Biochemical Analysis of the Role of Transmethylation in the Methionine Dependence of Tumor Cells

Jean Gabriel Judde, Martha Ellis, and Philip Frost

 Departments of Cell Biology [J. G. J., M. E., P. F.] and Medicine [P. F.], The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

ABSTRACT

The effects on methionine metabolism of the substitution of homocysteine for methionine in vitro were investigated in normal and tumor cell lines differing in their ability to utilize homocysteine for growth. The major finding of this study was that methionine-independent (Met-Indep) cell lines had much lower basal transmethylation rates than methionine-independent (Met-Dep) cell lines. This was particularly evident in the parent SP1 cell line and its Met-Indep revertant, SP1-R. SP1-R compensated for a lack of methionine by reducing both its transmethylation and growth rates. An analysis of other potential differences in methionine metabolism between Met-Dep and Met-Indep cell lines failed to demonstrate any consistent abnormalities in all but the absolutely Met-Dep MDAV-D2 cell line. Thus, protein, S-adenosylhomocysteine, and polyamine synthesis were the same in Met-Dep and Met-Indep cell lines.

These results indicate that the major regulatory step in determining the Met-Dep phenotype is an inherent increase in the rate of transmethylation reactions. Cell lines with high basal transmethylation rates cannot compensate for a relative deficiency of methionine and either cease growing (MDAV-D2) or generate revertants (SP1-R) for which the basal rate of transmethylation is considerably reduced.

INTRODUCTION

Methionine auxotrophy (Met-Dep) is defined as the inability of a cell line to grow in medium devoid of methionine but containing its metabolic precursor homocysteine along with folic acid and vitamin B12 (Met-Hcy* medium). The defect appears to be exclusively associated with transformation (though most transformed cells are Met-Indep) since all normal cell lines tested thus far, including fibroblasts (1), liver and kidney cells (2, 3), and highly replicating mitogen-stimulated lymphocytes (4), can grow normally in Met-Hcy* medium.

The biochemical basis of Met-Dep is only partially understood. In cultured mammalian cells methionine biosynthesis is accomplished by remethylation of homocysteine by Met synthase (Fig. 1). It has been shown in some systems that Met-Dep cells retain Met synthase activity at a level as high as normal cells (Met-Indep) (5, 6) and that an increased level of methionine synthesis is not responsible for cells reverting from Met-Dep to Met-Indep (7). However, Met-Dep SV40-transformed fibroblasts incubated in Met-Hcy* medium labeled with [35S]homocysteine have reduced pools of free [35S]methionine compared to normal fibroblasts, even though similar amounts of [35S]-methionine are incorporated into proteins (8). In addition, (isotopic) AdoMet levels are decreased and AdoHcy levels are increased in Met-Dep as opposed to Met-Indep cells (9).

These studies indicate that defective methionine biosynthesis is not the basis for Met-Dep in the cell lines examined and suggest that the Met-Indep or Met-Dep phenotype may be determined by the ability or inability of the cell to maintain a normal AdoMet/AdoHcy ratio in Met-Hcy* medium.

As shown in Fig. 1, the activity of AdoMet synthase, which catalyzes the transfer of the adenosyl moiety of ATP to the sulfur atom of methionine, and the transmethylation and polyamine pathways are central to the regulation of cellular AdoMet levels. In fact, elevations of AdoMet-dependent transmethylation reactions (10) and polyamine synthesis (11) have been observed in tumor cell lines as have deficiencies in MTA phosphorylase (12), a key enzyme in the salvage of methionine through the polyamine pathway. Alterations in any of these steps in the methionine pathway could result in increased AdoMet and methionine requirements in tumor cells.

To assess which step(s) are responsible for the acquisition of the Met-Dep phenotype by transformed cells, we undertook a more comprehensive analysis of methionine metabolism in normal and tumor cell lines the abilities of which to proliferate in Met-Hcy* medium we recently characterized (4). Among these cell lines, a Met-Indep revertant isolated from a Met-Dep tumor cell line provided us with a particularly good model for identifying the biochemical basis for methionine auxotrophy.

