Submitted: 28 August, 2024 Accepted: 06 November, 2024 Published: 30 September, 2025 DOI:10.22514/j.androl.2025.035

ORIGINAL RESEARCH

REV INT ANDROL
Revista Internacional de Andrología

A global screen for testicular proteins and RNAs interacting with STRBP

Jingyan Yu[1,†], Minyan Yu[2,†], Yufei Chen[1], Chaoye Ma[1], Xiaoning Zhang[1,*], Yuan Gao[3,*], Pengyuan Dai[1,*]

[1]Institute of Reproductive Medicine, Medical School, Nantong University, 226019 Nantong, Jiangsu, China
[2]Center of Reproductive Medicine, The Affiliated Hospital of Nantong University, Nantong University, 226019 Nantong, Jiangsu, China
[3]Experimental Animal Center, Nantong University, 226001 Nantong, Jiangsu, China

***Correspondence**
pengyuandai@ntu.edu.cn
(Pengyuan Dai);
dayuanzi@ntu.edu.cn
(Yuan Gao);
zhangxn@ntu.edu.cn
(Xiaoning Zhang)

† These authors contributed equally.

Abstract

Background: Spermatid perinuclear RNA-binding protein (STRBP) facilitates RNAs transportation and translational activation during spermatid development. STRBP deficiency impairs male fertility and causes premature death of mouse offspring. However, the mechanisms underlying the regulation of spermiogenesis remain elusive. **Methods**: RNA immunoprecipitation sequencing and immunoprecipitation, followed by liquid chromatography-mass spectrometry, were used to explore how RNAs and proteins interact with STRBP. **Results**: These results suggest that STRBP is involved in spermiogenesis, mainly through precursor messenger RNA (mRNA) maturation by the spliceosome, RNAs transportation, translation-activating or translation-inactivating processes, and interaction with long noncoding RNAs. The transport action exerted by STRBP involves interactions with RNAs and proteins, including centromere protein E (*Cenpe*), dynein axonemal intermediate chain 3 (*Dnai3*), kinesin family member 23 (*Kif23*), kinesin family member 20b (*Kif20b*), dynein axonemal intermediate chain 1 (*Dnai1*), tubulin tyrosine ligase like 3 (*Ttll3*), DExH-Box helicase 9 (DHX9) and heterogeneous nuclear ribonucleoprotein A2/B1 (HNRNPA2B1). Transformer 2 beta homolog (TRA2B), HNRNPA2B1, serine and arginine rich splicing factor 2 (SRSF2), apoptotic chromatin condensation inducer 1 (ACIN1), sin3A associated protein 18 (SAP18), la ribonucleoprotein 7 (*Larp7*) and transcription elongation regulator 1 (*Tcerg1*), may mediate nuclear precursor mRNA maturation. Poly(RC) binding protein 2 (PCBP2), DHX9, synaptotagmin binding cytoplasmic RNA interacting protein (SYNCRIP), heterogeneous nuclear ribonucleoprotein D (HNRNPD) and N-acetyltransferase 10 (NAT10) are involved in the translation process, whereas UPF1 RNA helicase and ATPase (UPF1) and ribosomal protein L13a (RPL13A) probably impose a delayed action on mRNA translation. **Conclusions**: Our study not only provides preliminary evidence on STRBP-regulated processes involved in sperm physiology but also indicates potential candidates for investigating their roles in spermatogenesis.

Keywords

STRBP; Spermiogenesis; RNA transportation; Cytoplasmic translation; lncRNAs; Spermatogenesis

Búsqueda global de proteínas y ARN testiculares que interactúan con STRBP

Resumen

Antecedentes: La proteína de unión al ARN perinuclear de los espermatozoides (STRBP) facilita el transporte de ARN y la activación traslacional durante el desarrollo de los espermatozoides. La deficiencia de STRBP afecta a la fertilidad masculina y causa la muerte prematura de las crías de ratón. Sin embargo, los mecanismos subyacentes a la regulación de la espermiogénesis siguen siendo esquivos. **Métodos**: En este trabajo se utilizó la secuenciación por inmunoprecipitación de ARN y la inmunoprecipitación, seguidas de cromatografía líquida-espectrometría de masas, para explorar cómo interactúan los ARN y las proteínas con la STRBP. **Resultados**: Estos resultados sugieren que la STRBP interviene en la espermiogénesis, principalmente a través de la maduración del ARNm precursor por el espliceosoma, el transporte de ARNs, los procesos de activación o desactivación de la traducción y la interacción con ARN no codificantes largos. La acción de transporte ejercida por la STRBP implica interacciones con ARN y proteínas, entre ellas la proteína E del centrómero (*Cenpe*), la cadena intermedia axonemal 3 de la dineína (*Dnai3*), el miembro 23 de la familia de la kinesina (*Kif23*), el miembro 20b de la familia de la kinesina (*Kif20b*), la cadena intermedia axonemal 1 de la dineína (*Dnai1*), la tubulina tirosina ligasa como 3 (*Ttll3*), la helicasa 9 DExH-Box (DHX9) y la ribonucleoproteína nuclear heterogénea A2/B1 (HNRNPA2B1). El homólogo beta del transformador 2 (TRA2B), la HNRNPA2B1, el factor de empalme 2 rico en serina y arginina (SRSF2), el inductor 1 de la condensación apoptótica de la cromatina (ACIN1), la proteína 18 asociada a sin3A (SAP18), la ribonucleoproteína 7 (*Larp7*) y el regulador 1 de la elongación de la transcripción (*Tcerg1*), pueden mediar en la maduración del ARNm precursor nuclear. La proteína de unión a poli(RC) 2 (PCBP2), DHX9, la proteína de interacción con el ARN citoplasmático de unión a sinaptotagmina (SYNCRIP), la ribonucleoproteína nuclear heterogénea D (HNRNPD) y la N-acetiltransferasa 10 (NAT10) participan en el proceso de traducción, mientras que la ARN helicasa y ATPasa UPF1 (UPF1) y la proteína ribosómica L13a RPL13A probablemente imponen una acción retardada en la traducción del ARNm. **Conclusiones**: En conclusión, nuestro estudio no sólo aporta pruebas preliminares sobre los procesos regulados por STRBP implicados en la fisiología espermática, sino que también indica posibles candidatos para investigar sus funciones en la espermatogénesis.

Palabras Clave

STRBP; Espermiogénesis; Transporte de ARN; Traducción citoplasmática; lncARNs; Espermatogénesis

1. Introduction

RNA-binding proteins (RBPs) bind to RNAs at special sites and further alter the function or fate of the binding RNAs [1]. The originally transcribed RNA, known as precursor messenger RNA (pre-mRNA), can be affected by RBP to a large extent after binding to 3′-untranslated regions generally, and are involved in RNA splicing, storage, transportation and translation [2]. During spermatogenesis, *de novo* RNA transcription is silenced in leptotene/zygotene spermatocytes and elongating spermatids, and a large number of RNAs are synthesized, stored, and translated to be available at specific stages. RBPs are closely associated with these post-transcriptional events, ensuring the production of fertile spermatozoa [2].

