Wobble Inosine tRNA Modification Is Essential to Cell Cycle Progression in G1/S and G2/M Transitions in Fission Yeast*

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Inosine (I) at position 34 (wobble position) of tRNA is formed by the hydrolytic deamination of a genomically encoded adenosine (A). The enzyme catalyzing this reaction, termed tRNA A:34 deaminase, is the heterodimeric Tad2p/ADAT2-Tad3p/ADAT3 complex in eukaryotes. In budding yeast, deletion of each subunit is lethal, indicating that the wobble inosine tRNA modification is essential for viability; however, most of its physiological roles remain unknown. To identify novel cell cycle mutants in fission yeast, we isolated the tad3-1 mutant that is allelic to the tad3+ gene encoding a homolog of budding yeast Tad3p. Interestingly, the tad3-1 mutant cells principally exhibited cell cycle-specific phenotype, namely temperature-sensitive and irreversible cell cycle arrest both in G1 and G2. Further analyses revealed that in the tad3-1 mutant cells, the S257N mutation that occurred in the catalytically active Tad2 subunit affected its association with catalytically inactive Tad3 subunit, leading to an impairment in the A to I conversion at position 34 of tRNA. In tad3-1 mutant cells, the overexpression of the tad3+ gene completely suppressed the decreased tRNA inosine content. Notably, the overexpression of the tad2+ gene partially suppressed the temperature-sensitive phenotype and the decreased tRNA inosine content, indicating that the tad3-1 mutant phenotype is because of the insufficient I34 formation of tRNA. These results suggest that the wobble inosine tRNA modification is essential for cell cycle progression in the G1/S and G2/M transitions in fission yeast.

The transfer RNAs (tRNAs) are adapter molecules, which decode mRNA into protein and thereby play a central role in gene expression. There exist far fewer tRNA genes than the 61 codons specifying amino acids, hence many tRNAs must be capable of recognizing more than one codon. It has been known that inosine (I) occurs at position 34 (the first anticodon position or wobble position) of tRNA (1), and it is believed that when present at the wobble position, inosine can pair degenerately with U, C, or adenosine, enabling a single tRNA to recognize up to three different codons (2). This modification occurs in eight cytoplasmic tRNAs in higher eukaryotes (seven in budding yeast Saccharomyces cerevisiae) and in tRNAH18528 and tRNAH11001 from prokaryotes and plant chloroplasts (3). Inosine at the wobble position is formed by the hydrolytic deamination of a genomically encoded adenosine (A), and the enzyme catalyzing this reaction is termed tRNA A:34 deaminase. In budding yeast, it has been shown that tRNA A:34 deaminase is a heterodimer formed by the sequence-related subunits Tad2p and Tad3p, both of which contain cystidine deaminase motifs (C/H)EXExPCXCC (where X denotes any amino acid, and n is any number of residues) (4). According to mechanistic and structural studies of bacterial and eukaryotic cytidine deaminases, the active site of these enzymes has a zinc ion tetrahedrally coordinated to either a histidine and two cysteine residues or three cysteine residues; the fourth ligand is a zinc-activated water. Proton shuttling during the hydrolytic deamination reaction is mediated by a conserved glutamate (5–7). The absence of the essential glutamate residue in the putative Tad3p deaminase domain suggests that Tad3p is catalytically inactive and that Tad2p is the catalytic subunit of the enzyme. Each subunit is encoded by an essential gene (TAD2 and TAD3), indicating that 11 of tRNAs is an essential base modification for viability. However, most of its physiological roles remain unknown.

In this study using fission yeast Schizosaccharomyces pombe, we screened for mutants that showed temperature-sensitive growth arrest but did not accumulate phloxin B (a red dye staining dead cells), and we isolated a tad3-1 mutant that was allelic to the tad3+ gene encoding a homolog of budding yeast Tad3p. Surprisingly, the phenotypic characterizations of the tad3-1 mutant indicated that cell cycle arrest in G1 and G2 was the principal outcome in the tad3-1 mutation. Further analyses revealed that in the tad3-1 mutant cells, the S257N mutation that occurred in Tad3 deaminase domain destabilized a Tad2-Tad3 enzyme complex, leading to a reduced activity of tRNA A:34 deaminase. These findings suggest that wobble
inosine tRNA modification plays an important role in cell cycle progression.

