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

Permalink
https://escholarship.org/uc/item/9g19h36n

Journal
Molecular Cell, 32(2)

ISSN
1097-2765

Authors
Kotlajich, MV
Hertel, KJ

Publication Date
2008-10-24

DOI
10.1016/j.molcel.2008.10.008

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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.

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

Matthew V. Kotlajich and Klemens J. Hertel

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

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**REFERENCES**


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 splicing 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 homologous 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 splicing assembly, during the process of splice-site recognition (Figure 1). Surprisingly, Bonnal et al. (2008) found that RBM5 stalls splicing assembly after A complex formation, a splicing 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 splicing 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

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**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.

Molecular Cell 32, October 24, 2008 ©2008 Elsevier Inc. 163
Targeting a TAF to Make Muscle

Daniel O. Hart¹ and Michael R. Green¹,*

¹Howard Hughes Medical Institute, Programs in Gene Function and Expression and Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605, USA
*Correspondence: michael.green@umassmed.edu
DOI 10.1016/j.molcell.2008.10.004

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.