Spermiogenesis is the terminal phase of spermatogenesis, which is a highly orchestrated developmental process that consists of the differentiation of newly formed spermatids into spermatozoa by the formation of acrosomes, flagellation, and head shaping, with three primary stages: round, elongating and elongated spermatids [3]. During spermiogenesis, most of the transcription terminates and many RNAs are stored in the cytoplasm as ribonucleoprotein (RNP) complexes until they are recruited for translation. The spermatid perinuclear RNA-binding protein (STRBP) is initially detected in step 9 spermatids (the transitional stage from round to elongating spermatids) [4]. Mice deficient in STRBP exhibit abnormalities in spermatogenesis and sperm physiological functions, as shown by thin seminiferous epithelium, decreased sperm motility, and defective transportation in oviducts [5]. Moreover, STRBP is highly expressed in the brain in various forms and exhibits neurological abnormalities upon mutation [5]. Additionally, protein transportation to the head and tail is essential for spermiogenesis. The manchette is a microtubular-based protein transportation platform surrounding the elongating head that is only detected in the spermatid elongation process [6]. STRBP maintains manchettes in their normal state, as indicated by spatial or temporal immunolocalization, which coincides with manchette formation [7]. Furthermore, direct binding of STRBP to manchette microtubules or indirect interaction through microtubule-associated protein (MAP) was observed, and the binding of STRBP to microtubules has also been observed *in vitro* [7]. Two STRBP motifs with binding activities have been well established. First, Schumacher *et al.* [4] showed STRBP binding to the 3′-untranslated region (3′-UTR) of Protamine 1 (*Prm1*) mRNA and that is associated with Prm1 transportation and translational activation in elongating spermatids. Second, STRBP also interacts with protein kinase R (PKR), which mediates translation initiation by phosphorylating eukaryotic initiation factor 2 alpha (eIF2α) in stress responses [8].

To date, the proteins or RNAs interacting with STRBP in the male reproductive system have not been systematically identified. We determined STRBP-binding RNAs and proteins using RNA immunoprecipitation sequencing (RIP-seq) and immunoprecipitation (IP) combined with liquid chromatography-mass spectrometry (LC-MS), respectively. The primary investigation will provide an insight into the mechanism of STRBP involvement in protein transportation and translation, and the candidate genes and proteins will be screened to establish

a foundation for uncovering the genetic mystery occurring during spermatogenesis.

2. Materials and methods

2.1 Testicular sample collection

Adult (56 days) wild-type C57BL/6J mice were prepared for testis collection. Briefly, mice were euthanized by cervical dislocation, the testes were isolated and cryopreserved at −80 ℃ for RIP-seq and LC-MS analyses. Six mixed samples (two samples from the (immunoprecipitation, IP) group, two samples from the immunoglobulin G (IgG)-IP group, and two samples from the input group) were collected for RIP-seq. Four mixed samples (two for the STRBP group, and two samples for the IgG group) were prepared for LC-MS analysis. Each mixed sample contained eight samples.

2.2 RIP-seq

The RIP assay was performed as previously described with some modifications [9]. Briefly, 500–600 mg testis tissue was homogenized in radioimmunoprecipitation assay (RIPA) lysis buffer (P0013B, Beyotime Biotechnology, Shanghai, China) containing 1× protease inhibitors (11206893001, Roche, Basel, Switzerland) and 100 U/mL RNase inhibitor (TAKARA.2313A, TaKaRa, Kusatsu, Japan). The suspension was incubated in an ice bath for 30 min and then centrifuged at 12,000 g, 4 °C, for 5 min to remove debris. Lysates were ultraviolet (UV)-irradiated at 254 nm (400 mJ/cm^2) for 1.5 min and allowed to lyse at 4 ℃ for 30 min. The lysates were fragmented by disruptive sonication (10 s ON and 15 s OFF) for 5 min. After the lysate was pre-cleared with protein A/G beads binding IgG at 4 °C for 30 min, it was incubated with STRBP (17362-1-AP, Proteintech, Wuhan, Hubei, China) or an IgG (30000-0-AP, Proteintech, Wuhan, Hubei, China) antibody at 4 °C overnight on an inverse rotator. The beads were subsequently washed five times in RIP buffer 150 mM potassium chloride (KCl), 0.5 mM dithiothreitol (DTT), 25 mM Tris pH 7.5, 5 mM ethylene diamine tetraacetic acid (EDTA), 0.5% NP-40, 1× protease inhibitors, and 100 U/mL RNase inhibitor). Finally, RNA was extracted using TRIzol (15596-026, Invitrogen, Carlsbad, CA, USA) and RNA sequencing was conducted by Novogene (Beijing, China).

2.3 RNA isolation, RIP-quantitative polymerase chain reaction (qPCR)

Total RNAs from the STRBP complexes were extracted using the TRIzol reagent (15596026CN, Thermo Fisher, Waltham, MA, USA) according to the manufacturer's instructions. Then RNA was reverse-transcribed to cDNA using PrimeScript™ RT reagent Kit with gDNA Eraser (RR092A, TaKaRa, Osaka, Japan). qPCR was performed by TB Green® Premix Ex Taq™ (TIi RNase H Plus) (RR420A, TaKaRa, Osaka, Japan) with an ABI StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA, USA), with primers specific for transcripts. The primers matching exons were randomly selected. The $2^{-\Delta\Delta Ct}$ quantification method combined with the β-actin level was employed to calculate the gene expression levels. The PCR assay was as follows: 98 ℃ for 2 min, followed by 40 cycles of 98 ℃ for 10 s, 60 ℃ for 20 s, and 72 ℃ for 20 s, with a final extension at 72 ℃ for 2 min. All primers were designed using the national center of biotechnology information (NCBI) for Biotechnology Information Primer-BLAST tool and sent to Genewiz Inc. (Suzhou, China) for synthesis. Primer sequences for the detected genes are listed in **Supplementary Table 1**. Selected genes including (*Hspb2*, *Olfr1281*, *mt-Atp8*, *mt-Nd3*, *Gm26594*, *Gm7976* and *Inc00700*) were quantified three times.

2.4 Protein identification by LC-MS

Testis proteins were isolated using RIPA buffer (Beyotime Biotechnology). After the lysate was pre-cleared with IgG and protein A/G beads, the supernatant was incubated with STRBP antibody-magnetic bead conjugate at 4 °C under rotary agitation for 4 h. Beads that combined the STRBP complexes were washed five times with lysis buffer to remove non-specific binding. Finally, the proteins were eluted and denatured using 2× sodium dodecyl sulfate (SDS) loading buffer. The total protein extracted from the STRBP complex was separated using SDS-polyacrylamide gel electrophoresis (PAGE). The stained protein bands were excised, sliced into 0.5–1 mm^3 pieces, and destained with 50% acetonitrile in 50 mM triethylammonium bicarbonate. The gel pieces were dehydrated by washing with 100% acetonitrile, followed by natural drying. Proteins were reduced with 10 mM DTT for 40 min at 56 °C and subsequently alkylated with 50 mM iodoacetamide for 30 min in the dark. The dehydrated proteins were digested with 10 ng/μL trypsin. The digested peptides were desalted using a C18 cartridge and dried using vacuum centrifugation. Shotgun proteomic analyses were performed using an EASY-nLCTM 1200 UHPLC system (Thermo Fisher, Waltham, MA, USA) coupled with an Orbitrap Q Exactive HF-X mass spectrometer (Thermo Fisher, Waltham, MA, USA) operating in data-dependent acquisition mode. The identified peptides were aligned to STRBP using BLAST and analyzed with the Uniprot database using the Proteome Discoverer 2.2 software (PD 2.2, ThermoFisher, Waltham, MA, USA).

2.5 Bioinformatics analysis

The raw data produced by the Illumina HiSeq™ 4000 platform (Illumina, San Diego, CA, USA) with a pair-end 150 bp strategy was subjected to quality control tests, including removing the adaptors and empty reads before filtering the low-quality reads. BWA (Burrows-Wheeler Aligner) was used to align the reads to reference sequences [10]. Peak calling analysis was performed using MACS2 software ($p < 0.05$) [11]. To elucidate the sequence-binding preferences of STRBP, motif enrichment was performed after peak calling. MEME and Dreme [12] were used to detect the consensus sequences (motifs) within the peak sequences of all samples. Subsequently, a position-specific probability matrix (PSPM) was assigned to each motif. Tomtom [13] was used to compare the query motif sequences with a database of known motifs. mRNAs and long noncoding RNAs (lncRNAs) were analyzed and identified as previously described [14]. After aligning the reads to reference sequences using TopHat2 [15], the transcripts

were quantified using HTSeq [16]. Gene expression levels were calculated using the FPKM method. Gene Ontology (GO; http://www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) pathway analyses were performed to determine the functional roles of STRBP interaction genes and proteins using DAVID tools (https://david.ncifcrf.gov/home.jsp). The heatmap and protein-protein interaction network were analyzed using Rstudio version 4.2.3 and the STRING database at http://string-db.org respectively. NPInter v5.0 [17] was used for lncRNA target prediction at http://bigdata.ibp.ac.cn/npinter5/.

