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Pre-mRNA splicing: where and when in the nucleus

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Abstract

Alternative splicing is a process to differentially link exon regions in a single precursor mRNA to produce two or more different mature mRNAs, a strategy frequently used by higher eukaryotic cells to increase proteome diversity and/or enable additional post-transcriptional control of gene expression. This process can take place either co-transcriptionally or post-transcriptionally. When and where RNA splicing takes place in the cell represents a central question of cell biology; co-transcriptional splicing allows functional integration of transcription and RNA processing machineries, and could allow them to modulate one another, whereas post-transcriptional splicing could facilitate coupling RNA splicing with downstream events such as RNA export to create additional layers for regulated gene expression. This review focuses on recent advances in co- and post-transcriptional RNA splicing and proposes a new paradigm that some specific coupling events contribute to genome organization in higher eukaryotic cells.

Introduction

Pre-mRNA splicing is essential for gene expression in mammalian cells in which most protein-coding genes are disrupted by intervening sequences (introns). The process to remove introns is efficient and precise, thus constituting the vast majority of constitutive splicing events in the cell. However, most transcripts in higher eukaryotic cells also contain regions that are subjected to alternative selection, resulting in the production of different mRNA isoforms. This process, known as alternative splicing, has been recognized as a mechanism to increase the functional diversity of the proteome, and to introduce additional layers for regulated gene expression, because different mRNA isoforms often alter gene coding capacity or exhibit distinct RNA stability. Because different mRNA isoforms are often produced in different cell types or tissues, alternative splicing has been increasingly linked to important biological pathways in development and disease [1,2].

RNA splicing, whether constitutive or alternative, is catalyzed by the macromolecular machinery known as the spliceosome consisting of U1, U2, U4/U6, and U5 small nuclear ribonucleoprotein particles (snRNPs) and numerous protein factors [3]. Many years of research have established the canonical pathway for stepwise assembly of the spliceosome on pre-mRNA, beginning with binding of U1 snRNP to the 5′ splice site and U2 snRNP

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binding to the branchpoint at the 3′ splice site, followed by the joining of U4/6:U5 tri-snRNPs to establish the mature spliceosome for RNA-based catalysis [4,5].

Although spliceosome assembly has been traditionally studied in cell-free nuclear extracts, this process appears intertwined with other key steps in gene expression in the nucleus, including transcription and RNA export [6,7]. In contrast to 5′ capping, which is tightly coupled with transcription reinitiation [8], and to 3′ end formation, which is closely linked to transcription termination [9,10], RNA splicing can proceed either during transcription (co-transcriptional splicing) or after transcription and release of the transcript from template DNA (post-transcriptional splicing). This distinction is functionally important because co-transcriptional splicing could subject splicing to regulation by diverse transcription-dependent mechanisms, whereas post-transcriptional splicing might allow additional regulatory mechanisms to operate or couple splicing with other downstream events such as RNA export. This raises a series of questions: (i) how frequently does splicing take place co- versus post-transcriptionally in the cell; (ii) what determines that a splicing event proceeds in a specific mode; and (iii) do co- and post-transcriptional splicing have distinct impacts upon the regulation of alternative splicing or gene expression in general. In this review we address these questions in light of recent advances in the field.

Co-transcriptional splicing: a general rule, with exceptions

Co-transcriptional RNA splicing for most constitutive splicing events has become a consensus in the field based on multiple lines of evidence: (i) electron microscopy (EM) reveals looped RNAs attached to chromatin [11]; (ii) spliced mRNAs are associated with mechanically dissected or biochemically fractionated chromatin [12,13]; (iii) RNA *in situ* hybridization with splice-junction probes detects spliced mRNAs on their gene loci [14]; and (iv) introns are removed from nascent RNA before the completion of transcription in transcriptionally synchronized cells [15]. These model gene-based studies have now been extended to most genes in yeast using fractionated chromatin coupled with tiling array analysis [16]. The evidence for co-transcriptional intron removal is also consistent with co-transcriptional recruitment of splicing factors in both yeast [17,18] and mammalian cells [19] and with numerous physical interactions and functional interplays between the transcription and splicing machineries [6].

