FUNCTIONAL ADAPTATION OF tRNAs TO PROTEIN BIOSYNTHESIS IN A HIGHLY DIFFERENTIATED CELL SYSTEM. III. INDUCTION OF ISOACCEPTOR tRNAs DURING THE SECRETION OF FIBROIN IN THE SILKGLAND OF *BOMBYX MORI* L.

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1. Introduction

We have previously demonstrated that, in a differentiated cell system, a linear correlation exists between the amino acid distribution of a major protein and the corresponding tRNAs, e.g. the crystallines in the internal cortical zone of the lens [1] and the secreted fibroin in the posterior part of the silkgland of the silkworm *Bombyx mori* L [2]. This regulation process also concerns the intracellular level of aminoacyl-tRNA ligases (EC.6.1.1.) in the silkgland; their concentrations are almost proportional to the tRNA population during the secreted period [3].

For a better understanding of the regulation mechanism of protein biosynthesis at the translational level, tRNAs specific for the four major amino acids of the fibroin (Ala, Gly, Ser and Tyr) were individually subjected to a fractionation procedure. Chromatography on methylated albumin-kieselguhr (MAK) columns demonstrated qualitative differences among isoacceptor tRNAs, particularly for $tRNA^{Ala}$ and $tRNA^{Gly}$ from the end of the cell growth period (4th day) to the secretion period (8th day) during the Vth instar of the silkworm. These qualitative differences correspond to a differential 8 fold increase for the first isoacceptor tRNA compared with a 20–30 fold increase for the 2nd or/and the 3rd isoacceptor species.

2. Materials and methods

The extraction of tRNA from the silkglands and the preparation of the crude aminoacyl-tRNA ligases from rat liver were performed as previously described [4]. Special kinetic conditions are given by Chavancy et al. [3]. The MAK column chromatography procedure was simplified according to Garel et al. [5]. The relative amounts of each isoacceptor species were calculated allowing for a background of 100 cpm. Furthermore, the amount for a given tRNA at various times is taken from our results [2] and the evolutive quantity of total tRNA in one silkgland from the work of Chavancy et al. [3].

3. Results and discussion

The chromatographic profiles of the four major tRNAs are given in fig. 1. The ratios of the different isoacceptor species are shown in table 1. From these data it can be seen that the polydispersity of specific tRNA is greater at the 8th day of the Vth instar than at the end of the growth period (4th day). This fact is particularly clear with a relative loss of $tRNA_I^{Gly}$ and the appearance of 2.5 fold amount of a $tRNA_{II}^{Gly}$. With $tRNA^{Ala}$ and $tRNA^{Tyr}$, we observed a similar

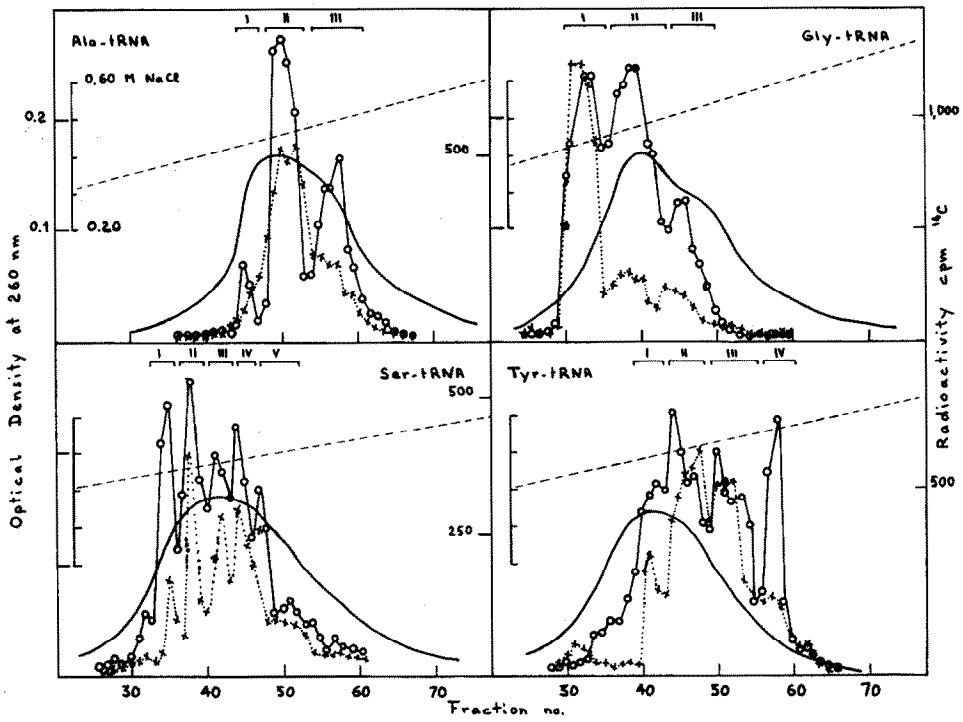

Fig. 1. Methylated albumin-kieselguhr (MAK) column chromatography of aminoacyl-tRNA from the posterior part of the silkgland. 4th day x...x, 8th day o—o, optical density at 260 nm —, NaCl gradient - - - -. Incubation conditions [5]: tris-HCl buffer 100 μmoles, ATP 50 μmoles, tRNA 100-200 μg, ^{14}C-amino acids from C.E.A., France 10 μCi/50–200 nmoles, rat liver enzymatic solution 12 mg, total volume 2.5 ml, final pH 7.2, temperature 37°, incubation time 15 min. Before application to the MAK column, the solution is adjusted to 0.05 M with EDTA, stirred for 5 min in the water bath, cooled, then adjusted to 0.1 M with NaCl and directly applied to the column. Two columns were simultaneous eluted using a linear gradient of NaCl from 0.20–0.65 M (total volume 400 ml). Fractions of 2–3 ml were precipitated with trichloracetic acid, the precipitate collected on Whatmann GF/C glass filter and the radioactivity measured using a liquid scintillation spectrometer (Intertechnique ABAC-SL 40, France).

evolution in the isoacceptor pattern. The situation is more complex with the multiple profile of $tRNA^{Ser}$. These results are also confirmed by chromatography on reversed-phase columns (RRC-2) and tRNAs acylated by homologous silkgland ligases [6].

