Regulation of Methionine Adenosyltransferase in Normal Diploid and Simian Virus 40-Transformed Human Fibroblasts 1,2

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ABSTRACT—Methionine adenosyltransferase activity in normal diploid and simian virus 40 (SV40)-transformed human fibroblasts increased severalfold when cell monolayers were cultured in medium deficient in l-methionine. This increase in methionine adenosyltransferase activity required RNA and protein syntheses and probably represented a derepression of the enzyme’s biosynthesis. Furthermore, studies with RNA synthesis inhibitors suggested that the regulation of this enzyme activity in human fibroblasts involved posttranscriptional mechanisms. The inclusion of homocysteine thiolactone, a metabolic precursor of methionine, in the methionine-deficient medium inhibited the derepression in normal human fibroblasts but augmented the derepression in fully transformed fibroblasts. These differences in derepression patterns thus appeared related to altered metabolism of homocysteine and/or methionine in SV40-transformed human fibroblasts and as such may serve as a transformation marker in SV40-transformed cells.—JNCI 65: 1237–1244, 1980.

A frequent component of the transformed phenotype of cultured neoplastic cells is the inability of cells to proliferate when methionine is replaced in the culture media by its immediate metabolic precursor homocysteine (7–5). We recently observed met in SV40-transformed hamster and human fibroblasts (6, 7). In contrast to BHK fibroblasts, cells of an SV40-transformed BHK line were unable to proliferate in methionine-deficient medium. However, subclones of two independently derived SV40-transformed human fibroblast lines were able to utilize homocysteine thiolactone, at least with respect to requirements for cell division (7). Using positive selection techniques we were able to isolate rare transformed cells that had regained the ability to divide in the homocysteine thiolactone-supplemented medium and were thus phenotypically similar to the met diploid fibroblasts with regard to this property (9). This reversion of the transformed cells from met to met was accompanied by a shift toward the untransformed phenotype for other transformation parameters (10), which suggests that an understanding of the biochemical events responsible for met might broaden our understanding of the process of cell transformation.

In vitro and in vivo measurements of the methionine biosynthetic capacity of the human cells did not distinguish normal diploid and transformed revertant fibroblasts (both met) from transformed fibroblasts exhibiting the met phenotype (7, 9). Another important aspect of methionine metabolism, not dealt with in previous studies of met in cultured cells, is the utilization of methionine. Methionine is a methyl source for transmethylations and is utilized in the biosynthesis of polyamines, cysteine (transsulfuration pathway), and proteins. With the exception of protein synthesis, these pathways share a common metabolite more immediate than methionine: AdoMet. MetAT (EC 2.5.1.6) catalyzes the formation of AdoMet from methionine and ATP. In this communication the activity and regulation of MetAT are examined in normal and SV40-transformed human fibroblasts.

MATERIALS AND METHODS

Cell lines and culture conditions.—Five diploid fibroblast strains were used: MGF287 and MGF292, fibroblasts from biopsy specimens of skin from adult males; MGF390, fibroblasts from a biopsy specimen of foreskin of a 5-week-old human; MGF786, fibroblasts from a biopsy specimen of skin from a male infant; and WI-38, embryonic lung fibroblasts obtained from the American Type Culture Collection, Rockville, Maryland. In addition, we used WI-38 VA 13, met SV40-transformed WI-38; P1 and P5, met subclones of the transformed lines WI18VA2 and SV80, respectively.

ABBREVIATIONS USED

AdoHcy = S-adenosylhomocysteine; AdoMet = S-adenosylmethionine; a-Am = a-amaminit; AMD = actinomycin D; BHK = baby hamster kidney; MetAT = methionine adenosyltransferase; met = methionine auxotroph; met = methionine-independent; MPB = mercaptopropylidyl benzimidazole; PBS = phosphate-buffered saline; SV40 = simian virus 40; TCA = trichloroacetic acid.

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8 We dedicate this paper to the memory of George Halikas.
centrifuged, the supernatant was aspirated, and the pellet was suspended in 1 ml of cold TCA and transferred to a scintillation vial. To the vial was added 15 ml of Aquasol II (Beckman Instruments, Inc., Palo Alto, Calif.), and radioactivity was determined in a liquid scintillation spectrometer (Beckman).

