Interactions between the 5' UTR mRNA of the *spe2* gene and spermidine regulate translation in *S. pombe*

WENXIA SUN,¹ XUHUI ZHANG,¹ DONGRONG CHEN, and ALASTAIR I.H. MURCHIE

Fudan University Pudong Medical Center, Pudong and Key Laboratory of Medical Epigenetics and Metabolism, Institute of Biomedical Sciences, Shanghai Medical College, Fudan University, Shanghai 200032, China

Key Laboratory of Metabolism and Molecular Medicine, Ministry of Education, School of Basic Medical Sciences, Fudan University, Shanghai 200032, China

ABSTRACT

The 5' untranslated regions (5' UTR) of mRNAs play an important role in the eukaryotic translation initiation process. Additional levels of translational regulation may be mediated through interactions between structured mRNAs that can adopt interchangeable secondary or tertiary structures and the regulatory protein/RNA factors or components of the translational apparatus. Here we report a regulatory function of the 5' UTR mRNA of the *spe2* gene (SAM decarboxylase) in polyamine metabolism of the fission yeast *Schizosaccharomyces pombe*. Reporter assays, biochemical experiments, and mutational analysis demonstrate that this 5' UTR mRNA of *spe2* can bind to spermidine to regulate translation. A tertiary structure transition in the 5' UTR RNA upon spermidine binding is essential for translation regulation. This study provides biochemical evidence for spermidine binding to regulate translation of the *spe2* gene through interactions with the 5' UTR mRNA. The identification of such a regulatory RNA that is directly associated with an essential eukaryotic metabolic process suggests that other ligand-binding RNAs may also contribute to eukaryotic gene regulation.

Keywords: regulatory RNA; spermidine

INTRODUCTION

Eukaryotic translation initiation is a complex process in which mRNA is translated into protein by coordinated assembly of initiation complexes through interactions between the 5' mRNA untranslated regions (UTR) and components of the translation apparatus (the ribosomal subunits and eukaryotic initiation factors [eIFs]) (Jackson et al. 2010; Hinnebusch and Lorsch 2012; Sokabe and Fraser 2019). Although it is widely believed that translation within the 5' UTR of eukaryotic mRNA does not encode functional protein, it plays an important regulatory role in translation of the main ORF (Hinnebusch et al. 2016; Leppek et al. 2018); the 5' UTR of mRNA is physically located before the start codon and its potential for adopting interchangeable secondary or tertiary structures offers an extra level of regulation to translation. Currently regulatory elements within the 5' UTR of mRNA include diverse RNA structures and/or binding sites for RNA binding proteins or uORFs. For example, a conserved iron responsive RNA stem-loop close to the cap of the mRNAs of the iron storage

protein ferritin and the iron transporter ferroportin bind to iron regulatory proteins to regulate translation initiation (Gray and Hentze 1994; Muckenthaler et al. 1998, 2017); Guanine-tetraplex RNAs may bind proteins to repress translation (Schaeffer et al. 2001; Didiot et al. 2008; Agarwala et al. 2014; Lightfoot et al. 2018); An antisense RNA base pairs with the mRNA of mouse ubiquitin carboxyl-terminal hydrolase L1 (*Uchl1*) to regulate translation of (*Uchl1*) (Carrieri et al. 2012); A pseudo-knot structure in the 5' UTR of human interferon gamma regulates its own translation (Ben-Asouli et al. 2002; Cohen-Chalamish et al. 2009); eIF4A requires specific RNA structures or sequence motifs (Leppek et al. 2018); cellular Internal Ribosomal Entry Sites (IRES) in the 5' UTRs of mRNA in the homeobox gene family function to regulate translation in response to stress (Xue et al. 2015) and upstream open reading frames (uORFs) in the 5' UTR of S-adenosylmethionine decarboxylase (SAMDC) mRNA regulate translation through uORFs (see below in detail) (Miller-Fleming et al. 2015). Emerging evidence from gene-by-gene based studies

¹These authors contributed equally to this work.

Corresponding authors: aihmfudan.edu.cn, drchen@fudan.edu.cn

Article is online at <http://www.rnajournal.org/cgi/doi/10.1261/rna.072975.119>.

© 2020 Sun et al. This article is distributed exclusively by the RNA Society for the first 12 months after the full-issue publication date (see <http://majournal.cshlp.org/site/misc/terms.xhtml>). After 12 months, it is available under a Creative Commons License (Attribution-NonCommercial 4.0 International), as described at <http://creativecommons.org/licenses/by-nc/4.0/>.

demonstrates the importance of structured RNA in the regulation of translation (Leppek et al. 2018). Furthermore, recent advances in genome-wide analysis of RNA structure profiling have revealed the prevalence of structured RNA in cells (Kertesz et al. 2010; Ding et al. 2014; Rouskin et al. 2014; Mustoe et al. 2018; Tapsin et al. 2018).

Polyamines are essential to all kingdoms of life (Tabor and Tabor 1984). They are subject to tight homeostatic control, and are strictly regulated at the levels of transcription, translation, catabolism, protein turnover, and transport (Wallace et al. 2003; Casero et al. 2018; Lightfoot et al. 2018). Biosynthesis of polyamines occurs through the coordinated action of two decarboxylases (ornithine decarboxylase (ODC) and SAMDC) and two synthases (spermidine synthase [SPDS] and spermine synthase [SPMS]) as shown in Figure 1. SAMDC is one of the key regulatory points in the polyamine biosynthesis pathway.

In mammals and plants SAMDC is regulated at the level of translation and polyamine levels are the key regulators of SAMDC. One uORF that encodes a hexapeptide is located in the 5' UTR of SAMDC mRNA and is involved in modulation of SAMDC translation in mammals (Hill and Morris 1993). At high spermidine (Spd) concentrations, the ribosome stalls close to the termination site of the uORF, blocking access to the translation initiation site of the downstream SAMDC gene (Law et al. 2001; Raney et al. 2002; Ivanov et al. 2010; Miller-Fleming et al. 2015). In plants, two uORFs (named "5' tiny" and "3' small") have been identified in the 5' UTR of SAMDC. Through a coordinated polyamine responsive homeostatic mechanism, the uORF pair regulate translation of the SAMDC enzyme (Hanfrey et al. 2005; Ivanov et al. 2010). However, to date no uORF in *Saccharomyces cerevisiae* has been identified in the UTR of SAMDC mRNA (Mize and Morris 2001). In the fission yeast *Schizosaccharomyces*

pombe (*S. pombe*), although two uORFs are predicted, only one uORF encoding a tetrapeptide was detected by ribosome profiling in the UTR of SAMDC (SPE2) (Duncan and Mata 2014). The overall regulatory mechanism of SAMDC in translation seems divergent across species. The protein sequences of SAMDC between yeast and mammals also shares little identity (Kashiwagi et al. 1990). The mechanism of SAMDC (encoded by *spe2*) translation and function of the 5' UTR mRNA of *spe2* in translation regulation in *S. pombe* has not yet been reported.

Here we identified the 5' UTR mRNA of *spe2* as a functional RNA in translation and translational regulation in fission yeast. Reporter gene expression, cross-linking, and chemical probing, combined with mutational analysis demonstrate that the 5' UTR of *spe2* mRNA plays an important role in the regulation of translation.

