FUNCTIONAL ADAPTATION OF AMINOACYL-tRNA SYNTHETASES TO FIBROIN BIOSYNTHESIS IN THE SILKGLAND OF *BOMBYX MORI* L.*

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1. Introduction

The involvement of tRNA and aminoacyl-tRNA synthetases as a regulating factor at the translational level has been tested in several biological systems.

The quantitative adaptation of the tRNA population demonstrated in the silkgland of the silkworm *Bombyx mori* L. [1,2] has also been found in other differentiated tissues such as lens [3] and reticulocytes [4,5]. This regulation phenomenon can be applied to other cell systems [6]. All these data show that in a given tissue the relative amounts of each free tRNA are directly related to the amino acid composition of the proteins synthetized in that tissue (fibroin and sericin, crystallins, haemoglobin). Furthermore, during development and differentiation, qualitative changes occur in the distribution of isoacceptor tRNA species (in insects [7–10], for a general review, see [11], suggesting that the adaptation is closely dependent upon codons recognition.

Changes in the activities of aminoacyl-tRNA synthetases have also been found in the sea urchin [12,13], mammary gland [14] and pea root [15]. Other studies [16–19] suggest, moreover, a dynamic correlation between the level of a given aminoacyl-tRNA and the formation of the corresponding aminoacyl-tRNA synthetase. The posterior part of the *B. mori* silkgland, wich is suitable for analyzing this interaction, has been used in the investigation of a quantitative adaptation of the aminoacyl-tRNA synthetases population. We considered the possibility of a coordinated regulation of the concentration of tRNAs and synthetases adjusted to the amino acid composition of the fibroin.

Our results show clearly that aminoacyl-tRNA synthetases, like tRNAs, are quantitatively related to fibroin biosynthesis in the silkgland. Both soluble enzymes (from 150 000 *g* supernatant) and ribosomes associated ones, are involved in the phenomenon. This adaptation appears to be very strict and suggests a regulatory mechanism at the level of biosynthesis of each of these enzymes.

2. Material and methods

tRNA and aminoacyl-tRNA synthetases (EC 6.1.1) from the posterior part of *Bombyx mori* L. silkglands were extracted as previously described [2]. Two fractions of synthetases were obtained: the soluble one (150 000 *g* supernatant) and that associated with the ribosomal pellet which was resuspended in Tris–HCl buffer 0.05 M pH 7.4, KCl 0.025 M, $MgCl_2$ 0.01, β-mercaptoethanol 0.01, glycerol 10% vol.

Reversed-phase chromatography on capillary columns (called micro-RPC) was carried out according to Hentzen et al. [20]. We used heterologous tRNAs (Brewer's Yeast tRNA purchased from Boehringer, Mannheim, Germany) as well homologous tRNAs acylated with synchronous synthetases from the posterior part of the silkgland from *B. mori* L. By synchronous we mean synthetases extracted from gland the same day from the Vth instar of the laval life as those used to prepare tRNAs. Under previously described condi-

* Communication in part at the tRNA Symposium (Société de Chimie biologique), Strasbourg, December 1971. Part VIII of a series on Functional Adaptation of tRNAs to Protein Biosynthesis in high differentiated cell systems. Part VII. Garel, J. P., Hentzen, D. and Daillie, J. (1974) FEBS Lett. 39, 359–363.

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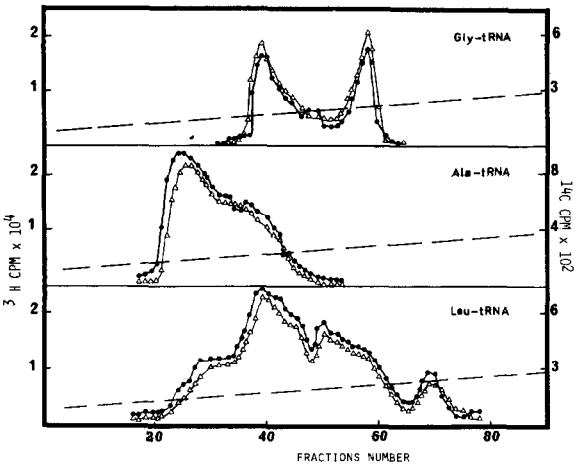

Fig.1 Reversed-phase chromatography on capillary columns of yeast tRNAs acylated by silkgland synthetases, technical procedures are described in detail by Hentzen et al. [20]. NaCl gradient 0.20–0.60 M. (●–●–●) [^{3}H]aminoacyl-tRNA synthetases from the second day. (△–△–△) [^{14}C] aminoacyl-tRNA synthetases from the eigth day.

