Alteration in tRNA Methyltransferase Activity in Mengovirus Infection: Host Range Specificity

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The tRNA methyltransferase activity in mengovirus-infected L cells, HeLa cells, and Maden Derby bovine kidney cells has been examined during the course of infection. The first two cell lines yield a productive infection, but have different kinetics of inhibition of host RNA synthesis, whereas the bovine kidney cells are a restrictive host. In infected L cells the enzymes show altered capacity and base specificity throughout the infection. In infected HeLa cells and in infected bovine kidney cells less marked changes were seen. No inhibitors or activators of the enzymes were detected in any of the infected cell lines. Labeling experiments in infected cells indicated that in infected L cells synthesis of RNA was inhibited to a greater degree than was methylation of RNA. The consequence of this would be a hypermethylation of RNA. The methylated derivatives synthesized in infected L cells showed changes in relative proportions. Infection of HeLa cells and bovine kidney cells did not show such marked effects on methylation of RNA.

Infection of mammalian cells with picornaviruses leads to a general impairment of host protein and RNA synthesis (10). The degree and kinetics of inhibition of host macromolecular syntheses can vary widely for a given virus over a range of host cell types (6, 16). Also, the yield of virus is greatly reduced in a restrictive host, even though host RNA and protein synthesis is shut off and cell death occurs (6). The mechanism of host restriction of viral replication is not well understood. In the case of restrictive infection of mengovirus in cells Maden Derby bovine kidney (MDBK), it appears that single-stranded viral RNA is degraded in the restrictive host 3 to 4 h after infection (22). It has also been suggested that the degree of differentiation of a host cell line affects its ability to replicate viral genes or translate viral message (6).

Poliovirus and foot-and-mouth disease virus cause an inhibition of host methylation of RNA as well as host RNA synthesis after infection. Grado et al. (11) have shown that after infection of HEP-2 cells with poliovirus, synthesis and methylation of ribosomal RNA are almost completely shut off, and the rate of tRNA methylation is decreased by about 60%. They also noted the appearance of a novel methylated derivative which was not present in the tRNA of control cells.

Ascione and Vande Woude (1) noted that in

baby hamster kidney cells infected with foot-and-mouth disease virus, and while total host RNA synthesis is not drastically inhibited, methylation of RNA drops sharply. They traced this partly to an inability of the infected cells to methylate 45S ribosomal precursor RNA. They also observed an inhibition of tRNA methylation.

The mechanism of inhibition of host tRNA methylation is not clear. The methyltransferase enzymes in infected cells have not, as yet, been examined to determine whether they are altered, inhibited, or destroyed.

It seems of interest, therefore, to study the effect of a picornavirus on the methylating enzymes of a spectrum of host cells which exhibit different responses to the virus. We report here the effect of mengovirus infection on the tRNA methyltransferase activity in L cells in which host RNA synthesis is almost immediately inhibited, in HeLa cells in which host RNA synthesis continues until late in infection, and in MDBK cells in which host RNA synthesis ceases and which results in an abortive infection with mengovirus (6, 16).

MATERIALS AND METHODS

Materials. Mouse L cells (L929) and mengovirus stocks were kindly supplied by W. K. Roberts (Department of Microbiology, University of Colorado

Medical Center). HeLa cells and MDBK cells were the gift of M. W. Taylor (Department of Microbiology, Indiana University). Eagle minimum essential medium, fetal calf serum, and calf serum were obtained from Grand Island Biological Co. (Berkeley, Calif.). Lyophilized trypsin came from Worthington Biochemical Corp. (Freehold, N. J.).

S-adenosyl-methyl- ^{14}C methionine (specific activity 54 mCi/mmol) was purchased from International Chemical and Nuclear Corp. L-methionine-methyl- ^{14}C (specific activity 54 mCi/mmol) was supplied by Amersham/Searle. L-methionine-methyl- ^3H (specific activity 2 Ci/mmol) and ^{32}P -phosphate, carrier free, were obtained from Schwarz/Mann.

