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Original research

The SNAPc complex mediates starvation-induced trans-splicing in *Caenorhabditis elegans*

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ABSTRACT

Dietary restriction usually suppresses biosynthesis but activates catabolic pathways in animals. However, the short-term starvation enhances biosynthetic activities and promotes ribosomal biogenesis in adult *Caenorhabditis elegans*. The mechanism underlying the processes remains largely unknown. Here, we find that the short-term starvation enhances the SL1 trans-splicing of translation-related genes in adult *C. elegans* by transcriptome analysis. The small nuclear RNA-activating protein complex (SNAPc) promotes SL RNA production and mediates starvation-induced trans-splicing. TOFU-5, a core factor in the upstream sequence transcription complex (USTC) essential for piRNA production, is also involved in the starvation-induced trans-splicing processes. Knocking down components of the SNAPc complex and *tofu-5* extends worm survival under starvation conditions. Taken together, our study highlights the importance of SL trans-splicing in the nutrition response and reveals a mechanism of the survival regulation by food deprivation via SNAPc and TOFU-5.

Introduction

Organisms have evolved a variety of mechanisms to endure severe living conditions. Upon nutrient depletion, animals can reprogram their metabolic activity, endocrine system, and other physiological machinery to promote survival and growth (Codogno and Meijer, 2005; Baumeister et al., 2006; Angelo G, 2009). In response to nutrient stress, cellular metabolic pathways are altered. Anabolic pathways, including protein and fat synthesis, are suppressed. Catabolic pathways, such as the autophagy/lysosomal degradation of cellular proteins, are activated during the starvation period to provide alternative energy sources (Scott et al., 2004; Codogno and Meijer, 2005; de Lange et al., 2007; Kraft et al., 2008; Chang et al., 2009).

The soil nematode *Caenorhabditis elegans* has provided a wealth of insight into mechanisms governing the regulation and adaptation to starvation. In addition to reducing its basal metabolism to preserve energy during nutrient deprivation, adult *C. elegans* exhibits a two-phase metabolic response to nutrient restriction (Tan et al., 2011). In the initial short phase of starvation, both biosynthetic and degradation activities are greatly enhanced. If the stress persists, metabolic activities are repressed in the second long phase. During short-term or early starvation, both ribosomal biogenesis and translation activities are increased. Mutations in *rrp-8* and *rsks-1*, or inhibition of rDNA transcription using actinomycin D, lead to impaired rRNA processing and ribosome assembly (Hannan et al., 2003; Zhu et al., 2018), cause excessive lipid accumulation and extend worm survival under starvation conditions (Wu et al., 2018). Thus, ribosome biogenesis could be a key regulator of metabolic pathways, starvation responses, and survivability of *C. elegans*. However, the precise mechanisms of how animals alter ribosomal biogenesis and translation activities upon food-deprivation remains largely unknown.

Approximately 70% of *C. elegans* mRNAs are covalently modified at their 5′ end by the addition of 22-nt trans-spliced leader RNA sequences (Blumenthal, 2012). Trans-spliced leaders are donated by independently transcribed SL RNAs of ~100–110 nt in length, which

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are mainly classified into two groups, SL1 RNAs and SL2 RNAs (Stricklin et al., 2005). SL1 is the major spliced leader in nematodes and is used primarily for trans-splicing at the 3′ splice sites following outrons, whose sequences resemble introns but occur at the 5′-most ends of pre-mRNAs (Conrad, 1991; Saito et al., 2013). Approximately half of the *C. elegans* genes are estimated to contain outrons and are consequently trans-spliced into SL1 RNAs (Allen et al., 2011). SL2 RNAs are trans-spliced at trans-splice sites between genes in polycistronic pre-mRNAs produced from operons (Spieth et al., 1993; Zorio, 1994; Blumenthal et al., 2002; Allen et al., 2011).

Ribosomal biogenesis and translation are greatly affected by SL1 trans-splicing in nematodes (Yang et al., 2017). SL1 trans-splicing likely enhances translational efficiency by shortening native 5′ UTRs (Yang et al., 2017). Under environmental stress, SL1 trans-splicing increases the translational efficiency of essential genes. SL2 trans-splicing is involved in the expression of operon genes that are required for rapid recovery from growth-arrested states into a fast-growing state. Operon genes that are trans-spliced into SL2 RNAs are significantly upregulated in the first hour of recovery from L1 arrest. Thus, SL trans-splicing may act as a key regulator of ribosomal biogenesis and translation upon food deprivation.

In *C. elegans*, a small nuclear RNA (snRNA)-activating protein complex (SNAPc), consisting of SNAPC1 (worm SNPC-1.1,−1.2,−1.3 and −1.4), SNAPC3 (worm SNPC-3.1,−3.2,−3.3 and −3.4) and SNAPC4 (worm SNPC-4), binds to the proximal sequence element (PSE) of snRNA genes and promotes snRNA transcription (Henry, 1995; Yoon, 1995; Su, 1997; Wong, 1998; Ma and Hernandez, 2002; Jawdekar and Henry, 2008). Recently, our lab revealed that an upstream sequence transcription complex (USTC) consisting of PRDE-1, TOFU-4, TOFU-5, and SNPC-4 is required for piRNA transcription (Weng et al., 2019). Interestingly, while PRDE-1 and TOFU-4 are restricted to piRNA clusters, SNPC-4, and TOFU-5 bind both piRNA gene promoters and canonical SNAPc targets, which include RNA Pol II- and RNA Pol III-transcribed noncoding RNA (ncRNA) genes (Kasper et al., 2014; Weng et al., 2019). However, the regulation of the SNAPc complex and TOFU-5 on canonical SNAPc targets remains largely unclear. In addition, elevated temperature suppresses piRNA production by depleting the binding of USTC complex to piRNA genes (Huang et al., 2021), suggesting that SNAPc and USTC complexes may contribute to stress responses of ncRNA transcription and physiological adaption.

Here, We find that the SNAPc complex mediates starvation-induced trans-splicing by promoting SL RNA production. Intriguingly, the core factor of the USTC complex, TOFU-5, is also involved in this process. Furthermore, knocking down the SNAPc complex or *tofu-5* promotes worm survival under starvation conditions. Taken together, our findings highlight the importance of SL trans-splicing during food deprivation and reveals a mechanism regulating trans-splicing via the SNAPc complex and TOFU-5.

Results

Short-term starvation increases the expression of translation-related genes

A previous study showed that short-term starvation in adult nematodes significantly increased the expression of factors required for rRNA processing and ribosome assembly, export and maturation (Tan et al., 2011). To investigate short-term starvation-induced alterations in gene expression, we performed the mRNA-seq of adult *C. elegans* after 6 h of starvation (Fig. 1A). Starvation induced dramatic changes in global gene expression (Fig. 1B). The upregulated genes identified in starved worms were enriched for the Gene Ontology (GO) terms "steroid hormone mediated signaling pathway" and "regulation of transcription, DNA-templated" (Fig. S1A). Genes associated with the "innate immune response" and "oxidation-reduction process"were enriched in the downregulated gene group (Fig. S1B). To assess whether starvation enhances the expression of translation-related genes, we focused on genes annotated with the GO term "translation", which includes genes encoding both ribosome proteins and essential translational regulators. In contrast to non-translation genes, the mRNA abundance of highly expressed translation-related genes likely increased upon starvation (Fig. 1C and 1D). We divided all genes based on their expression levels. Based on Fig. 1D, the RPKM of 1000 was applied as the cutoff. However, we did not observe a starvation-induced significant change of either lowly- or highly-expressed all gene groups (Fig. 1E). We focused on translation-related genes. We divided the translation-related genes into two groups based on their expression levels (Fig. 1F). Within the low-expression group, including genes with RPKM values below 1000, starvation did not significantly change expression levels. Intriguingly, in the high-expression group, including genes with RPKM values greater than 1000, starvation significantly increased expression levels (Fig. 1F).

These results suggested that *C. elegans* may deploy a distinct mechanism to respond to nutrition depletion and regulate translation-related genes.

