Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Splicing activator RNPS1 suppresses errors in pre-mRNA splicing: A key factor for mRNA quality control

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ARTICLE INFO

Article history:
Received 10 January 2018
Accepted 18 January 2018
Available online xxx

Keywords:
Pre-mRNA splicing
RNPS1
Aurora kinase B
Aberrant splicing
Splicing fidelity
mRNA quality control

ABSTRACT

Human RNPS1 protein was first identified as a pre-mRNA splicing activator *in vitro* and RNPS1 regulates alternative splicing *in cellulo*. RNPS1 was also known as a peripheral factor of the exon junction complex (EJC). Here we show that cellular knockdown of RNPS1 induced a reduction of the wild-type aurora kinase B (AURKB) protein due to the induced aberrant pre-mRNA splicing events, indicating that the fidelity of AURKB pre-mRNA splicing was reduced. The major aberrant AURKB mRNA was derived from the upstream pseudo 5′ and 3′ splice sites in intron 5, which resulted in the production of the non-functional truncated AURKB protein. AURKB, is an essential mitotic factor, whose absence is known to cause multiple nuclei, and this multinucleation phenotype was recapitulated in RNPS1-knockdown cells. Importantly this RNPS1-knockdown phenotype was rescued by ectopic expression of AURKB, implying it is a major functional target of RNPS1. We found RNPS1 protein, not as a component of the EJC, binds directly to a specific element in the AURKB exon upstream of the authentic 5′ splice site, and this binding is required for normal splicing. RNPS1-knockdown induces a parallel aberrant splicing pattern in a fully distinct pre-mRNA, MDM2, suggesting that RNPS1 is a global guardian of splicing fidelity. We conclude that RNPS1 is a key factor for the quality control of mRNAs that is essential for the phenotypes including cell division.

1. Introduction

Pre-mRNA splicing is catalyzed by a large ribonucleoprotein complex called the spliceosome that contains five kinds of small nuclear ribonucleoprotein particles (snRNPs), U1, U2, U4, U5 and U6 snRNPs, and at least 170 kinds of protein factors (reviewed in Ref. [1]). The higher eukaryotes, ultimately humans, have evolved to gain more, and larger, introns, which provide complexity and flexibility in the splicing process, and generate numerous isoforms from most genes by alternative splicing (reviewed in Ref. [2]). Both constitutive and alternative splicing must be exquisitely accurate to produce multiple functional proteins for proper physiological phenotypes and mis-regulation in this process causes disorders in cell functions often with severe clinical consequences (reviewed in Ref. [3]). However, the general mechanism to ensure fidelity of human pre-mRNA splicing is poorly understood.

After splicing is complete, the exon junction complex (EJC) is assembled onto spliced mRNAs upstream of exon–exon junctions. This complex is composed of four core components, eIF4AIII, Y14, MAGOH and MLN51, and several weakly associated peripheral factors including RNPS1. The EJC plays important roles in multiple post-splicing gene expression events, such as mRNA export, surveillance, localization, and translation (reviewed in Ref. [4]). Recently, we have demonstrated that the EJC assembled on pre-mRNA, not spliced mRNA, is critical for efficient and faithful splicing of a subset of short introns in mitotic cell cycle-related genes, in which RNPS1 is a key player [5].

Here we investigated the mechanism by which RNPS1 ensures splicing fidelity. We originally identified RNPS1 as a general splicing activator *in vitro* [6]. Moreover, we demonstrated that RNPS1 modulates various types of alternative splicing *in cellulo* [7], and the

Abbreviations: ASO, antisense oligoribonucleotides; AURKB, aurora kinase B; CLIP, UV-crosslinking and immunoprecipitation; CPC, chromosome passenger complex; DAPI, 4′, 6-diamidino-2-phenylindole; EJC, exon junction complex; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; INCENP, inner centromeric protein; NMD, nonsense-mediated mRNA decay; PTC, premature termination codon; snRNP, small nuclear ribonucleoprotein particle.

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https://doi.org/10.1016/j.bbrc.2018.01.120
0006-291X/

activity was regulated by the specific phosphorylation of RNPS1 [8]. We therefore postulate that RNPS1 activates and/or modulates the selection of *bona fide* splice sites to achieve precise and essential splicing outcomes.

2. Methods summary

Full descriptions are provided in the Supplementary Materials and methods.

2.1. Transfection of HeLa cells and RT–PCR analysis

HeLa cells were transiently transfected with the indicated siRNAs and expression plasmids. Total RNAs were extracted, the splicing products were reverse transcribed with oligo-dT and random primers, and cDNAs were analyzed by end-point PCR using the indicated primer sets. The PCR products were visualized by 6% polyacrylamide gel electrophoresis. Further quantitation was achieved by real-time quantitative PCR (qPCR). Protein levels were examined by Western blotting with the indicated antibodies.

2.2. Construction of expression plasmids

The expression plasmids for the Flag/Myc-tagged fusion proteins were constructed by subcloning the PCR-amplified fragments into pcDNA3-Flag/Myc vector. The AURKB/E5-E6 mini-gene plasmid was constructed by subcloning the PCR-amplified fragment into pcDNA3 vector. Deletion (Δ#3) and mutation (K106R) were introduced into the AURKB/E5-E6 mini-gene plasmid by overlap extension PCR.