MATERIALS AND METHODS

Materials. Radioactive compounds were purchased from Amersham Corporation, Arlington, IL. [methyl-3H]-S-adenosyl-L-methionine (83 Ci/mmole) by acid hydrolysis and purified by HPLC (13, 14). The Dowex resins used for product separation in the enzyme assays were obtained from Bio-Rad Laboratories (Richmond, CA). P81 ion exchange chromatography paper was purchased from Whatman (Clinton, NJ) and 3-deazaadenosine was from the Southern Research Institute (Birmingham, AL). Other biochemical reagents were obtained from Sigma Chemical Co. (St. Louis, MO). Media and materials for tissue culture were purchased from Flow Laboratories (Rockville, MD).

Cell Lines. The human HE lung embryo fibroblast cell line was obtained from Flow Laboratories. The mouse SP1 mammary adenocarcinoma (15), MDAV-D2 lymphoma (16), and human A375 melanoma (17) have been described elsewhere. SP1-R is a spontaneous Met-Indep revertant isolated from the SP1 cell line in this laboratory (4). For these studies, cells were grown in methionine-deficient RPMI 1640 supplemented with penicillin (100 units/ml), streptomycin (0.1 mg/ml), 10% dialyzed fetal calf serum (shown to be free of methionine by high-voltage paper electrophoresis), 100 μg/L folinic acid, 1.5 μg/L cyano-cobalamin, and 200 μM DL-methionine (Met-Hcy* medium). The SP1-R cell line was maintained in the same medium where methionine was replaced by 200 μM DL-homocysteine thiosaltate (Met-Hcy* medium).

The cultures were kept in humidified 95% air-5% CO2 at 37°C and subcultured with 0.25% trypsin every 3 or 4 days. To study the effects of homocysteine substitution for methionine, cells were plated in Met-Hcy* medium and on the following day the medium was poured off and replaced by Met-Hcy* medium. The cells were then harvested at various times for analysis.
METHIONINE DEPENDENCE AND TRANSMETHYLATION IN NEOPLASIA

Fig. 1. Methionine metabolism in mammalian cells. The numbers represent the enzymatic reactions and pathways which have been examined in this study: 1, Met synthase; 2, AdoMet synthase; 3, AdoMet-dependent transmethylation reaction; 4, AdoMet decarboxylase; 5, methythioladenosine phosphorylase; 6, utilization of methionine for protein synthesis; 7, cystathionine synthase. PUT, putrescine; ORN, ornithine; MTR-1-P, 5-methylthioribose 1-phosphate; THF, tetrahydrofolate.

Measurement of Intracellular AdoMet and AdoHcy. Cell cultures were harvested by trypsinization and washed twice in ice-cold phosphate-buffered saline. The cell pellet was extracted on ice for 30 min in 0.4 M perchloric acid (2 x 10^6 cells/ml) containing 0.15% (w/v) Na_2S_2O_5 and 0.05% (w/v) EDTA. After centrifugation, the acid supernatant was filtered through disposable 0.2-µm Spin-X filter units (Costar, Cambridge, MA) and stored at −70°C for up to 1 month without appreciable loss of activity. Perchloric acid extracts were analyzed by HPLC as described previously (18) using a Beckman Ultrasphere ion-pair column (particle size, 5 µm). Samples were eluted at 1.5 ml/min using a linear gradient of 0.1 M NaH_2PO_4-acetonitrile (98:2, v/v) and 0.15 M NaH_2PO_4-acetonitrile (74:26, v/v) containing 8 x 10^{-4} M octanesulfonic acid sodium salt (Kodak, Rochester, NY). AdoMet and AdoHcy were quantified by monitoring absorbance at 254 nm. External standards were injected every ten samples and used for calibration.

