THE METHYLATION OF TRANSFER RIBONUCLEIC ACID DURING REGENERATION OF THE LIVER

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ABSTRACT
Transfer ribonucleic acid is methylated after the molecule is synthesized; at least eight enzymes are involved in the transfer of methyl groups (derived from methionine). The time courses of methylation and synthesis of tRNA during rat liver regeneration have been compared in an in vivo radioisotopic study, using 6- orotic acid-14C and 3H-methyl-L-methionine as precursors in double label pulses. Liver regeneration is a synchronized system in which biochemical events of the cell cycle are separable. Transfer RNA methylation increase precedes by several hours tRNA synthesis during regeneration, although the curves overlap. A ratio of the relative rate of methylation to the relative rate of synthesis has been made; that curve positively correlates with the rise and fall of protein synthesis during regeneration. It is clear that methylation and synthesis of tRNA are only weakly coupled; changing methyl content of the tRNA “pool” resulting from differential tRNA methylase and polymerase activities may regulate the rate of protein synthesis in the cell cycle at the translational level. The “pool sizes” of uridine monophosphate (UMP) and S-adenosylmethionine (SAM) were measured indirectly; UMP and SAM were isolated from perchloric acid supernatants and their specific activities were computed. Differential changes in radioactivity available to tRNA methylases and polymerases are not a source of artifact. That is, the control of both the synthesis and methylation of tRNA is at the enzyme level in vivo, rather than at some enzymatic step prior to those enzymatic reactions.

INTRODUCTION
Alteration of protein or nucleic acid by methylation (1, 2), acetylation (3–5), or phosphorylation (6, 7) changes its primary sequence, essentially. Primary sequence influences tertiary structure, and tertiary structure regulates function. It is appropriate, then, to ask what is the function associated with the addition of methyl groups to a completed transfer ribonucleic acid molecule. There has been speculation that methylation regulates tRNA function in the aminoacyl synthetase reaction (27, 8) and there has been evidence to the contrary (9–11). It is thought that tRNA methylation acts at the translational level in protein synthesis to increase ambiguity in reading the genetic message (12), but there is evidence to the contrary (13, 14). According to Capra and Peterkofsky (13, 14), methylated bases in tRNA are required for specific codon recognition.

No clear-cut, unified, working hypothesis has yet been proposed which agrees with experimental results from many laboratories. It is the aim of this paper to give such a hypothesis and provide
preliminary evidence for its validity. The methylation and synthesis of tRNA are only weakly coupled in the cell cycle, represented by regenerating rat liver. This weak coupling gives rise to a changing methyl content in the tRNA "pool." It may be that the changing methyl content of the tRNA "pool," therefore, is responsible for controlling the rate of protein synthesis at the translational level in regenerating rat liver. Evidence for the timing of the synthesis and methylation of transfer-RNA during regeneration of the liver is presented and is consistent with the working hypothesis.

MATERIALS AND METHODS

Radioactive precursors were purchased from New England Nuclear Corp., Boston, Mass.; DEAE-cellulose from Schleicher & Schuell, Keene, N. H.; concentrated liquid scintillator from Packard Instruments, Downers Grove, Ill.; NCS reagent from Nuclear-Chicago Corporation, DesPlaines, Ill.

Rats

Male albino rats, weighing 225-250 g, were purchased from Blue Spruce Farms, Inc., Altamont, N. Y. The animals were maintained on Purina chow ad libitum. The animals were hepatectomized by removing two-thirds of the liver, the left lateral and median lobes; all rats were sacrificed between 9:30 and 10:30 a.m. Sham-operated animals served as controls.

Isotope Injection

Either 6- orotic acid-14C or both 6- orotic acid-14C and tritiated-methyl-L-methionine were injected intraperitoneally. The isotope was injected as a 20-, 30-, 40- or 60-min pulse just prior to sacrifice, depending on the experiment.

