Accumulation of 2'-O-Methylguanosine Deficient tRNA^{Trp} in Tryptophan Limited *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae synthesizes one major tryptophan transfer ribonucleic acid (tRNA^{Trp}) species (isoacceptor A) carrying characteristic base modifications. Under tryptophan limited growth conditions wild-type strain X2180-1A exhibited a second important tRNA^{Trp} species (isoacceptor B). The total amount of tRNA^{Trp}, approximately 2.5 pmol (mg dry wt)⁻¹, stayed essentially constant during amino acid shift-down experiments. The amount of isoacceptor B relative to total tRNA^{Trp} was 10 to 15% during amino acid sufficient exponential growth conditions, but increased during tryptophan limitation three- to four-fold. Analysis of the base compositions showed that isoacceptor B differed from isoacceptor A in one respect only: 2'-O-methylguanosine, a modified guanosine base occurring at position 17 of the major isoacceptor A tRNA^{Trp}, was not detectable in hydrolysates of purified isoacceptor B. The biological significance of isoacceptor B is discussed.

INTRODUCTION

Saccharomyces cerevisiae copes efficiently with imbalances of its amino acid supply. Besides specific regulatory mechanisms affecting single enzymes of certain amino acid biosyntheses, a cross pathway regulation designated 'general control' of amino acid biosynthesis has been described (Schürch *et al.*, 1974; Delforge *et al.*, 1975; Wolfner *et al.*, 1975). This general regulatory system affects the synthesis of enzymes of a number of amino acid biosynthetic pathways: the aspartate family including leucine and valine, the aromatic and the basic amino acids (Niederberger *et al.*, 1981). Limitation of growth by any one of these amino acids leads to a non-coordinate derepression of most enzymes of the pathways involved. However, as shown by Messenguy & Delforge (1976), Miozzari *et al.* (1977) and Stäheli *et al.* (1981) the general control is not directly dependent on amino acid limitations, but on the degree of charging of the cognate tRNAs. No studies are reported on possible alterations of tRNA occurring during amino acid limited growth in yeast. Changes in amounts of tRNA species for histidine (Litt & Weiser, 1978), leucine and tryptophan (Weiser & Litt, 1979) during amino acid starvation have been reported for Friend leukaemia cells in culture.

In prokaryotic micro-organisms such as *Escherichia coli*, the expression of the amino acid biosynthetic pathways for tryptophan, phenylalanine, histidine, threonine, isoleucine and leucine is subject to attenuation (for review, see Yanofsky, 1981). This regulatory mechanism does not only depend on the degree of tRNA charging, but is also influenced by mutations reducing tRNA modification (Yanofsky & Soll, 1977; Blasi & Bruni, 1981). On the other hand, an accumulation of new, presumably modified isoaccepting tRNAs was also observed in wild-type strains of *E. coli* after amino acid starvation (Kitchingman & Fournier, 1977; Thomale & Nass, 1978).

In order to investigate a possible contribution of reduced or altered modification of tRNAs in the general control of amino acid biosynthesis in *S. cerevisiae* we have searched for tRNA isoacceptors that occur only under conditions of amino acid limited growth. Our work concentrated on tRNA^{Trp}, because the tryptophan biosynthetic enzymes are subject exclusively to the general control system (Niederberger *et al.*, 1981) and because its primary sequence has been determined by Keith *et al.* (1971*a*). In addition the unspliced pre-tRNA^{Trp} has been characterized by Odgen *et al.* (1979). We present evidence for the preferential synthesis of a tRNA^{Trp} isoacceptor devoid of 2'-*O*-methylguanosine, and occurring under all conditions leading to decreased charging levels of tRNA^{Trp}.

METHODS

Organisms. All experiments were carried out either with the wild-type strain of *Saccharomyces cerevisiae* X2180-1A, obtained from T. Manney, Manhattan, Kansas, U.S.A., or with mutant strains derived from it. Their main characteristics are summarized in Table 1.