Short-term food deprivation increases the SL1 trans-splicing of translation-related genes

Approximately 70% of *C. elegans* genes are trans-spliced (Allen et al., 2011). Highly expressed genes show a greater propensity for trans-splicing (Allen et al., 2011). Strikingly, nearly all translation-related genes (307 out of 335) were shown to be trans-spliced (Fig. S2A). Therefore, we speculated that starvation may modulate trans-splicing of the highly expressed translation-related genes and increase the expression.

To test this hypothesis, we quantified SL trans-splicing events based on the mRNA-seq dataset. Trans-splicing in *C. elegans* is mainly classified into two types: SL1 and SL2, which affect 62% and 12% of genes, respectively (MacMorris et al., 2007; Allen et al., 2011; Blumenthal, 2012). We used a bioinformatics pipeline adapted from a previously described method to assess SL1-and SL2-bearing transcripts (Allen et al., 2011; Maxwell et al., 2012; Yague-Sanz and Hermand, 2018). Briefly, SL1 and SL2 sequences were identified with high sequence specificity and trimmed off from the input reads, followed by remapping to the reference genome (see Materials and methods for details). Upon nutrition deprivation, the overall levels of SL1-bearing transcripts were not significantly changed (Figs. 2A and S2B). However, among highly expressed genes with RPKM values greater than 1000, the levels of SL1-bearing transcripts were significantly elevated (Figs. 2A and S2B), suggesting that SL1 trans-splicing is enhanced in genes that are highly expressed during starvation.

We conducted gene ontology (GO) enrichment analysis of the up- and down-regulated (>1.5-fold) SL1-bearing transcripts by using DAVID. Among the upregulated transcripts, "embryo development ending in birth or egg hatching", "reproduction" and "translation" were enriched (Fig. S2C). GO terms associated with "embryo development ending in birth or egg hatching" and "reproduction" were enriched in the group of downregulated transcripts (Fig. S2D). The group of genes related to embryo development and reproduction were enriched in both up- and down-regulated SL1 transcripts. However, the group of translation-related genes were specifically enriched in upregulated transcripts, suggesting starvation activated SL1 trans-splicing of translation-related genes. Accordingly, box-plots and MA plots indicated that the SL1-bearing transcripts of translation-related genes increased significantly upon short-term starvation (Figs. 2B and S2E).

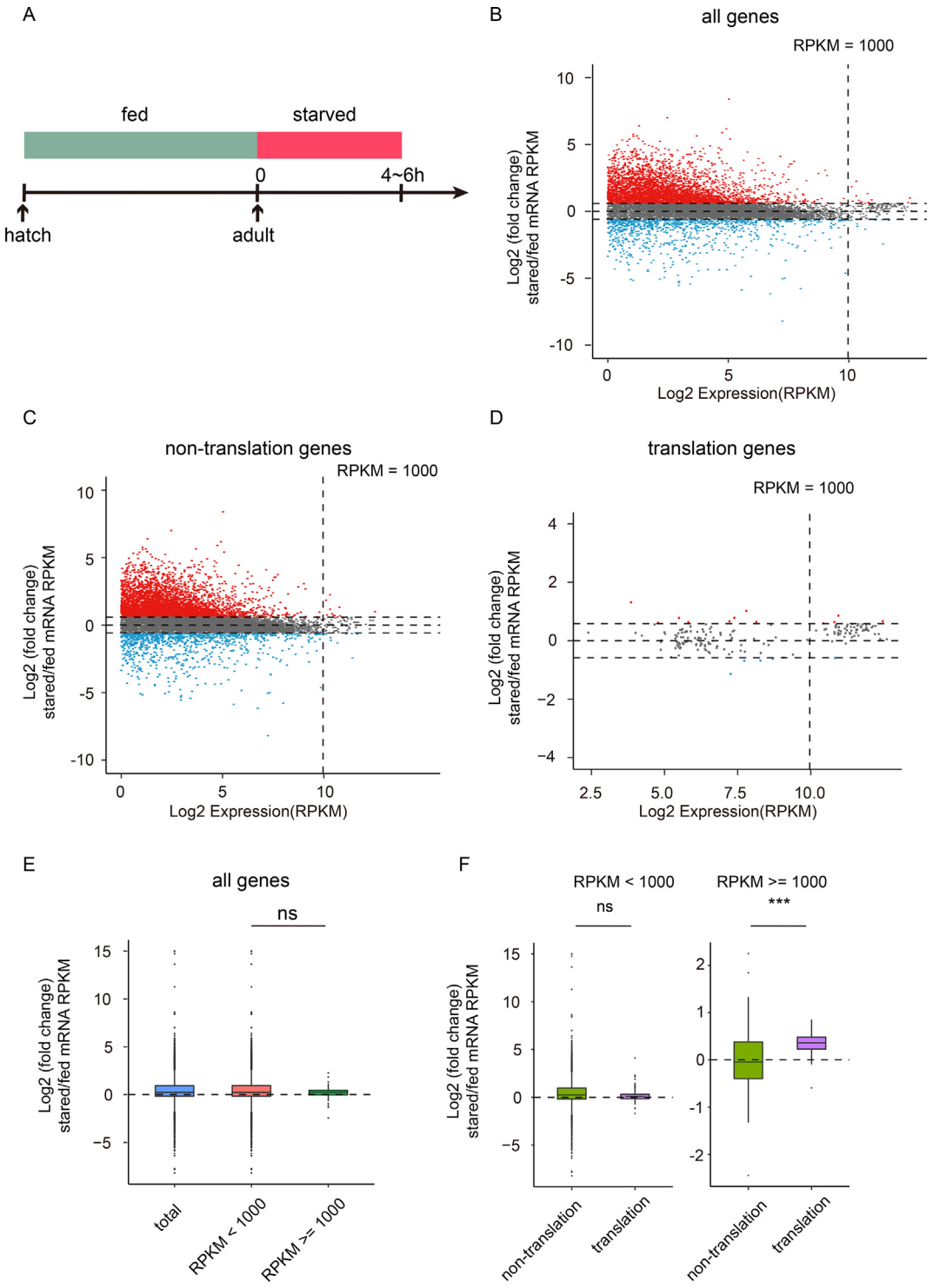

Fig. 1. Short-term starvation increases the levels of highly expressed translation-related genes. **A**: Schematic diagram of the starvation treatment of *C. elegans*. **B**–**D**: MA plot of mRNA RPKM values of all genes (**B**), non-translation-related genes (**C**), and genes annotated with the GO term "translation" (**D**) in starved versus fed animals. Red and blue dots indicate upregulated and downregulated (fold change > 1.5) transcripts, respectively. **E** and **F**: Boxplot showing $\log_2$-fold change of mRNA RPKM values of the indicated gene class in starved versus fed animals. Significance was tested using the unpaired Wilcoxon test. ***, $P < 0.001$; ns, not significant.

To exclude a possibility that the increase in SL1 trans-splicing of translation-related genes resulted from an elevation of the expression levels of all genes in mRNA deep sequencing experiments, we analyzed non-trans-spliced transcripts from the mRNA-seq data. The non-trans-spliced transcripts of all highly expressed genes were decreased upon short-term starvation (Fig. 2C). Meanwhile, the short-term starvation modestly reduced the expression of both lowly- and highly-expressed non-trans-spliced translation-related gene transcripts (Fig. 2D). Furthermore, the ratio of SL1/non-trans-spliced transcripts of translation-related genes and genes that are highly expressed were both elevated upon starvation (Fig. S2F–S2G). In addition, we performed quantitative reverse transcription polymerase

