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Role of the ATP-dependent chromatin remodeling enzyme Fun30/Smarcad1 in the regulation of mRNA splicing

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Abstract

The yeast ATP-dependent chromatin remodeling enzyme Fun30 has been shown to regulate heterochromatin silencing, DNA repair, transcription, and chromatin organization. Although chromatin structure has been proposed to influence splice site recognition and regulation, whether ATP-dependent chromatin remodeling enzyme plays a role in regulating splicing is not known. In this study, we find that pre-mRNA splicing efficiency is impaired and the recruitment of spliceosome is compromised in Fun30-depleted cells. In addition, Fun30 is enriched in the gene body of individual intron-containing genes. Moreover, we show that pre-mRNA splicing efficiency is dependent on the chromatin remodeling activity of Fun30. The function of Fun30 in splicing is further supported by the observation that, Smarcad1, the mammalian homolog of Fun30, regulates alternative splicing. Taken together, these results provide evidence for a novel role of Fun30 in regulating splicing.

Keywords

Fun30; Smarcad1; Pre-mRNA splicing; Alternative splicing; Chromatin remodeling

1. Introduction

The bulk of chromatin is composed of nucleosomes as its fundamental repeating unit. Each nucleosome contains approximately 147 base pairs (bp) of DNA and two each of the histone

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2020.02.175.

H2A, H2B, H3, and H4 proteins. While chromatin remodeling is known to promote or inhibit transcription, its role in regulating other nuclear events, such as pre-mRNA splicing, has begun to emerge. Histone modifications such as H3K36me3, H3K4me3, H3K27me2, H2BK123ub, H3K9ac as well as histone variant H2AZ (*Drosophila*) are enriched specifically in the exons at the intron-exon junctions [1]. In addition, nucleosome mapping studies revealed that nucleosome density is much higher in exons, which are flanked by relatively nucleosome-depleted intron regions [2,3]. The function of these distinct patterns of histone modifications and nucleosome positioning is thought to facilitate recognition of exon-intron-exon junctions by splicing factors.

The *S. cerevisiae* ATPase Fun30 (mammalian homolog Smarcad1) belongs to the Snf2 family of the ATP-dependent chromatin remodeling enzymes. This class of enzymes breaks histone-DNA contacts to slide the histone octamer along DNA, transfers the histone octamer or H2A-H2B dimer, or facilitates an exchange between canonical and variant histone dimers [4,5]. Fun30 has been shown to remodel nucleosomes by histone dimer transfer [6] or nucleosome sliding [7] as a single-component enzyme. Studies have shown that Fun30 and its *S. pombe* (fft3) and mammalian (Smarcad1) homologs play important roles in multiple biological processes, including transcriptional regulation [7,8], heterochromatin maintenance [9–14], double-strand DNA repair [15–19], and mismatch repair [20], presumably through its chromatin remodeling activity.

Here we report a novel function of Fun30 in promoting pre-mRNA splicing. We observe increased pre-mature mRNA/full length total mRNA ratio and reduced spliceosome recruitment in Fun30-depleted cells, suggesting that the splicing efficiency in Fun30-depleted cells is compromised. We next demonstrate that Fun30 is localized at intron-containing genes (ICGs) and its ATPase activity is required for efficient pre-mRNA splicing. We further show that alternative splicing was altered in Fun30's mammalian homolog Smarcad1 knockdown primary cortical neurons. Our results indicate a role of the yeast Fun30 as well as its mammalian homolog Smarcad1 in regulating splicing.

2. Material and methods

Strains used in this study are listed in Supplementary Table 1. Primers used in this study are listed in Supplementary Tables 2–5. Data were collected from a minimum of biological triplicates and technical duplicates. Gene deletion or tagging yeast strains were constructed and confirmed using a PCR-based approach [21]. Yeast total RNA was isolated using the standard hot acid phenol extraction procedure [22]. cDNA was synthesized by reverse transcription using the total RNA. Quantitation of the "pre-mRNA" and the "total mRNA" (as defined in Fig. 1a) was achieved by quantitative PCR of cDNA. The primers for qPCR of cDNA are listed in Supplementary Table 2. Ratios of pre-mRNA and total-mRNA for individual loci in the fun30 mutant were normalized by those in the wild-type strain. Chromatin immunoprecipitation was performed as described previously [23] and on the Tsukiyama Lab website (http://research.fhcrc.org/tsukiyama/en/protocols.html). Antibodies for immunoprecipitation were M2 anti-FLAG (F1804, Sigma, 4 μg *per* sample) for the Flag-tagged Fun30 or individual subunits of spliceosome. The intronless *PMA1* locus was used as a control for signal normalization for spliceosome ChIP [24]. A nucleosome-free region of

the *HSC82* locus [7] was used as a control for signal normalization for Fun30 ChIP. Primary cortical neurons were prepared from embryonic day 18 (E18) C57BL/6 mice. Dissociated neurons were plated on poly-D-lysine-coated plates in neurobasal medium (Life Technologies) supplemented with B27 and L-glutamine as previously described [25]. Mouse total RNA was isolated using TRI re-agent (T9424, Sigma) following the manufacturer's instruction. Further details available in the Extended Experimental Procedures.