Co-transcriptional RNA splicing at the 5′ end of genes makes sense from a kinetic point of view because typical genes in eukaryotic cells contain short exons and long introns. This could provide the splicing machinery sufficient time to recognize the 5′ and 3′ splice sites associated with a transcribed exon while the elongation complex proceeds through the downstream intron and the rest of the gene. Therefore, it is entirely conceivable that most introns at the 5′ end of genes are co-transcriptionally removed. However, a significant fraction of introns near the 3′ end of genes appears to be post-transcriptionally spliced, and this has been documented by comparing chromatin-associated RNA versus released RNA [12,13]. In addition to the general gradient along the direction of gene transcription, with increasing transition from co- to post-transcriptional splicing, there are exceptions in which internal splicing events take place after transcription and, as a result, splicing order does not strictly follow the 5′ to 3′ direction [20]. In fact, several dendrite-associated pre-mRNAs

appear to be exported to the cytoplasm where their splicing is activated by Ca^{2+} signaling [21,22]. An extreme example of post-transcriptional splicing is the interleukin-1β pre-mRNA, a fraction of which remains unspliced even after the cell becomes anucleate and can be induced to splice in response to signaling in anucleate platelets [23]. These observations suggest that some splicing events are regulated by specific developmental cues or external signals long after the completion of transcription.

Therefore, although co-transcriptional splicing might generally apply to most introns, it is not an obligatory process for all introns. The problem is that we currently lack a global picture on how many splicing events, especially those subject to regulation, occur co-versus post-transcriptionally. This crucial question requires future investigation using global approaches, especially those based on deep sequencing.

Global approaches to constitutive and regulated splicing

Three general types of global approaches are particularly relevant to splicing research (illustrated in Figure 1) by allowing genome-wide sampling of regulatory mechanisms and functional consequences. Chromatin immunoprecipitation coupled with deep sequencing (ChIP-seq) has been widely used to study genetic and epigenetic regulation of transcription. This technique can now be applied to specific components of the RNA processing machinery to understand their roles in catalyzing co-transcriptional splicing and imposing various regulatory strategies [24,25]. For example, the SR family of splicing factors is required for committing pre-mRNA to the splicing pathway [26], and there is clear evidence that these and other splicing factors are co-transcriptionally recruited to the elongating RNA polymerase II (Pol II) complex to facilitate co-transcriptional splicing [17–19].

Recent work advancing our understanding of transcription/splicing coupling has established two important concepts. The first concerns the direct impact of regulated transcription upon alternative splicing because both promoter identity and Pol II processivity appear to have profound influence on splicing outcomes [27,28]. This concept also potentiates the regulation of RNA splicing by epigenetic strategies. Indeed, both chromatin remodeling factors, particularly components of the SWI/SNF complex [29], and specific chromatin marks [30,31] have been implicated in modulation of alternative splicing in specific gene models. The second concept is that co-transcriptional splicing can, in turn, affect transcription [32–34]. Although it is possible that specific splicing factors could have a dual role in transcription and splicing, recent work in yeast has reinforced the notion of mutual influence by demonstrating that splice sites can function as a checkpoint for the elongating Pol II complex [35]. It is now crucial to determine how these regulatory principles might be generally applied to regulated splicing by using global approaches such as ChlP-seq in combination with global analysis of alternative splicing (see below).

The second global approach that we have witnessed as having a major impact on splicing research is crosslinking immunoprecipitation (CLIP) to study genome-wide RNA–protein interactions [36]. CLIP is distinct from ChIP in a crucial aspect: CLIP isolates crosslinked RNA–protein adducts by immunoprecipitation followed by SDS-PAGE rather than by immunoprecipitation alone as in ChIP, and this further ensures specificity in mapping protein binding sites in RNA. Since its original development CLIP has now been coupled

with high-throughput sequencing (CLIP-seq or HTS-CLIP) and several modifications have been introduced to the protocol to increase the crosslinking efficiency (PAR-CLIP [37]) or elevate the precision for mapping RNA binding sites (iCLIP [38]).

These techniques have been applied to unveil binding patterns as well as binding consensus for multiple RNA binding proteins and, more importantly, to generate an 'RNA map' for specific splicing regulators by relating functional consequences (i.e. induction of specific alternative splicing events) to their binding profiles [39–42]. A striking principle that has emerged from these studies is the positional effect on alternative splicing, where binding of splicing regulators in intron regions upstream of the alternative exon generally suppresses selection of the exon, whereas binding of the same factor in intron regions downstream of the alternative exon promotes inclusion of the exon (Figure 2). Although the precise mechanism for this positional effect has not yet been elucidated, it could be related to a general regulatory principle of splice-site selection where strong splicing signals on flanking constitutive exons compete with the weak signals on the internal alternative exon to cause exon skipping. Thus, the relative strength of splicing signals on the alternative exon dictates its level of inclusion, as recently demonstrated on model genes [43]. In this regard the observed positional effect could reflect a polarity in which a splicing regulator bound to intron sequences can suppress the downstream 3′ splice site, as in the case of the splicing regulator PTB [44]. Although greater understanding of the positional effect awaits further mechanistic dissection, the important message here is that the global CLIP-seq strategy can and should be extended to all RNA-binding proteins in mammalian genomes.

