Biochem. J. (1992) 281, 533–538 (Printed in Great Britain)

Deficiency of 5′-deoxy-5′-methylthioadenosine phosphorylase activity in malignancy

Absence of the protein in human enzyme-deficient cell lines

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The absence of 5′-deoxy-5′methylthioadenosine phosphorylase (MTAase) activity in malignant cells, and the putative localization of its gene, suggest that this enzyme deficiency might be due to a genomic alteration also involving a tumour-suppressor gene. We studied the possible occurrence of inactive forms of the protein in two MTAase-negative cell lines, namely K562 and Jurkat, by immunochemical methods. Two highly specific antisera, directed against different epitopes of the phosphorylase [Della Ragione, Oliva, Gragnaniello, Russo, Palumbo & Zappia (1990) J. Biol. Chem. **265**, 6241–6246], were used to carry out immunotitration and immunoblotting analyses, as well as to investigate the biosynthesis of the enzyme. No MTAase protein was detected by Western-blotting technique performed under conditions where all the phosphorylase-positive samples gave a clear band at the MTAase subunit molecular mass. No cross-reacting material was observed by a sensitive immunotitration method which permitted the detection of as low as 0.5 ng of protein. Moreover, the results obtained by [^{35}S]methionine-labelling experiments ruled out phosphorylase biosynthesis in the negative cell lines. Altogether, these data suggest that an alteration at the gene level hampering the specific mRNA biosynthesis or resulting in an untranslatable mRNA is the cause of the enzyme deficiency in the MTAase-negative cell lines studied.

INTRODUCTION

Malignant transformation is due to mutations that modify the mechanisms regulating normal cellular growth and development. These alterations include the somatic activation of cancer-promoting genes (cellular oncogenes; Bishop, 1987) and the germ-line or somatic inactivation of tumour-suppressor genes, also known as anti-oncogenes or recessive oncogenes (Weinberg, 1989).

The identification of oncogenes is greatly facilitated by their ability to transform appropriate host cells (Reddy *et al.*, 1988), but the search for anti-oncogenes is remarkably complicated by the lack of strong selection procedures. However, detailed molecular-genetic studies (Friend *et al.*, 1986; Lee *et al.*, 1987; Baker *et al.*, 1989; Rose *et al.*, 1990; Fearon *et al.*, 1990; Call *et al.*, 1990) have demonstrated that the loss of function of tumour-suppressor genes is involved in the pathogenesis of a large number of malignancies.

In this scenario, it is of interest that more than 20% of human cell lines derived from various tumours (Kamatani *et al.*, 1981) and about 10% of acute lymphoblastic leukaemias (Kamatani *et al.*, 1982; Traweek *et al.*, 1988) are devoid of 5′-deoxy-5′-methylthioadenosine phosphorylase (MTAase) activity. Conversely, this enzymic activity has been found in all normal tissues and cell lines of non-malignant origin investigated so far (Toohey, 1977; Zappia *et al.*, 1978; Kamatani *et al.*, 1981; Della Ragione *et al.*, 1986).

MTAase (5′-deoxy-5′-methylthioadenosine:orthophosphate methylthioribosyltransferase, EC 2.4.2.28) catalyses the phosphorolytic cleavage of 5′-deoxy-5′-methylthioadenosine (MTA), a sulphur-containing adenosyl nucleoside formed from *S*-adenosylmethionine by several independent pathways (Zappia *et al.*, 1980; Williams-Ashman *et al.*, 1982; Della Ragione *et al.*, 1989). The reaction products, namely adenine and 5-methylthioribose 1-phosphate, are then recycled to AMP and to methionine respectively (Backlund & Smith, 1981; Williams-Ashman *et al.*, 1982). Therefore, the enzyme presumably plays a key role in a purine salvage pathway and in the recycling of methylthio groups (Della Ragione *et al.*, 1989).