2.6 Statistical analysis

Differential mRNAs and lncRNAs were screened when $p < 0.05$ (Fig. 1A). Results were expressed as the mean ± standard error of mean (SEM) (Fig. 1B). GO terms and KEGG pathways with corrected $p < 0.05$ were considered significantly enriched. All statistical analyses were performed using SPSS 19.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1 Genes interacting with STRBP

Total RNA isolated from RIP complexes (Fig. 1A) was used to identify STRBP-interacting RNAs by RNA-sequence (RNA-seq). After the raw data were trimmed and filtered, peak calling was performed using MACS2. Numerous peaks were observed for the STRBP-IP group (Fig. 1B). Among the upregulated peaks, 359 overlapped with specific genes. Interestingly, most peaks were located in the intronic region. The higher ratio of STRBP interacting with the introns of pre-RNAs is likely due to its involvement in pre-RNA splicing. A total of 155 protein-coding RNAs and 60 long non-coding RNAs (lncRNAs) were significantly enriched in the STRBP group (Fig. 1C, **Supplementary Table 2**). Seven transcripts were selected randomly to confirm our results using RIP, including *Hspb2*, *Olfr1281*, *mt-Atp8*, *mt-Nd3*, *Gm26594*, *Gm7976* and *Inc00700*, followed by qPCR. These genes were found to be significantly enriched in the STRBP database (Fig. 1D).

3.2 The characterization of mRNAs interacting with STRBP by GO and KEGG annotations

GO analysis based on RNA-seq data is used to reduce genome complexity and enhance the biological processes, molecular functions, and cellular components through gene function annotation [18]. Many of the interacting RNAs encode proteins involved in cilium assembly, microtubule-based movement, spermatid development, regulation of protein ubiquitination, axoneme assembly, RNA processing, and intracellular transport (Fig. 2A). KEGG mapping is applied to reveal the high-level functions and reconstruct the biological system using the cellular or organism genome sequences [19]. In the current study, we found that motor protein and nucleocytoplasmic transport signal cascades were mediated by protein-coding RNAs attached to STRBP (Fig. 2B). Dynein 2 intermediate chain 2 (*Dync2i2*), *Dnai3, Ttll3*, intraflagellar transport 140 (*Ift140*), cilia and flagella associated protein 53 (*Cfap53*) and *Dnai1* mediated cilium assembly, cilium organization, and microtubule-based process. Kinesin-like genes including *Cenpe, Kif20b* and *Kif23* present in microtubule-based movement. *Dnai3*, *Ttll3* and *Dnai1* also perform indispensable functions in cilium movement, axoneme assembly, and microtubule bundle formation. TATA element modulatory factor 1 (*Tmf1*), *Ttll3*, cilia and flagella associated protein 58 (*Cfap58*), *Cfap53* and *Dnai1* were found to functionally mediate flagellated sperm motility. *Tmf1*, adhesion regulating molecule 1 26S proteasome ubiquitin receptor (*Adrm1*), H3.3 histone A (*H3f3a*), *Cfap58*, meiosis specific with coiled-coil domain (*Meioc*), calcium and integrin binding 1 (*Cib1*) and eukaryotic translation initiation factor 4 gamma 3 (*Eif4g3*) were annotated during spermatid development. Siah E3 ubiquitin protein ligase 2 (*Siah2*), SUMO/sentrin specific peptidase 2-like 1 (*Semp2l1*), ubiquitin protein ligase E3A (*Ube3a*), SUMO/sentrin specific peptidase 2-like 2A (*Semp2l2a*), autophagy related 7 (*Atg7*), and GTP binding protein 4 (*Gtpbp4*) have been implicated in the regulation of protein ubiquitination. Cytidylate tRNA unprecedented 2 (*Ctu2*), polyribonucleotide nuclease Nedd4 (*Pnn*), *Tcerg1*, DEAD box helicase 49 (*Ddx49*), ribosomal protein S8 (*Rps8*), zinc finger CCCH type containing 3 (*Zc3h3*), *Larp7*, serine/threonine kinase receptor associated protein (*Strap*), nucleolar and occasional coiled body associated protein 10 (*Nol10*), CBF1 interacting corepressor 1 (*Cir1*) and GTP binding protein 4 (*Gtpbp4*) are shown in RNA processing (Fig. 2C). A total of 60 lncRNAs were identified in the STRBP complex, of which 24 lncRNAs had an established gene name while the others were novel lncRNA transcripts (**Supplementary Table 3**). A total of 58 potential targets were identified from the 19 lncRNAs (**Supplementary Table 4**). Five targets, CUGBP, elav-like family member 1 (*Celf1*), YTH domain containing 2 (YTHDC2), deleted in azoospermia-like (*Dazl*), lin-28 homolog B (LIN28B) and ELAV like RNA binding protein 1 (*Elavl1*) were annotated in spermatogenesis or germ cells, as shown in **Supplementary Table 4**.

3.3 The profiling analysis of proteins interacting with STRBP

We identified 80 proteins in the STRBP complex (**Supplementary Table 5**). Multiple biological events are driven by proteins attached to STRBP (Fig. 3A). The annotated metabolic pathways extracted from the KEGG database comprised the ribosome and spliceosome signaling pathways (Fig. 3B). Thyroid hormone receptor associated protein 3 (THRAP3), U2 small nuclear ribonucleoprotein auxiliary factor 2 (U2AF2), TRA2B, HNRNPA2B1, SRSF2, ACIN1, and SAP18 regulate RNA splicing via spliceosomes. DHX9, SYNCRIP, HNRNPD, PCBP2, *etc.* are involved in cytoplasmic translation. NAT10, WD repeat domain 3 (WDR3), NOP56 ribonucleoprotein (NOP56), *etc.* were enriched in ribosomal small subunit biogenesis. Splicing factor proline and glutamine rich (SFPQ), small RNA binding exonuclease protection factor La (SSB), THRAP3, DHX9 and HNRNPA2B1 were associated with nucleic acid transport. The negative regulation of translation was mediated by

