Molecules and Cells

Minireview

The Role of Splicing Factors in Adipogenesis and Thermogenesis

Yadanar Than Naing and Lei Sun*

Cardiovascular and Metabolic Disorders Program, Duke-NUS Medical School, Singapore 169857
*Correspondence: sun.lei@duke-nus.edu.sg
https://doi.org/10.14348/molcells.2023.2195
www.molcells.org

Obesity is a significant global health risk that can cause a range of serious metabolic problems, such as type 2 diabetes and cardiovascular diseases. Adipose tissue plays a pivotal role in regulating energy and lipid storage. New research has underlined the crucial role of splicing factors in the physiological and functional regulation of adipose tissue. By generating multiple transcripts from a single gene, alternative splicing allows for a greater diversity of the proteome and transcriptome, which subsequently influence adipocyte development and metabolism. In this review, we provide an outlook on the part of splicing factors in adipogenesis and thermogenesis, and investigate how the different spliced isoforms can affect the development and function of adipose tissue.

Keywords: adipogenesis, adipose tissue, splicing, splicing factors, thermogenesis, thermogenic adipocytes

INTRODUCTION

Adipose tissue is a vital metabolic organ and plays a critical role in energy homeostasis by coordinating lipogenesis, lipolysis, and fatty acid oxidation. Adipose tissue also functions as an endocrine organ by secreting adipose tissue-derived hormones, such as leptin and adiponectin (Hui et al., 2015; Trayhurn et al., 1999). There are at least three major adipose tissues, distinct anatomically and physiologically: white adipose tissue (WAT), brown adipose tissue (BAT), and beige adipose tissue. While WAT is specialized for modulating lipid storage, beige and BAT contribute to energy expenditure through Ucp1-dependent and -independent thermogenesis (Sakers et al., 2022).

Adipose tissue dysfunction underlies the development of many metabolic disorders such as obesity, insulin resistance, type 2 diabetes, atherogenic dyslipidemia, non-alcoholic fatty liver disease and cardiovascular diseases (Gesta et al., 2007). Adipose tissue can increase its mass through two mechanisms: hyperplasia (increased adipocyte numbers) and hypertrophy (increased adipocyte size). The hyperplastic WAT has a relatively mild detrimental effect on metabolic health, as adipocytes maintain normal metabolic function. In contrast, the hypertrophic WAT is often accompanied by adipocyte dysfunction and contributes more to metabolic abnormalities (Morigny et al., 2021). Beige and BAT, known as thermogenic adipocytes, are distinguished from WAT in their multilocular lipid droplets, higher mitochondrial activity, and abundant expression of the uncoupling protein 1 (UCP1). Beige adipocytes are dispersed in subcutaneous WAT and resemble white adipocyte morphology under a dormant state. However, they are activated upon cold or adrenergic stimulation to take on brown adipocyte-like appearances and functions (Sakers et al., 2022). It was reported that a reduced mass and activity of thermogenic adipose tissues could contribute to the development of obesity (Becher et al., 2021).

To date, an increasing number of studies indicate that alternative splicing plays an essential role in adipocyte biology (Chao et al., 2021). Most transcripts in mammals undergo

Received December 27, 2022; revised February 27, 2023; accepted March 3, 2023; published online April 2, 2023

eISSN: 0219-1032

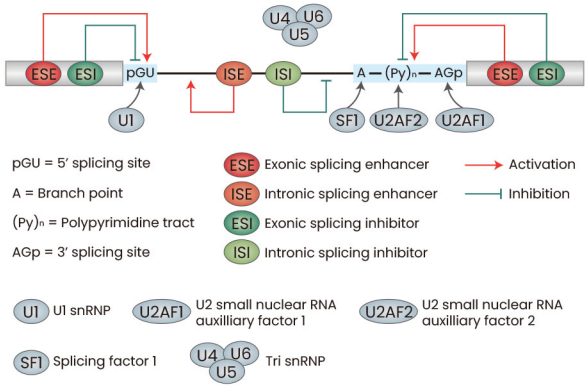


Fig. 1. Schematic representation of splicing mechanisms in precursor messenger RNA (pre-mRNA). The 5′ splice sites are recognized by U1, the branch points by SF1, the polypyrimidine tract by U2AF2, the 3′ splice sites by U2AF1. Tri snRNP (U4/U6 and U5) acts as the core catalytic machinery. Splicing factors regulate the spliceosome components recognition of 5′ and 3′ splice sites through binding to ESE, ESI, ISE, and ISI cis elements on pre mRNA. snRNP, small nuclear ribonucleoprotein.

splicing, a post-transcriptional mechanism that has evolved to expand protein diversity. Splicing can be largely classified into a few types, including exon inclusion/exclusion, intron retention, mutual exclusion of adjacent exons, or alternation in 5′ or 3′ splice sites (Pan et al., 2008). Alternative splicing is executed by spliceosome (U1, SF1, U2AF1, U2AF2), the core machinery of splicing (U4/U6 and U5), together with splicing factors (Chao et al., 2021; Ule et al., 2019) (Fig. 1) that often modulate the core splicing machinery by affecting their site specificity (Hang et al., 2015; Lim et al., 2011; Liu et al., 2017). Splicing factors are RNA binding proteins that act as activators or repressors of splicing by binding to pre-mRNA exonic or intronic enhancer or silencer elements (Fig. 1). Splicing factors are reported to play essential roles in cell differentiation, tissue identity, and organ development (Baralle and Giudice, 2017), and their functions in adipocytes are being increasingly appreciated. Some excellent reviews have been covered on implication of splicing in adipogenesis and obesity (Chao et al., 2021; Wong et al., 2018). This review will focus on the splicing factors involved in adipogenesis and thermogenesis. These studies are providing important new insights into the mechanism of obesity and related metabolic abnormalities.

SPLICING FACTORS IN ADIPOGENESIS

SRC associated with mitosis of 68 kDa (Sam68)

Sam68 recognizes the 5′-[AU] UAA-3′ rich regions and promotes exon inclusion (Feracci et al., 2016; Lin et al., 1997; Ray et al., 2009). Sam68 has been reported to promote mTOR splicing by interacting with U1 snRNP (small nuclear ribonucleoprotein) in mouse embryonic fibroblast (Subramania et al., 2019). It also contributes the exon 7 skipping of survival of motor neuron 2 (SMN2) in spinal muscle atrophy and regulates alternative exon splicing in neurogenesis (Chawla et al., 2009; Pedrotti et al., 2010).

Several recent studies have shown that Sam68 regulates adipogenesis via alternative splicing of mTOR and S6K1 mRNA (Huot et al., 2012; Song and Richard, 2015). Huot et al. (2012) showed that Sam68$^{-/-}$ mice developed lower body mass with fewer adipogenic progenitors. The deletion of Sam68 protected mice from diet-induced obesity due to increased energy expenditure and impaired white adipocyte differentiation with reduced Pparγ expression and lipid accumulation (Huot et al., 2012). The phenotypes of Sam68 knockout may be partially explained by the influence of Sam68 on mTOR splicing in WAT. Huot et al. (2012) reported an increased retention of intron 5 in mTOR transcript (mTOR^{I5}) and a decreased mTOR protein expression in WAT of Sam68 deficient mice. Mechanistically, the intron retention of intron 5 can produce a premature stop codon and unstable mTOR transcripts (Figs. 2A and 2B, Fig. 3). Knockdown of Sam68 using RNA interference in 3T3-L1 preadipocytes impaired insulin-stimulated Akt phosphorylation and inhibited adipocyte differentiation. Ectopic expression of a full-length mTOR transcript in the Sam68-depleted 3T3-L1 cells partially rescued the impaired adipogenesis, supporting that the dysregulated mTOR splicing is a key downstream event of Sam68 deficiency (Huot et al., 2012).