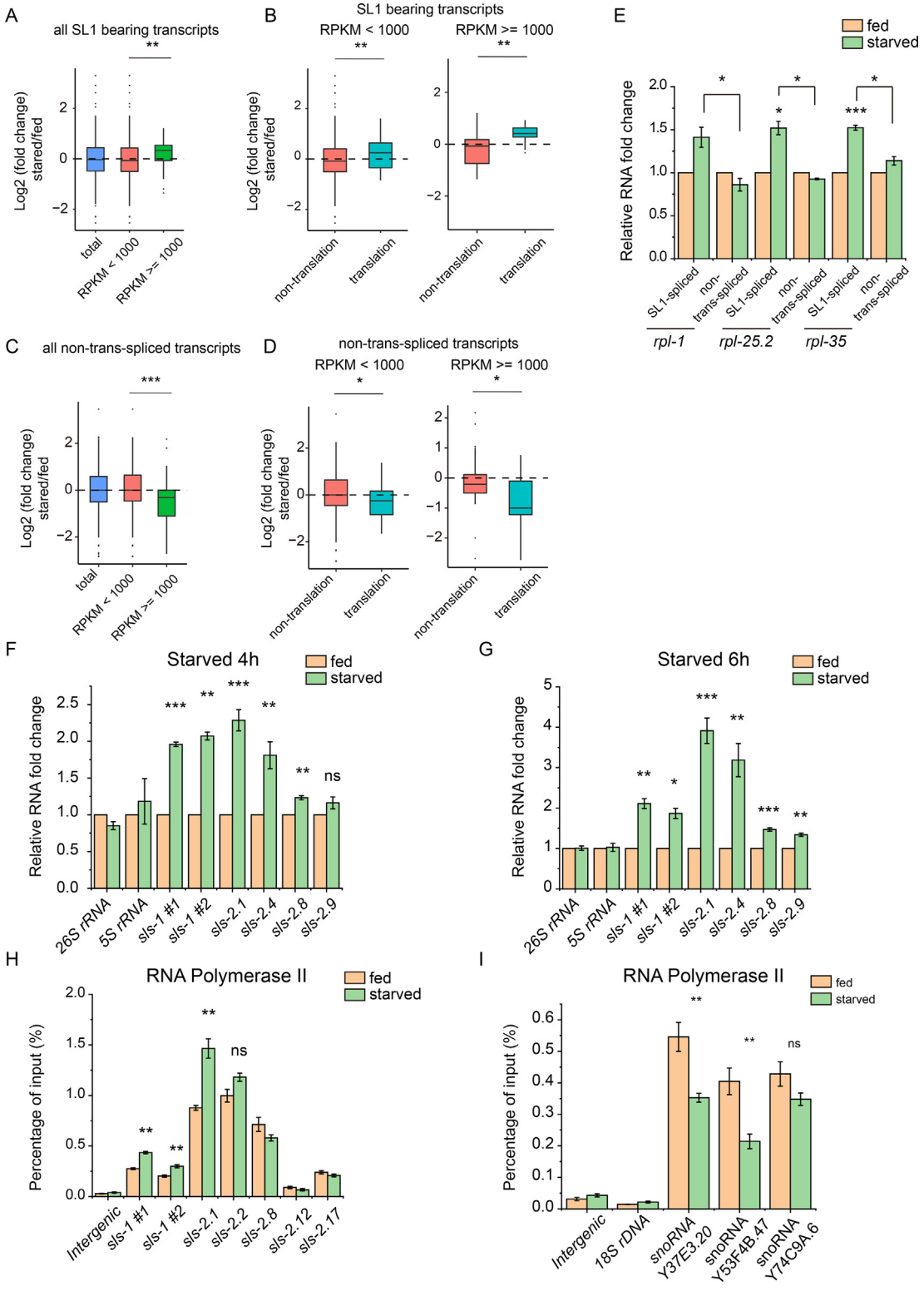

Fig. 2. Short-term starvation induces the production of SL RNAs and SL1 trans-splicing. **A**–**D**: Boxplot showing the $\log_2$-fold change of SL1-bearing transcripts of the indicated gene class in starved versus fed animals. The numbers of trans-spliced reads were firstly normalized to the number of total reads in the mRNA-seq dataset and then compared. Significance was tested using the unpaired Wilcoxon test, ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. **E**: Levels of trans-spliced and non-trans-spliced isoforms of the indicated genes detected by qRT-PCR. Mean ± SD; ***, $P < 0.001$; *, $P < 0.05$; $n = 3$. **F** and **G**: SL RNA levels measured by qRT-PCR in fed and starved worms. Mean ± SD; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant; $n = 3$. **H**: Relative enrichment of RNA Polymerase II according to ChIP-qPCR assays of the indicated genes in fed and starved animals. Mean ± SD; **, $P < 0.01$; ns, not significant; $n = 4$. **I**: Relative enrichment of RNA Polymerase II according to ChIP-qPCR assays of the indicated genes in fed and starved animals. Mean ± SD; **, $P < 0.01$; ns, not significant; $n = 4$.

chain reaction (RT-qPCR). Notably, the SL1 trans-spliced but not the non-trans-spliced isoforms of *rpl-1*, *rpl-25.2*, and *rpl*-35 mRNAs increased significantly upon starvation (Fig. 2E).

Approximately 15% of *C. elegans'* genes are located in operons (Allen et al., 2011). SL2 trans-splicing mainly occurs among genes located at position 2 or beyond within operons. Under short-term starvation, the overall SL2-bearing transcripts were not significantly changed (Fig. S3A–S3C). However, among highly expressed genes, SL2-bearing transcripts were significantly increased relative to those of low-expression genes (Fig. S3A–S3C), suggesting that starvation activated SL2 trans-splicing of highly expressed genes. In contrast to the findings regarding SL1 trans-splicing, the SL2-bearing transcripts of translation-related genes were not significantly elevated by starvation (Fig. S3D). By subjecting up- and down-regulated (>1.5-fold) SL2-bearing transcripts to gene ontology (GO) term enrichment analysis, we observed the GO term "translation" among both up-regulated and down-regulated genes (Fig. S3E and S3F). Collectively, these data suggest that SL2 trans-splicing is important for highly expressed genes but is not limited to translation-related genes upon short-term starvation.

Taken together, these data suggest that short-term starvation enhances the SL1 trans-splicing of translation-related genes.

Short-term nutrition deprivation induces the production of SL RNAs

The *C. elegans* genome encodes 110 SL1 RNA genes on a 1 kb tandem repeat that also contains the genes encoding 5S rRNA (Nelson DW, 1985; Krause and Hirsh, 1987; Blumenthal, 2012). The genome contains 18 dispersed SL2 RNA genes, which specify a number of SL2 RNA variants (Blumenthal, 2012). To test whether nutrition deprivation increased the production of SL RNAs, we performed qRT-PCR measurements of SL1 and SL2 RNAs (*sls-2.1*, *sls-2.4*, *sls-2.8* and *sls-2.9*). Both SL1 and SL2 RNAs were significantly increased after 4 h and 6 h of starvation (Fig. 2F and 2G). As controls, the expression of snoRNAs (Y74C9A.6, F59C6.15, F25H2.15) and other ncRNAs (F54D8.7, *rpr-1*) did not change significantly upon starvation (Fig. S4A and S4B). These results suggested that short-term nutrition deprivation specifically promotes the expression of SL RNAs. SL1 RNAs are transcribed adjacent, and in an opposite orientation, to the 5S ribosomal gene (Krause and Hirsh, 1987). To test whether starvation also promotes the expression of 5s rRNA, we measured the levels of 5s rRNA by qRT-PCR after short-term starvation. However, short-term starvation did not significantly change the levels of 5s rRNA (Fig. 2F and 2G).

In nematodes, SL RNAs are transcribed by RNA Polymerase II (Hastings, 2005). Upon food depletion, a rapid response to maintain energy homeostasis has been shown to elicit RNA Pol II accumulation at growth and development genes (Baugh et al., 2009; Maxwell et al., 2014). To test whether the starvation-induced SL RNA elevation is mediated by RNA Pol II, we performed chromatin immunoprecipitation (ChIP) of RNA Pol II using the antibody 8WG16, followed by qRT-PCR. Gravid adult animals were starved for 4 h and harvested. We observed significant enrichment of Pol II at both SL1 and SL2-1 RNA genes in starved animals (Fig. 2H). As controls, the presence of Pol II at two snoRNA genes, Y37E3.20 and Y53F4B.47, modestly decreased upon short-term starvation (Fig. 2I).

Therefore, these data suggest that short-term starvation activates the production of SL RNAs.

