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Cellular stress and RNA splicing

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In response to physical and chemical stresses that affect protein folding and, thus, the execution of normal metabolic processes, cells activate gene-expression strategies aimed at increasing their chance of survival. One target of several stressing agents is pre-mRNA splicing, which is inhibited upon heat shock. Recently, the molecular basis of this splicing inhibition has begun to emerge. Interestingly, different mechanisms seem to be in place to block constitutive pre-mRNA splicing and to affect alternative splicing regulation. This could be important to modulate gene expression during recovery from stress. Thus, pre-mRNA splicing emerges as a central mechanism to integrate cellular and metabolic stresses into gene-expression profiles.

Constitutive and alternative pre-mRNA splicing

Pre-mRNA splicing is the process by which introns are removed from pre-mRNAs to form the mature mRNA. This reaction is carried out by a complex macromolecular machine, the 'spliceosome', which consists of five small nuclear ribonucleoprotein particles (snRNPs) U1, U2, U4, U5 and U6, and ~100 non-snRNP splicing factors (for review, see Ref. [1]). Spliceosome assembly is a highly coordinated multistep process that involves the sequential recognition of conserved sequences in the pre-mRNA and the successive organization of distinct, well-characterized ribonucleoprotein complexes (RNPs; see Glossary) [2] (Box 1). This stepwise remodeling of the splicing complex entails a large number of weak protein–protein and protein–RNA interactions that are the targets of specific post-translational modifications and that might integrate the various signals coming from within or outside the cell. Intriguingly, with the exception of the first and last di-nucleotides at the intron boundaries, splice sites correspond to short, loose consensus sequences. This raises several questions about the molecular mechanisms underlying the recognition of proper splice sites by the spliceosome. By contrast, this flexibility of the spliceosome in recognizing different splice-site sequences, which can potentially lead to missplicing events (e.g. in tumors), has been exploited during evolution for an important phenomenon known as alternative splicing. In this process, different combinations of splice sites can be joined to each other, resulting in the synthesis of several structurally and functionally distinct protein isoforms thereby enabling large proteomic complexity from a limited number of genes (for review, see Ref. [3]). Initially considered as a peculiarity of a few genes involved in sex determination in the fruit fly, it is now clear that alternative splicing is an important mechanism to control gene expression; indeed, at least 75% of human genes undergo this regulation. Exons undergoing alternative splicing are usually flanked by splice sites that poorly match the consensus sequence. Usually, an interplay of *cis*-acting sequences and *trans*-acting factors modulates the splicing of regulated exons, most frequently in tissue-specific and/or developmentally regulated patterns [4]. Thus, splicing factors recognize either positive (splicing enhancers) or negative (splicing silencers) *cis*-acting sequence elements, which are either exonic or intronic, leading to exon inclusion or skipping, respectively. Usually, splicing enhancers are bound and activated by serine/arginine-rich proteins (SR proteins), which comprise a family of structurally related proteins that are highly conserved throughout evolution and are involved in both constitutive and alternative-splicing regulation [5]. In these proteins, one or two N-terminal RNA-recognition motifs (RRMs) are followed by a C-terminal domain rich in alternating serine and arginine residues, which is known as the RS domain. The activity of SR proteins in alternative splicing can be antagonized by another group of RNA-binding proteins, the heterogeneous nuclear ribonucleoprotein proteins (hnRNPs) (for review, see Ref. [6]). The relative abundance and activity of these proteins might control the splicing profile of a large number of genes. However, in certain cases, tissue-specific splicing factors also have a prominent role in alternative-splicing regulation, as in the case of Nova proteins, which are neuron-specific alternative-splicing factors [7].

Glossary

hnRNP: heterogeneous nuclear ribonucleoprotein (hnRNP) family of proteins, which in humans consists of at least 20 different polypeptides. These proteins are associated with pre-mRNA in the nucleus and affect many different RNA processing events, both in the nucleus and in the cytoplasm.
HSF1: heat-shock factor 1 is a transcriptional regulator of the stress response.
PP1: protein phosphatase 1; belongs to the PPP family of serine/threonine protein phosphatases and participates in the regulation of many physiological processes.
PTB: the polypyrimidine tract binding protein (PTB, also known as hnRNP I) is a ubiquitous RNA-binding protein with a role in pre-mRNA splicing. It binds to the polypyrimidine tract at the 3′ splice site. As shown for some SR proteins, PTB participates in some post-splicing activities including mRNA stability and translation initiation.
RNP: ribonucleoprotein, a complex of RNA and bound proteins.
SR proteins: this family of proteins comprises several phylogenetically conserved and structurally related proteins with a characteristic domain rich in arginine and serine residues, known as the RS domain. They have a dual role in pre-mRNA splicing: they affect not only constitutive splicing but are also important alternative splicing regulators. In addition, they participate in post-splicing activities, such as mRNA nuclear export, nonsense-mediated mRNA decay and mRNA translation.
Stress granules: cytoplasmic domains that harbor translationally arrested mRNAs that accumulate in cells exposed to a broad range of stresses, including oxidative, genotoxic, hyperosmotic or heat shock.

