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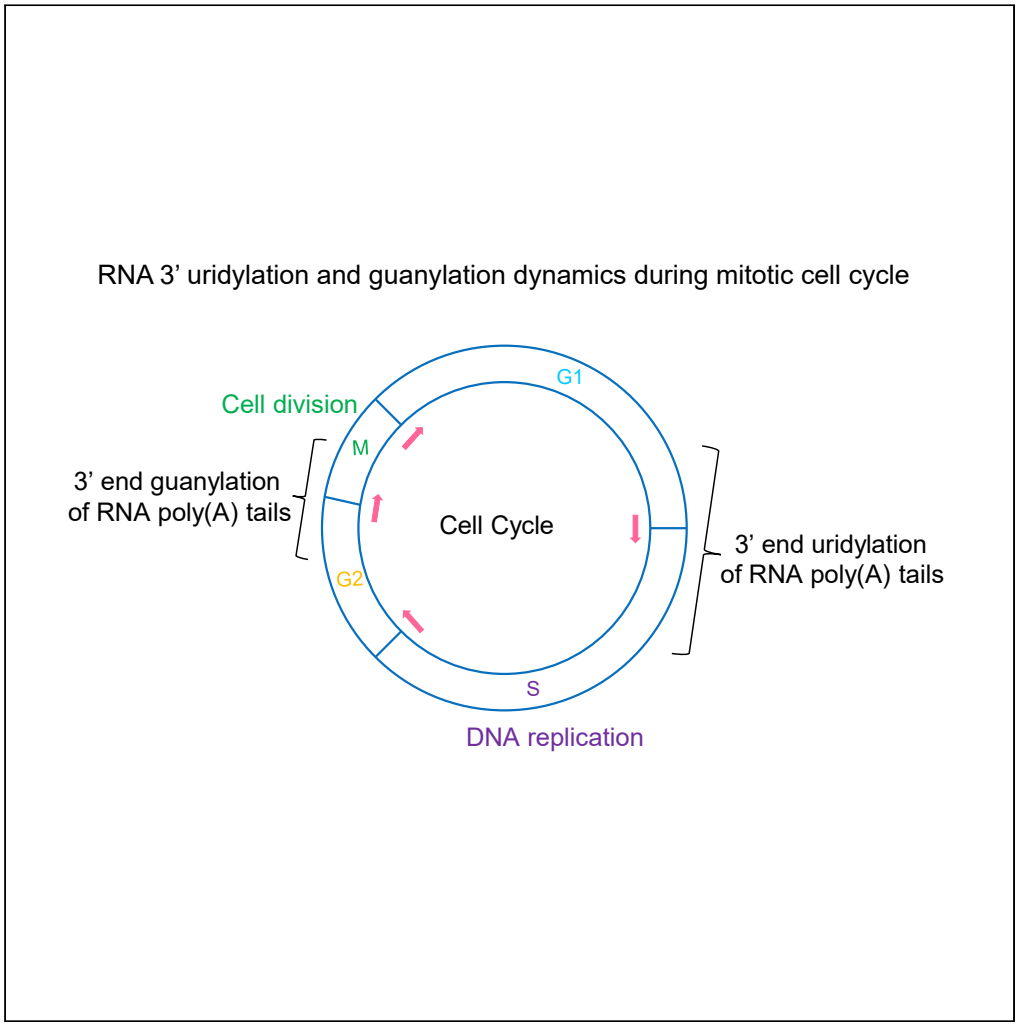

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HIGHLIGHTS

Global RNA 3′ uridylation associated RNA degradation takes place in S phase

Global RNA 3′ guanylation accumulates in M phase

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Dynamic RNA 3′ Uridylation and Guanylation during Mitosis

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SUMMARY

Successful cell division involves highly regulated transcriptional and post-transcriptional control. The RNA poly(A) tail represents an important layer of RNA post-transcriptional regulation. Previous TAIL-seq analysis of S phase and M phase poly(A) tail information showed that only a small number of genes showed more than 2-fold change in their poly(A) tail length between the two cell cycle stages. In addition, the changes in poly(A) tail length between these two stages showed minimal impact on the translation of the genes as long as the poly(A) tails were longer than 20 nt. Therefore, the significance of poly(A) tail dynamics during the cell cycle remains largely unknown. Here, by re-analyzing the S phase and M phase TAIL-seq data, we uncovered an interesting global dynamics of RNA poly(A) tails in terms of their terminal modifications, implying global RNA regulation between mitotic cell cycles through poly(A) tail terminal modifications.