However, the full-length mTOR protein could not fully rescue the effects from Sam68 ablation on adipogenesis as Sam68 also regulates the alternative splicing of many other transcripts including S6K1 (Song and Richard, 2015). Rps6kb1, the gene encoded for S6K1 proteins, produced three isoforms: p70S6K, p85S6K, and p31S6K (Tavares et al., 2015). Song and Richard (2015) showed that knockdown of Sam68 in 3T3-L1 cells and Sam68 knockout in WAT increased the expression of p31S6K (Rps6kb1-002), a truncated protein lacking the region of mTOR- and PDK-phosphorylation (Ben-Hur et al., 2013) (Figs. 2C and 2D, Fig. 3). Interestingly, ectopic expression of p31S6K in 3T3-L1 impaired adipogenesis by lowing lipid storage and inhibiting the expression of Pparγ, CEPBα and Glut4. To further support this, the author proved that p31S6K depletion by siRNA in Sam68-deficient 3T3-L1 cells enhanced the lipid accumulation and the expression of differentiation markers. Thus, the reduced adipogenesis in Sam68 deficient cells was partially rescued by the loss of p31S6K expression (Song and Richard, 2015). Using cross linking and immunoprecipitation (CLIP) assays in preadipocytes, the authors showed Sam68 can bind to the intron 6 of Rps6kb1 transcript, preventing Serine/arginine-rich splicing factor 1 (SRSF1) from this region and facilitating the canonical Rps6kb1 splicing. In the absence of Sam68, SRSF1 recognizes exon 6 and promotes the inclusion of three extra exons to generate the p31S6K-encoding isoform (Song and Richard, 2015). Taken together, Sam68 regulates adipogenesis at least partially via alternative splicing of mTOR and S6K1 mRNAs. However, whether Sam68 regulates other mRNAs and how other targets influence adipogenesis remains unclear.

Serine arginine rich splicing factor 1 (SRSF1)

SRSF1, also referred to as SF2/ASF, has multiple functional impacts, including nonsense-mediated mRNA decay (NMD),

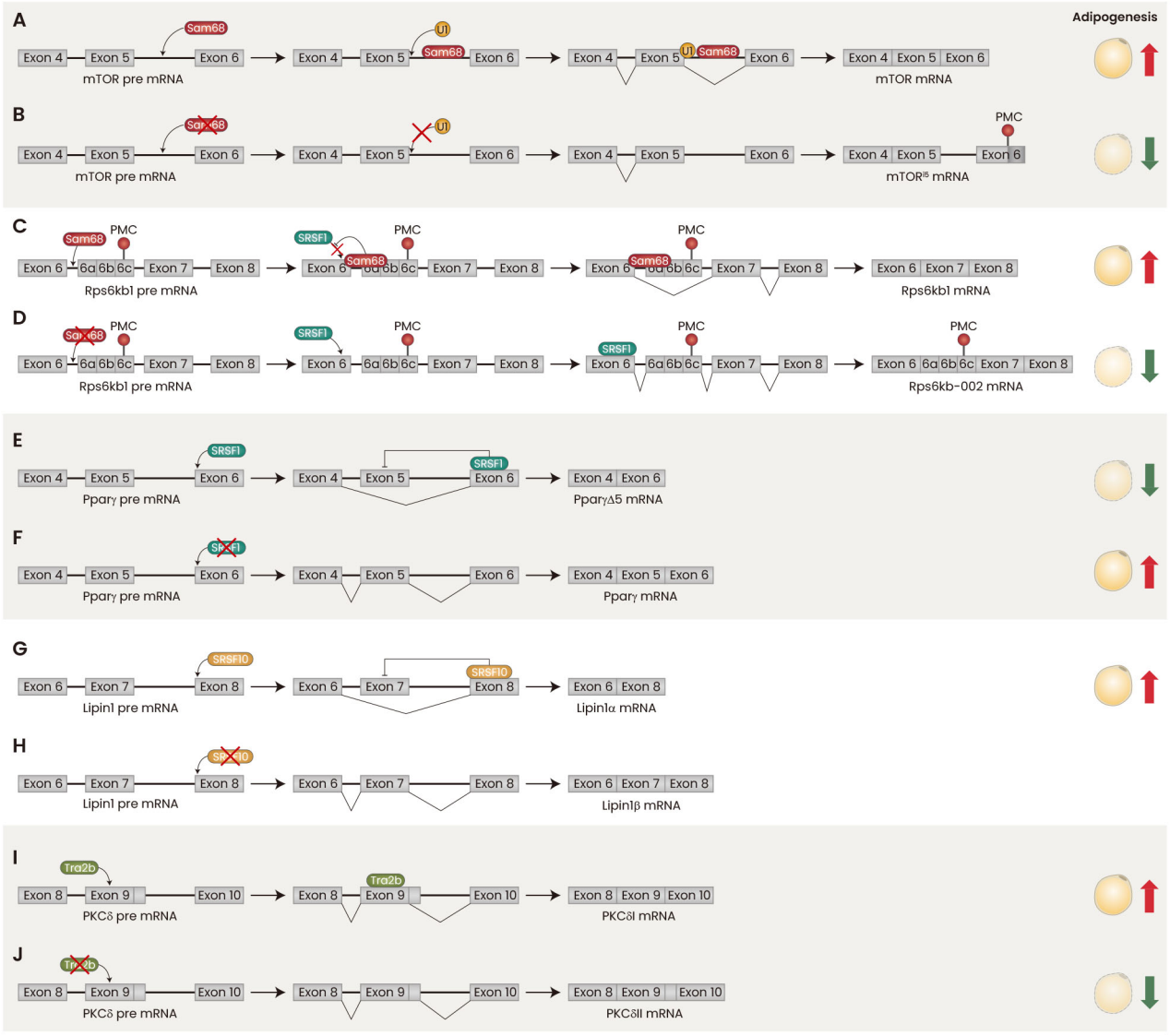
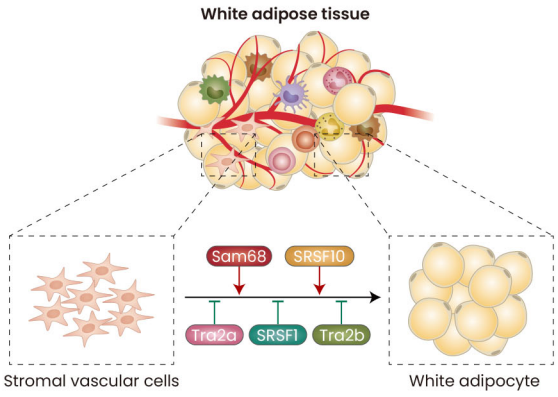


Fig. 2. Schematic representation of canonical and alternative splicing of mTOR, ribosomal Rps6kb1, Pparγ, lipin1, and PKCδ in WAT. (A) Sam68 binds to intron 5 and U1 binds to exon 5. Canonical mTOR transcript is produced. (B) mTOR$^{\Delta 5}$ is produced without Sam68. (C) Sam68 blinding to intron 5 blocks SRSF1 binding and canonical Rps6kb1 mRNA is expressed. (D) SRSF1 binds to exon 6 in the absence of Sam68 and Rps6kb1-002 transcript is produced. (E) SRSF1 binds to exon 6 of Pparγ and Pparγδ5 mRNA is produced. (F) Canonical Pparγ mRNA is formed in the absence of SRSF1. (G) SRSF10 binds to exon 8 and Lipin1α is produced. (H) Lipin1β mRNA is formed in the absence of SRSF10. (I) Tra2b binds to exon 9 of PKCδ and PKCδI is produced. (J) PKCδII is produced in the absence of Tra2b.

mRNA nuclear transport, mRNA translation, miRNA processing, genome stability, chromatin association, protein sumoylation, and nuclear stress (Das and Krainer, 2014; Karni et al., 2007; Loomis et al., 2009; Pelisch et al., 2010; Pradeepa et al., 2012). SRSF1 plays a pivotal role in adipocyte biology by regulating the alternative splicing events of Rps6kb1 and Pparγ (Aprile et al., 2018; Karni et al., 2007; Song and Richard, 2015) (Figs. 2C-2F, Fig. 3). As discussed above, SRSF1 enhances the inclusion of three extra exons between exons 6 and 7 and favours the formation of Rps6kb1-002 transcript which encodes p31S6K, a truncated S6K1 lacking the region of mTOR and PDK-phosphorylating (Fig. 2D, Fig. 3). SRSF1 knockdown with siRNA in both HEK293 and 3T3-L1 cells reduced the P31S6K expression. Overexpression of P31S6K in 3T3-L1 cells impaired adipogenesis with decreased lipid accumulation and lower expression of Pparγ, C/EBPα and GLUT4. Conversely, siRNA knockdown of P31S6K enhanced the lipid accumulation in 3T3-L1 cells where Sam68 was knocked down (Song and Richard, 2015).

