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**Commentary**

Chromatin modification influences gene expression by either repressing or activating genes, depending on the specific histone mark. Chromatin structure can also influence alternative splicing of transcripts; however, the mechanisms by which epigenetic marks influence splicing are poorly understood. A report in the current issue of the *JCI* highlights the biological importance of the coordinated control of alternative pre-mRNA splicing by chromatin structure and transcriptional elongation. Yuan et al. found that mutation of the histone methyl transferase SEDT2 affects alternative splicing fates of several key regulatory genes, including those involved in Wnt signaling. As a consequence, loss of SEDT2 in the intestine aggravated Wnt/β-catenin signaling effects, thereby leading to colorectal cancer.

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Epigenetics at the base of alternative splicing changes that promote colorectal cancer

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Chromatin modification influences gene expression by either repressing or activating genes, depending on the specific histone mark. Chromatin structure can also influence alternative splicing of transcripts; however, the mechanisms by which epigenetic marks influence splicing are poorly understood. A report in the current issue of the JCI highlights the biological importance of the coordinated control of alternative pre-mRNA splicing by chromatin structure and transcriptional elongation. Yuan et al. found that mutation of the histone methyltransferase SEDT2 affects alternative splicing fates of several key regulatory genes, including those involved in Wnt signaling. As a consequence, loss of SEDT2 in the intestine aggravated Wnt/β-catenin signaling effects, thereby leading to colorectal cancer.

One gene, multiple products

Human genes, as well as those of most multicellular eukaryotes, are organized as mosaics of exons and introns. RNA polymerase II (RNAPII) transcribes the DNA sequence of each gene to generate a precursor mRNA molecule (pre-mRNA) that contains both exons and introns and serves as the substrate for splicing, the process by which introns are removed and exons are joined, yielding the mature mRNA that will eventually be translated to protein. One of the evolutionary advantages of pre-mRNA splicing is the ability to alter mature mRNA via alternative splicing, which allows generation of multiple mRNA and protein variants from each gene, thereby greatly expanding the coding capacity of the genome. Indeed, the number of protein-coding genes in humans is not radically different from that in the worm Caenorhabditis elegans (~20,000). However, alternative splicing is prevalent in vertebrates (1), strongly suggesting a key role in the higher complexity of these organisms. As a compelling example, alternative splicing occurs in more than 95% of mammalian genes (2). In alternative splicing, a single type of pre-mRNA can be spliced in different ways, leading to similar but not identical mature mRNA species. A simple metaphor is that of a textile industry (transcription) that generates many copies of a fabric piece of the same length and quality (pre-mRNAs) and the tailor (the spliceosome) who makes different suits (mRNAs) with each piece of fabric, depending on where he/she cuts and sews (splicing) and on the remnants (introns) that are discarded.

Splicing is carried out by the spliceosome, a multimolecular complex composed of dozens of proteins and ribonucleoproteins. A single spliceosome assembles at every intron to be excised on each pre-mRNA as soon as it emerges from RNAPII during transcription (3). Indeed, accumulating evidence indicates that splicing, or at least the binding of spliceosome and splicing factors to the pre-mRNA that enables it, occurs cotranscriptionally (4). In turn, this cotranscriptionality allows for a complex molecular interplay between the transcription and splicing machineries so that both processes are mutually coordinated and the kinetics and molecular mechanisms intrinsic to each of them influence each other. As a consequence of this coupling, splicing, like transcription, is highly regulated by chromatin structure and the quality and distribution of posttranslational histone modifications (histone marks).

Two models

Two nonmutually exclusive models have been proposed for the role of chromatin in alternative splicing. In the transcription kinetics model, the preferential positioning of nucleosomes in exons (5, 6) or deployment of specific histone marks within the gene body hinder or facilitate RNAPII progression. Changes in overall elongation rates of RNAPII have been shown to promote or inhibit the inclusion of alternative exons in the mature mRNA, depending on the identity of the particular alternative splicing event (7–9). Intragenic histone modifications, including H2B monoubiquitylation (10), H3K9 acetylation and methylation (11, 12), and H3K4 trimethylation (13), that affect nucleosome organization have been shown to correlate with changes in elongation. Independently of their role in elongation, certain histone modifications, such as H3K79me1, H2BK5me1, H3K27me1, H3K27me2, and H3K27me3, are more abundant throughout internal exons compared with introns and clearly correlate to exon expression (see ref. 14 for a review). In the second model, histone marks are thought to affect alternative splicing by recruiting splicing factors through chromatin-binding proteins (15). For example, H3K36me3 has been shown to recruit MRG15, a chromodomain-containing protein that is part of several histone-modifying complexes. MRG15, in turn, drafts the splicing factor polyprymidine tract binding protein 1 (PTB), which controls alternative splicing decisions (16). There are many additional examples in the recent literature of histone modification–splicing factor associations that affect alternative splicing and evidence of a reverse process through which splicing factors and
the splicing reaction itself participate in the writing and stabilization of histone marks that affect chromatin structure (14, 15).