Media and growth conditions. MV-medium [0.145% (w/v) Difco yeast nitrogen base without amino acids and ammonium sulphate, 0.525% (w/v) ammonium sulphate, 2% (w/v) glucose] was buffered to pH 4.0 by the addition of 1% (w/v) succinic acid and 0.28% (w/v) potassium hydroxide. Mpro-medium contained no ammonium sulphate, but contained 0.1% (w/v) L-proline instead. Amino acid analogues were filter-sterilized and added to the autoclaved media before use, and amino acids were sterilized with the medium at 121 °C for 20 min.

Cells were grown at 30 °C either in Erlenmeyer flasks on a rotary shaker or in a 141 P.E.C. glass fermenter (type GF 0014, Chemap AG, Männedorf, Switzerland). Growth was followed turbidimetrically at 546 nm with a 1 cm light path (A_{546}). Growth rate, μ (h⁻¹), was defined as $\mu = (\ln x_2 - \ln x_1)/(t_2 - t_1)$.

Determination of the tryptophan pool. The intracellular tryptophan pool was determined with the microbiological assay using *Lactobacillus plantarum* ATCC 8014 as described by Fantes *et al.* (1976). Dry weight calculations were based on the following relation: 1 litre of cells at $A_{546} = 1$ contains 165 mg dry wt.

Preparation of cell extracts with tryptophanyl-tRNA synthetase activity. Crude extract or particle-free S-100 extract, both dialysed extensively to eliminate amino acids, of wild-type strain X2180-1A was used as a source of tryptophanyl-tRNA synthetase. Crude extract was prepared as described earlier (Stäheli *et al.*, 1981). S-30 and S-100 extracts were prepared essentially according to Keith *et al.* (1971*b*), omitting the final DEAE-cellulose chromatography step.

Determination of the degree of in vivo tRNA^{Trp} aminoacylation. tRNA extraction was carried out according to Folk & Berg (1970). Periodate oxidation of the tRNA was done according to Ehresmann *et al.* (1974), but the concentration of sodium periodate was 10 mmol l⁻¹ instead of 100 mmol l⁻¹. The tryptophan aminoacylation capacities of the oxidized and the control tRNA preparation were determined with the assay described below.

tRNA aminoacylation assay. A reaction mixture according to Keith *et al.* (1971*b*) was used for assaying tryptophanyl-tRNA synthetase (EC 6.1.1.2), leucyl-tRNA synthetase (EC 6.1.1.4) and phenylalanyl-tRNA synthetase (EC 6.1.1.20) with the following modifications: the buffer was Tris/HCl (100 mmol l⁻¹, pH 7.0) and the ¹⁴C-labelled amino acids (1.85×10^9 Bq mmol⁻¹) were used at a final concentration of 0.05 mmol l⁻¹. The mixture was incubated for 45 min at 30 °C and then stopped either by DEAE-cellulose column separation of the tRNA from the reaction mixture (see next paragraph) or by transferring the mixture to ice-cold TCA. The TCA-

Table 1. *Strains of Saccharomyces cerevisiae*

Strain	Phenotype	Reference
X2180-1A	Wild-type strain	} Miozzari <i>et al.</i> (1978)
RH 218	Tryptophan auxotrophic (<i>trp1</i> ⁻)	
RH 570	Tryptophan bradytrophic (Trp [±])	
RH 596	Phenylalanine bradytrophic (Phe [±])	
RH 654	Lysine bradytrophic (Lys [±])	
RH 657	Leucine bradytrophic (Leu [±])	} Niederberger <i>et al.</i> (1981)
Regulatory mutant strains		
RH 375	} Non-derepressible (Ndr)	Schürch <i>et al.</i> (1974)
RH 487		
RH 558		
RH 771		
RH 774	} Constitutively derepressed (Cdr)	Miozzari <i>et al.</i> (1978) and unpublished data from M. Aebi (this laboratory)
RH 775		
RH 777		

precipitated material was filtered through Whatman GF/C filters, washed extensively with ice-cold 5% (w/v) TCA, containing 100 mg of unlabelled amino acid l⁻¹ and finally with ice-cold ethanol. The filters were dried and counted in a toluene based scintillation mixture.

