A very poorly expressed tRNA^{Ser} is highly concentrated together with replication primer initiator tRNA^{Met} in the yeast Ty1 virus-like particles

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ABSTRACT

The analysis of the tRNAs associated to the virus-like particles produced by the Ty1 element revealed the specific packaging of three major tRNA species, in about equal amounts: the replication primer initiator tRNA^{Met}, the tRNA^{Ser}AGA and a tRNA undetected until now as an expressed species in yeast. The latter tRNA is coded by the already described tDNA^{Ser}GCT. This tRNA is enriched more than 150 fold in the particles as compared to its content in total cellular tRNA where it represents less than 0.1% (initiator tRNA^{Met} and tRNA^{Ser}AGA being 11 and 4 fold enriched respectively). This tRNA is the only species coded by the tDNA^{Ser}GCT gene which is found in three copies per genome since no other corresponding expressed tRNA could be detected. This gene is thus very poorly expressed. The high concentration of tRNA^{Ser}GCU in the particles compared to its very low cellular content led us to consider its possible implication in Ty specific processes.

INTRODUCTION

Transposition of *Saccharomyces cerevisiae* Ty1 elements has been shown to involve reverse transcription which takes place in intracytoplasmic virus-like particles (Ty1-VLPs). These particles contain Ty encoded reverse transcriptase, core proteins, genomic Ty RNA and Ty reverse transcripts (for review, see ref.1). In Ty, like in retroviruses, a specific tRNA is implicated in priming the minus DNA strand. The tRNA species used by Ty1 reverse transcriptase was identified as being initiator tRNA^{Met} (2,3). Its packaging into Ty1-VLPs was shown by ourselves at the tRNA Workshop which was held in Rydzyna (Poland) on May 4–9, 1991 (Abstract book p.37) and more recently by Chapman et al. (3).

In several retroviral systems that have been studied, the tRNA counterpart which is packaged into the virion was found to be not only the primer tRNA, generally the major species, but also

a subpopulation of host cellular tRNAs (4,5,6). However the reasons for such a selective packaging of other tRNAs than the primer tRNA are not understood yet. Moreover, attempts to attribute a role to non-primer packaged tRNAs in retroviral processes only led to speculations.

We show in this paper that, besides initiator tRNA^{Met}, Ty1-VLPs also contain several other tRNAs. Among them, we found in the same relative amount as that of initiator tRNA^{Met} a tRNA species not yet detected in expressed yeast tRNAs whose abundance in the cell was found to be much lower than that of any minor species characterized until now.

MATERIAL AND METHODS

Strains: MW16 (α , his 3 Δ 200, leu Δ 1, ura 3-167, GAL⁺). JB224 (α , his 3 Δ 200, ura 3-167, GAL⁺) transformed by the pGTy1-H3 plasmid that contains a Ty1 element under the transcriptional control of a GAL1 promoter (7). These strains were kindly provided by G.R.Fink.

The culture medium contained 0.67% yeast nitrogen base without amino acids supplemented with 2% galactose (or glucose), uracile and aminoacids depending on the strain.

Ty1-VLPs were isolated from the strain JB 224 using a method described by Eichinger and Boeke (8) with some minor modifications. A further purification included a gel filtration (9). Yeast cells were grown at 22°C in 2% galactose. Cultures (2l) were harvested at a density of 3.10⁷ cells per ml. Cell breakage in buffer B/Mg (10mM Hepes-KOH pH7.8, 15mM KCl, 3mM DTT, 5mM MgCl₂ and aprotinin 10 μ g/ml) and centrifugation of the cytoplasmic fraction on sucrose gradients were as described (8) except that the sucrose gradient contained one more layer and was prepared in Buffer B without aprotinin and MgCl₂ but with 1mM EDTA. The sucrose gradient was made of 5 ml each of 70%, 50% and 35% sucrose and 15 ml of 20% sucrose. Purification of Ty-VLPs was monitored by reverse transcriptase activity (10). The peak of activity was located at the border between 35% and 50% sucrose. Fractions of that peak were

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Figure 1. Autoradiograms of 2D gel electrophoresis of 3' end labelled total yeast tRNA and tRNAs packaged into Ty1-VLPs. A: tRNA from the strain JB224 grown in galactose. B: labelled Ty1-VLPs tRNA coelectrophoresed with not radioactiv tRNA from strain JB224. Identifications of tRNA species in spots 1, 2, 3, 4, 6 and 7 were performed by sequencing their 3' ends (see figure 2). Spot 5 was assigned to be tRNA^{Ser}CGA and tRNA^{Ser}UGA from hybridization experiments.