SRSF1 regulates the alternative splicing of Pparγ, the master regulator of adipocyte biology (Figs. 2E and 2F, Fig. 3). Aprile et al. (2018) reported that Pparγ expresses several natural isoforms and one of them excludes exon 5, referred to as PparγΔ5, which lacks the ligand-binding domain. Over-



Splicing factors	Target genes	Preferential isoforms	Antagonistically catalize splicing factors	Phenotypic outcome for loss of function
Sam68	mTOR	Canonical mTOR		Impaired adipogenesis
	Rps6kb1	Canonical Rps6kb1	SRSF1	
Srsf1	Rps6kb1	Rps6kb1-002	Sam68	Enhanced adipogenesis
	Pparγ	PparγΔ5		
Srsf10	Lipin	Lipin1α		Impaired adipogenesis
Tra2a	CPT1	CPT1-6α		Impaired β oxidation Enhanced lipid storage
Tra2b	PKCδ	PKCδ-1		Impaired adipogenesis

Fig. 3. Splicing factors and their targeted isoforms in adipogenesis.

expressing PparγΔ5 in hTERT-immortalized adipose tissue-derived human mesenchymal stem cells (hMSCs) and primary human adipocyte progenitors negatively impacted adipogenesis with decreased lipid accumulation during differentiation, showing a negative role of PparγΔ5 on adipogenesis. In addition, SRSF1 ablation using siRNA alleviated the production of PparγΔ5 in hMSCs. The regulation of SRSF1 on Pparγ splicing is not limited to adipocytes since siRNAs knockdown of SRSF1 in HEK293cells can also enhance the exon 5 inclusion and reduce the production of PparγΔ5. CLIP-seq in HEK293 cells revealed a SRSF1-binding site in Pparγ exon 6, and the binding was confirmed by RNA immunoprecipitation (RIP) using anti-SRSF1. Taken together, SRSF1 is likely to negatively regulate adipogenesis by enhancing the p31S6k1 protein and PparγΔ5 expression.

Serine arginine rich splicing factor 10 (SRSF10)

SRSF10 plays a critical role in tumour cell growth and glucose metabolisms (Wei et al., 2015; Zhou et al., 2014). In Hela cells, the function of SRSF10 is regulated by its phosphorylation state as its acts as a splicing repressor upon dephosphorylation (Cowper et al., 2001; Shin et al., 2004). Whole body SRSF10 knockout mice could not survive beyond the embryonic stage due to several cardiac defects, highlighting SRSF10 as a vital splicing factor (Feng et al., 2009).

Li et al. (2014) showed an impairment of axillary subcutaneous WAT development in SRSF10 knockout embryonic mice besides heart defects. SRSF10 knockout primary MEF cells exhibit decreased adipogenic capacity and lowered PPARγ and Adiponectin expressions. Consistently, shRNA SRSF10 knockdown in C3H10T1/2 resulted in down-regulated expression of Pparγ and Adiponectin and impaired lipid accumulation during C3H10T1/2 differentiation (Li et al., 2014). They further showed that SRSF10 binds to exon 8 of lipin1 in a sequence specific manner by ^{32}P-labeled RNAs -gel shift assay and causes exon-skipping of exon 7 in primary MEF cells to favour the production of Lipin1α (Figs. 2G and 2H, Fig. 3). Conversely, knockdown of SRSF10 in C3H10T1/2 cells increased Lipin1β relative to Lipin1α. Overexpressing Lipin1α and Lipin1β respectively in SRSF10 knockdown C3H10T1/2 cells showed Lipin1α can rescue adipogenesis more efficiently than Lipin1β, evidenced by more lipid accumulation and higher expressions of Pparγ and Adiponectin in Lipin1α-infected cells (Li et al., 2014). Thus, the decreased usage of Lipin1α isoform may partially account for the comprised adipogenesis in SRSF10-deficient cells.

Transformer 2 (Tra2)

Tra2, a regulator of sex determination in Drosophila, was reported as a sequence-specific splicing factor in Hela cells (Tacke et al., 1998). Mikoluk et al. (2018) showed that siRNA-mediated Tra2a knockdown in female Drosophila resulted in an increased fat body and longer survival rates on starvation. Tra2a deficiency can induce the usage of CPT1-6β over CPT1-6α (Fig. 3). As CPT1-6β is the less active in catalysing the formation of acyl carnitines, the Tra2a deficiency-induced CPT1-6β usage may lead to lower β-oxidation activity, resulting in increased lipid storage (Mikoluk et al., 2018).

Tra2b, also known as SFRS10, is a vital splicing factor, as the whole body Tra2b knockout caused embryonically lethal in mice (Pihlajamäki et al., 2011). Tra2b was reported to regulate alternative splicing of Wnt 11b in *Xenopus*, and lipin1 alternative splicing in HepG2 cells (Dichmann et al., 2015; Pihlajamäki et al., 2011). Patel et al. (2014) showed that Tra2b can regulate alternative splicing of PKCδ in 3T3-L1 cells to modulate the usage of PKCδ-1 (Patel et al., 2014), an apoptotic isoform, and PKCδ-2, a cell survival isoform (Figs. 2I and 2J, Fig. 3). Tra2b knockdown resulted in a decrease in PKCδ-1 while Tra2b overexpression increased the usage of PKCδ-1 in 3T3-L1 cells. The authors further utilized minigene and RIP assays to confirm that Tra2b regulated PKCδ splicing by affecting the 5′ splice site selection of the intron 9 (Patel et al., 2014) (Figs. 2I and 2J, Fig. 3). Consistent with these findings, during 3T3-L1 cell differentiation, the expression of Tra2b is gradually decreased, concomitant with a switch from PKCδ-1 to PKCδ-2. What about the role of PKCδ-1 in adipogenesis? siRNA knockdown of PKCδ-1 at day 1 of differentiation improved lipid accumulation and the expression of Pparγ and Adiponectin during the subsequent differentiation (Patel et al., 2014). Carter et al. (2013) reported that the