One of the great challenges in relating RNA binding to functional consequences is precise and large-scale determination of induced alternative splicing events. Traditionally, this has been approached by interrogating known alternative splice junctions to construct splice-junction arrays placing oligonucleotides on individual splice junctions and/or on unique sequences associated with individual alternative splicing events [45–47]. The challenge of this approach is the highly variable efficiency of probe hybridization, and this can be further complicated by cross-hybridization with different probes. Although useful information can and has been deduced from the technology platform, the discovery rate is generally low and the results need extensive validation by RT-PCR, a 'gold standard' in the field for quantitative analysis of alternative splicing. In fact, this gold standard has been directly employed to profile alternative splicing via massive parallel analysis of PCR amplicoms by automated capillary electrophoresis [48].

A common limitation of these methodologies is that the survey is still based on documented splicing events. Deep sequencing (RNA-seq) coupled with development of bioinformatics tools is now gaining momentum for the unbiased analysis of RNA isoform production [49,50]. With technical improvements allowing longer and more accurate sequencing it has become feasible to sequence 75–100 nt from both ends of RNA fragments. This dramatically increases the number of tags that cover splice-junction sequences for quantitative analysis. However, it is important to point out the limitation of the sequencing-based approach, which is the tag density required for inferring quantitative differences for low-abundance transcripts. Because RNA abundance can differ by three to four orders of magnitude and their isoforms by a further two orders of magnitude, many low-abundance mRNA isoforms

could be overwhelmed by high-abundance housekeeping gene transcripts, and thus become difficult to detect or quantify. Even so, with rapid technological advance in increasing the tag density and reducing cost the sequencing-based approach is expected to become the major method of choice for splicing research. With respect to co-and post-transcriptional splicing, the sequencing-based approach could be coupled with cell fractionation to analyze separately RNAs released into the nucleoplasm and those that remain attached to chromatin; this would permit global survey of the temporal dynamics of pre-mRNA splicing in higher eukaryotic cells.

Co-transcriptional splicing commitment versus post-transcriptional RNA catalysis

RNA splicing is a multi-step reaction. Although it is conceivable that regulated splicing primarily takes place during initial splice-site recognition, conversion of initial complexes to splicing-competent spliceosomes could follow distinct kinetics at particular splice-site pairs to allow co-transcriptional commitment to splicing, but post-transcriptional execution of the actual splicing reaction [51]. It has been demonstrated on specific genes that the first intron can actually be removed last [52,53]. This leaves room for regulation, such as if a key factor(s) is missing or needs to be activated, or if a splicing repressor must be dismantled under particular cellular conditions. In addition, some paired splice sites might undergo rearrangement to allow alternative pairing, given the reversibility of initial splicing complexes [54]. Therefore, the temporal regulation of splicing is an important mechanistic issue in understanding splicing regulation.

Because alternative splicing is frequently associated with weak splice sites, are alternative splicing events generally slower than constitutive ones and, if so, does alternative splicing take place co- or post-transcriptionally? These questions were recently addressed in several cell types where the well-studied cellular *Src* and fibronectin *(Fn1)* genes show dramatic differences in the selection of their alternative exons [13,55]. These studies revealed that unspliced exon–intron and intron–exon junctions were detectable at higher levels on the alternative exons N1 in *Src* and EIIIB in *Fn1* than on flanking constitutive exons, especially in cells where these alternative exons are largely skipped. These observations are consistent with the possibility that the introns associated with the alternative exons are removed with slower kinetics relative to constitutive introns in the same gene. By fractionating chromatin-bound and released RNA it was found that most splicing intermediates are associated with the chromatin fraction, and not with released RNA in the nucleoplasm, suggesting that both constitutive and alternative splicing take place co-transcriptionally.