**EXPERIMENTAL PROCEDURES**

**Strains, Media, and Genetic and Molecular Biology Methods**—Fission yeast strains used in this study are listed in Table 1. The complete medium, yeast extract-peptone-dextrose (YPD) and the minimal medium, Edinburgh minimum medium (EMM), have been described (8, 9). Standard genetic and recombinant DNA methods (8) were used except where noted.

**Bioinformatics**—Data base searches were performed using the National Center for Biotechnology Information BLAST network service (www.ncbi.nlm.nih.gov), the Sanger Center S. pombe data base search service (www.sanger.ac.uk), and the genomic tRNA data base (rna.wustl.edu/GtRDB/Sp/).

**Isolation of KP186 Strain**—The KP186 strain was isolated in a screen of cells that had been mutagenized with nitrosoguanidine (8). Cells of HM123 strain were mutagenized with 300 mM nitrosoguanidine (Sigma) for 60 min (~10% survival) as described previously. Mutants, in appropriate dilutions, were seeded onto YPD plates and incubated at 27 °C for 4 days. The plates were then replica-plated onto YPD plates containing 5 µg/ml phloxin B red dye, which accumulated in dead cells (10) and incubated at 36 °C for 1–2 days. Mutants that showed temperature sensitivity but were not stained red by phloxin B were selected. The original mutants isolated were backcrossed three times to wild-type strains HM123 or HM528.

**Gene Cloning**—To clone the mutated gene, KP186 cells were transformed with a fission yeast genomic DNA library constructed in the pDB248 vector and were grown at 27 °C. The Leu+ transformants were replica-plated onto YPD plates at 36 °C, and the plasmid DNA was recovered from the transformants that showed a plasmid-dependent rescue. By DNA sequencing, the suppressing plasmids fell into a single class that contained the tad3+ gene (SPAP27G11.04).

To investigate the genetic relationship between the mutated gene and the tad3+ gene, linkage analysis was performed as follows. The tad3+ gene was subcloned into the pUC-derived plasmid containing budding yeast LEI12 gene. Using this construct, the LEI12 gene was integrated by homologous recombination into the tad3+ gene locus of the genome of KP207. The integrant was mated with KP186 strain, and the resultant diploid was sporulated. When the tetrads were dissected, only parental ditype tetrads were found, indicating that the mutated gene was tightly linked to the tad3+ gene locus.

**Microscopic Analysis**—Techniques in fluorescence microscopy such as the localization of the GFP-tagged proteins were performed as described previously (11). Calcofluor staining was performed as described previously (11).

**FACS Analysis**—Cells were collected by centrifugation and fixed in ethanol at 4 °C overnight. After washing with 1 ml of 50 mM sodium citrate, the fixed cells were resuspended in 1 ml of a solution containing 50 mM sodium citrate and 0.1 mg/ml RNase A and incubated at 37 °C for at least 2 h. Before analysis, the cells were briefly sonicated and stained with propidium iodide at a final concentration of 8 µg/ml. Ten thousand cells were collected with a FACS Calibur flow cytometer (Becton Dickinson), and data were analyzed with Cell Quest 4.0 software.

Nitrogen source starvation was done as described previously (12). Briefly, cells were cultured in EMM medium to mid-log phase at 27 °C, washed four times with EMM without NH₄Cl, and then cultured in EMM without NH₄Cl at 27 °C for 36 h. The FACS analysis confirmed that the cells were arrested in the G1 phase. Arrested cells were released from the G1 block by switching them to YPD medium.

**Sequence Analysis of tRNAAla**—Total RNA was isolated from cultures of cells grown to mid-log phase at 27 °C by the method of Kohrer and Domdey (13). RNA was further treated with RNase-free DNase. RT was performed using 1 µg of total RNA as a template with tRNAAla(AGC)-specific reverse primer (5' -CGC GGA TCC TGG ACA AGG AAC TCG GAT AAC C-3') and PCR was then performed using 1 µl of the RT reaction as a template with tRNAAla(AGC)-specific forward (5' -CGC GGA TCC TGG ACA AGG AAC TCG GAT AAC C-3') primer. PCR products were subcloned into pBluescript SK(+)(Stratagene), and the individual clones were sequenced.