3. Results

3.1. Pre-mRNA was accumulated in fun30Δ cells

To explore the potential role of Fun30 in splicing, we examined levels of pre-mRNA and full-length total mRNA (full-length pre-mRNA + full-length mature mRNA) (Fig. 1a) in the wild-type and *fun30Δ* strains. We then calculate the ratio of pre-mRNA to total full-length mRNA. While there are several ways to define splicing efficiency, we define this ratio as an indicator of splicing efficiency, the higher the ratio, the lower the efficiency. This study was initially conducted in the *rrp6Δ* background to prevent the pre-mRNA from being degraded [26]. We found that the ratios of pre-mRNA to full-length total mRNA were increased in 12 out of 15 ICGs in Fun30-depleted (*fun30Δrrp6Δ*) cells compared to those in the wild-type Fun30 (*rrp6Δ*) cells. The fold ranged from ~1.3 to ~1.9 (Fig. 1a and b). The effect on pre-mRNA splicing was not due to a decrease in RNA decay in the *rrp6Δ* background as we observed a comparable level of increased pre-mRNA in the *fun30Δ* mutant alone (Fig. 1c and Supplementary Fig. 1). Furthermore, the steady-state levels of total mRNA at individual ICG loci in *fun30Δ* was similar to that in the wild-type strain as measured by qPCR (Fig. 1d). Thus, Fun30 deletion mutant renders splicing less efficient.

It is important to note that the effect of Fun30 depletion on pre-mRNA splicing efficiency is independent of Fun30's role in dere-pression of a subset of genes [7] because we do not observe significant changes in the expression of RNA processing-related genes, including genes encoding snRNP components, other splicing factors and components in the RNA degradation pathways, as well as genes encoding chromatin remodeling enzymes or factors that are known to regulate RNA processing [7]. Second, the effect of Fun30 depletion on pre-mRNA splicing efficiency is unlikely due to competition between pre-mRNAs for the splicing machinery upon the global activation of ICGs [27] because there are only two ICGs in the 223 up-regulated Fun30-dependent genes (>1.3 fold in the mutant) [7]. Taken together, these results suggest that Fun30 may play a direct role in regulating splicing efficiency.

3.2. Localization of Fun30 at ICGs

The major class of introns, "the U2 type intron", defined by the canonical GT-AG boundaries, contains sequence elements that are essential for intron recognition and removal by the splicesome, including the 5′ splice site (5′SS), 3′ splice site (3′SS), and branch point sequence and polypyrimidine tract that are within 50 bp upstream of the 3′SS. In yeast, there are ~300 U2 type ICGs with annotated sequence features [28]. We next tested whether Fun30 is enriched at ICGs by conventional chromatin immunoprecipitation (ChIP) analysis. We found that Fun30 is enriched in the gene body of individual ICGs (Fig. 2a–c). In

addition, we analyzed the recently published Fun30 ChIP-seq data [10] for Fun30 enrichment at 277 ICGs. We found that Fun30 is enriched at the 5′SS, 3′SS and open reading frames (ORFs) of 277 ICGs, comparing to the Fun30's ChIP signals at 5774 ORFs, with slightly more enrichment toward the 3′SS (Fig. 2d).

3.3. Splicing efficiency is dependent on the chromatin remodeling activity of Fun30

Previous studies have shown that the yeast strain containing a mutation (K603R) in the ATPase domain of Fun30 exhibits pheno-types similar to the *fun30Δ* mutant, including the loss of heterochromatin silencing, G2/M arrest, and failure in checkpoint adaptation [9,29], suggesting that the K603 residue is critical for Fun30's ATPase activity and its function. We have further shown that Fun30 remodels mononucleosomes *in vitro* and the chromatin remodeling activity is abolished by the Fun30 ATPase K603A mutation that eliminates its ATPase activity [7]. To examine whether Fun30's role in splicing efficiency is related to its chromatin remodeling activity, we next test splicing efficiency in the ATPase mutant *fun30 K603A*. Comparing the wild-type Fun30 with the *fun30 K603A* mutant, the ratios of pre-mRNA to total mRNA at individual ICGs were increased in the range of 1.2–1.6 fold (Fig. 2e), consistent with what we observed in the *fun30Δ* mutant. These results demonstrate that splicing efficiency is facilitated by the ATPase activity of Fun30, indicating that the chromatin remodeling activity of Fun30 plays an important role in regulating splicing efficiency.

3.4. The recruitment of snRNPs was compromised in fun30Δ cells

Given that splicing efficiency was impaired in *fun30Δ* cells, we next examined whether the recruitment of spliceosome was also affected. Previous studies have shown that it is feasible to examine recruitment of U1, U2 and U5 snRNPs by conventional ChIP, with enrichment peaks slightly shifted downstream of their actual binding sites [24,30,31]. We found that U1 snRNP (Yhc1–3Flag) enrichment at two ICGs was moderately, yet significantly, reduced (Fig. 3a). In addition, the enrichment of U2 (Lea 1–3Flag) and U5 (Prp8–3Flag), which are components of the active spliceosome, were also significantly reduced in *fun30Δ* cells (Fig. 3b and c). Taken together, our findings of (1) increased pre-mRNA/total full-length mRNA ratio in *fun30Δ* cells, (2) Fun30 enrichment at ICGs and (3) reduced recruitment of snRNPs in *fun30Δ* cells, link Fun30 to pre-mRNA splicing efficiency in yeast.