A potential caveat to cell-fractionation studies on mammalian cells, however, is the inability to separate chromatin from so-called ‘nuclear matrix’, which does not contain much DNA but is highly enriched in splicing factors (see below). Consequently, it remains unclear where splicing exactly occurs in the nucleus, and *in situ* analysis using splice junction probes is needed to determine whether there is any difference in spatial localization of co- versus post-transcriptional splicing. Despite such caveats, it will be interesting to use global approaches coupled with cell fractionation in future studies to determine how frequently splicing takes place co-versus post-transcriptionally, what structural features are associated with slow splicing events, and whether alternative splicing might proceed post-

transcriptionally more frequently than most constitutive splicing events. These questions are important and interesting because slow splicing might be generally associated with regulated splicing and such regulated events could induce feedback regulation on transcription (if they occur co-transcriptionally) or allow coupling with other mechanisms regulating RNA metabolism in the cell (if they proceed post-transcriptionally, see below).

Intriguingly, the analysis of intron removal kinetics associated with the alternative *Fn1* EIIIB exon revealed that the intron downstream of the alternative exon is excised before the intron upstream of the alternative exon [13,55]. Although the phenomenon remains to be extended to other alternative splicing events, the observation suggests a revised model for kinetic coupling between transcription and splicing, emphasizing 'first-come, first-recognized,' but not necessarily 'first-finished'. In other words, the splicing signals associated with alternative exons could be recognized first and the splice sites could be paired in the order of transcription; their conversion to splicing-competent complexes could then follow kinetics distinct from the surrounding introns.

These findings stress the possibility that steps after the assembly of initial splicing complexes (i.e. the relatively stable commitment complex consisting of U1 base-paired with the 5′ splice site and crucial factors bound to the downstream 3′ splice site [56]) might not necessarily be rate-limiting for splicing. Currently, the mechanism remains entirely elusive with respect to why some splicing events are slower than others and which factors cause slowing of particular splicing events. One possibility is that some splice sites might be tied up in unproductive complexes which must be rearranged or have specific splicing inhibitory factors removed before functional spliceosomes can be formed – a challenging question that will need to be addressed in future biochemical studies. This temporal control of splicing could reflect a key feature in the regulation of alternative splicing, and this could be intimately linked to the spatial control of many splicing events in the nucleus of mammalian cells.

Where does splicing take place if not co-transcriptionally executed?

Post-transcriptional intron removal could contribute to nuclear morphology, and vice versa, in eukaryotic cells. In yeast, actively transcribed genes tend to localize closer to the nuclear envelope, which is thought to facilitate rapid export of spliced mRNAs [57]. The opposite is true in higher eukaryotic cells where active genes tend to move toward the interior of the nucleus [58]. Where newly released mRNAs go, especially those that still contain an unspliced intron(s), is little understood in higher eukaryotic cells. This could be pertinent to the morphology of nuclear speckles, which are enriched with most components of the splicing machinery and with hyperphosphorylated Pol II [59]. Nuclear speckles also show intensive poly(A) signals detected by oligo-dT *in situ* hybridization, but it has been unclear whether this signal reflects a transient path for nuclear mRNA before export or the localization of particular stable polyA-containing RNAs which could serve as the organizer of this nuclear domain. This would be analogous to processes taking place in para-speckles (a domain spatially adjacent to nuclear speckles with unknown function) where the long non-coding RNA *NEAT1* provides an organization function for this nuclear domain [60]. However, whether something similar is occurring in nuclear speckles has not been

established because a long non-coding RNA associated with nuclear speckles does not seem to have such an organizational function [61].

The speckled nuclear domain has been traditionally considered as a storage site for splicing factors which are recruited to active genes for co-transcriptional splicing [59]. However, debate on the formation and dynamics of nuclear speckles is still continuing, partly because of poorly defined architecture of the nuclear domain. Under EM, the core region of nuclear speckles corresponds to interchromatin granules, which do not seem to show any gene activity, whereas the periphery of these granules contains perichromatin fibrils with abundant nascent RNA, indicative of active transcription [62,63]. Because these fine nuclear structures cannot be resolved at the fluorescence microscopy level, it has been unclear in most studies whether an ascribed nuclear speckle-associated activity is linked to interchromatin granules, to perichromatin fibrils, or to both. Nevertheless, the question is why nascent RNAs accumulate around interchromatin granules and whether these nascent RNAs are still attached to their genes or correspond to spliced RNAs that have been released from their genes. A recent study revealed that, when an intron-containing gene is expressed from a plasmid, the expressed pre-mRNA becomes intimately associated with nuclear speckles where it is spliced before export out of the nucleus and, interestingly, RNAi inactivation of a key mRNA nuclear export factor, UAP56, causes the accumulation of spliced mRNA on nuclear speckles [64]. These findings suggest that post-transcriptional splicing could take place at or near nuclear speckles before export from the nucleus, at least for a fraction of the mRNA in the cell.

