CODON RESPONSES OF $tRNA^{Ala}$, $tRNA^{Gly}$ AND $tRNA^{Ser}$ FROM THE POSTERIOR PART OF THE SILKGLAND OF *BOMBYX MORI* L.*

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1. Introduction

We can now consider that the population of tRNA in the silgland of the silkworm *Bombyx mori* L. is quantitatively adjusted, in order to translate with maximal efficiency the mRNA of fibroin ($mRNA_F$) in the posterior part [1–3] and the mRNA of sericin in the middle part of the gland [3]. Such an adjustment, called 'functional adaptation' (see [4] for a review), does not affect equally all the isoacceptor tRNA species (iso-tRNAs) occurring in the gland cells. We have observed [5] a differential increase of some iso-tRNA species specific for the major tRNAs for alanine and glycine during the fibroin secretion phase (5–8th day from the Vth instar). Thus, it would be possible to distinguish between 'common or basic' iso-tRNAs involved in synthesis of many proteins including the fibroin mRNA translation and 'modulator' iso-tRNAs which would preferentially decode the $mRNA_F$, so that the ratio of iso-tRNAs reflects the frequency of synonym codons in the mRNA of fibroin [5].

From the work of Suzuki and Brown [6] about the oligonucleotide distribution of the isolated $mRNA_F$, it appears that this messenger mainly contains the codon GCU for alanine, the two condons GGA and GGU with the ratio 1:1:4 for glycine and the codon UCA for serine.

Our present study on the codon response of isolated iso-tRNAs from the posterior part of the silkgland is in good agreement with their results, so that the iso-tRNA distribution is qualitatively adapted to its translational function of $mRNA_F$. It will be shown that $tRNA_1^{Ala}$ recognizes GCC and GCU while GCA, GCC and GCU are recognized by $tRNA_2^{Ala}$; $tRNA_1^{Gly}$ recognizes GGC and GGU, $tRNA_2^{Gly}$ GGA and GGU; $tRNA^{Ser}$ interacts with the UCN codons group.

2. Materials and methods

tRNAs were extracted from the posterior silkgland of *Bombyx mori* L. silkworm, hybrid from two European strains 200 and 300, at the 8th day from the Vth instar as described by Chavancy et al. [3] and submitted to a countercurrent distribution with 320 transfers in a salt solvent system [7] (see fig. 1). Purified iso-tRNAs specific for the major amino acids of fibroin (alanine, glycine and serine) have been used directly for ribosomal binding tests.

Alanine codons (GpCpN) were purchased from Miles–Seravac (Lausanne). Glycine and serine codons were prepared with primer-dependent polynucleotide

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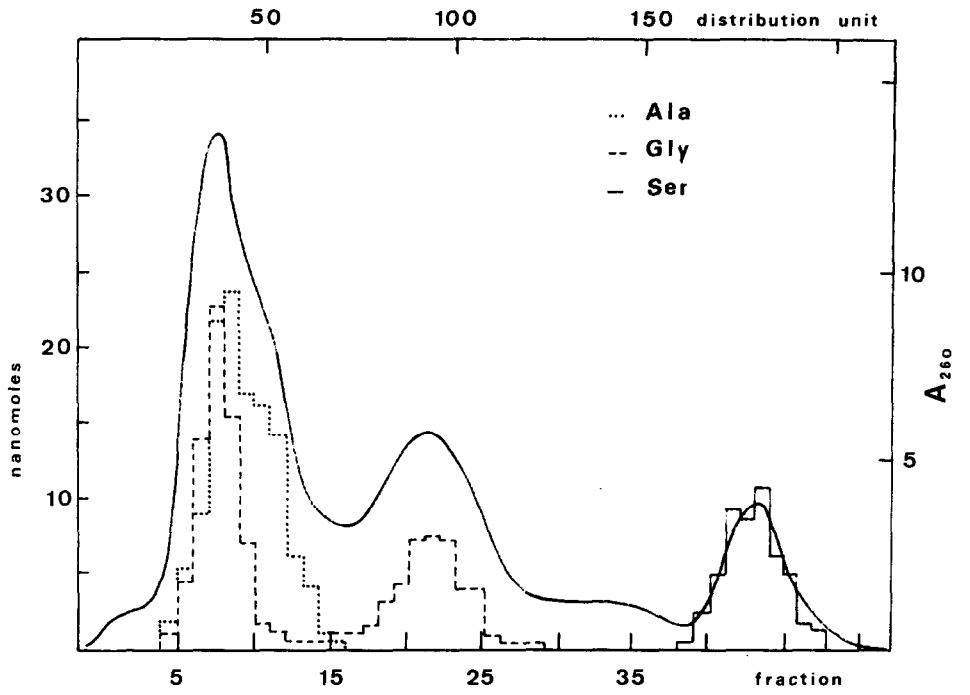

