Volume 4 Number 12 December 1977 Nucleic Acids Research

RNA methylation and control of eukaryotic RNA biosynthesis : processing and utilization of undermethylated tRNAs in CHO cells

François Amalric*, Jean-Pierre Bachellerie* and Michel Caboche +

*Centre de Recherche de Biochimie et Génétique Cellulaires du C.N.R.S. 118, Route de Narbonne, 31077 Toulouse-Cedex and +Laboratoire de Génétique Cellulaire, I.N.R.A. Toulouse, 31320 Castanet-Tolosan, France

Received 25 October 1977

ABSTRACT

The role of RNA methylations in the control of tRNA production and utilization for protein biosynthesis has been investigated through a study of the effects in vivo of cycloleucine a specific and potent inhibitor of S adenosyl-methionine mediated methylation.

During the cycloleucine treatment, the rate of appearance of newly synthetized tRNAs into the cytoplasm is markedly reduced (about 50 %). These molecules are extensively (more than 90 %) undermethylated and are integrated into polysomes, but at a slower rate than normally methylated tRNAs.

INTRODUCTION

Like most of prokaryotic or eukaryotic genes, the genes for tRNAs are not directly transcribed as functional molecules, but as precursors which are subsequently processed into mature forms by a series of specific post-transcriptional events, including enzymatic cleavages of the primary transcript and modifications of the conserved chain [1]. Among these modifications that could play a prominent role in the control of genomic expression, RNA methylation is a process of a general occurrence concerning altogether ribosomal, messenger and transfer RNAs. In order to precise the role of methylation, it has been investigated in several studies how the production of mature tRNA was affected when the methylation process was inhibited, in various conditions of altered growth. However, as most of these treatments (among them methionine starvation [2], treatments by ethionine [3], by 5-azacytidine[4]) altered too, in the same time, other major biosynthetic pathways, like protein of RNA biosynthesis, an unambiguous interpretation of the results was difficult to be drawn. On the other hand, the selection of mutant clone, deficient for a specific tRNA methylase [5] is far from being yet available in eukaryotic cells. We therefore have reexamined

this problem with the aid of a more selective tool, cycloleucine, a reversible inhibitor of nucleic acid methylation. In vitro, cycloleucine was shown [6] to competitively inhibit an enzyme (ATP : L-methionine S-adenosyltransferase) involved in the production of S-adenosyl methionine, the donor of methyl group for RNA methylation. We have shown recently [7] that a cycloleucine treatment of C.H.O. cells in vivo rapidly blocks the synthesis of S-adenosyl methionine without drastic alteration of protein and nucleic acid synthesis.

In these conditions, we demonstrated [7] that a normal level of methylation is not stringently required for the completion of ribosomal RNA maturation although the degree of RNA methylation could modulate the global efficiency of the maturation process.

In the present study, we show that, in C.H.O. cells, tRNAs can be synthesized and transferred to cytoplasm, although at a reduced rate, during an extensive inhibition (more than 90 %) of RNA methylations. These undermethylated tRNAs appear to be integrated into polysomes and to participate to the protein biosynthesis with a probability very similar to that of normal tRNA molecules.

MATERIALS AND METHODS

Materials and methods are the same as previously described [7] with the following additions and modifications.

^{32}P labelling conditions : exponentially growing C.H.O. cells in monolayer were washed with a prewarmed phosphate free Hanks basal saline solution and incubated for 2 hours in prewarmed phosphate-free low methionine medium (10 µM). Cells were labelled for 2 hours in the same medium containing (^{32}P) ortho-phosphate (140 µCi/ml; 50 Ci/mg, Amersham England). Cycloleucine treatments (2 mg/ml) were begun 30 min. before labelling.

Cell fractionation : cells in monolayer were immediately detached with glass beads (Ø 0.25 - 0.30 mm; B. Braun Melsungen) in R buffer (40 mM Tris-HCl pH 7.7, 25 mM KCl, 4 mM $MgCl_2$ and 10 µg/ml polyvinylsulphate), in the presence of 0.5 % (v/v) cemulsol NPT 6 and 500 µg/ml collagenase. After 2 min handshaking in the cold (4°C), glass beads were discarded by decantation and the suspension containing detached cells submitted to the action of an Ultra-Turrax to prepare nuclei as previously described [8]. After elimination of mitochondria, supernatant was added with sodium deoxycholate

1 % (w/v) and layered on a 30 % sucrose cushion in the same medium (1:2, v/v) and centrifuged 100 min at 50,000 rev/min in a Spinco Ti 50 rotor. The resulting pellet was essentially composed of polysomes. The supernatant was called "soluble" cytoplasmic fraction.