**Gene Expression**—The full coding sequences of Tad2 and Tad3 were amplified by PCR using genomic DNA from wild-type strain as a template. The PCR primers used were as follows: Tad2F (5' -CGC GGA TCC TGG ACA AGG AAC TCG GAT AAC C-3') and Tad2R (5' -CGC GGA TCC TTA TTA GAT AAA AGC AGA TAA ATC CAA AC-3'); Tad3F (5' -CGC GGA TCC ATG GAA ACT AAT ATT TCT AA-3') and Tad3R (5' -CGC GGA TCC TCA GAC ATG ATT TTC CCT TCT-3'). The Tad3S257N coding sequence was also amplified by PCR using the genomic DNA from KP186 strain as a template. These PCR fragments were ligated to the C terminus of GFP or GST and subcloned into the pREP1 expression vector, containing a thiamine-repressible nmt1 promoter (14). Expression was

### Table 1

| Strain   | Genotype | Reference |
|----------|----------|-----------|
| HM123    | h+ leu1-32 | Our stock |
| HM528    | h+ his2  | Our stock |
| KP186    | h+ leu1-32 tad3-1 | This study |
| KP207    | h+ his2 leu1-32 | Our stock |
| KP1248   | h+ leu1-32 ura4-294 | This study |
| KP2886   | h+ leu1-32 ura4-294 ura4-294-nmt1-GFP-tad2* | This study |
| KP3360   | h+ leu1-32 tad3-1 ura4-tad3-1 chl1::ura4* | This study |
| KP3361   | h+ leu1-32 sad1-1-GFP-LEI12 | Our stock |
| KP3364   | h+ leu1-32 ura4 tad3-1 chl1::ura4* | This study |
| KP3365   | h+ leu1-32 ura4 tad3-1 chl1::ura4* | This study |
induced by the incubation of the cells in EMM lacking thiamine.

To obtain cells expressing GFP-Tad2 from the chromosomally integrated gene, the fused gene was subcloned into the vector containing a nmt1 promoter and a urad+ marker, and was integrated into the chromosome at the urad+ gene locus of KP1248 cells as described (11, 15).

GST Pull-down Assay—Cells expressing GFP-Tad2 from the chromosomally integrated gene were transformed with pREP1-GST, pREP1-GST-Tad3, or pREP1-GST-Tad35257N, and were cultured at 27 °C for 20 h in EMM without thiamine to induce the gene expression. Cells were resuspended in ice-cold homogenizing buffer, 50 mM Tris-HCl, pH 7.5, containing 2 mM EDTA, 1 mM dithiothreitol, and a mixture of the protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM sodium metabisulfite, 0.1 μg/ml aprotinin, 1 μg/ml pepstatin A, 1 μg/ml phosphoramidon, and 0.5 μg/ml leupeptin). Glass beads were then added, and the cells were homogenized using a Mini-Beadbeater (Bio-Spec Products) at 5,000 rpm for 30 s, after which the tubes were placed on ice for 1 min. Homogenization and cooling were repeated three times, after which the glass beads and cellular debris were removed by centrifugation at 15,000 rpm for 5 min. GST-fused proteins in the protein extracts were purified with glutathione-Sepharose beads (Amersham Biosciences) and were subjected to immunoblot analysis with anti-GFP and -GST antibodies.

Mass Spectrometry—Crude tRNA was isolated from cultures of cells grown to mid-log phase at 27 °C as described previously (16). Briefly, cells were resuspended in 500 μl of lysis buffer (20 mM Tris-HCl, pH 7.5, 10 mM MgCl2), and treated with 500 μl of neutral pH phenol/chloroform by shaking for 3 h at room temperature. RNA was recovered by ethanol precipitation from the aqueous phase and then dissolved in sterile water. 1-μg aliquot of the RNA sample was separated on a 10% polyacrylamide gel of the temperature-sensitive growth arrest (Fig. 2A). Nucleotide sequencing of the cloned DNA fragment revealed that the mutated gene was SPAP27G11.04, which encoded a 315-amino acid deaminase. Linkage analysis also identified two complementation groups (data not shown). The mutant KP186, belonging to one of the two complementation groups, was selected to identify the mutated gene. KP186 mutant cells grew at 27 °C, but cells failed to grow at 33 °C or at a higher temperature (Fig. 1A).

