DEFICIENCY OF THE PEROXY-Y BASE IN OOCYTE PHENYLALANINE tRNA

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1. Introduction

The activity of genes coding for 5 S ribosomal RNA has been shown to differ between oocytes and somatic cells of amphibians and fishes. This has been done by comparing in *Xenopus laevis* [1,2] and in *Tinca tinca* [3] the primary sequences of somatic 5 S RNA with its major counterpart in oocytes. Somatic 5 S RNA differs by 8 bases from the major oocyte species. Whereas probably all the 5 S genes are transcribed in the oocyte in *Xenopus* and *Tinca*, only ~10% is active in somatic cells.

Since transfer RNA accumulates synchronously with 5 S RNA in the oocytes of these species [4,5], a difference in the activity of somatic and oocyte tRNA genes could also be looked at by comparing the sequences of the corresponding RNA transcripts.

Other differences between somatic and oocyte tRNAs could also exist at the level of modified bases, which are formed after transcription.

We have compared the chromatographic properties of tRNA from previtellogenic oocytes and somatic cells [6]. Except for $tRNA^{met_1}$, all tRNA species we tested on RPC_5 or BD cellulose columns had a chromatographic behaviour characteristic of their somatic or oocyte origin. Such differences also occur between tRNA of normal and tumoral cells [7–12]. So far, the only well documented difference is the deficiency of the Y base in tumoral $tRNA^{phe}$ [13–15]. This residue, a tricyclic imidazo derivative of guanine to which is attached a 4 carbon side chain [17], is a highly fluorescent and hydrophobic base. It has been found in all normal eukaryotic cells, next to the 3′-end of the anticodon [16]. Some variations occur between species in terms of the sidechain [17].

Here I report the deficiency of this base in a normal eukaryotic cell, namely the oocyte of the teleost fish *Tinca tinca*, the fresh-water european tench.

2. Materials and methods

2.1. *Materials*

BD-cellulose was obtained from Boehringer (Mannheim). L-[^{3}H]phenylalanine (25–33 Ci/mM) and L-[^{14}C]phenylalanine (374 mCi/mM) were purchased from CEA (Saclay).

2.2. *Origin of RNA*

Small (previtellogenic) oocytes of *T. tinca* were obtained from immature females (10–15 cm). Ovaries of mature females (25–30 cm) were used as a source of large (vitellogenic) oocytes. No attempt was made to separate the oocytes from the accessory cells of the ovary. We assumed that the amount of RNA extracted from these cells was negligible compared to the amount present in oocytes. Transfer RNA was prepared by the method in [6]. In some cases, the extraction was performed at pH 7.3 in Tris 10 mM, $MgCl_2$ 10 mM, to prevent possible degradation of the Y base [22]. After 2 phenol extractions and 1 ethanol precipitation, the RNA was layered on a DEAE cellulose (DE 52 Whatman) in ammonium acetate 50 mM (pH 7.3), $MgCl_2$ 5 mM and NaCl 100 mM at 0°C. It was then eluted with NaCl 1.2 M in the same buffer.

2.3. *Preparation of aminoacyl-tRNA synthetases*

Crude aminoacyl-tRNA synthetases were prepared

either from the liver of *Xenopus laevis*, *T. tinca* or rat. Fresh tissues (5–10 g) were homogenised in 30 ml cacodylate 50 mM (pH 7.5), KCl 25 mM, $MgCl_2$, 10 mM, β-mercaptoethanol 10 mM. After centrifugation for 15 min at 12 000 × *g*, the supernatant was centrifuged for 45 min at 100 000 × *g* and to the new supernatant 10% glycerol was added and the solution was passed through a DEAE-cellulose column (DE52) adjusted with the extraction buffer without glycerol. The acylation procedure was as in [6], except that it was performed at 37°C for 25 min. The acylated tRNA was re-extracted.