Fig. 1. Countercurrent distribution of tRNA from the posterior silkgland of *Bombyx mori* L. The countercurrent distribution was performed with a countercurrent apparatus EV-800 of 160 elements or distribution units containing 3 ml of each phase (Wright Scientific, Kenley, England) on 320 transfer with 2400 A_{260} of tRNA extracted at the 8th day from the Vth instar of the posterior part of silkglands of *Bombyx mori* L., as indicated by Chavancy et al. [3], at 15°C in the salt solvent system 'PMB': potassium phosphate buffer pH 7.0, 1.50 M (3 vol), 2-methoxyethanol (1 vol) and 19.2% of 2-butoxyethanol [6]. The solute in the mobile phase having run out the last distribution unit is collected and stored until use. The content of both phases of each four neighbouring elements are pooled into fractions numbered from 1 to 50, washed three times with diethylether, dialyzed over night, lyophilized and then dissolved in 2 ml of 5 mM $MgCl_2$. These fractions can be tested for accepting amino acids, ribosome binding or subjected to further purification. (. . . .) Acceptor activity of $tRNA^{Ala}$ in nanomoles/fraction. (- - - -) Acceptor activity of $tRNA^{Gly}$ in nanomoles/fraction. (——) Acceptor activity of $tRNA^{Ser}$ in nanomoles/fraction. (▬) Absorbance at 260 nm/2 ml of tRNA of both phases.

phosphorylase (EC 2.7.7.8) of *Micrococcus luteus* on adequate MpN primer and nucleoside diphosphate, as indicated by Schetters et al. [8]. Triplets were diluted to 100 A_{260}/ml water.

For tRNA acylation, rabbit reticulocyte enzyme was used, prepared according to Lin et al. [9]. Only the fraction precipitated between 40–70% of ammonium sulphate was recovered and stored at −20°C. Preparative acylation of silkgland tRNA was performed in the following reagent mixture for 500 µl final solution: 1 A_{260} tRNA in 10 µl, 60 µg enzyme in 50 µl, 50 µCi ^{3}H-labelled amino acid or 10 µCi ^{14}C-labelled amino acid (CEA, Saclay, France), 200 µl stock solution composed of 150 mM Tris–HCl pH 7.4, 50 mM KCl, 10 mM $MgCl_2$, 7 mM 2-mercaptoethanol, 2 mM ATP, 1 mM CTP, 8 mM phosphoenolpyruvate and 10 µg pyruvate kinase (EC 2.7.1.40) (Boehringer, Mannheim, Germany). After 30 min incubation at 37°C, the solution is extracted three times with phenol previously saturated with water, dialyzed in the cold 2–3 hr against sodium acetate buffer 5 mM pH 4.2, 5 mM $MgCl_2$ and finally stored at −20°C. The specific activities of acylated tRNAs were: ^{3}H-labelled-Ala-tRNA 2.10^6 cpm/A_{260}, ^{3}H-labelled-Gly-tRNA 130000 cpm/A_{260} and ^{14}C-labelled-Ser-tRNA 60000 cpm/A_{260}.

Binding activity tests were carried out with *E. coli* ribosomes prepared according to Matthaei [10] in the

following conditions for 100 µl final solution: 60 mM KCl; 16 mM $MgCl_2$; 10 mM Tris–HCl pH 7.2; 5–10 µl triplet (0.5–1 A_{260}), 10 µl (2.7 A_{260}) *E. coli* ribosomes, 10–30 µl (5–15 mA_{260}) acylated tRNA. Incubation was performed during 20 min at 37°C. Bound aminoacyl-tRNA were assayed by adsorption of the ribosomal complex to cellulose nitrate membrane filters (Sartorius, Göttingen), washed with about 30 ml of 10 mM Tris–HCl pH 7.2, 16 mM $MgCl_2$, dried and counted in liquid scintillation spectrometer.