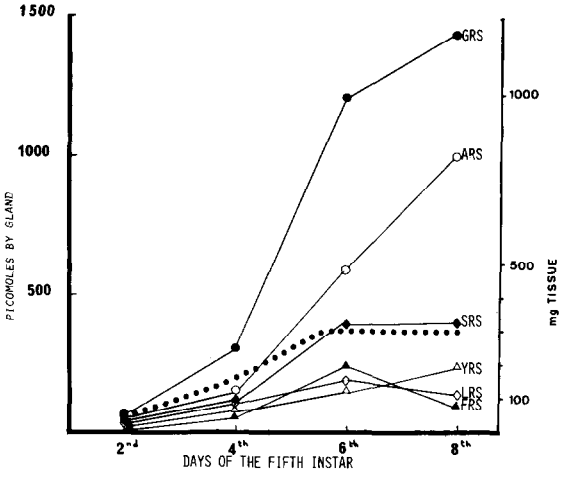

Fig.2. Changes of the 'soluble' aminoacyl-tRNA synthetases activities during the Vth instar in the posterior part of the silkgland of *Bombyx mori* L. Results are expressed in pmoles of [^{14}C] amino acid fixed on yeast tRNA after 25 min by aminoacyl-tRNA synthetases from silkgland at different days of the last larval instar. Acylation conditions are given according Chavancy et al. [2] using saturating amounts of tRNA (100 μg for 300 μg enzyme proteins). (●–●–●) Glycyl-tRNA synthetase (GRS), (○–○–○) Alanyl-tRNA synthetase (ARS), (◆–◆–◆) Seryl-tRNA synthetase (SRS), (△–△–△) Tyrosyl-tRNA synthetase (YRS), (◇–◇–◇) Leucyl-tRNA synthetase (LRS),(▲–▲–▲) Phenylalanyl-tRNA synthetase (FRS), (●●●) Weight of posterior part of the silkgland according to Daillie [21].

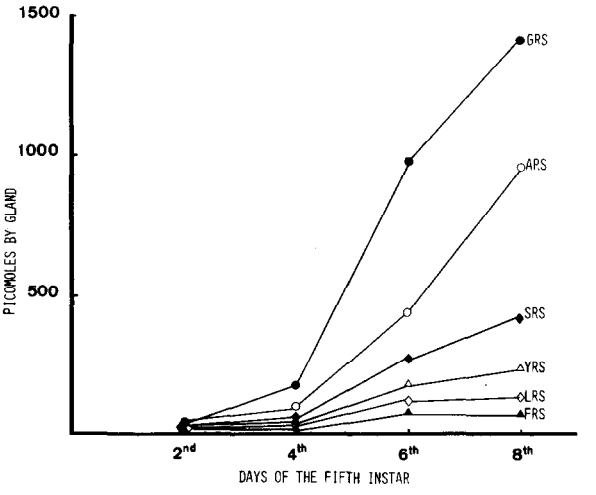

Fig.3. Changes of the 'particular' aminoacyl-tRNA synthetases activities during the Vth instar in the posterior part of the silkgland of *Bombyx mori* L. See figure 2.

tions [2], we first checked that all isoacceptor yeast tRNAs are acylated to the same extent by silkgland synthetases and by yeast synthetases (fig.1). Furthermore, the quantities of tRNA used in these experiments were always saturating (100 μg yeast tRNA and 60 μg silkgland tRNA for 300 μg enzyme proteins). We also verified that in all cases the acylation plateau is reached within 25 min.

3. Results and discussion

The activity of several aminoacyl-tRNA synthetases was measured during the Vth larval instar of the silkworm using yeast tRNAs as substrate. The results (figs. 2 and 3) show that the activity increases with time from the 2nd to the 8th day for each species of enzyme. Before the 5th day, this is related to the cellular growth; after the 5th day on, there is no more growth but the synthesis of fibroin predominates [21] leading to an increasing concentration of the major aminoacyl-tRNA synthetases in the cell.

The increases in the various synthetase activities are not all identical however. Glycyl-tRNA synthetase activity increases more rapidly than that of alanyl-, seryl-, tyrosyl-, leucyl- or phenylalanyl-tRNA synthetase. The enzymatic activity pattern changes greatly

Table 1
Composition of the tRNAs and aminoacyl-tRNA synthetases populations in the posterior part of the *Bombyx mori* L. silkgland during the Vth instar

Amino acid	Fibroin (%)[a]	tRNA (%)[b]			Supernatant synthetases[c]			Pellet-associated synthetases[c]		
		2nd	4th	8th day	2nd	4th	8th day	2nd	4th	8th day
Alanine	30	14.1	17.5	22.6	22.2	20.9	32.4	26.5	25.7	29.6
Glycine	48	13.2	21.8	42.4	27.1	43.3	42.8	24.3	39.9	41.4
Leucine	0.5	8.5	0.4	–	12.3	8.9	2.7	14.7	10.4	3.2
Phenylalanine	0.5	3.8	0.4	6.0	17.4	6.8	2.3	16.8	5.9	1.9
Serine	13	9.1	10.9	14.0	12.9	14.4	12.9	11.9	13.5	13.7
Tyrosine	6	4.3	13.7	9.7	8.1	5.7	7.9	6.8	4.6	10.2

[a] % Of the total of six amino acids.
[b] % Of the total tRNA pool according to Chavancy et al. [2].
[c] % Of the total six studied synthetases taken respectively from fig. 2 and 3.

