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Yeast serine isoacceptor tRNAs: variations of their content as a function of growth conditions and primary structure of the minor $tRNA^{Ser}GCU$

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Abstract

The primary structure of *Saccharomyces cerevisiae* $tRNA^{Ser}GCU$ is presented (EMBL database accession No. X74268 *S. cerevisiae* tRNA-Ser). In addition, quantitation of the relative amounts of serine isoaccepting tRNAs in yeast grown on different media showed that the minor $tRNA^{Ser}GCU$ decreased while the major $tRNA^{Ser}AGA$ increased as the growth rate and the cellular protein content increased. The minor species, $tRNA^{Ser}CGA$ and $tRNA^{Ser}UGA$, were not separated by our gel system, however, taken together they appeared to vary in the same way as $tRNA^{Ser}GCU$. These data suggest a growth rate dependence of yeast tRNAs similar to that previously described for *E. coli* tRNAs.

Key words: *Saccharomyces cerevisiae*; $tRNA^{Ser}GCU$ primary structure; Serine isoaccepting tRNA abundance

1. Introduction

The analysis of tRNAs associated with the yeast cytoplasmic Ty1 virus-like particles revealed the selective packaging therein of several tRNAs [1]. Among them, one tRNA species, according to partial sequencing, was assumed to be the transcription product of the already described $tDNA^{Ser}GCT$ gene [2]. Despite the description of the corresponding precursor tRNA [3] the mature corresponding tRNA has not yet been detected. According to our earlier work [1] this tRNA was found to be a species of very low abundance in yeast. It was estimated to correspond to less than 0.03% of total tRNA in commercial baker's and brewer's yeast and about 0.1% in laboratory grown yeast cells. The present paper deals with the primary structure determination of $tRNA^{Ser}GCU$ (accession no. at the EMBL database is X74268 *S. cerevisiae* tRNA-Ser). In addition, we report the relative abundance of the serine tRNAs in yeast as a function of growth conditions.

2. Materials and methods

Saccharomyces cerevisiae MW16 (α, *his3Δ200*, *leu2Δ1*, *ura3-52*, GAL^+) was kindly provided by G.R. Fink and J. Boeke. Strain a/α (homozygous for all markers except at the MAT locus) was derived from MW16 after transformation by an YCP50 plasmid that contains the HO gene which induces the mating type switching. The a/a and α/α strains were derived from a/α strains by γ-irradiation and screening for mating competent colonies.

Complex medium (YP) containing 1% yeast extract, 2% bactopeptone was supplemented with 2% glucose (YP-glucose). Synthetic minimal medium containing 0.67% yeast nitrogen base without amino acids (YNB; Difco) was supplemented with 0.002% of each uracil, histidine and leucine and 2% glucose (YNB-glucose) or 2% galactose (YNB-galactose).

Cultures were grown at 30°C and harvested in exponential phase at cell concentrations of about 2×10^7 cells per ml. Growth rates were determined from cell counts after ultrasonic dissociation of the aggregates.

Cellular protein content was determined as described by Stickland [4].

Purification of total yeast tRNA, electrophoresis on one-dimensional polyacrylamide gels (1D PAGE) and hybridization assays were performed as described [1]. Total commercial brewer's yeast tRNA was from Boehringer, Mannheim.

The tRNA specific probes were synthetic 19mer DNA oligonucleotides complementary to the aminoacyl stem, TΠ stem and loop sequences of the tRNAs studied [1]. These were the following: (i) SerGCU for $tRNA^{Ser}GCU$ (S6281), 5′ GTCACAGAGAGGATTCGAA 3′; (ii) SerAGA for $tRNA^{Ser}AGA$ (S6280), 5′ GACAACTGCAGGACTCG-AA 3′; and (iii) Ser CGA/UGA for $tRNA^{Ser}CGA$ (S6400) and $tRNA^{Ser}UGA$ (S6401), 5′ GACACCAGCAGGATTTGAA 3′.