Actinomycin D was purchased from Merck, Sharp and Dohme (Rahway, N. J.) and cycloheximide and puromycin were purchased from Sigma Chemical Co. Pancreatic ribonuclease A was obtained from Calbiochem. Methylated purine derivatives for chromatography came from Cyclo Chemical Corp. "Methyl-deficient *Escherichia coli*, Borek strain, tRNA" was bought from General Biochemicals. Precoated cellulose thin-layer chromatography plates were purchased from Eastman Kodak, as was no-screen X-ray film.

Cell culture and mengovirus infection. L cells and MDBK cells were grown in Eagle medium supplemented with 10% fetal calf serum, and HeLa cells were grown in Eagle medium with 10% calf serum. Cells were grown as monolayers and maintained in the "log" phase of growth by subculturing 1 to 2 times weekly by using a 0.025% trypsin solution.

For mengovirus infection, confluent monolayers were treated at 20 C for 30 min with 100 PFU/cell in a minimum volume of medium, usually 2.0 ml/60 cm². Fresh medium was then added, and incubation continued at 37 C until the cells were harvested. L cell-grown mengovirus stocks were passaged twice on HeLa cells before being used to infect HeLa cells in experiments. MDBK cells were infected using virus grown in L cells.

Labeling of RNA. When cellular RNA was to be labeled with methyl groups, the cells were preincubated for 6 h in methionine-deficient medium. The cells were then infected with virus and methyl-labeled methionine was added for the desired period along with medium supplemented with 10^{-4} M adenosine, guanosine, and thymidine to suppress purine ring labeling. Uninfected control cells were treated similarly.

Treatment of cells in double-label experiments was the same, except that the preincubation medium was both methionine- and phosphate-deficient.

Extraction of RNA. Labeled monolayers of control and infected cells were washed with 0.9% NaCl and harvested by scraping. The cells were allowed to swell in hypotonic buffer (0.01 M Tris, 0.01 M NaCl, and 0.0015 M MgCl₂, pH 7.4) and homogenized. The nuclei were deposited by centrifugation at $800 \times g$, and the supernatant fluid was used for extraction of RNA.

For total RNA determination, the supernatant was treated with an equal volume of cold 10% trichloroacetic acid. The resulting precipitate was washed extensively with 5% trichloroacetic acid, followed by chloro-

form-methanol (3:1), ethanol-ether (2:1), and ether. The RNA was then solubilized by heating the precipitate to 90 C for 15 min in 5% trichloroacetic acid. The soluble fraction was counted in a scintillation fluid containing per liter: Triton X-100, 350 ml; toluene, 650 ml, and 10 g of 2,5-diphenyloxazole.

When it was desired to separate ribosomal RNA from tRNA, the cell supernatant fluid was extracted with an equal volume of water-saturated phenol containing 0.1% 8-quinolinol, and the RNA was precipitated with 2 volumes of 95% ethanol and 0.1 volume of 20% potassium acetate, pH 5. tRNA was separated from ribosomal RNA by solubilization in 1 M NaCl and reprecipitated with ethanol. The tRNA was stripped of amino acids by incubation in 1.8 M Tris-hydrochloride, pH 9, at 37 C for 90 min.

The tRNA isolated in this manner from L-methionine-methyl- ^{14}C -labeled cells was used for analysis of methylated bases and oligonucleotide maps.

Double-stranded RNA from mengovirus-infected L cells was isolated and purified according to the procedure of Cordell-Stewart and Taylor (8).

Preparation of enzyme extracts. Monolayers of uninfected and mengovirus-infected cells were washed with 0.9% NaCl solution and then with hypotonic buffer. The cells were scraped from the bottle surface and homogenized in hypotonic buffer. The homogenate was centrifuged at $105,000 \times g$ for 60 min. The high-speed supernatant fraction was used for protein estimation (15), assay of ribonuclease activity, and as a source of tRNA methyltransferase enzymes.