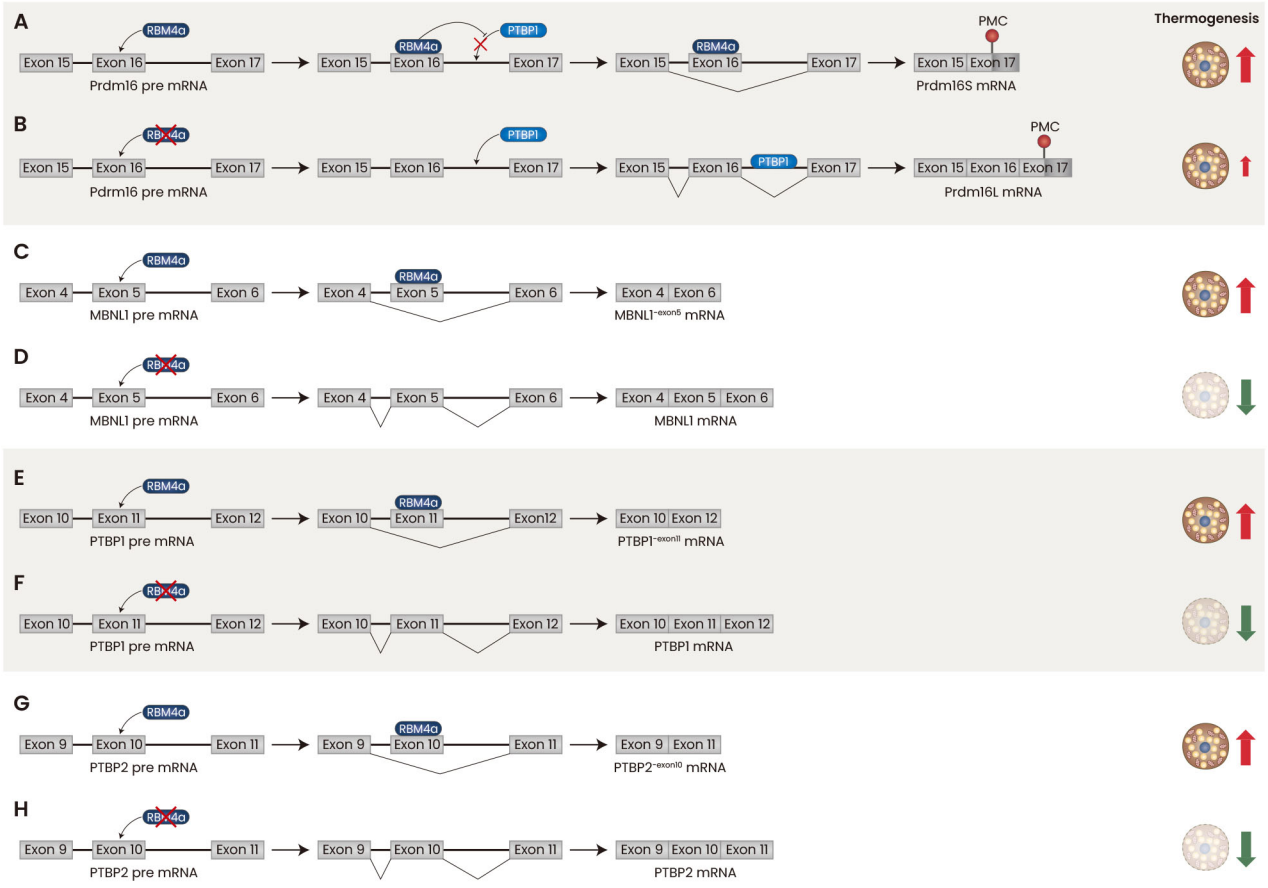


Fig. 4. Schematic representation of canonical and alternative splicing of Prdm16, MBNL1 and PTBPs in BAT. (A) RBM4a binds to exon 16 and Prdm16S mRNA is produced. (B) PTBP1 binds to intron 16 in the absence of RBM4a and Prdm16L mRNA is formed. (C) RBM4a binds to exon 5 and MBNL^{-exon5} mRNA is produced. (D) Canonical MBNL1 transcript is formed in the absence of RBM4a. (E) RBM4a bind to exon 11 of PTBP1 pre mRNA and PTBP1^{-ex11} is produced. (F) Canonical PTBP1 transcript is produced in the absence of RBM4a. (G) RBM4a bind to exon 10 of PTBP2 pre mRNA and PTBP2^{-ex10} is produced. (H) Canonical PTBP2 transcript is produced in the absence of RBM4a. BAT, brown adipose tissue.

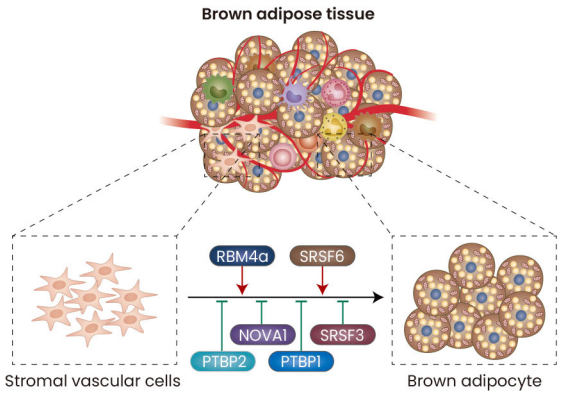
preadipocytes from subcutaneous and omental adipose tissue of obese individuals showed a decrease in PKCδ-1 relative to PKCδ-2, which may partially explain their higher resistance to apoptosis than the preadipocytes from lean individuals. Thus, the Tra2b is likely to exert a negative influence on 3T3-L1 adipogenesis through favouring the generation of PKCδ-1 isoform, although the function of Tra2b in adipogenesis was not tested directly in these studies.

SPLICING FACTORS IN THERMOGENIC ADIPOCYTES

RNA binding motif protein 4a (RBM4a) and polypyrimidine tract binding proteins (PTBP)

RBM4a was reported to regulate alternative splicing events in malignant cells as well as in cells differentiation. It acts as a splicing regulator of several other splicing factors such as MBNL1, PTBP1, PTBP2, NOVA1 and SRSF3 (Lin et al., 2016a; 2016b; Lin and Tarn, 2011; Peng et al., 2018; Wang et al., 2014). RBM4a whole-body knockout mice displayed low insulin production and hyperglycaemia phenotypes (Lin et al., 2013). The expression of RBM4a is gradually increased along BAT differentiation, implying a role of RBM4a in BAT development. Indeed, the BAT of RBM4a deficient mice exhibited lower levels of brown adipocyte markers such as Myf5, Bmp7, Prdm16, and Ucp1. Furthermore, RBM4a-deficient BAT developed impaired lipid accumulation, decreased oxygen consumption, and reduced energy expenditure. Conversely, RBM4a overexpression in C3H10T1/2 cells enhances the expression of brown adipocyte markers and oxygen consumption rates (OCRs) (Lin et al., 2014).

The transcriptome profiling analysis of postnatal RBM4a KO BATs showed differential alternative splicing patterns of 186 genes (Lin et al., 2016a). One of the RBM4a-regulated alternative splicing events in adipose tissue occurs in Prdm16 (PR domain containing 16) (Figs. 4A and 4B, Fig. 5). Prdm16 is a BAT-enriched protein and play a key role in brown and beige adipocytes development (Cohen et al., 2014; Harms et al., 2014). Prdm16 expresses an exon 16-exclusive iso-



Splicing factors	Target genes	Preferential isoforms	Antagonistically catalize splicing factors	Phenotypic outcome for loss of function
RBM4a	Prdm16	Prdm16S	PTBP1	Impaired thermogenesis
	MBNL1	MBNL1^{-exon5}		
	PTBP1	PTBP1$^{-exon11}$		
	PTBP2	PTBP2$^{-exon10}$		
	SRSF3	SRSF3$^{+exon4'}$	PTBP2	
	Map4k4	Map4k4-Iso4	SRSF3	
NOVA1	SRSF6	SRSF6$^{+intron2}$		Enhanced thermogenesis
	Mapk8	Mapk8β		Impaired JNK signaling pathway
	Mapk9	Mapk9α		

Fig. 5. Splicing factors and their targeted isoforms in thermogenesis.

form (Prdm16S) and an exon 16-inclusive long (Prdm16L) isoform. Chi and Lin (2018) reported that overexpressing RBM4a enhanced the usage of Prdm16S while shRNA knockdown of RBM4a promoted the usage of Prdm16L isoform in C3H10T1/2. Prdm16S has a stronger effect on inducing the expression of PGC1-α and C/EBP-β than Prdm16L during the adipogenic differentiation of C3H10T1/2. Consistently, Prdm16S is also more effective in promoting OCR, ATP production, and lipid droplet formation than Prdm16L. Therefore, RBM4a-mediated Prdm16 isoform usage may be a critical mechanism to sustain BAT adipogenesis and thermogenesis (Chi and Lin, 2018).