Determination of concentration of tRNA^{Trp} and of its isospecies. tRNA extraction was done according to Ehresmann *et al.* (1974). The crude tRNA was immediately deacylated in 2 ml Tris/HCl buffer (100 mmol l⁻¹, pH 9.0) for 60 min at 37 °C. After two ethanol precipitations, tRNA was redissolved in Tris/HCl buffer (10 mmol l⁻¹, pH 7.0, 1 mol NaCl l⁻¹) to discard rRNA. After a further ethanol precipitation tRNA was dissolved in the same buffer lacking NaCl. The concentration of tRNA^{Trp} was determined by an *in vitro* amino acylation assay with ¹⁴C- or ³H-labelled L-tryptophan (7.4 × 10⁸ Bq mmol⁻¹ and 2.1 × 10⁹ Bq mmol⁻¹, respectively; for further details of the assay see above). Concentrations of isoacceptors A and B were determined after fractionation on an RPC-5 column as described in the next paragraph.

Large scale tRNA extraction and purification of the tRNA^{Trp} isoacceptors. Crude tRNA was isolated essentially as described by Holley (1963). The cells (80 g wet weight) suspended in 2 vol. Tris/HCl (10 mmol l⁻¹, pH 7, 10 mmol magnesium chloride l⁻¹, and 5 mmol 2-mercaptoethanol l⁻¹) were shaken with 2 vol. phenol for 30 min at 25 °C. The aqueous phase was reextracted with phenol. After ethanol precipitation, the nucleic acids were extracted with 1 mol NaCl l⁻¹ in the above buffer. The insoluble residue was discarded. The tRNA was deacylated in Tris/HCl (100 mmol l⁻¹, pH 9, 5 mmol 2-mercaptoethanol l⁻¹) at 37 °C for 1 h. After ethanol precipitation, the tRNA was dissolved in Tris/HCl (10 mmol l⁻¹, pH 7, 2 mmol 2-mercaptoethanol l⁻¹) and applied to a DEAE-cellulose column (2 × 5 cm), equilibrated with the same buffer. The columns were first washed with the same buffer containing 0.2 mol NaCl l⁻¹ until the absorbance at 260 nm (*A*₂₆₀) of the effluent was practically zero. Then the tRNA was eluted with buffer containing 1 mol NaCl l⁻¹ and precipitated with ethanol. The precipitate was dissolved in distilled water and, after dialysis against distilled water, the tRNA (90 mg) was stored frozen.

As a first step for purification of tRNA^{Trp}, unfractionated tRNA was subjected to column chromatography on Sepharose 4B (1.6 × 27 cm) (Holmes *et al.*, 1975). After extensive dialysis of the eluate fractions against distilled water, samples of selected fractions were tested for tryptophan acceptance using the tRNA aminoacylation assay. The most active fractions were pooled. The tRNA in this pool was precipitated with ethanol and enzymically aminoacylated with ¹⁴C-labelled L-tryptophan. After removal of the reaction reagents and the protein by DEAE-column chromatography (see above) the two tRNA^{Trp} isoacceptors were separated and further enriched by chromatography on an RPC-5 column (0.6 × 56 cm) according to Pearson *et al.* (1971). Samples of the effluent were counted in a toluene-Triton X-100 based scintillation mixture. The fractions containing tRNA^{Trp} isoacceptor A or isoacceptor B, respectively, were pooled and the tRNAs were precipitated with ethanol. After deacylation in Tris/HCl (100 mmol l⁻¹, pH 9.0, 2 mmol 2-mercaptoethanol l⁻¹) the tRNA was rechromatographed on the RPC-5 column.

Purification of the tRNA^{Trp} isoacceptors to homogeneity was achieved by two-dimensional PAGE using a modified Ikemura & Dahlberg (1973) system. An acrylamide-urea gel (10%, w/v acrylamide, 7 mol urea l⁻¹) was used for the first dimension, followed by a gel (20%, w/v acrylamide, 4 mol urea l⁻¹) in the second dimension. The gel (20 cm × 40 cm × 3 mm) was loaded with 1.2 to 1.5 *A*₂₆₀ units of tRNA. After electrophoresis and staining with ethidium bromide, the single major spots of the separate gels were excised. The gels were homogenized and the tRNA eluted with Tris/HCl (50 mmol l⁻¹, pH 7.5, 300 mmol NaCl l⁻¹, 1 mmol EDTA l⁻¹). Ethidium bromide was extracted with *n*-butanol and the tRNA was stored lyophilized after extensive dialysis against distilled water.

