The tRNA *N*2,*N*2-dimethylguanosine-26 methyltransferase encoded by gene *trm1* increases efficiency of suppression of an *ochre* codon in *Schizosaccharomyces pombe*

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Received 16 November 1999

Edited by Horst Feldmann

Abstract In the majority of eukaryotic tRNAs, the guanosine at position 26 is modified by a dimethyl group, but so far a function of this modification has not been detected. We isolated the *Schizosaccharomyces pombe* gene, *trm1*, encoding the tRNA *N*2,*N*2-dimethylguanosine-26 methyltransferase. Strains having the gene deleted completely lack *N*2,*N*2-dimethylguanosine. In strains carrying the weak *ochre* tRNA suppressor *sup3*-i, deletion of *trm1* abolishes suppression indicating that the *trm1* deletion acts as an antisuppressor mutation. The result suggests that in vivo *N*2,*N*2-dimethylguanosine-26 increases the capacity of the *sup3*-i serine tRNA to translate the UAA (*ochre*) codon.

Key words: tRNA modification; Antisuppression; Dimethylguanosine-26 methyltransferase; *Schizosaccharomyces pombe*

1. Introduction

*N*2,*N*2-Dimethylguanosine at position 26 (m_2^2G26) is a modified base that is present in many tRNAs of eukaryotes and archaebacteria [1]. In eukaryotic cells, it is found in cytoplasmic as well as in mitochondrial and chloroplast tRNA. In 80% of 129 tested nuclear-coded eukaryotic tRNAs having a guanine at position 26, the base has been found to be modified to m_2^2G, indicating that the G26-dimethyl-group is a quasi ubiquitous modification of nuclear-coded eukaryotic tRNAs. Methylation of the guanine is accomplished by an S-adenosylmethionine-dependent *N*2,*N*2-dimethylguanosine-26 methyltransferase [1]. The function of m_2^2G26 is not known. *Saccharomyces cerevisiae* cells having the gene encoding the tRNA *N*2,*N*2-dimethylguanosine-26 methyltransferase, *TRM1*, deleted lack m_2^2G26 but otherwise exhibit no phenotype [2,3]. It has been hypothesized that the dimethyl-group is involved in determining the flexibility of the tRNA molecule and that by this function, it facilitates the interaction with various macromolecules in the cell [1]. In this communication, we report isolation of the *Schizosaccharomyces pombe* gene encoding *N*2,*N*2-dimethylguanosine-26 methyltransferase and show that in a strain containing a weak *ochre* suppressor, its deletion causes antisuppression.

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2. Materials and methods

2.1. Media, strains and genetic methods

The strains used in this study have been cultivated in solid yeast extract medium (YEA) or in solid minimal medium (MMA) supplemented with uracil (80 mg/l) or adenine (100 mg/l) if necessary [4]. The strains *ura4*-D18, *ade7*-413, *ade6*-704, *ade7*-413*sup3*-i and *ade6*-704*sup8*-e are from our or from the Leupold strain collection in Bern, Switzerland. The deletion strain Δ*trm1*::*ura4* has been constructed as described below and strains *ade7*-413*sup3*-i Δ*trm1*::*ura4* and *ade6*-704*sup8*-e Δ*trm1*::*ura4* were obtained by crossing the appropriate heterothallic strains with each other and by selecting the strains with the desired genotypes. The standard genetic techniques for *S. pombe* such as crossing, sporulation and tetrad analysis have been described by Gutz et al. [5].

2.2. Isolation of the trm1 gene

The *trm1* gene was isolated as a cDNA clone (pACT-trm1) in a two hybrid screen [6]. As bait, we used plasmid pAS2-2 containing the sequence encoding amino acids 1–408 of the ksg1 protein [7] fused in frame with the HA tag and the DNA binding domain of the yeast gal4 protein. The *S. pombe* cDNA library was ligated into vector pACT2 in which the cDNA was fused to the gal4 transcription activation domain. The trm1 protein co-immunoprecipitated with the ksg1 protein, but in the reciprocal experiment, a co-immunoprecipitation of the ksg1 protein with the the trm1 protein was not observed. It is likely that the positive response of clone pACT-trm1 in the two hybrid system is an artefact. Physical localization, subcloning and sequencing of the *trm1* gene was achieved by standard methods [7,8].