RESULTS

The 5' UTR mRNA of *spe2* mediates reporter gene expression on addition of spermidine

The 5' UTR mRNAs have important roles in eukaryotic translation regulation through diverse mechanisms (Agarwala et al. 2014; Leppek et al. 2018). Here the translational function of the 5' UTR mRNAs of the key enzymes in the polyamine biosynthetic pathway (including the 5' UTR of SAMDC) in *S. pombe* fission yeast is investigated. Under physiological conditions the concentration of endogenous spermidine in the cell is precisely regulated through its biosynthetic pathway. It is not feasible to study the effects of Spd on gene expression in wild-type strains of *S. pombe*. We therefore developed a reporter system based on $\Delta spe2$ deletion mutants of SAMDC as a host strain in *S. pombe* for the reporter plasmid. The $\Delta spe2$ strain cannot synthesize Spd endogenously and requires supplementation with Spd to restore cell growth (Chattopadhyay et al. 2002). Addition of Spd to the growth medium allows the relative levels of Spd to be controlled in $\Delta spe2$ cells. The reporter plasmid REP81X-lacZ, contains a thiamine-repressive nmt81 promoter (Pnmt81), cloning sites, and the β -galactosidase (β -gal) reporter gene (Forsburg 1993). The 5' UTR of *spe2* was mapped by 5'RACE (Supplemental Fig. S1A), and the DNA sequence (198 nt including 21 nt coding sequence) was cloned in between Pnmt81 and the β -gal reporter gene of the plasmid (Fig. 2A). The reporter plasmid was transformed into the $\Delta spe2$ strain, and β -gal activity measured in solution in response to increasing doses of Spd (0, 0.001, 0.1, 10, and 1,000 μ M) with or without thiamine. In the absence of thiamine, where the Pnmt81 promoter is not repressed, the 5' UTR RNA was transcribed and we observed a progressive increase in β -gal expression as Spd concentration increased from 0 μ M to 10 μ M, 10 μ M Spd resulted in the highest β -gal expression (323.3 Miller units compared

FIGURE 1. A brief illustration of polyamine biosynthesis pathway. Dark blue indicates the regulated decarboxylases, the polyamine biosynthesis pathway is shown in light blue, and S-adenosyl methionine metabolites are shown in green.

FIGURE 2. Spd control of reporter gene expression through the 5' UTR of *spe2*. (A) The lacZ reporter constructs, incorporating the 5' UTRs of *spe2*, *aru1*, *srn1*, and *sam1*. (B) β-gal expression in the presence of 0, 10, and 1000 μM of Spd with (±) thiamine in *Δspe2* cells transformed by empty reporter vector or the *spe2* 5' UTR reporter plasmid. Statistical significance was calculated compared to controls using Student's t-test, (**) $P < 0.01$. The error bars are the standard deviation of three independent experiments. (C) β-gal expression measured in response to increasing doses of Spd without thiamine in *Δspe2* cells that were transformed with the reporter plasmid containing the 5' UTRs, as listed in A. Statistical significance was calculated compared to other controls using Student's t-test, (**) $P < 0.01$. The error bars are the standard deviation of three independent experiments. (D) The GFP reporter constructs with the 5' UTRs of *spe2*. (E) Reporter activity of *Δspe2* cells transformed with the *spe2* 5' UTR GFP reporter plasmid, in response to 10 μM of Spd with (+) and without (–) thiamine (thi). GFP was detected by western blot. GFP expression is enhanced approximately fivefold by adding 10 μM of Spd, relative to no Spd normalized against actin.

with 51.7 Miller units without Spd, ~6.3-fold induction). At higher Spd concentrations from 10 μM to 1000 μM, β-gal expression was repressed. As a control, in the presence of thiamine, where the promoter is repressed and the UTR RNA not made, no β-gal expression was observed on Spd titration (Fig. 2B). As an additional control, cells transformed with the empty vector with no UTR sequence inserted, showed no β-gal expression on Spd titration with or without thiamine (Fig. 2B). We also cloned the DNA sequences of 5' UTRs of other enzymes (Wilhelm et al. 2008) in the polyamine biosynthesis pathway of *S. pombe* including arginase (*Aru1*), spermidine synthase (*Srn1*) and SAM synthetase (*Sam1*) into the reporter construct (Fig. 2A). Basal levels of β-gal expression were detected with these constructs in the absence of Spd, and no significant change of β-gal expression was observed on Spd titration (Fig.

2C). In summary, from the construct where the *spe2* UTR RNA was made, we observed progressive induction and repression of β-gal expression in response to Spd titration, in contrast, the controls that lack the *spe2* UTR RNA, display only basal levels of β-gal expression with or without Spd. When the *spe2* UTR RNA is replaced by the UTR of other enzymes in the pathway, low basal levels or no regulation of β-gal expression was seen on Spd titration. These careful controls rule out the possibility that the regulation observed upon Spd titration was due to general Spd dependent translational stimulation (Igarashi and Kashiwagi 2010, 2015). We further replaced β-gal with the alternative reporter GFP and measured GFP expression by western blotting in the presence or absence of thiamine with or without 10 μM Spd (Fig. 2D,E). With 10 μM Spd, the GFP induction was comparable to that of β-gal. All

these data confirm that the presence of both the 5' UTR of *spe2* and Spd are required for translation and regulation of reporter gene translation.

Spermidine binds to the 5' *spe2* UTR RNA in vitro

We speculated that direct interactions between Spd and the *spe2* UTR may be responsible for the regulation of reporter gene translation. Binding of Spd and related ligands to *spe2* RNA was measured by surface plasmon resonance spectroscopy (SPR) (Hendrix et al. 1997; Jia et al. 2013). The *spe2* UTR RNA was prepared by in vitro transcription using T7 RNA polymerase. The in vitro transcribed RNA was biotinylated and immobilized onto a streptavidin SA-biosensor chip, and the binding of Spd and analogs was measured by flowing them over the immobilized RNA. Both Spd and Spermine (Spm) bound to the in vitro transcribed RNA with affinities of 78.9 and 88.1 μ M, respectively, which is in the physiological range (Fig. 3A–C; Igarashi and Kashiwagi 2010), indicating that the shared chemical groups within the structure between Spd and Spm are important for binding. However, other related ligands putrescine (Put), ornithine (Orn), cadaverine (Cad), and methionine (Met)

showed no obvious binding to the in vitro transcribed RNA by SPR (Fig. 3C).

To further identify potential Spd binding sites in the 5' UTR RNA, a photoactive diazirine (Fig. 4A) derivative of Spd (DA-Spd) was synthesized (Gomes and Gozzo 2010). The in vitro transcribed 5' UTR RNA was irradiated with UV light in the presence of DA-Spd, and the sites of specific DA-Spd crosslinks were mapped by reverse transcriptase primer extension and sequenced directly by capillary electrophoresis with fluorescence detection. Small molecule–RNA UV crosslinks can be identified by primer extension (Jia et al. 2013). The sites of specific DA-Spd crosslinks on *spe2* RNA were identified by the position of abortive reverse transcripts when compared to two controls; the 5' UTR RNA UV irradiated with DA-Spd, and a 10-fold excess of unlabeled Spd and the 5' UTR RNA irradiated with inactive DA-Spd. With 10 μ M photo-activated DA-Spd, we detected some strong crosslinks, which are absent in the control containing photo-inactivated Spd or in the control when 100 μ M Spd competed with 10 μ M photo-activated DA-Spd. These crosslinks indicated that the reverse transcription indeed stopped at the location of the Spd–RNA crosslinking site. We identified strong crosslinks at nucleotides such as A29, C36, C96, C136,

FIGURE 3. The polyamines bind to the *spe2* UTR RNA. (A) Change in SPR signal in response units (RU) on Spd bound to immobilized *spe2* UTR RNA. Hill coefficient is shown. (B) Change in SPR signal in RU on Spm bound to immobilized *spe2* UTR RNA. Hill coefficient is shown. (C) The relationship between polyamine structure, binding affinity, and reporter gene induction. NB represents no binding.