3-fold diluted with Buffer B/EDTA, then centrifuged at 40 000 g for 1 h. The pellets were resuspended in 1 ml of the same buffer with 0.05% Nonidet-P40 added and loaded on a Sephacryl S1000 gel filtration column (bed size 2.6×70 cm). The flow rate was 10 ml.h⁻¹. The peak of reverse transcriptase activity appeared after 75 ml of Buffer B/EDTA with 10% glycerol added had run through the column. The active fractions were pooled and centrifuged at 40.000 g for 1 h.

Nucleic acids were extracted from VLPs using 0.5% SDS, 0.1 M NaCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA for 30 min at room temperature and addition of proteinase K (300 µg/ml) for 30 min at 37°C, followed by two phenol treatments, ether extraction and ethanol precipitation. tRNA was separated from Ty1 nucleic acids by chromatography on DEAE-cellulose column and finally recovered by ethanol precipitation with glycogen added as carrier.

Total yeast tRNA was purified as described by Wilhelm *et al.* (11). The two dimensional polyacrylamide gel (2D gel) electrophoresis was performed at 5°C on 9.6% acrylamide in presence of 6M urea (first dimension) and on 20% acrylamide in presence of 4M urea (second dimension) both containing 20mM Tris-borate buffer, pH 8.3, 2.5mM EDTA. tRNA deacylation, 3' end specific labelling using tRNA nucleotidyl transferase and quantification of ³²P tRNA from gel spots were performed as described (12).

For hybridization experiments, the transfer of tRNA from polyacrylamide gels on nylon membrane (Hybond N, Amersham) was performed by electroblotting in 0.01 M Tris acetate buffer pH 8 (applied current 5 mA/cm² for 1.5 h at 15°C). Specific oligomers were 5' labelled using T4 polynucleotide kinase. Dot blots on Hybond N membranes and hybridization conditions were as described (13).

To sequence the 3' labelled end oligonucleotides of the tRNAs, we have used electrophoresis-homochromatography as previously described (14).

Commercial baker's and brewer's yeast tRNA were purchased from Boehringer, Mannheim, GFR.

tRNA oligonucleotide probes

The selected sequences complementary to the tRNA aminoacyl stem, TΨ stem and loop were according to the tRNA and tDNA sequences given in ref. 15 and specified here by the indicated code numbers. These were the following:

- 1—tRNA^{Ser}GCU (S 6281) : 5' GTCACAGAGAGGATTGCGAA 3'
- 2—tRNA^{Ser}AGA (S 6280) : 5' GACAACTGCAGGACTCGAA 3'

Figure 2. Electrophoresis-homochromatography and structural assignment of 3' end labelled tRNAs packaged in Ty1-VLPs (see figure 1). The results shown are: spot 1, tRNA^{Leu}U* mAA; spot 2, tRNA^{Ser}GCU; spot 3, tRNA^{Ser}AGA; spot 4, tRNA^{Leu}CAA; spot 6, tRNA^{Tyr} (right track) contaminated by tRNA^{Val} (left track); spot 7, initiator tRNA^{Met} (note the characteristic long shift due to Ar(p) resistant bond).