Enzyme assays. tRNA methyltransferase activity was assayed as previously described (14), except that the reaction volume was reduced to 0.1 ml, 50 μg of methyl-deficient *E. coli* tRNA was used as the substrate and, in most cases, assays were done in the presence of 2 mM spermidine. Incubation was at 37 C for 60 min. A unit of tRNA methyltransferase activity is defined as the amount of enzyme that incorporated 1 pmol of ^{14}C -labeled methyl group into tRNA in 60 min. When total capacity of the enzymes was to be determined, the tRNA was limited to 5 μg , and the reaction was allowed to proceed for 120 minutes. This measures the absolute number of methyl groups that the enzymes from a given source can introduce into a limiting amount of a heterologous substrate.

Ribonuclease activity was determined according to the method of Nestle and Roberts (17), except that the reaction volume was reduced to 0.3 ml, and methyl-deficient *E. coli* tRNA was used as the substrate. A unit of ribonuclease activity is defined as that amount of enzyme which causes an increase of 1.0 in absorbance at 260 nm of 2% perchloric acid-soluble material in 60 min.

Analysis of methylated bases. For base analysis ^{14}C -methylated-labeled tRNA was isolated from the enzyme reaction mixtures after phenol extraction by ethanol precipitation. The tRNA was hydrolyzed in 1 N HCl at 100 C for 30 min and dried in vacuo. This procedure yields the free purine bases and pyrimidine nucleotides. The samples were spotted on thin-layer cellulose plates, and the derivatives were separated by two-dimensional chromatography according to Bjork

and Svensson (4). The methylated bases were located by autoradiography and identified by co-chromatography with authentic marker compounds.

Oligonucleotide maps. ^{14}C -methyl-labeled tRNA (5 to 10 U, A_{260}) were digested with 20 μg of ribonuclease A in 0.2 M ammonium acetate, pH 7.6. The digests were fractionated on thin-layer cellulose plates by two-dimensional electrophoresis and chromatography according to Bergquist (3). Radioactive oligonucleotides were located by autoradiography.

RESULTS

Methylation of RNA. Infection of L cells or HeLa cells with mengovirus led to an inhibition of methylation of cytoplasmic RNA. However, the kinetics were somewhat different in the two cell lines. In infected L cells, where host RNA synthesis is shut off almost immediately (16), inhibition of methylation of RNA did not begin until between 2 and 4 h after infection and reached about 50% by 6 h postinfection. In infected HeLa cells, where host RNA synthesis continues at the normal rate for almost 3 h (16), methylation of RNA continued normally until 4 h after infection, but then decreased sharply. These data are presented in Table 1. This effect is somewhat different from that observed in the case of foot-and-mouth disease virus infection in which methylation of RNA was inhibited by 50% within 60 min (1).

The observation of lack of inhibition of methylation of RNA early after mengovirus infection, particularly in L cells, suggested a differential effect of virus infection on the rate of RNA synthesis and RNA methylation. This was investigated by a double-labeling experiment using ^{32}P -phosphate to detect RNA synthesis and methionine-methyl- ^3H to detect methylation of RNA. Cells were pulsed for 60 min at hourly intervals after infection for up to 6 h. An uninfected culture was also pulsed for 1 h at the start of the experiment as a control. The results are expressed in Table 2 in terms of the ratio of

TABLE 2. Ratio of ^3H -methyl and ^{32}P incorporation into RNA in mengovirus-infected L cells and HeLa cells

Hours ^a postinfection	^3H -methyl- ^{32}P incorporation			
	L cells		HeLa cells	
	tRNA	rRNA	tRNA	rRNA
0	10 $\pm$ 0.1	1.7 $\pm$ 0.1	20 $\pm$ 5	4 $\pm$ 0.2
2	14 $\pm$ 0.3	4.0 $\pm$ 0.5	20 $\pm$ 2	3 $\pm$ 0.5
3	30 $\pm$ 5	4.0 $\pm$ 0.2	19 $\pm$ 2	5 $\pm$ 0.6
4	30 $\pm$ 6	4.5 $\pm$ 0.5	14 $\pm$ 0.6	5 $\pm$ 1.0
5	19 $\pm$ 2	3.8 $\pm$ 0.6	7 $\pm$ 0.8	3 $\pm$ 0.1
6	10 $\pm$ 1	2.8 $\pm$ 0.2	3 $\pm$ 0.4	2 $\pm$ 0.1