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Box 1. Spliceosome assembly

During spliceosome assembly, the 5′ and 3′ splice sites are recognized by the U1 snRNP and the heterodimeric splicing factor U2 snRNP auxiliary factor (U2AF), respectively, to form the E complex. The U2 snRNP is then recruited by U2AF to the branch point, in an ATP-dependent manner, generating the A complex. The pre-catalytic B complex is formed by the recruitment of the U4–U6–U5 tri-snRNP to the pre-spliceosome. This is followed by a series of complex rearrangements that lead to the displacement of U1 and U4 snRNP [65]. Subsequent formation of the activated B* spliceosome promotes the first step of splicing that gives rise to the cleaved 5′ exon and intron–3′-exon lariat intermediates. Finally, the C complex catalyzes the second step of splicing, during which the intron is excised and the 5′ and 3′ exons are ligated to form mRNA [66] (Figure I).

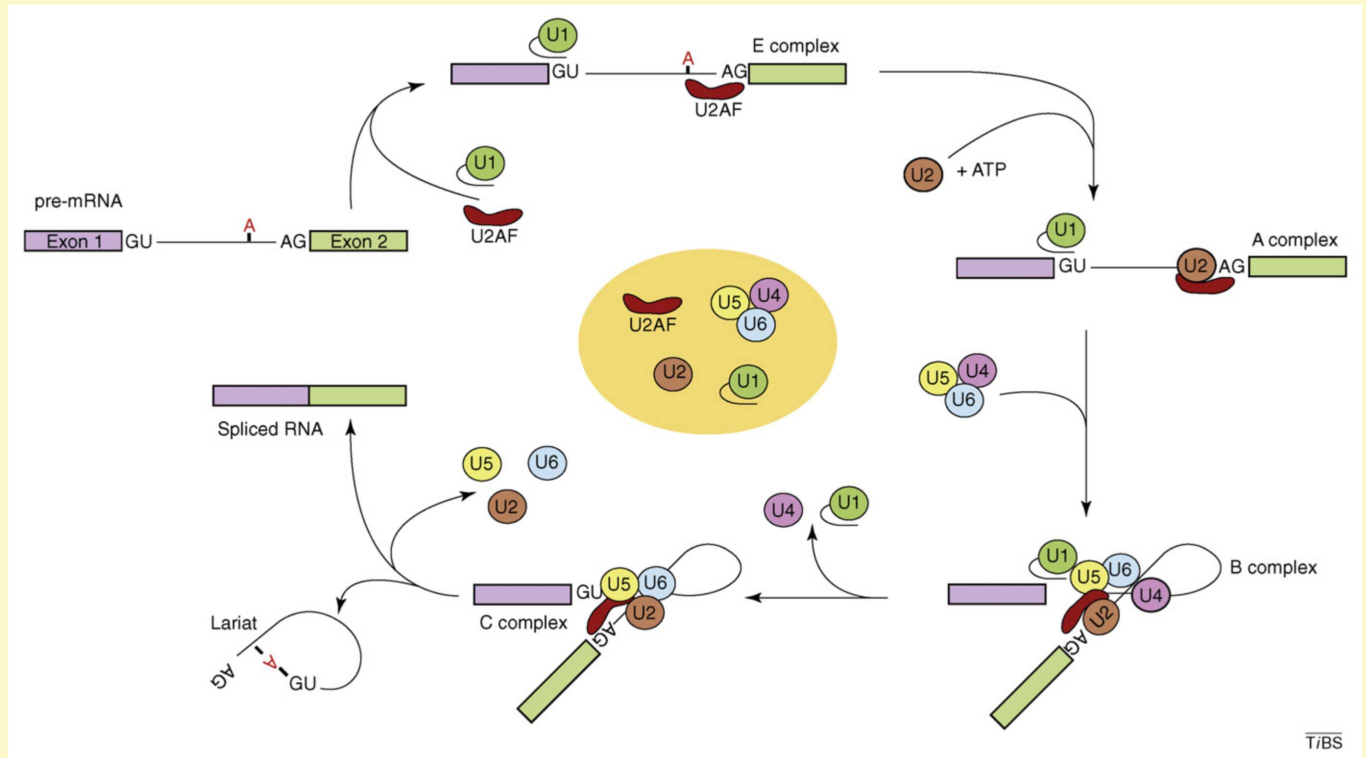

Figure I. Cartoon depicting spliceosomal assembly.