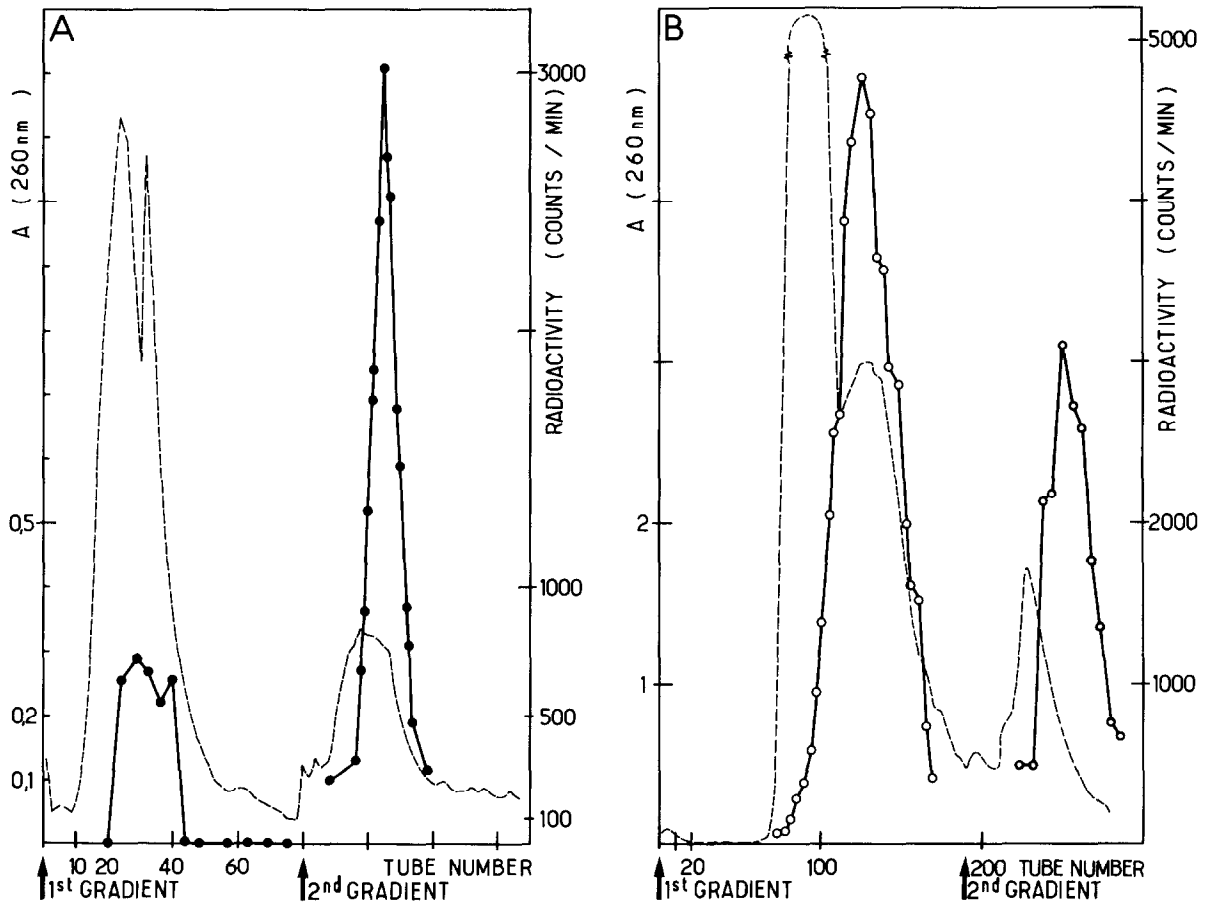

Fig.1a. BD-cellulose chromatography of tRNA from *T. tinca* liver. A column of 2 cm diam. × 7.5 cm ht of well-tamped BD-cellulose was first washed with 100 ml NaCl 2 M, $MgCl_2$ 10 mM, $CH_3COOONa$ 20 mM (pH 4.5) and then with NaCl 0.4 M in the same buffer. About 10 mg tRNA dissolved in 0.2 M NaCl in the above buffer were laid on the column. Chromatography was at pH 4.5. The first gradient ranged from 0.4–1 M NaCl (2 × 100 ml) followed by NaCl 1 M (200 ml). The second gradient ranged from 1–1.2 NaCl and 0–20% ethanol (2 × 75 ml). Acylation was performed as indicated in section 2 on 50 μl of each fraction. Charging in the ethanol peak reached 850 pmol amino acid/*A* unit of tRNA. From this column we can estimate that 80% of tRNA elutes in the second gradient. (- - -) A_{260}, (•—•) radioactivity ([^{3}H]Phe).