RNA extraction and analysis : polysomal pellets were resuspended at 4°C in Tris 50 mM (pH 7.4) -EDTA 10 mM then sodium dodecyl sulfate was added to a final concentration of 1 % and the solution directly extracted with phenol-chloroform or incubated 1 hour at 20°C in presence of 200 µg/ml Proteinase K (Merck). An equal volume of water satured phenol-chloroform (1:1, v/v) was added and the mixture vigourously shaken for 10 min at 20°C. After centrifugation, the aqueous phase was reextracted one more time with phenol-chloroform. After addition of NaCl (0.2 M final), RNA was precipitated with 2 volumes of cold ethanol and allowed to stand overnight at -20°C before analysis. "Soluble" cytoplasmic fraction was directly extracted by the phenol-chloroform mixture as described above. RNA was then fractionated into poly $(A)^-$ and poly $(A)^+$ RNAs by two consecutive passages through oligo (dT)-cellulose columns [9], one of which after heat denaturation of RNA (5 min at 70°C at low ionic strength).

Gel electrophoresis of RNA samples was performed according to Loening [10] using 12 % acrylamide gels in acetate buffer. Samples of 100 µl (50 µg RNA) were layered on top of 21 x 0.6 cm cylindrical gels. Separation of 4S and 5S RNA was usually achieved by a 5 h migration, using 5 mA/gel. Radioactive profiles were determined after a 3 mm slicing of gels and counting in a Packard Tricarb liquid scintillation spectrometer.

DEAE Column analysis of alkaline RNA digests : (^{32}P) tRNAs from polysomal or soluble fractions were first purified by passage through a Sephadex G 50 column equilibrated with 0.02 M phosphate buffer (pH 5.0) at 2°C, then run on a sucrose gradient (5 - 20 %) in acetate buffer. Recovered 4S tRNAs were exhaustively hydrolysed in 0.33 M KOH for 20 hours at 37°C. The digest was neutralized with 1 M Tris pH 7.2, diluted 10 times, adjusted to 20 mM Tris-HCl (pH 7.4), 4 mM EDTA and 7 M urea and absorbed to a 0.4 x 50 cm DEAE Sephadex column [11]. Nucleotides were eluted with a 160 ml linear gradient of 0.05 - 0.30 M NaCl in 20 mM Tris-HCl (pH 7.4), 7 M urea. Positions of mono, di, tri... nucleotides were determined by running RNase A digests of tritiated rRNA.

RESULTS

Cycloleucine effects on biosynthesis and processing of tRNA.

After a 30 min pulse with labelled uridine, most (90 %)of 4S labelled RNA was recovered in the "soluble" cytoplasmic fraction, which corresponded to the supernatant of the polysomal pellet. In both treated and control cells, labelled low molecular weight RNAs were resolved in three main discrete species: two well defined components, 5S rRNA and 4S RNA and 4.5S species which could correspond to tRNA precursors [2]. In control cells, after the 30 min pulse, about 65 % of the radioactivity recovered in the low molecular weight components was localized in the 4S area, while 18 % was found in the 4.5S RNA species. During the chase in the presence of unlabelled uridine, the part of label in the 4S peak increased continuously in control cells, up to 90 % after 210 min. of chase, while the percentage of low molecular weight counts recovered in the 4.5S peak decreased conversely. These kinetic data are consistent with the existence of a precursor-product relationship between these two components.

As shown in fig. 1a, in cycloleucine treated cells, the distribution pattern of labelled low molecular weight RNA after a 30 min pulse was clearly different. Only 38 % of the radioactivity was recovered in the 4S species, 51 % being in the 4.5S. After 210 min chase, the part of 4S labelling increased up to 75 %. Furthermore, the cycloleucine treatment resulted in marked decrease (approximatively 50 %) in the amount of 4S RNA labelled during a 30 min pulse. This deficiency in labelled 4S RNA remained nearly constant through out the chase experiment. This significant decrease suggests that production of mature tRNA could be controlled in some way by the level of RNA methylation as will be discussed latter.

Integration of tRNA into polysomes during a cycloleucine treatment

The ability of tRNA molecules synthetized in the presence of cycloleucine to be incorporated into polysomes was examinated in two ways.