Hybridization signal intensities were quantified on autoradiograms by photometric analysis using the RAG200 system (Biocom, France). The optical density of each band was defined by the formula $OD = -\log (T_d/T_{max})$ where T_d is the gray level of the band and T_{max} the maximum of transmission determined in a reference area of the autoradiogram. The signal intensity was calculated by multiplying the averaged optical density by the area of the band.

The primary structure of $tRNA^{Ser}GCU$ was determined using single hit hydrolysis and ^{32}P post-labelling as already described [5,6].

3. Results

3.1. *Purification and primary structure of the yeast $tRNA^{Ser}GCU$*

The strategy to purify $tRNA^{Ser}GCU$ had to accurately eliminate the major $tRNAs^{Ser}$ and other tRNAs, like $tRNAs^{Leu}$, with long extra arms that, in most classical methods (BD-cellulose and Sepharose 4B column chro-

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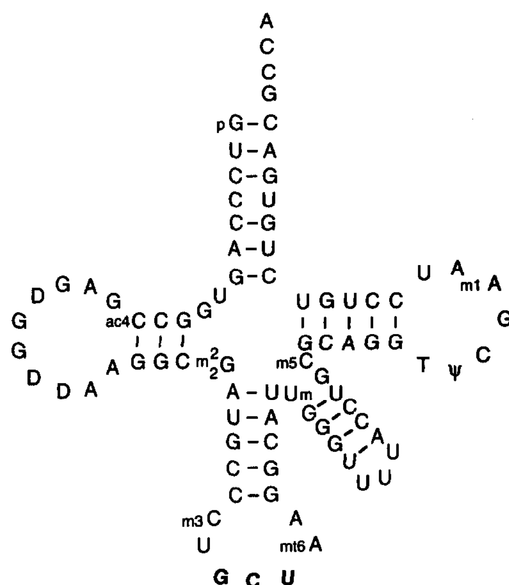

Fig. 1. Primary structure of the minor brewer's yeast tRNA^{Ser}GCU.

matographies, PAGE), migrate in its neighbourhood. To get the few nanomoles of the tRNA^{Ser}GCU necessary for its sequencing we used counter-current distribution (ccd) fractions [7] where this tRNA was found to elute in the aqueous phase whereas all other tRNAs with long extra arms eluted in the organic phase. Its primary structure was determined as mentioned in section 2: it is shown in its cloverleaf drawing in Fig. 1. It is worth mentioning that tRNA^{Ser}GCU has at the 3′ end of the anticodon (position 37) the hypermodified nucleotide, *N*-((9-*β*-D-ribofuranosylpurine-6-yl)*N*-methylcarbamoyl)threonine (mt^{6}A) instead of *N*6-isopentenyl-adenosine (i^{6}A) found at that place in the major tRNAs^{Ser}. The presence of mt^{6}A could be responsible for the elution of this tRNA in the ccd's water phase instead of the organic phase: i^{6}A could give hydrophobic properties to tRNAs whereas mt^{6}A could give hydrophilic ones. This tRNA^{Ser}GCU has the same anticodon loop, among other similarities, as tRNA^{Ser}GCU from mammals [8]: the latter also has mt^{6}A in position 37 instead of i^{6}A in the other mammalian tRNAs^{Ser}.

3.2. Relative amounts of tRNA^{Ser}GCU in various yeast tRNA preparations

We previously observed that the tRNA^{Ser}GCU content was significantly lower in commercial tRNA preparations than in tRNAs that we extracted from the JB224 and MW16 [1] strains. Given that commercial tRNA was extracted from polyploid cells grown on rich media whereas strains JB224 and MW16 are haploid cells and were cultivated on minimal medium (YNB), we compared the relative abundance of tRNA^{Ser}GCU in haploid α cells and diploid a/α, a/a and α/α cells grown on media leading to different growth rates. These media were either glucose-based rich medium (YP) or minimal media (YNB) containing either glucose or galactose (the latter being also fermented by the strain MW 16).

