ANTICODON LOOP SEQUENCES OF TRANSFER RNA$^{Ser}_{CGA}$ AND TRANSFER RNA$^{Ser}_{IGA}$ FROM THE POSTERIOR SILKGLAND OF Bombyx mori L.*

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SUMMARY : The adaptive level of tRNAs to their use for synthesizing silk proteins involves three isoaccepting serine tRNAs . The two lipophilic $tRNA_{2a}$ and $tRNA_{2b}$ species from the posterior silkgland of the silkworm Bombyx mori, which are able to decode the UCG and UCU,UCC,UCA respectively , have been purified by counter-current distribution . They have been subjected to pancreatic and T_1 ribonucleases digestion . Resulting oligoribonucleotides have been analyzed and partially sequenced . The IGA anticodon found in the dodecanucleotide : Y-A-G-A-m^3C-U-I-G-A-i^6A-A-Ψp for the preponderant $tRNA_{2b}$ is consistent with the occurrence of UCA as the main serine codon in fibroin mRNA . A CGA anticodon has been detected in the homologous fragment of a minor isoaccepting $tRNA_{2a}$.

INTRODUCTION

We have previously shown that the intracellular concentration of the cognate tRNAs is proportional to the composition of amino acids of proteins being synthesized (1) . A correlation also exists between isoaccepting tRNAs (or iso-tRNAs) and the distribution of synonymous codons in mRNAs being translated (2). From the work of Suzuki and Brown (3) on the oligonucleotide distribution of purified fibroin mRNA , it appears that this messenger contains mainly the codon GCU for alanine , two codons GGU : GGA in a ratio 1.4 to 1 for glycine , and the codon UCA for serine . The preponderant iso-$tRNA^{Ser}$ species or $tRNA^{Ser}_2$ is found in a lipophilic peak and is 90 % pure after 1,500 transfer counter-

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Abbreviations : R = purine , Y = pyrimidine , N or W = R or Y (see Table I) .

current distribution (4) . While this $tRNA^{Ser}$ fraction is capable of decoding at least the UCU codon , we suggested that $tRNA_2^{Ser}$ could have an IGA anticodon in order to decode the main UCA codon of fibroin mRNA (5) . We provide direct evidence that the anticodon for the main $tRNA_{2b}^{Ser}$ species is IGA . We also show the presence of a minor lipophilic $tRNA_{2a}^{Ser}$ species , which has a CGA anticodon and is therefore able to decode the UCG codon .

MATERIALS AND METHODS

The crude tRNA and amino acid:tRNA ligase (EC 6.1.1) were extracted from the posterior silkgland of the silkworm Bombyx mori L. , a hybrid from two European strains 200 and 300 , at the 8th day from the Vth instar as described by Chavancy et al.(6) . Assay of the amino acid acceptor activity was performed as previously described (5) . The basic purification of $tRNA_2^{Ser}$ species consists of a 1,500 transfer counter-current distribution with the Phosphate-Formamide-Isopropanol (PFI) solvent system in the conditions used by Garel et al.(4). Reversed-phase chromatography (RPC-4) at 37° according to Miller et al.(7) achieves fractionation into one major component $tRNA_{2b}$ and one minor $tRNA_{2a}$ (D.Hentzen , unpublished results) . The purity of these $tRNA_2^{Ser}$ fractions was estimated to be over 90 % by means of nucleoside composition and acceptor activity (4) .

The pancreatic and T_1 ribonuclease digestions of $tRNA_2^{Ser}$ as well as the separation of the oligoribonucleotides on neutral DEAE-cellulose columns and by high voltage electrophoresis , the nucleoside analysis by thin-layer chromatography and all subsequents steps for further sequence analysis were carried out as described by Keith et al.(8) .

RESULTS

An examination of homologies in the nucleotide sequence of five homodecoding $tRNA^{Ser}$ species ($tRNA_{WGA}^{Ser}$, which WGA being the anticodon) clearly indicates the nature of the eukaryotic anticodon loop and some additional bases of the anticodon stem as shown in Table I : R-G-A-Y-U-W-G-A-i^6A-A-Ψ-C-Yp . From the nucleoside composition of an enriched $tRNA_2^{Ser}$ fraction (4) , we know that two 3-methyl cytidines , one N^6-isopentenyl adenosine and a little less than one inosine are present per $tRNA_2^{Ser}$ molecule . These nucleosides may belong in the sequence of the anticodon loop .