In a first experiment, cells were pulsed for 30 minutes with labelled uridine and chased for various periods of time in presence of cold uridine. In order to eliminate errors due to fluctuations in the yield of the cytoplasmic fractions, treated and control cells labelled with (^{3}H)-uridine and (^{14}C) uridine respectively, were mixed before fractionation. The $^{3}H/^{14}C$ count ratios were determined in the 4S peak of the gel profiles from "soluble" cytoplasmic fraction and from polysomes. Results are shown

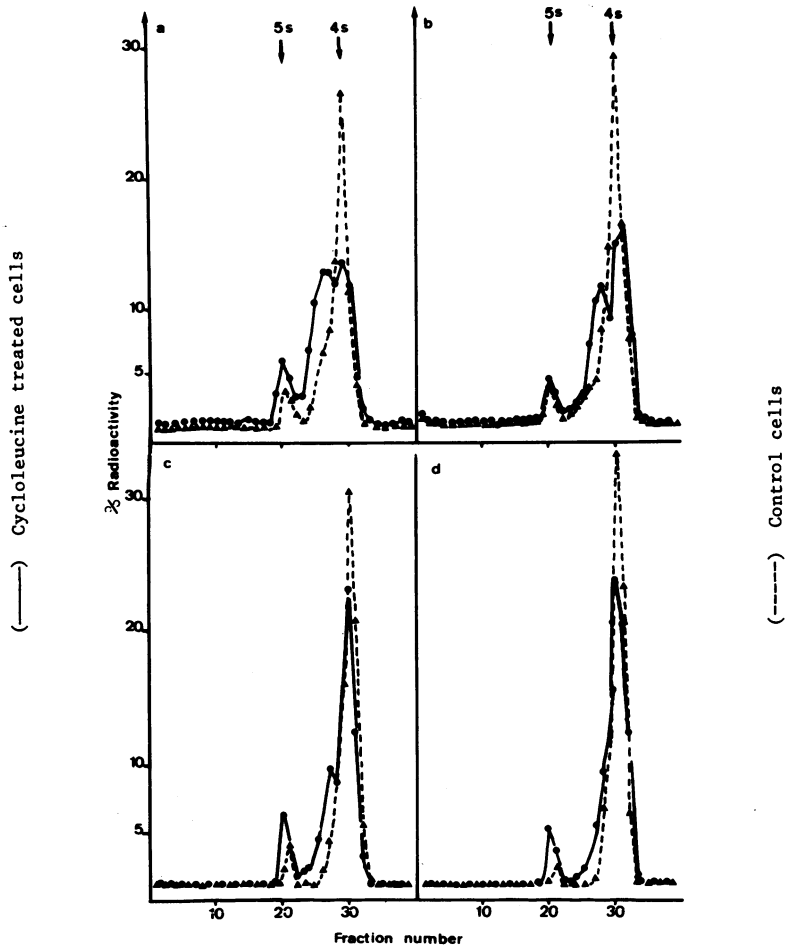

FIGURE 1 - Kinetics of tRNA production during a cycloleucine treatment
Exponentially growing cells were pulsed for 30 min. with (^{3}H) uridine (40 µCi/ml, 40 Ci/mmol) and chased in cold medium containing 200 µM unlabelled uridine. At different times after the beginning of the chase (a: 0; b: 30 min; c:90 min; d: 210 min.) cells were harvested: RNA was extracted from the "soluble" cytoplasmic fraction and analysed by electrophoresis onto 12 % acrylamide gels (see Materials and Methods). In order to facilitate the quantitative comparison between treated and control profiles the plotted values corresponded to percentages of total radioactivity recovered on each gel. Total counts recovered in the low molecular weight areas of the gels varied from 57,600 to 125,000 cpm for control cells and 43,200 to 65,000 cpm for cycloleucine treated cells.

in Table 1. This approach should allow an accurate detection of even minor changes in the relative distribution of tRNA among polysomal and "soluble" cytoplasmic fractions, by comparison of the ^{3}H/^{14}C count ratios. In fact,

for the various periods of chase that we have studied, this ratio was significantly lower for polysomes than for the "soluble" cytoplasmic fraction, corresponding approximatively to half the control value, thus indicating a significant inhibition of cytoplasmic tRNA integration into polysomes. However, for longer periods of chase, this discrepancy between the two $^{3}H/^{14}C$ ratios diminished significantly, thus suggesting that the cycloleucine treatment resulted in a delayed utilization of cytoplasmic tRNA into polysomes. This possibility was further examined through a second experiment.