Given the prominent phenotype of BAT defect in RBM4a knockout mice, it is no surprising to find that RBM4a targets some key BAT/beige regulators such as Prdm16. However, it is more intriguing that it also regulates some the splicing of some other splicing factors such as MBNL1 (Hung and Lin, 2020), PTBP (Lin et al., 2016b), and Nova1 (Lin et al., 2016a). Hung and Lin (2020) reported that RBM4a repressed MBNL1 exon 5 inclusion during BATs development *in vivo* and 3T3-L1 differentiation (Figs. 4C and 4D, Fig. 5). RBM4a knockout enhanced the inclusion of exon 5 of MBNL1 in BAT and siRNA-mediated RBM4a knockdown in 3T3-L1 increased the usage of the MBNL1^{+exon5} isoform. Conversely, overexpressing the MBNL1^{-exon5} isoform during 3T3-L1 differentiation, compared to the MBNL1^{+exon5} isoform, induced a more prominent effect on beige cell-related splicing events, expression of Prdm16 and UCP1, lipid accumulation, and oxygen consumption (Hung and Lin, 2020). Thus, RBM4a can regulate brown adipogenesis at least partially by modulating the alternative splicing of MBNL1 exon 5 skipping.

PTBP proteins are known to be involved in alternative splicing of pyruvate kinase in C2C12 cells, NIH-3T3 cells and Brain Tumour cells (David et al., 2010). PTBP has two paralogous genes PTBP1 and PTBP2. Each of these two genes can generate an NMD transcript, PTBP1$^{-exon11}$ (exon 11 skipping) and PTBP2$^{-exon10}$ (exon 10 skipping), respectively, and the usage of both isoforms increase from embryonic to neonatal BAT. RBM4a depletion promotes the usage of the canonical PTBP1 and PTBP2 transcripts during BAT development (Lin et al., 2016b), while RBM4a overexpression in C3H10T1/2 cells represses the usage of PTBP1 and PTBP2 canonical splicing by promoting exon 11 and exon 10 skipping, respectively, in C3H10T1/2 (Figs. 4E and 4G). Furthermore, the authors showed that PTBP2 overexpression reduced the Prdm16 and UCP1 transcripts during adipogenic differentiation of C3H10T1/2. Conversely, shRNA-mediated PTBP2 knockdown increased Pdrm16 and UCP1 expression and enhanced the lipid accumulation, which phenocopies RBM4a overexpression and suggests that PTBP2 is a major downstream effector of RBM4a.

A downstream target of PTBP2 is NOVA1. PTBP2 can bind to the 3′ UTR region of NOVA1 to stabilize the NOVA1 transcript, while the PTBP2 ablation destabilizes the NOVA1 mRNA. Overexpressing NOVA1 represses and shRNA-knockdown of NOVA1 promotes the expression of Prdm16 and Ucp1 in C3H10T1/2 cells, suggesting a negative role of NOVA1 in brown/beige adipogenesis (Lin et al., 2016a).

Based on the results described above, it is postulated that PTBP1 and RBM4a may play antagonistically roles in regulating BAT development at alternative splicing levels. While RBM4a is necessary for normal BAT development, PTBP functions as an adipogenic repressor (Chi and Lin, 2018; Lin et al., 2016b). However, further biochemistry analysis will be needed to illustrate the detailed molecular mechanism of how these factors regulate BAT development program.

Neuro oncological ventral antigen 1 (NOVA1)

NOVA1 recognizes RNA in a 5′-YCAY-3′ sequence-specific manner and was originally reported to play a critical role in neuronal viability (Jensen et al., 2000). Vernia et al. (2016) showed that NOVA is also an important regulator in adipose tissue thermogenesis. They analysed RNA splicing changes in adipose tissue during diet-induced obesity and connected the alternative splicing changes with NOVA1 splicing factor. To test the function of NOVA in adipose tissue, they generated adipose tissue-specific knockout mice to delete NOVA1 and NOVA2 at the same time. NOVA-deficient mice demonstrated increased adipose tissue thermogenesis and improved glycemia. During the cold challenge, high fat diet (HFD)-fed

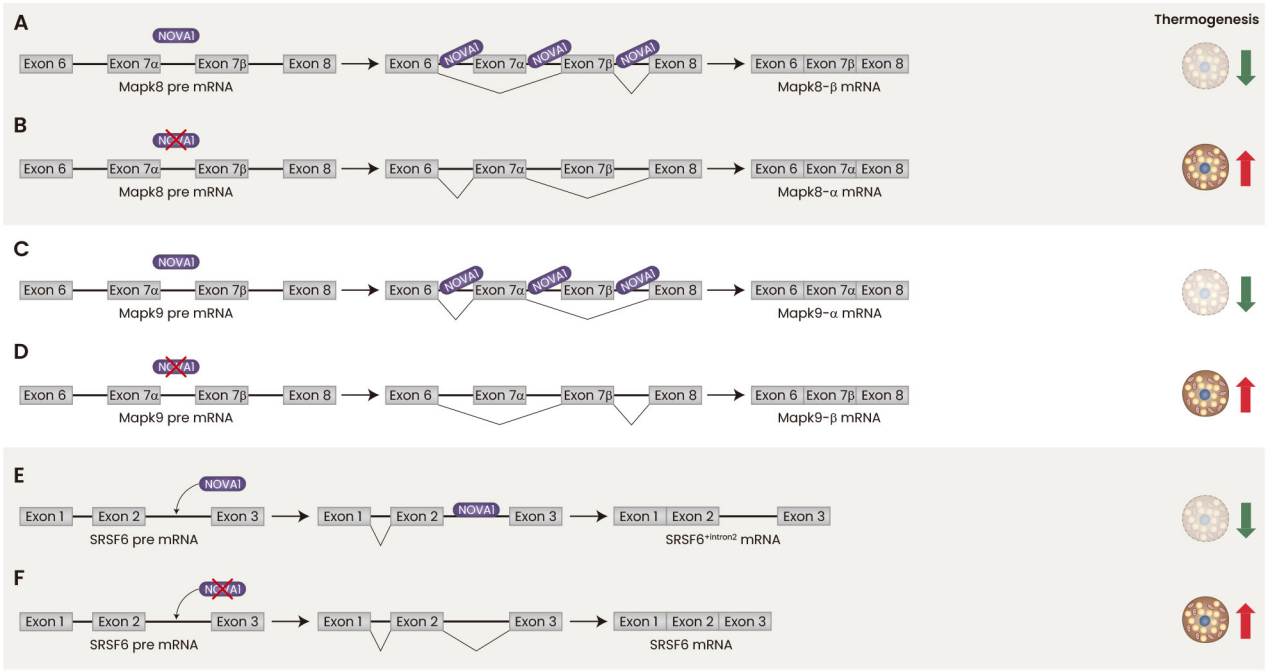


Fig. 6. Schematic representation of the canonical and alternative splicing of Mapk8, Mapk9 and SRSF6 in BAT. (A) NOVA1 binds to the intron 6, 7α and 7β and Mapk8β is produced. (B) Mapk8α is produced in the absence of NOVA1. (C) NOVA1 binds to the intron 6, 7α and 7β of Mapk9 and Mapk9α is produced. (D) Mapk9β is formed in the absence of NOVA1. (E) NOVA1 bind to intron 2 of SRSF6 and $SRSF6^{+in2}$ is formed. (F) Canonical SRSF6 is produced in the absence of NOVA1. BAT, brown adipose tissue.

NOVA deficient mice had significantly less body and fat mass and higher core body temperature than the control mice at HFD (Vernia et al., 2016).