The chromatographic analysis of the pancreatic RNase digest of a fraction of $tRNA_2^{Ser}$ (later shown to contain 65 % of $tRNA_{2b}^{Ser}$ and 35 % of $tRNA_{2a}^{Ser}$) is shown on Figure 1 . The elution profile of the T_1 RNase digest on a neutral DEAE-ceelulose column is indicated in Figure 2 for $tRNA_2^{Ser}$.

TABLE I

STRUCTURAL HOMOLOGIES IN THE ANTICODON ARM OF HOMODECODING $tRNA^{Ser}_{WGA}$

T 4	CCGGU CmU	NGA	A*AA CCGG (9)
E.coli	CCGGU CmU	oac^5UGA	ms^2i^6A AA CCGG (10,11)
Yeast	AAAGA ΨU	IGA	i^6A AA CUUU (12)
Rat liver	AΨGGAm^3C U	IGA	i^6A AΨmCCAU (13-16)
Rat liver	ΨΨGGAm^3C U	CGA	i^6A AΨmCCAA (15,17)
eukaryotic homologies	--RGA Y U	WGA	i^6A AΨ*CY--
B.mori silkgland	-YAGAm^3C U	$\begin{matrix}I\\C\end{matrix}$GA	i^6A AΨ ----

Abbreviations : Cm = 2'-O-methylcytidine , A* = unknown modified adenosine , oac^5U = 5-oxyacetic uridine, ms^2i^6A = methylthio-2 N^6-isopentenyl adenosine , Ψ = pseudouridine , i^6A = N^6-isopentenyl adenosine , m^3C = 3-methyl cytidine , Ψm = 2'-O-methyl pseudouridine , Ψ* = modified pseudouridine .

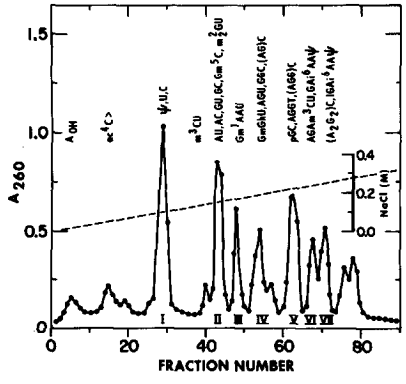

Figure 1. Chromatography of pancreatic RNase digest of $tRNA_2^{Ser}$ from posterior silkgland of Bombyx mori L. Serine $tRNA_2$ (2/3 $tRNA_{2b}$ and 1/3 $tRNA_{2a}$) was prepared by 1,500 transfer counter-current distribution using the Phosphate-Formamide-Isopropanol (PFI) solvent system (4) . 120 A_{260} units were digested with 0.5 mg of pancreatic RNase in 1 ml 0.1 M Tris-HCl buffer pH 7.5 during 2 h at 37°. The digest was made 7 M urea and applied into a DEAE-cellulose column (1 x 40 cm) . Elution was carried out with a linear gradient of 0-0.40 M NaCl in 0.02 M Tris-HCl pH 7.3 , 7 M urea (total volume 0.8 liter) .

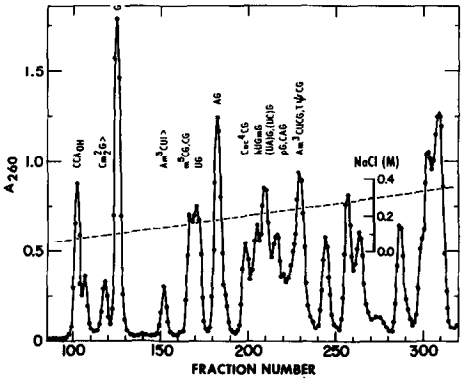

Figure 2. Chromatography of T_1 RNase digest of $tRNA_2^{Ser}$ from posterior silk-gland of Bombyx mori L. 120 A_{260} units of serine $tRNA_2$ prepared as indicated above (see fig. 1) were digested with 1,000 units of T_1 RNase in 1 ml 0.1 M triethylammonium bicarbonate buffer pH 7.5 during 4 h at 37°. The digest was made 7 M urea and applied into a DEAE-cellulose column (0.6 x 110 cm) . Elution was carried out with a linear gradient of 0-0.40 M NaCl in 0.02 M Tris-HCl pH 7.3 and 7 M urea (total volume 1 liter) . Fractions of 2.5 ml were collected at a flow rate of 10 ml/h at room temperature .