RNA Extraction and Purification

tRNA was extracted and purified as described by Brunngraber (15): livers were minced, weighed, and added to 1.5 volumes (w/v) of a 0.05 mM Tris, 1 mM EDTA, 3 mM β-mercaptoethanol buffer, pH 7.5. Livers were blended in a Sorvall Omni-Mixer (Ivan Sorvall, Inc., Norwalk, Conn.) for 1 min, and an equal volume of redistilled, 80% phenol was added. The mixture was shaken for 30 min at 4°C and centrifuged for separating the phases. The recovered aqueous phase was precipitated with 2 volumes of absolute ethanol after the addition of 3/4 volume of 20% potassium acetate, 0.01 M EDTA, pH 6.5, and left overnight at -10°C. The phenol extraction and alcohol precipitation steps were repeated. The crude RNA preparation was added to a DEAE-cellulose column; tRNA was eluted from the resin with a 0.1 M Tris, 1.0 M NaCl, pH 7.6 buffer, after washing overnight with 1 liter of 0.1 M Tris-HCl, pH 7.6. The tRNA eluate was extracted again with phenol and precipitated with ethanol as described above. The tRNA precipitate was washed with 80% ethanol and ether, and redissolved in 3 ml of glass-distilled water.

Measurement of Specific Activity of tRNA

Absorbancy at 260 mµ was measured for determining tRNA concentration. The $A_{260}/A_{280}$ ratio of the tRNA's was 1.96 ± 0.03. An aliquot of each tRNA extract was taken for measurement of radioactivity. E. coli DNA was added as a "carrier" to the tRNA, and the mixture was made 5% in TCA and allowed to stand at 0°C for 15 min. The precipitate was filtered on Millipore filters (Millipore Filter Corp.), washed three times with ice-cold 5% TCA, three times with absolute ethanol, and dried. Filters were counted in 10 ml of a 24:1 mixture of toluene: concentrated liquid scintillator; a Packard Scintillation Counter (Packard Instruments) was adjusted to measure tritium and 14C radioactivity simultaneously. Efficiency was 8% for tritium and 54% for 14C.

Measurement of UMP and CMP "Pool Sizes"

The specific activity of UMP and CMP recovered from rat liver homogenates was determined, after obtaining a 5% PCA-soluble fraction, by the technique of Kammen and Harlbert (16). A Dowex-1 (J. T. Baker Chem. Co., Phillipsburg, N. J.)/(AG-1X8, 200-400 mesh) formic acid column was prepared, and nucleotides were separated by a discontinuous formic acid gradient. Absorbancy of eluates was measured; the eluates were then dried by vacuum desiccation in liquid-scintillation vials, and radioactivity was measured as described above.

Measurement of Biological Activity of Isolated tRNA's

An in vitro assay of aminoacyl synthetase activity was made of tRNA's isolated from regenerating rat liver. The method for obtaining crude synthetase preparations was essentially that described by Goldman, Johnston, and Griffin (17). Standard assays were made according to the procedure of Cantoni et al. (18). After incubation at 37°C for 30 min, counts were recovered by TCA precipitation, as described above; radioactivity was measured by liquid-scintillation counting. External standards were included for determining µµmoles uptake.
Electrophoresis Procedure

The technique described by Peacock and Dingman (19, 20) was followed for the electrophoresis of radioactive tRNA preparations, with only minor modifications. For measurement of specific staining of gels, not more than 100 µg of tRNA per slot was used in the eight-slot, 3-mm gels prepared in the E.C. Corp. polyacrylamide block electrophoresis apparatus. For measurement of radioactivity, 500-µg samples could be used.

10% gels were prepared by mixing stock solutions: (a) 80 ml of 20% acrylamide (19 g of acrylamide and 1 g of bisacrylamide in 100 ml of water); (b) 10 ml of 6.4% solution of dimethylaminoproprionitrile; (c) 16 ml of Tris-boric acid buffer, pH 8.5, containing 108 g of Tris, 9.3 g of disodium-EDTA, and 55 g of boric acid, in 1 liter; (d) water to 150 ml. The gel mixture was stirred under vacuum for 20 min, 10 ml of 1.6% ammonium persulfate (w/v) was added, and the solution was poured into the electrophoretic cell.

At 200 v (approximately 10 v/cm) there was a current of about 80 ma. The run of 3.5 hr was made with cooling at 6°C, and the buffer was recirculated. The gels were stained with methylene blue as described by Peacock and Dingman (19, 20).

After electrophoresis, appropriate parts of gels were either sliced or stained. Each 1-mm slice was incubated overnight in 0.2 ml of 1.0 N NaOH at room temperature in a scintillation vial. 1.0 ml of NCS reagent (Nuclear-Chicago Corp.) was added, and incubation was continued for 1 hr. Finally, 10 ml of toluene-liquiflor scintillation fluid was added, and vials were counted in a Packard Liquid Scintillation Spectrometer. The efficiency for 14C counting was 40% and recovery of counts 99%. The color yield of specific bands was measured by integrating the area under curves resulting from densitometric traces of gels (Densicord Recording Electrophoresis Densitometer, Photovolt Corp., N.Y.). A ratio of the color of each band to total color was calculated. The amount of RNA per sample was corrected by using this ratio, resulting in an estimate of the amount of RNA per band. Specific activities were then calculated and expressed as cpm per mg of tRNA.