INTRODUCTION

The RNA poly(A) tail is an essential component for most mRNA required for their stability, nuclear export, and translation efficiency (Eckmann et al., 2011; Groisman et al., 2001, 2002; Nicholson and Pasquinelli, 2019; Richter, 1999; Villalba et al., 2011; Weill et al., 2012). The global dynamics and function of mRNA poly(A) tail-mediated post-transcriptional regulation is highly underestimated owing to technical difficulties in sequencing long homopolymeric sequences on standard second-generation sequencing platforms. TAIL-seq and PAL-seq (poly(A)-tail length profiling by sequencing) methods with customized base-calling algorithm or sequencing chemistry on Illumina platform were developed to estimate the lengths of poly(A) tails (Chang et al., 2014; Subtelny et al., 2014). In addition, TAIL-seq revealed that the 3′ ends of mRNA poly(A) tails can be modified by other bases (Chang et al., 2014; Lim et al., 2014, 2018). Short RNA poly(A) tails can be terminally U modified by TUT4/7, which can promote the degradation of the marked mRNA (Lim et al., 2014). In contrast, mixed tailing of RNA poly(A) tails by TENT4A/B can introduce G modifications that attenuate the degradation of the mRNA poly(A) tails, resulting in stabilized RNA with G modifications at the end of the tails (Lim et al., 2018).

Interestingly, studying the meiotic cell cycle of oocytes at multiple stages in multiple species, including *Drosophila*, *Xenopus*, and mouse, revealed drastic dynamics of global RNA poly(A) tails during the meiotic cell cycle with a clear link that longer poly(A) tails were generally associated with increased efficiency of mRNA translation (Eichhorn et al., 2016; Lim et al., 2016; Luong et al., 2020; Morgan et al., 2017; Subtelny et al., 2014; Yang et al., 2020). Some of the cell cycle-related genes are periodically expressed, whereas most of the genes show relatively stable steady-state transcript level throughout the mitotic cell cycle (Bertoli et al., 2013; Park et al., 2016). However, little is known about the dynamics and roles of RNA poly(A) tails during mitotic cell cycle. Combination of poly(A) tail analysis by TAIL-seq and translation efficiency by ribosome profiling revealed that less than 2% genes showed more than 2-fold change in their poly(A) tail length (Chang et al., 2014; Park et al., 2016). In addition, the length change of a poly(A) tail minimally affects mRNA translation efficiency in somatic cell cycle as long as the tails are longer than 20 nt (Park et al., 2016). Therefore, the significance of poly(A) tail dynamics during somatic cell cycle awaits further exploring. Here, we re-analyzed the S phase and M phase TAIL-seq data and found that the terminal modifications of RNAs were globally dynamic during somatic cell cycle.

RESULTS

RNA poly(A) tail 3′ end uridylation can mark the RNA for rapid degradation (Lim et al., 2014; Morgan et al., 2017; Zuber et al., 2016). First, we looked into the uridylation frequency of RNA poly(A) tails during different