After further fractionation by means of a high voltage electrophoresis on DEAE-cellulose paper and analysis of their base composition , we have selected six oligoribonucleotides : two contain inosine , four have 3-methyl cytidine and two have a N^6-isopentenyl adenosine residue . Table II summarizes details of their sequences .

1.Isolation and sequencing of the 5'-part of the anticodons . Two pancreatic and two T_1 RNase digests each containing one 3-methyl cytidine residue are candidates for the 5'-part of the anticodon loops of both $tRNA_2^{Ser}$ species : m^3C-Up , A-G-A-m^3C-Up , A-m^3C-U-I> and A-m^3C-U-C-Gp found with a ratio of about 0.7 , 1.0 , 0.65 and 0.35 respectively (Table II) .

The dinucleotide m^3C-Up (fragment $P_{II,3}$) , resistent to pancreatic digestion , must be preceded by a pyrimidine residue . This Y-m^3C-Up cannot belong to the anticodon loop since m^3C-Up detected in the other three oligonucleotides is associated with an adenosine residue at the 5'-end . Additional information suggests that Y-m^3C-Up is located in the loop of the variable arm according to the sequences of homodecoding $tRNA_{WGA}^{Ser}$ from Rat liver (13,17) .

TABLE II

IDENTIFICATION OF PANCREATIC AND T_1 RIBONUCLEASE DIGESTS CONTAINING m^3C, I AND i^6A OF LIPOPHILIC $tRNA_2^{Ser}$ SPECIES FROM THE POSTERIOR SILKGLAND OF Bombyx mori L.

Fragment		R_B	Methods of analysis	Products of digestion
$P_{II,3}$	m^3C-Up	1.78	BAP / SV	m^3C , pU
$P_{VI,14}$	A-G-A-m^3C-Up	0.19	T_1	A-Gp , (I) A-m^3C-Up
	(I) A-m^3C-Up		BAP / SV	A , pm^3C , pU
$P_{VI,15}$	G-A-i^6A-A-Ψp	0.23	T_1	Gp , (II) A-i^6A-A-Ψp
	(II) A-i^6A-A-Ψp		BAP / SV	A , pi^6A , pA , pΨ
			MN	A-i^6Ap , A-Ψp
$P_{VII,17}$	I-G-A-i^6A-A-Ψp	0.15	BAP / SV	I ,pG,2 pA,pi^6A,pΨ
			partial T_1	I-Gp , (II) A-i^6A-A-Ψp
$T_{III,6}$	A-m^3C-U-I	0.57	BAP / SV	A , pm^3C , pU , pI
			P	(I) A-m^3C-Up , Ip
$T_{IX,24}$	A-m^3C-U-C-Gp	0.64	BAP / SV	A , pm^3C , pU , pC , pG
			P	(I) A-m^3C-Up , Cp , Gp

P and T refer to pancreatic and T_1 ribonucleasic digestions . Roman numerals refer to the oligonucleotides peak after the first neutral chromatography on DEAE-cellulose (see Fig. 1 and 2) . Arabic numerals number the spots on DEAE-cellulose paper after a high voltage electrophoresis in 7 % HCOOH . R_B is the relative mobility of the oligonucleotide to the blue marker (xylene-cyanol FF) on DEAE-cellulose paper . (I,II) indicates a fragment further analyzed . P = pancreatic RNase , T_1 = RNase from Takadiastase , BAP / SV = alkaline phosphatase followed by snake venom phosphodiesterase after removal or inactivation of phosphatase , MN = micrococcal nuclease incubated 6 h (15) .

It belongs to the larger T_1 oligonucleotide not yet sequenced .

The overlapping of the three remaining oligonucleotides leads to the unambigous reconstruction of the 5'-part sequences : Y-A-G-A-m^3C-U-Ip for the major $tRNA_{2b}^{Ser}$ species and Y-A-G-A-m^3C-U-C-Gp for the minor $tRNA_{2a}^{Ser}$ species .

The unique fragment Y-A-G-A-m^3C-Up is common to both iso-$tRNA_2^{Ser}$ species .