- 3—tRNA^{Ser}CGA (S 6400) and tRNA^{Ser}UGA (S 6401) : 5' GACACCAGCAGG-
ATTTGAA 3'
- 4—tRNA^{Ser}CGA (S 6402) : 5' GACCACAGCAGGGATTGAA 3'
- 5—initiator tRNA^{Met} (X 6280) : 5' TAGCGCCGCTCGGTTTCGAT 3'

RESULTS

Two dimensional polyacrylamide gel electrophoresis of the tRNAs packaged in Ty1-VLPs and total cellular tRNA

The electrophoretic patterns of 3' end labelled tRNA extracted from Ty1-VLPs showed three major spots, 2, 3 and 7, and three weaker spots, 1, 4 and 6 (Fig. 1B) of which the intensity varied from one preparation to another. While most spots were found in 2D gels of total tRNA (Fig. 1A), one of them (spot 2) was undetectable in both autoradiography and staining. Overexposure of autoradiograms of Ty1-VLPs tRNAs also revealed a random assortment of all other cellular tRNAs.

Identification of the tRNAs packaged in Ty1-VLPs

The tRNAs extracted from Ty1-VLPs were 3' labelled, separated on 2D gels, eluted from gels and analysed by electrophoresis-homochromatography using the techniques described in Material and Methods (Fig. 2). Their identification was obtained by

Table 1. Relative amounts of the main tRNA species packaged in Ty1-VLPs in these particles and in total cellular tRNA.

Spot n°	tRNA	Total cellular tRNA (strain JB224)	Ty1-VLPs tRNA	Ty1-VLPs tRNA/ total cellular tRNA
1	Leu U*mAA	2.0	7.3	3.6
2	Ser GCU	0.1	15	150
3	Ser AGA	4.0	15	3.7
4	Leu CAA	3.3	5	1.5
6	Tyr	3.6	4.6	1.3
7	initiator ^{Met}	1.1	12	10.9

The relative amounts of tRNAs in Ty1-VLPs and of tRNA^{Leu}U*mAA, tRNA^{Leu}CAA, tRNA^{Tyr} and initiator tRNA^{Met} in total cellular tRNA were determined from the radioactivity of individual spots in 2D gels (see figure 1 C and D) relative to the total radioactivity recovered from the gel. The tRNAs were 3' end labelled using tRNA nucleotidyl transferase. Quantitation of tRNA^{Ser}GCU and tRNA^{Ser}AGA in total cellular tRNA was obtained by comparing dot-blot hybridization signals revealed by specific probes with that revealed by an initiator tRNA^{Met} probe. Their relative amounts were calculated by assigning to initiator tRNA^{Met} the relative value of 1% (being that of spot 7 in 2D gel).

Figure 3. Partial sequence of tRNA^{Ser}GCU (B) (from Ty1-VLP tRNAs) as compared to tDNA^{Ser}GCT sequence (A) (18). The presumed anticodon of the tDNA is indicated in italic lettering and its intron is shown between arrowed dots.

comparing their 3' end sequence to already published tRNA sequences (15). Spots 3 and 7 were found to correspond to tRNA^{Ser}AGA and initiator tRNA^{Met} respectively. The characteristic long shift (two purine nucleotides forming an alkali resistant dinucleotide bond) observed in the pattern of initiator tRNA^{Met} after the UCGCGGC sequence (Fig. 2) indicated that initiator tRNA^{Met} packaged into the particles carried the O- β -riboseyl-(1''-2'')-adenosine 5' phosphate (position 64) previously determined by one of us (16). Spot 1 corresponds to tRNA^{Leu}U*mAA recently identified by one of us in yeast tRNA (17), whereas spots 4 and 6 corresponded to tRNA^{Leu}CAA and tRNA^{Tyr} respectively, the latter being slightly contaminated by tRNA^{Val}.

The partial sequence of the tRNA species contained in spot 2 is shown in figure 3. The part of the sequence which can be read on the electrophoresis-homochromatography pattern is identical to that of already published tDNA^{Ser}GCT (18). Spot 2 tRNA migrated on denaturing gels like tRNAs^{Ser} or tRNAs^{Leu}

which have long extra arms. It has tRNA^{Ser} characteristics: the presence of ac⁴C, i⁶A and Um which have been identified as constituents by trying to sequence the 'tRNA material' eluted from its presumed place in 2D gels of non radioactive cellular tRNA (where no other tRNA could be seen). The same experiment together with accurate wandering spot readings in the lower part of the electrophoresis-homochromatographies allowed us to extend the sequence assignment to 3 nucleotides farther than the characteristic T- Ψ (not shown).