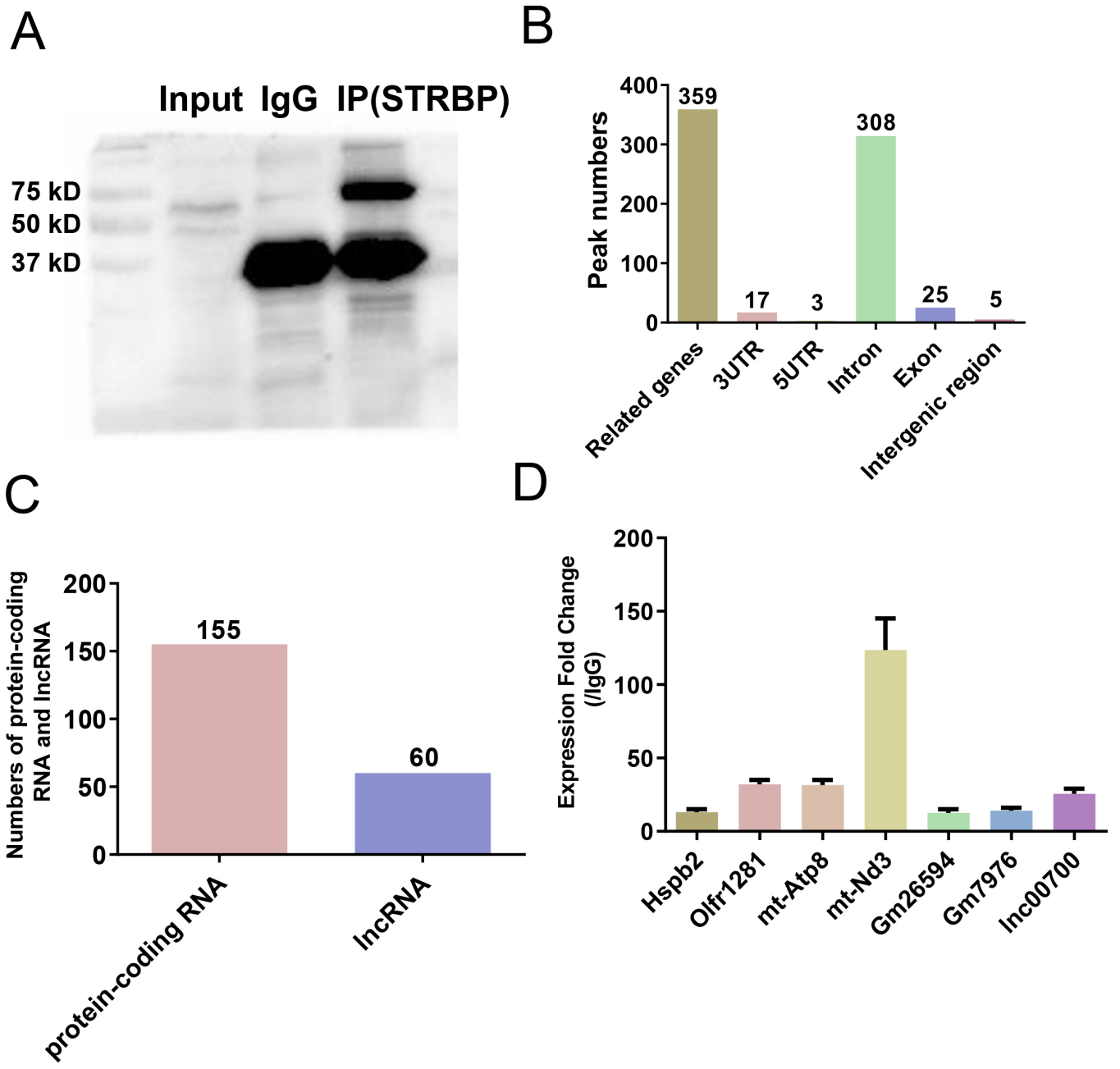


FIGURE 1. The identification and characterization of RNAs and proteins expression in the spermatid perinuclear RNA-binding protein (STRBP) complex. (A) RNA immune-precipitation (RIP) of STRBP. The STRBP-RNA complexes were isolated by RIP with STRBP antibody. IgG was used as a negative control. (B) Peak-related genes. Peak-related genes were counted on the genome and the location of the enriched peaks in genes was analyzed. (C) Numbers of the STRBP-interacting protein-coding RNAs and long noncoding RNAs (lncRNAs). (D) Validation of sequencing data using quantitative PCR (qPCR). Total RNA was isolated from STRBP complexes, and then qPCR was performed to detect the RNA expression levels. The *β-actin* gene was used as a reference to normalize RNA expression levels of desired genes. IP: immunoprecipitation; UTR: untranslated region; IgG: immunoglobulin G.

SYNCRIP, UPF1, interleukin enhancer binding factor 3 (ILF3), DHX9, HNRNPA2B1, HNRNPD, RPL13A and heterogeneous nuclear ribonucleoprotein R (HNRNPR). Ribosomal protein L5 (RPL5), RPS14 and RPS19 are involved in multiple processes, including RNP complex biogenesis and assembly, ribosome assembly, ribosomal RNA (rRNA) metabolic processes, and rRNA processing. SYNCRIP, DHX9, HNRNPD, HNRNPR, heterogeneous nuclear ribonucleoprotein C (HNRNPC), *etc.* are involved in the regulation of mRNA stability (Fig. 3C). As shown in Fig. 3D, an interaction network was formed by the detected enrichment of KEGG annotations, with all protein links present interactively and visually emphasized in the network.

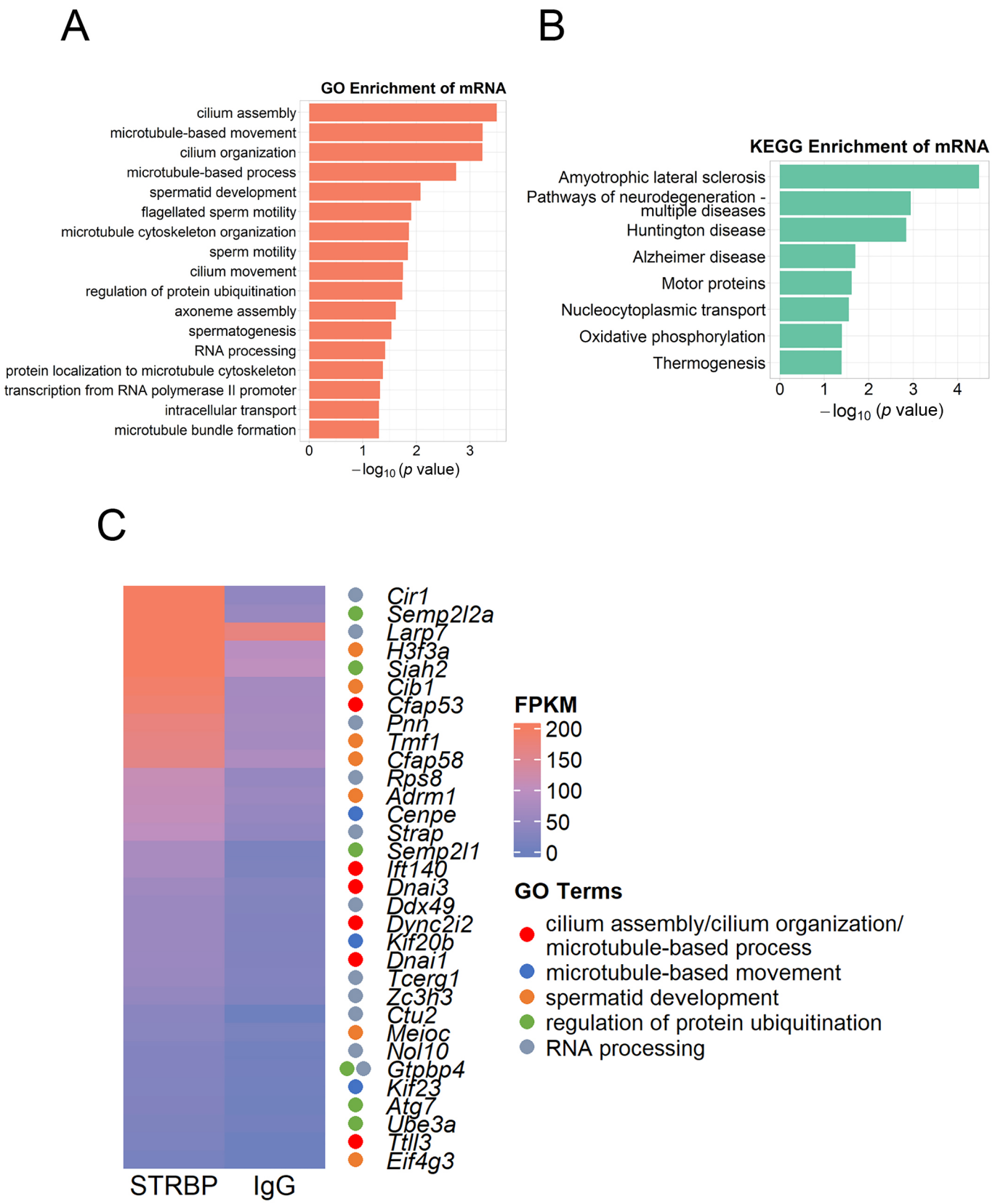
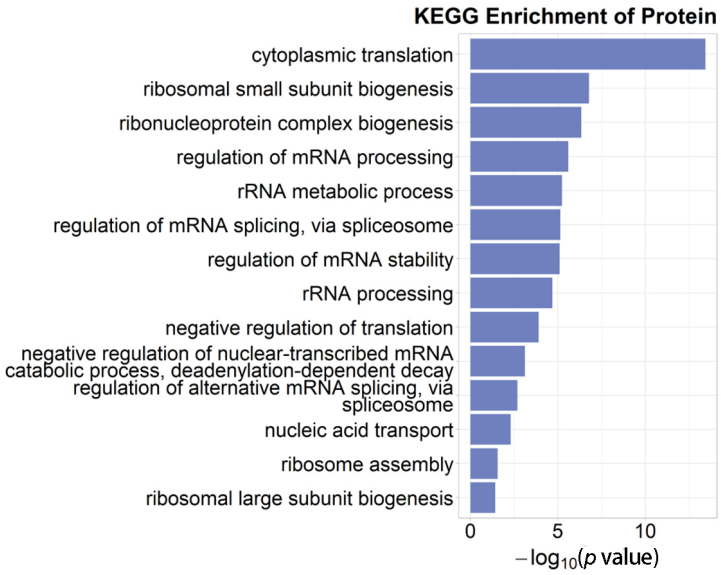