A potential RNA secondary structure was predicted by computational folding of the 5' UTR RNA using Vienna fold (Fig. 4D; Hofacker 2003), and to test the predicted structure, we performed structural covariance analysis based on mutagenesis. We prepared random mutants with mutational locations spanning the entire RNA. The effect of each mutation on the reporter gene expression in

the presence or absence of Spd was measured and compared with the wild-type construct. Screening by reporter gene expression of the mutants identified 23 functional mutants and 17 mutants that had lost their function. The inactive mutants are analyzed in the final section. The 23 functional mutants, whose levels of reporter gene expression are close to that of the wild-type construct (Supplemental Fig. S1B), were used for structural covariance analysis by CMfinder () (Fig. 4D; Supplemental Fig. S1C; Yao et al. 2006). The predicted secondary structure of the 5' UTR RNA by Vienna fold was identical to the one from the covariance analysis by CMfinder.

To further investigate the secondary structure of the 5' UTR RNA, we performed dimethyl sulphate (DMS) chemical probing (Tijerina et al. 2007; Jia et al. 2013; Ding et al. 2014; Rouskin et al. 2014). In DMS-probing experiments, the products of primer extension were detected by capillary electrophoresis with fluorescence detection. The results from the DMS probing are largely consistent with the predicted structure by Vienna fold and the structural covariance analysis based on mutagenesis (Fig. 4C,D; Supplemental Fig. S1C). The secondary structure of the 5' UTR RNA is composed of junction, stems, and loops. The identified crosslinks, based on this secondary structure (Fig. 4D), imply that a tertiary structure fold of the 5' UTR RNA may be important for its function.

Osmium tetroxide (OsO₄) detects tertiary structural changes in the *spe2* UTR RNA upon spermidine binding

We performed DMS analysis on the 5' UTR RNA on titration of Spd. No obvious secondary structure change was detected in the in vitro transcribed 5' UTR RNA upon Spd binding (data not shown). We further investigated if a tertiary structure change occurs within the 5' UTR RNA in the presence of Spd. The OsO₄/pyridine modifies RNA at the 5–6 double bond of pyrimidines, predominantly at unstacked uracil residues that are conformationally exposed on the surface of the folded RNA and accessible to out-of-plane attack by OsO₄/pyridine (Zhang et al. 2017). We therefore probed the 5' UTR RNA with OsO₄ in the presence of increasing doses of Spd and its analogs. In vitro transcribed RNAs were incubated with ligands, followed by OsO₄/pyridine modification. The OsO₄-modified RNA was reverse-transcribed with a fluorescein (FAM)-labeled primer and analyzed by capillary gel electrophoresis. The modification sites were detected by the position and incidence of abortive reverse transcripts. The OsO₄ modification of RNA yielded a set of electropherograms composed of a series of fluorescent peaks (Fig. 5A). The fluorescence signals reflect the reactivity of OsO₄ modification at each nucleotide. A number of fluorescent peaks were detected, the majority of which corresponded to OsO₄-modified uracils (Fig. 5A; Supplemental

Fig. S2). Spermidine titration led to changes in OsO₄ reactivity at specific nucleotides over the range of Spd concentrations (Fig. 5A; Supplemental Fig. S2). The most noteworthy change generated on Spd titration was at nucleotides 59U–61U within a predicted loop. Without Spd, high fluorescent peaks were observed at nucleotides 59U–61U, and upon increasing doses of Spd, the OsO₄ reactivity at 59U–61U became progressively reduced, consistent with a *K_D* of 14.88 μ M (at U59) in a similar range to that measured by SPR (Figs. 3A, 5A–C). This is consistent with the 5' UTR RNA adopting a less constrained and “open” conformation in the absence of Spd, such that the loop nucleotides 59U–61U are ostensibly unstacked and accessible to OsO₄ modification. In contrast, upon Spd binding the 5' UTR RNA adopts a more compact structure in which access to the 5–6 double bonds of 59U–61U become screened by the tertiary structure of the 5' UTR RNA rendering them inaccessible to out-of-plane attack by OsO₄/pyridine with consequent low reactivity.

Mechanism of *spe2* UTR RNA in regulation of translation

Ligand-sensing RNAs can control gene expression at the level of transcription or translation (Henkin 2008). We analyzed the mRNA abundance of the β -gal reporter in response to titration of Spd by real-time PCR. The results showed that the abundance of reporter gene mRNA did not obviously correlate with the Spd-mediated regulation of expression on Spd titration (Figs. 2C, 6A), suggesting that Spd–RNA binding probably regulates Spd-dependent gene expression through interactions with the translational machinery (Igarashi and Kashiwagi 2010, 2015).

Reporter assay data showed that translational regulation of the reporter gene required both *spe2* UTR RNA and Spd. Direct binding of Spd to RNA was measured and the tertiary structure transition upon Spd binding was detected by OsO₄ probing. The 5' UTR RNA adopts tertiary structures which may interact with the translation apparatus to maintain sustainable levels of translation in the absence of Spd but can undergo a structural transition upon Spd binding to regulate translation of *spe2*. To explore the relationship between the Spd induced structural transition in the 5' UTR RNA and the functional role of the 5' UTR RNA and to identify regulatory components within the 5' UTR RNA structure, inactive mutations were selected from the mutagenesis screen (described above) for further investigation. These mutants were analyzed in the reporter assay and compared to the wild-type RNA (Fig. 6B,C; Supplemental Fig. S3A,B). The mutants could be separated into two groups based on expression levels; mutants (M24–30) with very low levels of β -gal expression (Fig. 6C) and mutants (M31–41) (Supplemental Fig. S3B) that exhibited reduced levels of β -gal expression compared to the wild-type RNA with or without Spd (Supplemental

FIGURE 5. OsO₄ probing of tertiary structure of *spe2* RNA on polyamine titration. (A) Analysis of OsO₄ modification electropherogram of RNA with 30 μ M Spd (blue trace), compared with 0 μ M Spd (red trace). (B) Histogram of OsO₄ modification of the in vitro transcribed RNA nucleotides (58–62) on titration of Spd. The reactivity of the nucleotide is calculated as the ratio of the height of the nucleotide to the height of all nucleotides. (C) Fitting curve for the ratio of OsO₄ modification signal of position U59 to that of full length on titration of Spd; KD is shown.

Fig. S3B). In the absence of Spd these mutants (M24–30) across the entire RNA caused a reduction in levels of translation suggesting that the whole RNA structure contributes to basal translation (Fig. 6D). These mutations (M25–26, M28, M30, M32, M34, and M36–37) lead to a loss of response to high concentrations of Spd, (Fig. 6E). At higher Spd concentrations (10 μ M to 1000 μ M), translation of the wild-type RNA was repressed, but in those mutants translation repression was lost, which indicates that the position of the mutation may be involved in translation repression in response to high doses of Spd (Fig. 6E). Although we also note that the position (59U–61U) on the in vitro transcribed 5' UTR RNA that shows the biggest Spd dependent changes in reactivity to OsO₄ also overlaps the site of the M25 mutation (60U–64U to 60A–64A). This analysis reveals that the positions of the mutants are important for translation regulation (Fig. 6B; Supplemental Fig. S3A).