^a Time of isolation postinfection. Cells were labeled 60 min before harvest with 10 μCi of ^{32}P and 20 μCi of methionine-methyl- ^3H . RNA was phenol extracted, ethanol precipitated, and separated into tRNA and ribosomal RNA by using 1.0 M NaCl. Data are the means of two separate experiments.

^3H -methyl incorporation to ^{32}P incorporation. Transfer RNA and ribosomal RNA were separated by using 1.0 M NaCl and analyzed individually. It can be seen that in L cells the relative rate of methylation of tRNA increases threefold over the control value between 2 and 4 h postinfection and then returns to the control value by 6 h postinfection. The rate of methylation of ribosomal RNA follows a similar pattern. In mengovirus-infected HeLa cells, however, no such changes were observed until late in infection, when it would appear that the rate of methylation is inhibited to a somewhat greater extent than the rate of RNA synthesis. In the case of the ribosomal RNA this probably reflects the increasing ^{32}P incorporation as viral-specific RNA synthesis begins. Viral RNA is not methylated and would be included in the ribosomal RNA fraction.

tRNA methyltransferase activity. The specific activities and capacities of the tRNA methyltransferases were examined in L cells and HeLa cells after mengovirus infection to see if they showed altered activity or specificity. The enzyme activities in a restrictively infected host, MDBK cells, were also determined (Table 3). In L cells no significant differences in specific activity of the enzymes were observed up to 4 h after infection, although there was some variation in the capacity throughout the infection cycle. MDBK cells showed no differences in specific activity or capacity of the enzymes up to 4 h after infection. HeLa cells showed a drop in specific activity 3 h after infection, which is at approximately the same time that inhibition of methylation of RNA in

TABLE 1. Methylation of RNA in mengovirus-infected L cells and HeLa cells^a

Hours postinfection	Percent of control	
	L cells	HeLa cells
2	102	109
4	65	97
6	46	19

^a Uninfected and infected cultures were labeled continuously with 10 μCi of methionine-methyl- ^3H per ml. Total RNA was extracted at the times indicated and counts per minute incorporated per 10^6 cells were determined.

TABLE 3. *Specific activities and capacities of tRNA methyltransferases in mengovirus-infected cells^a*

Cell line and hour postinfection	Specific activity (U/mg of protein)	Capacity (pmoles of ¹⁴ C-methyl/5 µg of tRNA/120')
L-cells ^b		
1 ^c	6.0 ± 0.5	84.0 ± 5.0
2	6.5 ± 0.4	100.0 ± 6.0
3	5.0 ± 0.6	73.0 ± 4.0
4	5.4 ± 0.6	73.0 ± 2.0
	6.3 ± 0.2	102.0 ± 3.0
HeLa cells ^b		
1	13.5 ± 1.5	95.0 ± 1.0
2	11.6 ± 1.0	93.0 ± 2.0
3	15.3 ± 2.0	92.0 ± 2.5
4	10.0 ± 0.5	94.0 ± 2.0
6	8.6 ± 1.0	93.0 ± 3.0
	8.4 ± 1.0	92.0 ± 2.0
MDBK cells ^d		
1	1.86 ± 0.5	12.0 ± 0.6
2	N.D. ^e	11.0 ± 0.5
3	2.14 ± 0.7	10.6 ± 0.6
4	N.D.	10.8 ± 0.4
	1.88 ± 0.6	10.0 ± 0.8

^a Data are the results of three separate experiments. All figures have been corrected for ribonuclease degradation of substrate.