FIGURE 2. mRNAs interacting with STRBP by GO and KEGG annotations. (A,B) show GO and KEGG terms for protein-coding RNAs, respectively. (C) The levels of prime GO terms are shown by the heatmap. "FPKM" is defined as Fragments per kilo base of transcript per million mapped fragments. mRNA: messenger RNA; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

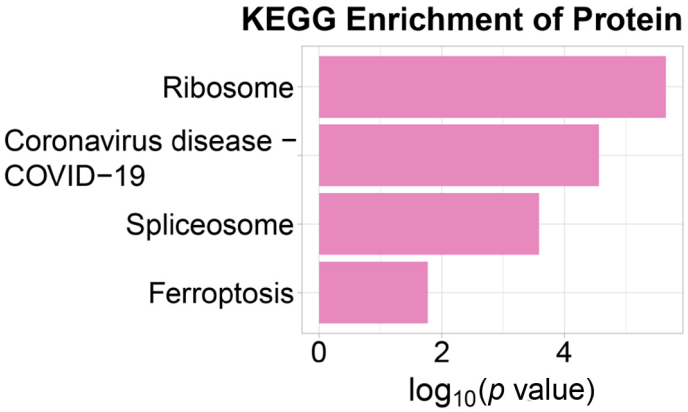
A



C

GO term	Proteins
Regulation of mRNA splicing, via spliceosome	THRAP3, U2AF2, TRA2B, HNRNPA2B1, SRSF2, ACIN1, SAP18
Ribosome assembly	RPL5, RPS14, RPS19
Ribosomal large subunit biogenesis	RSL1D1, RPL5, RPL26
Cytoplasmic translation	RPL5, SSB, RPL21, DHX9, RPL13A, RPS14, SYNCRIP, RPS17, RPS15A, RPS19, PCBP2, HNRNPD, RPL26, RPS13
Ribosomal small subunit biogenesis	NOP56, RPS14, RPS17, RPS15A, RPS19, WDR3, NAT10, RPS13
Regulation of mRNA stability	SYNCRIP, UPF1, THRAP3, DHX9, HNRNPD, HNRNPR, HNRNPC
Nucleic acid transport	SFPQ, SSB, THRAP3, DHX9, HNRNPA2B1
Negative regulation of translation	SYNCRIP, UPF1, ILF3, DHX9, HNRNPA2B1, HNRNPD, RPL13A, HNRNPR

B



D

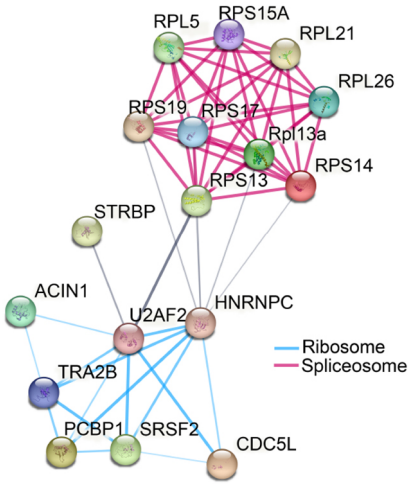


FIGURE 3. Proteins interacting with STRBP. (A,B), The distribution of annotated GO and KEGG terms of proteins. (C) Proteins in specific GO terms. (D) The potential ribosome and spliceosome signaling pathways were linked with STRBP. Proteins with KEGG annotations were shown as a sphere. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

4. Discussion

The manchette forms in step 8 spermatids, extends to the cauda around the developing flagellum, and disappears in elongated spermatids before being released into the lumen. The mechanical forces generated by the manchette are necessary for nuclear shaping and for promoting the movement of cellular components with cytoplasmic extension. The kinesin and dynein moving unidirectionally along microtubules are both localized in the manchette, as are the intermediate filament proteins SAK57 and MAP Tau [7]. RBP can attach to either dynein or kinesin after forming a special complex with STAUFEN, an mRNA transportation protein [20], and the study indicated that STRBP is expressed in the manchette and is tightly linked to manchette microtubule concentration [7]. Our data showed that the kinesin-like genes *Cenpe*, *Kif20b* and *Kif23* and dynein functionally dependent genes, including *Ttll3*, *Dnai3* and *Dnai1*, interacted with STRBP. The interaction of STRBP with kinesin- and dynein-related genes provides new insights into the processes of manchette formation and disassembly, which further regulate proteins and RNAs transport. TTLL3 is a tubulin glycine ligase mediating

flagellar beat activity [21]. Impaired dynein motors in the axonemum have been observed following *Dnai1* and *Dnai3* mutations [22]. With evidence that the extended manchette was observed in the cauda of developing flagella in spermatids [7] and that mice show abnormal sperm morphology and motility abnormalities after STRBP deletion [5], we assume that STRBP is necessary for the maintenance of flagellar function. Furthermore, IFT140 is necessary for cilia and flagella assembly in most mammals [23]. *Cfap58* loss could result in disorders of the mitochondrial sheath and flagellar axoneme [24]. The interactions of STRBP with *Ift140* and *Cfap58* identified in this study provides new insights into spermiogenesis. RNA metabolism under stress showed a vital function in the pathogenesis of some neurodegenerative disorders, such as Alzheimer's disease and amyotrophic lateral sclerosis, and RBP regulated mRNA employment under stress by regulating the formation and post-translational modifications of stress granules (SGs, membrane-less organelles) to some degree [25]. The study also indicated that STRBP deficiency can cause brain anatomical abnormalities and aberrant clutching reflex in mice [5]. Besides, multiple common proteins were found in the testis and brain, sharing biochemical characteristics reflected by similar signal pathways, receptors, as well as exocytosis process [26]. Our data present the KEGG mapping of pathways related to neurodegeneration associated with STRBP-binding RNAs, suggesting a potential pathological role of STRBP in neurodegenerative diseases, and identifying STRBP as a candidate regulator for further investigation.

Spliceosomes are well-established mRNA-RNP complexes (mRNP) in the nucleus, the function of which is to ligate the exons together after cutting out the intron in pre-mRNAs to produce functionally mature transcripts [27]. During alternative splicing, various mRNA isoforms are produced through the removal of distinct introns. RBPs, including STRBP bound to pre-mRNA, are also known as heterogeneous nuclear RNP (hnRNPs). These exert a stimulating or inhibitory action on the splicing of immature RNA, eventually forming an alternative splice [2]. RNA processing and mRNA splicing via spliceosomes were enriched in GO annotations. TRA2B, which is expressed in round and elongating spermatid, is involved in pre-RNA splicing events via direct RNA binding [28]. HNRNPA2B1 is mainly found in round spermatid and contributes to multiple nuclear RNA processes including splicing, transportation, and translation [29]. SRSF2, localized in round spermatid maintain RNA splicing under normal conditions [30]. ACIN1 and SAP18 can be fused with NUT midline carcinoma family member 1 (NUTM1), which is exclusively expressed in the mid-spermatid stage in the testis [31]; this interaction may be involved in regulating transcription and RNA splicing [32]. *Tcerg1* is expressed strongly in spermatocytes and moderately in spermatids [33]. *Larp7* is detected in the nucleus of round spermatid and maintains the stability of the alternative splicing process [34]. STRBP interacts with proteins or genes that are RNA splice regulators, suggesting an indispensable role in pre-RNA processing.