Colorectal cancer

Despite an overwhelming accumulation of evidence, there are two persistent questions about the role of chromatin in alternative splicing. One, do changes in chromatin only correlate with changes in splicing, or is there a clear cause and effect relationship between chromatin structure and splicing? Two, most importantly, what are the physiopathological roles of such a complex coupling mechanism? In this issue, Yuan and colleagues (17) present compelling evidence that answers both questions. Specifically, the authors focus on the histone H3K36 methyltransferase SETD2, which has been shown to be mutated in a variety of human tumors. In general, H3K36 methylation is a permissive mark that promotes gene transcription, while repressive marks, such as H3K9me and H3K27me, inhibit transcription. Yuan et al. showed that SEDT2 expression and H3K36 methylation are both downregulated in human colorectal cancer (CRC) tissues compared with normal counterparts. Reduction of SEDT2 expression in cultured cell lines promoted tumorigenesis, revealing that SEDT2 is a tumor-suppressor gene whose role depends on the catalytic activity of the enzyme. Furthermore, intestinal cell-specific inactivation of the Sedt2 gene in an engineered mouse model surprisingly had no detectable phenotypic or viability effects, suggesting that SEDT2 is dispensable for intestinal physiology. However, in animals harboring one mutated allele of the tumor-suppressor gene Apc (Apc<sup>min</sup> mice), intestinal SEDT2 ablation aggravated the adenoma-promoting role of APC deficiency, significantly shortening life span and greatly increasing the number of CRC tumors. These effects were determined to be the consequence of stimulation of Wnt-dependent transformation and stemness programs, as revealed by the up- and downregulation of the abundance of several signature mRNAs of Wnt signal transduction pathways. Many studies would have stopped at this result and assumed that perturbation of gene homeostasis in the absence of SEDT2 was exclusively due to changes in the transcription of key Wnt genes. However, Yuan and colleagues investigated global changes in mRNA-splicing variants and discovered more than 700 genes, many of which were syndicated as cancer associated, in the intestines of SEDT2-deficient animals with significant alterations in alternative splicing compared with intestines of control mice. ChIP followed by deep sequencing analyses (ChIP-seq) showed that, upon SEDT2 depletion, there is an outstanding overlap between genes with a reduction of the H3K36me3 mark in their bodies and those with changes in alternative splicing. Moreover, a subset of these genes displayed intragenic enrichment of RNAPII at alternative splicing sites, consistent with a reduction in RNAPII elongation.

One of the prevalent alternative splicing modes regulated by SEDT2 is intron retention. In particular, retention of intron 2 of the mRNA encoding DVL2 decreases with SEDT2 ablation. DVL2 is a member of the dishevelled (dsh) protein family, which is a key component of Wnt/β-catenin–dependent carcinoma progression.
triggers degradation through a mechanism known as nonsense-mediated mRNA decay (NMD) in WT cells. Therefore, retention of intron 2 in normal cells reduces DVL2 levels, whereas removal of intron 2 in CRC cell precursors, through the epigenetic mechanism elucidated by Yuan et al. (17), increased DVL2 levels, augmenting Wnt signaling and leading to malignant transformation (Figure 1).

Conclusions
Together, the results of this study highlight the importance of the coupling among the deployment of specific histone marks, alternative splicing regulation, and cell fate. Interestingly, another histone methyl transferase, G9a, that is specific for dimethylation of H3K9 has recently been shown to participate in an alternative splicing positive loop that enhances neuron differentiation (18). It is now more evident that when studying the molecular bases of cancer, the view should not be restricted to transcriptional regulation or to epigenetic alterations at the promoter and regulatory regions of genes. Instead, the scope must be widened to fully see how epigenetic changes within gene bodies regulate splicing decisions.

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