METHYLATION OF THE GUANINE BASES OF TRANSFER RNA

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SUMMARY: The extent of methylation of the guanine bases of tRNA was assessed by labeling KB cells with [2-^{14}C]-guanosine under a variety of pulse and pulse-chase conditions. Approximately 95% of the radioactivity incorporated into tRNA was recovered and identified as [2-^{14}C]-guanine and 1-, 7-, N^2- and N^2N^2-methyl-[2-^{14}C]-guanine. Analysis of the distribution of these constituents under long-pulse and pulse-chase conditions revealed that a maximum of 9.6% of the guanine bases of tRNA became methylated. As reflected by short pulses, the formation of N^2-methylguanine occurred predominantly at a later stage of tRNA maturation than did 1-, 7- and N^2N^2-methylguanine.

INTRODUCTION: As reviewed by Söll (1), newly synthesized tRNA molecules undergo a variety of modifications prior to maturation. Recently, several investigations have indicated that at least some of these modifications occur at specific times during the maturation process (2-8). Evidence for such specificity was provided by structural analysis of precursor tRNAs (2-4), measurements of the content of various uridine modifications on both precursor- and mature-tRNA populations (5-7) and determination of the resulting methylation products from actinomycin D-chased tRNA preparations (8).

In the present study the extent to which specific types of guanine modifications occur during the maturation of tRNA was examined. This parameter was investigated by determining the distribution of [2-^{14}C]-guanine constituents derived from tRNA preparations previously labeled with [2-^{14}C]-guanosine for progressively longer periods of time.

EXPERIMENTAL: KB cells, a clonal line of human carcinoma cells, were grown in suspension culture at 37°C to densities of 3 to 5 x 10^5 cells/ml in MEM medium (GIBCO) supplemented with 5% horse serum. The labeling of cells and their processing following various pulse and pulse-chase experiments with [2-^{14}C]-guanosine (51.2 mCi/mm, Schwarz Mann) are described in the legends of the appropriate Tables and Figures.

The RNA of KB cell homogenates was extracted with phenol-SDS at 65°C and the tRNA subsequently purified via successive fractionation treatments with 1.2 M NaCl and with isopropyl alcohol as previously described (8). Acrylamide gel electrophoretic characterization of the [2-^{14}C]-guanosine-labeled tRNA prepared in this manner indicated that greater than 95% of the radioactivity migrated as tRNA.

Procedures employed for determining the patterns of [2-^{14}C]-guanine constituents consisted of hydrolyzing tRNA in 88% formic acid (Mallinckrodt) for 2 hr at 100°C and separating the resultant guanine bases in the hydrolysate by two-dimensional thin-layer chromatography (9). Routinely greater than 90% of the radioactivity incorporated into tRNA was recovered and identified as guanine and 1-, 7-, N^2- and N^2N^2-methylguanine.

RESULTS AND DISCUSSION: In assessing the maximum extent of methylation of the guanine residues of tRNA, KB cells were labeled with [2-^{14}C]-guanosine under a variety of pulse and pulse-chase conditions and the ratio of the sum of the methylated [2-^{14}C]-guanines (ΣmeG) to the total [2-^{14}C]-guanines (G + ΣmeG) of tRNA determined. The results of these experiments are presented in Table 1 and indicated that the extent of methylation of the [2-^{14}C]-guanine bases of tRNA increased progressively during longer pulses to a maximum value of 9.6% (observed in

TABLE 1: EXTENT OF METHYLATION OF GUANINE BASES OF tRNA: EFFECTS OF PROGRESSIVELY LONGER PULSES WITH [2-^{14}C]-GUANOSINE AND OF PROGRESSIVELY LONGER CHASES[a].

Pulse Time (min)	ΣmeG/(G+ΣmeG)[b] S.D. ( )[c]
30	6.25 ± 0.40 (65)
60	7.76 ± 0.50 (81)
120	8.34 ± 0.28 (87)
180	9.67 ± 0.46 (101)
240	9.48 ± 0.39 (99)
30 + 30 min chase	7.82 ± 0.52 (81)
30 + 60 min chase	9.08 ± 0.34 (94)
30 + 90 min chase	9.45 ± 0.38 (98)
30 + 120 min chase	9.65 ± 0.25 (101)

[a]The RNA of KB cells (3.0 x 10^5 cell/ml) was labeled with [2-^{14}C]-guanosine at 0.025 μCi/ml and upon completion of the desired pulse 200 ml aliquots of cells were either rapidly chilled and collected by centrifugation or chased with unlabeled guanosine (3 mg/200 ml) for the indicated periods of time. Isolation of tRNA and subsequent determination of radioactive guanine bases as described in the Experimental section. [b]ΣmeG, represents the sum of the radioactivity recovered as 1-, 7-, N^2- and N^2N^2-methylguanines and ΣmeG/(G+ΣmeG) represents the percent of guanine bases of tRNA that become methylated. Each value represents the mean from 3 independent experiments. [c]( ), percent of maximum [2-^{14}C]-guanine methylations based on a common demoninator of 9.6%.

preparations of tRNA previously labeled for both 3- and 4-hr). The inability of labeling periods of less than 3 hr in duration to reflect this maximum value suggested the presence of immature tRNA populations, i.e., immature on the basis that the [2-^{14}C]-guanine residues of these preparations had not yet acquired their full complement of methyl groups. Further evidence for the existence of these immature tRNA populations (and their transition to mature forms) was provided by the appropriate pulse-chase experiments also listed in Table 1. Under these conditions the extent of methylation of the [2-^{14}C]-guanine bases incorporated into tRNA during a 30-min pulse progressively increased

to the maximum value of 9.6% (observed for chase periods of both 90- and 120 min). These results further implied that a period of approximately 90 min was required for newly synthesized tRNA molecules to acquire their full complement of methylated guanine constituents.