By means of mouse–human somatic-cell hybridization studies, the putative gene for the phosphorylase has been mapped at the 9pter–9q12 region (Carrera *et al.*, 1984). Since non-random abnormalities at 9p have been reported in several malignancies, including acute lymphoblastic leukaemia (Murphy *et al.*, 1989), non-Hodgkin lymphoma (Diaz *et al.*, 1988), melanoma (Cowan *et al.*, 1986) and malignant glioma (Bigner *et al.*, 1988), it can be hypothesized that a chromosomal aberration encompassing the phosphorylase gene and a tumour-suppressor gene might be responsible for the occurrence of the enzymic defect exclusively in malignant cells. It should be underlined that the presence of an anti-oncogene on the short arm of chromosome 9 has also been suggested by chromosomal microcell-transfer studies (Sager, 1989).

So far the phosphorylase deficiency has been investigated by enzymic assay *in vitro* or by autoradiographic methodologies *in vivo* which indirectly evidentiate MTA cleavage (Kamatani *et al.*, 1981, 1982; Traweek *et al.*, 1988). On the other hand, no detailed investigation has been carried out on the possible presence of inactive form(s) of the phosphorylase in cell lines lacking this enzymic activity.

In order to acquire further insight into the molecular mechanism(s) responsible for MTAase deficiency, we have purified the enzyme to homogeneity and raised high-titre antisera (Della Ragione *et al.*, 1990). The present paper describes an

Abbreviations used: MTA, 5′-deoxy-5′-methylthioadenosine; MTAase, 5′-deoxy-5′-methylthioadenosine phosphorylase; PBS, phosphate-buffered saline (120 mM-NaCl/2.7 mM-KCl/10 mM-sodium phosphate, pH 7.4); AKM1 serum, anti-(keyhole-limpet haemocyanin–MTAase) serum; APM serum, anti-(polymeric MTAase) serum; PNP, purine nucleoside phosphorylase.

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immunochemical study of the possible synthesis of modified forms of the protein in two human malignant cell lines lacking the phosphorylase activity, namely K562 and Jurkat. The results are discussed in the light of the possible chromosomal linkage between the MTAase gene and a recessive oncogene.

EXPERIMENTAL

Materials

S-Adenosylmethionine was prepared from *Saccharomyces cerevisiae* and isolated by ion-exchange chromatography (Zappia *et al.*, 1968). *S*-Adenosyl-L-[*methyl*-^{14}C]methionine, [^{35}S]methionine and [^{32}P]P_i were supplied by The Radiochemical Centre, Amersham, Bucks., U.K. MTA and [*methyl*-^{14}C]-MTA were prepared from unlabelled and labelled *S*-adenosylmethionine respectively (Schlenk & Ehninger, 1964) and purified by h.p.l.c. (Della Ragione *et al.*, 1981). Keyhole-limpet haemocyanin, Protein A–Agarose, alkaline-phosphatase-conjugated goat anti-rabbit IgG, 3-bromo-4-chloro-3-indolyl phosphate, Nitro Blue Tetrazolium, cycloheximide, actinomycin D, phenylmethanesulphonyl fluoride and leupeptin were supplied by Sigma, St. Louis, MO, U.S.A. All materials for cell culture were from Flow Laboratories, Irvine, Scotland, U.K.

Cells and cell lines

Cultures of human fibroblasts were established in our laboratory (A. Oliva, unpublished work). K562 and Jurkat cells were kindly given by Dr. G. Scala and Dr. C. Turco, University of Naples. HeLa cells were generously donated by Dr. A. Mogavero, University of Naples. A431 cells were obtained from the American Type Culture Collection. Samples of human leukaemic cells were from Dr. A. Pinto, Centro di Riferimento Oncologico, Aviano, Italy.

Cell culture conditions

Human fibroblasts, HeLa and A431 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal-calf serum and penicillin/streptomycin in a 5%-CO_2 atmosphere at 37 °C in a humidified incubator. K562 and Jurkat cells were grown in suspension culture under the conditions mentioned above, except that DMEM was replaced by RPMI 1640. The experiments carried out to investigate the half-life of MTAase were performed by incubating cell lines in a medium containing cycloheximide (100 μg/ml) or actinomycin D (5 μg/ml). At various times after addition of the compound, the cells were scraped off and the extract was prepared as described in the relevant section.