**Depression and preparation of extracts.**—Cells that had grown for several population doublings in stock medium supplemented with 100 μM folic acid and 1.5 μM hydrocortisol were refed with the same medium 12-24 hours before the start of an experiment. At time 0 subconfluent cell monolayers were washed twice with PBS (pH 7.2) and refed basal medium containing varying amounts of methionine, 200 μM Nα-homocysteine thiolactone, and inhibitors as indicated in the text. In all experiments cell extracts from the contents of one or two 145-mm petri dishes were sufficient for the MetAT assay.

Cultures were harvested by exposure to 0.25% trypsin for 5-10 minutes at 37°C. Trypsinization was terminated by the addition of cold PBS containing fetal bovine serum (10% vol/vol). Cells were recovered by centrifugation and enumerated in a Coulter counter, and the final washed pellet was suspended at 2×10^6 cells/ml in 0.25 M sucrose. Crude extracts were prepared by sonic disruption and centrifugation for 10 minutes in a Beckman Microfuge B at 4°C. The extracts were assayed for MetAT immediately after centrifugation. MetAT activity was not lost during storage of the sucrose-cell suspensions at −70°C for several weeks before cell disruption.

**MetAT assay.**—MetAT activity was measured by a modification of techniques of Mudd et al. (11) and Cabaco (12). The reaction mixture contained, in a final volume of 150 μl, 133 mM HEPES* buffer (pH 7.0), 13.3 mM ATP, 133 mM KCl, 67 mM MgCl₂, 1 mM β-mercaptoethanol, 100 μM L-[3^-3H]methionine (sp act, 2-5 μCi/μmol; Amersham/Searle Corp., Des Plaines, Ill.), and 25-150 μg of crude lysate protein. The reaction was initiated by the addition of the methionine substrate, the mixture was incubated at 37°C for 50 minutes, and the reaction was terminated by the addition of 150 μl of ice-cold 5% TCA. The precipitated protein was pelleted by centrifugation.

Small glass columns were packed with a slurry of the cation exchange resin AG50W-X4 (100-200 mesh, hydroxide form; Bio-Rad Laboratories, Richmond, Calif.) in water to yield a wet bed volume of 0.25 cm³/column. The resin was then equilibrated with approximately 8 volumes (2 ml) of 2 N HCl, and 200-μl aliquots of the reaction mixture supernatant were applied and allowed to absorb. The column eluate after application of 5 ml of 2 N HCl was discarded, and the eluate after the subsequent application of 2 ml of 6 N HCl was collected in a scintillation vial. Then 15 ml of a toluene-Triton X-100 Omnifluor (New England Nu-

* 4-(2-Hydroxyethyl)l-piperazineethanesulfonic acid.
clear) scintillation solution was added to each vial, and the radioactivity was measured in a liquid scintillation spectrometer (Beckman). Blank reaction tubes lacking enzyme contained less than 1% of the total radioactivity added to the reaction tube. Specific activities were expressed as nanomoles of AdoMet formed per 30 minutes per milligram of protein. The activity of MetAT increased linearly with increasing protein concentrations up to 150 µg/assay tube. The reaction was linear with time until 13% of the methionine substrate had been consumed. Reported specific activities were the average of paired determinations with the use of 15 and 30 µl of crude lysate.

Protein concentrations were measured spectrophotometrically (13).

RESULTS

MetAT activity in the extracts of the cultured human fibroblasts WI-38, MGF390, and MGF786 increased up to fifteenfold when the cells were shifted from stock medium containing 100 µM methionine to low-methionine medium. Text-figure 1 shows that MetAT activity increased for several days and then declined in MGF390 and MGF786. In experiments not shown, the MetAT activity in WI-38 fibroblasts similarly declined. The results of several MetAT activity determinations in fibroblasts cultured in high- and low-methionine media are summarized in table 1. After 48 hours in low-methionine medium the average increase in MetAT activity was 6.8-fold in MGF390, 4.6-fold in MGF786, and 4.4-fold for one determination in WI-38. As text-figure 1 shows, however, increases up to fifteenfold were observed. These variations are probably due to the fact that activity determinations at 48 hours did not always represent peak activities. In this regard we observed that MetAT activity reached maximum values 2–5 days after the shift to low-methionine medium (results not shown). Also, the linearity in the paired determinations was often poor in samples with low levels of MetAT activity.

