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et al. (2008) identified several additional methylation sites on FOXO1 that appeared to have no role in Akt-mediated phosphorylation. Methylation at these residues could potentially alter the accessibility of FOXO1 to other modifications, such as phosphorylation, acetylation, or ubiquitination. In this light, methylation could, therefore, play a very general role in the regulation of FOXO1 activity.

Finally, Yamagata et al. (2008) found that in addition to FOXO1, PRMT1 could also methylate other FOXO subfamily members: FOXO3, FOXO4, and FOXO6. As these proteins perform an array of individual functions, it remains to be determined how methylation alters the activity and posttranslational regulation of these proteins. Methylation of FOXOs might, therefore, be a master regulatory mechanism to dictate cell-fate decisions such as proliferation and survival. It is also possible that methylation is a common mechanism to regulate the ability of Akt to phosphorylate its substrates. It will be interesting to address these possibilities as the roles of methylation become apparent in the many functions of FOXO transcription factors and other Akt targets.

As an essential component of diverse growth and survival signaling pathways, FOXOs serve as a link between aging and age-related diseases such as diabetes and cancer. FOXO dysregulation has been reported in multiple tumor types, including rhabdomyosarcoma and leukemia (Huang and Tindall, 2007). By contrast, *FOXO1* deletion in hepatocytes protects against excessive glucose production and diabetes in insulin receptor null mice (Matsumoto et al., 2007). Therefore, proteins that regulate FOXOs form attractive targets for therapeutic intervention. The findings from Yamagata et al. (2008) suggest that PRMT1 could be a novel target for modulating the activity of FOXO proteins and, therefore, cell-fate decisions. This study demonstrates that PRMT1 can enhance FOXO activity to promote cell death, whereas decreased PRMT1 activity can promote cell survival. As FOXO proteins elicit such an array of effects on cells, pharmacologically modulating PRMT1 activity or expression might uncover novel approaches to target multiple FOXOs and alter survival and growth of cells in a variety of pathological conditions.

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Death by Splicing: Tumor Suppressor RBM5 Freezes Splice-Site Pairing

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In a recent issue of *Molecular Cell*, Bonnal et al. (2008) demonstrate that the tumor suppressor gene *RBM5* regulates alternative splicing of *Fas* pre-mRNA by interfering with splice-site pairing.

In eukaryotes, the process of pre-mRNA splicing is defined as the removal of intronic sequences along with the ligation of exons to form a mature message. As one way to diversify proteome evolution, gene expression developed a method to "mix and match" exonic sequences, thereby generating multiple transcripts from one gene. Alternative splicing is important for the regulation of gene expression in numerous cellular processes, including apoptosis and cancer progression. Interestingly, a large number of genes that function in the apoptosis pathway have multiple mRNA isoforms of opposing function that are generated through alternative splicing (Schwerk and Schulze-Osthoff, 2005). Although alternative splicing is prevalent during apoptosis, the mechanism and regulation of how it occurs is not very well understood. Recent work from Bonnal et al. (2008) shows that RBM5 (Luca-15/H37) regulates the splicing of the apoptotic factor *Fas* during a unique step of the splicing pathway.

