TURNOVER OF TRANSFER RNA SPECIES DURING DEVELOPMENT OF THE POSTERIOR SILKGLAND OF Bombyx mori L.*

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SUMMARY . Quantitative changes in total tRNA and in five individual tRNA species have been studied in the developing posterior silkgland during the fifth larval instar of the silkworm Bombyx mori L. . Using starvation and refeeding of the larvae at two distinct physiological stages - growth and secretion phases - half-lives and synthesis rates of tRNAs have been estimated . Half-life times are roughly similar for all tRNA species examined (130 h for tRNA Ser , 100 h for tRNA Asp , 97 h for tRNA Gly , 92 h for tRNA Ala and 70 h for tRNA Thr) while the rates of synthesis vary widely according to the intracellular levels of each tRNA species . Our results suggest that the adaptation of individual tRNA species takes place at the transcriptional level or at the pre-tRNA maturation level . They rule out a regulatory mechanism through a selective degradation of mature tRNA species .

INTRODUCTION

The differentiation in the silkgland of the silkworm Bombyx mori L. leads to the massive biosynthesis of the two silk proteins fibroin and sericin at the end of the fifth larval stage (1,2) . Of particular interest is the intracellular distribution of tRNA species and of amino acid-tRNA ligases for the preponderant amino acids of fibroin (glycine,alanine and serine) in the posterior part . Quantitative changes occur in the tRNA population during the fifth instar resulting in a distribution of tRNA species , which matches the amino acid composition of fibroin (3-9) . Amino acid-tRNA ligases also are quantitatively adapted to their cognate tRNA species (9,10) . This functional adaptation takes place in the middle silkgland for sericin synthesis (5,6,9) . It has also been observed in many other differentiated systems (11,12) .

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The adaptation of the tRNA population to the codon distribution of translated mRNAs implies a coordinated regulatory mechanism (13) . This may involve either a selective transcription of tRNA genes needed by a mRNA being decoded or a random transcription of all tRNA genes followed by a specific degradation at the precursor level or at the mature tRNA level . In the latter case the half-lives of tRNA species are expected bo be inversely proportional to the concentration of the tRNA . In order to distinguish between these possibilities , we examined the intracellular levels of total tRNA and five individual tRNA species in the posterior silkgland during the fifth instar under various feeding conditions . We found that starvation acts differentially upon the decrease of the tRNA in the two physiological phases : the growth stage during the first three days of the last instar and the following secretion stage . Refeeding leads to a rapid increase of tRNA content . Analysis of the data made it possible to estimate the turnover and synthesis rates of tRNA species .

MATERIALS AND METHODS

Silkworm larvae are hybrids of European strains 200 and 300 . Lots of 50 caterpillars are raised in closed boxes and fed ad libitum with mulberry leaves . At a temperature of 21 ± 1 C , the cocoon spinning occurs on the ninth day of the last larval instar . Silkworms have been divided into 47 groups . Twenty two were fed continuously and used as controls . The starvation starts after 48 h or 120 h of the fifth moult . These periods were chosen on the basis of two dietary stages according to Legay (14,15) : the larvae being starved during the first three days (necessary feeding stage) do not enter the final nymphosis whereas starving larvae after the third day (optional feeding stage) spin a lighter cocoon and undergo metamorphosis . Some groups of starving worms have been refed after different periods of fasting .

tRNA and amino acid-tRNA ligases have been extracted as previously described (5) . Aminoacylation was performed as indicated by Chavancy et al.(5).

RESULTS

1. Quantitative changes of total tRNA : the quantitative changes of the posterior silkgland tRNA in normal fed , fasted and refed silkworms are given in absorbance at 260 nm (fig.1A) and after in vitro aminoacylation using an hydrolysate of Chlorella ^{14}C-proteins (fig.1B) . The similarity between both profiles shows that the accumulated tRNA remains active under