TABLE 1.—MetAT activity in fibroblasts cultured in 100 µM and 1 µM methionine medium

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>MetAT activity</th>
<th>MGF390</th>
<th>MGF786</th>
<th>WI-38</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock medium, 100 µM</td>
<td>1.8, 1.7 (68)</td>
<td>1.0, 2.7 (75)</td>
<td>1.8 (5)</td>
<td></td>
</tr>
<tr>
<td>methionine</td>
<td>1.8, 2.1 (8)</td>
<td>3.4 (32), 4.8</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>Low-methionine medium, 1 µM</td>
<td>16.6, 9.9</td>
<td>15.5, 8.4</td>
<td>12.4</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>9.3, 15.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Two for the activity determinations of fibroblasts in high-methionine medium, cells were cultured a minimum of 2 days in stock medium before harvest. Activity determinations of fibroblasts in low-methionine medium were made 48 hr subsequent to the shift from stock medium to low-methionine medium.

The linearity in the paired determinations was often poor in samples with low levels of MetAT activity.

Average of paired determinations in which individual determinations vary from the average by <20%. In instances in which the variation is >20%, the magnitude is indicated in parentheses.

Text-figure 1.—MetAT activity in methionine-deficient medium.

At 0 hr, stock medium containing 100 µM methionine was replaced by low-methionine medium. WI-38, □—□ MGF390, O—O, and MGF786, △—△. Each data point represents the average of paired determinations in which either of the determinations varies from the average by <100% for MGF390 and <20% for MGF786 and WI-38, with the exception of the 4-hr point of MGF786 (27%). The 0-hr point of MGF390 and the 12-hr point of WI-38 represent a determination at one extract concentration only.

It was necessary to demonstrate that methionine, AdoMet, or some inhibitor was not present in cell extracts in sufficient quantity to account for the changes in MetAT activity described in text-figure 1 and table 1. When extracts of MGF390 cells cultured for 48 hours in high-methionine medium or low-methionine medium were mixed in various proportions, the MetAT activities were additive and yielded a linear relationship between the total activity of a mixture and the ratio of the two extracts. In addition, passage through a Sephadex G-25 column of an extract of MGF390 fibroblasts grown for 48 hours in high-methionine medium did not increase the activity. These two types of experiments made unlikely the possibility that the differences in enzyme levels illustrated in text-figure 1 were attributable to the presence or absence of low-molecular-weight substances in the cell extracts.

The increase in MetAT activity was dependent on RNA and protein syntheses. The protein synthesis inhibitor cycloheximide and the RNA synthesis inhibitors AMD, α-Am, and MPB were tested for their ability to inhibit the incorporation of [3H]leucine or [3H]uridine into TCA-insoluble cellular materials under the conditions of our experiments, i.e., growth in low-methionine medium. Table 2 illustrates that during a 4-hour period, 20–24 hours after exposure to inhibitor, the incorporation of labeled precursor into TCA-insoluble material was 18% of that of controls with cycloheximide, 19% of that of controls with AMD and 2 and 4% of that of controls (ethanol-containing) with AMD and MPB, respectively. MPB also inhibited the
TABLE 2.—Incorporation of [3H]uridine and [3H]leucine by MGF89 fibroblasts in the presence of RNA and protein synthesis inhibitors

<table>
<thead>
<tr>
<th>Additions to low-methionine medium</th>
<th>cpm [3H]leucine/10⁶ cells</th>
<th>cpm [3H]uridine/10⁶ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCA-insoluble</td>
<td>TCA-soluble</td>
</tr>
<tr>
<td>H₂O</td>
<td>5,674</td>
<td>ND</td>
</tr>
<tr>
<td>70% ethanol</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>AMD in 50% ethanol</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MPB in 70% ethanol</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>α-Am in H₂O</td>
<td>1,004</td>
<td>ND</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* See "Materials and Methods" for inhibitor concentrations and experimental details.

ND = not determined.