Authors suggested that NOVA may regulate adipose tissue thermogenesis by controlling the alternative splicing of Mapk8 (JNK1 protein kinase encoded gene) and Mapk9 (JNK2 protein kinase encoded gene) in beige adipocytes (Vernia et al., 2016) (Fig. 5, Figs. 6A and 6C). The NOVA-binding motif (YCAY) was found in the sequences around exon 7α and 7β of both Mapk8 and Mapk9 transcripts. NOVA knockout in adipose tissue caused an increase in the *Mapk8α/β* ratio and a decrease in the *Mapk9α/β* ratio, indicating that NOVA favours the usage Mapk8β (encodes JNK1β) and Mapk9α (encodes JNK2α) over the other isoform. Enzymic activity analysis using substrate c-Jun supported that JNK1β and JNK2α are the more active isoforms than Mapk8α-encoded JNK1α and Mapk9β-encoded JNK2β, respectively (Figs. 4A and 4C, Fig. 5). Consistently, NOVA KO adipocytes repressed the usage of Mapk8β (JNK1β) and Mapk9α (JNK2α) and showed markedly suppression in stress-induced phosphorylation of the JNK substrate $pSer^{63}$ c-Jun. Knock-down of *Mapk8β* mRNA and *Mapk9α* mRNA using shRNA caused increased expression of the *Prdm16* and *Ucp1* genes in 3T3-L1 adipocytes, suggesting a negative role of *Mapk8β* mRNA and *Mapk9α* in brown adipogenesis. Overall, these studies suggest that NOVA is a suppressor of thermogenesis, partially by promoting the usage of Mapk8β (JNK1β) and Mapk9α (JNK2α) (Vernia et al., 2016).

Another study by Lin et al. (2016a) provides more evidence for the negative effects of NOVA on brown adipogenesis. The authors found that NOVA1 overexpression reduced the expression of brown adipogenesis markers CEBP, PGC-1, and UCP1 and reduced the number of mitochondria during C3H10T1/2 differentiation (Lin et al., 2016a).The authors showed that NOVA1 can enhance SRSF6 intron2 retention to silence SRSF6 function in C3H10T1/2 (Fig. 5, Figs. 6E and 6F). siRNA-mediated SRSF6 knockdown lowered the abundance of mitochondria and the overexpression of SRSF6 improved lipid accumulation and OCR during C3H10T1/2 adipogenesis (Lin et al., 2016a). Thus, NOVA1 plays a negative role in brown/beige adipogenesis by regulating multiple targets, but which targets may play more important roles than others warrant further investigations.

Serine arginine rich splicing factor 3 (SRSF3)

SRSF3 is a multifunctional RNA binding protein as it is involved in alternative splicing, transcriptional termination, alternative RNA polyadenylation, translational regulation, and RNA export (Guo et al., 2015). SRSF3 can bind to m6A-modified nucleotides and promote exon inclusion in 293 cells (Xiao et al., 2016). Interestingly, SRSF3 was reported to autoregulate its own splicing by enhancing the inclusion of its exon 4′ (an alternative exon between the classic exon 4 and exon 5) with an in-frame stop codon and this inclusion is impaired in oral squamous cell carcinoma cells (Guo et al., 2015).

SRSF3 is a negative regulator of brown adipogenesis, as shRNA-medicated SRSF3 knockdown resulted in elevated

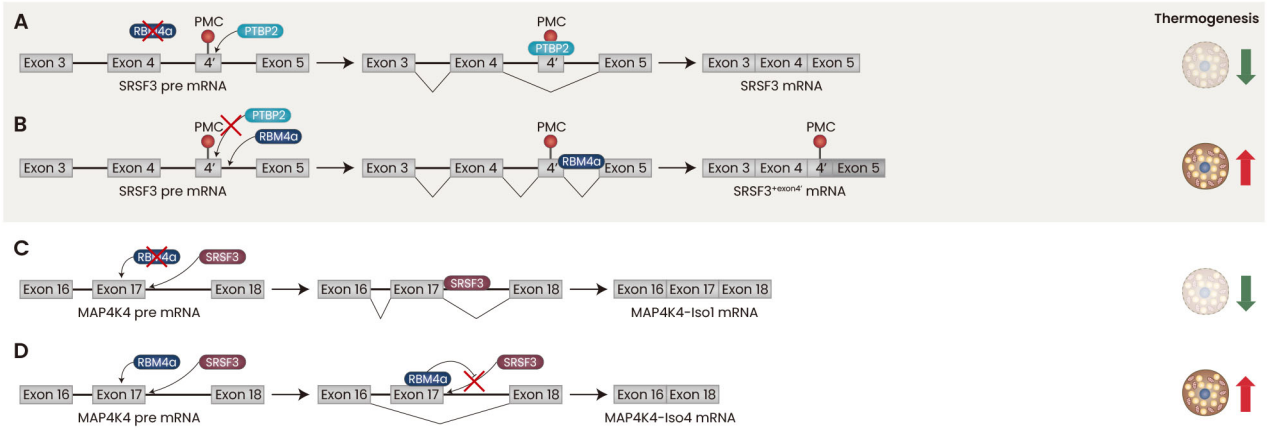


Fig. 7. Schematic representation of alternative splicing of SRSF3 and MAP4K4 in BAT. (A) PTBP1 binds to exon 4′ of SRSF3 and canonical SRSF3 transcript is produced in the absence of RBM4a. (B) RBM4a binds the intron 4′ and SRSF3-exon 4′ included transcripts is produced. (C) SRSF3 binds to intron 17 and MAP4K4-Iso1 is produced. (D) RBM4a binds to exon 17 and MAP4K4-Iso4 is produced. BAT, brown adipose tissue.

OCR and ATP production with up-regulated BAT markers such as Prdm16 and Ucp1, while SRSF3 overexpression reduced OCR, ATP, and BAT marker expression in C3H10T1/2 cells (Peng et al., 2018). The authors also observed an increase in SRSF3 exon 4′-containing transcript during BAT development and C3H10T1/2 differentiation. Interestingly, the inclusion of exon 4′ of SRSF3 is antagonistically catalysed by RBM4a and PTBP1 in C3H10T1/2 and BAT (Fig. 5, Figs. 7A and 7B). While RBM4a promotes exon 4′ inclusion by binding its intronic UUUCU element in postnatal BAT, PTBP1 represses this inclusion (Peng et al., 2018), which, again, supports an antagonistic role of RBM4a and PTBP1 in regulating the isoform usage program during brown adipogenesis. One of SRSF3′ targets is MAP4K4. SRSF3 binds the intron 17 of MAP4K4 and enhances its inclusion to form the isoform of MAP4K4-Iso-1 in BAT and C3H10T1/2 (Figs. 7C and 7D). Overexpression of MAP4K4-Iso-1 impaired OCR and ATP production during C3H10T1/2 differentiation. Thus, the SRSF3-mediated MAP4K4-Iso-1 production may partially explain the negative role of SRSF3 in brown adipogenesis (Peng et al., 2018).

CONCLUSION

Splicing factors are emerging as an essential class of regulators for adipocyte biology. The studies in the past decade have identified several splicing factors such as RBM4a, SRSF10, NOVA1 etc. that play regulatory roles in brown and white fat development. Moreover, several splicing events downstream these splicing factors were demonstrated to exert positive or negative influence on adipocyte differentiation. As expected, some of the downstream splicing events occur in known regulators of nutrient metabolism such as mTOR, Pparγ, Prdm16, etc. In addition, a significant portion of the downstream splicing events are also splicing factors such as MBNL1, PTBP2, NOVA1, etc. The regulated isoform usage of these splicing factors further affect adipocyte biology. These findings suggest a tentative model where splicing factors regulate the splicing of each other to form an interweaved network to regulate adipocyte development and metabolism, which is independent from but coordinated with gene expression-based regulations.

Several limitations were noted in earlier studies. First, most studies were conducted in cell lines *in vitro* and lack physiological relevance. Second, a majority of these studies focused on adipogenesis in white or brown/beige lineage, but the involvement of splicing factors or isoform usage events in complicated adipocyte metabolism in physiological and physiological contexts remain poorly explored. Last, while earlier studies have established the role of several specific splicing factor and splicing events in adipocytes, but how these factors and/or events are coordinated with each other and with the gene expression regulatory cascade in a spatiotemporal manner are unknown. These outstanding issues will need to be addressed in the future with more mouse genetic tools, deeper molecular biochemistry analysis, and more powerful high-throughput sequencing and bioinformatic analysis. Nonetheless, the studies about splicing in adipocytes have revealed important novel insights into the regulatory mechanism underlying adipocyte biology and will further provide the necessary information for developing new therapeutic approaches for clinical complications caused by adipose tissue dysfunction.