2.3. Deletion of the trm1 gene

Most of the *trm1* gene was replaced by the *ura4* gene as described by Kaur et al. [9]. Using a *ura4* containing plasmid as template, forward (STRM) and backward (ETRM) primers homologous to the ends of the *ura4* and the *trm1* gene as indicated in Fig. 2 were synthesized by PCR (sequence of STRM: 5′-GGATCTCAAATGCTGTGAATCACTTCTCAACCGCCATGATGTCGGGGGCCCCACTGGCTATATGTATGC-3′; sequence of ETRM: 5′-GGAGTATTTAAACTGTGCTCCACTTTACCTAAGATTTTGAGTCAACTTCAGCGGGTAATGTTGTAGGAGCATG-3′). With the PCR construct, the *ura4*-D18 h^{90} strain was transformed and stable *ura4* integrants were selected. Proper replacement was confirmed by Southern analyses.

2.4. Analysis of nucleosides

S. pombe cells were grown in YEL at 25°C to the mid log phase, washed with water, the cell pellet was resuspended in 5–10 volumes of TM buffer (10 mM Tris–HCl and 10 mM $MgCl_2$), tRNAs were extracted by adding the same amount of aquaphenol and by shaking cells for 10 min at room temperature. After centrifugation, 0.1 volume of 3 M potassium acetate and two volumes of ethanol were added to the aqueous phase and the RNA was precipitated at −20°C overnight, collected by centrifugation and washed with 70% ethanol. The nucleosides were determined by high performance liquid chromatography as described previously [10].