The tRNAs extracted from exponential phase growing cells and fractionated by 1D PAGE were hybridized to a tRNA^{Ser}GCU probe complementary to its 3′ end. The corresponding autoradiograms are shown in Fig. 2. Relative tRNA^{Ser}GCU amounts deduced from the photometric analysis of the autoradiograms and expressed as a ratio to that of tRNA from YNB-galactose grown a/α cells are presented in Table 1: the signals obtained for tRNAs from α, a/α and α/α cells (Fig. 2A) grown on YNB galactose medium were nearly similar, indicating that the content of tRNA^{Ser}GCU was not dependent on ploidy or on the mating type. It must be pointed out that the signal obtained for tRNA^{Ser}GCU from the a/a strain grown on YNB-galactose was about two-fold higher than that from other strains grown on the same medium. A longer exposure of the autoradiogram revealed in the pattern of a/α cells tRNA (Fig. 2B, lane 4) an upper band not seen in the pattern of a/a cells tRNA (Fig. 2B, lane 3) that could correspond to unprocessed species (other higher molecular weight species were not detected). However, this extra band is too faint to compensate for

Table 1
Relative abundance* of serine isoaccepting tRNAs in yeast cells grown on media leading to different growth rates

Culture medium	Strain	Doubling time (h)	Protein (μg/10^7 cells)	Total tRNA (ng/10^7 cells)	SerGCU$^{(A)}$	SerGCU$^{(B)}$	SerGCU$^{(C)}$	SerCGA/UGA$^{(C)}$	SerAGA$^{(C)}$
YP-glucose	α	1.5	65	500	0.34	–	–	–	–
	a/α	i.d.	–	689	0.30	0.19	0.35	0.65	1.71
	a/a	i.d.	98	650	0.28	0.37	0.42	0.62	2.26
	α/α	i.d.	–	570	0.11	–	–	–	–
YNB-glucose	a/α	2	77	370	–	0.55	–	–	–
YNB-galactose	α	2.7	27	221	0.74	–	–	–	–
	a/α	i.d.	–	172	1.0	1.0	1.0	1.0	1.0
	a/a	i.d.	43	212	1.8	1.90	1.6	1.1	1.13
	α/α	i.d.	–	315	0.85	–	–	–	–
Brewer's yeast tRNA	–	–	–	–	–	0.17	–	–	–

*Relative tRNA^{Ser} amounts were deduced from the photometric analysis of autoradiograms (Fig. 2) and are expressed as ratios to that of tRNAs^{Ser} from YNB-galactose grown a/α cells. A, B and C correspond to the autoradiograms shown in Fig. 2A–C, respectively.