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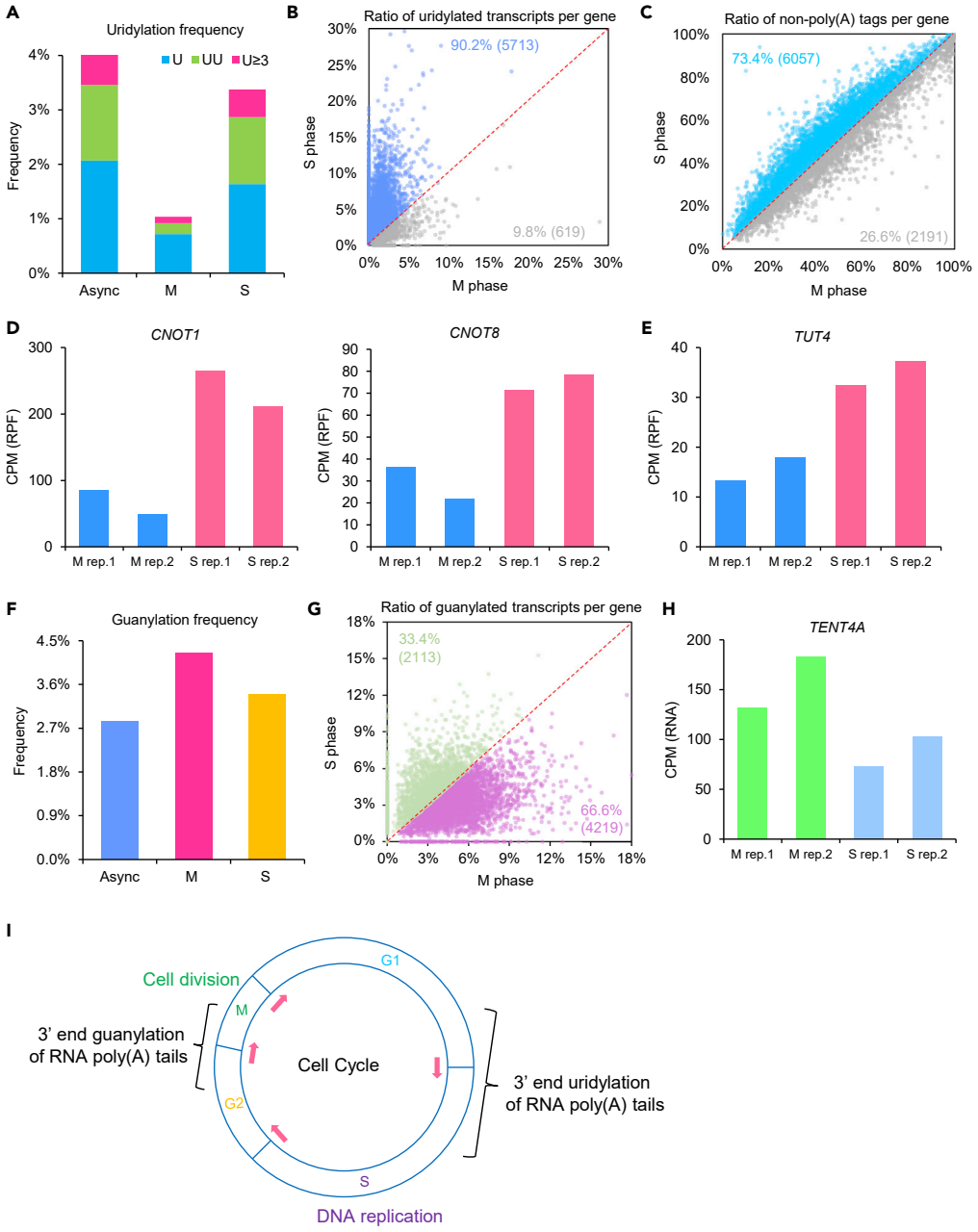

Figure 1. Poly(A) Tail Terminal Uridylation and Guanylation Cycle during the Mitotic Cell Cycle

(A) Frequency of uridylation is significantly lower at M phase compared with S phase. Frequency (y axis) is the fraction of detected reads with uridylation among all reads with poly(A) tails. Blue refers to mono-U, green refers to di-U, and red refers to three or more consecutive U. Async refers to asynchronized cells, M refers to M phase cells, and S refers to S phase cells.

(B) The fraction of transcripts with uridylation for each of the genes is significantly lower at M phase compared with S phase ($p = 0$, one-tailed Mann-Whitney U test). Genes with at least 30 reads with poly(A) tails in both samples are included in the analysis.

(C) The fraction of transcripts without poly(A) tails for each of the genes is significantly lower at M phase compared with S phase ($p = 7.87 \times 10^{-36}$, one-tailed Mann-Whitney U test). Genes with at least 30 total reads in both samples are included in the analysis.

(D) Ribosome protected fragment (RPF) counts for *CNOT1* and *CNOT8* are higher in S phase than those in M phase.

(E) Ribosome protected fragment (RPF) count for *TUT4* is higher in S phase than that in M phase.

Figure 1. *Continued*

(F) Frequency of guanylation is significantly higher at M phase compared with S phase. Frequency (y axis) is the fraction of detected reads with guanylation among all reads with poly(A) tails.