The SNAPc complex mediates starvation-induced SL RNA expression

The SNAPc complex binds to the proximal sequence element (PSE) and promotes the transcription of snRNA genes. We tested whether the SNAPc complex promotes SL RNA production upon starvation. We generated single-copy GFP-3xFLAG-tagged SNPC-1.1 and SNPC-3.4, (SNPC-1.1:GFP and SNPC-3.4:GFP, respectively) and mCherry-tagged SNPC-1.1 transgenic strains using Mos1-mediated single-copy insertion (MosSCI) technology (Frokjaer-Jensen et al., 2008). SNPC-1.1 and SNPC-3.4 were expressed in both soma and germline (Fig. S5A and S5B). In germline, SNPC-1.1 and SNPC-3.4 colocalized in the nucleus (Fig. 3Aand 3B). SNPC-4 has been shown to accumulate in the nucleus in germ cells and to be enriched at nuclear piRNA foci (Weick et al., 2014; Weng et al., 2019). SNPC-1.3 also colocalizes with the core piRNA transcription factor at nuclear foci in the male germline (Choi et al., 2021).

We performed ChIP-qPCR of SNPC-1.1 and SNPC-3.4 using gravid animals after 4 h of starvation. Remarkably, both SNPC-1.1 and SNPC-3.4 were significantly increased at SL1 RNA genes in starved animals (Fig. 3C and 3D). We then conducted a ChIP assay followed by deep sequencing (ChIP-Seq) of SNPC-1.1 and SNPC-4. Both SNPC-1.1 and SNPC-4 are associated with the promoters of protein coding, snRNA, snoRNA and ncRNA genes (Figs. 4A, S6A–S6D). Consistent with a previous report (Kasper et al., 2014; Weng et al., 2019), SNPC-4 also binds to piRNA clusters on chromosome IV. Starvation did not change the gene biotypes or gene regions bound by SNPC-1.1 and SNPC-4. However, starvation significantly increased the association of SNPC-1.1 and SNPC-4 with the SL1 and SL2 RNA genes (Fig. 4B and 4D).

Collectively, these data suggest that starvation enhances the association of the SNAPc complex with SL RNA genes.

TOFU-5 is involved in starvation-induced SL RNA production

SNPC-4 is also included in a USTC complex that binds piRNA gene promoters and promotes piRNA transcription (Kasper et al., 2014; Weng et al., 2019). The USTC complex consists of PRDE-1, TOFU-4, TOFU-5, and SNPC-4. Both TOFU-5 and SNPC-4, but not PRDE-1 or TOFU-4, have been shown to be enriched at snRNA and snoRNA genes (Weng et al., 2019).

To investigate whether TOFU-5 is involved in starvation-induced SL RNA expression, we performed a ChIP-seq analysis of TOFU-5 upon food deprivation. We generated a 3 × FLAG:GFP:TOFU-5 transgene by MosSCI technology and found TOFU-5 was expressed in both soma and germline (Weng et al., 2019) (Fig. S5C). We harvested 3 × FLAG:GFP:TOFU-5 gravid adult animals after 4 h of starvation. Consistent with previous work, TOFU-5 was associated with both piRNA clusters and canonical SNAPc targets (Kasper et al., 2014; Weng et al., 2019) (Fig. 5A). Starvation did not significantly change the gene biotypes or gene regions bound by TOFU-5 (Figs. 5A, S7A and S7B) but significantly increased the association of TOFU-5 with both SL1 and SL2 RNA genes (Fig. 5B and 5C).

SNPC-4 and TOFU-5 bind piRNA clusters and promote piRNA transcription (Weng et al., 2019); however, we did not observe significant changes in SNPC-4 or TOFU-5 binding to piRNA clusters on chromosome IV upon starvation (Fig. 5D). We deep sequenced small RNAs from fed and starved animals and only observed a modest reduction in type I and type II piRNAs and microRNAs in starved animals (Figs. 5E, S7C and S7D).

Collectively, our results suggested that food deprivation induces the association of TOFU-5 with SL RNA genes.

SNAPc complex and TOFU-5 are required for starvation-induced trans-splicing

To investigate whether SNAPc complex and TOFU-5 are required for SL RNA production and trans-splicing during starvation, we firstly knocked down *snpc-1.1*, *snpc-3.4*, *snpc-4* and *tofu*-5 by RNAi. We performed qRT–PCR to assay SL RNAs, but failed to observe significant changes of *sls-1* and *sls*-2 RNA levels upon

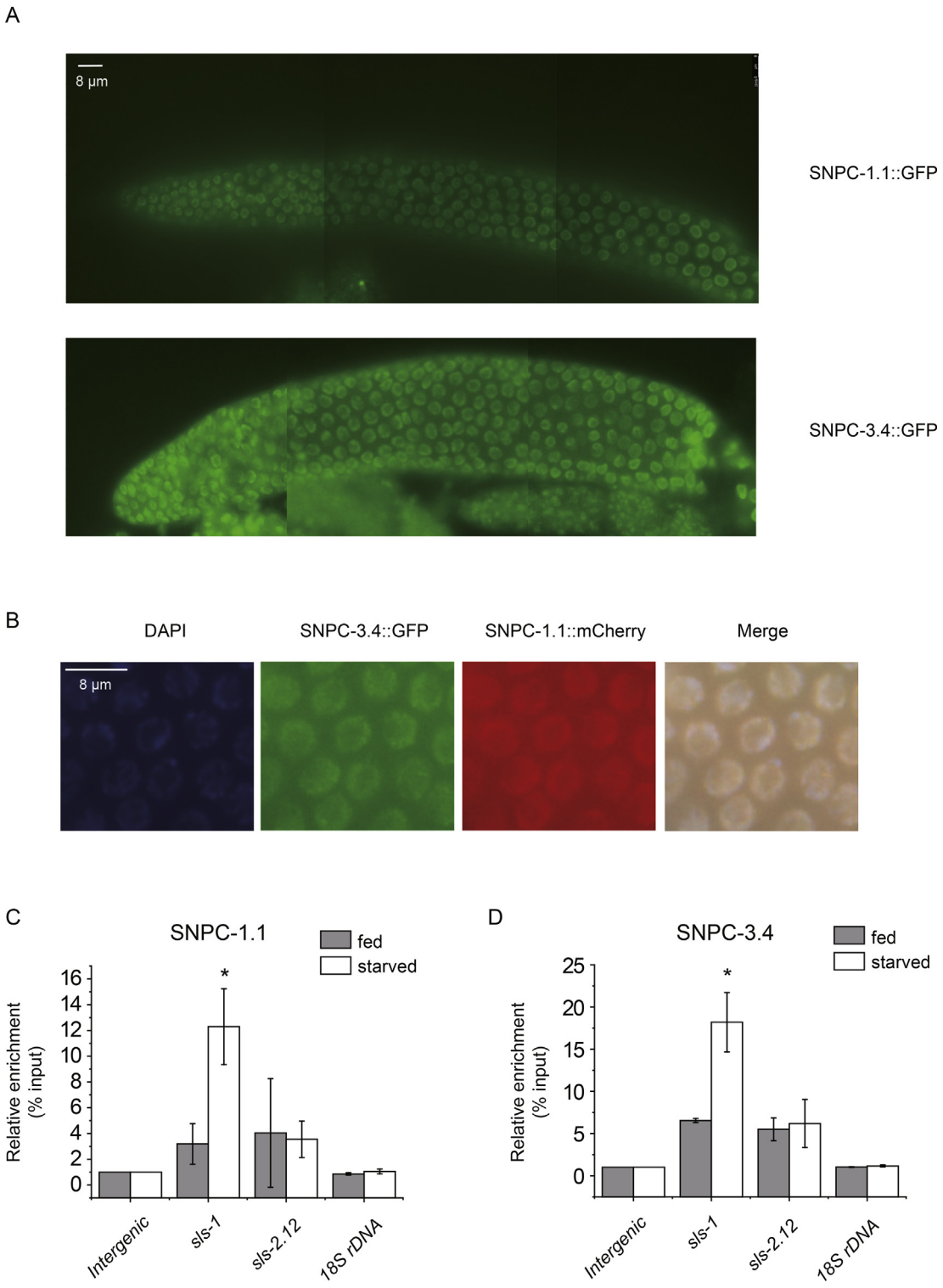

Fig. 3. SNPC-1.1 and SNPC-3.4 localize to the nuclei of germ cells. **A** and **B**: Images of the germlines of adult animals expressing the indicated transgenes. **C** and **D**: Relative enrichment of SNPC-1.1 (**C**) and SNPC-3.4 (**D**) according to ChIP-qPCR assays of the indicated genes in fed and starved animals. Mean ± SD; *, $P < 0.05$; $n = 3$.