Polyamine Analysis. Cells were trypsinized and washed twice with ice-cold phosphate-buffered saline. The cell pellet was extracted on ice for 1 h with 200 µl of 8% trichloroacetic acid for each 10^6 cells. The precipitate was separated by centrifugation at 10,000 x g for 10 min, and aliquots of the supernatant were immediately used for derivatization of polyamines. Fifty µl of supernatant were mixed with 50 µl of 20 µM 1,6-hexanediane in 0.1 N HCl (internal standard), 200 µl of a saturated Na_2CO_3 solution, and 200 µl of 10 mg/ml dansyl chloride in acetone. After agitation for 15 s on a vortex machine the mixture was heated for 10 min at 70°C. The derivatized polyamines were then adsorbed on C_8 Bond Elut columns (Analytichem International, Harbor City, CA), eluted with 0.5 ml absolute methanol, and stored at 4°C for up to 2 weeks without appreciable loss of activity.

The derivatized polyamines were assayed by HPLC as described previously (19) using a Beckman Ultrasphere octadecylsilane reverse phase column maintained at 50°C and perfused at 2 ml/min with a gradient of acetonitrile and 0.05 M NaH_2PO_4, pH 4.4. External standards of putrescine, spermidine, and spermine were derivatized and used for calibration.

Enzyme Assays. Cells were harvested by trypsinization and the pellet was resuspended at 2 x 10^6 cells/ml in 0.25 M sucrose. Sucrose-cell suspensions could be stored at −70°C for at least 3 weeks without loss of enzyme activity. Cell extracts were prepared just before use by three rounds of freeze-thawing followed by centrifugation at 10,000 x g for 15 min at 4°C. Determination of enzyme activities were done according to previously published assays for S-adenosylmethionine decarboxylase (20), MTA phosphorylase (21), Met synthase (22), AdoMet synthase (23), and cystathionine synthase (24). Enzyme assays were carried out under conditions in which the activity was proportional to the amount of protein added and to the time of incubation. Results are expressed as nmol of product obtained in a 1-h incubation per mg of protein.

Protein concentration was determined by the method of Lowry et al. (25) using lysozyme as standard.

Measurement of Transmethylation Rates. The rate of AdoMet-dependent transmethylation reactions was assayed by measuring the time-dependent accumulation of AdoHcy in cells treated with periodate-oxidized 3-deazaadenosine, a potent inhibitor of AdoHcy hydrolase (26). Periodate-oxidized 3-deazaadenosine was prepared just before use by the reaction of 3-deazaadenosine with sodium periodate in 0.1 M acetate, pH 4, as described previously (10). Cells in exponential growth were incubated with 10 µM inhibitor, a concentration that produces complete inhibition of AdoHcy hydrolase in cultured cells (10), and harvested every 30 min for HPLC analysis of cellular AdoHcy content.

RESULTS

Cell Growth Studies. As shown in Fig. 2, the five cell lines under study differed markedly in their ability to proliferate in Met^-Hcy^- medium. The HE lung fibroblasts showed no difference in growth rate when homocysteine replaced methionine in the medium. However, they had a doubling time of 130 h compared to 13.8 h for the SP1 mammary adenocarcinoma. The Met-Indep A375 human melanoma cell line had a slightly reduced growth rate during the first 3 days in Met^-Hcy^- medium and then resumed growth at a normal rate. In contrast, the absolutely Met-Dep MDAY-D2 lymphoma and SP1 adenocarcinoma did not grow in Met^-Hcy^-'. However, although we are unable to obtain Met-Indep revertants from MDAY-D2 despite repeated attempts at selection, SP1 Met-Indep revertants (SP1-R) were obtained that could proliferate in Met^-Hcy^- medium (SP1-R cell line). Although SP1-R cells could grow permanently in Met^-Hcy^- medium, their doubling time increased from 14.5 h in Met^-Hcy^- medium to 32 h in Met^-Hcy^- medium. Because of the significant degree of cell death when MDAY-D2 and SP1 (to a lesser extent) were placed in Met^-Hcy^- medium for 48 h, we limited our analysis to the first 2 days of in vitro culture in Met^-Hcy^- medium.