Analysis of modified nucleosides. Nucleoside mixtures, resulting from complete digestions of tRNA^{Trp} isoacceptor A or isoacceptor B by a combination of nuclease P1 and alkaline phosphatase, were analysed by HPLC chromatography as described earlier (Davis *et al.*, 1979).

***In vitro* tRNA methylation.** The assay allows for the simultaneous determination of tRNA aminoacylation and tRNA methylation. ³H-Labelled tryptophan and ¹⁴C-labelled *S*-adenosylmethionine were used as substrates. The test mixture contained: Tris/HCl (100 mmol l⁻¹), magnesium chloride (10 mmol l⁻¹), potassium chloride (30 mmol l⁻¹), dithiothreitol (2 mmol l⁻¹), ATP (10 mmol l⁻¹), ³H-labelled tryptophan (0.05 mmol l⁻¹, 3.7 × 10¹⁰ Bq mmol⁻¹), *S*-adenosyl-L-[methyl-³H]methionine (0.065 mmol l⁻¹, 2.15 × 10⁹ Bq mmol⁻¹), purified tRNA^{Trp} isoacceptors (0.6 *A*₂₆₀ units ml⁻¹), non-dialysed S-30 or S-100 cell extracts (2 mg protein ml⁻¹). The mixture was incubated at 30 °C. Samples were collected after 1, 2, 3 and 4 h. The tRNA was precipitated, washed and counted as described above.

Chemicals. DL-5-Methyltryptophan was obtained from Fluka AG, Buchs SG, Switzerland. DL-4-Methyltryptophan was synthesized by Bachem Feinchemikalien AG, Bubendorf, Switzerland. Other chemicals used were of analytical grade. All radioactively labelled amino acids were purchased from Amersham.

RESULTS

Synthesis of tRNA^{Trp} isospecies under conditions of insufficient aminoacylation of tRNA^{Trp}

On MV-medium the wild-type strain X2180-1A synthesized two major isospecies of tRNA^{Trp}, which could be separated by RPC-5 column chromatography (Fig. 1). The major isospecies

Fig. 1. RPC-5 profile of Trp-tRNA^{Trp} and influence of the tryptophan bradytrophism of strain RH 570 on the biosynthesis of tRNA^{Trp}. tRNA was extracted, deacylated and reacylated with labelled tryptophan as described in Methods before co-chromatography on an RPC-5 column. ●, ¹⁴C-Trp-tRNA^{Trp} of strain X2180-1A grown in MV-medium for 18 h; ○, ³H-Trp-tRNA^{Trp} of strain RH 570 grown in MV-medium for 18 h; ---, indicates the NaCl gradient.

(called isoacceptor A) eluted at a lower salt concentration than a minor isospecies (called isoacceptor B). A tiny shoulder on the left side of isoacceptor A possibly represented an additional isospecies, but this was not studied further. The two isoacceptors A and B were present in a ratio of 85% and 15%, respectively, under these conditions.

This change in the ratio between isoacceptor A and B could have been the result of their interconversion (without *de novo* tRNA^{Trp} synthesis) or alternatively a preferential *de novo* synthesis of isoacceptor B. Since total tRNA^{Trp} concentration before and after tryptophan limitation remained at a constant level of 2.5 pmol (mg dry wt)⁻¹, whereas concentration of isoacceptor B rose from 0.4 to 1.2 pmol (mg dry wt)⁻¹, a *de novo* synthesis could be postulated.