Radioisotopic labelling and immunoprecipitation

[^{35}S]Methionine labelling of monolayer cultures of HeLa cells was performed by incubating cells in serum-free methionine-free MEM supplemented with [^{35}S]methionine (200 μCi/ml) for 4 h. After radiolabelling, the plates were washed several times with phosphate-buffered saline (PBS)/0.4 mM EDTA. The cells were then scraped, pelleted at 800 *g* for 15 min and homogenized by freeze–thawing in 50 mM-Tris/HCl (pH 7.4)/200 mM-NaCl/50 mM-NaF/5 mM-EDTA/0.1% Triton X-100/0.1 mM-$NaVO_4$. The supernatant was then immunoprecipitated as described by Draetta *et al.* (1987). Cell lines growing in suspension (K562 and Jurkat) were similarly treated, except that each change of medium was carried out by centrifuging the cells at 800 *g* and resuspending them in the fresh medium.

Pulse-labelling experiments with [^{32}P]P_i were performed by the same protocol, except that phosphate-free medium with 300 μCi of labelled compound/ml was used.

Preparation of cell extracts

A431, HeLa and human fibroblasts were left to grow to 70% confluency in 100 mm-diam. dishes. After removal of the medium, the cultures were washed three times with cold PBS. The cells were then scraped from the dishes in 2 ml of PBS and centrifuged at 800 *g*. The cell pellet was resuspended at a density of approx. 15×10^7 cells/ml in 20 mM-potassium phosphate (pH 7.4)/1 mM-phenylmethanesulphonyl fluoride/1 mM-leupeptin/1 mM reduced dithiothreitol, and homogenized by three cycles of freeze–thawing. The 15000 *g* supernatant was employed for both immunotitration analysis and immunoblotting studies. In some of the experiments reported in Table 1, the proteinase inhibitors were omitted without any appreciable difference in the enzyme activity.

Protein purification and enzyme assays

Human placenta and bovine liver MTAases were purified to homogeneity by previously reported procedures (Della Ragione *et al.*, 1986, 1990). Highly purified preparation of purine nucleoside phosphorylase (PNP) from bovine spleen was supplied by Sigma. The activity of MTAase was determined by measuring the formation of 5-[*methyl*-^{14}C]methylthioribose 1-phosphate from [*methyl*-^{14}C]MTA (Della Ragione *et al.*, 1986). The activity of PNP was calculated by measuring the formation of hypoxanthine from inosine (Della Ragione *et al.*, 1990). One unit of the enzyme is defined as the amount of protein which catalyses the phosphorolytic cleavage of 1 μmol of the substrate at 37 °C in 1 min.

Preparation and purification of anti-MTAase antisera

Polymeric MTAase and MTAase linked to keyhole-limpet haemocyanin were prepared as reported by Della Ragione *et al.* (1990). The two antigens were injected subcutaneously in New Zealand female rabbits in complete Freund's adjuvant (0.1 mg of MTAase for each antigen). Three subsequent injections were given at intervals of 4 weeks, and the rabbits were bled 7 days after the last injection. The IgG fraction of each antiserum was purified on a Protein A–agarose column (Della Ragione *et al.*, 1990).

PAGE

SDS/PAGE was carried out at room temperature by using either 12.5%- or 15%-acrylamide resolving gels and 5%-acrylamide stacking gels (Weber *et al.*, 1982). Gradient gels were prepared as described by Draetta *et al.* (1987). After the electrophoretic separation, gels were either stained in 0.1% Coomassie Blue or used for immunoblotting. In some cases the gels were dried after the staining and used for autoradiography.

Immunoblotting

After separation by SDS/PAGE, the proteins were electrophoretically transferred overnight to a nitrocellulose sheet at a constant current of 40 mA in a Bio-Rad Trans-Blot cell. The transfer was performed in 20 mM-Tris/HCl (pH 8.3)/190 mM-glycine/20% (v/v) methanol. After blocking of the unbound sites with gelatin, the filter was incubated for 2 h with the antiserum against MTAase diluted 1:1000 with Tris-buffered saline containing 1% gelatin. Finally, the immunocomplexes were revealed by employing alkaline-phosphatase-labelled goat anti-rabbit IgG by following the manufacturer's instructions.