Fig.1b. BD-cellulose chromatography of tRNA from *T. tinca* previtellogenic oocytes. RNA was extracted at pH 7.3. Chromatography was at pH 7.3 (the same elution profile is obtained at pH 4.5). Other conditions as in fig.1A. Charging of the ethanol peak reached 150 pmol amino acid/*A* unit of tRNA. On this column, only 35% of the phenylalanine acceptance activity was found in the second gradient. (- - -) A_{260}; (•—•) radioactivity ([^{3}H]Phe).

2.4. *BD-cellulose chromatography*

BD-cellulose chromatography was by the general method in [18]. Chromatography was carried out either at pH 4.5 or at pH 7.3, as acid pH values possibly degrade the Y base [22].

2.5. *Fluorescence analysis of the Y base*

The specific fluorescence of the Y base was measured with a Jobin and Yvon HRS-II grating spectrofluorimeter equipped with a Xe arc lamp 450 W/4 (Osram), a diffraction grating of 70 × 100 mm, 1220 grooves/mm and a photomultiplier EM19558QA. The fluorescence emission spectrum of tRNA was measured from 310–500 nm upon excitation at 310 nm.

2.6. *Chemical treatment of the Y base*

The excision of the Y base from $tRNA^{phe}$ by acid treatment was by the method in [19].

Reaction of tRNA with 3-methyl-2-benzothiazolinone hydrazone (MBTH) was carried out as in [23] followed by analysis by chromatography on BD-cellulose columns.

3. Results

3.1. *BD-cellulose chromatography*

The elution profile of non acylated tRNA was different for oocyte and somatic $tRNA^{phe}$. The major part of oocyte $tRNA^{phe}$ eluted in the first (saline) gradient, whereas the major part of liver $tRNA^{phe}$ came out in the second (ethanol) gradient (fig.1a,1b).

When $tRNA^{phe}$ was charged before being chromatographed on BD-cellulose columns most of it eluted in the ethanol gradient. The major peak of oocyte phenylalanyl $tRNA^{phe}$ eluted before the major peak of somatic phenylalanyl $tRNA^{phe}$. The small peaks in the first (saline) gradient might correspond to altered molecules of $tRNA^{phe}$. This has not been investigated further (fig.2).

In order to make sure that the Y base is not lost during extraction of oocyte tRNA, oocyte homogenates of *T. tinca* were mixed with radioactive $tRNA^{phe}$ from *Xenopus laevis* kidney cells purified on a BD-cellulose column. This latter is known to possess the Y base [24]. After extraction, tRNA of oocyte and kidney cells were cochromatographed on a