(G) The fraction of transcripts with guanylation for each of the genes is significantly higher at M phase compared with S phase ($p = 4.36 \times 10^{-139}$, one-tailed Mann-Whitney U test). Genes with at least 30 reads with poly(A) tails in both samples are included in the analysis.

(H) RNA-seq quantification of transcript level for *TENT4A* is higher in M phase than that in S phase.

(I) A model for the transgenerational RNA cycle during mitosis.

stages of mitotic cell cycle. Very interestingly, the M phase RNA poly(A) tails showed much lower frequency of uridylation at their 3′ ends, especially the oligo-U, compared with that from the S phase cells as well as the asynchronized cells (Figure 1A). Consistently, when looking into individual genes, we can see more than 90% of genes showed lower terminal uridylation frequency in M phase compared with that in S phase (Figure 1B). We reason that the high level of uridylation in S phase may be correlated with increased global RNA degradation. TAIL-seq can capture RNA both with or without poly(A) tails (Chang et al., 2014; Park et al., 2016). As poly(A) tail deadenylation is one of the most important steps in RNA degradation, we looked into the amount of RNA with deadenylated tails to estimate the RNA degradation. Interestingly, 73% of genes showed higher percentage of deadenylated poly(A) tails in S phase compared with that in M phase (Figure 1C), confirming that higher global RNA degradation associated with uridylation in S phase. In supporting the global higher RNA degradation in S phase, we could see higher ribosome engagement of transcripts encoding core components of RNA deadenylation CCR4-NOT complexes, such as *CNOT1* and *CNOT8*, in S phase than that in M phase (Figure 1D). Consistent with uridylation of poly(A) tails deadenylated by the CCR4-NOT complex (Yi et al., 2018), *TUT4*, encoding the terminal uridylation enzyme in mammals is also highly translated in S phase (Figure 1E). These findings indicate that compared with M phase there is global RNA uridylation-associated RNA degradation taking place in S phase.

RNA poly(A) tail 3′ end guanylation catalyzed by TENT4A/B can shield the tails from active deadenylation (Lim et al., 2018), which is opposite to the poly(A) tail uridylation. Interestingly, we could see that the guanylation in M phase cells is higher than that in S phase cells or that in asynchronized cells (Figure 1F). When looking into individual genes, two-thirds of the genes showed lower terminal guanylation frequency in M phase compared with that in S phase (Figure 1G). This increased guanylation phenomenon in M phase may be associated with the higher RNA level of *TENT4A* (Figure 1H). These findings indicate that compared with S phase RNA guanylation may prevent the deadenylation of the RNA in M phase.

DISCUSSION

By reanalyzing the RNA poly(A) tail information from the S phase and M phase of the mitotic cell cycle, we revealed global RNA poly(A) tail terminal modification reconfiguration during the cell cycle (Figure 1I). The accumulation of terminal uridylation and high deadenylated transcripts at S phase suggest that there is global RNA decay happening around S phase. In turn, the accumulation of terminal guanylation and reduced deadenylated transcripts at M phase indicate that the majority of the transcriptome is protected from active deadenylation during M phase. Taken together, these data reveal that the RNA terminal guanylation and uridylation, two modifications of opposite molecular function in regulating RNA stability, are of complementary dynamics during mitotic cell cycle. Together with a recent report of m6A cell cycle dynamics (Fei et al., 2020), the findings here highlight the functional importance of RNA modifications in orchestrating the mitotic cell cycle.

Limitation of the Study

Here, we reanalyzed the previously published datasets and observed the cell cycle-associated global dynamics of RNA poly(A) tail terminal modifications. The function and regulation of this interesting global RNA terminal modification cell cycle dynamics needs further investigation in the future. Moreover, it will be interesting to see if the dynamics is differentially regulated in two daughter cells with distinct cell fates during asymmetric cell division. In addition, the TAIL-seq method used to generate the datasets analyzed here cannot measure the non-A residue modifications within the body of RNA poly(A) tails (Chang et al., 2014); new methods such as PAIso-seq and FLAM-seq may be able to reveal more information about the dynamics of poly(A) tail internal modifications during cell cycle (Legnini et al., 2019; Liu et al., 2019). Nevertheless, our findings here reveal a new layer of global post-transcriptional regulation during cell cycle, suggesting a general principle of RNA inheritance and clearance between somatic mother and daughter cells.

