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Beyond Transcription: Roles of Transcription Factors in Pre-mRNA Splicing

Xavier Rambout^{†,‡}, Franck Dequiedt^{*,§,||}, Lynne E. Maquat^{*,†,‡}

[†]Department of Biochemistry and Biophysics, School of Medicine and Dentistry, University of Rochester, Rochester, New York 14642, United States

[‡]Center for RNA Biology, University of Rochester, Rochester, New York 14642, United States

[§]Interdisciplinary Cluster for Applied Genoproteomics (GIGA-R), University of Liège, 4000 Liège, Belgium

^{||}GIGA-Molecular Biology of Diseases, University of Liège, 4000 Liège, Belgium

Abstract

Whereas individual steps of protein-coding gene expression in eukaryotes can be studied in isolation in vitro, it has become clear that these steps are intimately connected within cells. Connections not only ensure quality control but also fine-tune the gene expression process, which must adapt to environmental changes while remaining robust. In this review, we systematically present proven and potential mechanisms by which sequence-specific DNA-binding transcription factors can alter gene expression beyond transcription initiation and regulate pre-mRNA splicing, and thereby mRNA isoform production, by (i) influencing transcription elongation rates, (ii) binding to pre-mRNA to recruit splicing factors, and/or (iii) blocking the association of splicing factors with pre-mRNA. We propose various mechanistic models throughout the review, in some cases without explicit supportive evidence, in hopes of providing fertile ground for future studies.

Graphical Abstract

*Corresponding Authors: fdequiedt@ulg.ac.be. Phone: +32 4 366 90 28. lynne_maquat@urmc.rochester.edu. Phone: +1-585-273-5640.

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1. INTRODUCTION

The expression of protein-coding genes in eukaryotes is a highly complex process that integrates many nuclear and cytoplasmic steps. These steps include (i) gene transcription (i.e., the synthesis of pre-mRNA), (ii) pre-mRNA processing to generate the mature mRNA (i.e., 5'-end-capping, intron removal by splicing, and 3'-end cleavage and polyadenylation), (iii) nuclear mRNA export to the cytoplasm, (iv) mRNA translation (i.e., the synthesis of proteins), and (v) mRNA decay (Figure 1). Whereas these fundamental steps have been individually investigated for decades, findings from the early 1980s that the premature termination of mRNA translation due to frameshift or nonsense mutations can induce mRNA degradation demonstrated for the first time that steps of gene expression, in this case, pre-mRNA splicing, mRNA translation, and mRNA decay, can be mechanistically coupled (see, e.g., ref 1 for a review). Many other examples of how gene expression machineries are functionally connected have been described and reviewed over the past 15 years (for reviews, see, e.g., refs 2–14) (Figure 2).

Transcription factors (TFs) are defined as sequence-specific DNA-binding proteins that orchestrate gene transcription by binding to response elements (REs) located in promoters or enhancers, which are cis-acting DNA elements, the latter influencing the activity of distant promoters through chromatin looping.¹⁵ Typically, TFs orchestrate multiple processes leading to transcription initiation, such as the recruitment of preinitiation complexes to promoters,¹⁶ and/or the escape of RNA polymerase II (RNAPII) from promoter-proximal pausing.¹⁷ Evidence indicates that TFs might also influence more downstream events during the process of pre-mRNA synthesis, including RNAPII elongation beyond promoter-proximal pausing.^{18–20} RNAPII transcription rates are known to impact cotranscriptional pre-mRNA processing, in particular pre-mRNA splicing, the underlying mechanisms of which have been extensively investigated for the past 20 years (for review, see, e.g., refs 12, 13, and 21–27).

Pre-mRNA splicing is a multistep process during which the spliceosome, a ribonucleoprotein complex composed of five small nuclear ribonucleoproteins (snRNPs) and dozens of non-snRNP proteins, is recruited to the 5'- and 3'-ends of introns, known as 5'- and 3'-splice sites, and progressively assembled to catalyze intron removal.²⁸ Briefly, U1 small nuclear RNA (snRNA) in U1 snRNP base-pairs to the 5'-splice site, and SF1 binds to the branchpoint, namely, a residue upstream of the 3'-splice site that is attacked by the 5'-splice site during the first catalytic step of splicing, to form the E' complex. Subsequent recruitment of the large subunit of the U2 auxiliary factor (U2AF) heterodimer U2AF2 (also known as U2AF65) to the 3'-AG dinucleotide and the small U2AF subunit U2AF1 (also known as U2AF35) to the upstream polypyrimidine tract forms the E complex. The E complex is converted to the A complex once U2 snRNA replaces SF1 at the branchpoint. Recruitment of the U4/U6.U5 tri-snRNP leads to the formation of the B complex, which is extensively remodeled to form the mature and catalytically active C complex. Alternative splicing, that is, removal of alternative pre-mRNA regions defined by alternative 5'- and/or 3'-splice sites, is a crucial mechanism responsible for transcriptomic diversity. Proteins that impact spliceosome activity or efficiency are defined as splicing factors (SFs). SFs include spliceosome components and auxiliary cis-acting proteins, largely but not always RNA-

binding proteins (RBPs) that associate with the spliceosome to promote or inhibit recognition of the splice site.

Here, we review how TFs function beyond transcription initiation to control pre-mRNA splicing not only indirectly by influencing RNAPII elongation rates, as suggested since the late 1990s,²⁹ but also directly through “pre-mRNA imprinting” (Figure 3). The concept of pre-mRNA imprinting was introduced in the early 2010s to describe how multifunctional proteins cotranscriptionally associate with nascent transcripts, potentially affecting any step of gene expression downstream of transcription, for example, pre-mRNA splicing, mRNA export from the nucleus to the cytoplasm, mRNA translation, and mRNA decay.^{8,30} We begin by describing how TF binding to promoters (see section 2) or to gene bodies (see section 3) regulates pre-mRNA splicing by controlling RNAPII elongation rates (see sections 2.1 and 3.1) or by imprinting pre-mRNAs with SFs (see sections 2.2 and 3.2). Although this review focuses on roles for TFs in pre-mRNA splicing, when appropriate, we also mention cases of TF-mediated regulation of mRNA translation and/or mRNA decay, supporting the idea that TF-mediated imprinting of pre-mRNAs can extend beyond pre-mRNA splicing to downstream steps of gene expression. Then, we describe how TFs can regulate pre-mRNA splicing independently of their ability to bind DNA (see section 4). Finally, we present the possibility of additional mechanisms by which TFs could regulate pre-mRNA splicing, and we propose a unifying model integrating the different mechanisms discussed throughout this review (see section 5).

2. REGULATION OF PRE-mRNA SPLICING BY TRANSCRIPTION FACTOR BINDING TO PROMOTERS

Promoters are defined as proximal cis-acting DNA elements located upstream of transcription start sites. TF binding to specific promoter REs orchestrates the assembly of the preinitiation complex to initiate transcription and/or early steps of RNAPII elongation. The idea that promoter influence extends beyond transcription derives from experiments performed in the late 1980s showing that pre-mRNA splicing and polyadenylation are negatively affected when the RNAPII promoter of protein-coding genes is replaced by an RNAPI promoter,³¹ an RNAPIII promoter,³² or a bacteriophage T7 promoter.^{33,34} In the late 1990s, the observation that promoter-mediated control of alternative splicing was dependent on RNAPII promoter identity suggested that specific promoter sequences can influence splice site choice, pointing to TF REs as splicing regulators.^{21,29,35} Over the years, promoters have been shown to regulate alternative splicing by controlling transcription elongation rates (see section 2.1) or the recruitment of SFs to pre-mRNAs (see section 2.2). Considering the mutual interdependence of transcription elongation and pre-mRNA splicing, we cannot exclude the possibility that at least certain examples of TF-mediated splicing described below involve both control of transcription elongation and recruitment of SFs to pre-mRNA.³⁶ Promoters can also influence alternative splicing by regulating N⁶-methyladenosine (m⁶A) marks on pre-mRNAs (see section 5). In addition to the cases reviewed below, in which the responsible TF and the associated molecular mechanism have been determined or are strongly suspected, numerous additional studies have identified

promoter- or enhancer-mediated alternative splicing events in which the underlying mode of action of the promoter remains unclear.^{29,35,37–45}

2.1. Regulation of Pre-mRNA Splicing through Control of Transcription Elongation

RNAPII elongation rates are highly dynamic, both in promoter-proximal regions and within gene bodies.⁴⁶ Whether pre-mRNA splicing can be influenced by RNAPII promoter-proximal pausing and/or release from pausing, either of which can facilitate efficient promoter-dependent recruitment of SFs, remains uncertain.^{47,48} However, there is substantial evidence that transcription elongation rates in gene bodies can influence pre-mRNA splicing by temporally controlling the cotranscriptional assembly of the splicing machinery on splice sites.^{12,21–26} As an illustration, high rates of transcription elongation between a weak 3′-splice site of an alternative exon and a strong downstream 3′-splice site of a constitutive exon results in nearly simultaneous synthesis of both 3′-splice sites. This leads to the preferential utilization of the strongest 3′-splice site and exclusion of the alternative exon (Figure 4A). In contrast, low rates of transcription elongation or RNAPII pausing between a weak 3′-splice site of an alternative exon and a strong downstream 3′-splice site of a constitutive exon increases accessibility of the weak 3′-splice site compared to the strong downstream 3′-splice site that remains to be synthesized, selection of the weak site, and inclusion of the alternative exon (Figure 4B). The rate of RNAPII elongation can also regulate the accessibility of cis-acting elements within nascent pre-mRNAs. For example, low elongation rates promote skipping of an alternative exon within *CFTR* pre-mRNA by facilitating the recruitment of the SF ETR-3 to a 3′-splice site, which results in the displacement of U2AF2 from the overlapping polypyrimidine tract.⁴⁹

The initial finding that promoters can influence alternative pre-mRNA splicing by controlling transcription elongation rates derives from studies of the *FBI* promoter.²⁹ Deletion of the cAMP-responsive element (CRE) and Y/CCAAT-box from the promoter of an *FBI* minigene induced transcription and inclusion of an alternative exon in the resulting transcript.²⁹ These results suggested that TF binding to the CRE and/or Y-box in the *FBI* promoter impacts *FBI* pre-mRNA splicing.²⁹ More than 15 years later, binding of the TF CREB1 to the CRE of the *FBI* promoter was found to promote exon skipping by recruiting p300, a histone acetyl transferase (HAT).⁵⁰ HAT recruitment increased transcription elongation rates by inducing histone H4 acetylation of nucleosomes along the *FBI* gene body.⁵⁰ A nucleosome is a unit of chromatin that consists of a ~150-bp segment of DNA wrapped around two histone tetramers.⁵¹ Specific post-translational modifications of nucleosomal histones, particularly the acetylation of lysines on histone tails, loosen chromatin, facilitate RNAPII movement, and thus promote exon skipping.^{46,52} It is currently unclear how promoters control the acetylation of histones associated with gene bodies. However, numerous alternative splicing events have been found to involve chromatin looping between promoters and gene bodies, particularly exons,⁵³ and it was recently proposed that promoter-mediated control of RNAPII elongation-dependent splicing involves the formation of higher-order chromatin structures⁵⁴ (Figure 5).