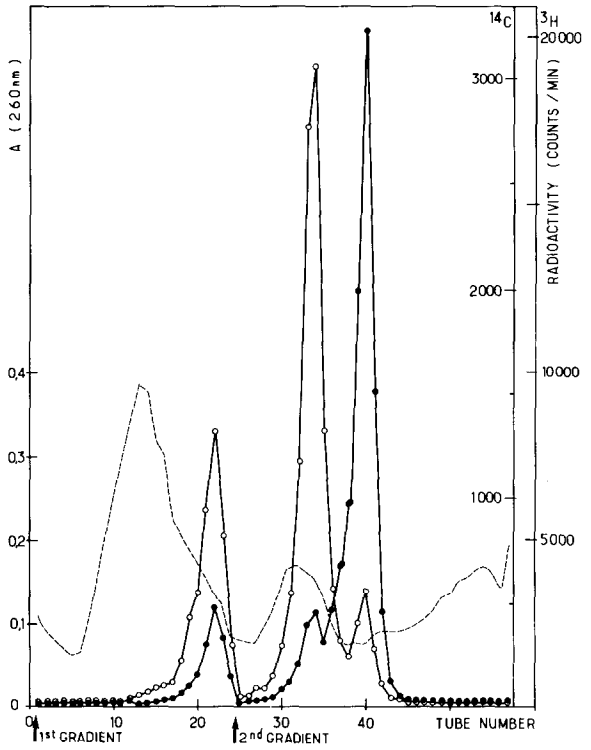

Fig.2. BD-cellulose chromatography of a mixture of tRNA from *T. tinca* liver (acylated with [^{3}H]Phe before chromatography) and tRNA from *T. tinca* previtellogenic oocytes (acylated with [^{14}C]Phe). A small column (0.5 × 2.5 cm ht) was washed as in fig.1a. Liver tRNA (470 μg) and oocyte tRNA (80 μg) were laid on the column. Chromatography was at pH 4.5; the first gradient ranged from 0.4–0.8 M NaCl (2 × 25 ml), the second from 0.8–1.2 M NaCl and from 0–20% ethanol (2 × 12.5 ml). Radioactivity of each fraction was measured on millipore filters after TCA precipitation with 50 μg yeast tRNA as carrier. (- - -) A_{260}; (○—○) ^{3}H radioactivity; (●—●) ^{14}C radioactivity.

BD-cellulose column. Only a small part of the $tRNA^{phe}$ of kidney cells eluted in the first gradient, whereas most of the $tRNA^{phe}$ of oocytes did (fig.3). From this experiment, I found that about 70% of oocyte $tRNA^{phe}$ molecules lack the Y base in vivo, but this varies from one experiment to another (from 65–85% in 8 experiments).

3.2. *Fluorescence emission of the Y base*

Liver $tRNA^{phe}$ purified by chromatography on BD-cellulose column had a maximum emission at

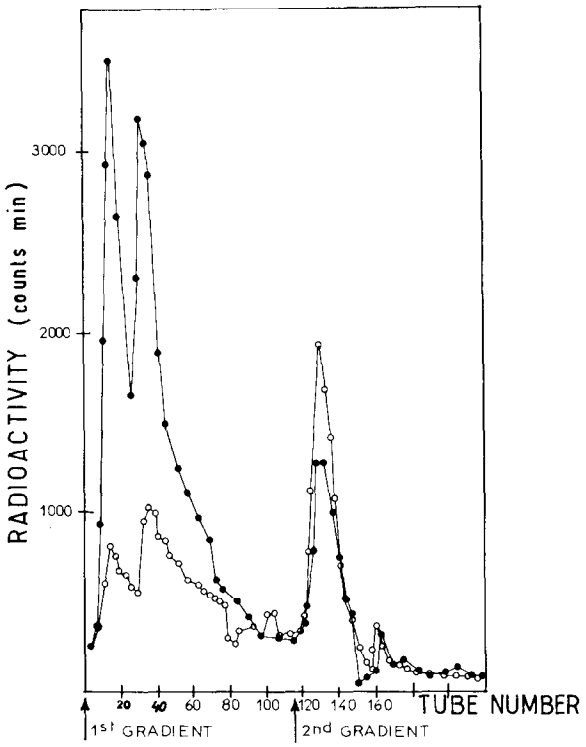

Fig.3. BD-cellulose chromatography of a mixture of RNA from *T. tinca* previtellogenic oocytes and $tRNA^{phe}$ of *Xenopus laevis* kidney cells. Cells were grown in Ham medium F12 supplemented with [^{3}H]uridine (30 Ci/mM, 15 μCi/ml) for 3 days and then for 1 day without labeling. RNA was phenol extracted, tRNA was purified on a Sephadex G-100 column and $tRNA^{phe}$ further purified on a BD-cellulose column as in fig.1B. This latter was added to 15 previtellogenic ovaries of *T. tinca* at the beginning of RNA extraction. After phenol extraction and ethanol precipitation, the total RNA was chromatographed on a BD-cellulose column. (○—○) trichloroacetic acid-precipitable ^{3}H cpm. (●—●) ^{3}H radioactivity on 50 μl of the fractions after acylation as in section 2, corrected for trichloroacetic acid precipitable cpm.