PII: S0014-5793(99)01679-8

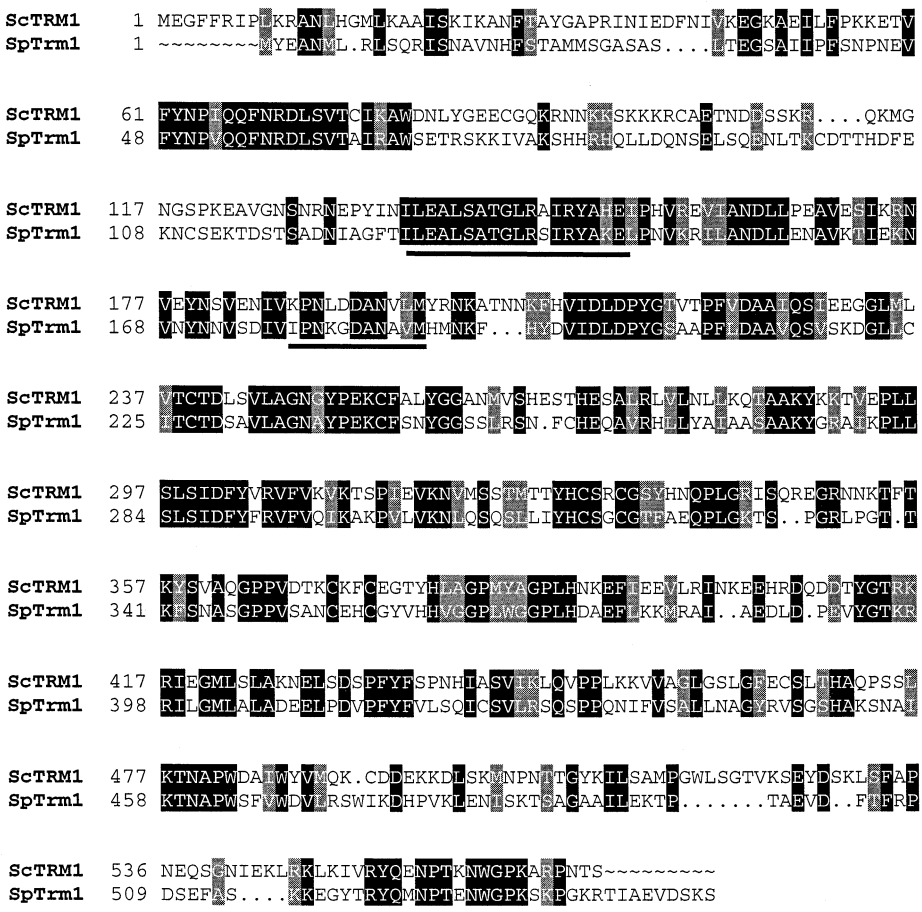

Fig. 1. Amino acid sequences from *S. cerevisiae* (Sc) S-adenosylmethionine-dependent tRNA *N*2,*N*2-dimethylguanosine-26 methyltransferase encoded by gene *TRM1* and the *S. pombe* (Sp)-derived trm1 protein. Regions of amino acid identity are in black and regions in gray denote amino acid similarity. The underlined boxed sequences indicate the putative S-adenosylmethionine binding motifs.

3. Results and discussion

3.1. Isolation of gene trm1

In a two hybrid screen, we isolated from a *S. pombe* cDNA library a prey plasmid which encoded a gene exhibiting homologies to the *TRM1* gene of *S. cerevisiae*. By filter hybridization, we localized the putative *S. pombe trm1* gene on a genomic cosmid as described for the *ksg1* gene [7]. It maps on the right arm of chromosome II closest to the published probe [11]. We subcloned the gene on a 9.4 kb insert into the *ura4* marker containing pUR19 shuttle vector [11] and sequenced the region (2441 bp) containing the putative *S. pombe trm1* gene (the nucleotide sequence of the gene has been deposited in GenBank under number AJ22400). It enbcodes an open reading frame of 548 amino acids. The homology to the budding yeast S-adenosylmethionine-dependent tRNA *N*2,*N*2-dimethylguanosine-26 methyltransferase encoded by gene *TRM1* (44% identity over 529 amino acids) and the two S-adenosylmethionine binding motifs described for tRNA specific methyltransferases is shown in Fig. 1.

3.2. Nucleoside content of tRNAs in a trm1 deletion strain

As shown in Fig. 2, we constructed a strain having most of the open reading frame of the *trm1* gene replaced by the *ura4* gene. From this strain and the wild-type we isolated the tRNAs and determined their nucleoside content. As shown in Table 1, the *trm1* deletion strain completely lacks dimethylguanosine, while all other tested modifications are normal. This result together with the sequence data leave little doubt that *trm1* represents the structural gene for *N*2,*N*2-dimethylguanosine-26 methyltransferase and indicates that *trm1* is the only functional gene encoding a tRNA dimethyltransferase in *S. pombe*. We tested growth, cell morphology and sporulation on MMA and YEA media at different temperatures (25°C,

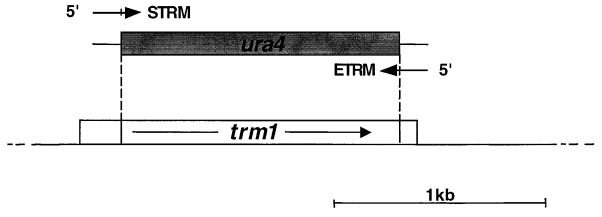

Fig. 2. The *trm1* gene and its disruption. The gene has been cloned and disrupted as described in the text. The white open bar denotes the coding region of the *trm1* gene. The arrow indicates the direction of transcription. The gray bar above symbolizes the ura4 gene and indicates which portion of the *trm1* gene is disrupted. The arrows STRM and ETRM denote the primers used for the construction of the disruption (see Section 2).

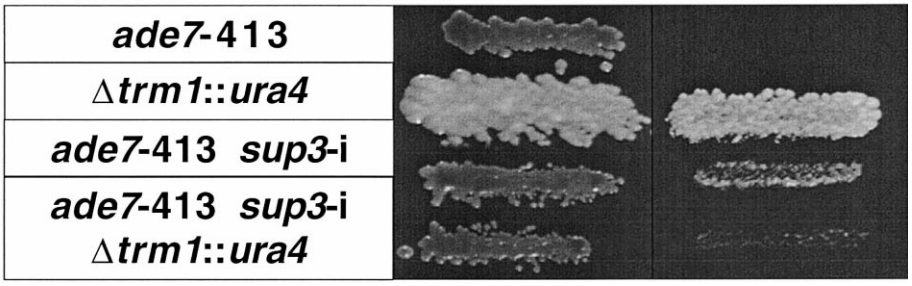

Fig. 3. Antisuppressor activity of a strain containing a *trm1* deletion as recorded by growth. Strains with different genotypes were streaked on YEA and MMA plates and incubated at 30°C for 5 days. The strains containing the *ade7*-413 allele are red on YEA, they differ however in color (not seen in the black and white reproduction). The strain containing the *sup3*-i allele and the *trm1* deletion is darker than the strain containing the suppressor alone.