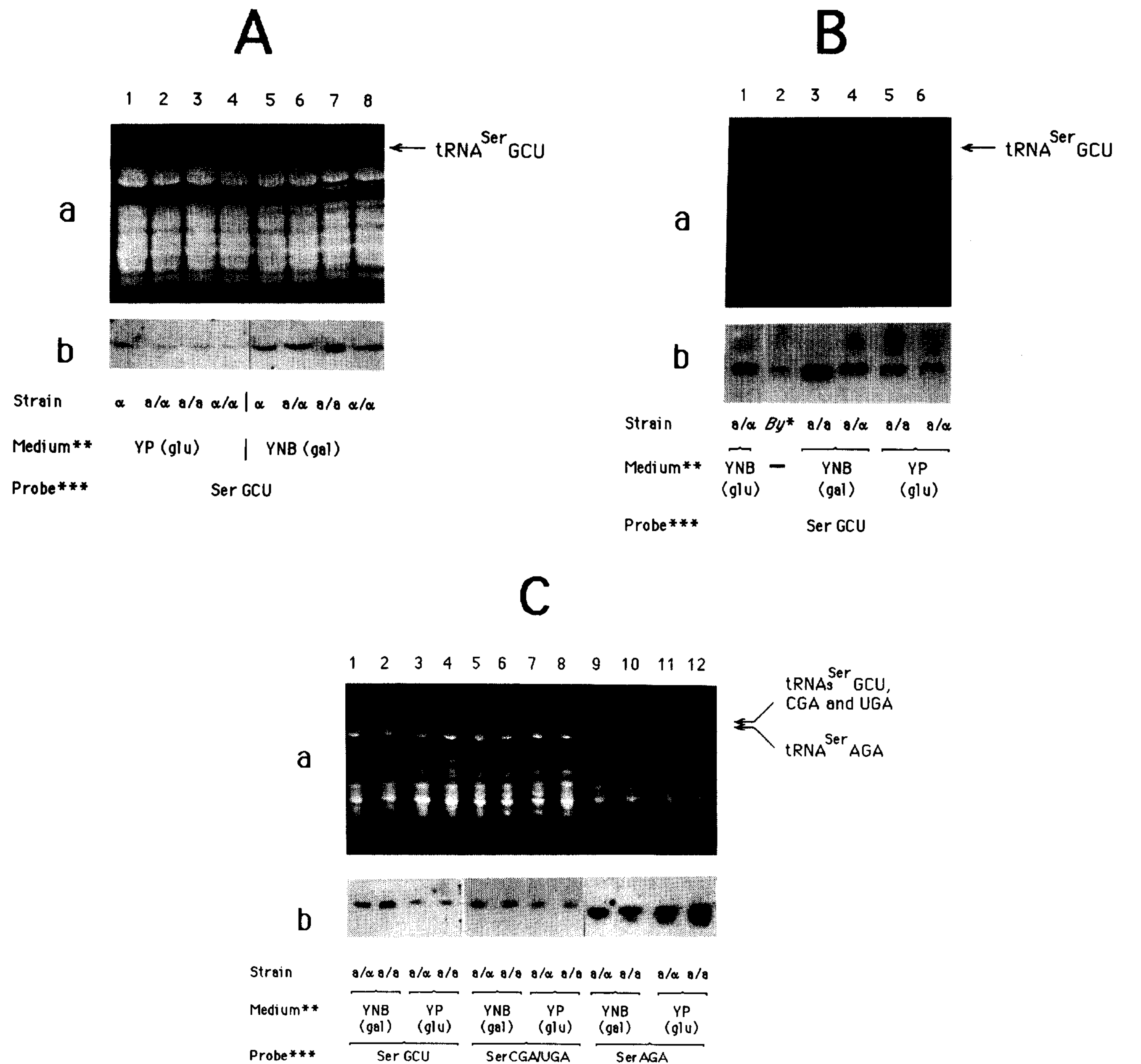

Fig. 2. Detection of serine isoaccepting tRNAs in yeast tRNA fractionated by 1D PAGE. Loaded total tRNA: panel A, 1 A_{260} OD unit; panels B,C, 0.5 A_{260} OD unit. (a) Ethidium bromide stained total tRNA. (b) Northern blot hybridization with ^{32}P-labelled oligonucleotide probes. **By*, commercial brewer's yeast tRNA. **Culture media (YNB, synthetic normal medium; YP, complex medium) and carbon sources (glu, glucose; and gal, galactose). ***Specific oligonucleotide probes as specified in section 2. The arrow(s) in a indicate the positions of the radioactive band(s) shown on the autoradiograms in b.

the difference in tRNA^{Ser}GCU contents between a/a and a/α cells which remains unexplained.

Moreover tRNA^{Ser}GCU from commercial brewer's yeast tRNA preparations (Fig. 2B, lane 2) and from cells grown on YP-glucose medium gave a 3- to 6-fold lower signal than tRNA from cells grown on YNB-galactose. The signal given by tRNA from cells grown on YNB-glucose (Fig. 2B, lane 1) was also lower than that of tRNA from cells grown on YNB-galactose.

Growth rates and cellular protein contents of the cell cultures described above are presented in Table 1. The corresponding values show that cells grown on glucose-based medium not only grew faster but also contained more proteins than cells grown on YNB galactose. The amounts of tRNA isolated from each culture indicated that the tRNA contents varied concomitantly with growth rates and protein contents (Table 1). These values are presented for information only, given that variable amounts of tRNA could be lost during the purification procedure.