Transmethylation Rates. AdoMet-dependent methyl transfer reactions consume about 70% of AdoMet in growing cells (27, 28). In addition, the overall rate of AdoMet-dependent tran-

![Fig. 2. Growth of the cell lines in Met^-Hcy^- (●) and Met^-Hcy^- (○) media.](https://cancerres.aacrjournals.org)
methylation reactions is increased in tumor cell lines compared to normal fibroblasts (10), as is the methylating capacity of DNA (29) and tRNA (30) methyltransferases. In order to test the hypothesis that Met-Dep could be related to an increase in AdoMet requirements through transmethylation reactions, we used the rate of AdoHcy accumulation in cells treated with the potent AdoHcy hydrolase inhibitor periodate-oxidized 3-deazaadenosine as a measurement of AdoMet-dependent transmethylation rates (10). As shown in Fig. 3, the Met-Dep SP1 and MDAY-D2 cells accumulated AdoHcy approximately 5-fold faster than the Met-Indep HE and A375 cells. Interestingly, the SP1-R Met-Indep revertant had a low transmethylation rate compared with the parent SP1 cell line.

Protein Synthesis. In preliminary experiments we had found that after a 4-h incubation of cells with [35S]methionine, 90% of the radioactivity was incorporated into proteins. We therefore could use the relatively safer radiolabeled [3H]methionine as a measure of methionine consumption in protein synthesis. Fig. 4A demonstrates that the incorporation of [3H]methionine into acid-insoluble material occurred at similar rates for all the cell lines tested. We next examined the effect of the substitution of methionine by homocysteine on the overall rate of protein synthesis as measured by the incorporation of [3H]leucine into acid-insoluble material from cells incubated in Met-Hcy medium. As shown in Fig. 4B, protein synthesis remained unaffected over a 24-h period for the Met-Indep HE and A375 cell lines. While [3H]leucine incorporation in the Met-Dep SP1 cell line decreased 50% at 4 h, it returned to normal at 24 h. In contrast, MDAY-D2 showed a continuous decrease in protein synthesis to 20% of background by 24 h correlating with cell death.

Effect of Met-Hcy Medium on AdoMet, AdoHcy, and Polyamine Pools. Previous studies using radioisotopic methods indicated that after 24 h incubation in Met-Hcy medium, AdoMet levels decreased and AdoHcy levels increased in Met-Dep tumors compared with Met-Indep tumors (9). We thus used a HPLC method to compare the AdoMet and AdoHcy pools of cell lines grown in Met-Hcy medium. The results are shown in Fig. 5. In the normal Met-Indep HE fibroblast cell line, AdoMet levels remained essentially constant for the first 8 h in Met-Hcy medium and then increased about 4-fold by 24 h. In the Med-Indep A375 and Med-Dep SP1 cell lines, AdoMet levels declined during the first 4–8 h and then increased about 2.5-fold by 24 h. In contrast, the AdoMet pool in the Med-Dep MDAY-D2 cell line was almost completely depleted by 8 h (20 pmol/10⁶ cells compared to 6400 pmol/10⁶ cells in Met-Hcy medium) and remained extremely low at 24 h (100 pmol/10⁶ cells).

For all cell lines the AdoHcy pool was increased 2–4-fold during the first 2–4 h in Met-Hcy medium. This increase may have resulted from a partial reversal of the hydrolysis of AdoHcy in the presence of excess homocysteine (31). After 24 h, AdoHcy levels were either normal in HE and MDAY-D2 cells or increased by about 3-fold in A375 and SP1 cells.