Table 2
Changes of the aminoacyl-tRNA synthetases population in the posterior part of the *Bombyx mori* L. silkgland during the Vth instar

Supernatant synthetases	2nd	4th	6th	8th day	(%)
Alanyl-tRNA synthetase	58	96	684	792	(24.8)
Glycyl-tRNA synthetase	85	320	1320	1650	(51.8)
Leucyl-tRNA synthetase	44	28	–	98	(3.1)
Seryl-tRNA synthetase	59	51	–	645	(20.2)

Results are expressed in picomoles of [^{14}C]amino acid fixed on synchronous extracted silkgland tRNA (for one gland).

Table 3
K_m values of four aminoacyl-tRNA synthetases at different days of the Vth instar in the posterior part of the *Bombyx mori* L. silkgland

Supernatant synthetases	K_m values in 10^{-6} M/l of yeast tRNA			
	2nd day	4th day	6th day	8th day
Alanyl-tRNA synthetase	1.2±0.3	1.4±0.3	1.5±0.2	1.3±0.3
Glycyl-tRNA synthetase	2.6±0.4	1.9±0.5	2.1±0.4	2.0±0.5
Leucyl-tRNA synthetase	1.3±0.5	1.7±0.3	1.8±0.4	1.9±0.4
Seryl-tRNA synthetase	0.7±0.2	0.5±0.1	0.7±0.2	0.6±0.2

Average of 3 K_m determinations.

from the 2nd to the 8th day (table 1). A similar pattern is found with homologous acylations (table 2).

In addition the K_m values measured with yeast tRNAs (table 3) are very similar during the last larval instar. Therefore we believe that our results show quantitative changes in intracellular concentration of synthetases and not merely a modification of the enzymatic activities.

The parallel evolution observed with the ribosome-associated enzymes and the soluble ones indicates that the whole enzyme pool is involved in this phenomenon. As the chromatography of acylated tRNAs obtained from the two fractions gives identical profiles (fig.4), it seems probable that the synthetase activities of the two fractions are similar. Moreover, there is likely a steady-state between the enzymatic populations of these two fractions. The subcellular repartition could be due solely to the proportion of molecules engaged in protein synthesis and associated in complexes. Similar results have been found by other authors [22, 23]. Yet, the proportion of the 150 000 *g* pellet-associated synthetases is higher in the silkgland than in rat liver. From an average of 30% for this fraction at the 2nd day of the last instar, it rises to 50% at the end of the secretion phase. This can be correlated with the fact that fibroin biosynthesis occurs on membrane-bound polysomes [24].

There is good correlation between the percentage of a given amino acid in the fibroin during the Vth larval instar and the relative amounts of the corresponding tRNA on one hand and the relative amounts of the corresponding aminoacyl-tRNA synthetase (and the supernatant and in the ribosomal pellet) on the other hand, as can be seen in tables 1 and 2. On the contrary, during growth phase there is no correlation of the major aminoacyl-tRNA synthetases (GRS, ARS and SRS) with the amino acid composition of fibroin. These three enzymes are, however, more abundant than all others; likewise the corresponding tRNAs predominate during this period [1].

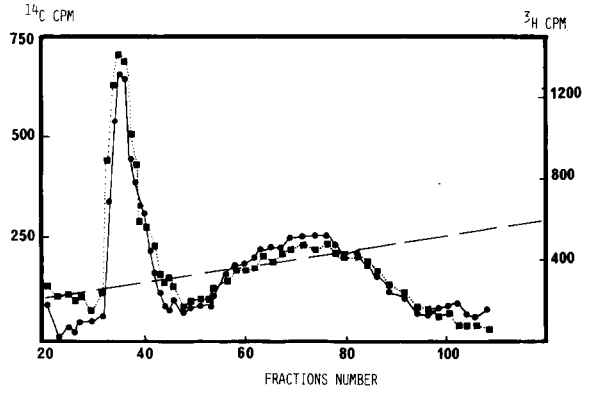

Fig.4. Reversed-phase chromatography on capillary columns on silkgland tRNAs acylated by the homologous glycyl-tRNA synthetases. NaCl gradient 0.25–0.60 M. (■–■–■) [^{3}H]aminoacyl-tRNA synthetase from 150 000 *g* ribosomal pellet. (●···●···●) [^{14}C]aminoacyl-tRNA synthetase from 150 000 *g* supernatant.

Our data suggest a quantitative adjustment or adaptation of synthetases population to fibrin biosynthesis. This adaptation occurs simultaneously with that for tRNAs. The ratio of the amounts of a given tRNA to the amounts of the corresponding synthetase varies little for the three major species during the Vth larval instar. We think that both tRNAs and synthetases play a role in the same coordinated regulatory process, called 'modulated biosynthesis' [6]. The data of the literature concerning procaryotic and yeast cells show that the formation of new aminoacyl-tRNA synthetases depends at least on the concentration of the corresponding aminoacyl-tRNA molecules, which could have a repressor-like action on the synthetase structural genes [16–19,25]. However, our results do not prove yet the formation of new enzymes, but only of new active ones. In fact, during the secretion phase of fibroin the transformation of inactive enzyme molecules already synthesized in active ones remains possible.

Further work is in progress to purify the three main synthetases from the silkgland and to study the relationship between acylated tRNA and synthetase biosynthesis during the silkworm last larval instar.

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