Measurement of the "Pool Size" of S-Adenosylmethionine

The specific activity of SAM recovered from rat liver homogenates was determined, after obtaining a 1.5 N PCA-soluble fraction, by the technique of Shapiro and Ehninger (28). Rats were injected with 400 µCi of tritiated-methyl-L-methionine, specific activity 5.20 Ci/mmol, 1 hr prior to sacrificing the rats. AG50-X8 Na+ columns were prepared, and preparations from regenerating and normal rat livers were chromatographed. The concentration of SAM was measured by the absorbancy of column eluates and radioactivity by liquid-scintillation counting. To 1 ml of each column eluate, 1 ml of NCS reagent (Nuclear-Chicago) was added. After mixing, 10 ml of toluene scintillation fluid was added; duplicate samples, external standards were added to allow the computation of specific activities in µmoles uptake.

RESULTS

Increased tRNA in Regeneration

The amount of extractable tRNA increased 18-24 hr post-hepatectomy (Table I) and is positively correlated with the increase in total nuclear RNA (1). Table I lists the increase in weight of the liver that accompanies the increase in amount of tRNA. These data indicate, in agreement with extensive studies on the biochemistry of regeneration, that synthesis predominates over degradation in regeneration events that prelude DNA synthesis, which occurs 24-30 hr post-hepatectomy (1, 21) in young adult rats (225 g).

Criteria of Purity of tRNA

In radioisotopic studies, it is essential to rule out contamination as a source of artifact. The isolation procedures which were used maximized the yield of tRNA over contaminants. Results of electrophoresis of tRNA preparations ruled out the

| Table I |
|---|
| Increase in the Weight of Rat Liver during Regeneration and Amount of Extractable tRNA |
| Time | Amt. liver per rat | Amt. tRNA per rat | Increase in tRNA per rat |
|---|---|---|---|
| Sham operation | 9.7 (3.2)* | 3.76 (1.18)* | — |
| 18 hr regeneration | 3.3 | 1.28 | 2.1 |
| 24 hr regeneration | 4.9 | 1.57 | 12.5 |

* Corrected for 2/3 hepatectomy.

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presence of a high specific activity contaminant (Fig. 1). There is negligible radioactivity in slowly moving bands. The specific activities of "4S" and "5S" RNA's are identical (Table II), and are, consequently, tightly coupled in appearance in the cell cycle. "5S" RNA contains no methyl group, and could not be a source of artifact in the methylation work. Further, the tRNA's are biologically active in a standard assay for aminoacyl acceptor activity (Table IV). So as to eliminate the possibility of artifacts due to the possible contamination of tRNA preparations with proteins, the following experiment was done. 5 mg of tRNA (isolated from normal and regenerating liver) was hydrolyzed at 110°C for 22 hr in 6 N HCl. Tubes were evacuated and sealed. Hydrolysates were dried, dissolved in a citrate buffer, pH 2.2, and chromatographed on a Beckman-Spinco Automatic Amino Acid Analyzer; the analyzer had been modified for increased sensitivity; 0.005 µmoles of methionine can be measured accurately. There was no detectable methionine, methionine sulfoxide, nor methionine sulfone in the hydrolysates. 5 mg is 50-fold in excess of the amount of tRNA in aliquots taken for precipitation on Millipore filters for counting.

**Figure 1** Densitometric scan of polyacrylamide gel electrophoresis of tRNA's isolated from regenerating rat liver and radioactivity recovered from gel slices. 100-200 µg of tRNA was applied to two of eight slots of the E. C. Corp. (Philadelphia, Pa.) polyacrylamide slab electrophoretic apparatus to measure methylene blue staining. 500-700 µg of tRNA per slot was applied to two slots to measure radioactivity. A 10% gel was used, and the run was 3.5 hr at 200 v and 80 ma. The dotted line is a densitometric trace of a scan on the Densicord Gel Scanner (Photovolt, N. Y.). The radioactivity trace represents counts per minute (originally derived from an in vivo pulse of orotic acid-14C) recovered from each 1-mm gel slice.