3. Results and discussion

The pattern of tRNA fractionation is indicated in fig. 1. $tRNA^{Ala}$ is eluted as a unique large peak, but its partial fractionation is sufficient for codon study. However, this peak has been fractionated into 3 isoacceptor species by chromatography on benzoylated DEAE-cellulose (D. Hentzen, unpublished). $tRNA^{Gly}$ is well separated in two peaks; the faster (peak 2) is broader than the peak 1 and probably contains more than one species. It is interesting to note that $tRNA_1^{Gly}$ has exactly the same mobility by partition procedure (countercurrent distribution and reversed-phase chromatography) as $tRNA_1^{Ala}$ (left part of the left peak in fig. 1). $tRNA^{Ser}$ appears as a very lipophilic species eluted near the front of the mobile phase. The absence of appreciable amounts of a second hydrophilic peak of $tRNA^{Ser}$, corresponding ordinarily to AG_U^C codons, leads to postulate that the $tRNA^{Ser}$ isolated from the posterior silkgland is part of the UCN codons group.

Twelve fractions of tRNA were assayed for their in vitro ribosomal binding in presence of adequate codons (table 1). The codon response of the five $tRNA^{Ala}$ fractions led us to presume the occurrence of two groups of $tRNA^{Ala}$: iso-$tRNA_1^{Ala}$ from the hydrophilic left part of the peak and iso-$tRNA_2^{Ala}$ from the right part (fractions 9–13). Both species recognize GCU codon occurring in the mRNA of fibroin. The nature of their anticodon must be different, as suggested in table 2. The weak but significant response to GCA and GCG codons of fraction 5 can be interpreted as due to a minor component of $tRNA^{Ala}$, the iso-$tRNA_{1a}^{Ala}$ species whose anticodon would contain U^xGC (U^x represents modified uridine). The iso-$tRNA_{1b}^{Ala}$ however recognizes predominantly GCC and GCU and could have a G^xGC anticodon (G^x modified guanosine). For the iso-$tRNA_2^{Ala}$ we find the well-known situation described for $tRNA^{Ala}$ from yeast, which contains a IGC anticodon [11, 12].

Table 1

Percentages of codon responses of tRNA fractions from the posterior silkgland of *Bombyx mori* L. after a countercurrent distribution.

Fraction of countercurrent distribution (see fig. 1)	GCU	GCC	GCA	GCG	Isoacceptor species
5	62	28	3	7	Ala 1a + Ala 1b
7	57	29	11	3	Ala 1 + Ala 2
9	54	28	18	0	Ala 2
11	52	31	17	0	Ala 2
13	52	30	18	0	Ala 2
	GGU	GGC	GGA	GGG	
7	46	54	0	–	Gly 1
21	49	0	51	–	Gly 2a + Gly 2b
24	60	0	40	–	Gly 2a + Gly 2b
	UCU	UCC	UCA	UCG	
39, 41, 43, 45	+	–	–	0	Ser

The hyphen(–) shows unrealized binding test because of non-available triplet.

Table 2
Presumed anticodon structure of iso-tRNAs from the posterior silkgland of *Bombyx mori* L.

Isoacceptor species	Possible anticodon
Ala 1a	U^XGC
Ala 1b	G^XGC
Ala 2	IGC
Gly 1	GCC or G^XCC
Gly 2a	ACC
Gly 2b	UCC
Ser	IGA or XGA