30°C and 35°C) and, as in the case of *S. cerevisiae* [2], found that the *trm1* deletion strain exhibited the same phenotype as the parent strain in the genetic background of the wild-type.

3.3. Detection of antisuppressor activity in a trm1 deletion strain

In *S. pombe*, several nonsense suppressor tRNAs are known which can read and translate the translation stop codons *ochre* and *opal* in protein encoding genes due to mutational alterations of the anticodon of tRNAs [12]. In a few cases, it has been shown that the efficiency of suppression is reduced by mutations (antisuppressor mutations) that affect tRNA modifications. One of these antisuppressor strains (*sin1* mutant) for example lacks the isopentenyladenosine next to the anticodon in tRNAs and two others (*sin3* and *sin4* mutants) exhibit reduced levels of 5-(methoxycarbonylmethyl)-2-thiouridine at the first anticodon position of tRNAs [13,14].

To test if the tRNA modification controlled by *trm1* affects suppression of translation stop codons, we focused on the two *S. pombe* suppressors *sup8*-e and *sup3*-i. *Sup8*-e is an *opal* suppressor and efficiently suppresses the *opal* codon in strain *ade6*-704 [12] whereas *sup3*-i inefficiently suppresses the *ochre* codon in strain *ade7*-413 [15]. *Sup8*-e encodes a mutated leucine tRNA with the anticodon U*CA (the star indicates a modified U) whereas *sup3*-i is a mutated serine tRNA with a U*UA anticodon [12]. Importantly, both tRNAs contain at position 26 m_2^2G. We crossed a *trm1* deletion strain with strain *ade6*-704 (*opal*) containing *sup8*-e and strain *ade7*-413 (*ochre*) containing *sup3*-i and tested growth. The *trm1* deletion has no effect on growth of the *opal* suppressor containing strain (data not shown) but in the background of the *sup3*-i suppressor, it abolishes growth on MMA (Fig. 3). We crossed the deletion strain Δ*trm1*::*ura4 ade7*-413*ura4*-D18*sup3*-i h^- with strain *ade7*-413*ura4*-D18*sup3*-i h^+ and dissected 16 tetrads. All showed a 2:2 segregation of the growth phenotype on MMA and all adenine prototrophic strains were ura^+ whereas the adenine auxotrophic progeny were ura^-, indicating that the *trm1* deletion causes antisuppression. This result shows that m_2^2G26 has a detectable function in vivo and suggests that it increases in vivo the capacity of the *sup3*-i suppressor tRNA to translate the UAA codon. The biochemical mechanism by which this is achieved remains to be elucidated.

Table 1
Determination and quantification of nucleosides in tRNAs of the wild-type and *trm1* deletion strain Δ*trm1*[a]

Nucleoside[a]	Wild-type[b]	Δ*trm1*
C	27 963	29 648
U	23 130	23 473
G	31 337	33 308
A	28 468	27 884
PsU	3 879	4 048
m1A	679	709
m5C	1 369	1 409
Cm	231	194
m7G+I	1 089	1 037
T	2 054	1 717
Um	374	320
m1I	131	144
m1G	986	1 063
ac4C	272	274
m2G	532	488
Tm	22	12
m22G	755	0
mcm5s2U	133	129
Am	21	15
t6A	476	529
m6A	62	99
i6A	156	151

[a]Abbreviations are from Limbach et al. [16].
[b]The numbers given represent pmol/AU RNA (1 AU corresponds to about 40 mg RNA).

Acknowledgements: We thank H. Grosjean for the help in the initial analysis of nucleosides, S.J. Elledge for the reagents used in the two hybrid system and I. Roditi for comments on the manuscript. This work was supported by the Swiss National Foundation.

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