The putative tumor suppressor gene *RBM5* is silenced in 70%–80% of lung
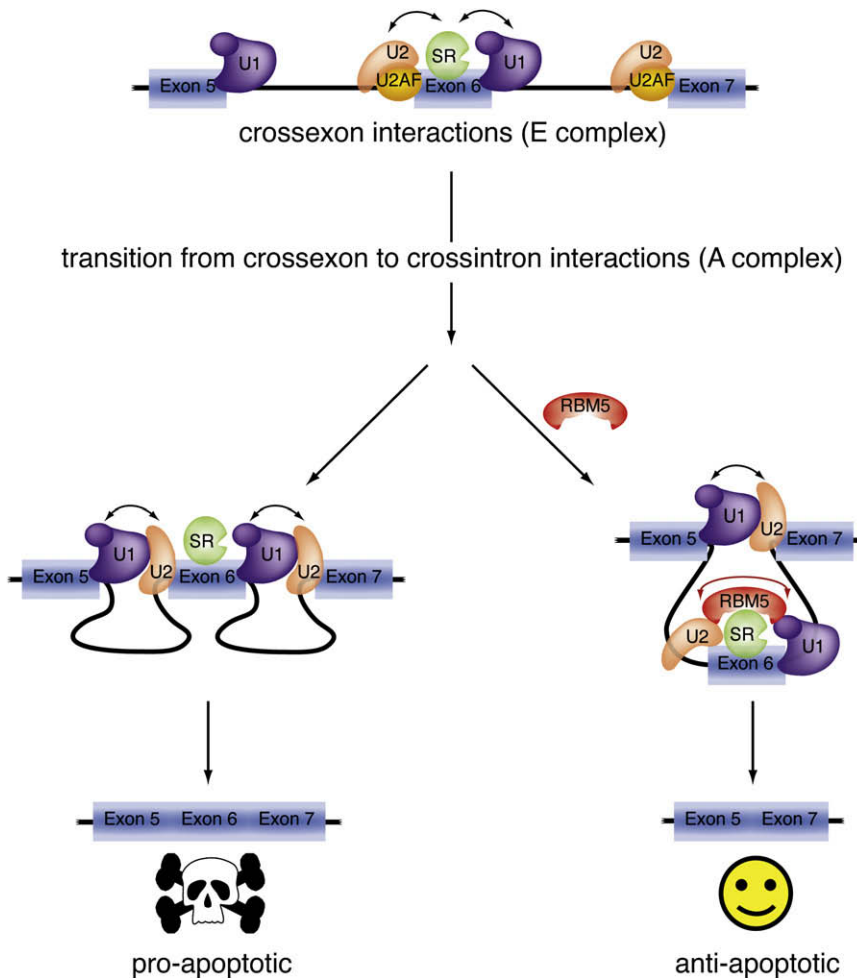
Figure 1. Regulation of RBM5-Mediated Alternative Splicing at the Splice-Site Pairing Step
In the E complex of spliceosome assembly, U1 snRNP and U2AF recognize the 5′ splice site and the polypyrimidine tract, respectively. SR proteins stabilize these crossexon interactions. In the A complex, crossexon interactions are replaced by crossintron interactions to establish splicing patterns. In the presence of RBM5 (right), the release from crossexon to crossintron interactions is blocked, presumably by strengthening crossexon interactions (red arrows). As a consequence, the regulated exon is skipped, generating the antiapoptotic isoform of Fas.

cancers and has been implicated in other types of cancers. Collectively, the data indicate that altered levels of RBM5 play a significant role in apoptosis and tumor progression (Mourtada-Maarabouni and Williams, 2006). Based on the presence of two RNA Recognition Motifs, two zinc fingers, and a serine-arginine-rich domain (SR domain), RBM5 was proposed to be involved in RNA processing. Indeed, proteomic analyses demonstrated that RBM5 copurified with the prespliceosomal A complex (Deckert et al., 2006). Bonnal et al. (2008) stepped up their investigations after RBM5 was identified as an interaction partner of U2AF65, a general splicing factor required for early

splice-site recognition. In a first set of follow-up experiments, Bonnal et al. (2008) established that knockdown of RBM5 and its family members RBM6 and RBM10 perturbed alternative splicing of the apoptosis-related genes *Fas* and *c-Flip*. Interestingly, the alternative splicing events induced upon RBM5 knockdown resulted in the preferential generation of proapoptotic Fas and c-Flip isoforms, regardless of whether the endogenous or minigenes were analyzed. As expected, RBM5 overexpression reversed these effects, which depended on the presence of a newly identified splicing enhancer and a weak 3′ splice site.

To address the specificity of RBM5-mediated alternative splicing, Bonnal et al. (2008) carried out a series of sequence swap experiments, testing heterologous pre-mRNAs derived from the regulatable *Fas* minigene and the RBM5 nonresponsive *α-globin* minigene. RBM5 could only confer regulation to *α-globin* pre-mRNAs if *Fas* exon 6 sequences and the distal splice sites were present. These results imply that RBM5-mediated alternative splicing is highly sequence specific, regulating presumably only a handful of target genes.