The variations in the AdoMet/AdoHcy ratios followed a similar pattern in HE, A375, and SP1 cells with a transient but significant drop during the first 2–4 h followed by a return to normal by 24 h. In MDAY-D2 cells, the AdoMet/AdoHcy ratio decreased dramatically from 119 in Met-Hcy medium to 1.0 after 8 h and 1.8 after 24 h in Met-Hcy medium. This pattern in MDAY-D2 resulted entirely from the depletion of the AdoMet pool. The Med-Indep revertant SP1-R had AdoMet and AdoHcy levels in Met-Hcy medium similar to those of the parent SP1 cell line (data not shown).

In addition to its role as a methyl donor in transmethylation
reactions, AdoMet provides the aminopropyl moiety necessary for the synthesis of the polyamines Spd and spermine. It is therefore possible that a polyamine sink could deplete AdoMet in Met-Dep cells. Alternatively, low AdoMet levels could result in polyamine depletion and inhibition of cell growth. To assess these possibilities we examined the levels of cellular polyamines in cells grown in Met- medium and determined their relationship to AdoMet levels. As shown in Fig. 6, all tumor cell lines had similar pools of Spd and Spm in Met- medium (between 6 and 7 mmol/10^6 cells), while the HE fibroblast cell line had a slightly lower polyamine content (4.3 mmol/10^6 cells). However, the ratio of Spd to Spm markedly differed between the cell lines. HE and A375 had a Spd/Spm ratio of about 1, compared to 3 for MDAY-D2 and SP1. The Spd/Spm ratio correlated with the growth rate: cells having a fast growth rate had a high Spd/Spm ratio. This higher Spd/Spm ratio in fast growing cells has been described previously in a comparative study of 3T3 and SV40-transformed 3T3 cells (32).

The fluctuations of the polyamine pools in response to methionine substitution by homocysteine followed a similar pattern for all cell lines. There was a rapid but transient decrease in both Spd and Spm between 2 and 4 h, one of which was marginal in HE cells and most pronounced in MDAY-D2 cells (59% of the Spd plus Spm content in Met- medium). The polyamine content then returned to normal or supranormal levels (60% increase in SP1 cells) by 4 to 8 h and remained at these levels during the 48-h observation period. In Met- medium, the SP1-R revertant had 6.4 nmol Spd/10^6 cells and 1.6 nmol Spm/10^6 cells compared to 7.0 nmol Spd/10^6 and 1.5 nmol Spm/10^6 for the parent SP1 cell line. Thus, the early decrease in the polyamine content corresponded to a decrease in AdoMet pools during the first hours in Met- medium and probably resulted from a lack of AdoMet substrate for AdoMet decarboxylase.

Enzymatic Studies. The activities of Met synthase, AdoMet synthase, and MTA phosphorylase in extracts from cells incubated for 24 h in either Met- or Met+ medium are shown in Table 1. All cell lines except MDAY-D2 had similar Met synthase activities in Met+ medium and showed a significant but modest increase in activity in Met- medium. The SP1-R revertant grown in Met- medium had an only slightly higher Met synthase activity than the parent SP1 cell line. Thus, the ability of HE, A375, and SP1-R cells to grow in Met- medium did not correlate with an increase in methionine biosynthesis when compared with the Met-Dep SP1 cells. However, MDAY-D2 cells had a very low basal Met synthase activity in Met- medium that further decreased in Met- medium. These differences in methionine biosynthesis could not be explained by differences in cystathionine synthase activity from the same cell extracts (Table 1).

The variations in AdoMet synthase activity for each cell line were consistent with the changes observed in the AdoMet pools. There was a 3-, 7-, and 6.6-fold increase in AdoMet synthase activity in HE, A375, and SP1 cells, respectively, after 24 h in Met- medium. The SP1-R revertant also had elevated AdoMet synthase activity in Met- medium, although it was only 60% of the activity in the parent SP1 cell line in the same medium. In Met- medium, SP1-R showed a decrease in AdoMet synthase activity to basal levels. In MDAY-D2, AdoMet synthase activity was depressed after 24 h in Met- medium, which accounted for the inability of this cell line to maintain its AdoMet pool in this medium.