Owing to its broad impact on gene-expression programs, alternative splicing provides an ideal means to modulate gene expression to the needs of the cells in response to stressing conditions. This is even more evident if it is considered that alternative-splicing events are direct targets of signal-transduction cascades. Thus, impacting on specific signaling pathways could have drastic effects on splicing of specific pre-mRNAs, sometimes giving rise to protein isoforms with unique properties upon stress stimuli. For example, 5′ splice-site selection in the 5′ non-coding region of the *HSP47* (heat-shock protein 47) pre-mRNA is affected by heat shock, giving rise to a splicing isoform that is translated more efficiently under heat-shock conditions [8]. Cold shock, but not heat shock or hyper-osmolarity, induces neurofibromatosis type 1 (*NF1*) pre-mRNA alternative splicing, which promotes the inclusion of a cryptic exon. Interestingly, this alternative-splicing event is not cell-type specific [9]. Alternative-spliced isoforms of MDM2 (murine double mutant 2), a negative regulator of p53, are induced upon genotoxic stress, resulting in p53 activation and triggering of the p53 damage response [10]. Finally, long-term neuronal hypersensitivity under stress is associated with stress-induced alternative splicing of neuronal acetylcholinesterase (*ACHE*) pre-mRNA [11]. This process has been linked with increased expression of the SR protein SC35, which promotes sustainable stress-induced alternative splicing of neuronal acetylcholinesterase mRNA [12]. Changes in splicing activity associated with stress are not exclusive to mammalian cells; indeed, recent work shows that the splicing of ribosomal protein-encoding genes is specifically inhibited in response to amino acid starvation in yeast [13]. Furthermore, stress-induced alternative splicing is of crucial importance for stress adaptation in plants (for review, see Ref. [14]).

Here, we focus on the impact of cellular stress, most notably heat shock, on the gene-expression cascade. In particular, we highlight how stress stimuli block pre-mRNA splicing and affect the regulation of alternative splicing. We present a few possible mechanisms by which stress stimuli inhibit RNA splicing, but we do not focus on the many connections between alternative splicing and cancer, which have been covered in some recent reviews [15,16].

Splicing is blocked in heat-shocked cells

Stressing agents, such as heat shock, lead to an immediate block of every important metabolic process, including DNA replication, transcription, mRNA export and translation, until the cells recover. Heat-shock proteins (HSPs),

which are induced by heat-shock transcription factors (HSFs), protect cells from injury and facilitate recovery and survival after a return to normal growth conditions (for review, see Ref. [17,18]) (Box 2). One of the best characterized changes in the response to stress is the shutdown of pre-mRNA splicing observed in heat-shocked cells [19–21]. Importantly, this change does not affect the expression of HSP genes because most do not contain introns. Intriguingly, however, introns in *HSP90a*, *HSP90b* and *HSP27* gene transcripts are properly spliced in heat-shocked human cells indicating that the block of splicing is not complete [22]. Many experiments have taken advantage of the fact that the inhibition of pre-mRNA splicing can be recapitulated in extracts from heat-shocked cells [20,23] and have shown that the splicing block after heat shock is caused by the inactivation of an activity termed HSLF (heat-shock labile splicing factor), which functions in the assembly of the U4–U6–U5 tri-snRNP [21]. However, it should be pointed out that despite the robust block in splicing of the few transcripts tested, the true extent of splicing inhibition upon heat shock has not been studied using global approaches. Therefore, it is not entirely clear whether the heat-shock-induced block in pre-mRNA splicing affects the majority of pre-mRNAs, whether all transcripts are affected to a similar degree, or whether heat shock targets only specific subsets of pre-mRNAs.

Box 2. Cellular stress

Stressing factors include various environmental (e.g. heat, cold, UV-light, oxygen, ion balance and heavy metals) and pathological factors (e.g. infections, inflammation, fever and ischemia). The cellular response depends on the nature and the intensity of the stressing condition. Some stressing agents directly challenge the integrity of the genome (genotoxic stress) by generating different types of DNA damage or perturbing the DNA replication process. Other agents cause protein denaturation, lipid peroxidation or a disturbance in the cellular redox state. The intensity of stress is also an important determinant for the type of response mounted by the cells; severe stresses are cytotoxic and can cause permanent growth arrest or cell death either by apoptosis or necrosis [67]. The best characterized stress-defense mechanism, which is triggered by a variety of stressing conditions, involves the transcriptional activation of a set of genes encoding molecular chaperones and those, for historical reasons, are called heat-shock genes. In vertebrate cells, heat-shock genes are under the control of a family of transcription factors: the so-called heat-shock factors, HSF1 to HSF4 [68]. Among them, only HSF1 is crucial for the activation of heat-shock genes after thermal stress. HSF1 is maintained in an inactive form in the cytoplasm. Its activation upon heat shock requires several events that ultimately lead to HSF1 trimerization and nuclear import, in which this transcription factor binds the heat-shock element in the promoters of heat-inducible genes [69]. In addition to the heat-shock response, other defense mechanisms are activated in a stress-specific manner. For example, the oxidative defense mechanism encompasses both enzymatic (superoxide dismutase, peroxidases and catalases) and non-enzymatic (glutathione and thioredoxin) detoxification mechanisms that destroy reactive oxygen species or restore the cellular redox balance [70,71]. The cellular defense against cadmium and heavy metals entails the synthesis of protective molecules such as metallothioneins and glutathione [72]. DNA-damaging agents (such as UV irradiation) trigger p53 and DNA-checkpoint-pathway activation. Finally, the cellular response to increased osmolarity can be divided in to immediate and delayed phases. The immediate response takes place within seconds and involves an increase in the intracellular concentration of charged ions such as potassium, sodium and chloride that are mediated by pre-existing ion transport systems [73]. The delayed or adaptive response, by contrast, is a slow process; it occurs over a period of hours and requires the activation of genes that enable ionic osmolytes to be replaced with non-ionic ones. The activation of these genes depends on the activity of transcription factor TonEBP (tonicity enhancer binding protein) [74].