The relative amount of each isoacceptor tRNA enables us to estimate the actual available quantity of these species in a dynamic state of the secreting cells. The results shown in table 2 were calculated using a value of 88 μg of tRNA 4 S at the 4th day and 165 μg at the 8th day [3]. From the growth period to the fibroin secretion period, the $tRNA_{II}^{Ala}$ and $tRNA_{I}^{Gly}$ species of the posterior part of the silkgland increase 8 fold; they are present finally in equal amounts. The other species ($tRNA_{III}^{Ala}$, $tRNA_{II}^{Gly}$, and $tRNA_{III}^{Gly}$) are specifically enriched among the tRNA population 16, 20 and 35 fold respectively. This parallel but not paired increase of all isoacceptor tRNAs makes interconvertible forms of species II to III (Ala) and species I to II and III (Gly) very improbable. Differential methylation, thiolation or specific CCA replacement are not absolutely excluded (see Sueoka [7] for a review).

In order to check this ambiguity directly, we are currently investigating the codon response [6] to the isoacceptor tRNA for alanine and glycine. Zaitseva et al. [8] have shown the existence of at least four anticodon responses to the $tRNA^{Ser}$ fractionated on a MAK column. Matsuzaki [9], in contrast to his early work on MAK column

Table 1
Relative amounts (%) between the isoacceptor tRNAs at the 4th and the 8th day of the Vth instar in the posterior part of the silkgland. Values are taken from the MAK column profiles (fig. 1).

Isoacceptor species	Ala			Gly			Ser					Tyr			
	I	II	III	I	II	III	I	II	III	IV	V	I	II	III	IV
4th day	2	78	20	65	20	15	8	23	22	26	21	10	50	34	6
8th day	4	62	34	33	48	19	22	24	20	20	14	18	34	30	18

chromatography of tRNA extracted at the 5th day of the Vth instar [10], has demonstrated the existence of multiple forms of tRNA differing at the oligonucleotide end. It appears that there are two different $tRNA^{Gly}$s, two or three $tRNA^{Ser}$s and three $tRNA^{Tyr}$s.

There is simultaneous increase in the amounts of some isoacceptor tRNAs and the specific protein, fibroin. These two increases are certainly related, and regardless of the nature of the tRNA induction mechanism, we propose the following scheme: the high rate of translation of the fibroin mRNA (or $mRNA_F$) requires a correspondingly high level of certain isoacceptor tRNAs (or iso-$tRNA_F$) which recognize synonym codons. These iso-$tRNA_F$ can be identified as the "modulator tRNA" according to the regulation model of Stent [11] and exist in the tRNA population at low concentrations during the growth period. They can participate in non-specific protein biosynthesis as well as in a constant low synthesis of fibroin in the silkgland.

From this point of view, the species Ala_{II} and Gly_I can be regarded as the "common, basic or major" isoacceptor tRNA involved in all protein synthesis. However, their intracellular enrichment at the 8th day compared to the two fold rise in the weight of the silkgland and the total tRNA content leads to the conclusion that they are *normally* involved in the $mRNA_F$ translation.

The situation is different for the other three iso-tRNAs (Ala_{III}, Gly_{II} and Gly_{III}). Compared to the first two (Ala_{II} and Gly_I), their content is 2–4 times higher. We think they are specific iso-$tRNA_F$ which *preferentially* decode the $mRNA_F$. Their ratio would reflect the frequency of synonym codons in the fibroin mRNA. The study of the oligonucleotide distribution or a partial sequence of enriched fraction of $mRNA_F$ would indicate if such a correlation exists between modulator tRNA and a non-random redundancy of the genetic code for a specific mRNA. Such a study would contribute to eludicate the role of the modulator tRNAs during differentiation process.

Table 2
Quantity in pmoles of each isoacceptor tRNA at the 4th and the 8th day of the Vth instar in the posterior part of *one* silkgland.

species	iso-$tRNA^{Ala}$		iso-$tRNA^{Gly}$	
	4th	8th	4th	8th
I	–	–	40	325
II	42	330	12	432
III	11	180	9	118

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References

[1] J.P. Garel, N. Virmaux and P. Mandel, Bull. Soc. Chim. Biol. 52 (1970) 987.
[2] J.P. Garel, P. Mandel, G. Chavancy and J. Daillie, FEBS Letters 7 (1970) 327.
[3] G. Chavancy, J. Daillie, P. Mandel and J.P. Garel, in preparation
[4] J.P. Garel and P. Mandel, European J. Biochem., 5 (1968) 474.
[5] J.P. Garel, G. Nullans and P. Mandel, J. Chromatog., in press.
[6] J.P. Garel, G. Chavancy, G. Beck and J. Daillie, in preparation.

[7] N. Sueoka and T. Kano-Sueoka, in: Progress in Nucleic Acid Research and Molecular Biology, Vol. 10 (Academic Press, New York, 1970).
[8] G.N. Zaitseva, V.P. Ermischki and L.O. Dynga, Dolk. Akad. Nauk SSSR 180 (1968) 488.
[9] K. Matsuzaki, J. Biochem. Tokyo 67 (1970) 851.
[10] K. Matsuzaki, Biochim. Biophys, Acta 114 (1966) 222.
[11] G.S. Stent, Science 144 (1964) 816.