RNAi (Fig. S8A). Meanwhile, knocking down SNAPc complex and TOFU-5 did not significantly change the levels of *rpl-1*, *rpl-25.2* and *rpl-35* either (Fig. S8B), suggesting that knocking down SNAPc complex and TOFU-5 are not sufficient to alter SL RNA production and trans-splicing in fed worms. Then we subjected adult worms after feeding RNAi to short term starvation (Fig. 6A). Surprisingly, knocking down *snpc-1.1*, *snpc-3.4*, *snpc-4* and *tofu-5* prohibited the starvation-induced increase of SL1, SL2 RNAs and trans-splicing of *rpl-1*, *rpl-25.2*, and *rpl*-35 mRNAs (Fig. 6B and 6C). Collectively, these data suggested that SNAPc complex and TOFU-5 were required for starvation-induced SL RNA production and trans-splicing.

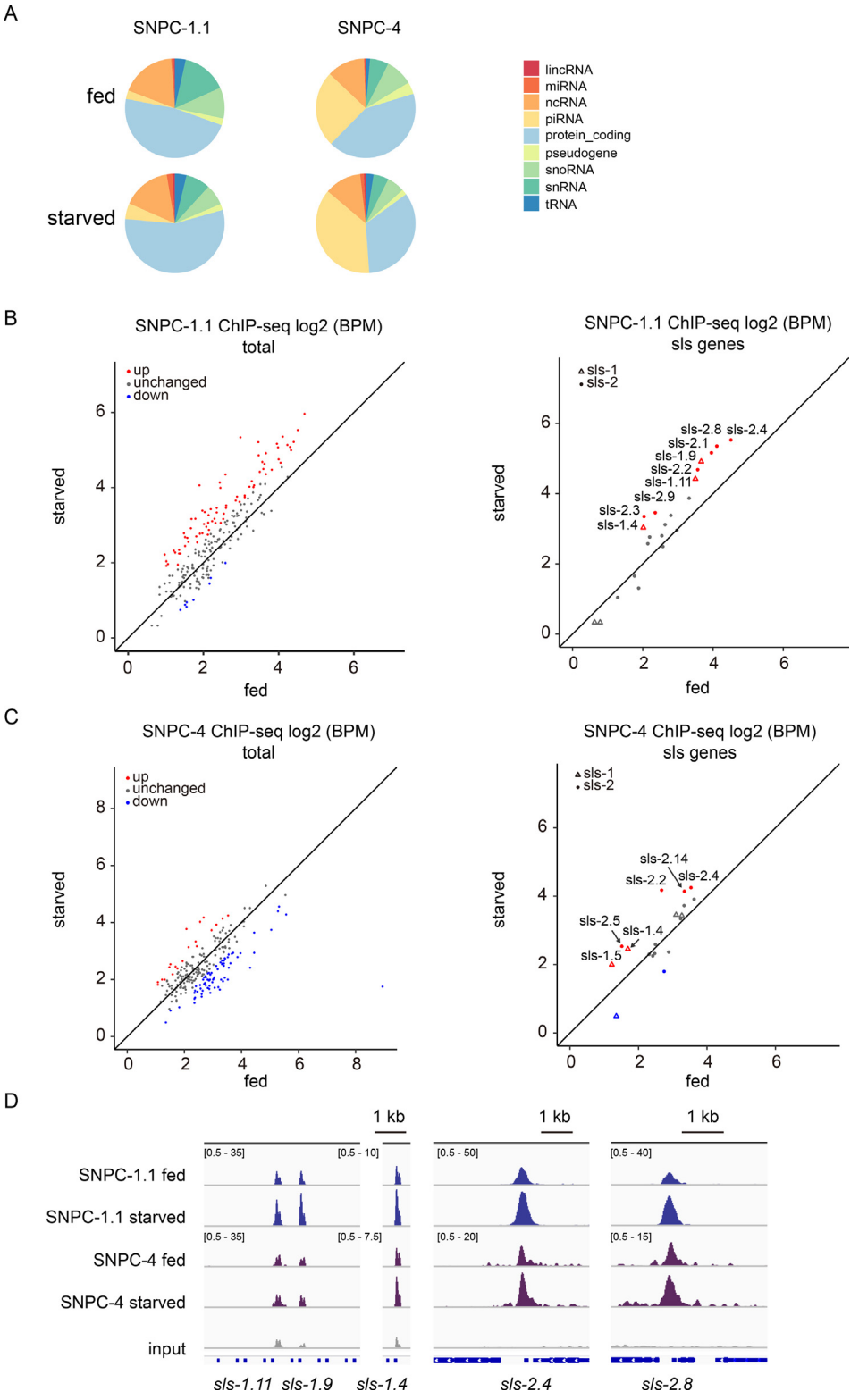

Fig. 4. Starvation induces the association of the SNAPc complex with SL RNA genes. **A**: Pie chart analysis of the gene biotypes bound by SNPC-1.1 and SNPC-4 in the indicated worms. **B** and **C**: Scatterplots comparing the SNPC-1.1 (**B**) and SNPC-4 (**C**) ChIP-seq binding signals of the indicated genes between fed and starved worms. The ChIP-seq binding signals were quantified as the number of reads per bin/sum of all reads per bin (in millions). **D**: Genome browser views of the SNPC-1.1 (blue) and SNPC-4 (purple) ChIP-seq binding profiles at SL genes in fed and starved worms.