SRp38

SRp38 is an SR protein family member with a single N-terminal RRM followed by a C-terminal RS domain that displays unique properties [24,25]. It was originally identified independently as a neuron-specific SR protein, NSSR-1 [26], and also in a two-hybrid screen using the human translocation liposarcoma (*TLS*) gene as bait [27]. When overexpressed in human cells it antagonizes the activity of classical SR proteins SF2/ASF (splicing factor 2; also known as alternative splicing factor) and SC35, and activates the more distal 5′ splice sites of the adenoviral *E1A* gene [24].

In vitro studies showed that, in contrast to classical SR proteins, phosphorylated SRp38 functions as a sequence-specific activator of splicing and that its activity requires the presence of an as yet unidentified cofactor. This unique activity stems from the ability of SRp38 to enter a ternary complex with U1 snRNP and pre-mRNA that strengthens the interaction of U1 and U2 snRNP with the pre-mRNA. However, in the absence of this cofactor, SRp38 promotes the formation of splicing complex A, but cannot progress to complexes B and C [28]. Another distinguishing feature of SRp38 is its ability to function as a potent and splicing repressor when dephosphorylated in M phase or in response to heat shock [25,29]. In agreement, SRp38 depletion results in cell-cycle alterations, specifically a prolonged G2/M phase, and poor recovery from heat shock (for review, see Ref. [30]). After mild heat shock (43 °C for 30 min), SRp38 phosphorylation is re-established *in vivo* within the first hour of recovery from stress, and this modification correlates well with a full restoration of splicing activity. Mechanistically, this inhibitory effect was proposed to be based on the interaction of dephosphorylated SRp38 (dSRp38) with the U1 snRNP-associated protein U1 70K, thereby preventing its association with other SR proteins [29].

These same authors elucidated the molecular mechanism underlying SRp38 dephosphorylation upon thermal stress. The serine/threonine phosphatases PP1 and PP2A have been shown to have a role in SR protein dephosphorylation [31,32]. Furthermore, it was recently shown that PP1 interacts directly with the RRM of several SR proteins, including SF2/ASF, SRp30c and transformer2-β1 [33]. The use of specific chemical inhibitors *in vitro* and RNA-interference-mediated depletion *in vivo* revealed that two PP1 isoforms, PP1β and PP1γ, are responsible for SRp38 dephosphorylation during heat shock. Interestingly, despite a role for these PP1 isoforms in dephosphorylating SR proteins, only SRp38 is found preferentially dephosphorylated after heat shock. This difference stems from the differential low kinase activity on SRp38 compared with other SR proteins. Thus, the activation of these PP1 isoforms upon heat shock due to their dissociation from their natural inhibitor NIPP1 (a nuclear inhibitor of PP1),

together with the differential activity of SR protein kinases on different SR proteins, establishes a complex pathway that controls the SRp38 phosphorylation status and regulates its activity with high specificity in response to heat shock [34]. Furthermore, the association of SRp38 with 14-3-3 proteins, a family of highly conserved proteins that bind to phospho-serine/threonine residues in a variety of proteins, protects it from dephosphorylation under non-stress conditions. Importantly, however, this complex dissociates upon heat shock, thereby enabling PP1, which dissociates from NIPP1 to act upon SRp38 and cause its dephosphorylation (Figure 1).

How is SRp38 phosphorylation re-established? Several reports indicate a role of HSPs in the recovery of splicing after heat shock. Thus, in cells that have accumulated HSPs as a result of a previous stress, splicing is less affected by a successive heat shock [19,20,35,36]. In yeast, Hsp70p and Hsp104p seem to repair splicing after heat shock both *in vitro* and *in vivo* although they are not involved in splicing thermotolerance. Moreover, Hsp70p associates with the U4–U6–U5 tri-snRNP in thermotolerant yeast cells [37]. Recently, exogenous Hsp27p expression was shown to enhance recovery from splicing after severe heat shock (1 h at 45 °C). This correlates with the ability of Hsp27p in facilitating SRp38 re-phosphorylation [38]. Contrary to other SR proteins, SRp38 is a poor substrate for two well-characterized SR protein kinases, the SR-specific protein kinase (SRPK)1 and the dual specificity kinase Clk (CDC-like kinase 1; also called Sty); therefore, it is likely that an as yet unidentified kinase is involved in this phenomenon.