As another example of TF-influenced splicing, VP16, a potent herpesvirus TF that stimulates both transcription initiation and elongation, can promote exon skipping when it is

artificially tethered to the promoter of an *FBI* minigene,¹⁸ possibly by promoting HAT-dependent acetylation of histones at the promoter and/or along the gene body⁵⁵ or by binding to positive elongation factor b (P-TEFb)⁵⁶ (Figure 5). In contrast to HAT-mediated loosening of chromatin to promote RNAPII elongation, P-TEFb promotes elongation by phosphorylating negative elongation factor (NELF), 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole sensitivity-inducing factor (DSIF), and the RNAPII C-terminal domain (CTD) at promoters so that RNAPII exits promoter-proximal pausing and transitions to productive elongation.⁴⁶ In support of a role for RNAPII elongation in VP16-mediated splicing, which notably requires the RNAPII CTD,⁵⁷ a VP16 variant that retains its ability to induce transcription initiation, but is unable to stimulate transcription elongation, manifests a reduced ability to induce exon skipping.^{18,58} Moreover, tethering to promoters other TFs such as SP1 or CTF/NF-1, which promote transcription initiation but not elongation, did not promote exon skipping unless they were coexpressed with a potent coactivator of transcription elongation.¹⁸

The B subunit of NELF coimmunoprecipitates with the TF androgen receptor (AR) and inhibits AR-mediated transcription and exon skipping of *CD44* pre-mRNA.⁵⁹ NELF cooperates with DSIF and binds RNAPII at promoters to induce promoter-proximal pausing and reduce productive RNAPII elongation.⁴⁶ We propose that NELF-dependent regulation of pre-mRNA splicing by AR involves the recruitment of NELF to AR-regulated promoters and its subsequent loading onto RNAPII (Figure 5).

2.2. Regulation of Pre-mRNA Splicing through Recruitment of Splicing Factors

In addition to impacting pre-mRNA splicing by controlling elongation rates, RNAPII also influences splicing through its CTD by serving as a docking platform for pre-mRNA processing factors that are carried over as transcription initiates, elongates, and terminates.¹³ A large body of literature indicates that nuclear receptors (NRs), and possibly other TFs, couple gene transcription and pre-mRNA splicing by recruiting to promoters either RNA-binding transcriptional cofactors that have splicing functions or bona fide SFs. The predominating model of TF-mediated splicing involves TFs and their associated factors, that is, transcriptional cofactors or SFs, hitching a ride with elongating RNAPII, encountering alternative splice sites, imprinting nascent pre-mRNA, and influencing alternative exon inclusion or exclusion^{60,61} (Figure 6, scenario i). Alternatively, in the same vein as discussed above, transcriptional cofactors or SFs recruited to promoters through NRs or other TFs might imprint nascent pre-mRNAs and regulate their splicing through DNA looping⁵³ (Figure 6, scenario ii). Whereas evidence globally converges toward this SF-recruitment model, we do not exclude the possibility that some of the examples cited below might also or alternatively rely on the regulation of transcription elongation rates (see section 2.1).

2.2.1. Nuclear Receptors and Their Transcriptional Cofactors Cooperate with RNAPII—Initial evidence that NR-mediated splicing relies on transcriptional cofactors derives from studies on the peroxisome proliferator-activated receptor γ (PPAR γ) coactivator 1 alpha (PGC-1 α). PGC-1 α induces the synthesis and exclusion of an alternative exon that derives from an *FBI* minigene driven by a PPAR γ -responsive promoter.⁶² Consistent with a direct function in splicing, PGC-1 α localizes to nuclear speckles,⁶² which

are dynamic interchromatin foci where SFs are stored, modified, and assembled in complexes to ensure efficient splicing at adjacent sites of active transcription.⁶³ PGC-1 α also coimmunoprecipitates with several serine/arginine- (SR-) rich proteins,⁶² a class of SFs whose roles in coupling transcription and alternative pre-mRNA splicing are well-documented.⁶⁴ Furthermore, a PGC-1 α variant that cannot associate with elongating RNAPII was unable to regulate pre-mRNA splicing, suggesting that PGC-1 α -mediated splicing involves binding to RNAPII.⁶² Genome-wide studies identified hundreds of alternative splicing events controlled by PGC-1 α in mouse primary myoblasts.⁶⁵ Some of these alternative splicing events rely on alternative promoter usage, strengthening the idea that PGC-1 α binding to promoters is linked to its function in splicing.⁶⁵

Subsequent work demonstrated that progesterone receptor or estrogen receptor (ER) α binding to different promoters driving *CD44* minigene transcription mediates either skipping⁶⁶ or inclusion⁶⁷ of the same alternative exon independently of transcription elongation rates. Consistent with this hypothesis, genome-wide studies indicated that estrogen promotes the alternative splicing of pre-mRNAs regulated by ER α -targeted promoters or enhancers.⁶⁷ Splicing assays in cells using an ER α -responsive promoter showed that different SF-related coactivators of ER α -mediated transcription had different effects on ER α -mediated splicing.⁶⁶ As examples, the thyroid hormone receptor-binding protein activator CoAA⁶⁶ and SF U2AF2-like proteins CAPER α and CAPER β ⁶⁸ each facilitates ER α -mediated exon skipping. CAPER α and CAPER β are large U2AF subunits that can functionally substitute for U2AF2.^{69–71} CoAA, CAPER α , and CAPER β , like PGC-1 α , are RNA-recognition-motif- (RRM-) containing RBPs, and at least one of their multiple RRM is critical for their NR-dependent splicing activity.^{68,72} Additionally, possibly like PGC-1 α , CAPER β directly binds an accessory subunit of RNAPII.⁷³ Binding to RNA and RNAPII, in particular its CTD, is a feature shared by several NR transcriptional cofactors.^{60,61} Therefore, it was hypothesized that RNAPII acts as a movable platform carrying NRs and their transcriptional cofactors from promoters to gene bodies so as to promote pre-mRNA imprinting with NRs and their cofactors at alternative splice sites and regulate spliceosome recruitment^{60,61} (Figure 6, scenario i).

Other examples of transcriptional cofactors that are components of functional spliceosomes and control NR-mediated splicing include the SNW/SKI-interacting protein (SKIP), which is a non-snRNP spliceosome component⁷⁴ that coordinates vitamin D receptor- (VDR-) dependent transcription and splicing.⁷⁵ The thyroid hormone receptor associated protein 3 (THRAP3, also known as TRAP150), an RNA-binding SF,⁷⁶ and the polypyrimidine tract-binding protein-associated SF PSF promotes and inhibits, respectively, thyroid receptor β - (THRB-) mediated inclusion of alternative exons.⁷⁷ Additionally, apoptotic chromatin condensation inducer in the nucleus (Acinus) is an RRM-containing spliceosome component^{74,78} that coordinates retinoic acid receptor- (RAR-) dependent repression of gene transcription and removal of retained introns.⁷⁹ Whereas each of these coactivators associates with the spliceosome, no evidence suggests that they associate with RNAPII. Thus, NR-mediated splicing through these particular coactivators might involve DNA looping rather than loading onto RNAPII (Figure 6, scenario ii).

In addition to NR cofactors, NRs that include the retinoic acid receptor α ,⁸⁰ the glucocorticoid receptor,^{81,82} and DAX1⁸⁰ have also been found to directly bind RNA in vitro and/or in vivo. It is possible that the coimprinting of NRs and their interacting cofactors might (i) increase the specificity with which they bind/recognize splice sites through cooperative binding to adjacent RNA sequences, as abundantly described in the literature for other RBPs;^{83–87} (ii) regulate spliceosome composition and/or formation; (iii) play roles in downstream events, such as pre-mRNA alternative polyadenylation,^{66,88} mRNA transport,⁸⁹ mRNA translation,^{80,89,90} and mRNA decay;^{81,82,91,92} or (iv) mediate any combination of these possibilities.

2.2.2. Other Transcription Factors—In addition to NRs, evidence suggests that promoter-dependent regulation of pre-mRNA splicing through recruitment of SFs can be extended to other TFs (Figure 6). For instance, minigene splicing assays demonstrated that the retinoic acid-corepressor Acinus promotes the splicing not only of transcripts under the control of NR-responsive promoters in a retinoic acid-dependent manner, but also of transcripts under the control of NR-independent promoters in a retinoic acid-independent manner.⁷⁹ This indicates that this NR cofactor also cooperates with non-NR TFs to control alternative splicing. We illustrate below how non-NR TFs impact splicing in a promoter-mediated manner through the recruitment of SFs to promoters and either loading onto RNAPII or DNA looping.

In fission yeast, the forkhead TF Mei4 promotes transcription of the *rem1* meiotic gene and removal by splicing of a stop-codon-containing intron from *rem1* pre-mRNA.^{93,94} Mei4 controls *rem1* pre-mRNA splicing in a mechanism whereby forkhead-binding sites within the *rem1* promoter are necessary and sufficient for the timely production of the Rem1 cyclin protein as cells enter meiosis.^{93,94} In addition to binding to the *rem1* promoter, chromatin immunoprecipitation (ChIP) followed by qPCR (ChIP-qPCR) demonstrated that Mei4 is also found across the entire *rem1* gene body.⁹⁴ We suspect that Mei4 association with the *rem1* gene body does not reflect direct binding to numerous DNA-binding sites but association with elongating RNAPII. Furthermore, Mei4 coimmunoprecipitates with several SFs that include the U1 snRNP subunit U1–70K, the U2 snRNP component Prp11, and the non-snRNP spliceosome assembly protein Cdc5, promoting their recruitment to both the *rem1* promoter and gene body, particularly around the targeted intron. Thus, it appears that, similarly to NR-mediated splicing, promoter-mediated recruitment of SFs by Mei4 is followed by coload of Mei4 and its associated SFs onto RNAPII, which delivers them to pre-mRNA splice sites. Another forkhead TF, Fkh2, which mediates *rem1* gene transcriptional repression and *rem1* pre-mRNA intron retention during vegetative growth,⁹⁴ might do so by competing with Mei4 for binding to the *rem1* gene promoter. Along the same lines, the mouse forkhead TF TFDPI/DRTF-1, which was identified in a functional screen for splicing regulators,⁹⁵ might function similarly to Mei4 or Fkh2.