In order to compare the rate of appearance into polysomes of newly synthetized tRNAs with that of more mature tRNAs, cells were labelled successively for 2 hours with (^{14}C) uridine then, after removal of the (^{14}C) precursor from the medium, for one hour with (^{3}H) uridine. Cycloleucine treated and control cells were labelled and processed in parallel for isolation of polysomal RNA. The gel profiles, for low molecular weight RNAs, are shown in fig. 2. For each labelling time, the 4S/5S count ratio was calculated, from the (^{14}C) and (^{3}H) profiles respectively. In control cells, the obtained values were very similar for either (^{3}H) or (^{14}C) labelling. On the contrary, in cycloleucine treated cells, the recovery of (^{3}H) labelled tRNAs (one hour pulsed), in the polysomal fraction, was markedly depressed as compared to that of 5S rRNA. However, for a longer time of labelling $\left[(^{14}C) \text{ label}\right]$, the relative recovery of polysomal tRNA was increased as the 4S/5S (^{14}C) count ratio reached a value very close to the control.

Degree of inhibition of tRNA methylation by cycloleucine.

The post transcriptional additions of methyl-groups on tRNA nucleotides are of two types : base methylations (with several possible positions for each base) and 2'O-ribose methylations.

In a first step, the overall degree of methylation of tRNAs (base + ribose) was determined through in vivo labelling experiments by simultaneous addition of (methyl-^{3}H) methionine and (^{14}C)-uridine, in the presence or absence of cycloleucine. As it will be discussed, we considered that the (^{3}H)/(^{14}C) count ratio in purified RNAs could be taken as a reliable index of the overall methylation degree.

Cells were pulsed for one hour with labelled isotopes and chased for two hours in the presence of cold precursors. The gel electrophoresis profiles of Figure 3 corresponded to the low molecular weight cytoplasmic RNAs extracted respectively from "soluble" cytoplasmic fraction (a, b) and from polysomes (c, d). In "soluble" cytoplasmic fraction of untreated cells

TABLE 1 - Integration of tRNA into polysomes

Chase time (min.)		0	30	90	210
$^3H/^{14}C$ in 4S RNA	"soluble" cytoplasm	5.5	5.2	5.3	5.8
	polysomes	2.5	2.5	2.7	3.7

Cells were labelled 30 min. either with (^3H) uridine (for cycloleucine treated cells) or (^{14}C) uridine (for control cells) and chased for various times in the presence of unlabelled uridine. Cycloleucine treated and control cells were mixed before fractionation and the count ratio of the two isotopes ($^3H/^{14}C$) was determined in the 4S peak after gel electrophoresis analysis for "soluble" cytoplasmic and polysomal RNAs.

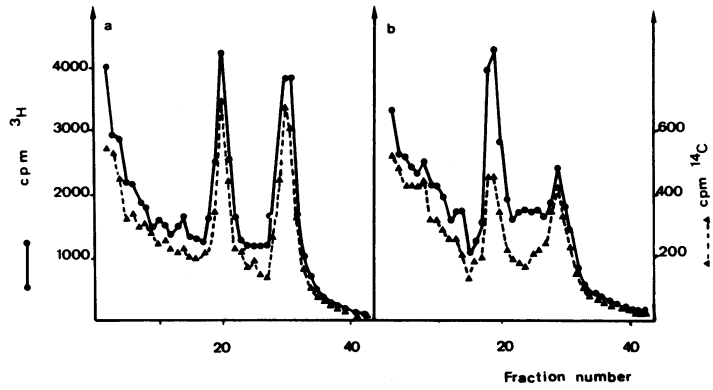

FIGURE 2- Incorporation of tRNA into polysomes

Cells were labelled for 2 hours with (^{14}C) uridine (1 µCi/ml, 0.4 Ci/mmol). The medium was then removed, replaced by fresh medium containing (^3H) uridine (20 µCi/ml, 20 Ci/mmol) and the incubation processed for one more hour at 37°C. Poly (A) containing RNA was removed by chromatography on oligo (dT) column and the unbound RNA was analysed by electrophoresis on 12 % acrylamide gels.