Bonnal et al. (2008) then carried out UV crosslinking and complex formation analyses to investigate the mechanism whereby RBM5 regulates *Fas* alternative splicing. Typically, regulation of alternative splicing occurs early in spliceosomal assembly, during the process of splice-site recognition (Figure 1). Surprisingly, Bonnal et al. (2008) found that RBM5 stalls spliceosomal assembly after A complex formation, a spliceosomal assembly step characterized by the stable association of U1 and U2 snRNPs with the 5′ and 3′ splice sites. Thus, even though the splice sites of the regulated exon are sufficiently recognized by components of the spliceosome, RBM5 somehow interferes with further progression of spliceosomal assembly (Figure 1).

This work adds to the growing support for the idea that control of alternative splicing can occur after splice-site recognition at the splice-site pairing step. In agreement with Bonnal et al. (2008) and kinetic evidence establishing that splice-site pairing occurs after splice sites are firmly associated with their respective snRNP (Lim and Hertel, 2004), two independent groups described alternative splicing regulation at the splice-site pairing step prior to the stable association of the tri-snRNP (House and Lynch, 2006; Sharma et al., 2008). To achieve this type of regulation, one can envision several plausible scenarios. The simplest explanation ascribes an inhibitory function to the regulator, in this case RBM5. It is possible that an interaction between RBM5 and the snRNPs at the 3′ and 5′ splice sites is so strong that it locks the snRNPs in a crossexon conformation. As a consequence, the necessary transition from crossexon to crossintron interactions cannot be carried out during

attempts to establish splice-site pairing (Figure 1). An alternative explanation is based on the recently described observation that SR proteins bound to an internal exon inhibit splicing of the flanking exons to each other (Ibrahim et al., 2005). A specific interaction between RBM5 and SR proteins bound to the regulated exon could mask this function, thus promoting exon skipping. Consistent with both interpretations is the fact that RBM5 is present in the prespliceosomal A complex (Deckert et al., 2006), but lost after the tri-snRNP has been incorporated.

From a biological point of view, the identification of RBM5 as a splicing regulator that induces the expression of antiapoptotic gene products opens several new avenues for further investigation. Although Bonnal et al. (2008) tested RBM5 specificity using functional assays, this analysis was limited to *Fas* splicing. In light of its copurification with prespliceosomal complexes, it remains possible that RBM5 is a more general splicing factor, a hypothesis easily testable using available high-throughput technologies. A previous study used expression microarrays to identify ~35 genes that change their expression levels upon RBM5 up- or downregulation (Mourtada-Maarabouni et al., 2006). Considering the newly established function of RBM5 as a specific regulator of alternative splicing, it is expected that the number of target genes will increase if similar analyses are extended to splicing sensitive microarrays. In addition, it should be investigated whether the up- and downregulation of the 35 identified genes are a consequence of alternative splicing that induce or prevent the activation of RNA surveillance mechanisms, such as nonsense-mediated decay. Another point of interest focuses on the question of whether the activity of RBM5 itself is regulated. Earlier work established that RBM5 comes in multiple flavors: through alternative splicing, *RBM5* mRNA isoforms are produced that either maintain their proapoptotic function or that induce antiapoptotic effects (Mourtada-Maarabouni et al., 2003). This dichotomy mirrors the biological consequence of *Fas* and *c-Flip* alternative splicing and raises the fascinating possibility that *RBM5* alternative splicing might be autoregulated. Irrespective of its regulation, the identification of RBM5 as a switch controlling the balance of cell survival and cell-cycle progression through modulating alternative splicing opens the door for the design and development of new therapeutic approaches combating cancer progression.

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Targeting a TAF to Make Muscle

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In a recent issue of *Molecular Cell*, Deato et al. (2008) elucidate the basis by which the muscle-specific activator MyoD recruits the core transcription machinery to the promoter of a key regulatory gene involved in myogenic differentiation.

The transcription of eukaryotic protein-coding genes is driven by interactions between sequence-specific activator proteins (activators) and one or more target proteins (targets) in the transcription machinery. A variety of activator targets have been proposed including general (or basal) transcription factors (GTFs), so-called coactivators and chromatin-modifying components. However, definitive demonstrations of activator-target interactions, particularly in vivo, have been elusive. In a recent issue of *Molecular Cell*, Deato et al. (2008) provide a compelling case for a somewhat unexpected target of the well-characterized activator MyoD, long known to be involved in muscle development. In addition, the study has some surprising implications for the global regulation of transcription of protein-coding genes in terminally differentiated cells.