One caveat to this plasmid-based strategy is that the expression unit is not anchored on chromatin and could be readily attracted via associated intron-containing RNA to any nuclear location with concentrated splicing factors. However, this could precisely reflect the fate of some post-transcriptionally spliced mRNAs. This question will need to be addressed by using single-molecule imaging approaches [65]. For example, specific RNA-binding sites could be engineered on both intron and exon regions of a pre-mRNA expressed from an expression unit integrated in the genome, and each of these sites could thus be tethered by different fluorescence-labeled RNA-binding proteins to monitor their expression and splicing in real time. This approach could be applied to both constitutively and alternatively spliced genes to study their splicing kinetics and nuclear structures associated with different modes of splicing. This represents a key question of cell biology concerning the relationship between splicing and potential nuclear structures in higher eukaryotic cells.

Implications of slow co- and post-transcriptional splicing in genome organization

The tendency for splicing complexes on unspliced RNAs to interact with one another could underlie the primary formation of nuclear speckles, and this interaction could be further aided or regulated by the long non-coding RNA *NEAT2/MALAT1* through its modulation of the phosphorylation state of SR proteins [66]. As recently proposed [67], many genes could contain slow introns in addition to the ‘normal’ fast introns that are efficiently spliced during transcription. The intron-containing nuclear RNA-protein complexes (or RNPs) could attract one another, thus providing a driving force for relocation of their attached genes to splicing-factor-concentrated regions (Figure 3). Indeed, a number of large genes, which might

contain some ‘slow’ introns, have been found to exhibit spatial proximity to nuclear speckles and the transcripts from those genes appear to enter the interior of nuclear speckles before export [68]. Furthermore, mutual attraction of RNPs that are still attached to elongating Pol II complexes could explain why nuclear speckles are also enriched with hyper-phosphorylated Pol II.

Attraction of a population of both co- and post-transcriptional splicing complexes and recycling of splicing factors upon removal of those slow introns to other active genes is fully consistent with the observed dynamics of nuclear speckles in actively transcribing cells [69]. If transcription is inhibited or splicing arrested, intron-containing splicing complexes could accumulate to form large rounded aggregates which are known to be readily reversible after the cell returns to the status of active transcription [70]. This is also consistent with enrichment of mRNA export factors, including Tap/NSFl, Aly and the TREX complex, in nuclear speckles [71,72]. Together, these observations suggest that nuclear speckles could function as a key gateway for nuclear export of post-transcriptionally spliced mRNAs.

A key argument for nuclear speckles as a storage site for splicing factors is their formation independent of transcription, some of which occasionally remain even during mitosis when transcription is shut down. However, it has been reported that some intron-containing RNPs remain unspliced even after the cell enters mitosis in yeast [51]. In addition, particular long-lasting intron-containing RNPs have been detected in the cytoplasm of neurons [21,73]. Furthermore, the ability to ‘trap’ unspliced RNPs to nuclear speckles could serve as a key mechanism to prevent export of unspliced RNA out of the nucleus. Yeast appears to lack such a mechanism; as a result, unspliced RNA becomes readily detectable in the cytoplasm when the RNA degradation machinery is compromised [74]. Therefore, whereas co-transcriptional splicing is likely to account for the majority of constitutive splicing events, post-transcriptional splicing could provide additional strategies for regulation. The attraction of RNP-associated genes to some common nuclear locations (i.e. around nuclear speckles) could contribute to unique nuclear morphology in higher eukaryotic cells, and this could in turn facilitate genome organization and potentiate inter-chromosomal interactions [75–77]. Key experimental proof for this idea will be the determination of whether slow splicing and/or post-transcriptional splicing events indeed exhibit a spatial relationship with nuclear speckles by using single-molecule imaging approaches.

Conclusions

Co-transcriptional splicing is likely to predominate for most introns in eukaryotic cells because it offers at least three advantages: (i) efficient recognition of splice sites as they emerge from the elongating Pol II complex; (ii) coupling with the transcription process permits splicing to be regulated by transcription factors and epigenetic regulators; and (iii) co-transcriptional splicing could also serve as an important mechanism to facilitate transcription. Therefore, co-transcriptional RNA processing is not just temporally and spatially convenient; it could have a profound impact upon multiple functional aspects of RNA processing in eukaryotic cells. By contrast, post-transcriptional splicing might offer additional regulatory control: (i) the use of splicing regulators concentrated in nuclear speckles could provide an additional strategy for regulated splicing; (ii) the association with

nuclear speckles could serve as a key mechanism to ensure that, in most cases, only spliced mRNA is exported out of the nucleus; and (iii) both slow and post-transcriptional splicing could contribute to genome organization and facilitate long-distance interactions within chromosomes or between chromosomes in the nucleus.

