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SPOTLIGHT

# Splicing under stress: A matter of time and place

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**Excision of introns during splicing regulates gene expression. In this issue, work by Sung et al. (https://doi.org/10.1083/jcb.202111151) demonstrates that the timing of intron removal in response to stress is coordinated in nuclear speckles, adding a component of spatial regulation to co-/post-transcriptional splicing.**

Eukaryotic gene expression is a tightly regulated, multi-step process in which the timing and spatial organization of each step play major roles (1). Transcription and co-transcriptional RNA processing take place on chromatin and within the timescale of transcription. A typical human gene is 30 kb long, corresponding to ∼10 min in total. Accumulating evidence indicates that most introns are removed co-transcriptionally, and 3′ end cleavage and polyadenylation is, also by definition, co-transcriptional. Nevertheless, post-transcriptional mRNA maturation steps can take place off chromatin, i.e., after a transcript has undergone 3′ end cleavage and before it is either exported to the cytoplasm or degraded through nuclear surveillance mechanisms. Interestingly, intron retention can be a specific regulatory step, usually during development. As examples, intron retention followed by carefully timed delayed splicing is required for proper development of granulocytes, neurons, and spermatocytes (see references in 1).

A variety of membraneless "nuclear bodies" compartmentalize functions such as pre-rRNA processing and pre-ribosomal subunit assembly in nucleoli and spliceosomal snRNP assembly in Cajal bodies (2). Nuclear bodies likely serve to increase the efficiency of nuclear processes, owing to the higher local concentration of components. Although this is difficult to show experimentally, existing data support this interpretation. Spliceosomal factors, splicing regulators, and 3′ end processing factors are all concentrated in nuclear speckles, which form an extensive network within the interchromatin spaces of the cell nucleus. The RNA-binding proteins (RBPs) present in nuclear speckles are highly dynamic, diffusing with the same rapid kinetics both within and outside of speckles. This dynamicity of components renders nuclear speckles responsive to conditions that change gene expression, such as cell cycle, the action of specific kinases (e.g., CDK1 and DYRK3), and/or transcriptional shut-off (2–4).

Now, a paper in this issue from Sung et al. demonstrates in human cells that ribotoxin-induced stress triggers remodeling of nuclear speckles concomitant with increases in splicing of intron-retained nuclear transcripts as well as increases in mRNA half-lives (5). Mycotoxins, such as anisomycin (ANI) and harringtonine (HT), blocked ribosome activity as well as cell proliferation. Immediate early genes, such as *FOS*, localize to the remodeled speckles wherein pre-mRNA splicing is enhanced. The study identifies a pivotal role for p38 mitogen-activated kinase (MAPK) activation in nuclear speckle reorganization. This new work reveals how nuclear speckles create functional spaces with the potential to change over time as cells are confronted with physiological stresses underpinned by the action of kinases (Fig. 1).

It was previously shown that the cyclin-dependent kinase 1 (CDK1) is inhibited under conditions of DNA replication stress by its localization to G2/M transition granules (GMGs) by the RNA binding protein TIAR (6). TIAR plays a key role in stress response, regulating translation in the cytoplasm and alternative splicing in the nucleus (7). Using high-resolution microscopy, Sung et al. (5) report that, upon stress, nuclear speckles are sites of enhanced recruitment of factors involved in splice site recognition such as TIAR, core spliceosome components, and Ser2 phosphorylated RNA polymerase II (Pol II). This suggests that nuclear speckles are important sites of post-transcriptional splicing also under stress conditions. Interestingly, TIAR recruitment to nuclear speckles is only transient and followed by its export to the cytoplasm, where it accumulates in G3BP1-positive cytoplasmic stress granules at longer time points. TIAR and Tia-1 proteins are well established participants in stress granule nucleation. This temporary localization to nuclear speckles was dependent on the activation of p38/MAPK signaling, as confirmed by the usage of specific inhibitors. These findings highlight how the activity of TIAR in the nucleus regulates the cell cycle in response to diverse sources of stress like ribotoxins, DNA damage, and blocks to replication.

Remarkably, this activity of TIAR seems to translate to cancer and chemotherapy resistance. HT is used as a cancer therapy drug to target proliferating cells, wherein therapy-induced ribotoxic stress leads to cell death. The challenge for pharmaceutic design remains to target quiescent cells with low DNA damage, reduced rate of DNA replication and mRNA translation. Therefore, the contribution of TIAR to cell cycle