We were interested in determining the physiological parameters upon which the concentration of isoacceptor B was dependent and if they could be related to the general control. The following conditions were studied: (1) variation in the tryptophan supply, (2) direct inhibition of tRNA^{Trp} charging, and (3) other amino acid limitations. Besides the growth rate, the tryptophan pool, the charging degree of tRNA^{Trp}, and the proportion of isoacceptor B were determined (Table 2). The basic values of the wild-type strain X2180-1A are a growth rate of 0.31 h⁻¹, 0.10 nmol tryptophan (mg dry wt)⁻¹, a charging degree of 95%, and an isoacceptor B proportion of 15% (line 1). Addition of tryptophan to the medium led to a 250-fold increase of the tryptophan pool and a drop in the proportion of isoacceptor B to 8% (line 2). A tryptophan limitation was induced either by 5-methyltryptophan (line 3), or by the tryptophan bradytrophism of strain RH 570 (line 5). This led to a tryptophan pool below 0.05 nmol (mg dry wt)⁻¹ in both cases, a charging degree of tRNA^{Trp} of 55% for wild-type and below 10% for the bradytroph, and ratios of isoacceptor B of 35% and 60%, respectively. A total tryptophan starvation in strain RH 218 (*trp1*⁻) did not increase the proportion of isoacceptor B any further (line 6).

4-Methyltryptophan was used to inhibit charging of tRNA^{Trp} directly (Miozzari *et al.*, 1977; Stäheli *et al.*, 1981). This analogue, applied in Mpro-medium on the wild-type strain, led to a drop of charging of the total tRNA^{Trp} to 25% (line 8) while the tryptophan pool was elevated 10-fold compared to the wild-type level on this medium. Repeated experiments showed, that under these conditions the proportion of isoacceptor B increased approximately twofold from 9% to 22%.

Table 2. Tryptophan pool, tRNA^{Trp} charging degree and concentration of isoacceptor B

Strain and phenotype	Medium	Growth rate μ (h ⁻¹)	Tryptophan pool [nmol (mg dry wt) ⁻¹]	tRNA ^{Trp} charging degree (%)	Proportion of isoacceptor B of total tRNA ^{Trp} (%)
1. X2180-1A (wild-type)	MV	0.31	0.10	95	15
2. X2180-1A (wild-type)	MV + Trp*	0.31	25.0	ND	8
3. X2180-1A (wild-type)	MV + 5MT*	0.24	<0.05‡	55	35
4. RH 570 (Trp [±])	MV + Trp*	0.30	ND	94	16
5. RH 570 (Trp [±])	MV*	0.05	<0.05‡	<10	60
6. RH 218 (Trp ⁻)	MV*	0	<0.05‡	ND	40
7. X2180-1A (wild-type)	Mpro	0.16	0.20	96	9
8. X2180-1A (wild-type)	Mpro + 4MT†	ND	2.0	25	22
9. RH 657 (Leu [±])	MV*	0.10	0.85	95	8
10. RH 654 (Lys [±])	MV*	0.18	0.27	93	12
11. RH 596 (Phe [±])	MV*	0.20	7.0	ND	9

ND, Not determined.

* Cells were cultivated for about 15 h under the conditions indicated, except for strain RH 218, which was harvested 6 h after tryptophan limitation. The media were supplemented with 1 mmol tryptophan l⁻¹ (line 2), 0.15 mmol tryptophan l⁻¹ (line 4) or 0.5 mmol DL-5-methyltryptophan (5MT) l⁻¹ (line 3).

† Cells were harvested 3 h after addition of DL-4-methyltryptophan (4MT) 0.2 mmol l⁻¹.

‡ Tryptophan pools below 0.05 nmol (mg dry wt)⁻¹ could not be determined by the method used.

Other amino acid limitations were studied in a leucine, a lysine and a phenylalanine bradytrophic strain (lines 9–11). These limitations led to a variable increase in the tryptophan pool but neither charging of tRNA^{Trp} nor the proportion of isoacceptor B were significantly affected.

These data suggested that isoacceptor B was synthesized preferentially under conditions of limited charging of tRNA^{Trp}.

Characterization of tRNA^{Trp} isoacceptor B

Isoacceptor A and B of tRNA^{Trp} were purified to determine molecular differences. The tRNA was extracted from the tryptophan bradytrophic strain RH 570 growing on MV-medium, since under these growth conditions both isoacceptors occurred in about equal amounts and the shoulder in the isoacceptor A peak was not present (see Fig. 1).