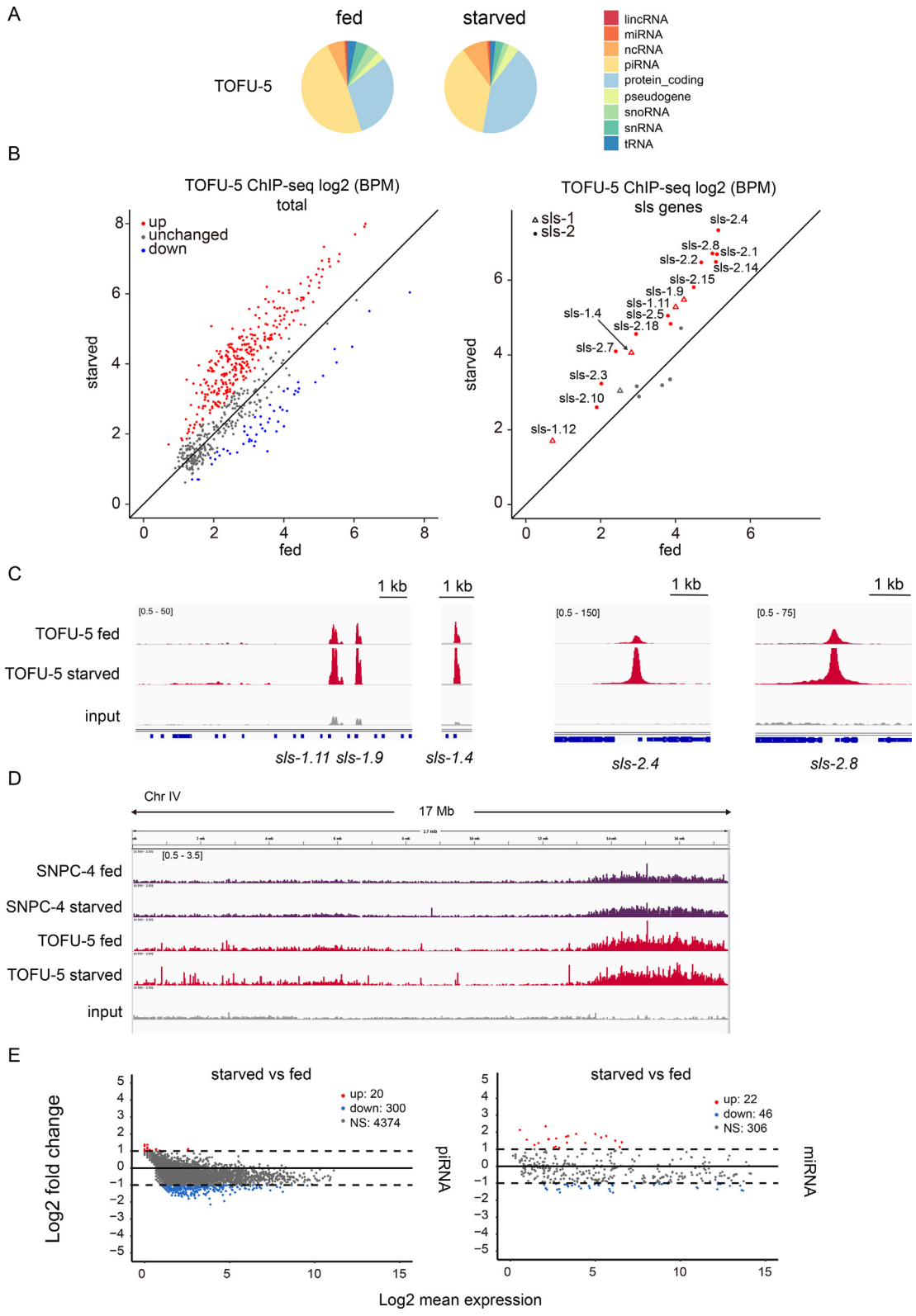

Fig. 5. Starvation induces the association of TOFU-5 with SL RNA genes. **A**: Pie chart analysis of the gene biotypes bound by TOFU-5 in fed and starved worms. **B**: Scatterplots comparing the TOFU-5 ChIP-seq binding signals at the indicated genes between fed and starved worms. Left, total genes; right, sls genes. The ChIP-seq binding signals were quantified as the number of reads per bin/sum of all reads per bin (in millions). **C**: Genome browser views of TOFU-5 (red) ChIP-seq binding profiles at SL genes in fed and starved worms. **D**: SNPC-4 (purple) and TOFU-5 (red) ChIP-seq binding profiles across chromosome IV in fed and starved worms. **E**: Scatterplots comparing the number of piRNA and miRNA reads between fed and starved worms.

Knocking down the SNAPc complex and *tofu-5* extends worm survival upon food deprivation

To assess the physiological roles of the SNAPc complex and TOFU-5-mediated trans-splicing regulation, we knocked down *snpc-1.1*, *snpc-3.4*, *snpc-4* and *tofu*-5 by RNAi. Knocking down *snpc-1.1*, *snpc-4* and *tofu-5* led to sterility in these worms (Fig. 6D), suggesting that these are essential genes.

To further investigate the biological roles of *snpc-1.1*, *snpc-3.4*, *snpc-4*, and *tofu-5* during starvation, synchronized adult worms that

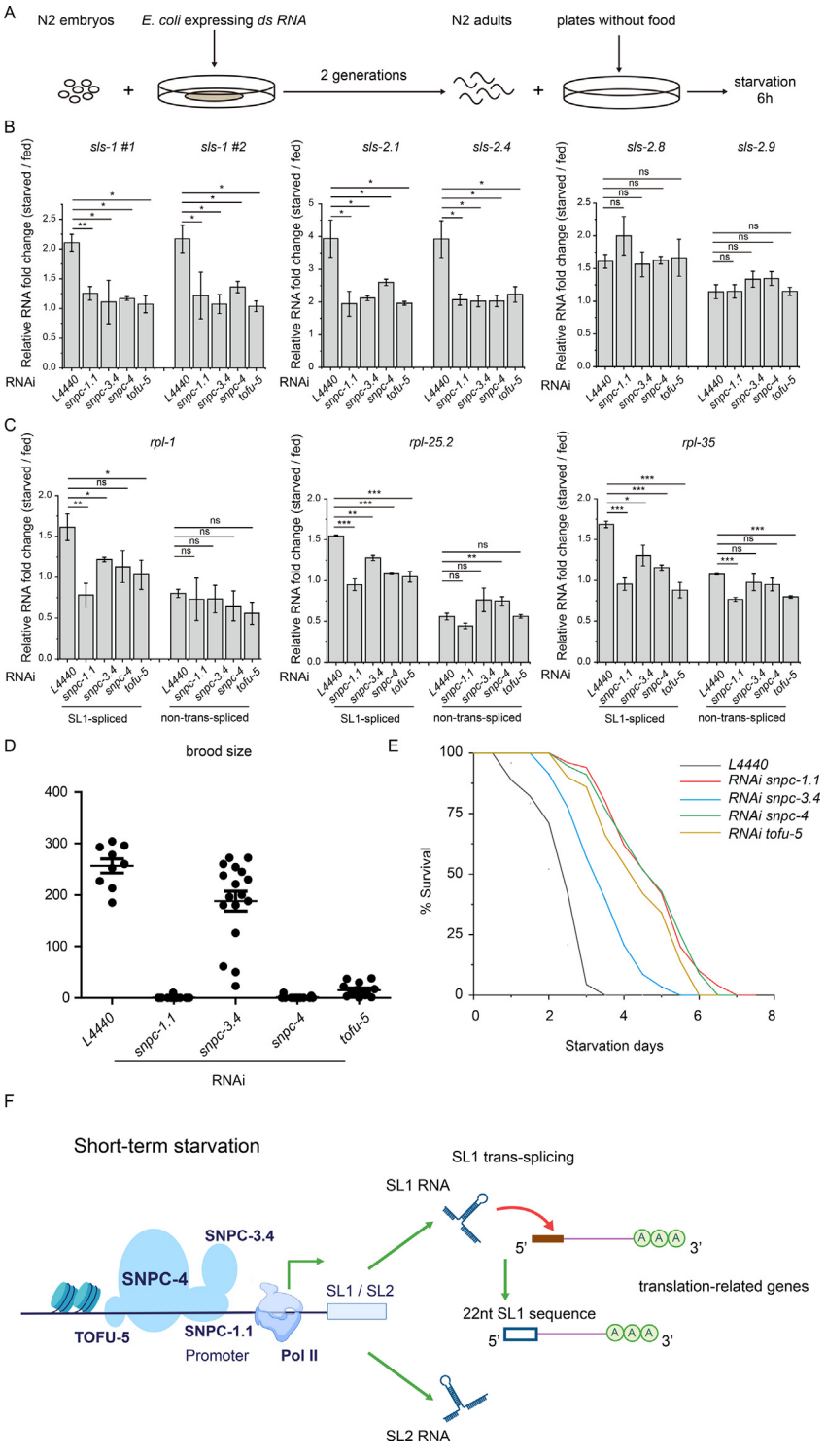

Fig. 6. The SNAPc complex and TOFU-5 are required for starvation-induced trans-splicing and limit worm survival upon food deprivation. **A**: Schematic diagram of RNAi feeding followed by starvation treatment. **B**: SL RNA levels measured by qRT-PCR in starved worms. **C**: Levels of indicated *rpl* and *rps* genes measured by qRT-PCR in starved worms. Mean ± SD; ***, $P < 0.001$, **, $P < 0.01$, *, $P < 0.05$; ns, not significant; $n = 3$. **D**: Bar graph displaying the brood size of worms treated with the indicated dsRNAs. Worms were grown at 20°C. Mean ± SD; $n > 8$. **E**: Representative survival curve of the indicated worms upon starvation. **F**: A working model of SNAPc complex and TOFU-5-mediated starvation-induced trans-splicing. Short-term starvation promotes the association of the SNAPc complex and TOFU-5 with SL RNA genes and increases SL gene expression, which further facilitates the trans-splicing of translation-related genes.

had been fed the corresponding dsRNAs for two generations were subjected to starvation. Knocking down *snpc-1.1*, *snpc-3.4*, *snpc-4*, and *tofu-5* significantly extended worm survival upon food deprivation (Fig. 6E).

Discussion

This work revealed a mechanism by which *C. elegans* responds to nutrient deprivation and increases the trans-splicing of translation-related genes (Fig. 6F). Upon starvation, the SNAPc complex and TOFU-5 associate with SL genes and promote the transcription of SL RNAs. Furthermore, knocking down the SNAPc complex and *tofu-5* extends worm survival upon food deprivation.