KP186 Mutant Cells Exhibited Temperature-sensitive Cell Cycle Arrest in G1 and G2—Next, we examined the phenotypic changes in the cells that were subjected to a temperature upshift. Wild-type and KP186 cells expressing Sad1-GFP to visualize spindle pole bodies (SPBs) were grown to mid-log phase at 27 °C in liquid YPD medium, and then each culture was divided into two portions. One portion was maintained at 27 °C, whereas the remaining portion was shifted to 36 °C for 8 h. The cells were collected and stained with Calcofluor to visualize septa (Fig. 1B). Two lines of evidence suggest that the KP186 phenotype is associated with cell cycle. First, even at 27 °C, KP186 cells were slightly longer than wild-type cells, and this cell elongation was more evident when temperature was upshifted to 36 °C (Fig. 1, B and C). Second, upon temperature upshift to 36 °C in KP186 cells, the frequency of mitotic cells characterized by duplicated SPBs and/or septa significantly decreased, whereas in wild-type cells, the frequency of mitotic cells remained unchanged (Fig. 1, B and D).

The above-mentioned phenotype prompted us to check whether the KP186 mutant had a defect in cell cycle progression. FACS analysis showed that KP186 cells incubated at 36 °C in liquid YPD medium consistently showed post-replicative DNA content (Fig. 1E), confirming that KP186 cells arrested in the G2 phase at the restrictive temperature. When first arrested in the G1 phase at 27 °C by nitrogen starvation and then released from the G1 block at 36 °C, KP186 cells, unlike wild-type cells, could not initiate the S phase and remained arrested in the G1 phase (Fig. 1F). In addition, we found that the longer preincubated at the restrictive temperature of 36 °C, the more KP186 cells failed to grow when shifted back to 27 °C (Fig. 1G), suggesting that growth arrest induced by temperature upshift in KP186 cells was irreversible. These results showed that the temperature-sensitive phenotype of KP186 cells was because of the irreversible cell cycle arrest in G1 and G2 at the restrictive temperature.

KP186/tad3-1 Is a Novel Allele of the tad3+ Gene Which Encodes a Non-catalytic Subunit of tRNA A:34 Deaminase—To clarify the mechanism of cell cycle arrest in KP186 cells, the mutated gene in KP186 strain was cloned by complementation of the temperature-sensitive growth arrest (Fig. 2A). Nucleotide sequencing of the cloned DNA fragment revealed that the mutated gene was SPAP27G11.04, which encoded a 315-amino acid homolog of budding yeast Tad3p, a catalytically inactive subunit of tRNA A:34 deaminase. Linkage analysis also indicated that the mutated gene was tightly linked to the
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SPAP27G11.04 locus (data not shown). Hence, we named this gene as *tad3* and the KP186 strain as the *tad3*-1 mutant. Sequence analysis of the genomic DNA from the *tad3*-1 mutant revealed that serine at 257 was mutated to asparagine by a *G* to *A* transition (*S257N*; AGT → AAT). This missense mutation is located in the evolutionarily conserved deaminase domain (Fig. 2B). The absence of the glutamate residue essential for catalysis in the Tad3 deaminase domain suggests that Tad3 is catalytically inactive. This raises the possibility that the mutation of Tad3 might indirectly affect the enzyme activity of tRNA A:34 deaminase.

Impaired Conversion of *A*<sub>34</sub> to *I*<sub>34</sub> in tRNA<sup>Ala</sup> from *tad3*-1 Mutant—Although fission yeast uses four alanine codons GCC, GCA, GCG, and GCA, its genome encodes only three different tRNA<sup>Ala</sup> genes with anticodons AGC, CGC, and TGC (Fig. 3A). This discrepancy implies that tRNA A:34 deaminase converts tRNA<sup>Ala</sup> (AGC) into tRNA<sup>Ala</sup> (IGC), which then pairs with and decodes an alanine codon GCC. Therefore, we performed the sequence analysis of tRNA<sup>Ala</sup> (AGC) as one of putative substrates of tRNA A:34 deaminase. With oligonucleotide primers specific for tRNA<sup>Ala</sup> (AGC) (Fig. 3B), RT-PCR was performed on total RNA isolated from wild-type and *tad3*-1 mutant cells grown at 27 °C. The resultant cDNAs were subcloned, and 15 different clones derived from *tad3*-1 mutant cells as well as 15 clones from wild-type cells were sequenced. Because 1 pairs with C during reverse transcription, A to I deamination changes the sequence from A to G. All clones (100%) derived from wild-type cells contained guanosine at position 34 (G<sub>34</sub>), demonstrating that the genomically encoded A at this position was deaminated to I. On the other hand, 1 out of 15 (7%) clones derived from *tad3*-1 mutant cells carried guanosine at position 34, whereas the other 14 clones carried adenosine at position 34 (Fig. 3C). These results indicate that the enzyme activity of tRNA A:34 deaminase is impaired in the *tad3*-1 mutant.