Changes in OsO₄ reactivity at U59–61 in the wild-type RNA indicated a tertiary structure change in the in vitro transcribed 5' UTR RNA upon Spd binding. To investigate if such a structural transition was responsible for the changes in β -gal expression, we implemented OsO₄/pyridine probing on three of the inactive mutants (M28–30). Compared to the wild-type RNA, OsO₄ probing of M29 showed that U59–61 reactivities remained unchanged upon Spd titration (Fig. 6F; Supplemental Fig. S4), indicating that no tertiary structural change occurred when Spd was added. For M28 and M30, OsO₄ reactivities at U59–61 also differ compared to the wild-type RNA (Fig. 6F; Supplemental Fig. S4). Therefore, there is a good association between specific tertiary structure of RNA and transla-

tion. The tertiary structure transition of RNA upon Spd binding appears to be essential for translation regulation. It is possible that the mutations M24–28 and M30 also interact directly with the translational apparatus, we note that mutations M25–27 introduce novel uORF sequences and that the mutants M24, M28, and M30 alter the sequence contexts of the initiation codons for uORF1 and uORF2 and for the main ORF, respectively; the possibility that direct translational effects contribute to the repression observed for these particular mutants cannot be excluded.

The uORFs in the UTR of SAMDC in mammals and plants show polyamine mediated regulation of translation (Hill and Morris 1993; Law et al. 2001; Raney et al. 2002; Ivanov et al. 2010; Miller-Fleming et al. 2015). No uORFs are present in the UTR of SAMDC in the budding yeast *S. cerevisiae* (Mize and Morris 2001). In *S. pombe*, two putative uORFs are present in the *spe2* UTR and one of them shows relatively low ribosomal occupancy (Duncan and Mata 2014). To investigate the function of this putative uORF, we mutated the first codons of the first uORF (M6) and second uORF (M22) to prevent peptide synthesis (Fig. 7A,B). Upon Spd titration, reporter gene expression for M6 and M22 was close to that of the wild-type cells (Fig. 7C). A series of mutations to the supposed peptide codons of the first uORF were further introduced (M42–M45), in which the 5' UTR RNA sequence was changed, but the amino acid sequence of the peptide remains unaltered (Fig. 7B; Jia et al. 2013). These mutations led to significant reductions in translation without Spd (with the exception of M44), and altered translation regulation with Spd (Fig. 7C). If the peptide played a critical role in

FIGURE 6. Functional and structural specificity of Spd binding to the *spe2* UTR RNA. (A) Real-time PCR analysis of mRNA abundance of the *spe2* UTR RNA relative to the internal control tubulin, showing the level of mRNA abundance of lacZ (representing *spe2*) remains unchanged on Spd titration. Error bars represent the standard deviation of three independent experiments. Statistical significance was shown compared with 0 μ M, respectively, using Student's *t*-test, (**) $P < 0.01$. (B) The positions (boxed dark blue) and identity of the mutations on the secondary structure of the *spe2* UTR RNA. The position boxed in purple indicates the mutation of the initiation codon of the uORF. (C) Reporter gene expression of seven inactive RNA mutations, responding to 0, 10, and 1000 μ M of Spd. The error bars are the standard deviation of at least three independent experiments. Statistical significance of each mutant was calculated compared to that of WT at 0, 10, and 1000 μ M, respectively, using Student's *t*-test, (**) $P < 0.01$. (D) Reporter gene expression of seven inactive RNA mutations at 0 μ M of Spd. The error bars are the standard deviation of at least three independent experiments. Statistical significance of each mutant was calculated compared to that of WT at 0 μ M, respectively, using Student's *t*-test, (**) $P < 0.01$. (E) Reporter gene expression of inactive RNA mutations M25–26, M28, M30, M32, M34, and M36–37, responding to 0, 10, and 1000 μ M of Spd. The error bars are the standard deviation of at least three independent experiments. Statistical significance of each mutant was calculated compared to that of WT at 0, 10, and 1000 μ M, respectively, using Student's *t*-test, (**) $P < 0.01$. (F) Histogram analysis of OsO₄ modification for nucleotides U59 of the wild-type and three inactive mutation RNAs on Spd titrations.

FIGURE 7. The uORFs in the *spe2* 5'UTR have no dominant translational function. (A) The locations of the micro-ORF in the *spe2* UTR; gray indicates peptide sequence, and black rectangle filled with yellow indicates start codon mutation M6 and M22 in the peptide. (B) Sequences of the wild-type peptide and the corresponding RNAs, M6 start codon mutant and their degenerate mutants M42–45 (mutations marked pink). The peptide RNA sequences of M42–45 were altered, whereas the peptide amino acid sequences remained unchanged. The sequences of the M22 start codon mutant are also shown. (C) β-gal activity measured in the presence of 0, 10, and 1000 μM of Spd without thiamine in Δ*spe2* cells that were transformed with the reporter plasmid containing mutants M6, M22, and M42–45. Statistical significance was calculated compared to WT at 0, 10, and 1000 μM, respectively, using Student's *t*-test, *P* < 0.01.

translation of the reporter gene, as is the case in mammals or plants (Hill and Morris 1993; Law et al. 2001; Raney et al. 2002; Hanfrey et al. 2005), such changes in translation and its regulation would not be expected. These data indicate that the uORF does not play a dominant role in the regulation of *spe2* expression and that the 5' UTR RNA of *spe2* is important for translation and its regulation upon Spd binding.

DISCUSSION

Here we show that the 5'UTR mRNA of the *spe2* gene plays a significant role in the regulation of translation through RNA structure transitions upon spermidine binding. We provide the following evidence to support this conclusion:

(i) Regulation of reporter gene expression is dependent on both the presence of spermidine and the UTR mRNA of the *spe2* gene (Fig. 2B,C). (ii) The 5'UTR mRNA of the *spe2* gene directly binds spermidine (SPR) (Fig. 3A, C). (iii) The secondary structure of the 5'UTR mRNA of *spe2* was determined by three independent methods: DMS probing, covariance analysis upon mutagenesis and computational folding (Vienna fold). OsO₄ probing reveals that the 5'UTR mRNA of *spe2* undergoes a tertiary structure transition upon spermidine binding (Fig. 5A,B). (iv) Mutational analysis showed that the entire 5'UTR mRNA of *spe2* was required for basal translation (Fig. 6C–E; Supplemental Fig. S3B). Mutational analysis also identified important RNA structural elements that contribute to the regulation of translation upon spermidine binding. In the wild-type RNA where translation regulation with Spd occurs, a tertiary structure transition can be detected by OsO₄ probing. However, in mutants where Spd-dependent translational regulation is impaired, this tertiary structure transition is not observed (Fig. 6F). Thus, regulation of translation in response to spermidine is dependent on the 5'UTR RNA tertiary structure transition upon spermidine binding.