(a) Control cells - (b) Cells treated with 2 mg/ml cycloleucine (——) ^3H (1h pulse) ; (-----) ^{14}C

(a), the main (^{14}C) uridine labelled component was the 4S tRNA species, with only a very small 5S peak, while the [methyl-(^3H)] labelling was restricted to the 4S peak. In the polysomal fraction of control cells (c), the (^{14}C) radioactivity profile revealed the two prominent peaks of 5S rRNA and 4S tRNA, the [methyl-(^3H)] labelling being restricted to the latter species, in good agreement with previous reports of an absence of methylation in the 5S rRNA molecule [12], and confirming furthermore that no methyl labelling occured via purine metabolism in our experimental conditions. The cycloleu-

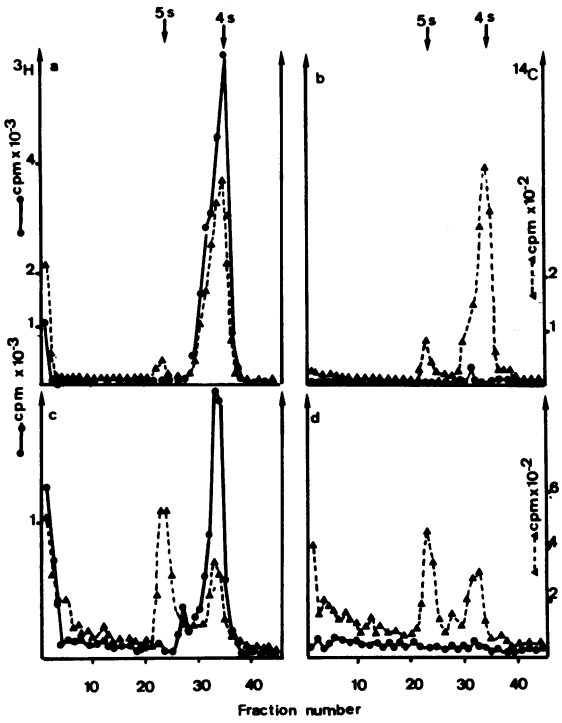

FIGURE 3 - Effects of cycloleucine on (methyl ^{3}H) methionine incorporation into cytoplasmic and polysomal tRNAs.

Cells were doubly labelled for 1 hour by simultaneous addition of (^{14}C) uridine (1 µCi/ml; 0.4 Ci/mmol) and (methyl ^{3}H) methionine (25 µCi/ml; 5 Ci/mM) either in the absence (a, c) or in the presence (b, d) of 2 mg/ml cycloleucine and the label was chased for two hours in the presence of cold uridine and methionine. RNA was extracted from cytoplasmic "soluble" and polysomal fractions and analysed by gel electrophoresis on 12 % acrylamide gels. (a) and (b) "soluble" cytoplasmic RNA; (c) and (d) polysomal RNA.
(---): (^{14}C); (——) : (^{3}H).

cine treatment of the cells (b, d) did not markedly modify the (^{14}C) label profiles.

However, a moderate decrease in the (^{14}C) specific activity of the 4S peak was observed either in "soluble" cytoplasmic fraction or in polysomes in good agreement with the results described in the preceeding section. Conversely, the [methyl(^{3}H)] labelling of the 4S RNA species was almost fully suppressed for both cytoplasmic fractions : the $^{3}H/^{14}C$ count ratio in the 4S species was more than twenty times lower for treated cells than for control cells (Table 2).

TABLE 2 - Cycloleucine effects on biosynthesis and methylation of tRNA

	Chase time	Total cpm in 4S peak			
		Soluble RNA		Polysomal RNA	
		(^{14}C)uridine	(^{3}H)methionine	(^{14}C)uridine	(^{3}H)methionine
Control cells	0	955 (100)	36 700 (100)	1070 (100)	13 000 (100)
	2 h	1585 (166)	18 100 (49)	1290 (122)	5 600 (43)
Cycloleucine treated cells	0	635 (67)	90 (0.5)	620 (58)	160 (1.2)
	2 h	720 (76)	110 (0.5)	900 (84)	200 (1.5)

Experiment similar to fig. 3 : after the one hour double labelling with (^{14}C) uridine and (^{3}H) methionine, cells were harvested either immediately or after a two hour chase. The values correspond to the total counts recovered in the 4S peak of the gel, normalized for equal amounts of input RNA. Values into brackets are percent of the one-hour control pulse in the corresponding column.