Starvation and trans-splicing

Approximately 70% of *C. elegans* mRNAs are trans-spliced by SL1 and SL2 RNAs. Between the two types of trans-splicing, SL1 trans-splicing plays the major role, affecting ~62% of genes, while SL2 affects ~12% of genes. Essential genes, such as highly expressed translation-related genes, are more likely to undergo SL1 trans-splicing (Blumenthal et al., 2002; Yang et al., 2017). Our results showed that both SL1 and SL2 RNAs are increased upon food deprivation. Short-term starvation increased the SL1-associated trans-splicing of translation-related genes. Further investigation will be required to identify the factors that specifically regulate translation-related genes.

Previous studies have reported that genes that are trans-spliced by SL2 are significantly up-regulated during recovery from L1 growth-arrested states (Zaslaver et al., 2011; Maxwell et al., 2012). We found that although short-term starvation increased the expression of SL2 RNAs, the SL2-associated trans-splicing of the translation genes was not significantly elevated, suggesting that SL2 is not biased toward translation genes.

Organisms usually show reduced basal metabolic activities upon nutrient deprivation to preserve energy (Tan et al., 2011). However, ribosome biogenesis and translation are enhanced in the initial phase of food depletion in adult *C. elegans*. It has been postulated that it is important to preserve physiological functions to ensure the immediate resumption of digestive and metabolic processes to endure a short period of stress (Tan et al., 2011). Thus, the activated ribosome biogenesis and translation in adult *C. elegans* might reflect an adaptive response to nutrient stress that ensures worms to resume growth and fertility if food is restored.

SL1 trans-splicing was previously reported to increase the translation efficiency of mRNAs. Therefore, the increased SL1 trans-splicing could contribute to elevated protein levels. To test whether SL1 trans-splicing increased the protein levels of translation-related genes, we tried to insert the fluorescent tag GFP into the endogenous *rpl* and *rps* genes. Unfortunately, we failed to obtain stably integrated strains. We speculated that the insertion of GFP disrupted the function of these RPL and RPS proteins. Further investigations are required to examine the roles of trans-splicing on protein translation.

One interesting observation is that knocking down the SNAPc complex and *tofu-5* extends worm survival upon food deprivation. The mechanism is unclear yet. Short-term adaptive changes and long-term fitness may adopt distinct regulatory mechanisms. Mutations in *rrp-8* and *rsks-1*, or inhibition of rDNA transcription using actinomycin D, lead to impaired rRNA processing and ribosome assembly (Hannan et al., 2003; Zhu et al., 2018), cause excessive lipid accumulation and extend worm survival under starvation conditions (Wu et al., 2018). We speculated that upon starvation, knocking down SNAPc and TOFU-5 prohibited the increase of SL1-trans-spliced translation-related genes and likely the increase of translation activity of the animals, which extended the viability under starving conditions. Whether and how SNAPc complex and TOFU-5 act in nutrient deprivation-induced life span extension requires further investigation.

We have described SNAPc- and TOFU-5-mediated increase in SL1 expression in gravid adults. However, it is unclear whether starvation modulates trans-splicing in soma, germline, or both. Since starvation can affect both survival and fecundity, it will be interesting to investigate tissue specific regulation of starvation on trans-splicing.

piRNA and SL RNA

The nematode piRNA transcription machinery likely evolved from snRNA transcription, and the upstream Ruby motif of the piRNA promoter is evolutionarily related to the SNAPc-binding motif (Beltran et al., 2019; Weng et al., 2019). The SNAPc complex is essential for the transcription of genes that encode snRNAs in humans. *C. elegans* encodes nine proteins (SNPC-4, SNPC-1.1, 1.2, 1.3, 1.4 and SNPC-3.1−3.4) that are orthologous to the mammalian SNAPc complex. Both SNPC-1.3 and SNPC-4 also bind piRNA clusters and are required for piRNA expression (Kasper et al., 2014; Weng et al., 2019; Choi et al., 2021). Among the subunits of the USTC complex, PRDE-1, SNPC-4, TOFU-4, and TOFU-5 bind the promoter sequences of each piRNA gene to promote piRNA transcription, while SNPC-4 and TOFU-5 are also enriched on other classes of ncRNAs (Kasper et al., 2014; Weick et al., 2014; Weng et al., 2019). Our data revealed that both the SNAPc complex and TOFU-5 are critical for the starvation response to selectively activate the expression of SL RNAs, suggesting a broad physiological role of the USTC complex beyond piRNA biogenesis.

Interestingly, another piRNA processing complex, the PICS complex (also known as the PETISCO complex), is required for both piRNA biogenesis and snRNA production (Cordeiro Rodrigues et al., 2019; Zeng et al., 2019). The PICS complex interacts with both piRNA precursors and SL1 RNAs. Whether there is potential competition between piRNA and SL RNA for PICS occupancy during short-term nutrition deprivation requires further investigation.

Materials and methods

Strains

Bristol strain N2 was used as the standard wild-type strain. All strains were grown at 20°C unless otherwise specified. The strains used in this study are listed in Table S1.

Feeding RNAi

RNAi experiments were performed at 20°C by placing synchronized embryos on RNAi feeding plates as previously described (Timmons et al., 2001). HT115 bacteria expressing the empty vector L4440 (a gift from A. Fire) were used as non-RNAi controls. Bacterial clones expressing dsRNAs were obtained from the Ahringer RNAi library and sequenced to verify their identity. All RNAi feeding experiments were performed for two generations.

Brood size

L4 hermaphrodites were fed the corresponding dsRNAs for two generations and were transferred to new plates daily as adults until embryo production ceased. The number of progenies were scored.

Starvation treatment

Synchronized adult worms were washed with 1× M9 buffer 3 times and transferred to empty NGM plates without any bacterial lawn. For the survival rate assay, starved worms were transferred to fresh empty plates daily and scored.

Construction of transgenic strains

For SNPC-1.1:GFP, SNPC-3.4:GFP, and SNPC-1.1:mCherry transgenes, endogenous promoter sequences, 3′ UTRs, ORFs, the coding sequence of *gfp::3xflag*, mCherry and a linker sequence (GGAGGTGGAGGTGGAGCT) (inserted between the ORFs and *gfp::3xflag* or mCherry) were fused and cloned into PCFJ151 vectors using a ClonExpress MultiS One Step Cloning Kit (Vazyme C113-02, Nanjing). SNPC-1.1:GFP and SNPC-3.4:GFP were integrated into *C. elegans*' chromosome II, and SNPC-1.1:mCherry was integrated into *C. elegans'* chromosome III by using MosSCI technology (Frokjaer-Jensen et al., 2008). The primers used for molecular cloning are listed in Table S2.

Microscopy and imaging

Images were collected on a Leica DM2500 microscope. Gonads were dissected in PBS (Phosphate-Buffered Saline) supplemented with 0.2 mM Levamisole. For DAPI staining, dissected gonads were fixed in 2% formaldehyde for 5 min, followed by 1 ng/ml of DAPI (4′,6-Diamidino-2-Phenylindole, Dihydrochloride) staining.

RNA isolation

Synchronized young adult worms were sonicated in sonication buffer (20 mM Tris-HCl [pH 7.5], 200 mM NaCl, 2.5 mM $MgCl_2$, and 0.5% NP40). The obtained eluates were incubated with TRIzol reagent followed by isopropanol precipitation and DNaseI digestion (Qiagen).

qRT−PCR

cDNAs were generated from the RNA using a GoScript Reverse Transcription System (Promega) according to the vendor's protocol. The reverse transcription primers used were random primers from the GoScript Reverse Transcription System (Promega) unless otherwise specified. qPCR was performed using a MyIQ2 real-time PCR system (Bio−Rad) with AceQ SYBR Green Master mix (Vazyme). The primers used in qRT-PCR are listed in Table S3.

mRNA deep sequencing and data analysis

Purified mRNAs were subjected to deep sequencing using an Illumina platform (Novogene Bioinformatics Technology Co., Ltd). First, the mRNAs were captured using oligo (dT)-coated magnetic beads and fragmented in fragmentation buffer. Second, the fragmented products were reverse transcribed using random hexamers. Third, the cDNAs were purified and ligated to 3′ and 5′ adapters. Finally, the samples were sequenced on the Illumina HiSeq platform.

The Illumina-generated raw reads were first filtered to remove adaptors, low-quality tags, and contaminants to obtain clean reads at Novogene. For mRNA-seq data analysis, HISAT2 version 2.1.0 (Kim et al., 2019) was used to map the clean reads to the *C. elegans* genome (WBcel235). Next, the read counts of the genes were estimated with StringTie version 2.1.1 (Pertea et al., 2015) using the default parameters. Differentially expressed genes were defined by a fold change >1.5.

