for splicing regulation during HS. In fact, cells manifest widespread inhibition of splicing that affects approximately one

fourth of all genes especially during severe HS (Shalgi *et al*, 2014), and hnRNP proteins as well as SR protein dephosphorylation have been implicated in HS-induced inhibition of global splicing (Gattoni *et al*, 1996; Shin *et al*, 2004). Does the sequestration of certain hnRNP and SR proteins drive splicing inhibition during HS? Are nSBs important for HS-induced dephosphorylation of SR proteins? The HSATIII RNA KD approach by Ninomiya *et al* (2019) indicates that this is not the case, at least not under mild HS conditions of 42°C. Rather, the laboratory of Ganesh reported that HSATIII RNA is important for suppressing transcription of certain genes during HS, which might be linked to recruitment of the histone acetyltransferase CBP to nSBs (Goenka *et al*, 2016). Hence, a picture is emerging where nSBs appear to regulate transcriptional repression during

the HS response while orchestrating specific splicing events by promoting intron retention during the recovery phase.

While a full HS response might occur only during episodes of high fever in mammals, it is interesting to note that the normal mammalian body temperature oscillates with the time of the day, which drives rhythmic phosphorylation of SR proteins together with concerted changes in splicing (Preußner *et al*, 2017). Hence, one may speculate that the molecular mechanisms underlying nSB function might also control physiologically oscillating gene expression patterns during the circadian rhythm.

Liquid–liquid phase separation underlies the formation of stress-induced membraneless compartments such as cytosolic stress granules and nSBs. Chromatin itself can be described as a molecular condensate generated by histone tail-driven phase separation, whereby acetylation of histones promotes transition to a distinct phase-separated chromatin state (Gibson *et al*, 2019). Interestingly, phosphorylation of RNA polymerase II (POL2) at its IDR (the C-terminal domain) appears to dictate whether POL2—in its hypophosphorylated form—preferentially associates with condensates that mediate transcription initiation or—in its hyperphosphorylated form—associates with condensates characterized by a high concentration of splicing factors

(Guo *et al*, 2019). We envision that nSBs represent a variation on this theme, where phase separation of nSBs during HS could be initiated by chromatin acetylation leading to a high rate of HSATIII transcription. During the recovery phase, the nSB condensate acquires new properties through re-phosphorylation of SR proteins, which promotes intron retention and may eventually cause disassembly of nSBs.

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