The actual undermethylation of tRNAs synthe ized during the cycloleucine treatment was confirmed by an approach independent of the methyl-labelled methionine incorporation, in a second series of experiments. The methyl group on the 2'-OH group of ribose makes the adjacent phosphodiester bond resistant to alkaline hydrolysis. We therefore have determined the proportion of tRNA nucleotides recovered in alkali-resistant dinucleotides, which was assumed to reflect principally the degree of ribose methylation. After an alkaline hydrolysis of (^{32}P) labelled tRNAs, mono-and di-nucleotides were separated on a DEAE Sephadex column. Fig.4 shows the elution profiles obtained for polysomal tRNAs of control (a) and treated cells (b).The radio-activity recovered in the dinucleotides (fractions 20 to 35) corresponded to 1.9 % and 0.4 % of the total eluted counts for control and treated cells respectively thus suggesting the presence of half amounts of 2'0 methyl groups : 0.95 and 0.2 per 100 nucleotides on the average for polysomal tRNA (Table 3).For the tRNA recovered in the "soluble" cytoplasmic fraction, we observed similarly an extensive undermethylation in cycloleucine-treated cells (0.3 % of 2'0 methyl-ribose instead of 1.50 % for control).

Differential extractibility of polysomal tRNAs.

The results of the previous sections demonstrated that undermethylated tRNAs could be transferred to cytoplasm and integrated into polysomes during a cycloleucine treatment.

During its association with ribosome, each functional tRNA molecu-

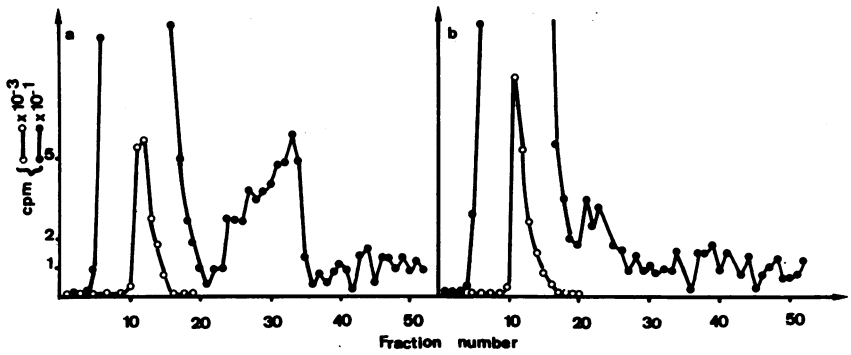

FIGURE 4 - Analysis of alkali-resistant dinucleotides in polysomal 4S tRNA on DEAE Sephadex column.

Polysomes were isolated and RNA extracted from (^{32}P) labelled cells as described in Materials and Methods. tRNAs were isolated by ultracentrifugation on sucrose gradient. After ethanol precipitation, 4S RNA was redissolved in 0.33 M KOH and hydrolyzed for 20 hours at 37°C. Resulting nucleotides were analyzed on a DEAE Sephadex column.
(a) control cells (b) cycloleucine treated cells

TABLE 3 - Analysis of alkaline digests of tRNAs

		Mono-nucleotides cpm	Di-nucleotides cpm	% cpm in di-nucleotides
4S in control cells	Polysomal	17,200	330	1.92 ± 0.4
	Soluble	42,150	1,300	3.1 ± 0.20
4S in cycloleucine treated cells	Polysomal	20,600	82	0.4 ± 0.4
	Soluble	59,800	360	0.6 ± 0.12

Data came from DEAE Sephadex column analysis of alkaline digests of tRNAs performed as described in fig. 4.

le is successively located in the so-called A (acceptor) and P (peptidyl) sites, the translocation being performed after completion of the new peptidyl bond. We therefore have assumed that the level of undermethylated tRNA located in the P site would reflect the functional ability of these molecules to participate to the protein synthesis process. In order to discriminate between the P and A location of tRNA molecules, we have taken advantage of the covalent linkage of the elongating peptide with tRNA in the P site,

which must result in different extractibility behaviour in well defined experimental conditions.

In fig. 5a, polysomal RNAs were extracted by the phenol-chloroform procedure, a treatment known to remove from the aqueous phase the proteinaceous components, and particularly the peptidyl-tRNAs located on the P site