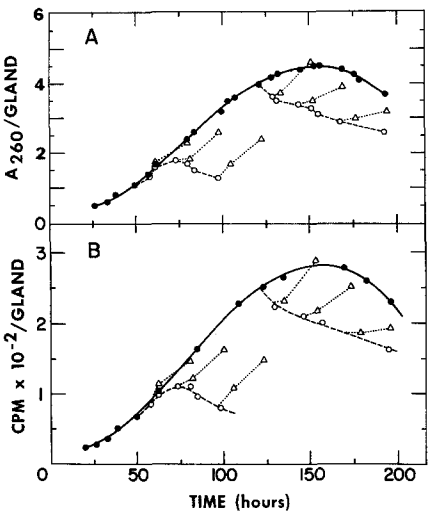

Fig. 1 . Quantitative changes of total tRNA in the posterior silkgland of Bombyx mori L. fasted and refed larvae during the fifth instar . The titration is based on ultraviolet absorbancy at 260 nm in panel A and on in vitro aminoacylation assays in panel B according to the conditions described by Chavancy et al.(5).The first meal taken by the silkworms after the fifth moult has been chosen as the origin of time . Each point indicates the moment of dissection and extraction of tRNA of a lot of 50 larvae : ●—● period of normal feeding (control animal) , o-----o period of fasting , ▲...▲ period of refeeding .

these physiological conditions . When the worms are normally fed , we observe a ten fold increase of total tRNA from the first up to the eighth day of the fifth instar as described earlier (5,9) . Starvation leads to a decrease in the amount of the tRNA . When fasting begins on the third day (end of the growth phase or necessary feeding stage) , there is a lag period during which the tRNA accumulation seems to be little affected ; the tRNA concentration then begins to decrease after 20 h . When starvation is initiated on the sixth day (secretory phase or optional feeding stage) , accumulation of tRNA stops immediately and the tRNA level decays exponentially. If worms are refed , the tRNA level increases in both phases . The amount of synthesized tRNA tends to reach the level observed in control animals , except when starvation is prolonged (80-100 h) .

The decays in tRNA concentration observed in the secretory stage (fig. 1-3) upon fasting represent only the result of degradative activities of preexisting mature tRNA molecules . In fact , essentially no de novo synthesis of tRNA occurs . A five hour pulse with ^{32}Phosphate showed that only 4 % of total tRNA remains when larvae are starved for 24 h beginning on the sixth day whereas 17 % synthesis occurs after 48 h of starvation beginning on the third day . It was thus possible to estimate the half-lives of tRNA listed in Table I (124 h based on absorbance , 119 h based on acceptor activity) . They are not significantly different from values estimated for total tRNA in mammalian organs : rat liver 80 h (16) , 90 ± 11 h (17) , 120 h (18,19) , rabbit liver 127 h , heart 132 h and uterus 144 h (20) .

TABLE I

TURNOVER AND SYNTHESIS RATES OF MATURE tRNAs IN THE POSTERIOR SILKGLAND OF Bombyx mori L. DURING THE FIFTH LARVAL INSTAR

	Half-life (h)	Synthesis rate (pmoles/h)
total tRNA based on A_{260}	124	
total tRNA based on acceptor activity	119	
$tRNA^{Ala}$	92	2.1
$tRNA^{Asp}$	100	0.4
$tRNA^{Gly}$	97	3.1
$tRNA^{Ser}$	130	1.5
$tRNA^{Thr}$	70	0.3

Half-lives and synthesis rates are computed from experimental data provided in fig. 1A for total tRNA based on absorbance , fig. 1B for total tRNA based on acceptor activity , fig. 2 for preponderant tRNA species (alanine, glycine and serine) and fig. 3 for the two minor tRNAs (aspartate and threonine) . The exponential decays following starvation during the secretory stage have been taken into account for calculating turnover rates and increases following refeeding during the optional nutritional stage for that of synthesis rates .

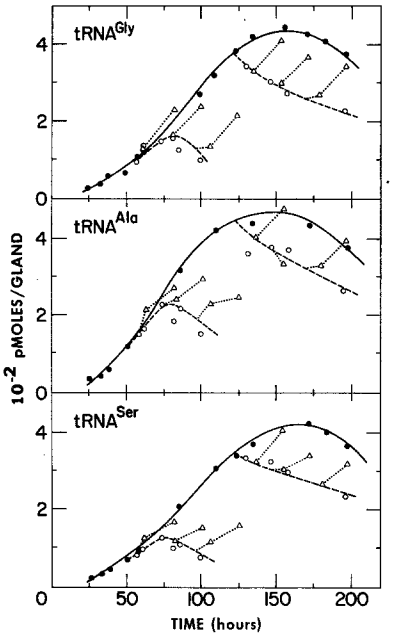

Fig. 2 . Quantitative changes of preponderant tRNA species (glycine, alanine and serine) in the posterior silkgland of Bombyx mori L. fasted and refed larvae during the fifth instar . Measurements are based on in vitro aminoacylation assays according to Chavancy et al.(5) . For symbols , see fig. 1. F = fasting , R = refeeding .