RESULTS

Specificity of antisera

Preliminary experiments demonstrated that native MTAase is scarcely immunogenic. Therefore three different modified forms

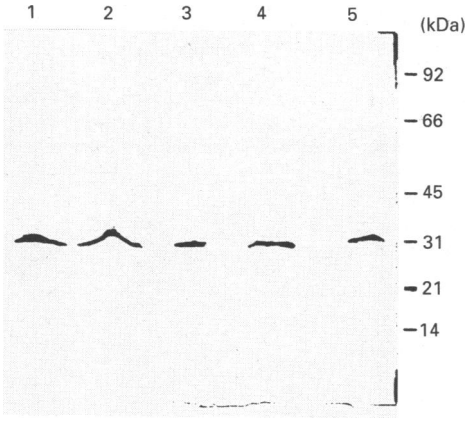

Fig. 1. Immunoblotting analysis of various bovine tissue extracts by AKM1 serum

Lane 1, liver; lane 2, spleen; lane 3, pure liver enzyme; lane 4, testis; lane 5, brain. Each lane contains about 100 μg of 15000 *g* supernatant, except lane 3, which contains 50 ng of homogeneous MTAase. Samples were run on a 12.5%-polyacrylamide gel under denaturing conditions, transferred to nitrocellulose paper and then processed as in the Experimental section.

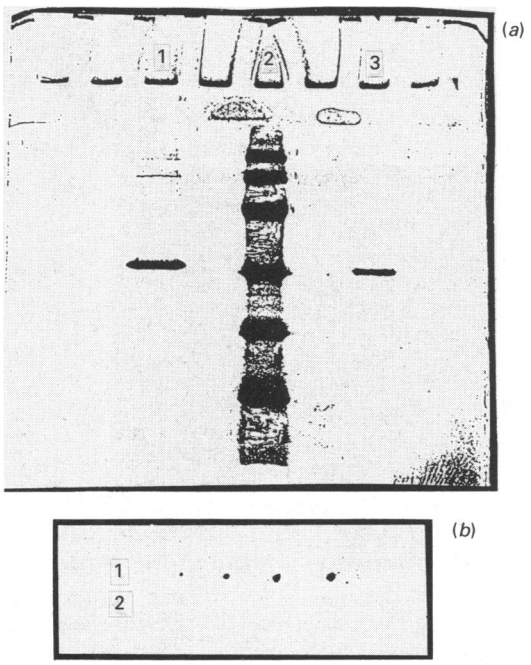

Fig. 2. (*a*) SDS/PAGE analysis of highly purified bovine spleen PNP and pure bovine liver MTAase, and (*b*) dot-blot analysis of bovine liver MTAase (row 1) and bovine spleen PNP (row 2)

(*a*) Lane 1, 5 μg of PNP; lane 2, molecular-mass standards (from top to bottom: 94 kDa, 67 kDa, 43 kDa, 30 kDa, 20 kDa, 14 kDa); lane 3, 3 μg of pure MTAase. (*b*) From left to right: 1 ng, 5 ng, 10 ng and 30 ng of enzyme sample.

of the bovine protein, namely polymeric MTAase, haemocyanin–MTAase and agarose-linked MTAase were employed to prepare polyclonal antibodies (Della Ragione *et al.*, 1990). Among the antisera obtained, two showed a remarkably high titre, i.e. one of those directed to haemocyanin–MTAase (AKM1) and the other raised against polymeric MTAase (APM). Moreover, an initial characterization demonstrated that these sera reacted with different epitopes of the protein, in that AKM1 was able to