From Spd titrations, expression levels of β-gal are induced at low Spd concentrations and repressed at higher Spd concentrations (Fig. 2B) and represent a logical mechanism for regulating cellular Spd levels. The Morris group have demonstrated a negative feedback mechanism in mammalian cells in the presence of high Spd levels (Hill and Morris 1993; Law et al. 2001; Raney et al. 2002) and this finding is consistent with our observation of repression of reporter gene expression at high doses of Spd. At limiting concentrations of Spd, *spe2* expression was up-regulated to increase Spd production and consequently maintain polyamine levels. Upon Spd starvation, Δ*spe2* cells display growth abnormalities (Chattopadhyay et al. 2002), but can survive on as little as 10^{−6} M supplementary Spd (Chattopadhyay et al. 2002). Therefore, under conditions in which Spd levels become limiting, cells need to synthesize sufficient amounts of Spd for survival. Fluctuations in Spd levels may occur in cells under various conditions; stresses associated with specific tissue types, developmental stages or diseases such as cancer (Eisenberg et al. 2009; Damiani and Wallace 2018; Madeo et al. 2018). Chemical probing of the *in vitro* transcribed RNA with OsO₄ shows that the 5'UTR RNA undergoes a tertiary structural change upon Spd binding, that is essential for the expression of the reporter gene. At low levels of Spd, the 5'UTR RNA adopts a more open structure; when Spd levels increase a more compact conformation of the 5'UTR RNA is induced by Spd binding and then, at higher Spd concentrations further tertiary interactions effectively block the structure, preventing translation. It is probable that inside the cell, the competing tertiary structures are in dynamic equilibrium and can

exchange from one conformation to another in response to fluctuations in the levels of cellular Spd, such that Spd binding drives the equilibrium between “induced” and “repressed” states of the *spe2* UTR to control *spe2* translation. Therefore, the *spe2* UTR effectively acts as a “sensor” of Spd to regulate the expression of the SAMDC gene. The data presented in this study offers insights into the features of the *spe2* 5′ UTR mRNA that regulate translation in response to spermidine binding.

An advantage of the reporter system is the simplification of a complex network of polyamine regulation, which allows us to study the translational regulatory function of the 5′UTR mRNA of *spe2* in response to controlled addition of exogenous Spd. In an independent study, the corresponding DNA sequence of a SAM-sensing RNA was cloned into the reporter construct of this work. In the presence of Met (SAM precursor), the translation of β-gal was progressively repressed (Zhang et al. 2020). The striking differences in the reporter gene expression between the two studies suggest that the reporter construct worked reliably for detection of small ligand interaction with RNA in vivo.

In mammalian and plant cells, polyamine responsive translation of SAMDC is regulated by uORFs in the leader RNA (Law et al. 2001; Hanfrey et al. 2005). The *spe2* UTR of the fission yeasts also has a small, putative uORF that encodes a short peptide. However, a number of mutations outside the uORF also have remarkable effects on reporter gene expression (e.g., M24–41, Fig. 6C; Supplemental Fig. S3B), while the mutation of the uORF start codon of M6 shows close to wild-type spermidine dependent reporter gene expression (Fig. 7C). Although the uORF may be involved in *spe2* expression through ribosomal recruitment, it does not appear to play a direct role in the regulation of *spe2* expression in response to spermidine and there is no obvious correlation between the putative uORF function and spermidine dependent reporter gene expression (Fig. 3A–C). In our experimental system, spermidine binding to the UTR RNA of *spe2* has a dominant effect on gene expression (Figs. 2B, 3A,C). The possibility that uORF encoded short peptides may have a regulatory function cannot be excluded, however, a regulatory role for such peptides would require further investigation.

In this study, we find that the eukaryotic 5′ UTR mRNA of *spe2* interact with the ligand spermidine to regulate translation in *S. pombe*. In bacteria, 5′ UTR mRNAs can also bind to small ligands to regulate translation (Jia et al. 2013; Serganov and Nudler 2013; Breaker 2018). The mechanism of translation regulation through 5′ UTR mRNAs is significantly different between prokaryotes and eukaryotes. Riboswitch regulation of translation depends on interactions between the 5′UTR RNA and ligand only and no protein factors are involved (Henkin 2008; Breaker 2012; Serganov and Nudler 2013). In contrast, eu-

karyotic 5′ UTR mRNAs regulate translation initiation by interacting with regulatory protein/RNA factors or components of the translational machinery. In *S. pombe* the mutations M28 and M30 reduced basal translation to very low levels, we speculate that the sites of these mutations may directly interact with the translational apparatus.

Overall identification and characterization of this unique spermidine binding eukaryotic 5′ UTR eukaryotic mRNA, which is involved in a pivotal metabolic process in fission yeast, suggests the possibility of a more widespread role for ligand-sensing regulatory RNAs in eukaryotic gene translation.

MATERIALS AND METHODS

Experimental strains

Spe2 was deleted in *h-leu1-32* strain (a kind gift from Jürg Bähler) by using a PCR-based approach, as described previously (Bähler et al. 1998), using the plasmid pFA6a-kanMX6 as the PCR template. The primer sequences can be found in Supplemental Table 1. The entire coding region of *spe2* was replaced by Kan^r in the Δ*spe2* strain. The deletion was initially verified by PCR and sequencing and further confirmed by genetic complementation. The Δ*spe2* strain cannot grow without added Spd in minimal medium, and this phenotype was rescued by transforming a plasmid that expressed the *spe2* protein.

Determination of UTR of *spe2* by 5′RACE

Total RNA was extracted from 10 mL of cell culture (972 h-) grown to OD 0.5, as described previously (Chen et al. 2003). The extracted RNA was treated with DNase I (Fermentas) to remove DNA from the RNA sample. The transcription start site was identified using SMART RACE cDNA amplification kit (Clontech), according to the manufacturer’s instructions. After PCR, the PCR products were subcloned into the TA cloning vector. The transcription start site was determined from the DNA sequence of the clones (Sangon Biotech).

Construction of the reporter plasmid and mutagenesis

The plasmid Rep81x-lacZ (Forsburg 1993) (a kind gift from the Forsburg laboratory), containing the Pnmt81 promoter, polylinker sites, and the in-frame lacZ coding sequence, was used as a vector to clone the reporter plasmids in this study. The polylinker site was modified by adding a BglII site. The DNA sequence corresponding to the 5′ UTRs of *spe2*, *aru1*, *srn1*, and *sam1* was individually amplified and inserted into the BglII and XhoI sites of the vector. Mutations to the *spe2* UTR were generated by using site mutagenesis with DpnI (NEB), following the manufacturer’s instructions. The forward primers for mutagenesis were listed in Supplemental Table S1. Reverse primers are the reverse complementary strand of the corresponding forward primers. All primers were supplied by Sangon Biotech. All clones were confirmed by DNA sequencing.

The fission yeast reporter assay

Δ Spe2 cells transformed with the reporter plasmid were initially grown on EMM plates with 10 μ M Spd for 3 d, followed by transfer to EMM liquid medium without Spd. Cells were diluted to OD 0.1 in 5 $\times$ 10 ml of EMM. To each diluted culture, Spd was added to a final concentration of 0, 0.001, 0.1, 10 μ M, and 1 mM and grown further for 16 h. For the control sample, 60 μ M of thiamine was added in parallel to the medium. Cells ($\sim 10^6$) were harvested and resuspended in 1 mL of Z buffer (60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 10 mM KCl, 1 mM MgSO₄, 50 mM 2-mercaptoethanol, pH 7.0). Cells were diluted thrice with Z buffer, and 600 μ L of cell suspension was mixed with 70 μ L of chloroform and 60 μ L of 0.1% SDS, followed by mixing for 10 sec and incubated at 30°C for 15 min, after adding 120 μ L of 4 mg/mL o-nitrophenyl β -D-galactopyranoside (ONPG), and further incubated for 15–20 min (30°C). The reaction was quenched by the addition of 400 μ L of 1 M sodium carbonate. The OD₄₂₀ and OD₆₀₀ were measured, and Miller units were calculated from the formula: $U = 1000 \times OD_{420}/(Time) \times (Vol) \times OD_{600}$ (Zhang and Bremer 1995). Data were presented as the Mean $\pm$ SD from three individual replicates.