**Table II**

| Regeneration time | RNA species | Specific activity | Methylene blue color |
|-------------------|-------------|-------------------|---------------------|
| hr                |             | cpm/mg X 10^-4    | % of Total          |
| 18                | "4S"       | 20.8              | 60.1                |
| 18                | "5S"       | 18.8              | 16.0                |
| 18                | Other*     | Trace             | 24.0                |
| 24                | "4S"       | 21.4              | 63.4                |
| 24                | "5S"       | 19.4              | 16.7                |
| 24                | Other*     | Trace             | 19.9                |

* Slowly migrating, methylene blue staining bands.

**Time Course of tRNA Synthesis and Methylation in Regeneration**

An experiment was designed for comparing the synthesis and methylation of tRNA in regeneration. Rats were doubly labeled with ³H-methylmethionine and 6-orotic acid-¹⁴C 30 min prior to sacrifice at appropriate regeneration periods. Fig. 2 A plots the relative specific activities of tRNA isolated at different times in the cell cycle. The peaks of both ¹⁴C and ³H radioactivity reach a
The uptake of 6 orotic acid-14C and 3H-methyl-L-methionine into tRNA isolated from regenerating rat liver. The in vivo pulse was 30 min; three rats per time point were injected. The 14C and 3H radioactivity was measured after precipitation of the tRNA on Millipore filters. Relative specific activity was calculated by dividing the specific activity of each preparation by the specific activity of nonregenerating tRNA. A relative specific activity of 4 represents a 400% increase over the specific activity of nonregenerating tRNA.

maximum before DNA synthesis reaches a maximum. Interestingly, the maxima of methylation and synthesis differ in time, with methylation increasing before synthesis.

The measurement of methyl uptake represents total methyl uptake; no base analyses to determine which bases were methylated were made. Rodeh, Feldman, and Litauer (22) showed that the methylases work in concert in regeneration; the ratio of N7-methylguanine to ribothymidine remains constant in regeneration. Consequently, measurement of total methyl uptake represents parallel increases in several methylase activities.

The relationships between the two activities, synthesis and methylation, can best be viewed by a plot of the ratio of the two activities (Fig. 2 B). A rise in the curve indicates when methylation predominates. It can be seen that methylation increases relative to synthesis at 18 hr post-hepatectomy. At this time, there is an increase of only 2% in the amount of extractable tRNA. Also in the figure is the indication that there is a balance between the two activities which is restored at the end of the cell cycle (32 hr). An upset in this balance, that is, a rise of the curve, is positively correlated with an increase (21, 1) in protein synthesis regeneration, and a decline with a decrease in protein synthesis.

**Rate of Methylation and Synthesis in Regeneration**

A simple uptake experiment alone does not give conclusive evidence of the rates of methylation and synthesis, owing to the possibility of "pool size" variation and "turnover" artifacts, in an in vivo study. Consequently, an experiment was designed which would answer the question, "What are the relative rates of methylation and synthesis of tRNA during regeneration?" and eliminate those variables as sources of artifact. Rats were pulse labeled for 20, 40, or 60 min prior to sacrifice with both 6-orotic acid-14C and tritiated-methyl-L-methionine. tRNA was isolated and purified, radioactivity was measured, and specific activities were calculated as above. Fig. 3 shows the results; a least squares line has been drawn through the experimental points. This line is proportional to the in vivo rate of methylation and synthesis of tRNA.

"Pool size" variation of orotic acid was estimated by measuring the specific activity of UMP. From each homogenate, an aliquot was taken to determine the specific activity of both UMP and CMP. Table III lists the specific activities of UMP and CMP isolated from a cold 5% TCA-soluble fraction of each homogenate. Each point on the graph in Fig. 3 was corrected for slight variation of the radioactivity of UMP during regeneration; the curves of Fig. 3 are, then, independent of "pool size" variations of precursors of tRNA synthesis. The specific activities of CMP are very low; orotic acid is converted mainly to UMP in rat liver. The very low specific activity of CMP makes it unlikely that "CCA turnover" could contribute significantly to total tRNA radioactivity.

The curves in Fig. 3 continue to rise over a period of an hour, and so "turnover" of tRNA and methyl radioactivity can be ruled out. Further, the experimental points lie close to
theoretical lines, demonstrating reproducibility of experimental results.

Is the "pool size" variation of S-adenosyl-methionine responsible for the experimental result rather than a variation of methylase activity? As can be seen from Table V, the specific activity of SAM does not change during the course of regeneration of the liver. The three specific activity values obtained from sham-operated and hepatectomized animals lie within the standard error of

**Figure 2 B** The ratio of uptake of $^3$H-methyl-L-methionine to 6-ortotic acid-$^{14}$C into tRNA of regenerating rat liver. A ratio of $^3$H/$^{14}$C specific activities was calculated for each time point from Fig. 2 A to yield a curve representing the relative uptake of methyl to orotic acid during regeneration.