In examining the extent of formation of specific methylated guanine residues, the ratio of each methyl-[2-^{14}C]-guanine constituent to the total [2-^{14}C]-guanines present in tRNA was determined following selected pulse and pulse-chase periods with [2-^{14}C]-guanosine. The results of these experiments are presented in Table 2 and in Figure 1 and show that the extent of formation of each methylated base increased during progressively

TABLE 2: EXTENT OF FORMATION OF THE METHYL-[2-^{14}C]-GUANINE BASES OF tRNA: EFFECTS OF PROGRESSIVELY LONGER PULSES[a].

Pulse Time (min)	G+ΣmeG[b] (cpm)	meG/(G + ΣmeG)[c]			
		1-meG	7-meG	N^2-meG	N^2N^2-meG
30	9500	1.75	1.80	1.32	1.43
60	17920	2.03	1.93	1.92	1.90
120	22450	1.87	1.83	2.72	1.92
180	28880	2.01	1.98	3.67	2.02
240	34850	2.02	1.94	3.55	1.95
30 + 30 min chase	9920	1.98	1.80	2.12	1.92
30 + 60 min chase	11060	2.00	1.83	3.28	1.96
30 + 90 min chase	10870	2.02	1.88	3.62	1.93

[a]KB cells were labeled under the conditions described in Table 1 and the tRNA was isolated and processed for determination of [2-^{14}C]-guanine constituents as described in the Experimental section. [b]G+ΣmeG, represents the amount of radioactivity recovered as [2-^{14}C]-guanines from one experiment. [c]meG/(G+ΣmeG) reflects the percentage of the total [2-^{14}C]-guanine radioactivity incorporated into tRNA and recovered as 1-methylguanine (1-meG), 7-methylguanine (7-meG), N^2-methylguanine (N^2-meG) and N^2N^2-dimethylguanine (N^2N^2-meG). The latter values reflect the mean from 3 independent experiments with standard deviations (not shown) of less than ±7.7% for each value listed.

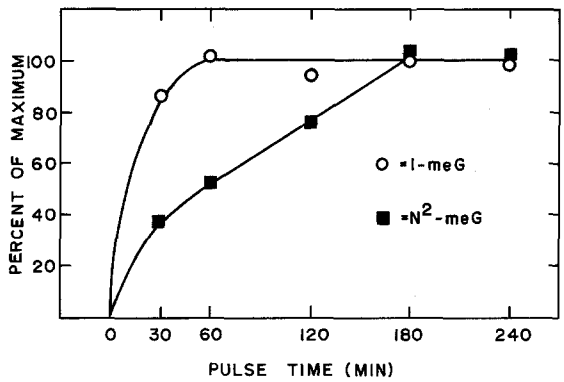

FIGURE 1. The extent of formation of 1-methyl-[2-^{14}C]-guanine and N^2-methyl-[2-^{14}C]-guanine on tRNA following progressively longer pulses with [2-^{14}C]-guanine. For experimental details see Tables 1 and 2.

longer pulses to maximum values of 2.0, 1.9, 3.6 and 1.9 for 1-, 7-, N^2-, and N^2N^2-methylguanine, respectively. Whereas maximum or near maximum values for 1-, 7- and N^2N^2-methylguanine were obtained following a 30-min pulse, pulses of 120- to 180-min in duration were required for N^2-methylguanine to approach its maximal value. These results demonstrate that newly synthesized tRNA molecules acquire their full complement of 1-, 7-, and N^2N^2-methylguanine constituents at a significantly earlier stage of maturation than that of N^2-methylguanine. A comparison of the relative extents of formation of 1-methylguanine and N^2-methylguanine (Figure 1) indicated that newly synthesized tRNAs acquire their full complement of 1-methylguanines (as well as 7- and N^2N^2-methylguanines) approximately 3-times faster than that of N^2-methylguanines (60 min vs 180 min). Furthermore, by the time the former methylated bases of tRNA reached their maximal values (60-min pulse) that of N^2-methylguanine was only 50% of maximum.

These results imply a certain specificity with regard to tRNA processing and the formation of specific types of methylated

guanine residues. Although, the reason for the delayed formation of N^2-methylguanine on tRNA relative to the other methylated guanines remains uncertain, it is reasonable to assume that the N^2-methylguanine methylase enzyme(s) may not be as accessible to tRNA precursors as are other methylases (compartmentalization) or that these tRNAs have to undergo other types of modifications prior to being recognized as suitable substrates.

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