Covariance analysis

A potential RNA secondary structure was predicted by computational folding of the 5' UTR RNA using Vienna fold <http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi> (Hofacker 2003) and to test the predicted secondary structure, we performed structural covariance analysis based on mutagenesis. We prepared random mutants with mutation locations spanning the entire RNA and singled out the mutants in which the in vivo β -gal expression was close to the wild-type construct. Based on these mutant RNA sequences, the covariance motifs for *spe2* UTR RNA were analyzed by CMfinder <http://bio.cs.washington.edu/yizhen/CMfinder/> (Yao et al. 2006). Then, the structure output was depicted by R2R software <http://breaker.research.yale.edu/R2R> (Weinberg and Breaker 2011).

DMS probing

An amount of 20 pmol RNA was dissolved in 50 mM Hepes-KOH buffer (pH 7.9) with 100 μ M MgCl₂. RNA was heated to 90°C and annealed. A total of 2 μ L 10 $\times$ Spd stock solution was added to a total volume of 20 μ L and incubated for 1 h at 25°C. DMS probing was performed by adding 1%–5% of DMS (Sigma) and incubated at 25°C for 5 min. DMSO instead of DMS was added as control sample. The reaction was stopped by addition of 475 μ L stop solution (30% [v/v] β -mercaptoethanol, 0.3 M sodium acetate) and 1 mL ethanol and recovered by ethanol precipitation. Primer extension was performed using SuperScript III Reverse transcriptase (Thermo Scientific) and was used to determine positions of modification with 5' FAM-labeled primer (Supplemental Table S1), abortive DNA sequences were analyzed by ABI 3730 DNA sequencer (Tijerina et al. 2007; Jia et al. 2013; Ding et al. 2014).

Crosslinking analysis of Spd with RNA

The photoactive DA-Spd (Gomes and Gozzo 2010) was prepared by incubating a final concentration of 1 mM of Spd with 1 mM of

LC-SDA (Thermo Scientific, 26168) at room temperature for 30 min in PBS buffer, according to the manufacturer's protocol. The reaction was quenched by the addition of Tris buffer to a final concentration of 50 mM. RNA was incubated with 10 μ M of DA-Spd in 50 mM Tris-HCl (pH 8.3), 100 mM KCl, at 30°C, and UV irradiated at 365 nm for 5 min. Additional crosslinking reactions were prepared in parallel as controls; RNA was UV-irradiated after incubation with 10 μ M of DA-Spd with a 10-fold excess of unlabeled Spd. RNA was also UV-irradiated with an inactivated diazirine Spd sample in which the active diazirine ester had been quenched before adding Spd. The presence of specific crosslinks between RNA and Spd was measured by the position of abortive reverse transcripts, compared with that of the control RNAs (Porse et al. 1999; Jia et al. 2013).

SPR

In vitro transcripts of 5' UTR RNA of *spe2* (198 nucleotides, including 21 nt of the *spe2* coding sequence) were 3' end labeled with biotin (Jia et al. 2013). SPR binding of RNA by polyamines or other ligands was performed on a Biacore X-100 instrument. Biotinylated RNA was coupled to a SA-chip (GE Healthcare), a series of increasing concentrations of Spd and its related analogs were perfused over the same regenerated surface in 10 mM Hepes-KOH (pH 7.4) 150 mM NaCl buffer with a flow rate of 10 μ L per minute (Hendrix et al. 1997; Jia et al. 2013). The equilibrium dissociation constant (KD) for each interaction was determined by fitting with the OriginPro 9.1 software.

Western blot

The experiment was performed with SDS-PAGE and MOPS SDS running buffer (Sun et al. 2011). A reporter strain expressing the *spe2* UTR fused to a GFP tag (Fig. 2B) was grown in 50 mL of EMM medium with or without 60 mM of thiamine at 30°C to an OD₆₀₀ of 0.5 in each condition with or without 10 μ M Spd. GFP levels for the same cell number were detected by the GFP antibody (Santa Cruz, sc-9996), with Actin (abcam, ab8224) as an internal control. The expression level of the fusion protein was quantified using the Image lab 4.0 software quantification tool, and normalized to the control sample actin.

Real-time PCR

Δ Spe2 cells transformed with the reporter plasmid were cultured in EMM medium on titration of Spd. Cells grown to OD_{0.5} were collected. Total RNA was extracted by hot phenol protocol and Dnase I digested. cDNA was synthesized using PrimeScript RT-PCR Kit (Takara, RR057) according to the manufacturer's manual. Messenger RNA abundance of lacZ (β -galactosidase reporter) from the reporter plasmid was detected by real-time PCR (PCR primers see Supplemental Table S1) using SYBR Premix Ex Taq Kit (Takara, RR041A) with increasing amounts of Spd, tubulin as an internal reference (Jia et al. 2013). Mean $\pm$ SD from three biological replicates was shown.

Osmium probing

Osmium probing was performed (Zhang et al. 2017). A series of concentrations of Spd and analogs were incubated with

~20 pmol of RNA in buffer (50 mM Hepes-KOH [pH 7.9], 100 μ M MgCl₂), and RNA was probed by 1 mM of OsO₄/pyridine at 25°C for 45 min. The reaction was stopped by ethanol precipitation, and the probing position was measured by reverse transcription and sequencing with a 5' FAM-labeled primer (Supplemental Table S1).

SUPPLEMENTAL MATERIAL

Supplemental material is available for this article.

ACKNOWLEDGMENTS

We thank Susan Forsberg for the gift of the plasmid Rep81x-lacZ, Jürg Bähler for the gift of the strain *h-leu1-32*, Gu Jungkai for assistance with capillary sequencing, and David Lilley (Dundee University) and members of the Murchie laboratory for discussion. This work was supported by Natural Science Foundation grants 2016YFA0500604, 31420103907, 31770873, and 31330022 to A.M., Natural Science Foundation grant 31370107 to D.C. and 31470777 to W.S.

Received August 22, 2019; accepted December 10, 2019.