**Figure 3** The rate of uptake of $^3$H-methyl-methionine (left) and 6-ortotic acid-$^{14}$C (right) into tRNA isolated from regenerating rat liver. Rats were doubly labeled with a precursor for both methylation and synthesis. Each point represents the specific activity of the tRNA isolated from the livers of three rats. Each point is corrected for "pool size" variation. Least squares lines and their slopes are plotted, representing the relative rates of synthesis and methylation of tRNA during regeneration.

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the mean of those values. Control of methylation is, consequently, at the enzyme level rather than at some enzymatic step prior to methylation.

These results can better be viewed by a plot of the slopes of the theoretical lines (Fig. 4). It is clear that the rate of methylation reaches a maximum at 18 hr and the rate of synthesis a maximum at 24 hr posthepatectomy. This parallels the finding in the time course experiment (Fig. 2 A). A plot is made of the ratio of synthesis to methylation, indicating when synthesis predominates (the reciprocal of the ratio plotted in Fig. 2 B). Clearly, the maxima of the rates of the enzymatic reactions differ in vivo; the functional implications of this are argued in the Discussion section of this paper.

**DISCUSSION**

One of the most intriguing problems in biology is the pattern of biochemical events which is responsible for initiating and terminating cell division. It seems likely that no single event triggers this process. It may be that the interphase cell can be regarded as a tenuous molecular steady state which, when upset, tends to undergo cell division. The pattern of molecular events preceding division should give insight into what upsets the steady state. Once cell division occurs, what returns the cell to a steady state? Perhaps the quantum jump is the dissolution of the chromosomes and reformation of the nuclear membrane.

One particularly interesting biochemical pattern in the cell cycle is the alteration of proteins and nucleic acids after they are made. Transfer-RNA, which is directly involved in protein synthesis, is modified in a number of ways after it is

| TABLE III |
| Specific Activity of Uridine-2',3'-Monophosphate Isolated from Regenerating Rat Liver Homogenates |
| --- |
| Regeneration time | Pulse time | Specific activity of CMP μmole | Specific activity of UMP μmole |
| --- | --- | --- | --- |
| hr | min | | |
| Sham operation | 20 | 3 | 18,070 |
| Sham operation | 40 | 8 | 25,450 |
| Sham operation | 60 | 14 | 29,600 |
| 12 | 20 | 7 | 24,460 |
| 12 | 40 | 29 | 62,120 |
| 12 | 60 | 35 | 60,090 |
| 18 | 20 | 80 | 71,640 |
| 18 | 40 | 60 | 62,800 |
| 18 | 60 | 51 | 66,180 |
| 24 | 20 | 61 | 73,690 |
| 24 | 40 | 59 | 40,790 |
| 24 | 60 | 160 | 73,690 |

| TABLE IV |
| Biological Activity of tRNA's Isolated from Regenerating Liver* |
| --- | --- | --- |
| Amino acid | Specific activity μmole/mg tRNA |
| --- | --- | --- |
| L-glutamic acid-14C | 469 |
| L-alanine-14C | 637 |
| 12 | L-glutamic acid-14C | 1240 |
| L-alanine-14C | 2243 |
| 18 | L-glutamic acid-14C | 1190 |
| L-alanine-14C | 1301 |
| 24 | L-glutamic acid-14C | 508 |
| L-alanine-14C | 521 |

* Aminocacyl-tRNA synthetase activity. Each assay contained in 0.25 ml the following: 1.56 μmoles of magnesium chloride, 5 μmoles of potassium phosphate buffer, pH 7.5, 1.25 μmoles of adenosine triphosphate (dipotassium salt), 400–600 μg of tRNA, synthetase (1/40 the synthetase extracted from one liver), amino acid-14C (an amount equal to 20 times the number of counts incorporated per assay after 30 min at 37°C). ‡ μmoles uptake per mg tRNA per unit of synthetase. One unit of synthetase is the amount of activity (at 37°C for 30 min) extractable from 1 g of liver.
TABLE V
Specific Activity of S-Adenosylmethionine Isolated from Regenerating Rat Liver Homogenates

| Regeneration time | Amount of methionine methyl incorporated* per A256 unit of S-adenosylmethionine (µµmoles/hr) |
|-------------------|-------------------------------------------------|
| Sham operation    | 1.34                                            |
| 10                | 1.09                                            |
| 24                | 1.15                                            |

* The mean and standard error of these values are 1.19 ± 0.24.