3.5. Alternative splicing was altered in Smarcad1-knockdown primary cortical neurons

To test whether the role of Fun30 in splicing is conserved, we examined twelve genes in which alternative splicing is often changed in patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTLD) [32,33]. We found that the alternative splicing of 9 out of 12 gene transcripts was altered in the Fun30 mammalian homolog Smarcad1 knockdown neurons (Fig. 4a–j, and Supplementary Fig. 2). Among the 9 Smarcad1 RNA targets, 7 showed increased exon skipping (Fig. 4b–h), while 2 had increased exon inclusion (Fig. 4i and j). The RNA processing proteins FUS/TLS and/or TDP-43 are known to regulate alternative splicing of these ALS genes [32,33]. We thus measured RNA levels of *Fus/Tls* and *TDP-43* in Smarcad1 shRNA-infected neurons and did not observe significant changes in the expression level (Supplementary Fig. 3), suggesting that Smarcad1 likely plays a direct role in alternative splicing. Furthermore, the expression levels of 4 out of 9 genes,

Grip 1, Nav 2, Tia1, and *Ttc3*, were increased in the range of 1.3–1.7 fold in either of the two Smarcad1 shRNA (shRNA1 or shRNA2) knockdown experiments, when compared to those of scramble shRNA-infected neurons, while expression of the other genes was not significantly changed (Supplementary Fig. 4). Taken together, these results suggest that Smarcad1, similar to its yeast homolog, plays an important role in splicing.

4. Discussion

In this report, we have established a novel role of Fun30 in regulating pre-mRNA splicing efficiency in yeast. We have observed a moderate yet consistent 1.3–1.9 fold decrease of splicing efficiency at individual loci in the fun30 mutant relative to the wild-type strain. We have found that Fun30 binds to ICGs. The chromatin remodeling activity of Fun30 is required for the regulation of splicing efficiency. Furthermore, the recruitment of snRNPs was compromised in Fun30 depleted cells. Lastly, we have shown that the role of Fun30 in splicing is conserved manifested by the mammalian homolog Smarcad1 playing a role in alternative splicing.

Using mass spectrometry analysis of Flag-Smarcad1 purification eluates from HEK293 nuclear extracts, a recent study showed that Smarcad1 associates with protein factors that either promote or repress splicing, including heterogeneous nuclear ribonucleoprotein particles (hnRNPs), U2 small nuclear RNA auxiliary factor 2 (U2AF2), pre-mRNA processing factor 19 (PRPF19), and serine/arginine-rich splicing factor 1 (SRSF1) [11]. The results corroborate with our findings that Smarcad1 regulates alternative splicing.

Potential mechanisms for the roles of histone modification and ATP-dependent chromatin remodeling in splicing include serving as marks for exon recognition, recruiting splicing factors directly, or controlling Pol II rate or kinetics, facilitating splicing factor recruitment by Pol II in co-transcriptional splicing. The yeast Gcn5 acetylates ICG promoters to facilitate the recruitment of splicing factors [31]. The chromatin remodeling enzyme CHD1, together with histone H3 Lysine 4 trimethylation (H3K4me3), has been shown to recruit U2 snRNP directly to promote splicing [34]. Previous studies have shown that histone hyperacetylation regulates exon inclusion/exclusion by increasing the Pol II rate through chromatin opening [35,36]. Our preliminary studies have found that Fun30 regulates Pol II CTD Ser 2, Ser 5 and Ser 7 phosphorylation patterns at ICGs (data not shown). Taken together, it is tempting to speculate that Fun30 remodels nucleosomes in introns near the splicing sites to modulate Pol II CTD phosphorylation patterns optimal for spliceosome recruitment and hence the splicing efficiency.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

[1]. Allemand E, Myers MP, Garcia-Bernardo J, Harel-Bellan A, Krainer AR, Muchardt C, A broad set of chromatin factors influences splicing, PLoS Genet. 12 (2016), e1006318, 10.1371/journal.pgen.1006318. [PubMed: 27662573]

[2]. Schwartz S, Meshorer E, Ast G, Chromatin organization marks exon-intron structure, Nat. Struct. Mol. Biol 16 (2009) 990–995, 10.1038/nsmb.1659. [PubMed: 19684600]

[3]. Tilgner H, Nikolaou C, Althammer S, Sammeth M, Beato M, Valcarcel J, Guigo R, Nucleosome positioning as a determinant of exon recognition, Nat. Struct. Mol. Biol 16 (2009) 996–1001, 10.1038/nsmb.1658. [PubMed: 19684599]

[4]. Narlikar GJ, A proposal for kinetic proof reading by ISWI family chromatin remodeling motors, Curr. Opin. Chem. Biol 14 (2010) 660–665, 10.1016/j.cbpa.2010.08.001. [PubMed: 20833099]

[5]. Hota SK, Bartholomew B, Diversity of operation in ATP-dependent chromatin remodelers, Biochim. Biophys. Acta 1809 (2011) 476–487, 10.1016/j.bbagrm.2011.05.007. [PubMed: 21616185]

[6]. Awad S, Ryan D, Prochasson P, Owen-Hughes T, Hassan AH, The Snf2 ho molog Fun30 acts as a homodimeric ATP-dependent chromatin-remodeling enzyme, J. Biol. Chem 285 (2010) 9477–9484, 10.1074/jbc.M109.082149. [PubMed: 20075079]

[7]. Byeon B, Wang W, Barski A, Ranallo RT, Bao K, Schones DE, Zhao K, Wu C, Wu WH, The ATP-dependent chromatin remodeling enzyme Fun30 represses transcription by sliding promoter-proximal nucleosomes, J. Biol. Chem 288 (2013) 23182–23193, 10.1074/jbc.M113.471979. [PubMed: 23779104]