REFERENCES

- Agarwala P, Pandey S, Maiti S. 2014. Role of G-quadruplex located at 5' end of mRNAs. *Biochim Biophys Acta* **1840**: 3503–3510. doi:10.1016/j.bbagen.2014.08.017
- Bähler J, Wu JQ, Longtine MS, Shah NG, McKenzie A, Steever AB, Wach A, Philippsen P, Pringle JR. 1998. Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. *Yeast* **14**: 943–951. doi:10.1002/(SICI)1097-0061(199807)14:10%3C943::AID-YEA292%3E3.0.CO;2-Y
- Ben-Asouli Y, Banai Y, Pel-Or Y, Shir A, Kaempfer R. 2002. Human interferon- γ mRNA autoregulates its translation through a pseudoknot that activates the interferon-inducible protein kinase PKR. *Cell* **108**: 221–232. doi:10.1016/S0092-8674(02)00616-5
- Breaker RR. 2012. Riboswitches and the RNA world. *Cold Spring Harb Perspect Biol* **4**: a003566. doi:10.1101/cshperspect.a003566
- Breaker RR. 2018. Riboswitches and translation control. *Cold Spring Harb Perspect Biol* **10**: a032797. doi:10.1101/cshperspect.a032797
- Carrieri C, Cimatti L, Biagioli M, Beugnet A, Zucchelli S, Fedele S, Pesce E, Ferrer I, Collavin L, Santoro C, et al. 2012. Long non-coding antisense RNA controls Uchl1 translation through an embedded SINEB2 repeat. *Nature* **491**: 454. doi:10.1038/nature11508
- Casero RA, Murray Stewart T, Pegg AE. 2018. Polyamine metabolism and cancer: treatments, challenges and opportunities. *Nat Rev Cancer* **18**: 681–695. doi:10.1038/s41568-018-0050-3
- Chattopadhyay MK, Tabor CW, Tabor H. 2002. Absolute requirement of spermidine for growth and cell cycle progression of fission yeast (*Schizosaccharomyces pombe*). *Proc Natl Acad Sci* **99**: 10330–10334. doi:10.1073/pnas.162362899
- Chen D, Toone WM, Mata J, Lyne R, Burns G, Kivinen K, Brazma A, Jones N, Bähler J, Botstein D. 2003. Global transcriptional responses of fission yeast to environmental stress. *Mol Biol Cell* **14**: 214–229. doi:10.1091/mbc.e02-08-0499
- Cohen-Chalamish S, Hasson A, Weinberg D, Namer LS, Banai Y, Osman F, Kaempfer R. 2009. Dynamic refolding of IFN- γ mRNA enables it to function as PKR activator and translation template. *Nat Chem Biol* **5**: 896–903. doi:10.1038/nchembio.234
- Damiani E, Wallace HM. 2018. Polyamines and cancer. *Methods Mol Biol* **1694**: 469–488. doi:10.1007/978-1-4939-7398-9_39
- Didiot M-C, Tian Z, Schaeffer C, Subramanian M, Mandel J-L, Moine H. 2008. The G-quartet containing FMRP binding site in FMR1 mRNA is a potent exonic splicing enhancer. *Nucleic Acids Res* **36**: 4902–4912. doi:10.1093/nar/gkn472
- Ding Y, Tang Y, Kwok CK, Zhang Y, Bevilacqua PC, Assmann SM. 2014. In vivo genome-wide profiling of RNA secondary structure reveals novel regulatory features. *Nature* **505**: 696–700. doi:10.1038/nature12756
- Duncan CDS, Mata J. 2014. The translational landscape of fission yeast meiosis and sporulation. *Nat Struct Mol Biol* **21**: 641–647. doi:10.1038/nsmb.2843
- Eisenberg T, Knauer H, Schauer A, Büttner S, Ruckenstein C, Carmona-Gutierrez D, Ring J, Schroeder S, Magnes C, Antonacci L, et al. 2009. Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol* **11**: 1305–1314. doi:10.1038/ncb1975
- Forsburg SL. 1993. Comparison of *Schizosaccharomyces pombe* expression systems. *Nucleic Acids Res* **21**: 2955–2956. doi:10.1093/nar/21.12.2955
- Gomes AF, Gozzo FC. 2010. Chemical cross-linking with a diazirine photoactivatable cross-linker investigated by MALDI- and ESI-MS/MS. *J Mass Spectrom* **45**: 892–899. doi:10.1002/jms.1776
- Gray NK, Hentze MW. 1994. Iron regulatory protein prevents binding of the 43S translation pre-initiation complex to ferritin and eALAS mRNAs. *EMBO J* **13**: 3882–3891. doi:10.1002/j.1460-2075.1994.tb06699.x
- Hanfrey C, Elliott KA, Franceschetti M, Mayer MJ, Illingworth C, Michael AJ. 2005. A dual upstream open reading frame-based autoregulatory circuit controlling polyamine-responsive translation. *J Biol Chem* **280**: 39229–39237. doi:10.1074/jbc.M509340200
- Hendrix M, Priestley ES, Joyce GF, Wong CH. 1997. Direct observation of aminoglycoside-RNA interactions by surface plasmon resonance. *J Am Chem Soc* **119**: 3641–3648. doi:10.1021/ja964290o
- Henkin TM. 2008. Riboswitch RNAs: using RNA to sense cellular metabolism. *Genes Dev* **22**: 3383–3390. doi:10.1101/gad.1747308
- Hill JR, Morris DR. 1993. Cell-specific translational regulation of S-adenosylmethionine decarboxylase mRNA. Dependence on translation and coding capacity of the cis-acting upstream open reading frame. *J Biol Chem* **268**: 726–731.
- Hinnebusch AG, Lorsch JR. 2012. The mechanism of eukaryotic translation initiation: new insights and challenges. *Cold Spring Harb Perspect Biol* **4**: a011544. doi:10.1101/cshperspect.a011544
- Hinnebusch AG, Ivanov IP, Sonenberg N. 2016. Translational control by 5'-untranslated regions of eukaryotic mRNAs. *Science* **352**: 1413–1416. doi:10.1126/science.aad9868
- Hofacker IL. 2003. Vienna RNA secondary structure server. *Nucleic Acids Res* **31**: 3429–3431. doi:10.1093/nar/gkg599
- Igarashi K, Kashiwagi K. 2010. Modulation of cellular function by polyamines. *Int J Biochem Cell Biol* **42**: 39–51. doi:10.1016/j.biocel.2009.07.009
- Igarashi K, Kashiwagi K. 2015. Modulation of protein synthesis by polyamines. *IUBMB Life* **67**: 160–169. doi:10.1002/iub.1363
- Ivanov IP, Atkins JF, Michael AJ. 2010. A profusion of upstream open reading frame mechanisms in polyamine-responsive translational regulation. *Nucleic Acids Res* **38**: 353–359. doi:10.1093/nar/gkp1037
- Jackson RJ, Hellen CUT, Pestova TV. 2010. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat Rev Mol Cell Biol* **11**: 113–127. doi:10.1038/nrm2838
- Jia X, Zhang J, Sun W, He W, Jiang H, Chen D, Murchie AIH. 2013. Riboswitch control of aminoglycoside antibiotic resistance. *Cell* **152**: 68–81. doi:10.1016/j.cell.2012.12.019