Nuclear stress bodies

The mammalian cell nucleus comprises a number of specialized domains and is highly dynamic (for review, see Refs [39,40]). The nuclear stress bodies (nSBs) were originally identified as the site of heat-shock factor 1 (HSF1) accumulation in stressed cells [41]. They transiently appear in human cells subjected to mild heat shock and have also been referred as nuclear stress granules, SAM68 nuclear bodies, HSF1 granules or HAP granules (for hnRNP-A1-associated protein) [42]. The nSBs are induced in human cells not only by heat shock but also by chemical and hypertonic stresses and are sites of accumulation for a subset of pre-mRNA processing factors [43]. In particular, three SR protein family members, SF2/ASF, 9G8 and SRp30c, are efficiently recruited to nSBs, whereas SC35 is unaffected. This selective recruitment leads to changes in the relative abundance of splicing factors in other nuclear districts with predictable effects on alternative-splicing profiles. So far, however, this has been verified only for one splicing reporter: the adenoviral *E1A* gene [43].

What is the mechanism underlying the recruitment of these splicing factors? First, it should be taken into account that nSBs are assembled on chromatin regions consisting of long tandem arrays of satellite III (SatIII) DNA, a human specific repetitive DNA element, found at pericentromeric heterochromatic regions of a few human chromosomes including human chromosome 9 [44,45]. Unexpectedly, after heat shock, these heterochromatic regions shift to euchromatin as shown by the presence of acetylated histone H4 and the lack of HP1 (heterochromatin protein 1) and/or histone H3 methylated on lysine 9 [46,47]. This chromatin reorganization is accompanied by the HSF1-driven transcription of SatIII RNAs that remain associated with transcription sites and have a fundamental role in the biogenesis of nSBs. Importantly, the recruitment of two SR proteins, SF2/ASF and SRp30c, requires the presence of stress-induced SatIII transcripts [48] and, consequently, downregulation of these transcripts blocks the recruitment of these splicing factors to the nSBs [49]. SF2/ASF recruitment is mediated by the second RRM of the protein, which seems to a have an important role in alternative-splicing decisions [50]. This is reminiscent of the situation observed in *Drosophila melanogaster* where upon stress stimuli hnRNP proteins are recruited to the non-coding hsr ω nuclear transcripts [51]. Based on these