Consecutive chromatographic purification procedures on Sepharose 4B and RPC-5 columns, followed by two-dimensional PAGE, yielded highly purified isoacceptor A and B, respectively (for details see Methods). For the analysis of the nucleoside modifications, each pure isoacceptor species was completely hydrolysed with nuclease P1 and alkaline phosphatase. The nucleoside mixtures were separated on a HPLC column (Fig. 2) and analysed quantitatively as described elsewhere (Davis *et al.*, 1979).

The nucleoside composition for tRNA^{Trp} isoacceptor A corresponded to the values deduced from the sequence of *S. cerevisiae* tRNA^{Trp} (Keith *et al.*, 1971a; Table 3). The chromatogram (Fig. 2) and composition (Table 3) of isoacceptor B, however, differed from that of isoacceptor A; the nucleoside 2'-O-methylguanosine (Gm) was missing in isoacceptor B. Instead an additional guanosine residue was found. Since the nucleoside Gm is present only once in the sequence of tRNA^{Trp} and located at position 17 of the D-loop (Keith *et al.*, 1971a), this can be postulated to be the only difference between the two tRNA isoacceptors.

The aminoacylation properties of both isoacceptors were studied *in vivo* and *in vitro*. Under the influence of 5-methyltryptophan, the degree of charging of tRNA^{Trp} fell from 95% to 55% (Table 2). RPC-5 fractionation of the periodate treated tRNA revealed that the decrease of the overall tRNA^{Trp} charging was distributed evenly to both isoacceptors (data not shown). On the other hand both isoacceptors could be aminoacylated *in vitro* with the same efficiencies (data not shown).

Fig. 2. HPLC analysis of the nucleosides of the tRNA^{Trp} isoacceptors A and B. Nucleoside abbreviations: A, adenosine; U, uridine; C, cytidine; G, guanosine; Ψ , pseudouridine; m¹A, 1-methyladenosine; Cm, 2'-O-methylcytidine; m⁷G, 7-methylguanosine; T, ribothymidine; m¹G, 1-methylguanosine; Gm, 2'-O-methylguanosine; m²G, 2-methylguanosine; D, dihydrouridine. dA, dG, dC, dT are the corresponding desoxy-nucleosides. IS denotes the internal standard and En denotes enzymes.

Conversion of isoacceptor B into mature tRNA^{Trp}

When the tryptophan bradytrophic strain RH 570 was cultivated on MV-medium, total charging of tRNA^{Trp} dropped below 10% and the proportion of isoacceptor B reached a value of 60% of the total tRNA^{Trp} after 15 h (Table 2). Although after cultivation over 48 h the total tRNA^{Trp} concentration of strain RH 570 was somewhat reduced [from 2.5 to 1.5 pmol (mg dry wt)⁻¹], the proportion of isoacceptor B of total tRNA^{Trp} was unaffected (Fig. 3), thus showing the stable nature of isoacceptor B under strong tryptophan limitations. The question arose whether the isoacceptor B was a dead end-product of tRNA^{Trp} biosynthesis or whether after release of tryptophan shortage it could be converted to isoacceptor A, which is thought to represent mature tRNA^{Trp}. To test this, tryptophan was readded to strain RH 570 which had been growing on MV-medium for 48 h. Further growth was prevented by the addition of cycloheximide. The conversion of isoacceptor B to isoacceptor A is shown in Fig. 3. Within 4–5 h after tryptophan addition, the isoacceptor B concentration dropped from 0.8 pmol to 0.18 pmol

Table 3. Nucleoside composition of two tRNA^{Trp} isoacceptors from *Saccharomyces cerevisiae*

Nucleoside*	Reference value†	Isoacceptor A	Isoacceptor B
Ψ	6	5.8	5.8
C	16	15.9	15.8
U	10	9.6	9.6
m ¹ A	1	0.81	0.93
Cm	2	1.74	1.43
m ⁷ G	1	0.88	0.73
T	1	1.01	1.09
G	16	16.8	17.7
m ¹ G	1	0.88	1.27
Gm	1	1.09	ND
m ² G	1	0.96	0.97
A	16	16.4	16.7
D	3	‡	‡
Number of bases analysed	75	72	72

ND, Not detected.