[8]. Okazaki N, Ikeda S, Ohara R, Shimada K, Yanagawa T, Nagase T, Ohara O, Koga H, The novel protein complex with SMARCAD1/KIAA1122 binds to the vicinity of TSS, J. Mol. Biol 382 (2008) 257–265, 10.1016/j.jmb.2008.07.031. [PubMed: 18675275]

[9]. Neves-Costa A, Will WR, Vetter AT, Miller JR, Varga-Weisz P, The SNF2-family member Fun30 promotes gene silencing in heterochromatic loci, PloS One 4 (2009), e8111, 10.1371/journal.pone.0008111. [PubMed: 19956593]

[10]. Durand-Dubief M, Will WR, Petrini E, Theodorou D, Harris RR, Crawford MR, Paszkiewicz K, Krueger F, Correra RM, Vetter AT, Miller JR, Kent NA, Varga-Weisz P, SWI/SNF-Like chromatin remodeling factor Fun30 supports point centromere function in S-cerevisiae, PLoS Genet. 8 (2012), e1002974, 10.1371/journal.pgen.1002974.

[11]. Rowbotham SP, Barki L, Neves-Costa A, Santos F, Dean W, Hawkes N, Choudhary P, Will WR, Webster J, Oxley D, Green CM, Varga-Weisz P, Mermoud JE, Maintenance of silent chromatin through replication requires SWI/SNF-like chromatin remodeler SMARCAD1, Mol. Cell 42 (2011) 285–296, 10.1016/j.molcel.2011.02.036. [PubMed: 21549307]

[12]. Stralfors A, Walfridsson J, Bhuiyan H, Ekwall K, The FUN30 chromatin remodeler, Fft3, protects centromeric and subtelomeric domains from euchromatin formation, PLoS Genet. 7 (2011), e1001334, 10.1371/journal.pgen.1001334. [PubMed: 21437270]

[13]. Yu Q, Zhang X, Bi X, Roles of chromatin remodeling factors in the formation and maintenance of heterochromatin structure, J. Biol. Chem 286 (2011) 14659–14669, 10.1074/jbc.M110.183269. [PubMed: 21388962]

[14]. Steglich B, Stralfors A, Khorosjutina O, Persson J, Smialowska A, Javerzat JP, Ekwall K, The Fun30 chromatin remodeler Fft3 controls nuclear organization and chromatin structure of insulators and subtelomeres in fission yeast, PLoS Genet. 11 (2015), e1005101, 10.1371/journal.pgen.1005101. [PubMed: 25798942]

[15]. Bi X, Yu Q, Siler J, Li C, Khan A, Functions of Fun30 chromatin remodeler in regulating cellular resistance to genotoxic stress, PloS One 10 (2015), e0121341, 10.1371/journal.pone.0121341. [PubMed: 25806814]

[16]. Costelloe T, Louge R, Tomimatsu N, Mukherjee B, Martini E, Khadaroo B, Dubois K, Wiegant WW, Thierry A, Burma S, van Attikum H, Llorente B, The yeast Fun30 and human SMARCAD1

chromatin remodellers promote DNA end resection, Nature 489 (2012) 581–584, 10.1038/nature11353. [PubMed: 22960744]

[17]. Chen X, Cui D, Papusha A, Zhang X, Chu CD, Tang J, Chen K, Pan X, Ira G, The Fun30 nucleosome remodeller promotes resection of DNA double-strand break ends, Nature 489 (2012) 576–580, 10.1038/nature11355. [PubMed: 22960743]

[18]. Eapen VV, Sugawara N, Tsabar M, Wu WH, Haber JE, The Saccharomyces cerevisiae chromatin remodeler Fun30 regulates DNA end resection and checkpoint deactivation, Mol. Cell Biol 32 (2012) 4727–4740, 10.1128/MCB.00566-12. [PubMed: 23007155]

[19]. Chakraborty S, Pandita RK, Hambarde S, Mattoo AR, Charaka V, Ahmed KM, Iyer SP, Hunt CR, Pandita TK, SMARCAD1 phosphorylation and ubiquitination are required for resection during DNA double-strand break repair, iScience 2 (2018) 123–135, 10.1016/j.isci.2018.03.016. [PubMed: 29888761]

[20]. Terui R, Nagao K, Kawasoe Y, Taki K, Higashi TL, Tanaka S, Nakagawa T, Obuse C, Masukata H, Takahashi TS, Nucleosomes around a mismatched base pair are excluded via an Msh2-dependent reaction with the aid of SNF2 family ATPase Smarcad1, Genes Dev. 32 (2018) 806–821, 10.1101/gad.310995.117. [PubMed: 29899141]

[21]. Knop M, Siegers K, Pereira G, Zachariae W, Winsor B, Nasmyth K, Schiebel E, Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines, Yeast 15 (1999) 963–972, 10.1002/(SICI)1097-0061(199907)15:10B<963::AID-YEA399>3.0.CO;2-W. [PubMed: 10407276]

[22]. Amberg DC, Burke DJ, Strathern JN, Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2005.