^b Assayed in the presence of 2 mM spermidine.

^c Time postinfection with mengovirus.

^d Assayed in the presence of 5 mM MgCl₂.

^e Not determined.

infected cells begins (Table 1). Capacity of the enzymes was not affected.

Ribonuclease activity. All extracts were examined for changes in ribonuclease activity before and after infection because these could affect the results of the tRNA methyltransferase assays. Both L cells and HeLa cells had low, but detectable, ribonuclease activity which did not change during the course of infection. However, the MDBK cells had appreciably greater ribonuclease activity than the other cell lines, and this activity rose almost threefold after mengovirus infection as shown in Table 4.

Base specificity. The *E. coli* tRNA from the reaction mixture was examined to determine the bases being methylated. Table 5 shows the results of three separate experiments with L cell extracts. The variations seen in the enzyme base specificities during the course of infection were quite reproducible within ±5%. What causes the activity of a particular base-specific enzyme, for example, 5-methylcytosine specific methyltransferase activity to go down at 1 h postinfection, to rise again at 2 h after infection, and then to fall again at 4 h postinfection,

is not known. However, hourly points during the infection were examined because such cyclic variations in enzyme activity have been observed in bacteriophage induction (21) as well as in inhibition of tRNA methylation in foot-and-mouth disease virus infection (1).

As shown in Table 6, the base specificities of the HeLa cell enzymes change very little by 4 h after infection. By 6 h after infection, a time when the cytopathic effect of the virus is quite drastic, marked changes are seen.

TABLE 4. *Ribonuclease activity in uninfected and infected MDBK cells*

Sample	Specific activity (U/mg of protein)
Uninfected	0.95
1 h postinfection ^a	2.7
3 h postinfection	3.75

^a Time postinfection with mengovirus.

TABLE 5. *Base specificity of tRNA methyltransferases from uninfected and infected L cells^a*

Methylated derivative	Uninfected	1 h ^b	2 h ^b	3 h ^b	4 h ^b
1-Methyladenine	15.0	12.0	13.0	13.7	14.0
5-Methylcytosine	16.0	9.2	16.0	14.7	7.0
1-Methylguanine	13.0	13.0	19.0	15.0	20.0
N ² -methylguanine	25.0	34.2	16.5	30.6	27.0
N ² , N ² -dimethylguanine	15.0	21.0	17.5	13.0	8.0
7-Methylguanine	16.0	10.6	18.0	13.0	24.0

^a Transfer RNA was isolated from enzyme assays for specific activity as described in Table 3. Results are expressed as the percentage of total recovered radioactivity associated with each base. Each figure is the mean of three separate experiments.

^b Time postinfection with mengovirus.

TABLE 6. *Base specificity of tRNA methyltransferases from uninfected and infected HeLa cells^a*

Methylated derivative	Uninfected	1 h	2 h	3 h	4 h	6 h
1-Methyladenine	7.2	12.5	11.6	19.0	13.2	9.4
5-Methylcytosine	25.4	22.1	28.6	23.6	29.5	41.2
1-Methylguanine	6.9	4.8	6.5	4.2	5.0	7.6
N ² -methylguanine	30.7	34.3	26.8	27.1	29.0	22.3
N ² , N ² -dimethylguanine	21.2	18.6	18.4	18.8	18.1	15.6
7-Methylguanine	8.3	7.4	7.7	7.0	5.0	3.6

^a Details are as in Table 5.

The enzymes from the MDBK cells show no significant differences in base specificities up to 4 h after mengovirus infection (Table 7).

tRNA methyltransferases cannot be assayed with homologous tRNA as the substrate because this tRNA has already been exposed to the enzymes in the cell. Thus, to examine the methylated base pattern of the host cell tRNA, labeling of the cells in culture with methionine-*methyl*- ^{14}C was carried out. The tRNA from infected and uninfected L cells and HeLa cells labeled in culture with methionine-*methyl*- ^{14}C was isolated, and the methylated bases were analyzed to determine whether the changes observed in the enzyme assays could be detected under the actual culture conditions. The results from L cell cultures are shown in Table 8, where it can be seen that after mengovirus infection the relative proportions of 5-methyluracil and N^2 -methylguanine underwent significant changes, whereas the relative proportions of the other methylated derivatives remained constant. The tRNA from HeLa cells labeled in culture did not show such large variations after infection (Table 9).

