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FUNCTIONAL ADAPTATION OF tRNAs TO FIBROIN BIOSYNTHESIS IN THE SILKGLAND OF *BOMBYX MORI L*

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1. Introduction

The regulation and economy of cells depend on the relationships between the tRNA pool and the amino acid composition of the protein synthesised. Results obtained in bacteria [1, 2] have not given unambiguous proof of a direct correlation. Animal cells, where a preferential synthesis of one type of structural or secreted protein occurs, provide more suitable conditions to study such a correlation and its regulation. Previous studies with the differentiating cell system of the calf lens [3, 4] indicate a direct correlation between the aminoacyl-tRNAs and the amino acid composition of the crystallins for the internal cortical zone. In the posterior part of the silk gland of *Bombyx mori* L., we find a linear correlation between the amino acid distribution of the fibroin and the corresponding tRNAs acylated during the secretion phase.

The posterior part of the silk gland synthesises essentially its own enzymatic and tissue proteins during the first half of the fifth instar. After the fifth day, cell growth ceases [5] and fibroin accumulates up to the spinning of the cocoon. Fibroin composition is specially suitable for our study: four amino acids (alanine, glycine, serine and tyrosine) represent 93% of the total amino acid composition [6]. Consequently we chose the silk gland to investigate if a relation exists between fibroin composition and the tRNA pool. We have compared acylation rates *in vitro* of specific tRNAs during the active growth of the gland (until 4th day of the 5th instar) and the active accumulation of fibroin (6–8th day of the 5th instar).

2. Materials and methods

The silk worms (*Bombyx mori* L.) were hybrids from two European strains 200 and 300. The silk glands were excised on the 4th, 6th and 8th day of the fifth instar, immediately frozen in liquid nitrogen, then treated by Garel and Mandel's method [7] to extract tRNA and aminoacyl-tRNA ligases (E.C.6.1.1.). A mean yield of 5 mg tRNA from 100 silk glands was obtained. Acylations were performed with 20 μg tRNA, at 30° for 20 min. These reactions were done once with silk gland ligases followed by two or three further incubations with rat liver ligases.

The results were analysed statistically by a linearity test between the amino acid distribution in the fibroin and the distribution of acylated tRNAs with every amino acid. The linear correlation coefficient for tRNA on the 6th and 8th days was also calculated.

3. Results and discussion

The results obtained for the two physiological phases of the posterior part of the silk gland are shown in table 1. Calculations were made to account for an aspecific contribution of the tRNAs to the biosynthesis of various enzymatic and tissue proteins. This contribution, evaluated at about 15 pmoles, was subtracted from the values of acylated tRNAs.

Within a 5% confidence limit, a linear correlation exists between the amino acid distribution and tRNAs on the 6th and 8th day of the fifth instar (table 2).

Table 1
Acylation of tRNAs in the posterior part of the silk gland.

Fibroin composition (%)		pmoles of tRNA (4th day)	pmoles of tRNA (6th and 8th day)
Alanine	28.5	12.5 ± 0.9	64.3 ± 25,3
Aspartic acid	1.0	9 2.0	10.6 1.7
Glutamic acid	0.6	5 1.5	8.6 3.6
Glycine	46.0	14 4.8	120.0 8.5
Leucine	0.7	5 3.4	5.0 2.6
Serine	11.7	23 6.9	39.6 8.7
Threonine	0.7	8 1.7	12.6 2.0
Tyrosine	6.3	14.5 4.0	27.7 11.8

Table 2

Statistical analysis	4th day	6th and 8th day
Deviation from linear trend (F_o = 2.74)	Not significant F_c = 1.06	Not significant F_c = 0.19
Effect of acylation factor (F_o = 2.66)	Not significant F_c = 0.91	Significant F_c = 9.53
Correlation coefficient	–	0.94
Conclusions	No linear correlation	Linear correlation

F_o = F from the statistical table for a 5% confidence limit
F_c = calculated F.

This correlation does not exist for tRNAs extracted during the growth phase. Nevertheless it is noteworthy to state the relative redundancy of the four tRNAs corresponding to the four main amino acids of the fibroin on the 4th day.

Our results agree with the observation of Matsuzaki [8] working on tRNA of the 6th day of the fifth instar. We also find the predominance of glycyl-tRNA and the nearly complete absence of leucyl-tRNA. The "non-quantitative correlation" by this author comes likely from the low acylation rate (about 6%) obtained with homologous ligases compared to our 40% yield with heterologous ligases. Furthermore, we do observe a quantitative correlation for other aminoacyl-tRNAs (Ala-, Ser- and Tyr-tRNA). We have not strictly proved that the distribution of the endogeneous tRNA of the silk gland translates one of the proteins synthesised during the two physiological states, but there appears to be a functional adaptation between tRNA level and the amino acids incorporated into the fibroin. Work is in progress to determine the actual qualitative evolution of the main tRNA species, the possible quantitative variations of the aminoacyl-tRNA ligases and their K_m-values [9].

During the first phase, acylation rates tend to the same level. For seryl-tRNA at least [9] the incorporation during the second day reaches a distribution similar to that found in liver or yeast (less differentiated cell type with regard to a specific protein production) where each tRNA is supposed to represent, on average, 4–6% of the total tRNA pool. The excess of $tRNA^{Ala}$, $tRNA^{Gly}$, $tRNA^{Ser}$ and $tRNA^{Tyr}$ on the 4th day may indicate an induction of the tRNAs specially needed for further function of the gland, unless

it denotes the very beginning of fibroin biosynthesis. The quantity of secreted fibroin is very important; the synthesised silk during the second phase of the fifth instar may represent 25% of the caterpillar weight [10].

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