[23]. Dudley AM, Rougeulle C, Winston F, The Spt components of SAGA facilitate TBP binding to a promoter at a post-activator-binding step in vivo, Genes Dev. 13 (1999) 2940–2945, 10.1101/gad.13.22.2940. [PubMed: 10580001]

[24]. Lacadie SA, Rosbash M, Cotranscriptional spliceosome assembly dynamics and the role of U1 snRNA:5'ss base pairing in yeast, Mol. Cell 19 (2005) 65–75, 10.1016/j.molcel.2005.05.006. [PubMed: 15989965]

[25]. Wei Z, Behrman B, Wu WH, Chen BS, Subunit-specific regulation of N-methyl-D-aspartate (NMDA) receptor trafficking by SAP102 protein splice variants, J. Biol. Chem 290 (2015) 5105–5116, 10.1074/jbc.M114.599969. [PubMed: 25555912]

[26]. Parker R, RNA degradation in Saccharomyces cerevisae, Genetics 191 (2012) 671–702, 10.1534/genetics.111.137265. [PubMed: 22785621]

[27]. Munding EM, Shiue L, Katzman S, Donohue JP, Ares M Jr., Competition between pre-mRNAs for the splicing machinery drives global regulation of splicing, Mol. Cell 51 (2013) 338–348, 10.1016/j.molcel.2013.06.012. [PubMed: 23891561]

[28]. Grate L, Ares M Jr., Searching yeast intron data at Ares lab Web site, Methods Enzymol. 350 (2002) 380–392. [PubMed: 12073325]

[29]. Eapen VV, Sugawara N, Tsabar M, Wu WH, Haber JE, The Saccharomyces cerevisiae chromatin remodeler Fun30 regulates DNA end resection and checkpoint deactivation, Mol. Cell Biol 32 (2012) 4727–4740, 10.1128/MCB.00566-12. [PubMed: 23007155]

[30]. Kotovic KM, Lockshon D, Boric L, Neugebauer KM, Cotranscriptional recruitment of the U1 snRNP to intron-containing genes in yeast, Mol. Cell Biol 23 (2003) 5768–5779. [PubMed: 12897147]

[31]. Gunderson FQ, Johnson TL, Acetylation by the transcriptional coactivator Gcn5 plays a novel role in co-transcriptional spliceosome assembly, PLoS Genet. 5 (2009), e1000682, 10.1371/journal.pgen.1000682. [PubMed: 19834536]

[32]. Ishigaki S, Masuda A, Fujioka Y, Iguchi Y, Katsuno M, Shibata A, Urano F, Sobue G, Ohno K, Position-dependent FUS-RNA interactions regulate alternative splicing events and transcriptions, Sci. Rep 2 (2012) 529, 10.1038/srep00529. [PubMed: 22829983]

[33]. Lagier-Tourenne C, Polymenidou M, Hutt KR, Vu AQ, Baughn M, Huelga SC, Clutario KM, Ling SC, Liang TY, Mazur C, Wancewicz E, Kim AS, Watt A, Freier S, Hicks GG, Donohue JP, Shiue L, Bennett CF, Ravits J, Cleveland DW, Yeo GW, Divergent roles of ALS-linked proteins

FUS/TLS and TDP-43 intersect in processing long pre-mRNAs, Nat. Neurosci 15 (2012) 1488–1497, 10.1038/nn.3230. [PubMed: 23023293]

[34]. Sims RJ 3rd, Millhouse S, Chen CF, Lewis BA, Erdjument-Bromage H, Tempst P, Manley JL, Reinberg D, Recognition of trimethylated histone H3 lysine 4 facilitates the recruitment of transcription postinitiation factors and pre-mRNA splicing, Mol. Cell 28 (2007) 665–676, 10.1016/j.molcel.2007.11.010. [PubMed: 18042460]

[35]. Zhou HL, Hinman MN, Barron VA, Geng C, Zhou G, Luo G, Siegel RE, Lou H, Hu proteins regulate alternative splicing by inducing localized histone hyperacetylation in an RNA-dependent manner, Proc. Natl. Acad. Sci. U. S. A 108 (2011) E627–E635, 10.1073/pnas.1103344108. [PubMed: 21808035]

[36]. Schor IE, Rascovan N, Pelisch F, Allo M, Kornblihtt AR, Neuronal cell de-polarization induces intragenic chromatin modifications affecting NCAM alternative splicing, Proc. Natl. Acad. Sci. U. S. A 106 (2009) 4325–4330, 10.1073/pnas.0810666106. [PubMed: 19251664]