* The nucleoside abbreviations are explained in the legend to Fig. 2.

† Keith *et al.* (1971 a).

‡ Dihydrouridine (D) could not be detected with the method used. The values given are based on calculations that took this fact into consideration.

Fig. 3. tRNA^{Trp} isoacceptor concentrations in strain RH 570 after release from tryptophan limitation. At time 0 tryptophan (1 mmol l⁻¹) and cycloheximide (0.35 mmol l⁻¹) were added to a culture of strain RH 570 grown in tryptophan limiting MV-medium for 48 h. At intervals, the overall tRNA^{Trp} concentration was measured from samples of the culture. The proportion of the two isoacceptors A and B was determined after RPC-5 chromatography. The dotted line indicates the growth behaviour of an untreated control culture. ▨, tRNA^{Trp} isoacceptor A; ▩, tRNA^{Trp} isoacceptor B; ○, A₅₄₆.

(mg dry wt)⁻¹, i.e. to about one fourth of the initial value. At the same time isoacceptor A concentration rose correspondingly.

These data suggested that isoacceptor B was not a dead end-product of tRNA^{Trp} biosynthesis but could be converted to mature tRNA^{Trp}. It was hypothesized that the conversion of the G in position 17 to Gm possibly occurred only after the isoacceptor B molecule had been aminoacylated. We tried to find direct evidence for this hypothesis by *in vitro* conversion of isoacceptor B to mature tRNA^{Trp} by crude cell extracts in the presence of *S*-adenosylmethionine as methyl donor. In our limited attempts conversion was not detected, when either aminoacylated or non-aminoacylated isoacceptor B was used.

Table 4. *Isoacceptor B concentrations in regulatory mutant strains of the general control*

Strain and genotype	Growth condition*	Proportion of isoacceptor B (% of total tRNA ^{Trp})
X2180-1A wild-type	MV	15
	MV + 5MT	35
RH 375 <i>ndr1-1</i>	MV	12
	MV + 5MT	44
RH 487 <i>ndr2-1</i>	MV	13
	MV + 5MT	53
RH 558 <i>cdr1-1</i>	MV	10
RH 771 <i>cdr2-1</i>	MV	10
RH 774 <i>cdr3-1</i>	MV	9
RH 775 <i>cdr4-1</i>	MV	11
RH 777 <i>cdr5-1</i>	MV	10

* Strains were grown for about 15 h under the conditions indicated. DL-5-Methyltryptophan (5MT) was used at a final concentration of 0.5 mmol l⁻¹.

Has isoacceptor B a regulatory function?

Tryptophan biosynthetic enzymes are regulated by the general control of amino acid biosynthesis (Schürch *et al.*, 1974; Miozzari *et al.*, 1978; Niederberger *et al.*, 1981). Isoacceptor B was found to accumulate under conditions of tryptophan limitation and shortage for charged tRNA^{Trp}. If isoacceptor B served as a signal for the general control, regulatory mutant strains of this system might differ in the relative or absolute amounts of isoacceptors A and B. However, the overall tRNA^{Trp} concentration of none of the regulatory mutant strains differed significantly from that of the wild-type strain, and both non-derepressible (Ndr) and all five constitutively derepressed (Cdr) mutant strains tested, showed isoacceptor B levels comparable to the wild-type strain (Table 4).

A screen for similar tRNA^{Leu} and tRNA^{Phe} isospecies accumulating during leucine or phenylalanine limitations, respectively, was carried out. tRNA^{Leu} from the leucine bradytrophic strain RH 657 and tRNA^{Phe} from the phenylalanine bradytrophic strain RH 596 were analysed by RPC-5 column chromatography but no new isospecies of these tRNAs were found (data not shown).