The utilization of AdoMet for polyamine synthesis yields the thioether nucleoside MTA, which is metabolized by MTA phosphorylase to adenine and 5-methylthioribose 1-phosphate (33). The 5-methylthioribose 1-phosphate is then reconverted into the methionine pathway, we also measured this enzyme activity. Methionine synthase, AdoMet synthase, and MTA phosphorylase in extracts from cells incubated for 24 h in either Met+ or Met- medium are shown in Table 1. All cell lines except MDAY-D2 had similar Met synthase activities in Met+ medium.

| Table 1 | Effect of methionine substitution by homocysteine on specific enzyme activities of the methionine pathway*
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HE</td>
<td>Met-</td>
</tr>
<tr>
<td>Met-</td>
<td>3.5</td>
</tr>
<tr>
<td>Met+</td>
<td>3.9</td>
</tr>
<tr>
<td>A375</td>
<td>Met-</td>
</tr>
<tr>
<td>Met+</td>
<td>7.0</td>
</tr>
<tr>
<td>SP1</td>
<td>Met-</td>
</tr>
<tr>
<td>Met+</td>
<td>3.7</td>
</tr>
<tr>
<td>SP1-R</td>
<td>Met-</td>
</tr>
<tr>
<td>Met+</td>
<td>5.3</td>
</tr>
<tr>
<td>MDAY-D2</td>
<td>Met-</td>
</tr>
<tr>
<td>Met+</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*Enzyme activities are expressed as nmol of product obtained in a 1-h incubation per mg of protein. The numbers are the means of duplicate determinations with less than 10% SD.

*Cells were grown in Met- medium or incubated for 24 h in Met- medium.

The SP1-R Met- indep revertant was grown in Met- medium or incubated for 24 h in Met- medium.

Fig. 6. Effect of methionine substitution by homocysteine on the intracellular pools of spermidine (•), spermine (○), and spermidine plus spermine (△). At zero time Met- medium was replaced by Met- medium and cellular polyamine pools were determined by HPLC. Each plot is the average of triplicate determinations with less than 10% SD.
In response to methionine substitution by homocysteine, the activity of AdoMet decarboxylase increased severalfold in all cell lines, reaching a peak at 8 h for HE, A375, and MDAY-D2 and at 24 h for SP1 (Fig. 7). AdoMet decarboxylase activity then returned to normal at 48 h except in SP1 where it remained elevated. SP1-R had a basal AdoMet decarboxylase activity similar to the parent SP1 cell line grown in Met-'Hcy'-medium. It thus appears that in Met-'Hcy'-medium, a rapid and significant increase in AdoMet decarboxylase activity allows cells to maintain their polyamine pools in the face of decreasing AdoMet levels.

Cystathionine synthase levels were also measured in Met-Dep and Met-Indep cells. No significant differences were found between the two phenotypes (Table 1).

**DISCUSSION**

We have presented evidence to indicate that the critical factor determining methionine dependence in tumor cells related to their inherent rates of transmethylation. In cells with high transmethylation rates, failure to increase AdoMet synthase activity in Met-'Hcy'-medium resulted in a rapid depletion of the AdoMet pool and cell death (MDAY-D2). In cells able to maintain AdoMet levels by increasing AdoMet synthesis, the balance between methionine synthesis from its precursor homocysteine and the methionine requirements for AdoMet-dependent transmethylation reactions ultimately determined the growth rate of tumor cells in Met-'Hcy'-medium.

Our ability to define the biochemical parameters of methionine dependency is due to the identification of specific cell lines with unique Met-Dep phenotypes (4). Thus, we were able to compare normal fibroblasts and Met-Indep tumor cells to absolutely Met-Dep cells that do not survive in Met-'Hcy'-medium (MDAY-D2) and to cells that although initially Met-Dep are able to generate Met-Indep revertants (SP1-S1-R).