It is important to emphasize here that many of these ideas are speculative and thus in great need of experimental evidence from future research. In particular, global approaches are needed to determine the frequency of co-versus post-transcriptional splicing and specific structural features that might be associated with each class. For example, it will be important to determine whether post-transcriptional splicing is more frequently associated with alternative splicing events due to the weak splice sites that are generally associated with them. It is obvious that we are only beginning to understand how the transcription machinery, including specific chromatin features, exerts regulatory functions on splicing, and this represents a new frontier for splicing research. Because of the functional integration between the transcription and splicing machineries, future research will also be directed towards understanding how splicing in turn regulates transcription. Last but not least, it is important to address the functional interplay between transcription and splicing in the nucleus with respect to specific nuclear structures because it is clear that our genome is not randomly packed in the nucleus. These problems for future research represent some of the central questions in understanding the mechanisms for regulated gene expression that play a role in normal development and in disease.

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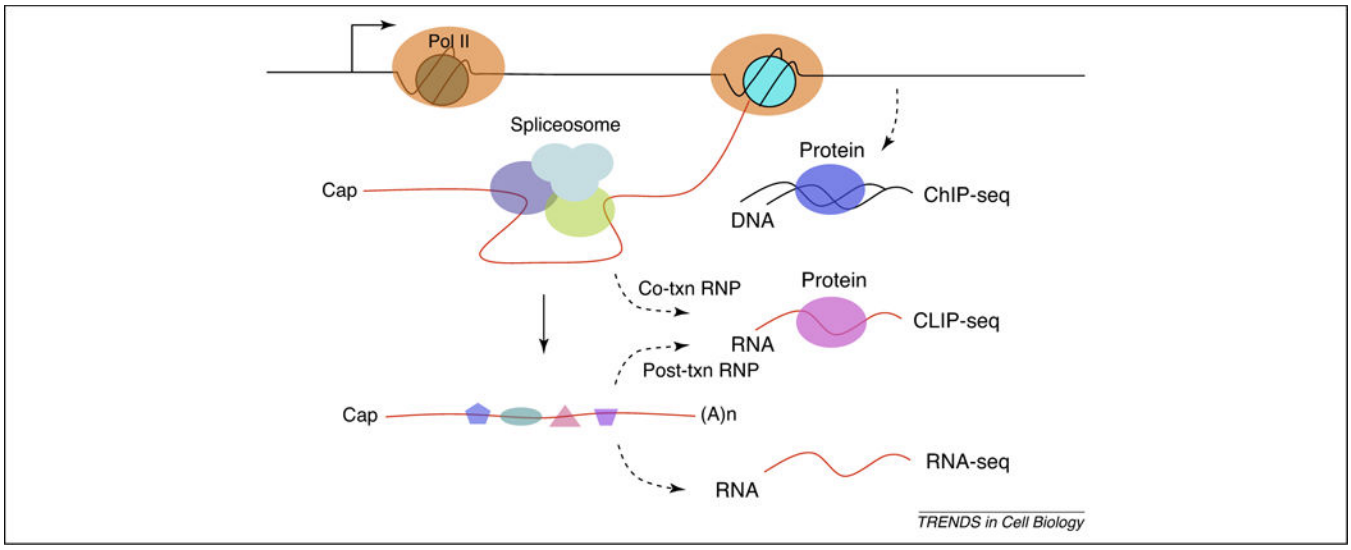

Figure 1.

Global techniques for analyzing coupling between transcription and splicing and the resulting functional consequences. Interaction of co-transcriptionally recruited RNA–binding proteins and other spliceosome components with genomic DNA can be detected with ChIP-seq following formaldehyde crosslinking. RNA-protein interactions can be assessed by CLIP-seq (also known as HIT-CLIP) or variation of the technology (PAR-CLIP or iCLIP) following UV-induced crosslinking. Coupling the CLIP method with cell fractionation could allow analysis of co-transcriptional (co-txn) and post-transcriptional (post-txn) RNPs. The functional consequences of constitutive and regulated splicing can be studied by RNA-seq or on various microarray platforms (tiling array, exon-array, splice junction array). In the illustration, the brown nucleosome represents the first nucleosome after the gene promoter, and this could be responsible for the initial Pol II pausing events on most eukaryotic genes; the light blue nucleosome illustrates potential internal Pol II pausing events; the dark blue protein indicates a splicing factor or regulator that can be in close proximity to DNA; the pink protein represents RNA-binding splicing factors on both nascent and released RNA; boxes of various shapes indicate hnRNP proteins associated with released RNA.