AUTHOR CONTRIBUTIONS

Y.T.N. wrote the manuscript. L.S. provided expretise and feedback.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

ORCID

Yadanar Than Naing https://orcid.org/0009-0004-2841-6363
Lei Sun https://orcid.org/0000-0003-3937-941X

REFERENCES

Aprile, M., Cataldi, S., Ambrosio, M.R., D'Esposito, V., Lim, K., Dietrich, A., Blüher, M., Savage, D.B., Formisano, P., Ciccodicola, A., et al. (2018). PPARγΔ5, a naturally occurring dominant-negative splice isoform, impairs PPARγ function and adipocyte differentiation. Cell Rep. *25*, 1577-1592.e6.

Baralle, F.E. and Giudice, J. (2017). Alternative splicing as a regulator of development and tissue identity. Nat. Rev. Mol. Cell Biol. *18*, 437-451.

Becher, T., Palanisamy, S., Kramer, D.J., Eljalby, M., Marx, S.J., Wibmer, A.G., Butler, S.D., Jiang, C.S., Vaughan, R., Schöder, H., et al. (2021). Brown adipose tissue is associated with cardiometabolic health. Nat. Med. *27*, 58-65.

Ben-Hur, V., Denichenko, P., Siegfried, Z., Maimon, A., Krainer, A., Davidson, B., and Karni, R. (2013). S6K1 alternative splicing modulates its oncogenic activity and regulates mTORC1. Cell Rep. *3*, 103-115.

Carter, G., Apostolatos, A., Patel, R., Mathur, A., Cooper, D., Murr, M., and Patel, N.A. (2013). Dysregulated alternative splicing pattern of PKCδ during differentiation of human preadipocytes represents distinct differences between lean and obese adipocytes. ISRN Obes. *2013*, 161345.

Chao, Y., Jiang, Y., Zhong, M., Wei, K., Hu, C., Qin, Y., Zuo, Y., Yang, L., Shen, Z., and Zou, C. (2021). Regulatory roles and mechanisms of alternative RNA splicing in adipogenesis and human metabolic health. Cell Biosci. *11*, 66.

Chawla, G., Lin, C.H., Han, A., Shiue, L., Ares, M., Jr., and Black, D.L. (2009). Sam68 regulates a set of alternatively spliced exons during neurogenesis. Mol. Cell. Biol. *29*, 201-213.

Chi, Y.L. and Lin, J.C. (2018). RBM4a modulates the impact of PRDM16 on development of brown adipocytes through an alternative splicing mechanism. Biochim. Biophys. Acta Mol. Cell Res. *1865*(11 Pt A), 1515-1525.

Cohen, P., Levy, J.D., Zhang, Y., Frontini, A., Kolodin, D.P., Svensson, K.J., Lo, J.C., Zeng, X., Ye, L., Khandekar, M.J., et al. (2014). Ablation of PRDM16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. Cell *156*, 304-316.

Cowper, A.E., Cáceres, J.F., Mayeda, A., and Screaton, G.R. (2001). Serine-arginine (SR) protein-like factors that antagonize authentic SR proteins and regulate alternative splicing. J. Biol. Chem. *276*, 48908-48914.

Das, S. and Krainer, A.R. (2014). Emerging functions of SRSF1, splicing factor and oncoprotein, in RNA metabolism and cancer. Mol. Cancer Res. *12*, 1195-1204.

David, C.J., Chen, M., Assanah, M., Canoll, P., and Manley, J.L. (2010). HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. Nature *463*, 364-368.

Dichmann, D.S., Walentek, P., and Harland, R.M. (2015). The alternative splicing regulator Tra2b is required for somitogenesis and regulates splicing of an inhibitory Wnt11b isoform. Cell Rep. *10*, 527-536.

Feng, Y., Valley, M.T., Lazar, J., Yang, A.L., Bronson, R.T., Firestein, S., Coetzee, W.A., and Manley, J.L. (2009). SRp38 regulates alternative splicing and is required for Ca2+ handling in the embryonic heart. Dev. Cell *16*, 528-538.

Feracci, M., Foot, J.N., Grellscheid, S.N., Danilenko, M., Stehle, R., Gonchar, O., Kang, H.S., Dalgliesh, C., Meyer, N.H., Liu, Y., et al. (2016). Structural basis of RNA recognition and dimerization by the STAR proteins T-STAR and Sam68. Nat. Commun. *7*, 10355.

Gesta, S., Tseng, Y.H., and Kahn, C.R. (2007). Developmental origin of fat: tracking obesity to its source. Cell *131*, 242-256.

Guo, J., Jia, J., and Jia, R. (2015). PTBP1 and PTBP2 impaired autoregulation of SRSF3 in cancer cells. Sci. Rep. *5*, 14548.

Hang, J., Wan, R., Yan, C., and Shi, Y. (2015). Structural basis of pre-mRNA splicing. Science *349*, 1191-1198.

Harms, M.J., Ishibashi, J., Wang, W., Lim, H.W., Goyama, S., Sato, T., Kurokawa, M., Won, K.J., and Seale, P. (2014). Prdm16 is required for the maintenance of brown adipocyte identity and function in adult mice. Cell Metab. *19*, 593-604.

Hui, X., Gu, P., Zhang, J., Nie, T., Pan, Y., Wu, D., Feng, T., Zhong, C., Wang, Y., Lam, K.S.L., et al. (2015). Adiponectin enhances cold-induced browning of subcutaneous adipose tissue via promoting M2 macrophage proliferation. Cell Metab. *22*, 279-290.

Hung, C.S. and Lin, J.C. (2020). Alternatively spliced MBNL1 isoforms exhibit differential influence on enhancing brown adipogenesis. Biochim. Biophys. Acta Gene Regul. Mech. *1863*, 194437.

Huot, M.É., Vogel, G., Zabarauskas, A., Ngo, C.T.A., Coulombe-Huntington, J., Majewski, J., and Richard, S. (2012). The Sam68 STAR RNA-binding protein regulates mTOR alternative splicing during adipogenesis. Mol. Cell *46*, 187-199.

Jensen, K.B., Musunuru, K., Lewis, H.A., Burley, S.K., and Darnell, R.B. (2000). The tetranucleotide UCAY directs the specific recognition of RNA by the Nova K-homology 3 domain. Proc. Natl. Acad. Sci. U. S. A. *97*, 5740-5745.

Karni, R., de Stanchina, E., Lowe, S.W., Sinha, R., Mu, D., and Krainer, A.R. (2007). The gene encoding the splicing factor SF2/ASF is a proto-oncogene. Nat. Struct. Mol. Biol. *14*, 185-193.

Li, H., Cheng, Y., Wu, W., Liu, Y., Wei, N., Feng, X., Xie, Z., and Feng, Y. (2014). SRSF10 regulates alternative splicing and is required for adipocyte differentiation. Mol. Cell. Biol. *34*, 2198-2207.

Lim, K.H., Ferraris, L., Filloux, M.E., Raphael, B.J., and Fairbrother, W.G. (2011). Using positional distribution to identify splicing elements and predict pre-mRNA processing defects in human genes. Proc. Natl. Acad. Sci. U. S. A. *108*, 11093-11098.

Lin, J.C., Chi, Y.L., Peng, H.Y., and Lu, Y.H. (2016a). RBM4–Nova1–SRSF6 splicing cascade modulates the development of brown adipocytes. Biochim. Biophys. Acta *1859*, 1368-1379.

Lin, J.C., Lu, Y.H., Liu, Y.R., and Lin, Y.J. (2016b). RBM4a-regulated splicing cascade modulates the differentiation and metabolic activities of brown adipocytes. Sci. Rep. *6*, 20665.