The spot 2 tRNA is the only species found on total yeast tRNA pattern which could be coded by the tDNA^{Ser}GCT gene. Indeed, hybridization to an oligomer complementary to tDNA^{Ser}GCT used as a ³²P labelled probe revealed only one single radioactive spot located at position of spot 2 in 2D gels. It thus can be assumed that this spot corresponds to tRNA^{Ser}GCU. The same experiment performed with a tRNA^{Ser}AGA specific probe revealed also only one single spot corresponding to spot 3 in 2D gels.

Only tRNA^{Ser}AGA and tRNA^{Ser}GCU were found in significant amount in Ty1 VLPs whereas the other tRNA^{Ser}CGA and tRNA^{Ser}UGA were not detected although they share strong homologies with the former. The latter tRNAs were localized in 2D gels of cellular tRNA by hybridization with specific probes (numbered 3 and 4 in Material and Methods). Both probes revealed a single radioactive spot located at the same place in a 2D gel, corresponding to spot 5 (Fig. 1A). This spot was not detected in a 2D gel of Ty1-VLPs tRNA.

Relative enrichment of specific tRNA species in Ty1-VLPs

Quantitation of 3' end labelled tRNAs fractionated on 2D gels (Table 1) showed that the main tRNA species detected in Ty1-VLPs were initiator tRNA^{Met} (12%), tRNA^{Ser}AGA (15%) and tRNA^{Ser}GCU (15%). The other tRNAs, tRNA^{Leu}U*mAA, tRNA^{Leu}CAA and tRNA^{Tyr} were important in the particles, but at lower quantities than the three main species (Tables 1 and 2).

These well resolved spots contained only one tRNA species (except spot 6 tRNA which is contaminated by tRNA^{Val}). Moreover, the relative amounts of tRNA^{Leu}CAA and initiator tRNA^{Met} correlated with those previously determined by others in yeast tRNA (19). However, in the case of total cellular tRNA, the quantitation of tRNA^{Ser}GCU and tRNA^{Ser}AGA corresponding to spot 2 and 3 respectively, required additional experiments. Spot 2 tRNA detected in 2D gels of Ty1-VLPs tRNA could only be detected in 2D gels of total cellular tRNA by hybridization to a specific probe. This hybridization occurs

Table 2. The relative amounts of the main tRNA species packaged into four different Ty1-VLPs preparations were determined from the radioactivity of 3' end labelled tRNAs separated on 2D gels. The amount of initiator tRNA^{Met} was normalized at 1.0.

	Relative amounts of tRNA packaged in different Ty1-VLPs preparations			
	A	B	C	D
initiator tRNA ^{Met}	1.	1	1	1
tRNA ^{Leu} U ^m AA	0.6	0.7	0.7	0.5
tRNA ^{Ser} GCU	1.2	1.0	1.1	1.6
tRNA ^{Ser} AGA	1.2	1.2	1.4	1.8
tRNA ^{Leu} CAA	0.2	0.4	0.5	0.2
tRNA ^{Tyr}	0.4	0.5	0.8	0.9

Figure 4. Detection of initiator tRNA^{Met} (two columns on left) tRNA^{Ser}GCU (two columns in middle) and tRNA^{Ser}AGA (two columns on right) in Ty1-VLPs tRNA and in total cellular tRNA by dot-blot hybridization with specific probes respectively. Spotted Ty1-VLPs tRNA samples (indicated as Ty in the lower part of the figure) were each extracted from the particles isolated from 200 ml cultures. *E. coli* tRNA was added to the yeast tRNA in order to apply 4 µg of total tRNA per spot. The two upper lines correspond to dots with different quantities of total cellular yeast tRNA: counts (minus control value corresponding to only *E. coli* carrier tRNA) of hybridized probes and µg amounts of spotted yeast tRNA are indicated for each dot, first and second values respectively. The activity per µg of the initiator tRNA^{Met}, tRNA^{Ser}GCU and tRNA^{Ser}AGA probes were 3.10^7 , $2.7.10^7$ and $3.9.10^7$ cpm respectively.