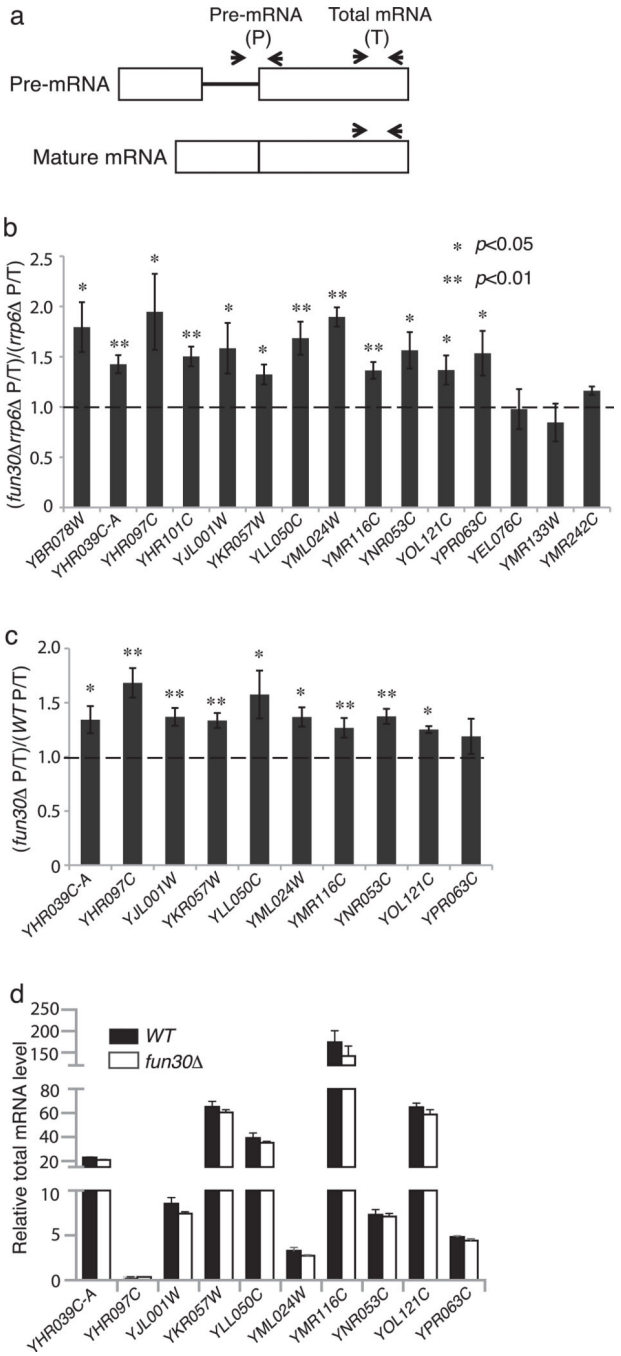

Fig. 1. Fun30 promotes pre-mRNA splicing efficiency.

Total RNA was extracted from the wild-type, *fun30Δ*, *rrp6Δ*, and *fun30Δ rrp6Δ* cells, followed by reverse transcription and qPCR. Splicing efficiency was measured as ratio of pre-mRNA to total mRNA. (**a**) Location of primers used for qPCR. The level of total mRNA was measured as both full-length pre-mRNA and full-length mature mRNA. (**b**) Splicing efficiency was impaired in the *fun30Δrrp6Δ* mutant. The pre-mRNA/total mRNA ratios for *fun30Δ rrp6Δ* were normalized by those for *rrp6Δ*. (**c**) Splicing efficiency was impaired in the *fun30Δ* mutant, independent of Rrp6. The pre-mRNA/total mRNA ratios for the *fun30Δ*

mutant were normalized to those for the wild-type strain. (**d**) Levels of total mRNA at individual ICG loci in the wild-type strain and *fun30Δ* mutant.