- Kashiwagi K, Taneja SK, Liu TY, Tabor CW, Tabor H. 1990. Spermidine biosynthesis in *Saccharomyces cerevisiae*. Biosynthesis and processing of a proenzyme form of S-adenosylmethionine decarboxylase. *J Biol Chem* **265**: 22321–22328.
- Kertesz M, Wan Y, Mazor E, Rinn JL, Nutter RC, Chang HY, Segal E. 2010. Genome-wide measurement of RNA secondary structure in yeast. *Nature* **467**: 103–107. doi:10.1038/nature09322
- Law GL, Raney A, Heusner C, Morris DR. 2001. Polyamine regulation of ribosome pausing at the upstream open reading frame of S-adenosylmethionine decarboxylase. *J Biol Chem* **276**: 38036–38043.
- Leppek K, Das R, Barna M. 2018. Functional 5' UTR mRNA structures in eukaryotic translation regulation and how to find them. *Nat Rev Mol Cell Biol* **19**: 158–174. doi:10.1038/nrm.2017.103
- Lightfoot HL, Hagen T, Cléry A, Allain FH-T, Hall J. 2018. Control of the polyamine biosynthesis pathway by G2-quadruplexes. *Elife* **7**: e36362. doi:10.7554/eLife.36362
- Madeo F, Eisenberg T, Pietrolola F, Kroemer G. 2018. Spermidine in health and disease. *Science* **359**: eaan2788. doi:10.1126/science.aan2788
- Miller-Fleming L, Olin-Sandoval V, Campbell K, Ralser M. 2015. Remaining mysteries of molecular biology: the role of polyamines in the cell. *J Mol Biol* **427**: 3389–3406. doi:10.1016/j.jmb.2015.06.020
- Mize GJ, Morris DR. 2001. A mammalian sequence-dependent upstream open reading frame mediates polyamine-regulated translation in yeast. *RNA* **7**: 374–381. doi:10.1017/S1355838201001972
- Muckenthaler M, Gray NK, Hentze MW. 1998. IRP-1 binding to ferritin mRNA prevents the recruitment of the small ribosomal subunit by the cap-binding complex eIF4F. *Mol Cell* **2**: 383–388. doi:10.1016/S1097-2765(00)80282-8
- Muckenthaler MU, Rivella S, Hentze MW, Galy B. 2017. A red carpet for iron metabolism. *Cell* **168**: 344–361. doi:10.1016/j.cell.2016.12.034
- Mustoe AM, Corley M, Laederach A, Weeks KM. 2018. mRNA structure regulates translation initiation: a mechanism exploited from bacteria to humans. *Biochemistry* **57**: 3537–3539. doi:10.1021/acs.biochem.8b00395
- Porse BT, Kirillov SV, Awayez MJ, Ottenheim HC, Garrett RA. 1999. Direct crosslinking of the antitumor antibiotic sparsomycin, and its derivatives, to A2602 in the peptidyl transferase center of 23S-like rRNA within ribosome-tRNA complexes. *Proc Natl Acad Sci* **96**: 9003–9008. doi:10.1073/pnas.96.16.9003
- Raney A, Law GL, Mize GJ, Morris DR. 2002. Regulated translation termination at the upstream open reading frame in S-adenosylmethionine decarboxylase mRNA. *J Biol Chem* **277**: 5988–5994. doi:10.1074/jbc.M108375200
- Rouskin S, Zubradt M, Washietl S, Kellis M, Weissman JS. 2014. Genome-wide probing of RNA structure reveals active unfolding of mRNA structures in vivo. *Nature* **505**: 701–705. doi:10.1038/nature12894
- Schaeffer C, Bardoni B, Mandel J-L, Ehresmann B, Ehresmann C, Moine H. 2001. The fragile X mental retardation protein binds specifically to its mRNA via a purine quartet motif. *EMBO J* **20**: 4803–4813. doi:10.1093/emboj/20.17.4803
- Serganov A, Nudler E. 2013. A decade of riboswitches. *Cell* **152**: 17–24. doi:10.1016/j.cell.2012.12.024
- Sokabe M, Fraser CS. 2019. Toward a kinetic understanding of eukaryotic translation. *Cold Spring Harb Perspect Biol* **11**: a032706. doi:10.1101/cshperspect.a032706
- Sun W, Wang Z, Jiang H, Zhang J, Bähler J, Chen D, Murchie AIH. 2011. A novel function of the mitochondrial transcription factor Mtf1 in fission yeast; Mtf1 regulates the nuclear transcription of *srk1*. *Nucleic Acids Res* **39**: 2690–2700. doi:10.1093/nar/gkq1179
- Tabor CW, Tabor H. 1984. Polyamines. *Annu Rev Biochem* **53**: 749–790. doi:10.1146/annurev.bi.53.070184.003533
- Tapsin S, Sun M, Shen Y, Zhang H, Lim XN, Susanto TT, Yang SL, Zeng GS, Lee J, Lezhava A, et al. 2018. Genome-wide identification of natural RNA aptamers in prokaryotes and eukaryotes. *Nat Commun* **9**: 1289. doi:10.1038/s41467-018-03675-1
- Tijerina P, Mohr S, Russell R. 2007. DMS footprinting of structured RNAs and RNA-protein complexes. *Nat Protoc* **2**: 2608–2623. doi:10.1038/nprot.2007.380
- Wallace HM, Fraser AV, Hughes A. 2003. A perspective of polyamine metabolism. *Biochem J* **376**: 1–14. doi:10.1042/bj20031327
- Weinberg Z, Breaker RR. 2011. R2R—software to speed the depiction of aesthetic consensus RNA secondary structures. *BMC Bioinformatics* **12**: 3. doi:10.1186/1471-2105-12-3
- Wilhelm BT, Marguerat S, Watt S, Schubert F, Wood V, Goodhead I, Penkett CJ, Rogers J, Bähler J. 2008. Dynamic repertoire of a eukaryotic transcriptome surveyed at single-nucleotide resolution. *Nature* **453**: 1239–1243. doi:10.1038/nature07002
- Xue S, Tian S, Fujii K, Kladwang W, Das R, Barna M. 2015. RNA regulations in Hox 5'UTRs confer ribosome specificity to gene regulation. *Nature* **517**: 33–38. doi:10.1038/nature14010
- Yao Z, Weinberg Z, Ruzzo WL. 2006. CMfinder—a covariance model based RNA motif finding algorithm. *Bioinformatics* **22**: 445–452. doi:10.1093/bioinformatics/btk008
- Zhang X, Bremer H. 1995. Control of the *Escherichia coli* *rrnB* P1 promoter strength by ppGpp. *J Biol Chem* **270**: 11181–11189. doi:10.1074/jbc.270.19.11181
- Zhang J, Li D, Zhang J, Chen D, Murchie AIH. 2017. Osmium tetroxide as a probe of RNA structure. *RNA* **23**: 483–492. doi:10.1261/rna.057539.116
- Zhang X, Sun W, Chen D, Murchie AIH. 2020. Interactions between SAM and the 5' UTR mRNA of the *sam1* gene regulate translation in *S. pombe*. *RNA* **26**: 150–161 (this issue). doi:10.1261/rna.072983.119

RNA

A PUBLICATION OF THE RNA SOCIETY

Interactions between the 5' UTR mRNA of the *spe2* gene and spermidine regulate translation in *S. pombe*

Wenxia Sun, Xuhui Zhang, Dongrong Chen, et al.

RNA 2020 26: 137-149 originally published online December 11, 2019

Access the most recent version at doi:[10.1261/rna.072975.119](https://doi.org/10.1261/rna.072975.119)

Supplemental Material

<http://rnajournal.cshlp.org/content/suppl/2019/12/11/rna.072975.119.DC1>

References

This article cites 59 articles, 20 of which can be accessed free at:
<http://rnajournal.cshlp.org/content/26/2/137.full.html#ref-list-1>

Creative Commons License

This article is distributed exclusively by the RNA Society for the first 12 months after the full-issue publication date (see <http://rnajournal.cshlp.org/site/misc/terms.xhtml>). After 12 months, it is available under a Creative Commons License (Attribution-NonCommercial 4.0 International), as described at <http://creativecommons.org/licenses/by-nc/4.0/>.

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

To subscribe to *RNA* go to:

<http://rnajournal.cshlp.org/subscriptions>