Resource Availability

Lead Contact

Further information and requests should be directed to and will be fulfilled by the Lead Contact, Falong Lu, (fllu@genetics.ac.cn).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

This study did not generate new datasets or code. The accession number of the published datasets analyzed in this study can be found in the Transparent Methods.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101402.

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AUTHOR CONTRIBUTIONS

Y.L. performed the data analysis with input from H.N.; Y.L. and F.L. interpreted the data and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

Bertoli, C., Skotheim, J.M., and de Bruin, R.A.M. (2013). Control of cell cycle transcription during G1 and S phases. Nat. Rev. Mol. Cell Biol. *14*, 518–528.

Chang, H., Lim, J., Ha, M., and Kim, V.N. (2014). TAIL-seq: genome-wide determination of poly(A) tail length and 3′ end modifications. Mol. Cell *53*, 1044–1052.

Eckmann, C.R., Rammelt, C., and Wahle, E. (2011). Control of poly(A) tail length. Wiley Interdiscip. Rev. RNA *2*, 348–361.

Eichhorn, S.W., Subtelny, A.O., Kronja, I., Kwasnieski, J.C., Orr-Weaver, T.L., and Bartel, D.P. (2016). mRNA poly(A)-tail changes specified by deadenylation broadly reshape translation in Drosophila oocytes and early embryos. Elife *5*, e16955.

Fei, Q., Zou, Z., Roundtree, I.A., Sun, H.L., and He, C. (2020). YTHDF2 promotes mitotic entry and is regulated by cell cycle mediators. Plos Biol. *18*, e3000664.

Groisman, I., Huang, Y.S., Mendez, R., Cao, Q., and Richter, J.D. (2001). Translational control of embryonic cell division by CPEB and maskin. Cold Spring Harb. Symp. Quant. Biol. *66*, 345–351.

Groisman, I., Jung, M.Y., Sarkissian, M., Cao, Q., and Richter, J.D. (2002). Translational control of the embryonic cell cycle. Cell *109*, 473–483.

Legnini, I., Alles, J., Karaiskos, N., Ayoub, S., and Rajewsky, N. (2019). FLAM-seq: full-length mRNA sequencing reveals principles of poly(A) tail length control. Nat. Methods *16*, 879–886.

Lim, J., Ha, M., Chang, H., Kwon, S.C., Simanshu, D.K., Patel, D.J., and Kim, V.N. (2014). Uridylation by TUT4 and TUT7 marks mRNA for degradation. Cell *159*, 1365–1376.

Lim, J., Kim, D., Lee, Y.S., Ha, M., Lee, M., Yeo, J., Chang, H., Song, J., Ahn, K., and Kim, V.N. (2018). Mixed tailing by TENT4A and TENT4B shields mRNA from rapid deadenylation. Science *361*, 701–704.

Lim, J., Lee, M., Son, A., Chang, H., and Kim, V.N. (2016). mTAIL-seq reveals dynamic poly(A) tail regulation in oocyte-to-embryo development. Genes Dev. *30*, 1671–1682.

Liu, Y., Nie, H., Liu, H., and Lu, F. (2019). Poly(A) inclusive RNA isoform sequencing (PAIso-seq) reveals wide-spread non-adenosine residues within RNA poly(A) tails. Nat. Commun. *10*, 5292.

Luong, X.G., Daldello, E.M., Rajkovic, G., Yang, C.R., and Conti, M. (2020). Genome-wide analysis reveals a switch in the translational program upon oocyte meiotic resumption. Nucleic Acids Res. *48*, 3257–3276.

Morgan, M., Much, C., DiGiacomo, M., Azzi, C., Ivanova, I., Vitsios, D.M., Pistolic, J., Collier, P., Moreira, P.N., Benes, V., et al. (2017). mRNA 3′ uridylation and poly(A) tail length sculpt the mammalian maternal transcriptome. Nature *548*, 347–351.

Nicholson, A.L., and Pasquinelli, A.E. (2019). Tales of detailed poly(A) tails. Trends Cell Biol. *29*, 191–200.

Park, J.E., Yi, H., Kim, Y., Chang, H., and Kim, V.N. (2016). Regulation of poly(A) tail and translation during the somatic cell cycle. Mol. Cell *62*, 462–471.