Studies of TGF- β signaling in alternative splicing identified hundreds of events coregulated by the TF SMAD3 and the RBP and SF PCBP1 independent of transcription elongation.⁹⁶ TGF- β signaling activates SMAD3 to promote its shuttling from the cytoplasm to the nucleus and its binding to PCBP1 in nuclear speckles. CLIP followed by RT-qPCR (CLIP-qPCR) revealed that both SMAD3 and PCBP1 directly and cooperatively bind introns

flanking the variable exons of *CD44* pre-mRNA. SMAD3 binding to *CD44* pre-mRNA inhibits PCBP1 binding to U2AF2 and the recruitment of U2AF2 to targeted intron–exon junctions. It is likely that SMAD3 promoter-binding regulates splicing given that *CD44* gene transcription is dependent on SMAD3 and that SMAD3 does not bind DNA encoding the alternative exon cassette of *CD44* pre-mRNA.⁹⁶

As a final example, the basic helix–loop–helix TFs HIF-1 α (hypoxia-induced factor-1 α) and HIF-2 α promote the synthesis and removal of intron 3 from hypoxia-induced *ADM* pre-mRNA⁹⁷ and also the inclusion of alternative exons in several other hypoxia-induced transcripts, including *PDK1* pre-mRNA.⁹⁸ Reporter assays demonstrated that the splicing functions of HIF-1 α and HIF-2 α are dependent on a responsive promoter and on their transcriptional activation domains (TADs), which could not be functionally replaced by the TADs of other TFs.^{97,98} This suggests a model similar to the model for NR-mediated splicing, in which promoters recruit specific transcriptional coactivators that manifest splicing functions. However, HIF-2 α binds (pre-m)RNA through its interaction with the sequence-specific RBP and SF RBM4 rather than directly.⁹⁹ Although the interaction between HIF-2 α and RBM4 on RNA was not investigated in the context of splicing, it was reported to control eIF4E2-mediated translation,⁹⁹ strengthening the idea that the physical association of TFs with their target transcripts has the potential to regulate multiple steps of gene expression.

2.2.3. The Elusive Case of Y-Box-Binding Transcription Factors—The Y-box-binding protein YB-1 (also known as YBX1) is a multifunctional protein regulating different levels of gene expression as well as DNA repair.^{100,101} It is characterized as a classical sequence-specific TF based on evidence from more than 25 years ago that it directly binds the Y/CCAAT-box of several promoters.^{102,103} However, neither the Y-box nor another specific DNA sequence was found to be bound by YB-1 in recent ChIP followed by deep sequencing (ChIP-Seq) experiments.¹⁰⁰ Moreover, binding of a *Chironomus* homologue of YB-1 to genes it regulates depends on RNA integrity,¹⁰⁴ suggesting that YB-1 regulates transcription through pre-mRNA-binding.¹⁰¹ Accordingly, systematic evolution of ligands by exponential enrichment (SELEX) coupled to quantitative in vitro binding data revealed that YB-1 binds RNA and single-stranded DNA with an affinity that is 2 orders of magnitude higher than the affinity with which it binds double-stranded DNA. Although the transcriptional roles of YB-1 are still debatable, YB-1 and its homologue Y-box-binding protein YBX3 are known to function not only in pre-mRNA splicing but also in mRNA translation and mRNA decay.^{101,105–108}

YB-1 and YBX3 are, to our knowledge, the only TFs identified in more than one study as pre-mRNA-bound spliceosome components^{74,109,110} that remain associated with mature spliceosomal B and/or C complexes.^{111,112} YB-1 promotes transcription and controls use of alternative 5'-splice sites in pre-mRNA from a *CMV* promoter-driven *E1A* minigene^{113,114} and promotes inclusion of potentially hundreds of camptothecin-dependent alternative exons in cellular pre-mRNAs.¹¹⁵ YB-1-dependent splicing relies on the RNA- and RNAPII CTD-binding transcriptional cofactors FUS or EWSR1, at least the latter of which mediates YB-1 association with RNAPII.¹¹⁵ Although it is not known if YB-1-mediated splicing depends on YB-1 binding to promoters, YB-1-mediated splicing does not depend on transcription

elongation rates,¹¹⁵ and appears to rely on YB-1 loading onto the RNAPII CTD (Figure 7A). These findings suggest a model for YB-1 function in splicing that is similar to that proposed for NRs (Figure 6).

YB-1 function in splicing requires not only its association with RNAPII through FUS and EWSR1 but also its ability to directly bind RNA. Whereas RNA binding is largely mediated by its C-terminal disordered region, specificity for the AACAU motif is mediated by its evolutionarily conserved nucleic acid-binding cold-shock domain, possibly through weak binding to AACAU and/or its dimerization or multimerization on RNA.^{116–119} YB-1 binding to RNA promotes alternative exon inclusion or skipping depending on its association with SR proteins or with heterogeneous nuclear ribonucleoproteins (hnRNPs), which are known splicing enhancers and inhibitors, respectively.^{26,64,120,121} For example, the SR protein TRA2B increases YB-1-mediated inclusion of an alternative exon in *CD44* pre-mRNA, possibly by facilitating YB-1 binding to an exonic-splicing enhancer.¹²² YB-1 binding to this splicing enhancer and to the upstream polypyrimidine tract facilitates recruitment of U2AF2 to the cognate 3'-splice site, formation of the spliceosomal A complex, and inclusion of the targeted alternative exon^{123,124} (Figure 7B). YB-1-mediated exon skipping through its cooperation with various hnRNPs is exemplified by its binding concomitantly with hnRNP C to an exonic-splicing suppressor in *MUSK* pre-mRNA so as to promote exon exclusion.¹²⁵ Nuclear hnRNP C promotes exon skipping by forming heterotetramers around which 150–250-nucleotide U-rich RNA stretches are wrapped so as to form “RNA nucleosomes” that hide specific splice sites and/or bring alternative 5'- and 3'-splice sites in close proximity.^{10,126,127} YB-1 also forms large multimeric structures that are encased by up to 600–700 nucleotides of pre-mRNA.¹²⁸ YB-1 could possibly cooperate with hnRNP C to form higher-order RNA nucleosomes, thereby promoting exon skipping (Figure 7C).

2.2.4. Summary—Overall, certain TFs, including a number of NRs, appear to regulate pre-mRNA splicing independently of their effects on transcription elongation. Instead, these TFs bind to promoters, where they recruit either RNA-binding transcriptional cofactors that have splicing functions or bona fide SFs. In addition to binding RNA, these cofactors or SFs often have the ability to associate with RNAPII and later with the spliceosome. In this model (Figure 6, scenario i), elongating RNAPII carries TFs and their associated SFs or cofactors from the promoter to the gene body. Subsequently, nascent pre-mRNA is imprinted with TFs and their cofactors or SFs, which promote or inhibit spliceosome formation on the targeted splice sites to regulate alternative splicing. Alternatively, promoter-recruited TFs might promote pre-mRNA imprinting through DNA looping rather than by loading onto RNAPII (Figure 6, scenario ii).

3. REGULATION OF PRE-mRNA SPLICING BY TRANSCRIPTION FACTOR BINDING TO GENE BODIES

3.1. Regulation of Pre-mRNA Splicing through Control of Transcription Elongation

3.1.1. C₂H₂-Zinc-Finger Transcription Factors Promote RNAPII Pausing—CTCF, VEZF1, and possibly MAZ, which are three TFs characterized by Krüppel C₂H₂-

type zinc fingers (C_2H_2 -ZnFs), bind gene bodies, namely, downstream of promoters, to temporally stall RNAPII elongation and promote alternative exon inclusion (Figure 8A). CTCF was initially found to augment the inclusion of alternative exon 5 of human *CD45* pre-mRNA by binding the DNA region that encodes exon 5, thereby promoting local RNAPII pausing.¹⁹ Additional genome-wide RNA sequencing (RNA-Seq) revealed that CTCF induces inclusion of alternative exons that ChIP-Seq experiments determined are preferentially located upstream of an intronic CTCF-binding site.¹⁹ In vitro runoff transcription assays using short synthetic double-stranded DNA harboring a CTCF RE demonstrated that CTCF binding to DNA promotes transcription arrest immediately upstream of the CTCF-binding site.¹⁹ These results indicate that CTCF acts as a roadblock to elongating RNAPII and that CTCF-mediated arrest of RNAPII might depend on the previously reported direct interaction of CTCF with the large subunit of RNAPII.^{129,130}

VEZF1 and MAZ belong to the MAZ-like family of TFs and share a 77% identical C_2H_2 -ZnF DNA-binding domain (DBD). ChIP-Seq using human HeLa S3 and mouse embryonic stem cells identified VEZF1-dependent enrichment of elongating RNAPII on more than half of the VEZF1 GC-rich DNA-binding sites in gene bodies.²⁰ Pre-mRNAs encoded by each of three selected genes harboring a VEZF1-binding site that triggers VEZF1-dependent RNAPII pausing undergo exon skipping upon VEZF1 knockout, suggesting that VEZF1 binding to gene bodies augments alternative exon inclusion by inducing local RNAPII pausing.²⁰ Interestingly, comparison of the anti-VEZF1 ChIP-Seq data with results from two independently performed anti-CTCF ChIP-Seq experiments indicates that VEZF1 and CTCF might cooperate to control transcription elongation-dependent splicing.²⁰ Regarding MAZ, insertion of the MAZ₄ DNA sequence, which harbors four MAZ DNA-binding sites, upstream of an intronic splicing silencer in a α -tropomyosin or *FGFR2* minigene promotes RNAPII pausing and inclusion of the adjacent alternative exon in the encoded pre-mRNA.^{131,132} These results indicate that MAZ, similarly to CTCF and the MAZ-like TF VEZF1, promotes inclusion of alternative exons through RNAPII pausing.

3.1.2. Regulation of Pre-mRNA Splicing through Chromatin Remodeling—

Regulation of alternative pre-mRNA splicing by TF binding to gene bodies can also involve cotranscriptional TF-mediated pre-mRNA imprinting with histone modifiers, that is, recruitment of histone modifiers to pre-mRNAs, and consequential changes in the rate of RNAPII elongation (Figure 8A,B). The RNAPII-bound TF complex DBIRD promotes local transcription elongation and skipping of those A/U-rich exons that it binds.¹³³ This could occur by (i) DBIRD acting as a bridge between DNA and nascent pre-mRNA through the C_2H_2 -ZnF TF ZNF326, which is a DNA- and RNA-binding subunit of DBIRD,^{134–136} and (ii) DBIRD inhibiting the transcriptional corepressor histone deacetylase 3 (HDAC3) through its DBC1 subunit¹³⁷ (Figure 8B). Similarly, the RBP HuR induces exon skipping in mouse *Nf1* and *Fas* pre-mRNAs by binding nascent pre-mRNA near the alternative exon so as to promote histone hyperacetylation and local chromatin loosening by inhibiting HDAC2.¹³⁸ This leads to increased RNAPII elongation rates and exon skipping of subsequently synthesized pre-mRNAs.¹³⁸ Conversely, HDAC1 and HDAC2 are recruited to the alternative exon 2 of *MCL1* pre-mRNA through the SF SRSF1 so as to deacetylate

histones H3 and H4 in nucleosomes bound to exon 2 of the *MCL1* gene, promote local chromatin condensation, reduce local transcription elongation rates, and increase inclusion of *MCL1* pre-mRNA exon 2¹³⁹ (Figure 8A).