Average of paired determinations in which individual determinations vary from the average by <10%. In instances in which the variation is >10%, the magnitude is indicated in parentheses.

accumulation of TCA-soluble label. This inhibitory effect of MPB, possibly related to uptake of [3H]uridine, reduced the accumulation of TCA-soluble label to 12% of that of the controls but by itself would not account for the 96% inhibition of incorporation of label into the TCA-insoluble material. Thus although MPB greatly reduced the ability of the cells to accumulate [3H]uridine, it apparently also blocked directly the incorporation of the labeled RNA precursor into TCA-

isoluble material. Similar findings were made previously by Levinson et al. [14]. Although α-Am has been reported to be a specific inhibitor of RNA polymerase II [15-17], the 81% inhibition of [3H]uridine incorporation into TCA-insoluble material 20-24 hours after exposure to the inhibitor could have resulted from indirect inhibition of rRNA synthesis via blockage of mRNA synthesis. In any event, α-Am did not inhibit the uptake of [3H]uridine but did inhibit its incorporation into TCA-insoluble material. The same appeared to be true with AMD under our experimental conditions. As shown in text-figure 2, cycloheximide added simultaneously with the shift of the cells from stock medium to low-methionine medium blocked the increase of the MetAT activity in MGF89 fibroblasts. Text-figure 2 also indicates that addition of cycloheximide at times subsequent to the shift inhibited further increases in activity. Similar results were obtained with puromycin (data not shown). RNA synthesis inhibitors AMD, α-Am, and MPB inhibited the increase in MetAT activity when added to cultures at the time they were shifted from stock medium to low-methionine medium: At 50 hours MetAT activity in cultures containing 0.3 μg AMD/ml, 2.5 μg α-Am/ml, and 40 μg MPB/ml was 39, 42, and 7%, respectively, of that in a parallel culture containing no inhibitors. These observations taken together provided evidence that the increases in MetAT activity observed when the supply of exogenous methionine was limited were the consequence of derepression of MetAT synthesis.

Activities remained essentially constant when the MGF890 or MGF786 fibroblasts were passaged in high-methionine medium at low density and MetAT activity was measured throughout the subsequent culture cycle. MetAT activity increased 60% (less than twofold) during log growth and subsequently returned to base-line levels as cell division ceased at confluence. These observations and the fact that cultures being monitored as in text-figure 1 were not rapidly dividing due to the limiting methionine concentration of the medium indicated that the depression did not require rapidly dividing cultures. Indeed, when confluent, nondividing cultures of MGF890 and MGF786 were shifted to low-methionine medium, MetAT activity increased to a maximum at day 5, after which it declined to a new steady-state value midway between base-line and peak activities (data not shown). Events associated with the cessation of growth when the cells were shifted to low-methionine medium were probably not a major cause of the increased MetAT activity. We found (unpublished data) that MetAT activity levels also increased in dividing fibroblasts cultured in methionine-free medium containing 200 μM DL-homocysteine thiolactone, the immediate metabolic precursor of methionine. Thus neither cell density nor replicative state was a primary determinant of derepressibility.

After the shift to low-methionine medium, the addition of 100 μM methionine up to 24 hours later blocked further increases in MetAT activity (text-fig.
Although RNA synthesis inhibitors were effective when added at the time of shift, the MetAT derepression thereafter became progressively less sensitive to such inhibition (text-fig. 3B-D). AMD and MPB added at 4 hours blocked the derepression, whereas α-Am was less effective. By 12 hours none of these three inhibitors retained its maximal effect, and by 24 hours AMD and α-Am produced no inhibition. In contrast, increasing the methionine concentration of the low-methionine medium to 100 μM prevented further increases of MetAT activity at all times of addition (text-fig. 3A).

Of particular interest was the inhibition of further increases in MetAT activity by 100 μM methionine at 24 hours, when the derepression was insensitive to α-Am and AMD and only partially sensitive to MPB. Similar results (not shown) were obtained with MGF90 when AMD and MPB were used. These observations agree with those of Caboche (12) and Caboche and Mulsant (18) who used AMD in BHK and Chinese hamster ovary cells. They suggest that in human diploid fibroblasts methionine or one of its metabolites can regulate MetAT at the posttranscriptional level.