Identification of SL1-and SL2-bearing transcripts

To identify SL1-and SL2-bearing transcripts, Bowtie2 version 2.3.5.1 (Langmead and Salzberg, 2012) was used to map the clean reads to the *C. elegans* genome and transcriptome (WBcel235). The SL1-and SL2-bearing transcripts were assessed by using a bioinformatics pipeline adapted from a previously described method (Allen et al., 2011; Maxwell et al., 2012; Yague-Sanz and Hermand, 2018). Briefly, reads that were not mapped to either the genome or the transcriptome were stripped of the first (5′) 6−22 nt sequence and remapped to determine if these reads came from the 5′ end of trans-spliced mRNAs. The stripped sequences that were mapped to the genome or transcriptome were further analyzed for SL1 or SL2 RNAs. Reads that were mapped after stripping and began with sequence GGTTTAATTACCCAAGTTTGAG were considered to be spliced into SL1 RNAs, whereas reads that started with sequence GGTTTTAACCCAGTTACTCAAG were considered to be spliced into SL2 RNAs. Genes with > 5 SL-bearing reads were selected for downstream analysis. Finally, the number of SL1-and SL2-bearing transcripts of each gene was normalized to the number of total reads in the mRNA-seq sample.

Identification of non-trans-spliced transcripts

To identify non-trans-spliced transcripts, Bowtie2 version 2.3.5.1 (Langmead and Salzberg, 2012) was used to map the clean reads to the *C. elegans* genome and transcriptome (WBcel235). Because of the existence of the 22-nt sequence donated by SL RNAs (Blumenthal, 2012), trans-spliced reads could not map to the genome. On the other hand, pre-mRNAs and reads with 5′outron sequence (Conrad, 1991; Saito et al., 2013) could map to the genome but not to the transcriptome. Therefore, reads that mapped to the genome but not to the transcriptome were considered pre-mRNAs or RNAs with 5′outron sequence (Saito et al., 2013). Then the selected reads were annotated by ChIPseeker (Yu et al., 2015). The reads located to 150 nt around the TSS sites were considered non-trans-spliced transcripts at 5′ end of the gene. Genes with > 5 non-trans-spliced reads were selected for downstream analysis. Finally, the number of non-trans-spliced transcripts of each gene was normalized to the number of total reads in the mRNA-seq data.

Small RNA deep sequencing

Small RNAs were subjected to small RNA deep sequencing using an Illumina platform (Novogene Bioinformatics Technology Co., Ltd). Briefly, small RNAs ranging from 18 to 30 nt in length were gel-purified and ligated to a 3′ adapter (5′-pUCGUAUGCCGUCUUCUGCUUGidT-3'; p, phosphate; idT, inverted deoxythymidine) and a 5′ adapter (5′-GUUCAGAGUUCUACAGUCCGACGAUC-3′). The ligation products were gel purified, reverse transcribed, and amplified using Illumina's sRNA primer set (5′-CAAGCAGAAGACGGCATACGA-3'; 5′-AATGATACGGCGACCACCGA-3′). The samples were then sequenced using the Illumina HiSeq platform.

Small RNA-seq data analysis

The Illumina-generated raw reads were first filtered to remove adaptors, low-quality tags, and contaminants to obtain clean reads at Novogene. Then, clean reads ranging from 17 to 35 nt in length were mapped to *C. elegans* transcriptome assembly WS243 using Bowtie2 version 2.3.5.1 (Langmead and Salzberg, 2012) with the default parameters. The number of reads targeting each transcript was counted using custom Perl scripts. The number of total reads that mapped to the transcriptome minus the number of total reads corresponding to sense rRNA transcripts (5S, 5.8S, 18S, and 26S)

and sense protein-coding mRNA reads was used as the number for normalization to exclude possible degradation fragments of sense rRNAs and mRNAs.

Chromatin immunoprecipitation (ChIP)

ChIP experiments were performed as previously described (Guang et al., 2010). Worm samples in the adult stage were cross-linked in 2% formaldehyde for 30 min. Fixation was quenched with 0.125 M glycine for 5 min at room temperature. Samples were sonicated for 20 cycles (30 s on and 30 s off per cycle) at medium output with a Bioruptor 200. Lysates were precleared and immunoprecipitated with 1.5 μL of a rabbit anti-GFP antibody (Abcam, ab290) for SNPC-1.1, SNPC-3.4, SNPC-4, and TOFU-5 overnight at 4°C. Chromatin/antibody complexes were recovered with DynabeadsTM Protein A (Invitrogen, 10002D) followed by extensive sequential washes with 150 mM, 500 mM, and 1 M NaCl. Crosslinks were reversed overnight at 65°C. The input DNA was treated with RNase (Roche) for 30 min at 65°C, and all DNA samples were purified using a QIAquick PCR purification kit (Qiagen, 28104).

ChIP-qPCR

ChIP-qPCR was performed using a MyIQ2 real-time PCR system with SYBR GREEN mix (Vazyme C112-01, Nanjing). The enrichment of immunoprecipitation was calculated relative to the input samples. Intergenic DNA regions were used as controls for sample normalization. The primer sequences are listed in Table S3.

ChIP-seq

The DNA samples from the ChIP experiments were deep sequenced at Novogene Bioinformatics Technology Co., Ltd. Briefly, 10–300 ng of ChIP DNA was combined with End Repair Mix and incubated for 30 min at 20 °C, followed by purification with a QIAquick PCR purification kit (Qiagen). The DNA was then incubated with A-tailing mix for 30 min at 37°C. The 3′-end-adenylated DNA was incubated with the adapter in the ligation mix for 15 min at 20°C. The adapter-ligated DNA was amplified through several rounds of PCR amplification and purified in a 2% agarose gel to recover the target fragments. The average length was analyzed on an Agilent 2100 Bioanalyzer instrument (Agilent DNA 1000 Reagents) and quantified by qPCR (TaqMan probe). The libraries were further amplified on a cBot system to generate clusters on the flow cell and sequenced via a single-end 50 method on a HiSeq1500 system.

ChIP-seq data analysis

ChIP-seq reads were aligned to the WBcel235 assembly of the *C. elegans* genome using Bowtie2 version 2.3.5.1 (Langmead and Salzberg, 2012) by Ben Langmead with the default settings. The SAMtools version 0.1.19 (Li et al., 2009) "view" utility was used to convert the alignments to BAM format, and the "sort" utility was used to sort the alignment files. ChIP-seq peaks were called using Peakzilla version 1.0 (Bardet et al., 2013) with a permissive enrichment cutoff of 3 (default 2) and a score cutoff of 2 (default 1) against the ChIP-seq input sample. Custom Shell scripts were used to convert BAM files to BigWig format. Finally, the Integrative Genomics Viewer genome browser (Robinson et al., 2011) was applied to visualize signals.

Statistics

The mean and standard deviation of the results are presented in bar graphs with error bars. All experiments were conducted with independent *C. elegans* animals for the indicated number (N) of times. Statistical analysis was performed with the two-tailed Student's *t*-test or unpaired Wilcoxon test as indicated.

Data availability

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics, 2021) in National Genomics Data Center (Nucleic Acids Res, 2021), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA005832) that are publicly accessible at https://bigd.big.ac.cn/gsa/browse/CRA005832 (Chen et al., 2021; Members and Partners, 2021).

CRediT authorship contribution statement

Xinhao Hou: Conceptualization, Data curation, Software, Formal analysis, Visualization, Investigation, Methodology, Writing - Original draft. **Chengming Zhu**: Conceptualization, Validation, Data curation, Methodology. **Mingjing Xu**: Data curation, Resources. **Xiangyang Chen**: Conceptualization, Validation. **Cheng Sun**: Data curation, Validation. **Björn Nashan**: Conceptualization, Supervision. **Shouhong Guang**: Conceptualization, Supervision, Project administration, Writing - Original draft, Writing - Review & Editing. **Xuezhu Feng**: Conceptualization, Supervision, Project administration, Writing - Original draft, Writing - Review & Editing.

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Supplementary data

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