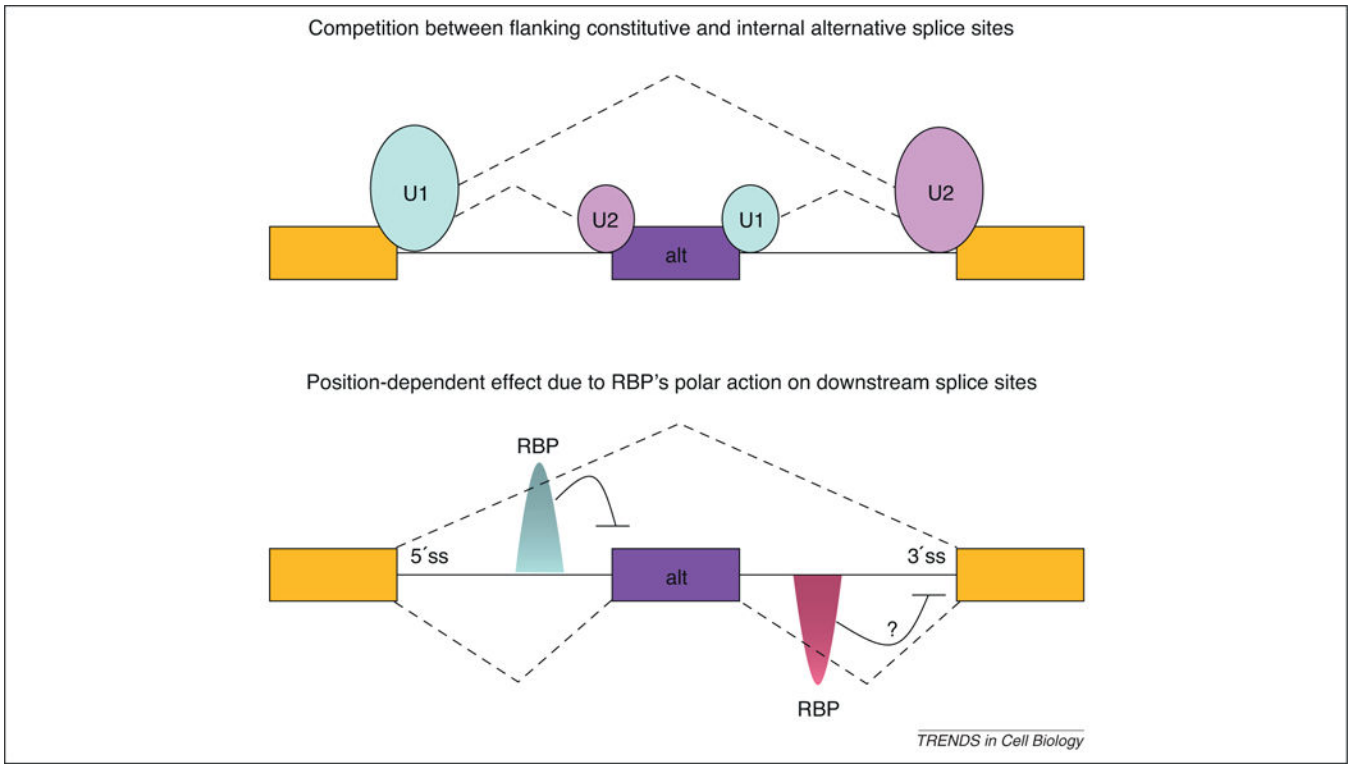

Figure 2.