For $tRNA^{Gly}$ the situation is apparently simple. With the iso-$tRNA^{Gly}_1$ (fraction 7) recognizing GGC and GGU, we found a similar behaviour to the one described for $tRNA^{Ala}_{1b}$. Its anticodon structure would be G^XCC or GCC because the interaction between the wobble base of the anticodon is stronger with C than with U. The relatively less polar peak of $tRNA^{Gly}_2$ (fractions 19–25) however is heterogenous showing two exclusive codon responses GGU and GGA. This peak of $tRNA^{Gly}$ must be composed of two distinct species in approximately equal amounts. Iso-$tRNA^{Gly}_{2b}$ recognizes GGA and would contain a unique UCC anticodon, iso-$tRNA^{Gly}_{2b}$ recognizing GGU would have a ACC anticodon. Let us give attention to the occurrence of iso-$tRNA^{Gly}_{2a}$ only one able to decode GGA in one hand and of iso-$tRNA^{Gly}_{2b}$ species decoding only GGU on the other hand, in opposition to iso-$tRNA^{Gly}_1$ reading both GGC and GGU. Actually these two tRNA species appear in the peak 2 of $tRNA^{Gly}$ which increases more rapidly than the first one during the secretion phase of fibroin [5,7]. Hence, we believe that isoacceptors species 2a and 2b would be preferential decoding species of $mRNA_F$ and could represent the previously described 'modulator tRNA'. When we consider the ratio of about 2:3 for iso-$tRNA^{Gly}_1$ to iso-$tRNA^{Gly}_2$, we can estimate the GGU reading capacity to 70% and that of GGA to 30%, so that the ratio of GGU:GGA decoding capacity of $tRNA^{Gly}$ in the posterior silkgland during the fibroin biosynthesis is up to 1.4 ratio of GGU:GGA codons found in $mRNA_F$.

Our preliminary results concerning the codon properties of $tRNA^{Ser}$ demonstrate that the lipophilic peak of $tRNA^{Ser}$ from the posterior silkgland recognizes the UCN codons group, since UCU is very well decoded. According to the findings of Staehelin et al. [13], it is possible that $tRNA^{Ser}$ which could respond to UCA (predominant codon found in mRNA of fibroin) [6] also decodes UCC, so that it corresponds to IGA anticodon. Another possibility would be that $tRNA^{Ser}$ does not decode UCC and could be identified with an additional $tRNA^{Ser}$ species, such as species IIb described by Staehelin et al. [13] in rat liver. We also must note that Zaitseva et al. [14] have carried out in vitro stimulation of polyserine with four fractions of $tRNA^{Ser}$ extracted from the whole silkgland and separated on methylalbumin–Kieselguhr column. They observed that poly UC (4:1) leads to a serine incorporation with the fractions 1 and 2 to which they assigned a GGA or IGA anticodon, poly UC (4:1) stimulates fraction 3 (presumed anticodon AGA) and poly AGU (1:1:1) fraction 4 (possible anticodon ACU or GCU). Unfortunately they did not test the poly UCA and poly UCG responses.

Our results could be related with the observations of Maenpää [15,16]. The induction of the synthesis of phosvitin, a serine-rich phosphoprotein in estrogen-treated avian liver [17] or in laying hens [18] is accompanied with concomitant alterations in the UCN-specific $tRNA^{Ser}$ fractions, concerning particularly the UC^A_U Seryl-tRNA.

The adaptation of tRNAs to protein biosynthesis is a general phenomenon, which one can easily observe in highly differentiated cells (plasmocytome, reticulocyte, lens) and occurs in all living systems (bacteria, yeast and mammalian tissues) [4]. Such a quantitative continuous modification of the concentration of tRNA has a meaning, if the various synthesized iso-tRNAs during the differentiation contribute to the optimal efficiency of the translational machinery. In other words the finality of newly occurring iso-tRNAs in the cytosol is to perfectoly decode the mRNA pool. From a logical point of view the population of isoacceptor species must mirror the codon redundancy of a given predominant mRNA. We can emphasize, that the quantitative adaptation of tRNAs overlaps a qualitative (selective or modulated) adaptation of tRNAs. Consequently the metabolic modifications of a tRNA population must be interpreted in terms of 'modulated biosynthesis of different tRNA species'.

Two problems are now to be solved: a) what is the precise nature of the structural differences between the usual and the 'modulator' iso-tRNA species, b) what is the regulatory mechanism of the 'modulated biosynthesis' of tRNA? Is there a direct or indirect relationship between the biosynthesis and relative accumulation of mRNA with long half-time (such as mRNA of fibroin) and the occurrence of preferential tRNAs in the cytosol?

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