at the same place where spot 2 tRNA of Ty1-VLPs tRNA migrates on 2D gels. In case of 3' end labelled total cellular tRNAs the radioactivity found in its presumed place accounted only for about 0.1 % of the total radioactivity. Such a value however has to be verified since it was close to that of the radioactivity background. By contrast, spot 3 corresponding to 8 % of total tRNA, a very high relative content, could result from the comigration of tRNA^{Ser}AGA with other species. Comparative estimates of initiator tRNA^{Met}, tRNA^{Ser}AGA and tRNA^{Ser}GCU were performed by using the dot-blot hybridization method. As shown in figure 4, Ty1-VLPs tRNA hybridized to about the same extent to initiator tRNA^{Met}, tRNA^{Ser}AGA and tRNA^{Ser}GCU probes, respective to their specific labelling. Hybridization values obtained in the case of cellular tRNA showed that tRNA^{Ser}GCU content was about 50 and 12 fold lower than that of tRNA^{Ser}AGA and initiator tRNA^{Met} respectively. tRNA^{Ser}AGA content was about 4 fold higher than that of initiator tRNA^{Met}. By assuming that the relative amount of initiator tRNA^{Met} is of 1 % in total cytoplasmic tRNA, tRNA^{Ser}AGA and tRNA^{Ser}GCU would account for about 4 % and 0.1 % respectively. Taken together, these results showed that initiator tRNA^{Met},

Figure 5. Detection of tRNA^{Ser}GCU in yeast tRNAs fractionated by 1D PAGE 1; tRNA from the strain JB224 grown in galactose. 2; tRNA from the strain JB224 grown in glucose. 3; tRNA from the strain MW 16 grown in galactose. 4; commercial baker's yeast tRNA. 5; commercial brewer's yeast tRNA. A: 1. gel electrophoresis of tRNAs. Ethidium bromide staining. B: Hybridization signal obtained with the tRNA^{Ser}GCU probe. The arrow in A indicates the position of the radioactive band.

tRNA^{Ser}AGA and tRNA^{Ser}GCU were enriched 11, 4 and about 150 fold, respectively, relative to cellular tRNA in Ty1-VLPs.

To determine whether such a low tRNA^{Ser}GCU content is specific to the strain JB224, hybridization assays were performed with tRNA from that strain, with tRNA from the non transforme strain MW16 and with commercial tRNA preparations. tRNA fractionated by 1D PAGE were hybridized to the tRNA^{Ser}GCU probe (Fig. 5). All the preparations gave a single radioactive band located at the position expected from the migration of tRNA^{Ser}GCU in 2D gel (Fig. 1). Nearly similar hybridization signals were given by tRNA from cells which overexpress the Ty1 element (strain JB224 grown in galactose), by tRNA from uninduced cells (strain JB224 grown in glucose) and by tRNA from the strain MW16. The tRNA^{Ser}GCU content was thus not affected by pGTy1-H3 expression. About 3 fold lower signal were given by both commercial baker's and brewer's yeast tRNAs indicating a relative amount of tRNA^{Ser}GCU of about 0.03 % in these preparations.

CONCLUSION

The tRNAs packaged into Ty1-VLPs are a select subpopulation of the cellular tRNAs. The main packaged species are initiator tRNA^{Met} which has previously been shown to be the primer for reverse transcriptase (3), tRNA^{Ser}AGA and probably tRNA^{Ser}GCU which has up to now only been found in its gen

form but never as an expressed tRNA. The particles contain in addition, tRNA^{Leu}U*_mAA, tRNA^{Leu}CAA and tRNA^{Tyr} in amounts significantly more important than all other yeast tRNAs which are present in the particles as trace amounts.