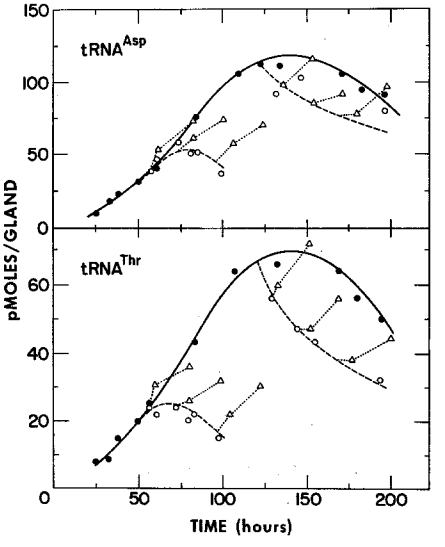

Fig. 3 . Quantitative changes of minor tRNA species (aspartate and threonine) in the posterior silkgland of Bombyx mori L. fasted and refed larvae during the fifth larval instar . Measurements are based on in vitro aminoacylation assays according to Chavancy et al.(5) . For symbols , see fig. 1 . F = fasting , R = reffeding .

2. Quantitative changes of individual tRNA species : in addition to the changes in total tRNA , we have also examined in detail changes of three tRNA species corresponding to the major amino acids of fibroin (glycine, alanine and serine) and of two other species representative of minor amino acids (aspartate and threonine) . The quantitative changes measured by in vitro aminoacylation assays are shown in figures 2 and 3 . The maximum levels of tRNA occur at the end of the secretory phase (7-8th days) for the preponderant three species ($tRNA^{Gly}$, $tRNA^{Ala}$ and $tRNA^{Ser}$) and earlier for the two minor species ($tRNA^{Asp}$ and $tRNA^{Thr}$) as shown previously by Chavancy et al.(5) . Fasting and refeeding have similar effects to those already observed with total tRNA (see fig. 1) .

Half-life times are roughly similar for all tRNA species examined (see Table I) . An additional information is given by the changes of tRNA level when silkworms are refed . A differential synthesis takes place . A synthesis rate for each five mature tRNA species can be computed . The rates of synthesis are widely distributed according to the adaptive levels of each tRNA required for an optimal translation of fibroin mRNA . $tRNA^{Gly}$ species have the highest rate of synthesis , ten times higher than that of the minor $tRNA^{Asp}$ or $tRNA^{Thr}$ species , followed by $tRNA^{Ala}$ and $tRNA^{Ser}$ species .

DISCUSSION

Of interest for a possible regulatory mechanism of tRNA biosynthesis is the observation of Hanoune and Agarwal (19) , that individual tRNA species from rat liver turn over at a constant rate with respect to one another and that the rate of biosynthesis of each tRNA species is indistinguishable from that of total tRNA as observed during regeneration (21) . However , in specialized tissue such as the silkgland this does not appear to be the case . The similarity of the turnover rates of the five tRNA species studied does not imply similar rates of synthesis . Our observations therefore rule out a selective degradation of mature tRNA species as the

main regulatory mechanism for maintaining an optimal steady-state concentration of individual tRNA species in order to ensure the maximum efficiency for decoding fibroin mRNA .

We suggest that a positive and specific regulation occurs at the tRNA biosynthesis level . In the posterior silkgland we know that there is no selective amplification either of total tRNA genes (22) or of $tRNA^{Gly}$ (23) . Regardless of the topography of these tRNA genes , the adaptation of tRNA population to the amino acid composition of silk proteins appears to be determined either from a selective transcription of DNA cistrons corresponding to the preponderant tRNA species or from a preferential processing of some pre-tRNA molecules accompanied by a rapid degradation of the other unused pre-tRNA transcripts . A distinction between these two regulatory processes can be made when quantitative distributions of pre-tRNAs and of tRNA genes become available .

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