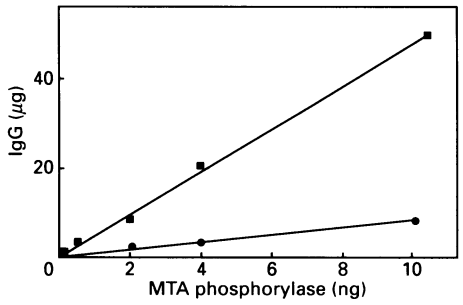

Fig. 3. Immunoprecipitation standard curve for MTAase estimation

Different amounts of human placenta (■) and bovine liver (●) MTAase were immunoprecipitated by different quantities of AKM1. The amount of IgG which gave 50% precipitation was then plotted against MTAase amounts. The results are averages of three different estimations, with a variability of about 4%.

recognize both the native and the denatured form of the enzyme, whereas APM reacted only with the denatured phosphorylase (Della Ragione *et al.*, 1990). Because of their different behaviour, both antisera were employed for the search for inactive forms of MTAase in enzyme-deficient cells by means of immunoblotting and immunotitration methods.

When AKM1 was used to analyse the 15000 *g* supernatant of several bovine tissues, only one band, at 30–32 kDa, was obtained, which corresponded perfectly to that of pure bovine MTAase standard (Fig. 1). A densitometric analysis of bands, which allows a rough estimation of the protein content, gave results comparable with those calculated by enzymic assay (not shown). This result suggested that the phosphorylase was the only protein detected by the antiserum.

However, since several structural features are shared by mammalian MTAase and PNP (Della Ragione *et al.*, 1990), cross-reactivity between anti-MTAase antibodies and PNP could be hypothesized. As shown in Fig. 2(*a*), SDS/PAGE analysis of pure bovine liver MTAase (lane 3) and a highly purified preparation of bovine spleen PNP (lane 1) showed an identical subunit molecular mass. In contrast, no significant cross-reactivity of the spleen phosphorylase towards APM (Fig. 2*b*, row 2) or AKM1 (results not shown) was observable by dot-blot analysis.

AKM1 serum, which also reacts with native MTAase, was used to set up a standard curve for quantification of the protein by immunotitration. In particular, increasing amounts of the pure bovine or human phosphorylase were immunoprecipitated with different quantities of immunoglobulins, and the results were plotted as the amount of IgG needed to obtain a 50% loss of activity (Fig. 3). The standard curve generated was linear in the range of 0.5–10 ng of MTAase, with a slope indicating the immunoprecipitation of 1 ng of human enzyme by 6 μg of IgG.

Immunological analysis of MTAase in human cell lines

Different mechanisms can explain the absence of MTAase activity in human malignant cell lines. First, the cells might contain some inhibitor interfering with the enzymic activity. Second, the protein might not be synthesized as a consequence of a genetic alteration hampering gene transcription or resulting in the production of untranslatable or unstable mRNA. Finally, the deficient cells might synthesize an inactive form of the protein. In the latter case, immunological analyses should show the occurrence of cross-reacting material in the negative cell lines.

As shown in Table 1, addition of large amounts of two MTAase-deficient cell extracts (K562 and Jurkat) to the pure

Table 1. Effect of MTAase-deficient cell-line extracts on the phosphorylase activity

Phosphorylase source: [a]1 ng of human pure placental enzyme; [b]500 μg of K562 or Jurkat cell extracts; [c]25 μg of HeLa cell extract; [d]samples preincubated for 2 h at 37 °C as reported in the Experimental section. The results are averages of two different estimations, with a variability of less than 5%.

Phosphorylase source	Cell extract added	Enzyme activity (μ-units)
Human placenta[a]	None	10.7
K562[b]	None	0
Jurkat[b]	None	0
Human placenta	K562	12
Human placenta	Jurkat	11
HeLa[c]	None	30
HeLa	K562+Jurkat	32
Human placenta[d]	None	8
Human placenta[d]	K562+Jurkat	8.3
HeLa[d]	None	28
HeLa[d]	K562+Jurkat	29