Initiator tRNA^{Met} in the particles is fully modified. It carries in particular in position 64 the modified nucleotide, O-ribosyl-phosphate adenosine, that has been determined by one of us (16). This modified nucleotide has been shown to inhibit the formation of the complex between the aminoacyl tRNA, GTP and the elongation factor thus restricting the functioning of that tRNA as protein synthesis initiator only (20). Its presence in initiator tRNA^{Met} associated to the particles would mean that it does not inhibit the formation of the complex between the RNAs and proteins which behave in reverse transcription initiation.

The other tRNAs which are packaged in the VLPs share common features: (i) tRNAs^{Leu} and tRNAs^{Ser} have long extra arms, (ii) the latter as well as tRNA^{Tyr} are hydrophobic species on countercurrent distribution (21), but not tRNA^{Ser}GCU (unpublished results). However these characteristics seem not to be the only reasons for which these tRNAs are packaged in the VLPs since neither tRNA^{Phe} a strongly hydrophobic species, nor the other tRNAs^{Ser} (tRNA^{Ser}CGA and tRNA^{Ser}UGA) could be detected in the particles in significant amounts. Moreover it is noteworthy that the tRNA^{Ser}GCU content in the particles is similar to that of tRNA^{Ser}AGA, although the latter is about 50 fold more abundant in the cell. These tRNAs would thus not compete for packaging.

The selective packaging of non-primer tRNAs does not appear to depend on Ty genomic RNA base pairing since their sequences do not show significant homologies with that of Ty. Moreover we observed that tRNA^{Ser}AGA and tRNA^{Ser}GCU, by contrast to initiator tRNA^{Met} are not associated with Ty nucleic acids normally extracted from VLPs with protein denaturant (unpublished results). We intend to investigate the possibility that one or more Ty coded proteins could be involved in the packaging of these tRNAs.

It must be pointed out that spot 2 tRNA must correspond to the species coded by the tDNA^{Ser}GCT gene previously described by Stücka and Feldmann (18) since no other spot hybridizing to a probe complementary to its 3' end could be found. To date, we did not succeed to purify amounts sufficient for complete sequencing. Enough material should however be obtained in a near future from overloading column chromatographies with enriched fractions obtained on BD-cellulose column chromatographies and on countercurrent distribution. Nevertheless we bring here the evidence that tRNA^{Ser}GCU is expressed, but at very low extent.

It has been reported that the amounts of many individual yeast tRNAs are roughly proportional to their gene copy numbers (19). These results led to the general idea that the number of gene copies might be the major factor in determining the contents of tRNAs in a cell. Such a correlation was observed in the case of initiator tRNA^{Met} which represents about 1% of total tRNA. This tRNA is encoded by four to five gene copies per haploid genome (22,23) i.e. 1 to 1.5% of the yeast tRNA genome. But in the case of tDNA^{Ser}GCT, there are three copies in the yeast genome (18) whereas the expressed tRNA^{Ser}GCU represents less than 0.1% of total tRNA from haploid strains that have been examined: this value is about eight fold lower than that expected from the constitutive expression of the corresponding three gene copies. An even lower content, three times less was found in commercial baker's and brewer's yeast tRNA preparations. Such

a low tRNA content could be due to a specific regulation of transcription i.e. a negative modulation. Another reason, such as uncomplete pre-tRNA processing, can also be considered.

The very low abundance of tRNA^{Ser}GCU in the cell gives rise to the problem of the translation efficiency of AGPy codons. These codons are mainly found in lowly expressed genes and therefore would be translated by a minor tRNA species (24). However, individual minor tRNAs preferably used for decoding of lowly expressed genes which have already been characterized, represent about 0.5 to 1% of total yeast tRNA (19). They represent thus much more than the quantity found for tRNA^{Ser}GCU. Therefore the tRNA^{Ser}GCU availability would act as a severe translational control element unless another tRNA species could recognize the AGPy codons. But then, what would be the cellular function of spot 2 tRNA? Finally, it is now essential to determine if that tRNA, which is highly concentrated in Ty1-VLPs but very lowly abundant in the cell, is involved or not in Ty specific processes. Solving these questions and sequencing tRNA^{Ser}GCU is our actual aim.

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