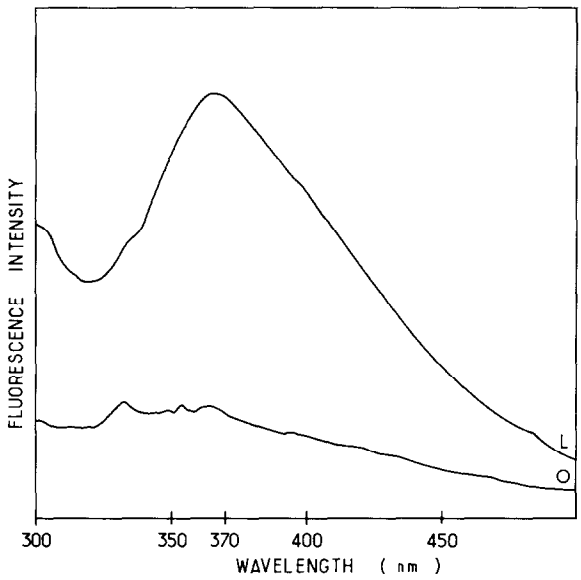

Fig.4. Fluorescence emission spectrum of liver and oocyte $tRNA^{phe}$. Fluorescence was measured in sodium acetate 0.01 N (pH 5) upon excitation at 310 nm, in a 1 cm cell (optical path). 6 A_{260} units $tRNA^{phe}$ from oocytes and from liver were purified on two different BD-cellulose columns after acylation as in fig.2. The major ethanol peaks were recovered and deacylated in the two cases. O, oocyte tRNA; L, liver tRNA.

370 nm. Under the same conditions purified oocyte $tRNA^{phe}$ gave > 300-times less fluorescence (fig.4).

3.3. *Acid treatment of the tRNA*

When treated at pH 2.9 as in [19], liver phenylalanyl $tRNA^{phe}$ was partly converted to a form which eluted in the ethanol gradient of BD-cellulose columns, at the position of the major oocyte phenylalanyl-$tRNA^{phe}$ peak, and partly to a form eluting in the saline gradient (fig.5a,5b). Under the experimental conditions used, conversion was ~50%. When the same treatment was applied to oocyte phenylalanyl-$tRNA^{phe}$, there was only about 17% conversion to a form eluting in the saline gradient (fig.5a,5b).

3.4. *Treatment with MBTH (see section 2.6.)*

Treatment of oocyte $tRNA^{phe}$ before chromatography on BD-cellulose column did not alter the elution pattern (exp. not shown).

4. Discussion

The experiments described above show that:

(1) Liver $tRNA^{phe}$ binds more tightly to BD-cellulose than does oocyte $tRNA^{phe}$.

(2) Oocyte $tRNA^{phe}$ does not exhibit the characteristic fluorescence of the Y base whereas liver $tRNA^{phe}$ does.

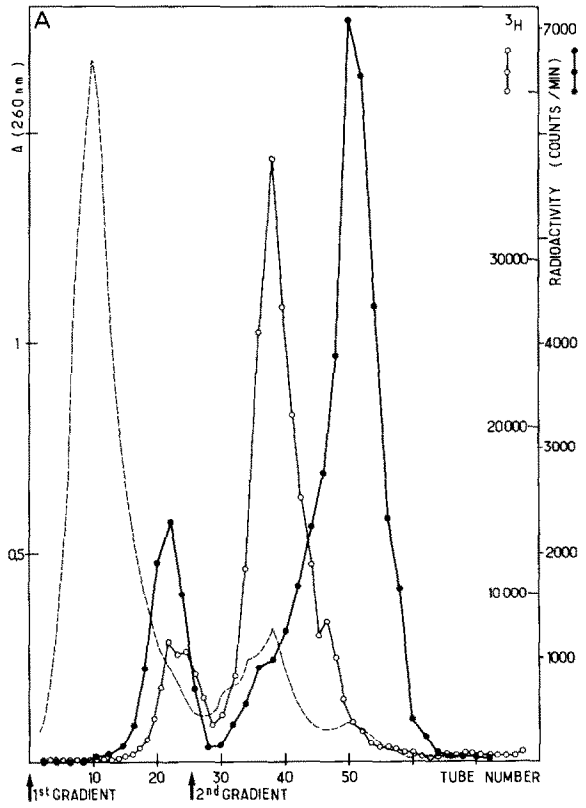

Fig.5a. BD-cellulose chromatography of oocyte and liver tRNA of *T. tinca* before acid treatment. On the same figure are shown the elution profiles of [^{3}H]phenylalanyl-tRNA^{phe} of liver (•—•) and of oocyte (○—○) chromatographed on two different columns as in fig.2. (- - -) A_{260}.