Some mRNPs are removed from mRNA before nuclear export, while others promote the export of related transcripts, primarily shuttling between the nucleus and cytoplasm through the nuclear pores [35]. Quality control steps are implemented prior to mRNA export, including mRNP complex packaging and mRNP surveillance signals that have been implicated in the degradation of aberrantly assembled or incorrectly processed mRNPs [35]. mRNA surveillance was among the KEGG database annotations, albeit this result exhibited no significant difference (data not shown). Furthermore, SIAH2 functionally upregulates the overall proteolytic activity, and was found in the STRBP complex. A previous study has indicated that SIAH2 [36], which is highly expressed in spermatid, triggers the degradation of proteins and contribute to multiple cellular physiological processes. One fate of the processed transcripts after being exported to the cytoplasm in conjunction with RBP, is immediately translation by ribosomes [2]. DHX9, HNRNPD, PCBP2 and SYNCRIP, which regulate cytoplasmic translation and ribosome-related protein NAT10, were enriched in this study (Fig. 3B). DHX9 signals are found in the cytoplasm and nucleus of pachytene spermatocytes and early round spermatid [37]; they are involved in diverse cellular processes including DNA replication and transcription, RNA stability, and translation [38]. HNRNPD appears initially in pachytene spermatocytes and is highly accumulated in round spermatid, functioning in RNA stability and decay, transportation, and translation [39]. SYNCRIP mainly mediates RNA processing and local protein synthesis [40]. PCBP2 is localized in round spermatid and regulates RNA translation [41]. NAT10 is localized in the zygotene and round spermatid stage, enhancing RNA stability and transcriptional efficiency [42]. Another fate for mRNAs is delayed translation by directing them to chromatoid bodies (CBs), which are perinuclear structures that predominantly form in round spermatids, storing transcripts and initiating translation according to demand [43]. Coincidentally, proteins related to the negative regulation of translation were found to interact with STRBP, including UPF1 and RPL13A. UPF1 is highly expressed in CBs [44]. The translational initiation complex is repressed by UPF1 phosphorylation after interacting with eIF3 [45]. The complex of RPL13A combined with glutamyl-prolyl tRNA synthetase (EPRS) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) can be bridged to eIF4G, which is bound to the 5′-UTR cap to inhibit translation [46]. This study showed that STRBP promotes translation initiation by phosphorylating eIF2α [8]. Therefore, we hypothesized that eIFs are more likely to be involved in STRBP-inhibited translation. Intracellular RNA content depends on the rate of transcription and mRNA decay. RBP binding is crucial either for maintaining RNA stability or promoting their unstable state [2]. Here, the annotated terms for the regulation of mRNA stability and deadenylation-dependent mRNA decay may reveal the dual roles of STRBP in the mRNA metabolic process of developing spermatids. Overall, our data highlighted the significance of STRBP in regulating mRNA translation during spermiogenesis. Moreover, RPL5, RPS14, RPS17, RPS15A, RPS19, RPL21, RPL13A, RPL26 and RPS13 interacting with STRBP were annotated in Coronavirus disease—COVID 19 by KEGG analysis. Ribosomal proteins (RPs), including ribosomal protein small unit (RPS) and ribosomal protein large unit (RPL), along with rRNA, are involved in translation. Protein biosynthesis, replication and infection of SARS-COV2 are initiated by the actions of

multiple RPs interacting with viral mRNA and proteins, and is vital for SARS-COV2 accumulation and activation [47].

In our study, the RIP-seq and LC/MS assays were conducted using the whole testis lysate, and RNA and protein profiles identified in this study indicate a global screen of testicular proteins and RNAs interacting with STRBP, rather than any specificity. A variety of molecules contribute to testicular development and spermatogenesis, and the current data strengthen the genetic regulatory systems regulated by STRBP during sperm production, primarily including RNAs transportation, pre-mRNA maturation by splicing, positive and negative regulation of translation, and lncRNAs interacting with STRBP. The genes and proteins involved are summarized in Fig. 4. Further localization and quantitative analysis in testis must be performed on the screened factors interacting with STRBP, some of which require special focus due to their limited description in male fertility, including HNRNPD, PCBP2, KIF20B, SYNCRIP, UPF1, DHX9, SAP18 and ACIN1. The highest signal of HNRNPD appears in the nuclei of Sertoli cells, and germ cells [39]. PCBP2 (also known as HNRNPE2) is found to increase substantially in P21 testis, and displays a declined level in P56 testis, suggesting its indispensable role during the first wave of spermatogenesis [39]. A functional study concentrating on the cellular signaling of HNRNPD and PCBP2 during spermatogenesis is warranted. A loss of function of KIF20B causes a development defect in the brain and reduced neurogenesis [48]; however, the phenotypes of testis from mutant mice have not been carefully investigated. In neuronal dendrites, SYNCRIP was found in mRNA granule. It is transported dependent upon microtubules and plays a part in mRNA stabilization and protein synthesis [49]. The characterization of SYNCRIP in testicular development is worthy of further investigation. It has been well-established that the nonsense-mediated mRNA decay regulated by UPF2 regulator of nonsense mediated mRNA decay (UPF2) is indispensable for spermatogenesis [50]. Considering that translation inhibition can be initiated by UPF1 [51], the negative influence on testicular protein synthesis exerted by UPF1 needs to be