DISCUSSION

Characterization of tRNA^{Trp} isoacceptors A and B

The tRNA^{Trp} from *Saccharomyces cerevisiae* was separable into two isospecies A and B on an RPC-5 column. The analysis of the nucleoside composition of the purified isoacceptors revealed only one difference: the modified nucleoside 2'-O-methylguanosine (Gm) at position 17 was not detectable in isoacceptor B. Keith *et al.* (1971a) have also described two isospecies of tRNA^{Trp} from bakers' yeast. These isoacceptors, however, differed in a pseudouridine modification at position 64 and their appearance was not linked to defined physiological conditions.

Regulation of tRNA^{Trp} isoacceptors

Tryptophan limitation had two consequences on tRNA^{Trp}: first it reduced its charging degree and secondly it stimulated the synthesis of isoacceptor B. The relative proportion of isoacceptor B varied from a basal level of about 15% in wild-type cells growing on MV-medium up to 60% of total tRNA^{Trp} in strongly tryptophan limited cells of strain RH 570 (Table 2). The accumulation of isoacceptor B was most likely dependent on a lack of charged tRNA^{Trp} since it was also stimulated by direct inhibition of tryptophanyl-tRNA synthetase by 4-methyltryptophan, i.e. under conditions of a high internal tryptophan pool. On the other hand, addition of external tryptophan (in the absence of 4-methyltryptophan) led to lower levels of isoacceptor B, but never to its complete disappearance.

The accumulation of isoacceptor B was dependent on the lack of charged tRNA^{Trp}. This led to the hypothesis that the modification of the guanosine at position 17 to 2'-O-methylguanosine

might occur only after a conformational change of tRNA^{Trp} promoted by aminoacylation. Such a mechanism was indeed proposed for tRNA^{Phe} by Potts *et al.* (1979). Experimental evidence in favour of this hypothesis came from the observation that isoacceptor B, accumulating in strain RH 570 growing under tryptophan limitation (and showing a very low charging degree of tRNA^{Trp}) was converted quantitatively to mature tRNA^{Trp} after readdition of tryptophan and restoration of a high tRNA^{Trp} charging degree (Fig. 3). However, no methylation of isoacceptor B was detectable *in vitro* in preliminary assays. The lack of Gm synthesis under conditions in which aminoacylation of tRNA^{Trp} is limited could be pleiotropic. The amount of Gm in unfractionated tRNA from tryptophan limited cultures is now being assessed in comparison to tRNA from controls. A pleiotropic effect would have consequences for control of this tRNA modification. Agris *et al.* (1973) reported a growth temperature dependent effect on the production of ribose methylated nucleosides in *Bacillus stearothermophilus*. Increased ribose methylation at higher growth temperatures indicated a need to protect the tRNA structure from endogenous nuclease activity.

Isoacceptor tRNAs as regulatory elements in the general control of amino acid biosynthesis?

We tried to find experimental evidence that isoacceptor B of tRNA^{Trp} served as a signal for the general control, to which the enzymes of the tryptophan biosynthesis are subject (Schürch *et al.*, 1974; Miozzari *et al.*, 1978; Niederberger *et al.*, 1981). Two lines of evidence did not support the idea, however. First, no altered proportions of isoacceptor B tRNA^{Trp} were observed in regulatory mutant strains of the Ndr- or the Cdr-phenotype in comparison to the wild-type strain (Table 4). Secondly, no accumulation of isoacceptor B was found in bradytrophic mutant strains of other amino acid biosynthetic pathways which also turned on the general control (Table 2).

One might expect that the bradytrophic strains would accumulate similar isoacceptors of their cognate tRNAs. But no 'isoacceptor B-like' tRNA^{Leu} or tRNA^{Phe} isospecies were observed, however, upon RPC-5 chromatography of extracts from the strains RH 657 and RH 596 growing on MV-medium.

Thus, we have no evidence for a participation of undermodified tRNAs, especially not of isoacceptor B tRNA^{Trp}, in the general control mechanism.

Note added in proof. No pleiotrophic effect with respect to the formation of Gm in unfractionated tRNA from strains X2180-1A (wild-type), RH 570 (Trp $\pm$) and RH 657 (Leu $\pm$) has been found in the meantime.

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