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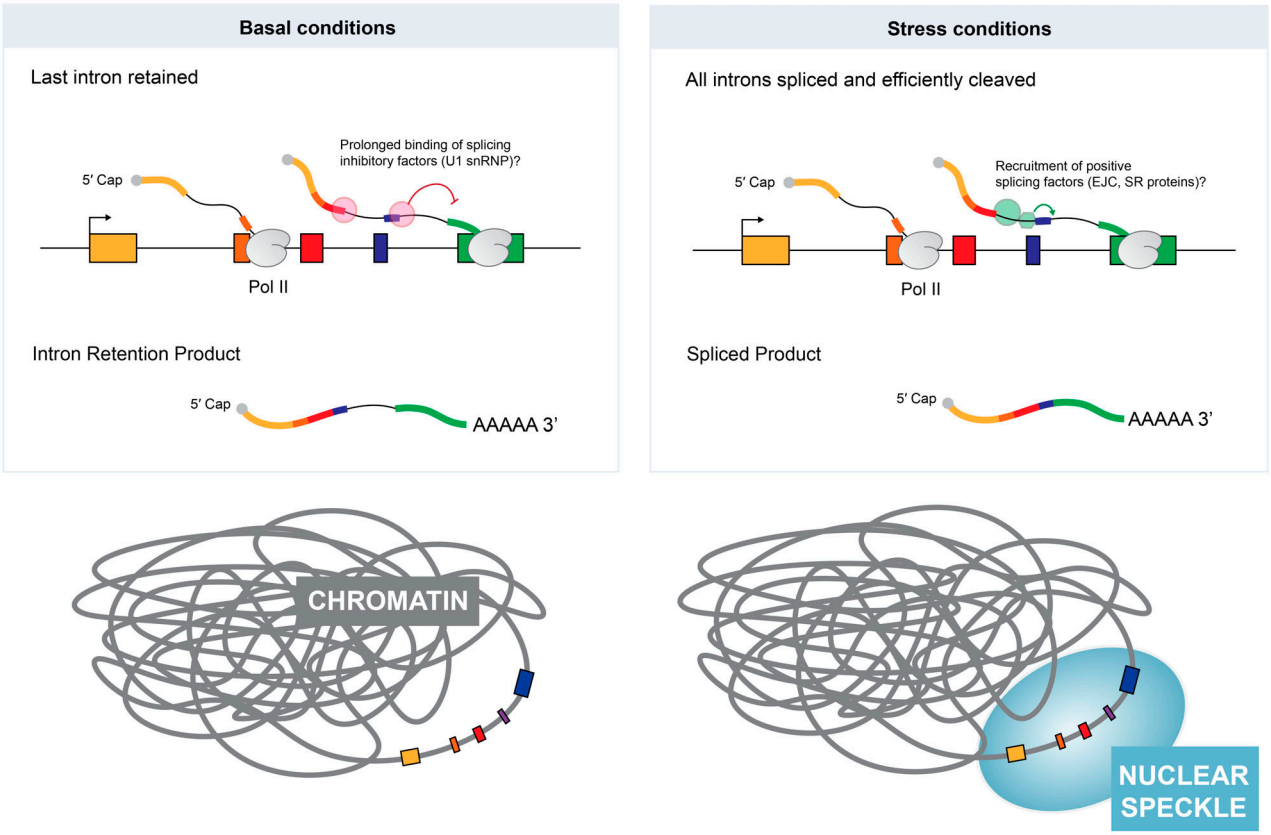


Figure 1. **Dependence of immediate early gene transcripts splicing on the cellular state.** Under basal conditions, excision of introns is delayed and becomes accelerated upon stress. This is accompanied by the formation of nuclear speckle around gene transcription upon stress.

arrest corroborates its inclusion in the essentials of chemotherapy resistance. Correspondingly, TIAR depletion reduces the survival of chronic myeloid leukemia cells upon HT treatment in hypoxic conditions, where reduced proliferation potential is the main founder of resistance (8). Additionally, Sung et al. implicate TIAR in the activation of immediate early genes, known to regulate processes detrimental to cell fate decisions, which expression is activated upon therapy-induced replication stress accompanied by chromatin remodeling and changes in splicing (9).

The authors analyze splicing by generating total RNA-seq datasets with and without transcriptional shut-off. It is known that post-transcriptional splicing takes place in nuclear speckles in the absence of stress (10). The latter dataset enabled a determination of mRNA half-lives revealing a change in the median half-life of mRNA from 5 to 9 h. Better splicing, detected by analysis of intron/exon read coverage—could contribute to longer half-lives upon ribotoxic stress. Changes in RNA stability alone do not explain the reduced detection of intron reads, suggesting a corresponding increase in the splicing of nascent transcripts as well as pre-existing mRNA. Gene-centered analysis revealed that the pool of post-transcriptionally spliced pre-mRNA was enriched in transcripts of immediate early genes. These were characterized by enhanced intron retention in basal conditions and accelerated intron removal upon ribotoxic stress.

What is more important? The time or the place? The ribotoxin-enhanced splicing could in principle occur co- or post-transcriptionally. Because authors detect Pol II in nuclear speckles, these genes could be undergoing co-transcriptional splicing due to the relocalization of genes themselves. Using RNA-fluorescence in situ hybridization (RNA-FISH) they show that transcription loci of *ZFP36* and *FOS* mRNA colocalized with nuclear speckles under ribotoxic stress but not basal conditions. This spatial rearrangement was reliant on p38/MAPK activity. Such a dependency was not observed in the case of *NR4A2* mRNA that upon stress was not in nuclear speckles. This demonstrated that acceleration of intron excision in nuclear speckles is specific for certain genes and their transcripts. The molecular mechanisms controlling this timing remain to be elucidated.

This dynamic remodeling of nuclear speckles in response to stress would also be consistent with the concept that membraneless RNP granules are driven by RNA itself (11). The spatial separation and timing of the excision of selected introns show the complexity of this process. The connection between p38 kinase activity and nuclear speckle formation leaves an intriguing open question of the underlying molecular mechanism. Phosphorylation of key components by cyclin-dependent kinases might orchestrate the dynamics of nuclear speckles in response to proliferation (12). The authors relate the excision of retained introns observed in transcripts encoding immediate early genes to nuclear speckle reorganization upon stress. Given the power of IEG to direct cellular fate, this spatial regulation of splicing allows precise fine-tuning of the level of mature mRNA. Considering post-transcriptional splicing,

detaining a backup pool of transcripts with retained introns would allow activation of expression when transcription is disturbed under stress conditions. Apart from that, differential excision of introns and exons could be a source of protein-coding isoforms. This expands the variability of the proteome, broadening the range of adaptive possibilities of cells and could play a pro-survival role in terms of stress response and chemotherapy resistance. Moreover, all of these considerations emphasize the likelihood that nuclear speckle form and function likely adapt to many cellular conditions beyond those currently analyzed.

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