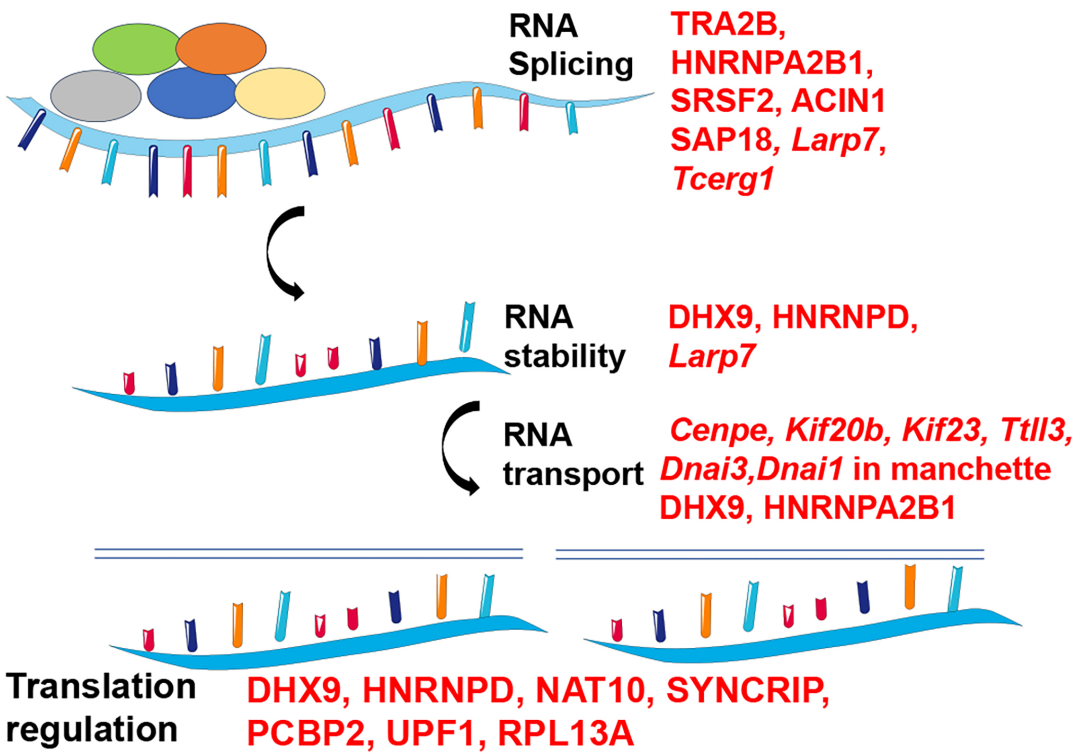


FIGURE 4. Schematic diagram showing the potential STRBP-interacting genes and proteins in multiple RNA metabolic processes. TRA2B, HNRNPA2B1, SRSF2, ACIN1, SAP18, *Larp7* and *Tcerg1* are likely involved in the RNA splicing mechanism. The RNA stability may be depending on the interaction of STRBP with DHX9, HNRNPD and *Larp7*. The enriched factors including *Cenpe*, *Kif20b*, *Kif23*, *Ttll3*, *Dnai3*, *Dnai1*, DHX9 and HNRNPA2B1 provided new insight into STRBP-mediated RNA transport. UPF1, RPL13A, DHX9, HNRNPD, NAT10, SYNCRIP and PCBP2 were assumed to regulate the translation inactivation process in the STRBP complex. TRA2B: transformer 2 beta homolog; HNRNPA2B1: heterogeneous nuclear ribonucleoprotein A2/B1; SRSF2: serine and arginine rich splicing factor 2; ACIN1: apoptotic chromatin condensation inducer 1; SAP18: sin3A associated protein 18 (SAP18); *Larp7*: la ribonucleoprotein 7; *Tcerg1*: transcription elongation regulator 1; DHX9: DExH-Box helicase 9; HNRNPD: heterogeneous nuclear ribonucleoprotein D; *Cenpe*: centromere protein E; *Kif20b*: kinesin family member 20b; *Kif23*: kinesin family member 23; *Ttll3*: tubulin tyrosine ligase like 3; *Dnai3*: dynein axonemal intermediate chain 3; *Dnai1*: dynein axonemal intermediate chain 1; UPF1: UPF1 RNA helicase and ATPase; RPL13A: ribosomal protein L13a; NAT10: N-acetyltransferase 10; SYNCRIP: synaptotagmin binding cytoplasmic RNA interacting protein; PCBP2: Poly(RC) binding protein 2.

carefully defined. The testis specialized protein transcription factor-like 5 (TCFL5) interacts with DHX9, serving a dual function by binding to both DNA and RNA, in which is essential for sustaining male fertility [37]. The actions of DHX9 need to be verified in future research.

Although this study provides comprehensive profiles of STRBP-interacting RNAs and proteins through RIP-seq and LC-MS, a primary limitation is the lack of experimental validation for the identified candidates. The interactions inferred from mass spectrometry and bioinformatic analyses remain putative without confirmation by complementary methods such as co-immunoprecipitation, western blotting, or in situ hybrization. Thus, the biological roles of these potential factors in spermiogenesis and their mechanisms alongside STRBP require further investigation.

5. Conclusions

In current assay, molecules interacting with STRBP involving in RNA splicing, stability, transport and translation process were screened, making a contribution to uncover the regulating mechanism during testicular development and spermatogenesis.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

PYD, YG and XNZ—designed the study. JYY and MYY—performed the research. YFC, CYM and XNZ—analyzed the data. PYD—wrote the manuscript. XNZ—provided financial support. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All animal protocols and procedures were approved by the ethics committee of Nantong University (S20210224-039).

ACKNOWLEDGMENT

Not applicable.

FUNDING

This work was supported by grants from the Basic Science Research Program of Nantong (No. JC22022086 to XNZ).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at https://files.intandro.com/files/article/1972835322084376576/attachment/Supplementary%20material.xlsx.

REFERENCES

[1] Kelaini S, Chan C, Cornelius VA, Margariti A. RNA-binding proteins hold key roles in function, dysfunction, and disease. Biology. 2021; 10: 366.

[2] Idler RK, Yan W. Control of messenger RNA fate by RNA-binding proteins: an emphasis on mammalian spermatogenesis. Journal of Andrology. 2012; 33: 309–337.

[3] Miyata H, Shimada K, Kaneda Y, Ikawa M. Development of functional spermatozoa in mammalian spermiogenesis. Development. 2024; 151: dev202838.

[4] Schumacher JM, Lee K, Edelhoff S, Braun RE. Spnr, a murine RNA-binding protein that is localized to cytoplasmic microtubules. The Journal of Cell Biology. 1995; 129: 1023–1032.

[5] Pires-daSilva A, Nayernia K, Engel W, Torres M, Stoykova A, Chowdhury K, *et al*. Mice deficient for spermatid perinuclear RNA-binding protein show neurologic, spermatogenic, and sperm morphological abnormalities. Developmental Biology. 2001; 233: 319–328.

[6] Lehti MS, Sironen A. Formation and function of the manchette and flagellum during spermatogenesis. Reproduction. 2016; 151: R43–R54.

[7] Schumacher JM, Artzt K, Braun RE. Spermatid perinuclear ribonucleic acid-binding protein binds microtubules in vitro and associates with abnormal manchettes *in vivo* in mice. Biology of Reproduction.1998; 59: 69–76.

[8] Coolidge CJ. A new double-stranded RNA-binding protein that interacts with PKR. Nucleic Acids Research. 2000; 28: 1407–1417.

[9] Elías-Villalobos A, Duncan C, Mata J, Helmlinger D. Quantitative analysis of protein-RNA interactions in fission yeast. STAR Protocols. 2022; 3: 101373.

[10] Oberc C, Li PCH. Next-generation DNA sequencing of Panax samples revealed new genotypes: burrows-wheeler aligner, python-based abundance and clustering analysis. Heliyon. 2024; 10: e29104.

[11] Hentges LD, Sergeant MJ, Cole CB, Downes DJ, Hughes JR, Taylor S. LanceOtron: a deep learning peak caller for genome sequencing experiments. Bioinformatics. 2022; 38: 4255–4263.

[12] Nystrom SL, McKay DJ. Memes: A motif analysis environment in R using tools from the MEME Suite. PLOS Computational Biology. 2021; 17: e1008991.

[13] Gupta S, Stamatoyannopoulos JA, Bailey TL, Noble WS. Quantifying similarity between motifs. Genome biology. 2007; 8: R24.

[14] Ye M, Xie L, Zhang J, Liu B, Liu X, He J, *et al*. Determination of long non-coding RNAs associated with EZH2 in neuroblastoma by RIP-seq, RNA-seq and ChIP-seq. Oncology Letters. 2020; 20: 1.

[15] Liu Y, Shen X, Gong Y, Liu Y, Song B, Zeng X. Sequence alignment/map format: a comprehensive review of approaches and applications. Briefings in Bioinformatics. 2023; 24: bbad320.

[16] Jiang G, Zheng JY, Ren SN, Yin W, Xia X, Li Y, *et al*. A comprehensive workflow for optimizing RNA-seq data analysis. BMC Genomics. 2024; 25: 631.

[17] Zheng Y, Luo H, Teng X, Hao X, Yan X, Tang Y, *et al*. NPInter v5.0: ncRNA interaction database in a new era. Nucleic Acids Research. 2023; 51: D232–D239.

[18] Wang S, You R, Liu Y, Xiong Y, Zhu S. NetGO 3.0: protein language model improves large-scale functional annotations. Genomics, Proteomics & Bioinformatics. 2023; 21: 349–358.

[19] Kanehisa M, Furumichi M, Sato Y, Kawashima M, Ishiguro-Watanabe M. KEGG for taxonomy-based analysis of pathways and genomes. Nucleic Acids Research. 2023; 51: D587–D592.