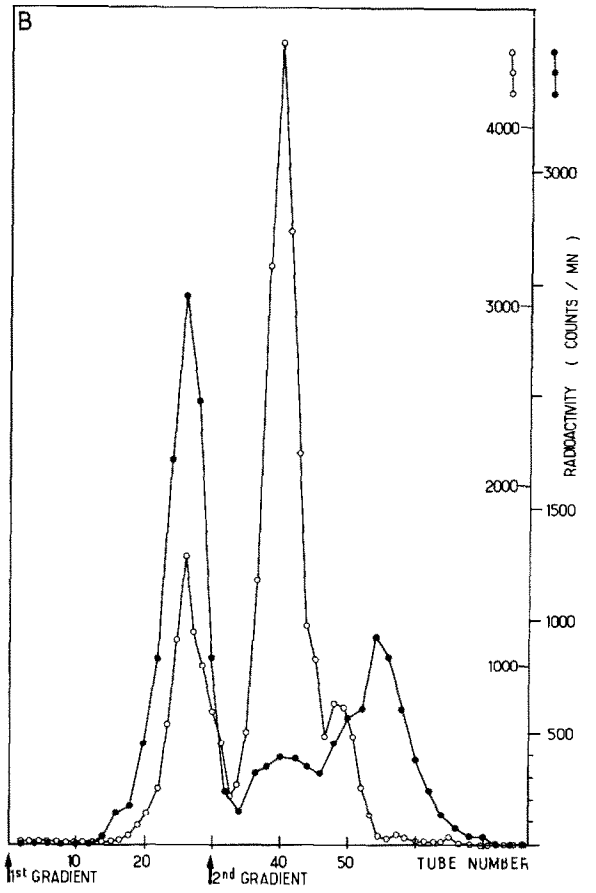

Fig.5b. BD-cellulose chromatography of oocyte and liver tRNA of *T. tinca* after acid treatment as in [20] on 66% of the second gradient peaks of fig.5a. On the same figure are shown the elution profiles of acid-treated [^{3}H]phenylalanyl-tRNA^{phe} of liver (•—•) and of oocytes (○—○) chromatographed on two different columns.

(3) Oocyte tRNA^{phe} is far less sensitive to acid treatment [19] than liver tRNA^{phe}.

From this we conclude that the Y base is absent in the predominant form of the tRNA of small previtellogenic oocytes of *T. tinca*. That this base is not lost during extraction and purification of tRNA^{phe} is shown by the following facts:

(i) Under the same conditions of preparation, liver tRNA^{phe} possesses the Y base whereas the oocyte does not.

(ii) in the presence of an oocyte homogenate of *T. tinca*, somatic *Xenopus laevis* tRNA^{phe} does not lose the Y base.

(iii) MBTH, which is known to react with the unsubstituted ribose in tRNA^{phe} whose Y base has been chemically excised [22], has no effect on oocyte tRNA^{phe}.

Oocyte tRNA^{phe} of *T. tinca* lacking the Y base may not be active in the oocyte since protein synthesis occurs at very low rate in oocytes, and since the major part of the tRNA is stored in ribonuclear particles sedimenting at 42 S in saccharose gradients [3,21]. In *T. tinca* it is possible that tRNA^{phe} is incorporated into 42 S particles immediately after transcription, and that it is not accessible to the enzymes implicated in Y base formation.

Alternatively the absence of the Y base could be

due to the absence or low activity in the young oocyte of the enzyme implicated in the formation of this base. More generally, the different tRNA species of the oocyte are perhaps less modified than somatic isoacceptors, due to the absence of modifying enzymes. The same explanation can perhaps also be given in the case of the $tRNA^{phe}$ that lacks the Y base in tumoral cells.

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