Lin, J.C. and Tarn, W.Y. (2011). RBM4 down-regulates PTB and antagonizes its activity in muscle cell–specific alternative splicing. J. Cell Biol. *193*, 509-520.

Lin, J.C., Tarn, W.Y., and Hsieh, W.K. (2014). Emerging role for RNA binding motif protein 4 in the development of brown adipocytes. Biochim. Biophys. Acta *1843*, 769-779.

Lin, J.C., Yan, Y.T., Hsieh, W.K., Peng, P.J., Su, C.H., and Tarn, W.Y. (2013). RBM4 promotes pancreas cell differentiation and insulin expression. Mol. Cell. Biol. *33*, 319-327.

Lin, Q., Taylor, S.J., and Shalloway, D. (1997). Specificity and determinants of Sam68 RNA binding: implications for the biological function of K homology domains. J. Biol. Chem. *272*, 27274-27280.

Liu, N., Zhou, K.I., Parisien, M., Dai, Q., Diatchenko, L., and Pan, T. (2017). N6-methyladenosine alters RNA structure to regulate binding of a low-complexity protein. Nucleic Acids Res. *45*, 6051-6063.

Loomis, R.J., Naoe, Y., Parker, J.B., Savic, V., Bozovsky, M.R., Macfarlan, T., Manley, J.L., and Chakravarti, D. (2009). Chromatin binding of SRp20 and ASF/SF2 and dissociation from mitotic chromosomes is modulated by histone H3 serine 10 phosphorylation. Mol. Cell *33*, 450-461.

Mikoluk, C., Nagengast, A.A., and DiAngelo, J.R. (2018). The splicing factor transformer2 (tra2) functions in the Drosophila fat body to regulate lipid storage. Biochem. Biophys. Res. Commun. *495*, 1528-1533.

Morigny, P., Boucher, J., Arner, P., and Langin, D. (2021). Lipid and glucose metabolism in white adipocytes: pathways, dysfunction and therapeutics. Nat. Rev. Endocrinol. *17*, 276-295.

Pan, Q., Shai, O., Lee, L.J., Frey, B.J., and Blencowe, B.J. (2008). Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. Nat. Genet. *40*, 1413-1415.

Patel, R.S., Carter, G., Cooper, D.R., Apostolatos, H., and Patel, N.A. (2014). Transformer 2β homolog (Drosophila)(TRA2B) regulates protein kinase C δI (PKCδI) splice variant expression during 3T3L1 preadipocyte cell cycle. J. Biol. Chem. *289*, 31662-31672.

Pedrotti, S., Bielli, P., Paronetto, M.P., Ciccosanti, F., Fimia, G.M., Stamm, S., Manley, J.L., and Sette, C. (2010). The splicing regulator Sam68 binds to a novel exonic splicing silencer and functions in SMN2 alternative splicing in spinal muscular atrophy. EMBO J. *29*, 1235-1247.

Pelisch, F., Gerez, J., Druker, J., Schor, I.E., Muñoz, M.J., Risso, G., Petrillo, E., Westman, B.J., Lamond, A.I., Arzt, E., et al. (2010). The serine/arginine-rich protein SF2/ASF regulates protein sumoylation. Proc. Natl. Acad. Sci. U. S. A. *107*, 16119-16124.

Peng, H.Y., Liang, Y.C., Tan, T.H., Chuang, H.C., Lin, Y.J., and Lin, J.C. (2018). RBM4a-SRSF3-MAP4K4 splicing cascade constitutes a molecular mechanism for regulating brown adipogenesis. Int. J. Mol. Sci. *19*, 2646.

Pihlajamäki, J., Lerin, C., Itkonen, P., Boes, T., Floss, T., Schroeder, J., Dearie, F., Crunkhorn, S., Burak, F., Jimenez-Chillaron, J.C., et al. (2011). Expression of the splicing factor gene SFRS10 is reduced in human obesity and contributes to enhanced lipogenesis. Cell Metab. *14*, 208-218.

Pradeepa, M.M., Sutherland, H.G., Ule, J., Grimes, G.R., and Bickmore, W.A. (2012). Psip1/Ledgf p52 binds methylated histone H3K36 and splicing factors and contributes to the regulation of alternative splicing. PLoS Genet. *8*, e1002717.

Ray, D., Kazan, H., Chan, E.T., Castillo, L.P., Chaudhry, S., Talukder, S., Blencowe, B.J., Morris, Q., and Hughes, T.R. (2009). Rapid and systematic analysis of the RNA recognition specificities of RNA-binding proteins. Nat. Biotechnol. *27*, 667-670.

Sakers, A., De Siqueira, M.K., Seale, P., and Villanueva, C.J. (2022). Adipose-tissue plasticity in health and disease. Cell *185*, 419-446.

Shin, C., Feng, Y., and Manley, J.L. (2004). Dephosphorylated SRp38 acts as a splicing repressor in response to heat shock. Nature *427*, 553-558.

Song, J. and Richard, S. (2015). Sam68 regulates S6K1 alternative splicing during adipogenesis. Mol. Cell. Biol. *35*, 1926-1939.

Subramania, S., Gagné, L.M., Campagne, S., Fort, V., O'Sullivan, J., Mocaer, K., Feldmüller, M., Masson, J.Y., Allain, F.H.T., Hussein, S.M., et al. (2019). SAM68 interaction with U1A modulates U1 snRNP recruitment and regulates mTor pre-mRNA splicing. Nucleic Acids Res. *47*, 4181-4197.

Tacke, R., Tohyama, M., Ogawa, S., and Manley, J.L. (1998). Human Tra2 proteins are sequence-specific activators of pre-mRNA splicing. Cell *93*, 139-148.

Tavares, M.R., Pavan, I.C., Amaral, C.L., Meneguello, L., Luchessi, A.D., and Simabuco, F.M. (2015). The S6K protein family in health and disease. Life Sci. *131*, 1-10.

Trayhurn, P., Hoggard, N., Mercer, J.G., and Rayner, D.V. (1999). Leptin: fundamental aspects. Int. J. Obes. Relat. Metab. Disord. *23* Suppl *1*, 22-28.

Ule, J. and Blencowe, B.J. (2019). Alternative splicing regulatory networks: functions, mechanisms, and evolution. Mol. Cell *76*, 329-345.

Vernia, S., Edwards, Y.J., Han, M.S., Cavanagh-Kyros, J., Barrett, T., Kim, J.K., and Davis, R.J. (2016). An alternative splicing program promotes adipose tissue thermogenesis. Elife *5*, e17672.

Wang, Y., Chen, D., Qian, H., Tsai, Y.S., Shao, S., Liu, Q., Dominguez, D., and Wang, Z. (2014). The splicing factor RBM4 controls apoptosis, proliferation, and migration to suppress tumor progression. Cancer Cell *26*, 374-389.

Wei, N., Cheng, Y., Wang, Z., Liu, Y., Luo, C., Liu, L., Chen, L., Xie, Z., Lu, Y., and Feng, Y. (2015). SRSF10 plays a role in myoblast differentiation and glucose production via regulation of alternative splicing. Cell Rep. *13*, 1647-1657.

Wong, C.M., Xu, L., and Yau, M.Y.C. (2018). Alternative mRNA splicing in the pathogenesis of obesity. Int. J. Mol. Sci. *19*, 632.

Xiao, W., Adhikari, S., Dahal, U., Chen, Y.S., Hao, Y.J., Sun, B.F., Sun, H.Y., Li, A., Ping, X.L., Lai, W.Y., et al. (2016). Nuclear m6A reader YTHDC1 regulates mRNA splicing. Mol. Cell *61*, 507-519.

Zhou, X., Li, X., Cheng, Y., Wu, W., Xie, Z., Xi, Q., Han, J., Wu, G., Fang, J., and Feng, Y. (2014). BCLAF1 and its splicing regulator SRSF10 regulate the tumorigenic potential of colon cancer cells. Nat. Commun. *5*, 4581.