MetAT activity was also observed to increase in extracts of diploid human fibroblasts cultured in low-methionine medium supplemented with 200 μM dl-

![Graph showing the effect of methionine and RNA synthesis inhibitors on MetAT activity in MGF786 fibroblasts.](image)

**DISCUSSION**

Characterization has not been achieved of the biochemical defect noted in many transformed and malignant cells whereby they cannot proliferate in medium in which methionine is replaced by its immediate metabolic precursor homocysteine (homocysteine thiolactone) in growth studies. The thiolactone is stable with regard to oxidation, is taken up readily by cultured human fibroblasts, and is utilized for growth by normal human fibroblasts. Homocysteine thiolactone will not, however, support the growth of subclones of two SV40-transformed human lines when it is substituted for methionine in growth medium, and previous attempts to characterize this defect in terms of impaired ability to synthesize methionine have been unsuccessful. We therefore looked at another aspect of methionine metabolism, S-adenosylmethionine synthesis, from the standpoint of regulation of MetAT. It seemed logical that comparative studies between met' and met’ cells be preceded by
investigation of the regulation of the enzyme in normal fibroblasts.

In normal diploid human fibroblasts MetAT activity increased up to fifteenfold when the cells were transferred to methionine-deficient medium. The MetAT activity of the fibroblasts increased when the only change in the medium composition was a decrease in the methionine concentration. Protein synthesis inhibitors cycloheximide and puromycin and RNA synthesis inhibitors AMD, MPB, and α-Am prevented the increase in MetAT activity when these inhibitors were added to the culture medium at the time of change to the methionine-deficient medium. These observations suggested that methionine or one of its metabolites was involved in the regulation of MetAT and that the increased MetAT activity observed was the result of a derepression of MetAT biosynthesis, although a more rigorous elimination of alternative explanations such as decreased degradation of MetAT would require turnover studies and direct measurements of enzyme protein and mRNA.

AdoMet exerts opposing effects on the rate of the MetAT reaction in vitro; at low (<50 μM) concentrations AdoMet increases reaction rates by stimulating the triphosphatase activity of the enzyme, whereas at higher concentrations MetAT reaction rates are inhibited presumably due to product inhibition (19, 20). Large amounts of AdoMet in the extracts of cells grown in medium containing 100 μM methionine might have been expected to inhibit reaction rates, thereby giving low MetAT activities when compared to extracts of cells that had similar amounts of enzyme protein but that were grown in methionine-deficient medium resulting in noninhibitory or even stimulatory concentrations of AdoMet. When cell extracts passed through a Sephadex G-25 column or mixtures of extracts from cells with base-line and elevated activities were assayed, the results obtained made unlikely the possibility that AdoMet or another low-molecular-weight substance in the cell extracts was affecting the measurement of MetAT activity in the assay. In addition, the calculated amount of AdoMet formed in the assay never exceeded 12 μM.

We also investigated further the effects of RNA...
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effects on the derepression of MetAT, our results provide evidence for a posttranscriptional component of MetAT regulation involving methionine or one of its metabolites in human fibroblasts. In vivo studies with RNA synthesis inhibitors should be interpreted cautiously, however, and these are only suggestive of the existence of posttranscriptional mechanisms. If posttranscriptional regulation can be confirmed more precisely when a system for in vitro protein synthesis is used, the availability of this type of derepressible enzyme should offer a useful approach for further studies. Indeed, it has been shown that mRNA in eukaryotic cells is relatively long lived (27); possibly, some control is effected at the translational level. MetAT in diploid fibroblasts has the additional advantage of providing a regulated system in a more normal cell type and may disclose quite different regulatory mechanisms than those identified in aneuploid lines.

The met phenotype has been observed in a number of SV40-transformed and malignant cells in culture (7–7). In the present study this phenomenon has been further investigated in met SV40-transformed human fibroblasts. We previously isolated rare met revertants of these met transformants, which had reverted toward normal with regard to several transformation parameters (9, 10). Furthermore, we were unable to distinguish as a class the met lines from met diploid fibroblasts or revertants when we measured the activities of methionine biosynthetic enzymes and the in vivo incorporation of labeled precursor into methionine (7, 9). Here we report that comparative studies of the regulation of MetAT in these various lines reveal differences in enzyme levels. Whereas the derepression of MetAT in methionine-deficient medium is noted in all of the cultures, supplementation of the low-methionine medium with homocysteine thiolactone results in an increased derepression in the met cells and an inhibition of the derepression in the normal diploid and revertant cells, both of which are met. One revertant line, R1, was variable in its response, but MetAT derepression inhibitory effects of homocysteine thiolactone were noted as early as 48 hours in experiments of the type described in text-figure 4. The simplest explanation for the inhibition of the derepression in these met cells in the presence of homocysteine thiolactone is increased methionine production, but when the response of the met cells is considered, the causes appear to be more complex because even extensively reduced homocysteine remethylation in these cells would not be expected to produce less available methionine for regulation than is produced in low-methionine medium alone.