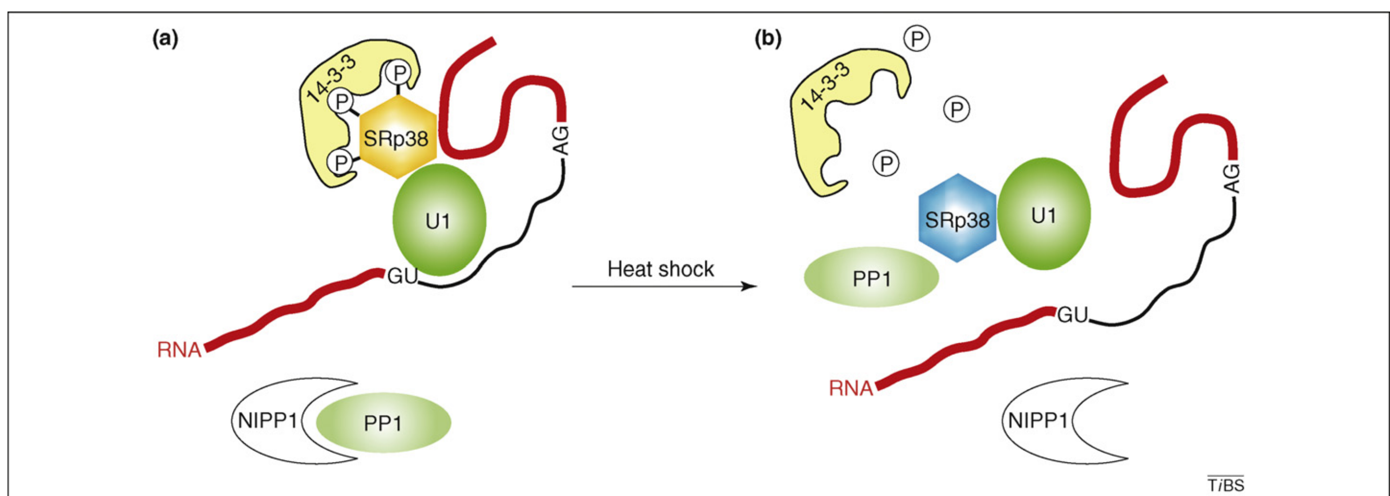

Figure 1. SRp38 phosphorylation affects the interaction of U1 snRNP with pre-mRNA. In unstressed cells, phosphorylated SRp38 (shown in gold) interacts with a specific RNA sequence element and stabilizes the interaction of U1 snRNP (green) with the pre-mRNA (red thick line indicates the exon and the thin black line indicates the intron, with the GU sequence denoting the 5′ splice site). **(a)** The interaction with 14-3-3 (yellow) protects SRp38 from dephosphorylation, and the PP1 (light green) phosphatase activity toward SRp38 is inhibited by the association with NIPP1 (white). Heat shock promotes the dissociation both of 14-3-3 from SRp38 and of NIPP1 from PP1. **(b)** As a result, SRp38 becomes dephosphorylated (denoted by the change to blue). Dephosphorylated SRp38 stably interacts with U1 snRNP, preventing the association of this snRNP with pre-mRNA. The net effect is inhibition of the splicing reaction.

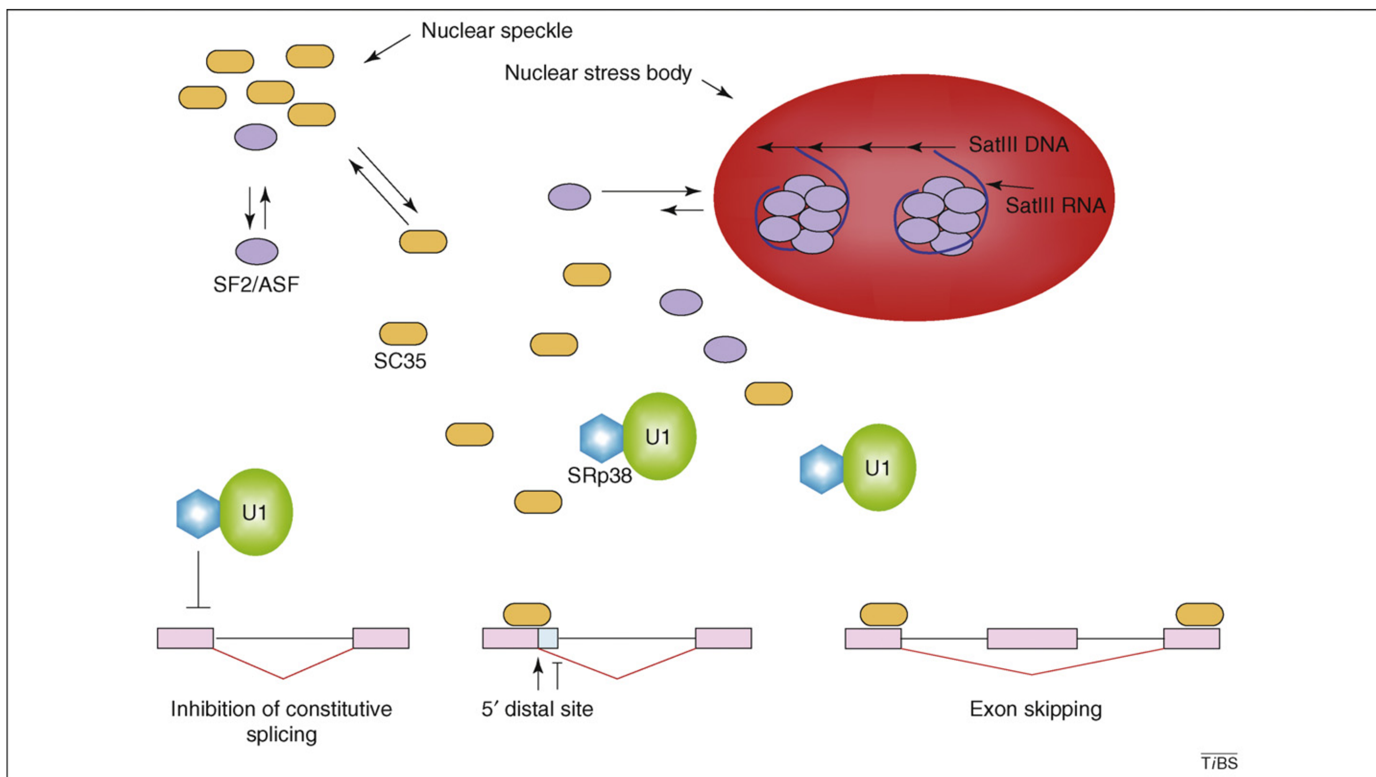

Figure 2. Different mechanisms control the splicing reaction and alternative-splicing profiles in heat-shocked human cells. During heat shock, dephosphorylated SRp38 (blue) stably interacts with U1 snRNP (green), thus affecting constitutive splicing. At the same time, heat shock induces the transcription of tandem arrays of SatIII repeats in the heterochromatic pericentromeric band of human chromosome 9. SatIII RNAs (dark blue) remain associated with sites of transcription and recruit a subset of pre-mRNA processing factors including the splicing factor SF2/ASF (purple), giving rise to the formation of nSBs (red). The distribution of other splicing factors such as SC35 (gold) is unaffected. The bottom panel shows dephosphorylated SRp38 binding to the U1 snRNP particle and inhibiting constitutive pre-mRNA splicing. The differential sequestration of SF2/ASF to the nSBs changes the ratio between splicing factors available for the splicing reaction, thus affecting alternative splicing profiles and affecting both 5′ splice-site selection and exon-skipping alternative-splicing events.

results, a model for nSB assembly and disassembly has been proposed, whereby nSBs regulate splicing activity during stress by sequestering a subset of pre-mRNA splicing factors to a particular subnuclear region where they bind to SatIII transcripts [42,43] (Figure 2).