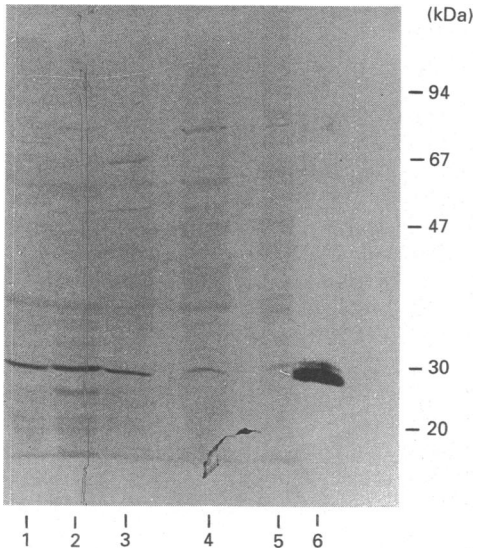

Fig. 4. Immunoblotting analysis of various MTAase-containing human cells by AKM1 serum

Lane 1, HeLa cells; lane 2, A431 cells; lane 3, human fibroblasts; lanes 4 and 5, human leukaemic samples; lane 6, human placenta pure enzyme. Each lane contains about 500 μg of 15000 *g* supernatant, except lane 6, which contains 200 ng of the homogeneous protein. On the right-hand side, masses of protein markers are indicated in kDa. The experiments were carried out as described in Fig. 1.

protein or to a MTAase-containing cell homogenate (HeLa) did not modify the enzymic activity. This was also observed when the various mixtures were preincubated at 37 °C for 2 h. These results permitted the exclusion of both a possible enzyme inhibitor and highly active proteinases in the enzyme-deficient cells.

When various MTAase-positive human cells were analysed by immunoblotting, a band at 30–32 kDa was clearly seen. Fig. 4 reports a Western-blot analysis of normal and malignant cells, i.e. HeLa cells (lane 1), A431 (lane 2), human fibroblasts (lane 3), two samples of blast cells from peripheral blood of leukaemic patients (lanes 4 and 5), and a phosphorylase standard (lane 6).

Table 2. MTAase content in cell-line extracts determined by enzymic assays and immunotitration method

MTAase activity was determined by enzymic assay; MTAase content calculated by immunotitration method. The results are averages of three different experiments with a variability of less than 5%.

Source	$10^4 \times$ MTAase activity (units/mg)	MTAase content (ng/mg)
HeLa	11	103
Human fibroblasts	7	60
A431	18	173
Jurkat	0	< 0.5
K562	0	< 0.5

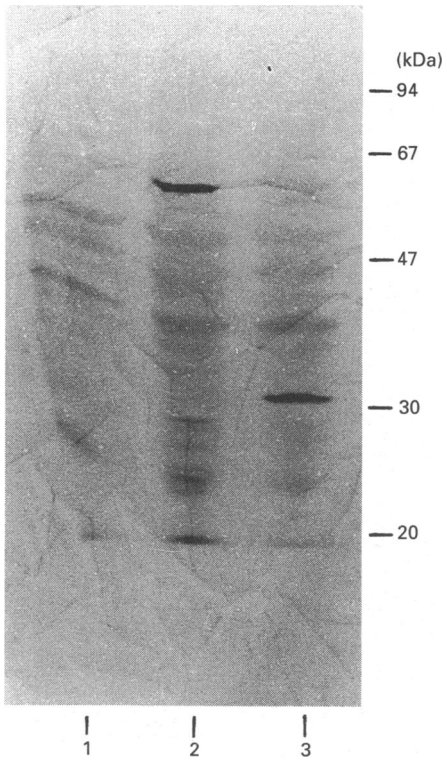

Fig. 5. Immunoblotting analysis of human cell lines by AKM1 serum

Lane 1, K562; lane 2, Jurkat; lane 3, HeLa. Each lane contains 500 μg of 15000 *g* supernatant. On the right-hand side, masses of protein markers are indicated in kDa. The experiments were carried out as in Fig. 1.

To obtain a precise estimation of MTAase content, an immunotitration analysis was carried out on enzyme-positive and -negative extracts by employing AKM1 serum (Table 2). The results obtained for the MTAase-containing samples are in good agreement with those of immunoblotting. Moreover, no cross-reacting material was demonstrable in K562 and Jurkat cells, at least within the sensitivity range of the method.