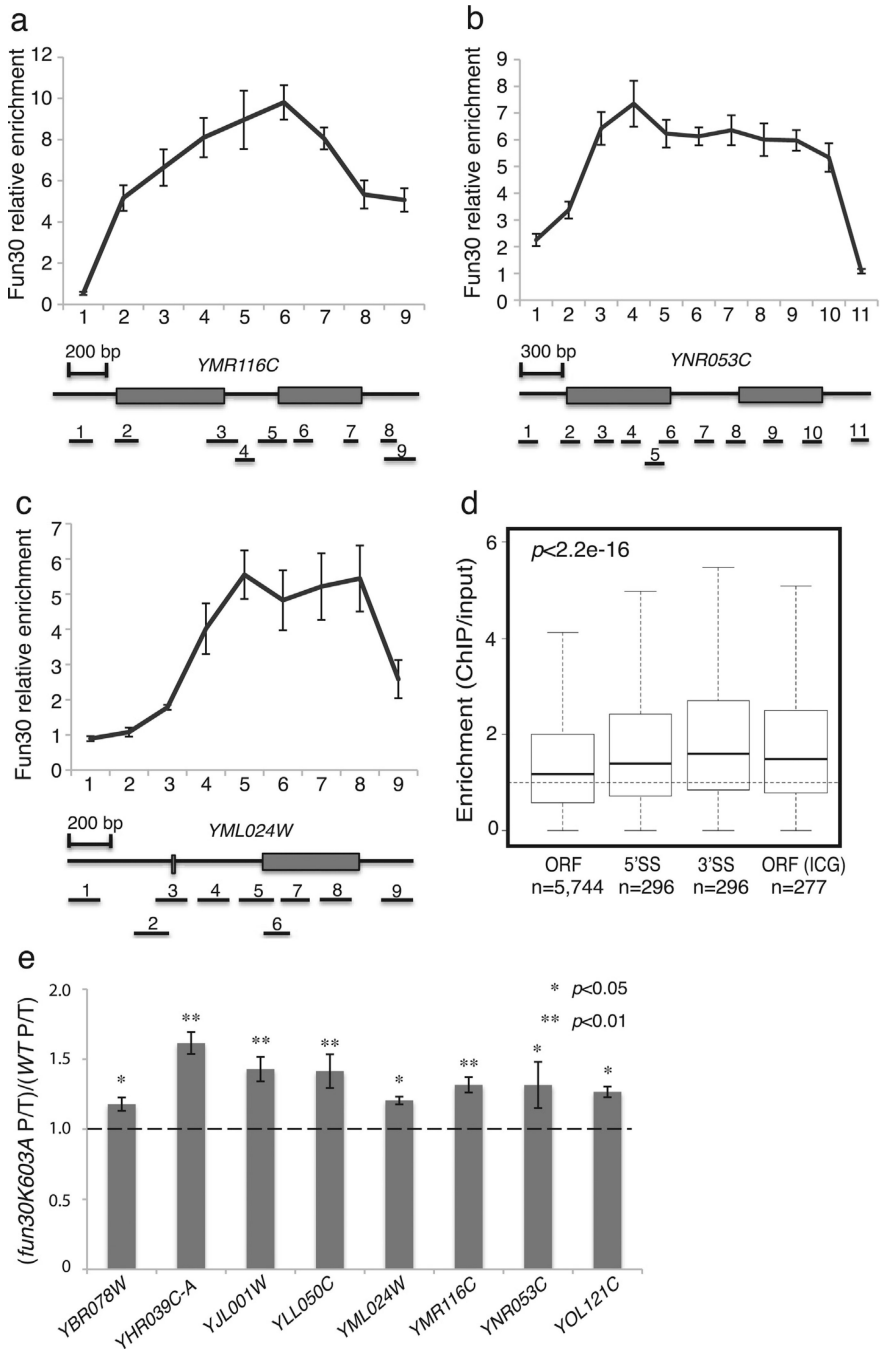

Fig. 2. Fun30 is enriched in ORFs of intron-containing genes and its function in splicing is dependent on the chromatin remodeling activity.

Fun30 is enriched in ORFs of (**a**) *YMR116C*, (**b**) *YNR053C* and (**c**) *YML024W* genes as detected by conventional ChIP. A nucleosome free region at the *HSC82* locus was used as control for signal normalization. (**d**) Fun30 is enriched at the 5′SS and 3′SS of ICGs. Ratios of ChIP-seq DNA signals over input DNA signals were aligned from start to end for ORF analysis or in a window of −/+250 bp for 5′SS or 3′SS analysis. Wilcox tests were performed for the difference between ORF and other chromosome features (*p*-value < 2.2e-16). SS, splicing sites; ICG, intron-containing gene; n, number of loci. The ChIP-seq

data was published by Durand-Dubief et al. [10]. (**e**) Splicing efficiency was impaired in the fun30 ATPase deficient (*fun30K603A*) mutant. The pre-mRNA/total mRNA ratio of fun30K603A was normalized to that of the wild-type cells.

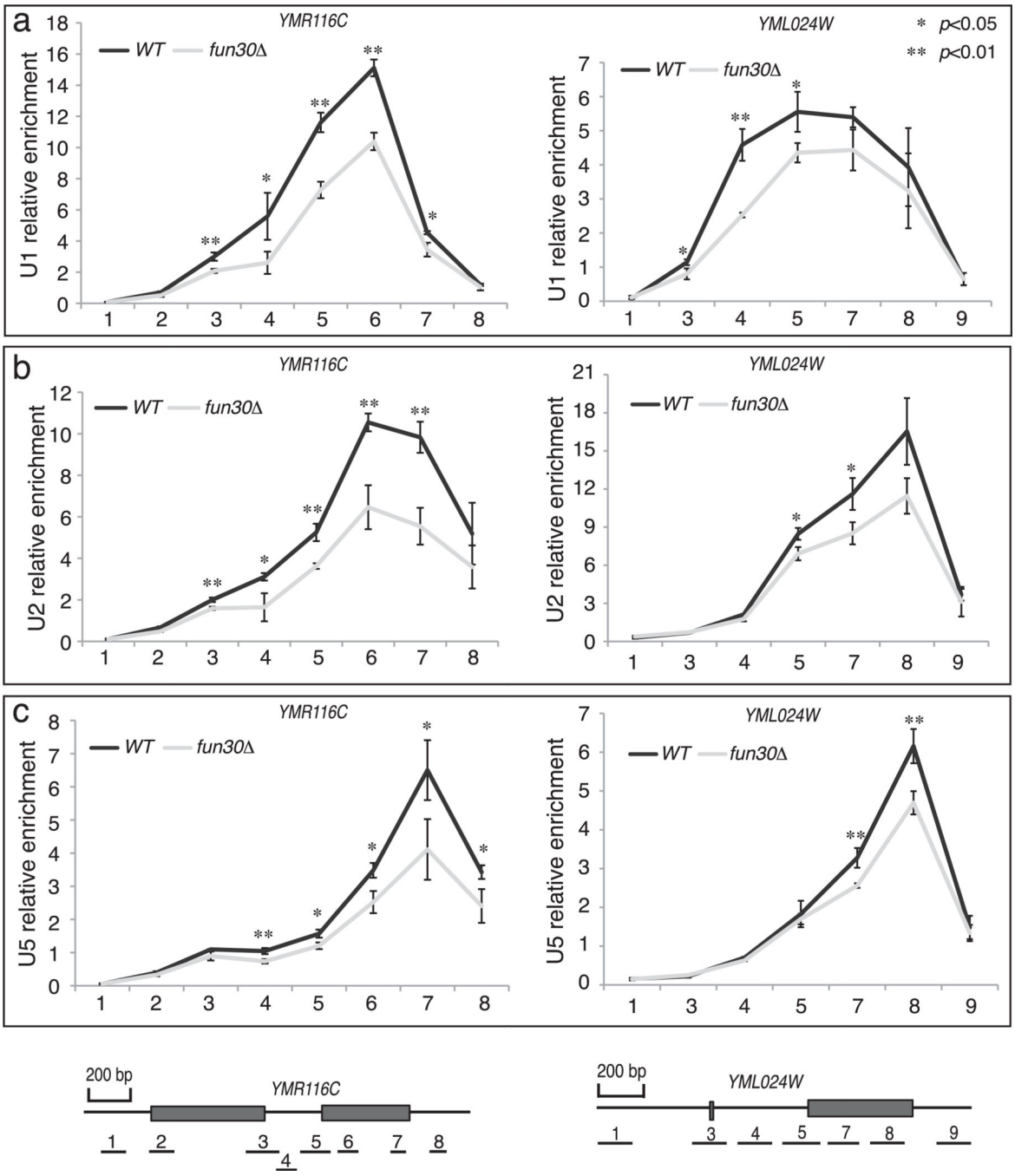

Fig. 3. Fun30 is required for the recruitment of snRNPs.