Stress and relocalization of splicing factors

There is a growing list of stress conditions that, through the activation of signaling pathways, lead to phosphorylation of target splicing factors and change their subcellular distribution, activity and/or association with multiprotein complexes. For instance, heat-shock-induced splicing inhibition is associated with a loss of hnRNP M from spliceosomal complexes, a finding which could further implicate this protein in constitutive splicing [52,53]. Another example comes from the phosphorylation of the polypyrimidine-tract binding protein (PTB; also known as hnRNP I) by protein kinase A that results in PTB cytoplasmic accumulation, which is most likely to affect its cytoplasmic functions [54]. In primary neuronal cultures, a rise in intracellular calcium levels promotes the cytoplasmic accumulation of the SR-related protein tra2-β 1 and consequently affects alternative-splicing patterns in the brain [55].

One of the most extensively characterized events is the stress-induced subcellular relocalization of hnRNP A1, a nucleo-cytoplasmic shuttling protein that antagonizes SR proteins during alternative splicing and is also involved in other aspects of mRNA metabolism. In response to different stress stimuli, including osmotic shock or ultraviolet-C (UVC) irradiation, hnRNP A1 accumulates in the cytoplasm localizing to the cytoplasmic stress granules (SGs). This event is linked to the activation of the mitogen-activated protein (MAP) kinase 3/6–p38 (MKK3/6) stress-signaling pathway and is mediated by the MAP-kinase-interacting serine/threonine kinase (Mnk1 and Mnk2) protein kinases that act downstream of p38 [56,57]. Stress-induced hnRNP A1 phosphorylation by the Mnk1/2 kinases abrogates the interaction with its import receptor, transportin, thus resulting in hnRNP A1 accumulation in the cytoplasm [58]. This accumulation causes an altered ratio of the antagonistic alternative splicing factors SF2/ASF and hnRNP A1 in the nucleus and consequently affects alternative splicing regulation. Thus, stress signals can control alternative splice-site selection *in vivo* by regulating the subcellular localization of antagonistic pre-mRNA splicing factors [56]. The physiological importance of hnRNP A1 in the stress response is underscored by the observation that cells lacking hnRNP A1 exhibit decreased viability rates both during stress and also after release from stress conditions [57]. In a similar manner, cell stress (e.g. arsenite exposure) induces phosphorylation of the splicing factor RBM4 (RNA-binding motif protein 4) and drives its cytoplasmic accumulation and targeting to SGs, via the MKK3/6–p38 signaling pathway, where it inhibits translation [59]. This mechanism