Notably, the genomically encoded A at position 37 was consistently changed to T in both RT-PCR products from wild-type and from *tad3*-1 mutant cells (Fig. 3C). As has been described in budding yeast tRNA<sup>Ala</sup> (18), this might be because A at position 37 is edited to N<sup>m</sup>-methyladenosine (m<sup>1</sup>A), which base pairs with reduced specificity in the RT reaction. This finding suggests that A<sub>37</sub> to m<sup>1</sup>A<sub>37</sub> editing in tRNA<sup>Ala</sup> is not impaired in the *tad3*-1 mutant.

The *tad3*-1 Mutant Is Hypersensitive to Cycloheximide—As insufficient I<sub>34</sub> formation of tRNA in the *tad3*-1 mutant may affect its decoding system and impair translation step, we then examined the effect of a protein synthesis inhibitor cycloheximide (CYH) that mainly blocks the elongation process of translation (19) on *tad3*-1 mutant (Fig. 3E). At a low concentration of CYH (10 μg/ml), the growth of *tad3*-1 mutant was significantly inhibited as compared with that of wild-type cells. At a higher concentration of CYH (20 μg/ml), the growth inhibition of *tad3*-1 mutant was further exacerbated. These results suggest...
that translation process is indeed impaired in the tad3-1 mutant.

**Temperature Sensitivity of tad3-1 Mutant Is Suppressed by the Overexpression of the tad2** Gene Encoding a Catalytic Subunit of tRNA A:34 Deaminase—To further study the mechanism as to how the S257N mutation of non-catalytic Tad3 impairs the enzyme activity of tRNA A:34 deaminase, we performed a genetic screen for multicopy suppressors of the temperature-sensitive phenotype of the tad3-1 mutant. This screen resulted in the identification of a single gene that suppressed the temperature sensitivity at 33 °C but not at 36 °C (Fig. 4 A). Interestingly, analysis of the nucleotide sequence revealed an open reading frame SPBC16D10.10 encoding a 367-amino acid homolog of budding yeast Tad2p, the catalytic subunit of tRNA A:34 deaminase. We therefore named this suppressor gene as tad2/H11001. Indeed, the C-terminal part of fission yeast Tad2 contains an evolutionarily conserved deaminase domain with a full consensus motif, suggesting that Tad2 is catalytically active (Fig. 4B). Given that budding yeast tRNA A:34 deaminase is a heterodimeric enzyme formed by Tad2p and Tad3p proteins (4), it is possible that Tad2 and Tad3 form similar complex in fission yeast, and that the S257N mutation of Tad3 might affect its association with Tad2, thereby decreasing the amount of the Tad2-Tad3 enzyme complex in the tad3-1 mutant.

**S257N Mutation of Non-catalytic Tad3 Destabilizes a Tad2-Tad3 Complex**—To test the above hypothesis, we investigated the effect of the S257N mutation of Tad3 on the Tad2-Tad3 interaction. Expression of GFP-Tad2 or GST-Tad2 suppressed the temperature sensitivity of tad3-1 mutant cells at 33 °C and at 36 °C, respectively, indicating that these fusion proteins were fully functional. In cells expressing GFP-Tad2 from the chromosomally integrated gene, GST, GST-Tad3, or GST-Tad3S257N was expressed from the harboring plasmid at 27 °C. The pull-down experiments showed that GFP-Tad2 associated with GST, GST-Tad3, or GST-Tad3S257N expressed from the harboring plasmid at 27 °C.
and Tad3. On the other hand, the pull-down for GST-Tad3S257N consistently showed less amount of GFP-Tad2 protein than was seen in the pull-down for GST-Tad3 (Fig. 5B), demonstrating that Tad3S257N was indeed defective in its association with Tad2. This result suggests that the overexpression of Tad2 in the tad3-1 mutant cells may partially suppress the decreased level of the enzyme complex and result in the partial suppression of the temperature-sensitive phenotype. Consistently, the overexpression of GST-Tad3S257N partially but significantly suppressed the temperature sensitivity of the tad3-1 mutant (Fig. 5A). These results also suggest that serine at position 257 in the deaminase domain of Tad3 plays an important role in its interaction with Tad2.