The disparity of the results from cultured cells and the in vitro enzyme assays was not unexpected. The substrates in the two systems are completely different. In the first instance, it is the homologous cellular RNA and in the second, it is a heterologous bacterial tRNA. Thus, the interaction of enzymes and substrates as well as the sites available for methylation in the two substrates are undoubtedly quite different.

Oligonucleotide analysis. Oligonucleotide maps were prepared from methyl-labeled *E. coli* tRNA isolated from enzyme assays as well as from tRNA isolated from uninfected and infected L cells and HeLa cells labeled in culture. This was done to explore the possibility of the appearance of enzymes with altered sequence

TABLE 7. Base specificity of tRNA methyltransferases from uninfected and infected MDBK cells^a

Methylated derivative	Uninfected	2 h	4 h
1-Methyladenine	19.0	19.5	16.7
5-Methylcytosine	32.4	30.8	34.1
1-Methylguanine	19.0	18.1	22.9
N^2 -methylguanine	10.0	11.2	10.0
N^2, N^2 -dimethylguanine	3.0	3.5	2.5
7-Methylguanine	16.0	16.6	13.5

^a Details are as in Table 5.

TABLE 8. Methylated derivatives in tRNA from uninfected and infected L cells^a

Methylated derivative	Uninfected	2 h ^b	4 h ^b
1-Methyladenine	4.9	6.0	5.5
5-Methylcytosine	35.1	28.9	32.7
1-Methylguanine	8.5	6.9	10.6
N^2 -methylguanine	9.7	15.7	17.1
N^2, N^2 -dimethylguanine	6.1	6.3	10.2
7-Methylguanine	6.9	9.0	8.7
5-Methyluracil	28.5	26.8	15.0

^a Cells were labeled for 2-h periods with 0.1 μCi of L-methionine-*methyl*- ^{14}C per ml. tRNA was extracted, hydrolyzed, and analyzed as described under Materials and Methods. Results are presented as the percentage of total recovered radioactivity associated with each base and are the means of three separate experiments.

^b Time postinfection with mengovirus.

specificity or the possibility that different sequences within the host tRNA might become available for methylation after virus infection. No gross, qualitative differences in oligonucleotide distribution were observed in tRNA either from enzyme assays or from labeled cultures for any of the cell lines. In the case of L cells and HeLa cells, it appeared there may have been some slight quantitative differences in the oligonucleotide pattern after mengovirus infection.

Absence of inhibitors. Cell extracts of infected and uninfected cultures were examined for the possible presence of either inhibitors or activators of the tRNA methyltransferases.

Enzyme extracts from infected and uninfected cultures were mixed, and in all cases enzyme activities were additive for each cell line.

Enzyme assays were carried out by using pH 5 precipitated fractions. In the case of mammalian liver and kidney, this procedure separates the tRNA methyltransferases from both small-molecular-weight inhibitors as well as from some competing enzyme systems (13, 14). However, in this instance enzyme activity was not increased by pH 5 precipitation in any of the cell lines before or after virus infection.

Double-stranded viral RNA has been implicated in mengovirus infectivity and pathogenicity (2, 8, 9). Neither double-stranded nor single-stranded mengovirus RNA isolated from infected L cells when added to the tRNA methyltransferase assay had any effect on the enzyme activity from uninfected L cells.