2.Isolation and sequencing of the 3'-part of the anticodons . We have identified an N^6-isopentenyl adenosine in two pancreatic digests $P_{VII,17}$ for the $tRNA_{2b}^{Ser}$ species and $P_{VI,15}$ for the $tRNA_{2a}^{Ser}$ species (Table II) in a ratio of 2 to 1 . As suggested above , this modified adenosine is adjacent to the adenosine of WGA anticodon of lipophilic Yeast and Rat liver $tRNA^{Ser}$ (12-17) . The sequence analysis of fragment $P_{VII,17}$ is interesting because it contains both characteristic nucleosides inosine and N^6-isopentenyl adenosine . Inosine was just shown to be located at the 5'-end of the anticodon of $tRNA_{2b}^{Ser}$ species. A partial digestion with the micrococcal nuclease yields the total sequence of this hexanucleotide : I-G-A-i^6A-A-Ψp . The pentanucleotide of fragment $P_{VI,15}$ can be also deduced : G-A-i^6A-A-Ψp . It belongs to the minor $tRNA_{2a}^{Ser}$.

3.Sequences of the anticodon loops of $tRNA_{2a}^{Ser}$ and $tRNA_{2b}^{Ser}$ species . The combination of sequences of the fragments $P_{VI,14}$, $P_{VI,15}$ and $T_{IX,24}$ provides sufficient overlapping to reconstruct the anticodon loop and a part of the anticodon stem of the minor $tRNA_{2a}^{Ser}$ or $tRNA_{CGA}^{Ser}$:

Y-A-G-A-m^3C-U-C-G-A-i^6A-A-Ψp

By combining the fragments $P_{VI,14}$, $P_{VII,17}$ and $T_{III,6}$, we can reconstitute the anticodon region of the major $tRNA_{2b}^{Ser}$ or $tRNA_{IGA}^{Ser}$:

Y-A-G-A-m^3C-U-I-G-A-i^6A-A-Ψp

DISCUSSION

The comparison of the first insect sequences for the anticodon region with homodecoding $tRNA_{WGA}^{Ser}$ species from T_4 bacteriophage (9) , E.coli (10,11) , Yeast (12) and Rat liver (13-17) shows some homologies . The occurrence of the i^6A seems a general feature for eukaryotic $tRNA_{WGA}^{Ser}$ whereas that of m^3C in the anticodon loop seems to be related only to highly differentiated eukaryotes . The nature of the pyrimidine nucleoside Y in 5'-part of the anticodon loop undergoes a great variability . Viral and prokaryotic $tRNA_{WGA}^{Ser}$ have an apolar

2'-O-methyl cytidine , Yeast a neutral pseudouridine and developed eukaryotic organisms , including $tRNA^{Ser}$ species from Drosophila (18) , carry a polar 3-methyl cytidine . Lastly , the anticodon regions of B.mori $tRNA^{Ser}_{WGA}$ reveal additional homologies with Yeast's $tRNA^{Ser}_{IGA}$ and with Rat liver $tRNA^{Ser}_{IGA,CGA}$ beyond the common features shown in Table I . This composite structure may be related to the intermediate position of Lepidopterans in the course of the evolution from lower eukaryotic organisms to mammals .

The occurrence of a preponderant UCA codon in fibroin mRNA (3) is consistent with the major iso-$tRNA^{Ser}_{IGA}$ found in the posterior silkgland and supports other evidence of a quantitative adaptation of iso-tRNAs to mRNA codons (2,5) . The identification of a 6-methylthreonyl adenosine (mt^6A) in the less lipophilic $tRNA^{Ser}_1$ (4) , adjacent to the 3'-end of the anticodon GCU in the homologous Rat liver $tRNA^{Ser}_3$ (17) , is in agreement with the decoding capacity of this third $tRNA^{Ser}$ species for AGU and AGC . The adaptive level of $tRNA^{Ser}$ is known in the posterior silkgland : 13 $\pm$ 3% of the total tRNA population (6,19-22) , in the middle part synthesizing sericin : 20 $\pm$ 5 % (6,19,21) as well in the carcass of the silkworm : 11 % (21) . Work is in progress to determine changes in the intracellular levels of the iso-$tRNA^{Ser}$ species in the silkgland during the last instar .

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