**Suppression of the tad3-1 Mutant Phenotype Depends on the Recovery of the Wobble Inosine tRNA Modification**—We next examined the relationship between the suppression of the tad3-1 mutant phenotype (Figs. 2A and 4A) and the extent of the wobble inosine tRNA modification (Fig. 6). Crude tRNA extracted from each transformant grown at 27 °C was analyzed by LC/MS to measure the tRNA inosine content. Consistent with the decreased wobble inosine modification of tRNAA^\text{AGC} (AGC), tRNA inosine content in tad3-1 mutant cells was decreased to 36% as compared with that in wild-type cells. The overexpression of the tad3S257N gene in tad3-1 mutant cells completely suppressed the decreased tRNA inosine content. On the other hand, the overexpression of the tad2S257N gene in tad3-1 mutant cells partially suppressed the decreased tRNA inosine content. These findings suggest that the phenotype of tad3-1 mutant cells is because of the insufficient $I_{34}$ formation of tRNA.

**DISCUSSION**

In the present study, in an attempt to identify novel cell cycle mutants in fission yeast, we isolated the tad3-1 mutant that exhibited temperature-sensitive growth arrest in G1 and G2. Further analyses revealed that in the tad3-1 mutant cells, the S257N mutation that occurred in Tad3 deaminase domain destabilized a Tad2-Tad3 complex, leading to the reduced activity of tRNA A:34 deaminase. Furthermore, we confirmed that such phenotype of the tad3-1 mutant was suppressed by the recovery of the wobble inosine tRNA modification. These findings demonstrate that the wobble inosine tRNA modification is essential for cell cycle progression in the G1/S and G2/M transitions. This is the first report to link the wobble inosine tRNA modification to cell cycle progression.

The tRNAs containing inosine at position 34 are major species, and all belong to the subset of amino acids represented by four codons (20). The architectural and functional significance of the base modification is not yet fully understood, however, it is believed that the modified nucleosides can influence the stability and structure of the tRNAs and thus improve the fidelity.
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and efficiency of the tRNAs in decoding the genetic message (20, 21). According to Crick’s wobble hypothesis (2), inosine at position 34 is believed to play a crucial role in protein synthesis by allowing alternative pairing with U, C, or A in the third position of appropriate codons in the mRNA. Moreover, inosine at position 34 in budding yeast tRNA^Ile has been shown to be a positive determinant for aminoacylation by isoleucyl-tRNA synthetase (22). Therefore, it is thought that a defect in the I_{34} formation of the tRNA should impair protein synthesis at different steps and produce a wide spectrum of phenotypes. Surprisingly, the phenotypic characterizations in this study indicate that cell cycle arrest in G_1 and G_2 is the principal outcome in the tad3^1 mutation.

One possible explanation for this unexpected result is that the status of tRNA base modification might be monitored by a kind of cell cycle checkpoint, the activation of which might produce the cell cycle-specific phenotype of tad3^1 mutant. But this checkpoint hypothesis is unlikely based on the following data. First, to examine whether the cell cycle arrest of tad3^1 mutant is dependent on the DNA damage or replication checkpoint, we constructed tad3^1 Δcds1 and tad3^1 Δchk1 double mutants (KP3364 and KP3365, respectively). Neither of the double mutations broke the cell cycle block at the restrictive temperature, verifying that the tad3^1 mutant cells arrested independently of the DNA damage and replication checkponts (data not shown). Second, from 10,000 nitrosoguanidine-treated tad3^1 mutant cells, we searched for mutants that grew at the restrictive temperature, but we failed to isolate such mutants (data not shown).

Hence based on the above findings, it is more likely that the cell cycle genes could not be efficiently translated in the tad3^1 mutant cells at the restrictive temperature. As mentioned above, the decoding system by inosine-bearing tRNAs contributes to decrease the number of isoacceptor tRNAs and thus enables the efficient translation. Translation is the most energy-expensive process occurring in the exponentially growing cells, and therefore its efficiency is under stringent selective pressure (23). Although no direct evidence has been presented, fission yeast might have in the process of evolution adopted the decoding system by inosine-bearing tRNAs toward cell cycle genes and have acquired the ability to grow faster by their efficient translation. The cell cycle-specific phenotype of tad3^1 mutant might reflect such a condition. Further studies are needed to test this hypothesis and to better understand the cell cycle-specific phenotype of the tad3^1 mutant.

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