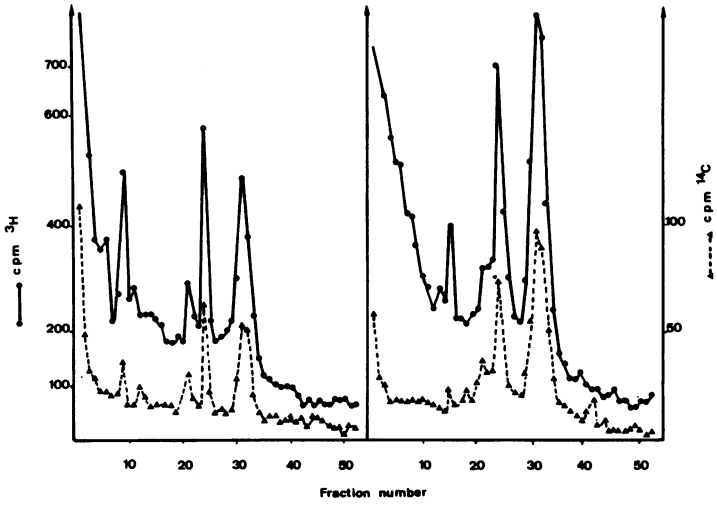

FIGURE 5 - Effects of proteinase K treatment on polysomal tRNA extractibility

Cycloleucine treated cells were labelled 1 hour with (^{3}H) uridine (20 µCi/ml; 20 Ci/mmol) while control cells were labelled 1 hour with (^{14}C) uridine (2 µCi/ml; 0.4 Ci/mmol). After labelling, the two cultures were mixed and polysomes prepared. After solubilization of polysomes into 2 ml of 1 % SDS, 1 ml was treated by proteinase K and 1 ml directly extracted as described in Materials and Methods. The purified RNAs were then analysed by electrophoresis on acrylamide gels. (A) Extraction without proteinase K. (B) Extraction with proteinase K.

TABLE 4 - Extractibility of polysomal RNA

	Extraction with proteinase K	
	Control cells	Cycloleucine treated cells
5S	+ 54 %	+ 33 %
4S	+ 115 %	+ 102 %

Data come from gel electrophoresis profiled described in fig. 5. Results were expressed as relative increase in recovery after the proteinase K treatment (fig. 5b) as compared to the extraction without proteinase K (fig. 5 a).

of the ribosome at the time of the extraction. In fig. 5b, a proteinase K treatment was performed on the resuspended polysomal material before the phenol-chloroform extraction in order to allow the recovery in the aqueous phase of the tRNA molecules which were linked to nascent peptides. The experiment was performed on a mixed batch of (^{3}H) uridine labelled cycloleucine treated cells and (^{14}C) uridine control cells.

As clearly seen in the gel profiles, taking as a reference the counts recovered in the 5S rRNA species, the second extraction procedure allowed an increased recovery of 4S control tRNA : this differential increase corresponded to about 60 % of the counts present when the proteinase K treatment was omitted (Table 4). A very similar increase was observed in the (^{3}H) radioactivity profiles of undermethylated polysomal tRNA, thus suggesting that undermethylated tRNAs in the polysomal fractions were distributed among A and P sites of the ribosome in proportions very similar to normally methylated tRNAs.

DISCUSSION

Cycloleucine, a competitive inhibitor of ATP:L-methionine-S-adenosyl transferase *in vitro* [6], induces *in vivo* a rapid decrease of S-adenosyl methionine intracellular pool, and consequently acts as a potent and reversible inhibitor of rRNA methylation, as shown in a previous paper [7]. In the present study, we have examined the effects of a cycloleucine treatment of CHO cells on tRNA methylations as well as on tRNA production and utilization.

tRNAs molecules, which are produced in the presence of cycloleucine, are extensively undermethylated as indicated by the level of (methyl-^{3}H) methionine incorporation. We consider that the amount of (methyl-^{3}H) radioactivity recovered in purified tRNAs can be taken as a reliable index of the overall (base + ribose) methylation degree of these molecules : in fact, we had previously verified that the cycloleucine treatment, besides its effects on the intracellular pool of S-adenosyl methionine, does not modify significantly the entry of methionine into CHO cells [7]. The drastic undermethylation of tRNAs synthetized during the cycloleucine treatment has been confirmed by an independent approach : the dosage of alkali-resistant dinucleotides indicates that methylation on the 2'O ribose is also stronghly inhibited too, although at a lower extent than total methylations; if significant, this discrepancy could indicate that bases and 2'O ribose are methylated with different efficiency when the pool of intracellular S-adenosyl methionine is reduced.

This result must be put together with previous reports describing a non-uniform sensitivity of the different methylation sites to ethionine treatment [14], as well as with results [15,16] demonstrating a definitive order of succession of the integration of the various methyl groups in the tRNA molecules.