The metabolism of methionine by normal HE fibroblasts is essentially the same whether the cells are grown in Met-'Hcy'- or Met-'Hcy' media. This is likely because these cells grow slowly and have low transmethylation rates. HE fibroblasts do, however, undergo several metabolic changes during their first 4 h of growth in Met-'Hcy'-medium. These changes are manifested as a decrease in AdoMet and polyamine levels and an increase in AdoHcy levels that may result from a partial reversal of the AdoHcy hydrolase reaction towards AdoMet synthesis, because of an increase in cellular homocysteine levels (31). The cells compensate for these changes with time by a 3-fold increase in AdoMet synthase activity that allows them to grow normally in Met-'Hcy'-medium.

Similarly, Met-Indep tumor cells such as A375 had low basal rates of transmethylation (and therefore low AdoMet requirements) and compensated for the substitution of homocysteine for methionine by increasing methionine and AdoMet synthase activities. These cells were thus able to survive and grow at a normal rate in Met-'Hcy'-medium. However, the AdoMet pools were initially much more affected in A375 than in the normal HE fibroblast cell line, and the compensatory increase in AdoMet synthase was also more significant, suggesting the AdoMet requirements in A375 are higher. Although both cell lines have low transmethylation rates, the polyamine levels, AdoMet decarboxylase activity, and rate of methionine incorporation into proteins were all slightly higher in A375 cells. This is probably due to the higher growth rate of A375 and may account for the more pronounced effect of Met-'Hcy'-medium on AdoMet synthesis in A375 cells.

In contrast, MDAY-D2 tumor cells are absolutely Met-Dep and manifest profound changes in many parameters of the methionine pathway when placed in Met-'Hcy'-medium. MDAY-D2 cells have 5-fold higher transmethylation rates than HE or A375 cells. Thus, the AdoMet requirement in MDAY-D2 cells is increased and the growth of such cells in Met-'Hcy'-medium would require dramatic compensatory biochemical changes so as to enable them to maintain their high transmethylation rate. In fact, MDAY-D2 cells grown in Met-'Hcy'-medium are unable to increase either methionine or AdoMet synthase activities resulting in a near total depletion of cellular AdoMet. The AdoMet/AdoHcy ratio in MDAY-D2 cells dropped from 119 in methionine-containing medium to 1.0 after 8 h in Met-'Hcy'-medium, presumably resulting in the inhibition of essential transmethylation reactions (35). This led to suppression of protein synthesis and cell death within 24 h. Our data do not provide an explanation for the inability of these cells to increase either Met synthase or AdoMet synthase activities in Met-'Hcy'-medium. However, the fact that the AdoMet decarboxylase activity of MDAY-D2 cells in Met-'Hcy'-medium increases in order to maintain polyamine levels and that MTA phosphorylase also increases indicates that there is no general inhibition of enzyme activity secondary to the observed decrease in protein synthesis.

Of particular interest are the SP1 cells which, when observed initially, were thought to be absolutely Met-Dep. These cells also have high transmethylation rates. However, SP1 cells placed in Met-'Hcy'-medium become cytostatic, but after approximately 1 week they begin dividing and generate a Met-Indep revertant cell line, SP1-R (4). The frequency of reversion in SP1 cells can be as high as 10%. Although SP1 cells stopped growing in Met-'Hcy'-medium, biochemical analyses showed that following an initial decrease, a considerable increase in AdoMet levels occurred after 24 h in this medium. This change was due to a 6–7-fold increase in AdoMet synthase activity and a significant but less dramatic increase in Met synthase activity. Thus, although SP1 cells cannot initially divide in Met-'Hcy'-medium, in contrast to MDAY-D2 cells they can survive via a compensatory increase in AdoMet synthase activity allowing them to maintain a high AdoMet/AdoHcy ratio.

Although our data do not identify the actual mechanism affecting cell division in SP1, several lines of evidence strongly suggest that it is related to the high rate of transmethylation of these cells. (a) The major biochemical difference between the SP1-R and parent SP1 cells is a marked decrease in the transmethylation rate in SP1-R that results in a decreased requirement for AdoMet. This seems to be the major compensatory mechanism allowing SP1-R cells to regain the ability to divide.