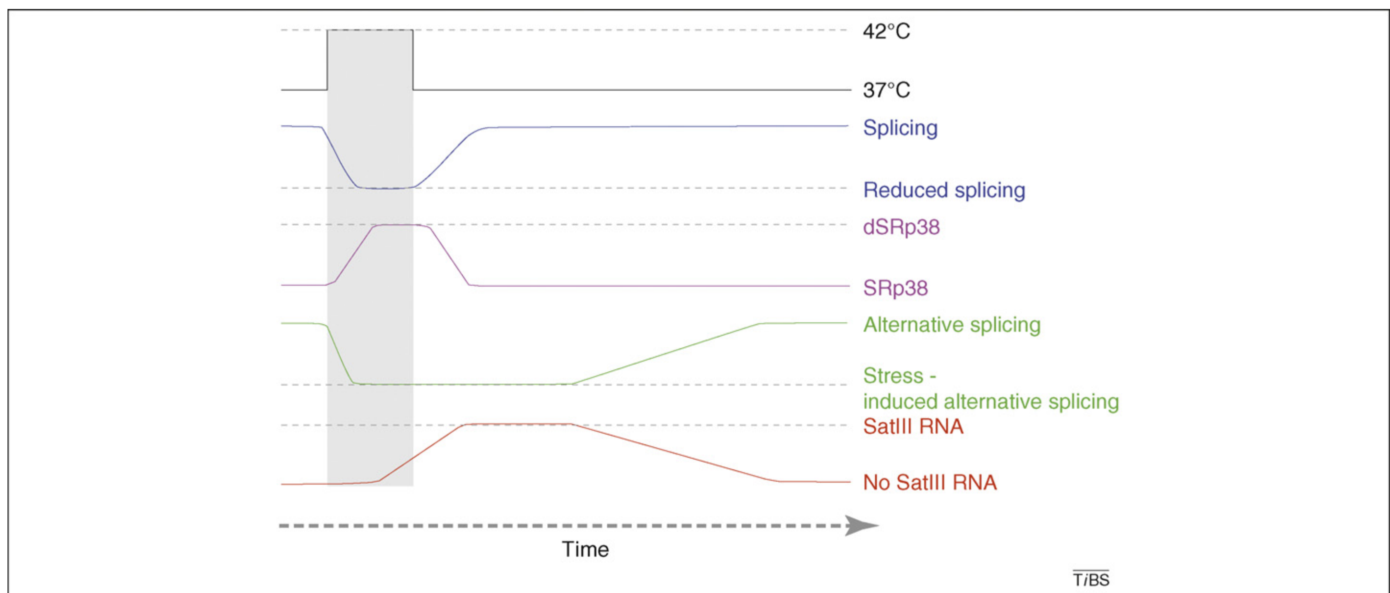

Figure 3. Kinetics of constitutive splicing and alternative-splicing profiles after heat shock. Mild heat shock (42 °C; black line in the upper part of the figure) has several effects on splicing, some of which occur during heat shock and others during the recovery phase at 37 °C. As soon as cells are shifted to 42 °C, SRp38 is rapidly dephosphorylated (dSRp38; purple line) and the splicing reaction is inhibited (blue line). SRp38 phosphorylation is re-established rapidly during the recovery from stress at 37 °C, and this is accompanied by the resumption of splicing. Heat shock also induces the production of SatIII RNAs (red line). This occurs mainly during the first part of the recovery from stress and is accompanied by the sequestration of a subset of splicing factors, including SF2/ASF, in nSBs. At longer recovery periods the level of SatIII RNAs decreases and splicing factors return to their physiological distribution. The selective sequestration of splicing factors, such as SF2/ASF to the nSBs, is expected to affect alternative-splicing profiles (green line).

seems not to be unique for hnRNP A1 or RBM4; stress stimuli influence the subcellular localization of several other RNA binding proteins, including the second step splicing factor hSlu7 [60] and HuR (human antigen R) [61]. hSlu7 is required for correct 3′ splice-site choice [62]. UVC light and heat shock alter its cellular distribution in a Jun N-terminal kinase-dependent signaling cascade and leads to changes in alternative-splicing regulation by decreasing the hSlu7 nuclear concentration [60].

Concluding remarks

The response to cellular stress involves extensive changes in gene expression causing, among other responses, a shutoff of pre-mRNA splicing. Is the SRp38 phosphorylation status the only and/or main determinant of splicing inhibition in stressed cells? A biochemical complementation assay determined that splicing inhibition in heat-shocked cells could be attributed to a deficiency in a biochemical activity required for tri-snRNP formation in stressed cells [21]. It is not clear, however, whether dephosphorylated SRp38 can interfere with tri-snRNP formation [30]. The heat-shock-mediated induction of nSBs, together with the associated titration of splicing factors, is likely to modulate alternative-splicing pathways. However, this mechanism operates only in primates, in which SatIII sequences are present. It is unclear whether (and how) a similar regulatory pathway is active in other mammals. Importantly, a role for dephosphorylated SRp38 and/or SatIII transcripts has not been documented for other types of stress, including genotoxic stress or hyperosmotic shock; this reinforces the view that other mechanisms could be involved in the response to diverse types of cellular stress.

The different kinetics of SRp38 dephosphorylation and recruitment of splicing factors to nSBs after mild heat shock of HeLa cells could provide a possible explanation for the existence of different mechanisms to control splicing after heat shock. SRp38 dephosphorylation occurs during stress treatments and is fully reversed during the first hour of recovery, in parallel with the splicing reaction. By contrast, the recruitment of splicing factors to nSBs occurs mainly during stress recovery (peaking at 3 h of recovery) and is progressively reversed over a period of ~10 h. It is possible that the stress-induced accumulation of splicing factors at nSBs could merely reflect their role in the splicing and/or stability of the SatIII RNA transcripts. A more likely interpretation is that the reactivation of constitutive splicing by SRp38 phophorylation is followed by the modulation of alternative-splicing profiles for genes whose products participate in cell recovery. Hopefully, future experiments will address this hypothesis (Figure 3). Finally, heat-shock factors and downstream target genes are activated in several cell differentiation processes. It is intriguing to speculate that this activation could perturb splicing and alternative-splicing profiles through the routes described here. It has been recently shown, both in *Saccharomyces cerevisae* and in *Drosophila*, that perturbation of core splicing factors can result in transcript-specific effects [63,64]. Thus, it seems possible that any stress-responsive signaling pathway that targets the splicing machinery can still display some transcript specificity. For example, this could be achieved if individual transcripts have different sequence-dependent sensitivities to dephosphorylated SRp38. The use of global approaches to tackle this issue will shed light on the specificity of the block to pre-mRNA splicing upon stress.

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