Richter, J.D. (1999). Cytoplasmic polyadenylation in development and beyond. Microbiol. Mol. Biol. Rev. *63*, 446–456.

Subtelny, A.O., Eichhorn, S.W., Chen, G.R., Sive, H., and Bartel, D.P. (2014). Poly(A)-tail profiling reveals an embryonic switch in translational control. Nature *508*, 66–71.

Villalba, A., Coll, O., and Gebauer, F. (2011). Cytoplasmic polyadenylation and translational control. Curr. Opin. Genet. Dev. *21*, 452–457.

Weill, L., Belloc, E., Bava, F.A., and Mendez, R. (2012). Translational control by changes in poly(A) tail length: recycling mRNAs. Nat. Struct. Mol. Biol. *19*, 577–585.

Yang, F., Wang, W., Cetinbas, M., Sadreyev, R.I., and Blower, M.D. (2020). Genome-wide analysis identifies cis-acting elements regulating mRNA polyadenylation and translation during vertebrate oocyte maturation. RNA *26*, 324–344.

Yi, H., Park, J., Ha, M., Lim, J., Chang, H., and Kim, V.N. (2018). PABP cooperates with the CCR4-NOT complex to promote mRNA deadenylation and block precocious decay. Mol. Cell *70*, 1081–1088 e1085.

Zuber, H., Scheer, H., Ferrier, E., Sement, F.M., Mercier, P., Stupfler, B., and Gagliardi, D. (2016). Uridylation and PABP cooperate to repair mRNA deadenylated ends in Arabidopsis. Cell Rep. *14*, 2707–2717.

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Supplemental Information

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TRANSPARENT METHODS

Data accession numbers

This study includes analysis of the following published data: TAIL-seq for asynchronized cells (GEO accession: GSE51299) and M/S phase cells (GEO accession: GSE79664), ribosome profiling data and RNA-seq data for M/S phase cells (GEO accession: GSE79664) (Chang et al., 2014; Park et al., 2016).

Statistical analyses and data visualization

The processed datasheets of the TAIL-seq, ribosome profiling and RNA-seq datasets were downloaded from the GEO accessions listed above.

Genes with at least 30 poly(A) tail containing reads were included in the analysis for Figure 1A and F. Then poly(A) tails containing U, UU or more than 2 consecutive U from these genes were counted and divided by the total number of poly(A) tails for Figure 1A. Then poly(A) tails containing G modifications from these genes were counted and divided by the total number of poly(A) tails for Figure 1F.

Genes with at least 30 poly(A) tail containing reads in both stages were included in the analysis for Figure 1B and G, for each gene the ratio of U or G containing poly(A) tails were calculated by the number of U or G containing poly(A) tails divided by the number of all poly(A) tails of the gene. Then the ratio of U or G containing poly(A) tails for each gene were plotted for the two cell cycle stages.

Genes with at least 30 total reads in both stages were included in the analysis for Figure 1C. TAIL-seq reads with less than 5 nt untemplated sequence measured by the TAIL-seq algorithm were considered as non-poly(A) tags as described in the method of the original paper by Park et al. (Park et al., 2016). For each gene the ratio of non-poly(A) tags were calculated by the number of non-poly(A) tags divided by the number of total reads of the gene. Then the ratio of non-poly(A) tags for each gene were plotted for the two cell cycle stages.

The values from two biological replicates were shown as bar plots for both replicates of the two stages for Figure 1D, E and H.

The statistical tests for Figure 1B, C and G are one-tailed Mann-Whitney U test according to the published literature studying the tail terminal U modifications between two genotypes (Lim et al., 2014). The processed data were visualized using plots generated in Microsoft Excel.

REFERENCES

Chang, H., Lim, J., Ha, M., and Kim, V.N. (2014). TAIL-seq: genome-wide determination of poly(A) tail length and 3' end modifications. Mol Cell *53*, 1044-1052.

Lim, J., Ha, M., Chang, H., Kwon, S.C., Simanshu, D.K., Patel, D.J., and Kim, V.N. (2014). Uridylation by TUT4 and TUT7 marks mRNA for degradation. Cell *159*, 1365-1376.

Park, J.E., Yi, H., Kim, Y., Chang, H., and Kim, V.N. (2016). Regulation of Poly(A) Tail and Translation during the Somatic Cell Cycle. Mol Cell *62*, 462-471.