Returning to CTCF, VEZF1, and MAZ, they too could trigger alternative splicing through their ability to bind RNA and control histone acetylation. First, reversing the orientation of the MAZ₄ element in the above-mentioned α -tropomyosin minigene partially inhibits its effect on alternative exon inclusion.¹³¹ MAZ₄-mediated pausing was also found to be orientation-dependent in its ability to promote transcription termination.¹⁴⁰ Considering that the orientation of TF DNA-binding sites does not interfere with the ability of TFs to bind DNA and to regulate transcription,^{141,142} inverting the MAZ₄ element might reduce MAZ binding to RNA^{135,136} so as to impede RNAPII elongation. Second, a comparison of CLIP-Seq and ChIP-Seq data revealed that CTCF binds pre-mRNA largely in introns that are encoded by DNA sequences in close proximity to CTCF DNA-binding sites.¹⁴³ This suggests that nascent pre-mRNAs might be cotranscriptionally imprinted with CTCF. As discussed below (see section 3.2), the finding that CTCF binds to DNA and RNA in a mutually exclusive manner¹⁴³ supports a DNA-to-RNA hand-off mechanism. Alternatively, because CTCF dimerizes in an RNA-dependent manner,¹⁴⁴ it might bind DNA and RNA as a homodimer. Third, because CTCF harbors deacetylase activity through its interaction with the SIN3A/HDAC(1/2)-corepressor complex¹⁴⁵ and VEZF1 associates with the MRG15 component of HAT and HDAC complexes,²⁰ CTCF, MAZ, and/or VEZF1 might regulate pre-mRNA splicing not only by forming a roadblock to RNAPII on DNA (see section 3.1.1) but also, as proposed in the case of DBIRD, by directly binding to pre-mRNA and controlling local histone acetylation levels (Figure 8A,B).

3.1.3. Regulation of Pre-mRNA Splicing through the Stabilization of R-Loops

—Another speculative mechanism by which CTCF, and possibly other TFs, might regulate transcription elongation-dependent splicing involves the stabilization of R-loops. R-loops are higher-order RNA:DNA structures that typically form upstream of elongating RNAPII. They are characterized by (i) an RNA:DNA hybrid generated by the annealing of the nascent pre-mRNA exiting from elongating RNAPII to the corresponding coding strand of the transcribed gene and (ii) the displaced noncoding strand of the gene¹⁴⁶ (Figure 9). The consequences of R-loop formation have been largely investigated in the context of gene transcription and genome stability.¹⁴⁶ However, one study that used the model plant *Arabidopsis thaliana* demonstrated that cotranscriptional R-loop formation induces RNAPII pausing and alternative exon retention.¹⁴⁷ This example of R-loop-dependent splicing involves the annealing of the DNA coding strand to its cognate circular RNA, which forms as a consequence of back-splicing. Additionally, R-loops formed from nascent pre-mRNA have been reported to promote RNAPII pausing in yeast, although effects on pre-mRNA splicing were not examined,¹⁴⁸ and to control pre-mRNA splicing in humans, although RNAPII pausing was not examined.¹⁴⁹ CTCF,¹⁵⁰ and possibly YB-1,¹⁵¹ preferentially binds R-loop-containing loci, and ER α and/or ER β stabilize R-loops in an estrogen-dependent manner.¹⁵² Therefore, one can imagine that the splicing functions of these TFs might also include control of RNAPII pausing by binding and stabilizing the RNA:DNA hybrid or the single-stranded DNA strand of an R-loop (Figure 9). Moreover, TF-binding to intronic or

exonic pre-mRNA sequences in the context of an R-loop might also influence spliceosome assembly or function (see sections 2.2 and 3.2).

Of note, the RNA:DNA hybrid of an R-loop that bears tandem guanine tracks can fold into an extremely stable secondary structure by forming RNA:DNA hybrid G-quadruplexes (G4s).¹⁵³ The MAZ-binding element MAZ₄ forms such RNA:DNA hybrid G4s and promotes RNAPII pausing and transcription termination.^{140,154,155} Considering that several SFs and TFs (including U2AF2, SRSF1, FUS, hnRNPs, and YB-1) bind RNA G4s,¹⁵³ it is conceivable that TF and/or SF binding to RNA:DNA hybrid G4s might control pre-mRNA splicing as discussed in the case of classical R-loops.

3.1.4. Role of DNA Methylation in TF-Mediated Pre-mRNA Splicing—

Methylation of DNA on cytosines is an important epigenetic modification, that is, a heritable modification that does not change the DNA sequence, that can either repress or activate gene transcription.¹⁵⁶ In vertebrates, DNA methylation occurs largely on CpG dinucleotides found in interspersed sequences called CpG islands. CpG islands are enriched in gene promoters and gene bodies, and their high levels of DNA methylation control gene transcription by inhibiting the binding of TFs to their REs and by recruiting methyl CpG-binding proteins, the latter of which can modify chromatin by promoting histone deacetylation and methylation.¹⁵⁷ It is therefore not surprising that DNA methylation is also emerging as an important regulator of pre-mRNA splicing.¹⁵⁸

CTCF provides the only known example of TF-mediated splicing controlled by DNA methylation (see section 3.1.1). DNA methylation was found to inhibit CTCF binding to its C-rich RE in exon 5 of the *CD45* gene, thereby promoting exon 5 exclusion by limiting RNAPII pausing upstream of the CTCF RE.¹⁹ Considering its critical role in the control of gene transcription, it is certain that DNA methylation will emerge as a regulator of additional TF-mediated splicing events.

Beyond classical TFs, it is worth mentioning that chromatin-binding proteins can influence alternative pre-mRNA splicing through DNA methylation-dependent mechanisms that appear to be similar to those orchestrated by TFs. As one example, binding of the methyl-CpG-binding protein MeCP2 to methylated DNA exons promotes the inclusion of these exons in the resulting mRNAs, presumably by decreasing RNAPII elongation rates in an HDAC-dependent manner.¹⁵⁹ Of note, DNA methylation influences histone modifications, including trimethylation of histone H3 on lysine 4 (H3K4me3), lysine 9 (H3K9me3), or lysine 36 (H3K36me3). These modifications are enriched in alternative exons¹⁶⁰ and regulate pre-mRNA splicing by recruiting diverse trimethylated histone-binding proteins that include HP1 γ ,^{161,162} which controls RNAPII elongation rates, as well as MRG15,^{163,164} PSIP1,¹⁶⁵ and HP1 α ,^{160,166} all three of which mediate the imprinting of SFs on nascent RNAs.

3.2. Regulation of Pre-mRNA Splicing through Recruitment of Splicing Factors

3.2.1. WT1 Transcription Factor: A Case Study

3.2.1.1. WT1 Localizes to Nuclear Speckles and Associates with Splicing Factors: The first indication that TFs could directly regulate pre-mRNA splicing emerged in 1995 when the laboratory of Nick Hastie investigated post-transcriptional functions of the Wilms tumor-suppressor protein WT1.¹⁶⁷ This was 2 years before the laboratory of Alberto Kornblihtt published the first evidence that promoters can control splicing through RNAPII elongation rates (see section 2.1). WT1 is a TF characterized by four C₂H₂-ZnFs, the last three of which are critical for WT1 binding to specific G-rich DNA sequences found in the promoters of numerous genes.¹⁶⁸ Use of an evolutionarily conserved alternative 5'-splice site in *WT1* pre-mRNA results in the insertion of three amino acids, lysine-threonine-serine (+KTS), between ZnFs #3 and #4 that reduces DNA-binding efficiency¹⁶⁹ and alters DNA-binding specificity.¹⁷⁰ Whereas the WT1 isoform lacking KTS, namely, WT1(-KTS), colocalizes largely with other TFs at sites of active transcription, WT1(+KTS) is mostly found with SFs in nuclear speckles.¹⁶⁷ Rather than being static, the two WT1 isoforms shuttle between active transcription sites and nuclear speckles, as demonstrated by the partial relocation of WT1(-KTS) to nuclear speckles upon DNase treatment and the partial relocation of WT1(+KTS) to transcription sites upon RNase treatment.¹⁶⁷

In agreement with its presence in nuclear speckles and potential function in splicing, WT1 coimmunoprecipitates with several SFs^{167,171} and cosediments with pre-mRNA bound to the spliceosomal B complex and/or the mature C complex.^{172,173} Furthermore, yeast two-hybrid screens identified three SFs as direct WT1 interactors: U2AF2¹⁷² and the regulatory SFs RBM4¹⁷³ and WTAP.¹⁷⁴ RBM4 and WTAP are RBPs¹⁷⁵⁻¹⁷⁷ and associate with U2AF2 in the spliceosome.^{110,178}

Evidence that WT1 directly regulates alternative splicing exists from studies of its interaction with RBM4.¹⁷³ RBM4 promotes or represses the splicing of alternative exons, possibly by targeting intronic pyrimidine-rich sequences such as the polypyrimidine tract.¹⁷⁵ Like WT1(+KTS), RBM4 localizes to nuclear speckles,¹⁷³ directly binds U2AF2, and is part of the mature spliceosomal C complex.¹¹⁰ The findings that WT1(+KTS) inhibits RBM4-mediated inclusion or skipping of minigene alternative exons¹⁷³ and that WT1(+KTS) does not impact splicing in the absence of RBM4 suggest that WT1(+KTS) binding to RBM4 inhibits RBM4 associating with the spliceosome, possibly by inhibiting its binding to pre-mRNAs. Additional evidence for a TF-SF inhibitory mechanism is presented below (see section 4.2). In contrast, WT1(-KTS), which does not bind RBM4, does not inhibit RBM4-regulated splicing, highlighting how WT1 isoforms differentially target and regulate the splicing of pre-mRNAs.¹⁷³

In agreement with the two WT1 isoforms functioning differently, WT1(+KTS) preferentially binds U2AF2,¹⁷² and WT1(-KTS) preferentially binds WTAP.¹⁷⁴ It was hypothesized that WT1 binding to U2AF2 or WTAP controls U2AF2- and WTAP-mediated splicing by either promoting or blocking early steps of spliceosome assembly.¹⁷⁹ Although direct evidence for WTAP impacting spliceosome formation on pre-mRNA is lacking, WTAP coimmunoprecipitates with many splicing regulators, including SR proteins and hnRNPs.¹⁸⁰

Moreover, its *Drosophila* ortholog, FL(2)D, coimmunoprecipitates with the *Drosophila* orthologs of U2AF1, U2AF2, and U1 snRNP constituent SNRP70, but not with a core component of U5 snRNP,¹⁷⁸ indicating that WTAP takes part in the early spliceosomal E complex or the subsequent A complex.