![Fig. 7. Effect of methionine substitution by homocysteine on the activity of AdoMet decarboxylase. At zero time Met-'Hcy'-medium was replaced by Met-'Hcy'-medium and cell cultures were harvested at different times for determination of specific enzyme activities in HE ( ), A375 ( ), SP1 ( ), and MDAY-D2 ( ) cell lines.](image-url)
in Met Hcy+ medium, albeit at a slower rate. (b) SP1 and A375 cells differ primarily in their rates of transmethylation, again suggesting that a lower AdoMet demand allows cells to compensate more readily for a relative methionine deficiency. It is possible that since SP1 cells must compensate for their high transmethylation rate by a corresponding increase in AdoMet synthesis, a relative state of ATP deficiency could occur, resulting in cytostasis. Such a mechanism would be consistent with the metabolic studies of Hardwick et al. (36) on methionine-induced toxicity in guinea pigs, which attributed methionine toxicity to hepatic ATP insufficiency resulting from increased AdoMet synthesis (36).

All the cell lines we studied maintained their polyamine content despite extensive AdoMet depletion (MDAY-D2 cells) by increasing AdoMet decarboxylase activity. This observation confirms that polyamine synthesis is tightly regulated and agrees with studies using AdoMet syntheses inhibitors, which indicated that depletion of AdoMet pools suppressed cell growth by inhibiting transmethylation reactions without affecting polyamine levels (37). In addition, besides having similar pools of Spd and Spm, none of the cell lines studied was deficient in MTA phosphorylase activity, making the existence of a polyamine sink unlikely. Therefore, the polyamine pathway does not appear to contribute significantly to the Met-Dep phenotype in the cell lines examined.

A question raised by these studies is why high rates of transmethylation deplete the AdoMet pool in Met Hcy+ medium, since the methionine pathway uses homocysteine obtained from AdoHcy hydrolysis for recycling into methionine via Met synthase. One possibility is that the cystathionine synthase reaction, by constituting the only outlet of homocysteine from the cycle, could shunt homocysteine toward cysteine production (38). However, we found no increase in the activity of this enzyme in Met-Dep cells. This suggests that the Met synthase reaction is unable to salvage excess homocysteine in cells with high transmethylation rates grown in Met Hcy+ medium. In support of this view are studies in patients with homocystinuria who lack cystathionine synthase and in whom homocysteine methylases are insufficient to metabolize homocysteine derived from transmethylation reactions (39). Furthermore, rats fed choline-deficient diets show a decrease in hepatic AdoMet levels and an increase in AdoHcy levels, indicating that the conservation of methionine by the methyltetrahydrofolate-homocysteine methyltransferase reaction alone, may not be possible (40).

Our finding that the critical event in Met-Dep is a basal increase in transmethylation rates confirms previous studies (in unrelated cell lines) suggesting a possible link between altered transmethylation in tumor cells and the Met-Dep phenotype (10, 41). Some cells with high basal transmethylation rates can compensate for the substitution of homocysteine for methionine by increasing AdoMet synthesis and ultimately generating revertants; others cannot. Cells with low transmethylation rates compensate even more readily for the substitution of homocysteine for methionine since their demands for AdoMet are considerably lower and they need not generate revertants to survive. We would emphasize that we have found very little evidence for absolute Met-Dep among tumor cells (4), and that most Met-Dep tumor cell lines are able to generate Met-Indep revertants (7). Thus, tumors like MDAY-D2 that do not compensate for high transmethylation rates are probably very rare. Although rare, in human cancer this phenotype could represent a target for therapy. Whether specific types or subtypes of tumors with a high frequency of Met-Dep exist remains to be determined.

REFERENCES


Biochemical Analysis of the Role of Transmethylation in the Methionine Dependence of Tumor Cells

Jean Gabriel Judde, Martha Ellis and Philip Frost