Figure 4. The rate of methylation and synthesis of tRNA during rat liver regeneration. The slopes of the lines from Fig. 3 are plotted. Also, the ratio of synthesis to methylation was calculated and graphed. This ratio was calculated inversely to the ratio graphed in Fig. 2 B; a drop in the curve here represents an increase of methylation over synthesis.

made. Borek (23, 24) and Hurwitz (25), among others, have elucidated the details of the process of tRNA methylation. Methyl groups are introduced into many sites in the molecule; there are at least eight methylases involved. The enzymes are species-specific and to some extent tissuespecific. Methylases from one species tend to over-methylate tRNA from heterologous but not homologous sources. Thus, the enzymatic process is quite elaborate. One particularly interesting finding is that the enzymes do not work very well on tRNA from the same homogenate. Transfer-RNA from "normal" tissues seems to have a full complement of methyl groups. Srinivasan and Borek (2) believe that altered methylase activity can be viewed as a naturally occurring carcinogen. The methylase enzymes from neoplastic tissues produce an altered pattern of methylation; in particular, N7-methylguanine is elevated (Magee and Farber, 26). In regenerating rat liver, partially synchronized in cell division, there is a threefold increase in methylation of tRNA, but no change in methylation pattern (22), the ratio of N7-methylguanine to ribothymidine remained the same during regeneration. This stands in contrast to the tumor systems, where the pattern changes. Littauer (22) concluded that increased methylation in regenerating rat liver was a consequence of increased synthesis of tRNA, although he did not measure synthesis.

Is there a time in the cell cycle when methylation predominates over synthesis? Or is increased methylation a consequence of increased synthesis of tRNA in the cell cycle? Can one find a time in the cell cycle when homologous enzymes will work on their own tRNA? From Fig. 4, it is clear that the peak of methylation of tRNA occurs before the peak of synthesis of tRNA; the two activities are only weakly coupled, and the methylation of tRNA is not necessarily a direct consequence of the synthesis of tRNA. This finding reinforces the possibility that the balance between methylation and synthesis of tRNA serves in some control capacity in the cell.

Capra and Peterkofsky (13, 14) and Gefter and Russell (11) have shown that tRNA function depends on its state of methylation in certain enzymatic reactions involved in protein synthesis. The ratio of methylation to synthesis of transfer-RNA rises (Fig. 2 B) at a time when protein synthesis rises (1, 21) in the cell cycle and falls when protein synthesis falls in the cell cycle; consequently, the methyl content of the tRNA pool may possibly be a regulatory mechanism for the rate of protein synthesis in the cell cycle. It is known that all components for protein synthesis are available at the time in the cell cycle when methylation occurs. The protein synthetic mechanism is present and poised.

Is the level of control of methylation of tRNA
at the methylase level, or at some enzymatic step lated in vivo by the availability of S-adenosyl-methionine? The answer to this question is no, for the specific activity of SAM isolated from rats previously injected with tritiated-methyl-L-methionine does not change during the course of regeneration. Therefore, the methylation is controlled at the enzyme level. This may implicate inhibitors or activators as methylase-activity regulators, although increased enzyme synthesis cannot be ruled out.

The specific ways in which the methyl content of the tRNA pool might exert control on the rate of protein synthesis are many since tRNA is involved in many steps in protein synthesis. One possibility is the amino-acylation step, and another, the way in which the tRNA is attached to the ribosome. Thus, tRNA modification and synthesis, loosely coupled reactions, could serve to prior to methylation? Is methylase activity regulated a strongly coupled enzymatic system, protein synthesis.

Note Added in Proof: The temporal sequence of uptake of radioactive precursors of methylation and synthesis into tRNA was repeated in another synchronized biological system. Chinese hamster fibroblasts were synchronized by Colcemid treatment as described by Stubblefield, E., and R. Kleece, Exp. Cell Res. 40:660 (1965). Cells were pulse labeled with tritiated-methyl-L-methionine and 2-uridine-14C for 30 min; tRNA was isolated and purified, with the techniques described under Materials and Methods, but on a microscale, and specific activities were calculated after TCA precipitation and Millipore filtration. Essentially the same result was obtained in the temporal uncoupling of methylation and synthesis of tRNA in the cell cycle, as was obtained in the rat liver system.

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