Fig. 5 shows a Western blot of negative cell lines and of HeLa cells. Although a clear band was detected in the positive cells (lane 3), no band was observed in the same area in K562 and Jurkat cell lines (lanes 1 and 2 respectively). In Jurkat cells a strong band was seen at higher molecular mass. However, the same band was detected with a control serum, and therefore it is not related to possible cross-reacting material (results not shown).

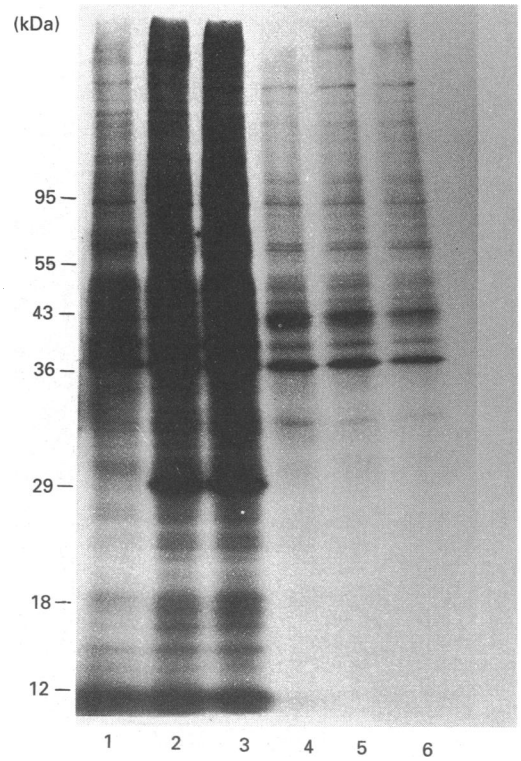

Fig. 6. Immunoprecipitation analysis of ^{35}S-labelled cell extracts

Lanes 1–3 contain labelled HeLa-cell extracts; lanes 4–6 contain labelled Jurkat-cell extracts. Lanes 1 and 4 were immunoprecipitated with a control serum, and lanes 2, 3, 5 and 6 with AKM1 serum. Each immunoprecipitate was run on a gradient (7.5–15%) polyacrylamide gel under denaturing conditions. The gel was then dried and analysed as described in the Experimental section. On the left-hand side, masses of protein markers are indicated in kDa.

Biosynthesis of MTAase protein

The absence of the phosphorylase protein in the deficient cells might be explained by several alterations involving any step of the protein-synthetic process, namely: (1) a genetic aberration that leads to a lack of MTAase gene expression, (2) synthesis of a non-functional mRNA, or (3) synthesis of an unstable protein. In order to evaluate the last possibility, the biosynthesis of MTAase was investigated in phosphorylase-positive (HeLa) and -negative cells (K562 and Jurkat) by pulse-labelling experiments with [^{35}S]methionine.

To select the labelling time, preliminary experiments on the half-life of the protein were carried out by employing actinomycin D and cycloheximide as inhibitors of protein synthesis. The extrapolated value in phosphorylase-containing cells, i.e. HeLa, A431 and human fibroblasts, was higher than 48 h (results not shown). Therefore, owing to the long turnover, a labelling period of 4 h was used.

HeLa and Jurkat cells were incubated with high-specific-radioactivity [^{35}S]methionine, and the cell extracts were precipitated with AKM1 serum. The immunoprecipitated material was then analysed by SDS/PAGE and made visible by autoradiography after a prolonged exposure. As shown in Fig. 6, a specific band was observed at 30 kDa (lanes 2 and 3) with the HeLa extracts. Conversely, no band was observed in Jurkat extracts at this molecular mass (lanes 5 and 6). Lanes 1 and 4 contain HeLa and Jurkat extracts immunoprecipitated with control sera. An identical result was obtained for the K562 cell line (results not shown).

In additional experiments, sub-confluent cultures of HeLa and Jurkat cells were grown in the presence of [^{32}P]P_i for 2 h, immunoprecipitated with AKM1 and analysed by SDS/PAGE. No labelling was observed in the 30–32 kDa area, thus indicating the absence of post-synthetic phosphorylation of the protein (results not shown).