Emerging principle of regulated splicing from recent global analysis. Upper panel: the selection of the alternative exon on this illustrated cassette-exon mode of alternative splicing is dependent on competition between the splice sites associated with the alternative exon and those with the flanking constitutive exons. The size of U1 and U2 illustrates their efficiency in recognizing specific 5′ and 3′ splice sites, respectively. Strong recognition of the splice sites associated with the flanking constitutive exons would cause exon skipping, whereas efficient recognition of the splice sites associated with the internal alternative exon would enhance the inclusion of the alternative exon. Lower panel: recent global analysis has revealed a position-dependent effect for most splicing regulators acting on intron sequences, where their binding to upstream sequences suppresses the alternative exon and their binding to downstream regions enhances the inclusion of the alternative exon. Although the precise mechanism for this positional effect remains to be determined, one possibility is the polarity of the splicing regulators in selectively suppressing the downstream 3′ splice site.

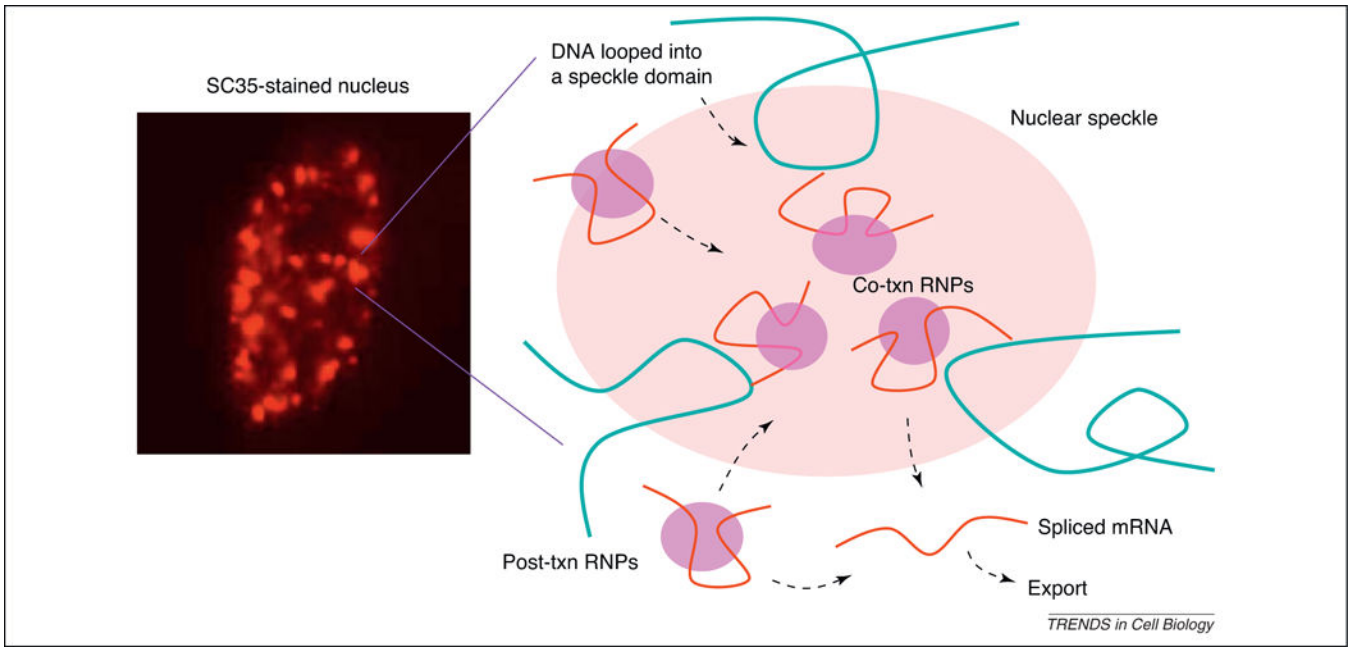

Figure 3.

Model for the formation and dynamics of nuclear speckles. Nuclear speckles (also known as the SC35 domain, as illustrated on the left) are enriched with most splicing factors, hyper-phosphorylated Pol II, various chromatin remodeling factors, and RNA export factors. Co-transcriptional RNPs could be attracted to one another if they are not temporally resolved during transcription, resulting in an elevation in local concentration of transcription and splicing factors. As a result, the associated DNA might be looped near the interchromatin space in the nucleus. Thus, the formation of nuclear speckles could influence genome organization in the nucleus because some post-transcriptional RNPs that contain slow introns could be attracted to nearby nuclear speckles. It is possible that a population of pre-mRNAs could be spliced within or at the periphery of nuclear speckles and spliced mRNAs are then released into the nucleoplasm for export. Upon completion of splicing, splicing factors can be recruited from nuclear speckles to initiate new rounds of spliceosome assembly and splicing on nascent RNA. The dynamics of nuclear speckles is thus likely to be dependent on the transcription status of the cell.