WTAP is also a regulatory subunit of the RNA m⁶A methylation complex that methylates adenosine at the N⁶ position.^{176,177,180,181} m⁶A within alternative exons promotes exon inclusion through cooperation between the m⁶A reader YTHDC1 and the SR protein SRSF3.¹⁸² In contrast, intronic m⁶A promotes skipping of an alternative exon of the *Drosophila Sxl* pre-mRNA through a mechanism involving cooperation of YT521-B, the *Drosophila* ortholog of human YTHDC1, and the SF Sxl.^{183,184} Interestingly, Sxl associates with and shares splicing targets with FL(2)D/WTAP.^{178,185} Thus, WT1 binding to WTAP might influence alternative pre-mRNA splicing in the capacity of WTAP to catalyze m⁶A formation on pre-mRNA. Additional evidence for this model is discussed below (see section 5).

In conclusion, isoforms of the TF WT1 preferentially bind distinct SFs and differentially influence pre-mRNA splicing, possibly by enhancing or inhibiting spliceosome recruitment to pre-mRNA in a mechanism that, in some cases, might reflect changes in pre-mRNA m⁶A content.

3.2.1.2. WT1 Binds RNA Secondary Structures: Hints of WT1 As a Bridge Between DNA and Nascent Pre-mRNA?: In addition to associating with SFs, both WT1(–KTS) and WT1(+KTS) bind nascent transcripts in *Xenopus* oocytes.¹⁸⁶ In support of a role for WT1 binding to pre-mRNA in splicing regulation, WT1(–KTS) and WT1(+KTS) promote and inhibit, respectively, the accumulation of an unspliced *CMV*-driven *HIV*RNA that contains the well-characterized *MPMV* constitutive transport element,¹⁸⁷ which is an extended RNA hairpin resembling other WT1 RNA-binding sites (Figure 10, top).

Additional evidence that WT1 directly binds RNA hairpins derives from experiments using SELEX that show that the ZnF DBD of WT1(±KTS) binds three distinct families of RNA ligands that form complex hairpins¹⁸⁹ whose structures are critical for binding¹⁹¹ (Figure 10, bottom, leftmost three). Similarly, surface plasmon resonance experiments demonstrated that the WT1 DBD binds a hairpin in mouse *Actn1* (pre-m)RNA (Figure 10, bottom, second rightmost) whose structure and flanking nucleotides are required for binding.¹⁹²

Additionally, yeast three-hybrid assays, which can better simulate mammalian-cell conditions than SELEX or surface plasmon resonance experiments, revealed that WT1 binding to the *Actn1* (pre-m)RNA hairpin requires both its DBD and its N-terminal region.¹⁷¹ The WT1 N-terminal region harbors a putative RRM that is similar to the RRM of the core snRNP SF U1A.¹⁹³ Thus, the yeast three-hybrid results¹⁷¹ suggest that both the DBD and the RRM of WT1 cooperatively bind RNA in vivo. Notably, WT1 binding to DNA and RNA through its ZnF region appears to be mutually exclusive in vitro.¹⁸⁹ Considering that the ZnF region of WT1 is strictly required for WT1 to bind RNA in cells¹⁷¹ and that WT1 was not reported to bind DNA through a domain other than its ZnF, binding of WT1 to DNA and RNA must also be mutually exclusive in cells.

When examining reports of WT1 binding to cellular RNAs,^{171,190} we noticed that WT1 RNA-binding sites are encoded by DNA that either harbors or resides upstream of a WT1 DNA-binding site (i.e., a WT1 RE). This arrangement typifies how other TFs bind genes and their encoded RNAs (see section 3.2.3). For example, we noticed a consensus WT1 RE on the antisense strand of the mouse *Actn1* gene situated ~300-bp upstream of the region encoding an exonic RNA-binding hairpin¹⁷¹ (Figure 10, bottom; Figure 11, top). These findings suggest the intriguing possibility that WT1 DNA- and RNA-binding activities might be functionally coupled in cells. In support of this possibility, the predicted WT1 RE within the mouse *Actn1* gene and the *Actn1* gene sequence encoding the RNA-binding hairpin are evolutionarily conserved relative to the intervening linker region (Figure 11, top). Moreover, this putative WT1 RE coincides with a WT1 ChIP-Seq peak in HEK293 cells (GEO:GSE92194, ENCODE Project¹⁹⁴), suggesting that WT1 actually occupies this location on mammalian-cell DNA. Considering that WT1 binding to DNA and RNA is mutually exclusive, we suggest that WT1 first binds a target gene and is later handed off to the nascent transcript of that gene. Alternatively, as suggested by others,¹⁷² in view that WT1 dimerizes on DNA,^{172,195,196} one DNA-bound WT1 molecule could cooperate with a second WT1 molecule that binds nascent pre-mRNA.

The WT1 RE in the mouse *Igf2* gene encodes a WT1 RNA-binding site in the alternative exon 2 of *Igf2* RNA,¹⁹⁰ which we suggest is bound by WT1 cotranscriptionally (Figure 11, bottom). Because this RNA-binding site would become accessible to WT1 only after RNAPII clears the corresponding DNA sequence, this arrangement is likewise consistent with the possibility of a DNA-to-RNA hand-off or dimerization-on-DNA mechanism. In support of either scenario, we noticed that the WT1 RNA-binding site is the tip of an extended hairpin structure within *Igf2* RNA (Figure 10, bottom, rightmost), and that the WT1 RE and the sequence of this RNA hairpin are evolutionarily conserved (Figure 11, bottom).

Extending observations made for the *Actn1* and *Igf2* genes, we found that WT1 binds at least 43 (pre-m)RNAs encoded by genes to which WT1 is recruited (Figure 12). Target genes were identified in mouse embryonic (E18.5) kidney using ChIP-Seq¹⁹⁷ and ChIP followed by microarray hybridization (ChIP-chip).¹⁹⁸ RNAs bound by WT1 were determined for mouse mesonephric M15 cells using both RNA-IP (RIP) followed by sequencing (RIP-Seq) and formaldehyde-assisted cross-linking, ligation, and sequencing of RNA hybrids (FLASH).¹⁹⁹ Despite significant overlap between WT1-binding genes and WT1-binding (pre-m)RNAs ($P = 4.49 \times 10^{-6}$), the vast majority of RNA-binding sites do not reside in (pre-m)RNAs encoded by the genes to which WT1 is recruited (Figure 12). This observation is unlikely to be due to incorrectly identified binding sites (i.e., false positives) because we analyzed genes and RNAs bound by WT1 using data derived from two independent methods in each case. Thus, the small percentage of WT1-binding RNAs that derive from WT1-binding genes can be partially explained by high false-negative rates due to the stringent criteria used to call target genes and RNAs. Additionally, WT1 may might bind pre-mRNAs through a mechanism that does not involve cotranscriptional imprinting.

FLASH identified a significant enrichment of hairpin structures among WT1 RNA-binding sites, particularly in 3'-untranslated regions (3'-UTRs).¹⁹⁹ It was proposed that WT1 binding to RNA promotes the formation of and/or stabilizes RNA hairpins,¹⁹⁹ as is evident when comparing the locations and predicted stabilities of hairpins in pre-mRNAs bound by WT1 using FLASH¹⁹⁹ and in cell lysates using psoralen analysis of RNA interactions.²⁰¹ Despite several lines of evidence pointing to a critical role for RNA hairpins in WT1 binding to RNA, how and to what extent RNA sequences contribute to binding requires further investigation. As discussed above in the case of NRs (see section 2.2.1), we propose that sequence specificity involves the interaction of WT1 with sequence-specific RBPs that include U2AF2, RBM4, and WTAP.

Although not a focus of this review, data indicate that WT1 binding to RNA hairpins also supports WT1-regulated steps of gene expression that occur after DNA transcription and pre-mRNA splicing, including mRNA translation¹⁸⁷ and mRNA decay.^{199,202} Such functions in mRNA metabolism are consistent with data indicating that WT1 remains associated with mature poly(A)⁺ RNAs^{203,204} and cosediments with polysomes.²⁰⁵

3.2.1.3. Two Models for Transcription-Factor-Mediated Pre-mRNA Imprinting: As presented above, WT1 might imprint nascent pre-mRNA to influence pre-mRNA metabolism through at least two different mechanisms. In the first, WT1 might be handed off from a target gene to its nascent transcript (Figure 13A), given that WT1 cannot bind DNA and RNA simultaneously. As an alternative, it is possible that one DNA-bound WT1 molecule might recruit a different WT1 molecule to the nascent pre-mRNA (Figure 13B), in view that WT1(–KTS) and WT1(+KTS) isoforms coexist in cells and are able to homo- and heterodimerize on DNA.^{172,195,196} In either model, considering the SF- and nucleic acid-binding specificity of the two WT1 isoforms, their expression ratio and/or accessibility might influence which SF would imprint which pre-mRNA, that is, influence the splicing outcome of WT1 target genes. Nevertheless, considering (i) the higher affinity for DNA of WT1(–KTS) relative to WT1(+KTS)¹⁶⁹ and (ii) the preferred localization of WT1(–KTS) at sites of active transcription and WT1(+KTS) in nuclear speckles with SFs,¹⁶⁷ the most common configuration would likely involve WT1(–KTS) binding to gene promoters so as to facilitate the WT1(+KTS)-mediated recruitment of SFs to nascent pre-mRNAs. Of note, the cooperation of WT1 with its partner SFs might also influence the RNA sequences to which WT1 is recruited. Further support of a model for gene-body-mediated imprinting of the encoded pre-mRNA is presented below. Notably, because many TFs form functional homo- and/or heterodimers on DNA,²⁰⁶ the TF molecule involved in gene transcription might not be the same as that involved in downstream events.

3.2.2. Other C₂H₂-Zinc-Finger Transcription Factors—A recent high-throughput functional genomic screen for splicing regulators in mouse cells revealed 137 ZnF proteins (i.e., 57% of the 242 tested), 49 of which are C₂H₂-ZnF proteins.⁹⁵ Among the C₂H₂-ZnF proteins identified as direct or indirect splicing regulators were TFs with the ability to bind RNA, including WT1, the ubiquitously expressed Yin Yang 1 (YY1), the general transcription factor TFIIIA, and the neuron-restrictive transcriptional repressor REST/NRSE. We predict that these four and probably other C₂H₂-ZnF TFs, including PEP, ZNF74, and

ZNF224, function in splicing through gene-body-mediated pre-mRNA-imprinting (Figure 13).