[20] Jansen RP. RNA-cytoskeletal associations. The FASEB Journal. 1999; 13: 455–466.

[21] Gadadhar S, Viar GA, Hansen JN, Gong A, Kostarev A, Ialy-Radio C, *et*

al. Tubulin glycylation controls axonemal dynein activity, flagellar beat, and male fertility. Science. 2021; 371: eabd4914.

[22] Wu H, Liu Y, Li Y, Li K, Xu C, Gao Y, *et al*. DNALI1 deficiency causes male infertility with severe asthenozoospermia in humans and mice by disrupting the assembly of the flagellar inner dynein arms and fibrous sheath. Cell Death & Disease. 2023; 14: 127.

[23] Man YH, Warmbrunn I, Zhang L, Zhang ZB. The roles of intraflagellar transport (IFT) protein 25 in mammalian signaling transduction and flagellogenesis. Asian Journal of Andrology. 2022; 24: 238–242.

[24] Sha Y, Sha Y, Liu W, Zhu X, Weng M, Zhang X, *et al*. Biallelic mutations of CFAP58 are associated with multiple morphological abnormalities of the sperm flagella. Clinical Genetics. 2021; 99: 443–448.

[25] Wolozin B, Ivanov P. Stress granules and neurodegeneration. Nature Reviews Neuroscience. 2019; 20: 649–666.

[26] Matos B, Publicover SJ, Castro LFC, Esteves PJ, Fardilha M. Brain and testis: more alike than previously thought? Open Biology. 2021; 11: 200322.

[27] Black CS, Whelan TA, Garside EL, MacMillan AM, Fast NM, Rader SD. Spliceosome assembly and regulation: insights from analysis of highly reduced spliceosomes. RNA. 2023; 29: 531–550.

[28] Grellscheid S, Dalgliesh C, Storbeck M, Best A, Liu Y, Jakubik M, *et al*. Identification of evolutionarily conserved exons as regulated targets for the splicing activator tra2β in development. PLOS genetics. 2011; 7: e1002390.

[29] Peng WZ, Zhao J, Liu X, Li CF, Si S, Ma R. hnRNPA2B1 regulates the alternative splicing of BIRC5 to promote gastric cancer progression. Cancer Cell International. 2021; 21: 281.

[30] Lei WL, Du Z, Meng TG, Su R, Li YY, Liu W, *et al*. SRSF2 is required for mRNA splicing during spermatogenesis. BMC Biology. 2023; 21: 231.

[31] Charlab R, Racz R. The expanding universe of fusions in pediatric cancer. Clinical and Translational Science. 2023; 16: 1331–1339.

[32] Murachelli AG, Ebert J, Basquin C, Le Hir H, Conti E. The structure of the ASAP core complex reveals the existence of a Pinin-containing PSAP complex. Nature Structural & Molecular Biology. 2012; 19: 378–386.

[33] Sinha N, Whelan EC, Tobias JW, Avarbock M, Stefanovski D, Brinster RL. Roles of Stra8 and Tcerg1l in retinoic acid induced spermatogonial differentiation in mouse. Biology of Reproduction. 2021; 105: 503–518.

[34] Wang X, Li ZT, Yan Y, Lin PH, Tang W, Hasler D, *et al*. LARP7-Mediated U6 snRNA modification ensures splicing fidelity and spermatogenesis in mice. Molecular Cell. 2020; 77: 999–1013.e6.

[35] De Magistris P. The great escape: mRNA export through the nuclear pore complex. International Journal of Molecular Sciences. 2021; 22: 11767.

[36] Dickins RA, Frew IJ, House CM, O'Bryan MK, Holloway AJ, Haviv I, *et al*. The ubiquitin ligase component Siah1a is required for completion of meiosis I in male mice. Molecular and Cellular Biology. 2002; 22: 2294–2303.

[37] Xu W, Zhang Y, Qin D, Gui Y, Wang S, Du G, *et al*. Transcription factor-like 5 is a potential DNA-and RNA-binding protein essential for maintaining male fertility in mice. Journal of Cell Science. 2022; 135: jcs259036.

[38] Lee T, Pelletier J. The biology of DHX9 and its potential as a therapeutic target. Oncotarget. 2016; 7: 42716–42739.

[39] Wang XL, Li JM, Yuan SQ. Characterization of the protein expression and localization of hnRNP family members during murine spermatogenesis. Asian Journal of Andrology. 2023; 25: 314–321.

[40] Chi W, Liu W, Fu W, Xia S, Heckscher ES, Zhuang X. RNA-binding protein syncrip regulates starvation-induced hyperactivity in adult drosophila. PLOS Genetics. 2021; 17: e1009396.

[41] Cao Y, Xu J, Liu J, Liang Y, Ao F, Wang S, *et al*. Bisphenol A exposure decreases sperm production and male fertility through inhibition PCBP2 expression. Environmental Science and Pollution Research. 2023; 30: 123309–123323.

[42] Ge JB, Wang ZX, Wu J. NAT10-mediated acC modification promotes ectoderm differentiation of human embryonic stem cells via acetylating mRNA. Cell Proliferat. 2024; 57: e13577.

[43] Fanourgakis G, Lesche M, Akpinar M, Dahl A, Jessberger R. Chromatoid body protein TDRD6 supports long 3′UTR triggered nonsense mediated mRNA decay. PLOS genetics. 2016; 12: e1005857.

[44] Guo R, Wu H, Zhu X, Wang G, Hu K, Li K, *et al*. Bi-allelic variants in chromatoid body protein TDRD6 cause spermiogenesis defects and severe oligoasthenoteratozoospermia in humans. Journal of Medical Genetics. 2024; 61: 553–565.

[45] Staszewski J, Lazarewicz N, Konczak J, Migdal I, Maciaszczyk-Dziubinska E. UPF1—from mRNA degradation to human disorders. Cells. 2023; 12: 419.

[46] Amorim IS, Kedia S, Kouloulia S, Simbriger K, Gantois I, Jafarnejad SM, *et al*. Loss of eIF4E phosphorylation engenders depression-like behaviors via selective mRNA translation. Journal of Neuroscience. 2018; 38: 2118–2133.

[47] Rofeal M, Abd El-Malek F. Ribosomal proteins as a possible tool for blocking SARS-COV 2 virus replication for a potential prospective treatment. Medical Hypotheses. 2020; 143: 109904.

[48] Janisch KM, Vock VM, Fleming MS, Shrestha A, Grimsley-Myers CM, Rasoul BA, *et al*. The vertebrate-specific Kinesin-6, Kif20b, is required for normal cytokinesis of polarized cortical stem cells and cerebral cortex size. Development. 2013; 140: 4672–4682.

[49] Bannai H, Fukatsu K, Mizutani A, Natsume T, Iemura S, Ikegami T, *et al*. An RNA-interacting protein, SYNCRIP (heterogeneous nuclear ribonuclear protein Q1/NSAP1) is a component of mRNA granule transported with inositol 1,4,5-trisphosphate receptor type 1 mRNA in neuronal dendrites. Journal of Biological Chemistry. 2004; 279: 53427–53434.

[50] Bao J, Vitting-Seerup K, Waage J, Tang C, Ge Y, Porse BT, *et al*. UPF2-dependent nonsense-mediated mRNA decay pathway is essential for spermatogenesis by selectively eliminating longer 3′UTR transcripts. PLOS genetics. 2016; 12: e1005863.

[51] Isken O, Kim YK, Hosoda N, Mayeur GL, Hershey JWB, Maquat LE. Upf1 phosphorylation triggers translational repression during nonsense-mediated mRNA decay. Cell. 2008; 133: 314–327.

How to cite this article: Jingyan Yu, Minyan Yu, Yufei Chen, Chaoye Ma, Xiaoning Zhang, Yuan Gao, *et al*. A global screen for testicular proteins and RNAs interacting with STRBP. Revista Internacional de Andrología. 2025; 23(3): 97-107. doi: 10.22514/j.androl.2025.035.