DISCUSSION

For several years, mutations of dominantly acting oncogenes have been the major, if not the only, object of studies on the molecular mechanisms responsible for malignant transformation. Conversely, as discussed above, several lines of evidence demonstrate that genetic alterations of tumour-suppressor genes are also critical steps in the route of cancer formation.

In this light, the observed lack of expression of specific genes in cell lines of malignant origin, as well as in tumour specimens, in contrast with the normal counterpart, acquires considerable interest, either if the gene involved is itself a recessive oncogene or if it is strictly linked to an anti-oncogene, as in the case of the linkage between the esterase D and the retinoblastoma tumour-suppressor gene. The absence of MTAase activity in a remarkable percentage of malignant cells and cell lines might therefore have some relevance in studies on the process of carcinogenesis.

This paper reports some investigations on the molecular basis of the phosphorylase deficiency in two malignant cell lines, namely K562 and Jurkat. In particular, by immunochemical techniques we demonstrate that the absence of enzymic activity is due to the lack of the protein itself and not to the occurrence of inactive or unstable forms of the enzyme.

Several pieces of evidence hint at this conclusion. Indeed, the cell lines did not contain any inhibitor or proteinase that might inactivate endogenous MTAase (Table 1). No MTAase protein was detected by Western-blotting analysis employing two highly specific antisera raised against different immunogenic forms of the protein (Fig. 5), and an identical result was obtained by using a sensitive immunotitration method (Table 2). No labelled protein was observed when the biosynthesis of MTAase was investigated by pulse-labelling experiments with [^{35}S]methionine (Fig. 6).

Two different mechanisms might explain the absence of phosphorylase protein in K562 and Jurkat cells: (i) the negative cells present an alteration of MTAase gene which prevents the phosphorylase synthesis, or (ii) the deficiency is due to some epigenetic mechanism(s) that hamper(s) the expression of a normal MTAase gene. It could indeed be hypothesized that the malignant deficient cells derive from a normal counterpart that is in a particular stage of differentiation lacking MTAase protein.

Although the latter hypothesis deserves further investigation, it appears quite unlikely, since MTAase must be envisioned as a housekeeping enzyme. Indeed, it shows a long turnover rate (Seidenfeld *et al.*, 1981; the present paper) and is scarcely inducible (Williams-Ashman *et al.*, 1982). Therefore the presence of primary genetic alteration(s) appears to be the most probable cause of the enzyme deficiency.

The MTAase gene has been localized in an area between 9pter and 9q12 (Carrera *et al.*, 1984). As reported by Diaz *et al.* (1988), K562 cells show a homozygous deletion of the α- and β-interferon genes that have been assigned to the 9p21–22 region. Therefore, it is likely that the phosphorylase gene maps in this chromosomal region, which also represents a known fragile site and the location of non-random abnormalities in several tumours (Murphy *et al.*, 1989).

The homozygous deletion of α- and β-interferon genes in a number of tumour cells (Diaz *et al.*, 1988; Miyakoshi *et al.*, 1990) suggests that the interferon gene(s) itself might be the putative tumour-suppressor gene (Diaz *et al.*, 1988). However, although

in several malignant cell lines the deletion of interferon genes is associated with the loss of MTAase activity, in some others the phosphorylase deficiency is observed in the presence of normal interferon genes (Diaz *et al.*, 1988). This finding suggests that the interferon and MTAase genes might be localized in a region also containing a putative tumour-suppressor gene, and that the phosphorylase gene is closer to the anti-oncogene than the interferon(s) coding sequences.

Work is needed to obtain molecular probes for investigating the genetics of MTAase and the possible relationship between its gene and interferon and tumour-suppressor genes.

We are grateful to Dr. Giulio Draetta for his help in the pulse-labelling experiments. This work was supported by grants from the Italian Ministry of Education and the National Research Council, Projects 'Oncologia' and 'Ingegneria Genetica'. R.P. is the recipient of a research fellowship from the 'Associazione Italiana per la Ricerca sul Cancro'.

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Received 12 June 1991/13 August 1991; accepted 3 September 1991