Multiple lines of evidence support the idea that YY1 controls pre-mRNA splicing through a DNA-mediated imprinting mechanism. First, YY1 binds the *UBC* gene in the same intron whose removal from human *UBC* pre-mRNA it promotes.²⁰⁷ Second, YY1 binds the mouse *Xist* gene and its encoded RNA at the same location, the latter of which consists of four recurring hairpins in *Xist* RNA.²⁰⁸ Similarly to WT1, YY1 is present in nuclear speckles²⁰⁹ and dimerizes on DNA,²¹⁰ and although it associates with cytoplasmic poly(A)⁺ mRNPs,^{211–213} its binding to DNA and RNA are mutually exclusive in vitro.²¹² Finally, YY1 associates with the U4/U6.U5 tri-snRNP component SNU13.²¹⁴ Thus, as for WT1, YY1 might mechanistically couple DNA- and RNA-targeting strategies by binding gene bodies so as to function in pre-mRNA splicing and possibly subsequent steps of mRNA metabolism.

Whereas direct roles for the C₂H₂–ZnF TFs TFIIIA, REST/NRSF, and PEP in splicing have not been investigated, these TFs share the same DNA- and RNA-binding strategy as WT1 and YY1.^{215–217} Moreover, PEP is a component of hnRNP-bound pre-mRNAs known as H complexes and associates with spliceosomal B and/or C complexes.¹¹² ZNF74 and ZNF224 share other features with WT1 and YY1 that also suggest that they operate similarly. For instance, transcriptionally inactive isoforms of ZNF74^{218,219} and ZNF224²²⁰ bind RNA; localize in nuclear speckles; and, in the case of ZNF224, associate with polysomes more than their transcriptionally active isoform.

Despite a large body of evidence pointing toward a model involving recruitment of SFs, we do not exclude the hypothesis that the above-mentioned C₂H₂–ZnF TFs also or alternatively regulate pre-mRNA splicing by controlling RNAPII elongation rates, as exemplified by CTCF, VEZF1, and MAZ (see section 3.1).

3.2.3. T-Box Family of Transcription Factors—Evidence suggests that the TBX3 and TBX5 members of the T-box family of TFs regulate alternative splicing by gene-body-mediated pre-mRNA imprinting and recruitment of SFs, similarly to C₂H₂–ZnF TFs. Genome-wide analyses of mouse embryos conditionally depleted for TBX3 revealed that T-box-binding elements (TBEs), identified using TBX3 ChIP-Seq, reside within either intron flanking TBX3-regulated alternative exons.²²¹ TBEs are enriched in repetitive short interspersed nuclear elements, which tend to form complex secondary structures.²²² In cells, studies of pre-mRNA that derived from an artificial *RHCGlo* minigene demonstrated that TBX3 overexpression or depletion inhibited or promoted, respectively, removal of retained introns flanking a small exon encoded by DNA harboring a TBE.²²¹ A TBX3 variant lacking its DBD had no effect on splicing, in support of TBX3 binding to a TBE in controlling splicing of the encoded pre-mRNA.²²¹ Strikingly, TBX5, another TF belonging to the same T-box subfamily as TBX3,²²³ was found to promote splicing of the sole intron of pre-mRNA encoded by a *CMV*-driven *NPPA* minigene.²²⁴ We noticed a perfectly conserved consensus TBX5-binding site, namely, AGGTGTGA,²²⁵ in the exon residing upstream of this intron (Figure 14A) that is embedded in a TBX5 ChIP-Seq peak in mouse cardiac HL1 cells (GEO:GSE21529²²⁶). Thus, data support the hypothesis that TBX5 and TBX3 binding to a gene can regulate splicing of the encoded pre-mRNA.

Additional splicing assays using cells that expressed TBX3 tagged with the MS2-coat protein and an *RHCglo* minigene demonstrated that tethering TBX3 to pre-mRNA promotes pre-mRNA splicing.²²¹ In light of these findings, electrophoretic mobility shift assays revealed that TBX3 binds an RNA that derives from DNA containing two TBEs and led to the proposal that TBX3 binds and controls the splicing of pre-mRNAs harboring the same nucleotide sequence as the one that TBX3 binds on the corresponding gene.²²¹ However, our finding that the RNA used in these assays likely forms a stable hairpin (predicted $G = -227 \text{ cal mol}^{-1} \text{ nucleotide}^{-1}$) that is likely to be disrupted (predicted $G = -32 \text{ cal mol}^{-1} \text{ nucleotide}^{-1}$) by mutations shown to abolish TBX3 binding²²¹ supports the idea that TBX3, like C_2H_2 -ZnF TFs (see sections 3.2.1.2 and 3.2.2), binds RNA hairpins rather than a primary RNA sequence (Figure 14B). In agreement with this idea, we noticed that RNA transcribed from the TBE-containing exon of the *RHCglo* minigene described above is predicted to constitute one strand of a very stable hairpin (predicted $G = -450 \text{ cal mol}^{-1} \text{ nucleotide}^{-1}$; Figure 14C). In fact, DNA sequences that bind T-box TFs often reside downstream of reverse complementary sequences.²²³ We imagine that these sequences encode highly stable RNA hairpins that TBX3 binds in a DNA-to-RNA hand-off mechanism so as to influence pre-mRNA splicing. Such a mechanism might also typify TBX5, which binds RNA through its DBD in a way that can be weakly outcompeted by a nonspecific double-stranded DNA.²²⁴ Of note, unlike the above-mentioned C_2H_2 -ZnF TFs, TBX3²²⁷ and TBX5²²⁵ bind DNA as monomers, which does not support the dimerization model for gene-body-mediated imprinting of pre-mRNAs.

Finally, different domains of TBX3 appear to have opposite effects on splicing, possibly because they bind to different SFs and/or RBPs, including hnRNPs and RNA helicases.²²¹ Supporting the concept that TBX3 recruits SFs to RNA, CLIP-qPCR has shown that TBX3 binding to RNA also promotes the recruitment of its partner SFs.²²¹

3.2.4. Summary—In summary, several C_2H_2 -ZnF and T-box TFs appear to bind the body of genes and their encoded pre-mRNAs, recruiting SFs to weak splice sites to modulate splicing (Figure 13). Such coupling of TF binding to DNA and RNA, which could involve either TF hand-off or TF homo- or heterodimerization, enables DNA-encoded information to be passed to the nascent pre-mRNA so as to control its splicing. The cooperation of RNA-binding TFs with RNA-binding SFs might also increase TF RNA-binding specificity. Several of these TFs appear to remain associated with the spliced product and control downstream post-transcriptional events, including mRNA decay or translation.

4. REGULATION OF PRE-mRNA SPLICING BY TRANSCRIPTION FACTORS INDEPENDENT OF THEIR BINDING TO DNA

4.1. Is SOX6 a General Splicing Factor Independent of Being a Transcription Factor?

All of the TFs mentioned so far appear to regulate alternative pre-mRNA splicing in a way that relies on their binding to the promoter (see section 2) or the body (see section 3) of the corresponding gene. In contrast, the sex-determining region Y-(SRY-) related high-mobility group box (Sox) TF SOX6 seems to promote constitutive splicing of various pre-mRNAs in

the absence of any chromatin context, indicating that SOX6 acts on splicing independently of its ability to bind DNA. This idea derives from reactions analyzing splicing of in vitro transcribed pre-mRNAs from different species, including human β -globin, chicken δ -crystallin, or fruitfly *ftz* transcripts, using nuclear extracts from human HeLa cells devoid of DNA coding for the tested pre-mRNAs.²²⁸ SOX6 was required at early steps of splicing, because immunodepletion of SOX6 from extracts prevented formation of spliceosomal B and C complexes. Although these findings led to the proposal that SOX6 is a general SF,²²⁸ SOX6 has never been found associated with spliceosomes.^{74,109–112}

Re-expressing only the DBD of SOX6, SOX9, or SRY rescued the splicing defects due to immunodepletion of SOX6.²²⁸ The DBDs of SOX proteins are atypical: They bind four-way DNA junctions and bend DNA so as to provide a three-dimensional chromatin geometry that facilitates the formation of active transcriptional complexes.²²⁹ Based on the ability of SOX6 to bind RNA,²³⁰ it was hypothesized that SOX6 could facilitate spliceosome maturation by interacting with and bending intermediate four-way-like RNA junctions.^{228,230} In particular, SOX6 could recognize the U2/U6 four-way RNA junction found in an immature spliceosomal B complex²³¹ so as to facilitate its remodeling to a mature spliceosomal B complex. This maturation step involves multiple RNP remodeling events that include the transition of the U2/U6 four-way RNA junction to a U2/U6 three-way RNA junction²³¹ and possibly the dissociation of SOX6. This model (Figure 15) is consistent with the positive role of SOX6 in forming mature spliceosomal B complexes²²⁸ and the absence of SOX6 in isolated spliceosomes^{74,109–112} given that immature spliceosomal B complexes are transient intermediates.²³¹

Additional in vitro splicing assays showed that expression of only the SOX6 DBD restored DAX1-mediated inhibition of splicing of the same pre-mRNAs whose splicing is inhibited by SOX6 immunodepletion and that overexpression of SOX6, SRY, or SOX9 inhibits DAX1 binding to U2AF2.²³² Because SOX6 does not bind DAX1 through its DBD, it was hypothesized that this inhibitory mechanism involves competitive binding of SOX6 and DAX1 to U2AF2,²³² which could facilitate SOX6-mediated formation of spliceosomes (Figure 15).

4.2. Regulation of Pre-mRNA Splicing through Inhibition of Splicing Factor Binding to RNA

Data indicate that antagonistic TF–SF interactions in which TFs inhibit SF binding to RNA and SF-mediated splicing exist independently of TF binding to DNA (Figure 16A). Although less certain, it is also possible that SFs might inhibit TF binding to DNA and TF-mediated splicing (Figure 16B). Because the causality between TFs inhibiting SF binding to RNA or TFs inhibiting SF-mediated splicing has not been demonstrated, we cannot rule out the possibility that at least some of the examples described below are due to other inhibitory mechanisms.

As a first example, the E26 transformation-specific (Ets) TF SPI1 binds the RNA-binding domains of p54nrb and FUS, blocks their interaction with RNA, and inhibits their effects on the splicing of *E1A* pre-mRNA.^{233–235} Conversely, FUS binds the DBD of SPI1, blocks SPI1 binding to DNA, and inhibits SPI1-mediated splicing.²³⁴ SPI1-mediated splicing of

E1A pre-mRNA was found to rely on its DBD and on SPI1-binding sites within the *FES* or *FLII* promoters.²³⁶ Taken together, these findings indicate that SPI1 inhibits p54nrb- and FUS-mediated splicing by preventing their association with their target pre-mRNAs and that, in return, p54nrb and FUS inhibit splicing of pre-mRNAs encoded by genes under the control of SPI-dependent promoters.

As a second example, the C₂H₂-ZnF TF FBI-1 promotes selection of a proximal 5'-splice site in *BCL-X* pre-mRNA by interacting with the SF SAM68 and preventing its binding to *BCL-X* pre-mRNA.²³⁷ Yeast two-hybrid and GST-pulldown experiments demonstrated that FBI-1 interacts with SAM68 through its DBD, which was sufficient to inhibit SAM68 function.²³⁷ Although not tested experimentally, SAM68 binding to the DBD of FBI-1 might block FBI-1 binding to DNA, recapitulating the type of functional inhibition proposed for SPI1, p54nrb and FUS. Of note, a mouse homologue of FBI-1, ZBTB7B, was recently identified as a splicing regulator⁹⁵ and might similarly regulate splicing.