The possibility that changes in tRNA methyltransferase activity after viral infection were

TABLE 9. *Methylated derivatives in tRNA from uninfected and infected HeLa cells^a*

Methylated derivative	Uninfected	3 h ^b	6 h ^b
1-Methyladenine	7.6	8.0	8.0
5-Methylcytosine	30.8	33.0	33.0
1-Methylguanine	4.5	4.8	5.2
N ² -methylguanine	16.0	18.0	20.5
N ² , N ² -dimethylguanine	7.9	8.0	8.5
7-Methylguanine	8.8	11.0	9.5
Methyluracil ^c	22.3	16.0	17.0

^a Cells were labeled for 3-h periods with 0.1 μ Ci of L-methionine-methyl-¹⁴C per ml. Other details are as in Table 8.

^b Time postinfection with mengovirus.

^c Two methylated uracil derivatives were observed in tRNA from labeled HeLa cells, and their combined radioactivity was determined.

due to a nonspecific response to the shutting off of protein and RNA synthesis was investigated by simulation with synthetic inhibitors of protein and RNA synthesis. The enzymes from cell cultures treated separately with 1 μ g of actinomycin D or 1 μ g of cycloheximide or puromycin per ml when assayed in vitro showed no change in activity or specificity for up to 6 h of exposure to these agents.

DISCUSSION

Mengovirus, like some other picornaviruses (1, 11), can affect the methylation of host cell RNA. The type and magnitude of the effect is quite dependent on the host cell line. In infected L cells there is greater inhibition of RNA synthesis than of methylation of RNA, the result of which could be hypermethylation of various RNA species. In infected HeLa cells, however, inhibition of RNA synthesis and methylation are more or less parallel.

These effects on methylation of RNA could be a mechanism by which the virus subverts the host protein synthetic apparatus. For instance, inhibition of methylation of ribosomal precursor RNA blocks the formation of functional ribosomes (20). Hypomethylation of tRNA can also affect its function in protein synthesis (7, 19).

The effect of possible hypermethylation of RNA, which appears in the infected L cells, is less easy to interpret. Chemical hypermethylation of tRNA can inactivate it (12, 18). Whether the degree of hypermethylation achieved in L cells is of this magnitude has not been determined.

Whether the changes in methylation of tRNA observed in infected cultures stem from virus-

induced alterations in tRNA methyltransferase activity is not yet clear. The changes observed in capacity for methylation and base specificity of the enzymes in infected L cells would suggest an altered specificity for the enzymes and the possibility of aberrant methylation of host RNA.

Whether these changes in base specificity result in altered tRNA's with altered functions has not yet been determined. This will require isolation and purification of specific tRNA species from both normal and infected cells. Novel, isoaccepting tRNA's have been observed in virally transformed cells (5).

Less marked changes in the enzymes were observed in HeLa cells, where host protein synthesis is not inhibited until late in infection, when severe cytopathic effects become apparent. In the abortively infected cell line (MDBK), host protein synthesis is inhibited early in infection, as in L cells (6), but no changes were observed in the tRNA methyltransferases. However, in vitro techniques of enzyme assay may not be sensitive enough to detect actual changes in infected cells. It is possible that viral infection could lead to disruption of cellular compartmentation, thus interfering with the proper processing of the RNA. The appearance of elevated ribonuclease activity in the infected cells would be expected to affect host RNA as well as viral RNA.

The mechanism of alteration in methylation of RNA by mengovirus infection has not been determined. No inhibitors or activators of the methylating enzymes could be detected in any of the infected cell lines. Viral RNA did not affect the enzyme reaction. The changes in base pattern observed in the case of infected L cells could stem from inactivation of specific enzymes although not to such a degree as to affect measurements of specific activity.

No common pattern in alterations in tRNA methyltransferase activity in mengovirus-infected cell lines has been detected. This is in contrast to cell lines transformed with oncogenic viruses, where invariably elevated levels of the tRNA methyltransferases activity have been observed (5). The involvement of the tRNA methyltransferases in nononcogenic viral infection would appear to depend on the particular form of disruption of host cell regulatory mechanisms which may or may not involve these enzymes.

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