3.3. *tRNA^{Ser}CGA and tRNA^{Ser}UGA content variations*

The closely related minor isoacceptors, tRNA^{Ser}CGA

and tRNA^{Ser}UGA, co-migrated in both 1D and 2D gels [1]. The signal detected by hybridization with the radioactive oligonucleotide probe thus corresponded to both species. As shown in Fig. 2C (lanes 5–8) and Table 1, the relative amounts of the tRNA^{Ser}CGA and tRNA^{Ser}UGA, taken together, are higher in tRNAs from cells grown on YNB-galactose than in tRNAs from cells grown on YP-glucose. These species thus varied in the same way as tRNA^{Ser}GCU.

3.4. *tRNA^{Ser}AGA content variations*

To determine whether the major isoaccepting species, tRNA^{Ser}AGA, varies like the minor tRNA^{Ser} species, we performed hybridization assays using a tRNA^{Ser}AGA-specific probe. As shown in Fig. 2C (lanes 9–12) and Table 1, the relative amount of tRNA^{Ser}AGA was significantly higher in cells grown on YP-glucose, i.e. at a higher growth rate than in cells grown on YNB-galactose, i.e. at a lower growth rate. The corresponding variations clearly indicated that the relative abundance of tRNA^{Ser}AGA varied in an opposite way to that of the minor tRNAs^{Ser} as far as growth conditions were concerned. In addition, it must be pointed out that the faster migrating band observed in the tRNA^{Ser}AGA pattern corresponds to the tRNA lacking the terminal AMP at the 3′ end since that extra band was not found in tRNA preparations treated by tRNA nucleotidyl transferase in the presence of ATP (not shown).

4. Discussion

Our results show the primary structure of the brewer's yeast minor tRNA^{Ser}GCU and indicate that the relative amounts of serine isoaccepting tRNAs in yeast cells are dependent on growth conditions. Two culture media, a glucose-based rich medium and a galactose-based minimal medium, were mainly used: the growth rate and the protein content per cell being about twofold higher in the first medium as compared to the second one. Using a quantitative Northern blotting technique, we observed that the relative amount of the minor tRNA^{Ser}GCU was at least threefold lower, while that of the major tRNA^{Ser}AGA was about twofold higher, in cells grown on rich medium than in cells grown on minimal medium. Although the other minor serine isoacceptors tRNA^{Ser}CGA and tRNA^{Ser}UGA were not separated by the gel system used, our results suggest that they varied in the same way as tRNA^{Ser}GCU. The decrease in the relative abundance of tRNA^{Ser}GCU when the growth rate and the cellular protein content increased would account for the very low abundance of tRNA^{Ser}GCU in commercial yeast tRNA, most likely isolated from cells grown on rich medium although other parameters such as the nature of the strains, the growth conditions and the harvesting time could be involved.

The variations of the yeast serine isoaccepting tRNAs described in this paper are similar to those previously reported by Emilsson and Kurland [9] in the case of *E. coli* tRNAs. These authors' studies showed that the abundance of minor species decreased while those of major species increased as the growth rates increased. Kurland et al. [9,10] suggested that these variations may be relevant to the growth rate-dependent variations of the protein population. Indeed they reported that major proteins, among them translational, transcriptional and membrane proteins, are far less dominant at the lowest growth rates than at the highest growth rates. In *E. coli*, as in yeast [10,11], the genes coding for major proteins mainly use a subset of codons that are those best recognized by major isoaccepting tRNAs. Conversely, lowly expressed genes use all 61 sense codons, the major codons as well as the minor codons, the latter being decoded by minor isoaccepting tRNAs. According to Kurland et al. [9,10], the relative abundance of individual *E. coli* tRNAs would be modulated in order to match the codon usage of the mRNA pool that is the more biased as the growth rate increased. Indeed the *E. coli* tRNA population shares a more even distribution at the lower growth rate although the relative amounts of the major species still exceed that of the minor ones [9].

Until now, there has been no evidence that in yeast, major proteins are relatively more abundant at high growth rates than at low growth rates. However, unless the yeast serine tRNAs that have been studied here are an exception, our results suggest that the response of yeast tRNAs to changes in the growth rates is similar to that of *E. coli* tRNAs.

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