(**a**) U1 (Yhc1^{3Flag}) enrichment at *YMR116C* and *YML024W* as determined by conventional ChIP in the wild-type and *fun30Δ* cells. (**b**) U2 (Lea 1^{3Flag}) enrichment at *YMR116C* and *YML024W* in the wild-type and *fun30Δ* cells. (**c**) U5 (Prp8^{3Flag}) enrichment at *YMR116C* and *YML024W*. Location of ChIP primers for *YMR116C* and *YML024W* genes was shown in schematic diagram. An intronless gene *PMA1* locus [24] was used as a control for signal normalization for snRNP ChIP.

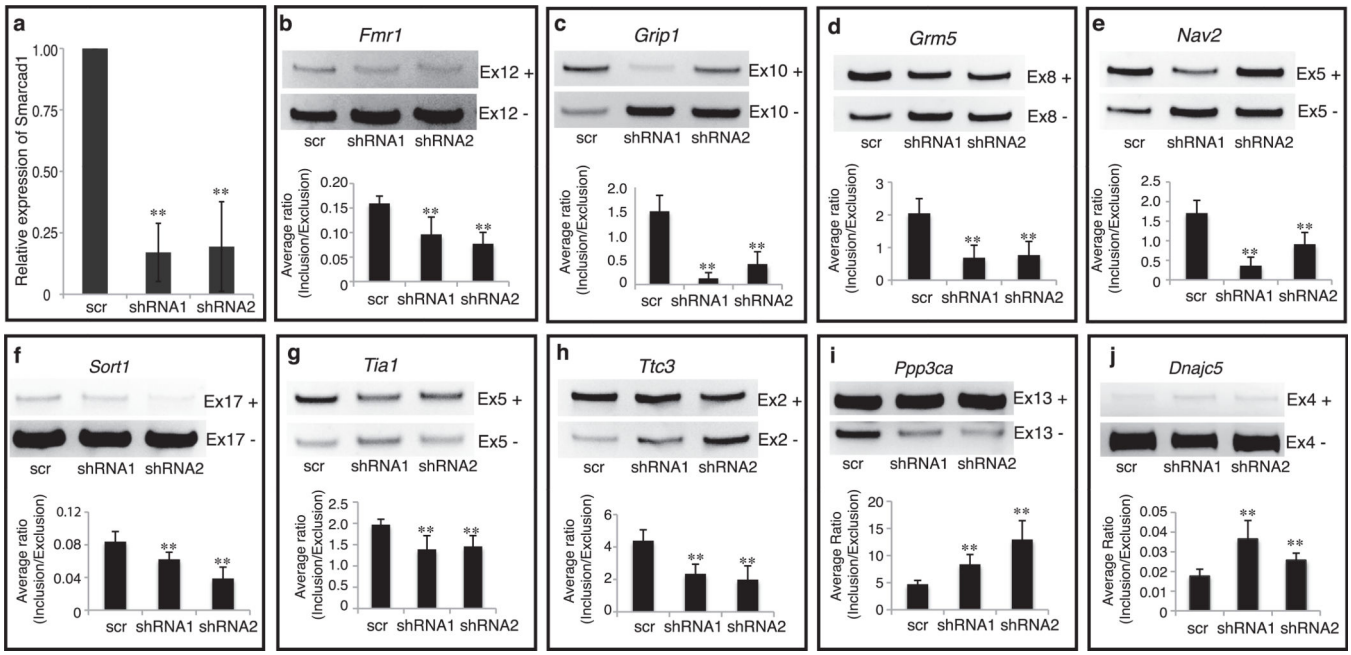

Fig. 4. Alternative splicing was altered in Smarcad1 shRNA knockdown primary cortical neurons.

(**a**) Smarcad1 protein expression determined by Western blot analysis of three independent Smarcad1 shRNA knockdown experiments. (**b–h**) Increased exon exclusion in Smarcad1 shRNA knockdown primary cortical neurons in indicated transcripts. (**i–j**) Increased exon inclusion in Smarcad1 shRNA knockdown primary cortical neurons in indicated transcripts. In each subfigure, top panel: 10% PAGE 0.5 X TBE gel and ethidium bromide staining results, showing indicated exon inclusion (Ex +) and exon exclusion (Ex −) events of indicated transcripts in scramble and shRNA knockdown samples; bottom panel: average of ratios of exon inclusion/exclusion from 3 independent knockdown experiments and 2 to 4 technical replicates for each knockdown experiment. Signals were scanned using ImageQuant LAS 4000 (GE Healthcare Life Sciences) and quantified using ImageQuant. shRNA1 and shRNA2 are two independent shRNA knockdown constructs. **, $p < 0.01$.