These undermethylated tRNAs are incorporated into polysomes and recovered in both A and P ribosomal sites, thus suggesting that they are functional in protein biosynthesis. This is in line with previous reports, showing no apparent differences between normal and hypomethylated bacterial tRNAs as to aminoacid incorporation efficiency in in vitro systems [17, 18]. However, a marked reduction in the amount of newly synthetized tRNAs recovered into polysomes is observed. This decrease corresponds altogether to a reduced production of cytoplasmic tRNA and to a delayed integration of these undermethylated cytoplasmic molecules into polysomes.

The reduction in the rate of tRNAs cytoplasmic appearance reaches about 60 %. It must be noted that the residual production of cytoplasmic tRNAs (40 %) is significantly higher than that observed for cytoplasmic rRNA (about 15 %) during the cycloleucine treatment. However, due to a lack of an unambiguous characterization of tRNA primary transcripts, it is difficult to discriminate between transcriptional and post-transcriptional levels for inhibition, as was done previously for rRNA species : the 85 % decrease of ribosome production resulted from a 30 % inhibition of the transcription rate and from further impairments of the maturation process [7]. However, if one assumes that the 4.5S species represents mainly tRNA precursors one can calculate that the rate of tRNA transcription (estimated from the sum of 4S + 4.5S counts for short term labelling) in treated cells comes approximatively to about 75 % of the control rate. Such a degree of inhibition would then be very close to what was observed for whole cellular RNA or ribosomal RNA precursor [7]. It must be stressed that the deficiency in the total labelling of 4S + 4.5S in treated cells becomes progressively more important as the chase proceeds : the inhibition reaches 60 % after a 210 min chase following a 30 min pulse. Such a result could indicate that either the efficiency of the maturation process of tRNA precursors, or the stability of newly synthetized undermethylated tRNAs were decreased as a result of the cycloleucine treatment.

Finally, our results indicate that a complete set of methylations is not obligatory for the production and utilization of tNRAs in mammalian cells. However, the undermethylation seems to result in a reduced probabili-

ty for the completion of maturational events as well as for participation in protein biosynthesis. Cycloleucine may provide a potent tool for a detailed study of the specific role of various methyl groups in the control of tRNA maturation and utilization, particularly through large scale isolations of individual undermethylated tRNAs and tRNA precursors.

ACKNOWLEDGEMENTS

We are grateful to Professor Zalta for his support and encouragements throughout this work. This investigation was financially supported by grants from C.N.R.S. (A_1 - 21 - 21), INSERM (74-1-226-2) and INRA.

+Present address : Laboratoire de Biologie Cellulaire, Centre National de la Recherche Agronomique, Route de Saint-Cyr, 78000 Versailles, France

REFERENCES

1. Perry, R. (1976) Ann. Rev. Biochem. 45, 605-629
2. Bernhardt, D. and Darnell, J.E. (1969) J. Mol. Biol. 42, 43-56
3. Wildenauer, D. and Gross, H.J. (1974) Nucleic Acid Res. 1, 279-288
4. Lu, L.W., Chiang, G.H., Medina, D. and Randerath, K. (1976) Biochem. Biophys. Res. Commun. 68, 1094-1101.
5. Björk, G.R. and Isaksson, L.A. (1970) J. Mol. Biol. 51, 83-100.
6. Lombardini, J.B., Coulter, A.W. and Talalay, P. (1970) Mol. Pharmacol. 6, 481-499.
7. Caboche, M. and Bachellerie, J.P. (1977) Eur. J. Biochem. 74, 19-29
8. Zalta, J., Zalta, J.P. and Simard, R. (1971) J. Cell Biol. 51, 563-568
9. Ojala, D. and Attardi, G. (1974) J. Mol. Biol. 82, 151-176.
10. Loening, V.E. (1967) Biochem. J., 102, 251-257
11. Schibler, U. and Perry, R. (1976) Cell 9, 121-130
12. Barrel, B.G. and Clark, B.G. (1974) Handbook of Nucleic Acid Sequences, Joynson - Bruwers, England Oxford.
13. Hobson, A.C. (1976) Molec. Gen. Genet. 144, 87-95.
14. Friedman, S. (1977) Nucleic Acid Res. 4, 1853-1871.
15. Munns, T.W. and Sims, H.F. (1975) J. Biol. Chem. 250, 2143-2149
16. Nau, F. (1976) Biochimie 58, 629-645.
17. Johnson, L., Hayashi, H. and Söll, D. (1970) Biochemistry 9, 2823-2831
18. Björk, G.R. and Neidhart, F.C. (1975) J. Bacteriol. 124, 99-111.