The biochemical basis of these apparent regulatory differences is unknown; possibly, changes in the activity and/or kinetics of one or more of the enzymes that metabolize homocysteine (homocysteine methyltransferase, S-adenosylmethionine hydrolase, and cystathionine synthetase) might result in elevated levels of AdoHcy. An increase in the intercellular concentration

Text-figure 5.—MetAT specific activity in normal diploid and SV10-transformed human fibroblasts. Cultures growing in stock medium containing 100 μM methionine were shifted to basal medium containing various concentrations of methionine. MetAT activity was determined 48 hr subsequent to the shift. Each data point represents the average of paired determinations in which either of the determinations varies from the average by ±15%, with the exception of a) in which the 10⁻⁷ M methionine point has a variation of 21%.

synthesis inhibitors on MetAT regulation, first described by Caboche in studies in which BHK cells were used (12). Using AMD, Caboche (12) and Caboche and Mulsant (18) obtained evidence for a posttranscriptional role for methionine or one of its metabolites in the regulation of MetAT. In several reported experiments AMD was shown to affect protein synthesis by mechanisms other than its presumed major effect as an inhibitor of transcription (21–24). Thus we believed that it was important to examine the effect of other RNA synthesis inhibitors acting by different mechanisms before drawing conclusions about these aspects of MetAT regulation. MPB, a purine analog, has been shown to rapidly inhibit RNA synthesis as judged by decreased incorporation of RNA precursors in mouse fibroblast cultures (25), rat hepatoma cell cultures (14), and fetal rat liver explants (26). The inhibitor α-AM is a toxin derived from the toadstool Amanita phalloides that has been reported to inhibit RNA synthesis by inhibiting the activity of RNA polymerase II (16). We have shown that all three of these inhibitors block the incorporation of [⁹H]uridine into TCA-insoluble material in diploid human fibroblasts cultured in low-methionine medium. We also found that methionine blocks the derepression of MetAT in diploid human fibroblasts even when it is added at times when this derepression is no longer inhibited by AMD and α-AM and is partially inhibited by MPB. Because these inhibitors have different modes of action but similar
of AdoHcy might lower the AdoMet/AdoHcy ratio, alter cellular methylation patterns (27), and result in the increase of MetAT activity observed in the fully transformed cells. Sometimes these changes might be accompanied by the met phenotype that we observed in two of the three transformed lines. Also, the cell's ability to utilize homocysteine thiolactone rather than homocysteine (or to convert the thiolactone to the reduced amino acid) might differ in met and met cells. Further progress in the clarification of the biochemical basis of the met phenotype requires measurement of the intracellular pools of AdoMet, AdoHcy, homocysteine thiolactone, homocysteine, and methionine and the determination of the relative abilities of these cells to utilize homocysteine and homocysteine thiolactone for growth and, more particularly, as a substrate for homocysteine-requiring enzymes.

One other observation has potential significance with regard to the existence of markers for cancer. One of the SV40-transformed lines investigated was WI-38 VA 13, an SV40-transformed WI-38 line that unlike P1 and P5 has a met phenotype. This example serves as a further reminder that the met phenotype and neoplastic state do not always occur together. However, all three of these fully transformed lines derepress MetAT to higher levels during the first 48 hours in low-methionine medium containing homocysteine than in low-methionine medium alone. This effect was never observed in diploid fibroblasts. SV40-transformed cells may possess abnormalities in homocysteine and/or methionine metabolism that are not always expressed as met.

The present study suggests that the regulation of MetAT biosynthesis differs in normal and some SV40-transformed human fibroblasts that are cultured under conditions that require the utilization of homocysteine thiolactone for methionine biosynthesis. Furthermore, the patterns of derepression observed under these conditions appear to be a sensitive indicator of the flux of sulfur metabolism intermediates, and changes in this flux may be causally related to the met phenotype and transformation.

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