Binding of the T-box TF TBX5 to the SF SC35 provides yet another example of a mutually inhibitory TF-SF interaction. Although TBX5 and SC35 individually promote splicing of *NPPA* pre-mRNA, expressing TBX5 and SC35 together results in their mutual inactivation.²²⁴ Whereas TBX5 inhibits SC35-mediated splicing most likely because it prevents binding of SC35 to *NPPA* pre-mRNA,²²⁴ how SC35 inhibits TBX5-mediated splicing remains unknown. As for other TF-SF interactions that we have presented above, SC35 possibly inhibits TBX5 by blocking its ability to bind DNA, which is key to T-box TF-mediated splicing (see section 3.2.3).

TF binding to and inhibiting SFs might typify other TF-SF interactions, such as those between GFI and U2AF26²³⁸ or p63 and hnRNP A/B.²³⁹ However, further investigation is necessary to validate this possibility.

5. SUMMARY AND FUTURE DIRECTIONS

TFs are widely and often solely recognized as orchestrators of transcription initiation. In this review, we curated the literature for evidence that TFs might influence gene expression beyond transcription initiation. We compiled a large body of published data supporting the idea that TFs can also control pre-mRNA splicing through various mechanisms and with differing degrees of specificity (Figure 3, Tables 1–3).

Evidence that RNAPII promoters can control pre-mRNA splicing was first provided in 1997,²⁹ after which it was found that promoter-mediated splicing can rely on the recruitment of TFs that control transcription elongation rates.¹⁸ Findings from the early 2010s revealed that TF binding to gene bodies can also regulate splicing by controlling elongation rates.^{19,20} The idea that transcription elongation and pre-mRNA splicing are functionally coupled is now widely accepted (for reviews, see, e.g., refs 12, 13, and 21–27). TF-mediated control of elongation rates could involve changes in chromatin accessibility through recruitment of chromatin-modifying enzymes, formation of roadblocks on DNA, and/or control of intrinsic RNAPII activity, all of which could be locally regulated through DNA looping and/or pre-mRNA imprinting (Figures 5, 8, and 9).

Physical connections of a TF with the spliceosome came to light in 1995 from the finding that WT1, a C₂H₂-ZnF TF, localizes to nuclear speckles.¹⁶⁷ Since then, WT1 and other C₂H₂-ZnF and T-box TFs have been shown to bind REs in the transcribed region of genes, cotranscriptionally imprint pre-mRNA, and/or recruit SFs that promote or inhibit splicing (Figure 13). Several studies of NRs strongly suggest that TF binding to a promoter RE can also result in TF imprinting nascent pre-mRNAs so as to control splicing. In this scenario, TFs might recruit transcriptional cofactors or SFs while promoter-bound and subsequently reach alternative splice sites by associating with elongating RNAPII or, possibly, by DNA looping (Figure 6).

The specificity of alternative pre-mRNA splicing relies on the “splicing code”, namely, cis-acting regulatory elements on RNA that SFs recognize cooperatively to either activate or repress the use of adjacent splice sites.²⁴⁰ Most TFs discussed in this review directly bind RNA and could increase specificity by cooperatively binding to RNA regulatory elements. Of note, TFs that do not bind RNA can also imprint pre-mRNA through their interactions with RBPs. We and others have shown that such a mechanism allows TFs to be loaded onto mRNA to control mRNA translation⁹⁹ or decay.²⁴¹ In addition to recognizing RNA-binding sites through their ability to directly bind RNA or interact with RBPs, we have proposed that TF recognition of DNA-binding sites provides an additional layer of specificity.²⁴¹ The cooperative binding of TFs to composite REs, which is known to lead to diverse types of TF-mediated transcriptional output,²⁴² could add to the splicing code as well.

Evidence suggests that TFs might additionally control pre-mRNA splicing by promoting or inhibiting SF binding to pre-mRNA independently of their ability to bind DNA (Figures 15 and 16). Although speculative, the specificity of TF-mediated splicing in these scenarios would be more limited and would largely rely on the RNA-binding specificity of the targeted SF.

m⁶A methylation is a type of epigenetic pre-mRNA imprinting that controls not only alternative splicing^{182,184} but also mRNA translation^{243–247} and decay.^{248,249} It is reasonable to think that TFs might regulate splicing by controlling pre-mRNA m⁶A content based on the observation that WT1 directly interacts with WTAP (see section 3.2.1.1).¹⁷⁴ In addition, ZNF217, another C₂H₂-ZnF TF, reduces the level of pre-mRNA m⁶A by binding to and inhibiting the N⁶-adenosine methyltransferase METTL3.²⁵⁰ ZNF217 also directly binds these pre-mRNAs and is enriched at their promoters.²⁵⁰ Curation of interatomic databases²⁵¹ revealed additional connections between TFs and the m⁶A machinery, suggesting that control of m⁶A is widespread among TFs. For example, CREB1, IKZF1, and IFI16 associate with WTAP,^{252–254} and MYC coimmunoprecipitates with METTL3.²⁵⁵ c-JUN associates with WTAP, the m⁶A methylation complex component KIAA1429, and the m⁶A eraser ALKBH5.²⁵⁶ Promoter-mediated m⁶A formation might also depend on RNAPII elongation rates.²⁵⁷ Whereas promoter-mediated m⁶A formation was investigated in the context of mRNA translation, it might similarly be involved in TF-mediated regulation of pre-mRNA splicing.

Despite the parsing of TF-mediated splicing regulation into individual models, as we have done here, single or multiple TFs could regulate the splicing of an individual transcript by

coupling multiple models that, in sum total, ensure appropriate splicing. The mechanisms by which TFs regulate pre-mRNA splicing might also pertain to other steps of gene expression, including alternative polyadenylation, mRNA translation, and mRNA decay.^{8,11,13,257–260} The complex web of regulatory events undertaken by TFs is surely more than we have evidence for today.

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Biographies

Xavier Rambout obtained a Bioengineering degree in Chemistry and Bio-Industries from the Faculty of Agronomy of Gembloux, Belgium, and an M.Sc. degree in Molecular Medicine at Cranfield University, U.K., in 2008. He earned a Ph.D. degree in 2014 from the University of Liège, where he studied how transcription factors regulate mRNA decay under the supervision of Prof. Franck Dequiedt. Afterward, he joined Prof. Lynne Maquat's laboratory at the University of Rochester Medical Center, where he is interested in understanding how transcription factors and cofactors coordinate gene transcription and pre-mRNA processing.

Franck Dequiedt obtained a Ph.D. degree in 1998 from the Gembloux Faculty of Agronomy in Belgium and then performed postdoctoral training under the supervision of Eric Verdin at the Gladstone Institutes, University of California-San Francisco. After working as a Research Associate at the Belgian National Fund for Scientific Research (FNRS) from 2002 to 2013, he became a Professor of Molecular Biology in the Department of Life Science at the University of Liège (ULiège) in Belgium. His research interests are in the control of eukaryotic gene expression, with a recent focus on the links existing between the transcriptional and post-transcriptional machineries.

Lynne Elizabeth Maquat is the J. Lowell Orbison Endowed Chair and Professor of Biochemistry & Biophysics in the School of Medicine and Dentistry, Director of the Center for RNA Biology, and Chair of Graduate Women in Science at the University of Rochester, Rochester, NY. After obtaining her Ph.D. in Biochemistry from the University of Wisconsin-Madison and undertaking postdoctoral work at the McArdle Laboratory for Cancer Research, she joined Roswell Park Cancer Institute before moving to the University of Rochester. Professor Maquat discovered human-cell nonsense-mediated mRNA decay (NMD) in 1981 and, subsequently, the exon-junction complex (EJC) and how the EJC marks mRNAs for a quality-control “pioneer” round of protein synthesis. She also discovered Staufen-mediated mRNA decay, which mechanistically competes with NMD and, by so doing, new roles for short interspersed elements and long noncoding RNAs. Additional current interests include microRNA decay and functional links between transcription factors and RNA-binding proteins. She is an elected Fellow of the American Association for the Advancement of Science (2006) and an elected Member of the American Academy of Arts & Sciences (2006), the National Academy of Sciences (2011), and the National Academy of

Medicine (2017). Lynne was a Batsheva de Rothschild Fellow of the Israel Academy of Sciences & Humanities (2012–2013) and has received the William C. Rose Award from the American Society for Biochemistry & Molecular Biology (2014), a Canada Gairdner International Award (2015), the international RNA Society Lifetime Achievement Award (2017), the Federation of American Societies for Experimental Biology (FASEB) Excellence in Science Award (2017), and the Vanderbilt Prize in Biomedical Science (2017).

ABBREVIATIONS

ChIP	chromatin immunoprecipitation
CLIP	UV-cross-linking and immunoprecipitation
CTD	C-terminal domain
DBD	DNA-binding domain
FLASH	formaldehyde-assisted cross-linking, ligation and sequencing of RNA hybrids
G4	G-quadruplex
HAT	histone acetyltransferase
HDAC	histone deacetylase
hnRNP	heterogeneous nuclear ribonucleoprotein
m⁶A	N ⁶ -methyladenosine
NR	nuclear receptor
RBP	RNA-binding protein
RE	response element
RNAPII	RNA polymerase II
RRM	RNA recognition motif
SELEX	systematic evolution of ligands by exponential enrichment
SF	splicing factor
snRNA	small nuclear RNA
snRNP	small nuclear ribonucleoprotein particle
SR	serine-rich
TAD	transcriptional activation domain
TBE	T-box-binding element
TF	transcription factor

UTR	untranslated region
ZnF	zinc-finger

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Figure 1. Elementary steps of eukaryotic protein-coding gene expression. Expression of protein-coding genes involves a succession of steps, including (i) gene transcription, (ii) pre-mRNA processing, (iii) nuclear mRNA export to the cytoplasm, (iv) mRNA translation, and/or (v) mRNA decay. Red “pacmen” indicate exoribonucleases.

Figure 2. Coupling of protein-coding gene expression machineries in eukaryotes. Examples of how gene expression machineries (colored boxes) are physically and/or functionally connected (arrows). Adapted from ref 2. Copyright 2002 Macmillian Publishers, Ltd.

Figure 3.

Model for TF-mediated pre-mRNA splicing. (A) TFs regulate pre-mRNA splicing dependent on TF binding to DNA, either to promoters or to gene bodies, in a mechanism that can involve either (i) control of RNAPII elongation rates by recruiting HATs, HDACs, P-TEFb, or NELF or by forming roadblocks to RNAPII or (ii) control of imprinting of SFs to nascent pre-mRNAs. (B) Alternatively, TFs might impact pre-mRNA splicing independently of their ability to bind DNA so as to recruit or inhibit SF binding to pre-mRNAs. Colored numbers specify the section describing the corresponding mechanism.