2.3. Immunoprecipitation assays

HeLa cells were co-transfected with the Myc-INCENP and Flag-AURKB series plasmids. Transfected cell extracts were immunoprecipitated with anti-Flag M2 affinity gel. The proteins associated with beads were detected by Western blotting with the indicated antibodies.

2.4. Immunofluorescence microscopic assays

HeLa cells, transfected with the indicated siRNAs, were fixed, permeabilized, and incubated with primary anti-GAPDH antibody and secondary Alexa Fluor 488 antibody (DNA was counter-stained with DAPI). The multinucleation phenotype was imaged by DAPI staining under a fluorescence microscope.

2.5. Mapping of RNPS1-binding site on the AURKB pre-mRNA

HeLa cells were transfected with antisense 2′-*O*-methyl oligoribonucleotides to cover specific AURKB pre-mRNA loci. Total RNAs were extracted and the endogenous splicing products were analyzed by RT–PCR using the indicated primers. The detected RNPS1-binding site was confirmed by RT–PCR assays of the splicing products from the ectopically expressed AURKB/E5-E6-Δ#3 mini-gene lacking RNPS1-binding site.

2.6. In cellulo UV cross-linking and immunoprecipitation (CLIP) assays

HeLa cells were co-transfected with Flag-RNPS1 expression plasmid and AURKB/E5-E6 mini-gene plasmid. The transfected cells were UV irradiated, lysed, and immunoprecipitated with anti-Flag M2 affinity gel. The precipitated RNAs were extracted and reverse transcribed using an SP6-promoter primer. The quantitation was achieved by qPCR.

3. Results

3.1. RNPS1-knockdown perturbs splicing and downregulates AURKB protein

We previously showed that siRNA-mediated knockdown of RNPS1 downregulates the expression of AURKB [5]. We thus examined the detailed mechanism of RNPS1 action using this target substrate. First, we confirmed that the AURKB protein product was markedly decreased by siRNA-mediated cellular knockdown of endogenous RNPS1 (Fig. 1A). At the RNA level, RNPS1-knockdown generated a remarkable array of aberrant mRNAs (II–VI) at the expense of normal AURKB mRNA (I, Fig. 1B). Sequence analysis confirmed that all the aberrant mRNAs (II–VI) use a common pseudo 5′ splice site in exon 5, in combination with five distinct 3′ splice sites (lower scheme). The most abundant aberrant mRNA (II) encode a truncated AURKB protein product that is likely functionally deficient because it lacks 48 amino acids from its catalytic domain (Fig. 1C). The other aberrant mRNAs (III–VI) are frame-shifted and have premature termination codons (PTCs) so are likely targeted by nonsense-mediated mRNA decay (NMD).

AURKB mRNA encodes aurora kinase B, a serine/threonine protein kinase, which is an essential factor in chromosome segregation and cytokinesis (reviewed in Ref. [9]). Aurora kinase B forms a multi-protein complex, termed the chromosome passenger complex (CPC), together with inner centromeric protein (INCENP), Survivin and Borealin, which are required for the kinase activity of AURKB and the proper localization to centromere regions (Fig. 1D). It is known that AURKB pre-mRNA is aberrantly expressed in many cancers, and interestingly, the protein-coding aberrant mRNA (II) is often found in hepatocellular carcinoma [10, and references therein].

Next, we examined whether the truncated AURKB (derived from mRNA II) can interact with INCENP, since it is known that AURKB interacts with C-terminal region of INCENP (Fig. 1D; reviewed in Ref. [9]). The dominant negative K106R mutant of AURKB (Fig. 1C) could be used as a positive control (together with wild-type AURKB) as it is catalytically inactive mutant but it interacts with INCENP [11]. We tested the interactions of myc-INCENP with normal Flag-AURKB (AURKB), mutant Flag-AURKB (K106R) or truncated Flag-AURKB (trAURKB) in transfected HeLa cells by immunoprecipitation followed by Western blotting (Fig. 1E). We found that both normal AURKB and K106R mutant AURKB proteins interacted with INCENP protein, but truncated AURKB could not (see arrows). We conclude that the truncated AURKB, generated from aberrant splicing induced by RNPS1-knockdown, loses the function.

3.2. RNPS1-knockdown cells have multiple nuclei and this phenotype is rescued by ectopic AURKB expression

Next, we focused on the cellular phenotype of RNPS1-knockdown that was deleterious to AURKB expression. AURKB-knockdown causes defects in chromosome segregation due to incorrect kinetochore–microtubule attachment, which results in multinuclear cells [11,12]. We thus checked whether the RNPS1-knockdown in HeLa cells causes a multinucleation either. Strikingly, RNPS1-knockdown in HeLa cells caused the same multinuclear phenotype as AURKB-knockdown, in ~20% and ~85% of cells, respectively (Fig. 2A). The higher frequency of unaffected cells in RNPS1-knockdown may be due to residual expression of normal AURKB protein.

To test whether the observed multiplication of nuclei in cells is

Please cite this article in press as: K. Fukumura, et al., Splicing activator RNPS1 suppresses errors in pre-mRNA splicing: A key factor for mRNA quality control, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.01.120