Figure 4.

Model for the regulation of pre-mRNA splicing through RNAPII elongation rates. (A) With rapid pre-mRNA elongation by RNAPII, a weak 3'-splice site and a strong downstream 3'-splice site are synthesized nearly simultaneously, resulting in the selection of the strong downstream 3'-splice site by the spliceosome and removal of the alternative exon in mature mRNA. (B) With slow pre-mRNA elongation by RNAPII, the weak 3'-splice site is utilized by the spliceosome before the strong downstream 3'-splice site has been synthesized, resulting in removal of the intron upstream of the weak 3'-splice and inclusion of the alternative exon in mature mRNA.

Figure 5.

Model for how TF binding to a promoter can influence pre-mRNA splicing by controlling RNAPII elongation rates. Promoter-bound TFs recruit diverse transcriptional cofactors: (i, orange) HATs, which induce histone acetylation near the promoter and, through chromatin looping, in the gene body so as to increase RNAPII elongation rates and promote exon exclusion; (ii, dark blue) P-TEFb, which phosphorylates and thereby inhibits NELF and DSIF (not shown) and also phosphorylates the RNAPII CTD (not shown) so as to increase RNAPII elongation rates and promote exon exclusion; and (iii, aqua) NELF, which cooperates with DSIF to bind RNAPII, inhibits RNAPII escape from promoter-proximal pausing, and decreases RNAPII elongation rates to promote exon inclusion. See Figure 4 for details on the kinetic model.

Figure 6.

Model for how TF binding to a promoter can influence pre-mRNA splicing by imprinting pre-mRNA with transcriptional cofactors or SFs. Promoter-bound TFs, including a number of NRs, recruit either RNA-binding transcriptional coregulators that function in splicing or bona fide SFs. (i) In one scenario, TF-associated factors, and possibly TFs themselves, associate with the RNAPII, which works as an anchoring platform to carry these TFs and their associated factors to newly synthesized splice sites during the process of transcription elongation, promoting or inhibiting pre-mRNA splicing. (ii) In an alternative scenario, TF-associated factors, and possibly TFs themselves, directly imprint pre-mRNA through DNA looping so as to promote or inhibit pre-mRNA splicing. The association of TFs with their cofactors or SFs may be fostered in nuclear speckles.

Figure 7.

Model for how YB-1 binding to a promoter can influence pre-mRNA splicing by cooperating with EWSR1 and with SR or hnRNP proteins. (A) Promoter-bound YB-1 recruits EWSR1, which interacts with the RNAPII CTD. Through this interaction, RNAPII carries YB-1 to alternative exons so as to promote their (B) inclusion or (C) skipping. (B) YB-1 and SR proteins cooperatively bind an exonic-splicing enhancer and recruit U2AF2 to a weak 3'-splice site, promoting alternative exon inclusion. (C) YB-1 and hnRNP C form a multimeric complex that configures the alternative exon, promoting its exclusion.

Figure 8.

Models for how TF binding to a gene body can influence pre-mRNA splicing by controlling RNAPII elongation. (A) Gene-body-bound TFs can bind and block RNAPII downstream of a weak 3'-splice site to promote spliceosome formation, removal of the upstream intron, and inclusion of the alternative exon in mature mRNA. Alternatively or simultaneously, gene-body-bound TFs imprint (i.e., bind to) nascent pre-mRNAs and recruit HDACs or inhibit HATs so as to increase histone deacetylation, slow RNAPII elongation, and promote exon inclusion. (B) Gene-body-bound TFs can imprint nascent pre-mRNAs and recruit HATs or inhibit HDACs so as to increase histone acetylation, increase RNAPII elongation rates, and promote exon exclusion.

Figure 9.

Speculative model for how TF binding to R-loops could influence pre-mRNA splicing by promoting RNAPII pausing. TFs can stabilize an R-loop by binding either to the RNA:DNA hybrid or to the displaced, i.e., single-stranded, DNA strand. TF-mediated stabilization of the R-loop can form a roadblock to subsequently elongating RNAPII complexes downstream of a weak 3'-splice site, promoting early spliceosome formation, removal of the upstream intron, and inclusion of the alternative exon in mature mRNA.

Figure 10.

WT1 binds hairpin structures in RNA. Secondary-structure predictions using the Mfold web server¹⁸⁸ of (top) the *MPMV* constitutive transport element that mediates WT1-induced accumulation of unspliced RNAs,¹⁸⁷ (bottom, leftmost three) a representative from each of the three RNA families identified by SELEX as WT1 ligands,¹⁸⁹ and (bottom, rightmost two) the mouse *Actn1* RNA region identified in yeast three-hybrid analyses¹⁷¹ and the mouse *Igf 2* RNA region identified by RNase footprinting¹⁹⁰ as WT1 ligands. Predicted free energies (G) calculated using the Mfold web server were normalized to the size of each RNA. RNA residues that were experimentally shown to bind WT1 are blue.

Figure 11.

WT1 binds sequences in the *Actn1* and *Igf2* genes that are upstream of or overlap with the sequences WT1 binds in the encoded RNAs. Clustal Omega-predicted phylogenetic tree and alignment of WT1-binding sites in the *Actn1* and *Igf2* genes (red rectangles) or transcripts (blue rectangles) from different mammals. WT1 binds GCG(T/G)(G/A)GGCG(G/T) on either DNA strand.¹⁹⁷ Percentages denote identity among the different species for each boxed region.

Figure 12.

Additional evidence that WT1 binds genes and their encoded RNAs. Overlap of the 232 protein-encoding genes (green) found to bind WT1 in embryonic (E18.5) mouse kidney in both ($\cap$) ChIP-Seq¹⁹⁷ and ChIP-chip assays¹⁹⁸ and the 2124 RNAs (red) found to bind WT1 in M15 mesonephric cells in both ($\cap$) RIP-Seq and FLASH analyses.¹⁹⁹ The P value was determined using a Pearson's chi-squared test, considering there are 21 948 protein-encoding genes in mouse as referenced in the January 2017 mouse GENCODE release (version M14).²⁰⁰ The 43 genes identified to bind WT1 and whose encoded RNAs were also identified to bind WT1 are boxed.

Figure 13.

Two models for how WT1 binding to a gene body can influence splicing by imprinting nascent transcripts. (A) Gene-body-bound WT1 transitions in a hand-off mechanism to the encoded nascent pre-mRNA once a WT1-binding RNA hairpin is synthesized and accessible. (B) Alternatively, gene-body-bound WT1 dimerizes with another WT1 molecule bound to the RNA hairpin of nascent pre-mRNA. In either model, we envision that the identity of the DNA-bound WT1 molecule, namely, whether it is WT1(+KTS) or WT1(-KTS), controls the specificity of DNA binding and that the isoform identity of the RNA-bound WT1 molecule controls the recruitment of specific SFs, for example, U2AF, RBM4, or WTAP, so as to promote or inhibit splicing. In this model, the recruitment of SFs by WT1 is fostered in nuclear speckles.

Figure 14.

Evidence that T-box TFs couple DNA and RNA binding to control pre-mRNA splicing. (A) Clustal Omega-predicted phylogenetic tree and alignment of an AGGTGTGA TBX5-binding site²²⁵ (red rectangle) in the *NPPA* gene from different mammalian species. (B,C) Secondary structure predictions and normalized free energies (ΔG) calculated using the Mfold web server¹⁸⁸ of (B) RNAs synthesized from wild-type and mutated TBX3-binding elements (TBEs) used in electrophoretic shift assays of TBX3 binding.²²¹ (C) RNA sequence synthesized from the TBE-containing exon in the *RHCglo* minigene used in splicing assays of TBX3 function. Wild-type TBE residues are blue; mutated TBE residues are red.

Figure 15.

Model for how the TF SOX6 can promote pre-mRNA splicing. In the immature spliceosome B complex, SOX6 can bind the U2/U6 four-way-like RNA junction and/or U2AF2 in complex with U2 snRNA, thereby facilitating formation of a mature B complex in which the four-way RNA junction has transitioned to a three-way RNA junction and SOX6 has dissociated.

Figure 16. Model for how TFs and SFs can mutually inhibit their splicing functions: (A) TFs inhibit SF-mediated alternative splicing by inhibiting SF binding to RNA. (B) SFs inhibit TF-mediated alternative splicing by inhibiting TF binding to DNA.

Table 1.

List of TFs That Control Pre-mRNA Splicing by Binding Gene Promoters and Their Demonstrated or Putative Effectors^a

TF	candidate effector	ref(s)
2.1. Control of Transcription Elongation		
CREB1	p300	50
VP16	HAT or P-TEFb	18, 55, 56
AR	NELFB	59
2.2. Recruitment of Splicing Factors		
PPAR γ	PGC-1 α	62
ER α	CoAA, CAPER α , CAPER β	66–68
VDR	SKIP	75
THRB	THRAP3, PSF	77
RAR α and/or RAR β	Acinus	79
Mei4	U1–70K, Prp11, Cdc5	93, 94
Fkh2	unknown	94
TFDP1	unknown	95
SMAD3	PCBP1	96
HIF-1 α and HIF-2 α	unknown	97, 98
YB-1	EWSR1, FUS, TRA2B, hnRNP C	74, 109–115, 123–125
YBX3	unknown	74, 110, 111

^aRelated to section 2.

Table 2.

List of TFs That Control Pre-mRNA Splicing by Binding Gene Bodies and Their Demonstrated or Putative Effectors^a

TF	candidate effector	ref(s)
3.1. Control of Transcription Elongation		
CTCF	RNAPII, SIN3A/HDAC(1/2)	19, 129, 130, 145
VEZF1	MRG15	20
MAZ	unknown	131, 132
ZNF326 (DBIRD)	HDAC3	133–137
3.2. Recruitment of Splicing Factors		
WT1(+KTS)	U2AF2	95, 167, 171–173
WT1(–KTS)	WTAP	95, 167, 171–174
YY1	SNU13	95, 207, 209, 214
TFIIIA	unknown	95
REST/NRSF	hnRNPs	95
PEP	unknown	112
ZNF74	unknown	218, 219
ZNF224	unknown	220
TBX3	hnRNPs, RNA helicases	221
TBX5	unknown	224

^aRelated to section 3.

Table 3.

List of TFs That Control Pre-mRNA Splicing Independently of Their Ability to Bind DNA and Their Demonstrated or Putative Effectors^a

TF	candidate effector	ref(s)
SOX6	U2AF2	228, 232
WT1(+KTS)	RBM4	95, 167, 171–173
SPI1	p54nrb, FUS	233–236
FBI-1	SAM68	237
TBX5	SC35	224
GFI	U2AF26	238
p63	hnRNP A/B	239

^aRelated to section 4.