Cellular Proteins That Associate with the Middle and Small T Antigens of Polyomavirus

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We have used two-dimensional gel electrophoresis to analyze in more detail the cellular proteins which associate with the middle and small tumor antigens (MT and ST, respectively) of polyomavirus. Proteins with molecular masses of 27, 29, 36, 51, 61, 63, and 85 kilodaltons (kDa) that specifically coimmunoprecipitated with MT were identified on these gels. The 36-, 51-, 61-, 63-, and 85-kDa proteins are probably the same as the proteins of similar sizes previously reported by a number of groups, whereas the 27- and 29-kDa proteins represent proteins that are heretofore undescribed. The 27- and 29-kDa proteins were abundant cellular proteins, whereas the others were minor cellular constituents. The association of each of these proteins with MT was sensitive to one or more mutations in MT that rendered it transformation defective. The association of the 85-kDa protein was the most sensitive indicator of the transformation competence of MT mutants. In addition, the 85-kDa protein was the only associated protein whose association with MT changed consistently in parallel with MT-associated phosphatidylinositol kinase activity. Furthermore, the fraction of the 85-kDa protein which was found associated with the MT complex contained 15 to 20% of its phosphate content on tyrosine. The 36- and 63-kDa proteins complexed with both polyomavirus MT and ST and comigrated on two-dimensional gels with two simian virus 40 ST-associated proteins originally described by Rundell and coworkers (K. Rundell, E. O. Major, and M. Lampert, *J. Virol.* 37:1090-1093, 1981). None of the other MT-associated proteins associated significantly with ST.

The early region of polyomavirus encodes three proteins, designated by size as the small tumor (ST), middle tumor (MT), and large tumor antigens. The action of MT is central to polyomavirus-mediated transformation and tumorigenesis (28, 31, 38, 39). MT is a plasma membrane-associated phosphoprotein of 421 amino acids, the amino-terminal 79 of which are identical to the amino termini of ST and the large T antigen (for a review, see reference 31). Although MT has no known intrinsic enzymatic activity, two different kinase activities have been found associated with it: a protein tyrosine kinase activity (11, 32, 36) and a phosphatidylinositol (PI) kinase activity (41). MT is known to associate with two cellular tyrosine kinases: pp60^{c-src} (9) and pp62^{c-yes} (18). The association results in the activation of the tyrosine kinase activity that is necessary but not sufficient for transformation (2, 7, 18). The PI kinase activity which is associated with the MT-pp60^{c-src} complex has received particular attention because it correlates extremely well with transforming ability (16, 41). However, the activity derives from neither MT nor pp60^{c-src} (15, 16, 27; unpublished data). In addition to the association with kinases, MT has also been reported to copurify with other proteins (12, 23, 30, 33-35).

To identify and analyze cellular proteins that can specifically coimmunoprecipitate with wild-type MT (MT-associated proteins or MTAPs), we used two-dimensional (2D) gel electrophoresis. 2D gels separate the MTAPs from proteins which comigrate on one-dimensional gels, thereby allowing the examination of all the MTAPs at once. In this article, we report the identification and analysis of two

previously unreported MTAPs and show that the 36- and 63-kilodalton (kDa) MTAPs reported in the polyomavirus literature associate with the STs and MTs of polyomavirus independently and that these two proteins comigrate on 2D gels with the 32- and 56-kDa simian virus 40 (SV40) ST-associated proteins previously described by Rundell and co-workers (30). Additionally, we show that the 85-kDa MTAP is the only one whose association with MT consistently changes in parallel with MT-associated PI kinase activity and that the 85-kDa MTAP is phosphorylated on tyrosine in the MT complex isolated from cells expressing MT.

MATERIALS AND METHODS

Cell culture. All cell lines were constructed by introducing cDNAs for individual T antigens into NIH 3T3 cells by means of retrovirus vectors (6). Cells that express wild-type MT and cells that express ST have been described previously (6). Cell lines that express polyomavirus *dl1015* (20), *dl23* (37), and NG59 (3) mutant MTs are described elsewhere (22). A control cell line was established by using a control retrovirus expressing only G418 resistance. The c3 and c14 mutant MTs are spontaneous deletions of yet undetermined location which originated upon infection of a rat embryo fibroblast line with the MT12 retrovirus vector for MT (6). Infectious retrovirus was rescued from the rat embryo fibroblast lines by fusing them with *psi2* packaging cells by using a fusion protocol described previously (5). Resultant retrovirus stocks were used to infect NIH 3T3 cells. Multiple G418-resistant colonies were pooled to generate populations expressing the c3 and c14 mutant MTs. These populations expressed mutant MTs which were indistinguishable from the products observed in the original REF52 clones. All cell

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lines were maintained in Dulbecco modified Eagle medium supplemented with 10% calf serum.

Radiolabeling and extraction of cells. For metabolic labeling with methionine, subconfluent 100-mm-diameter dishes of cells were labeled for 3 to 6 h with [^{35} S]methionine (300 μ Ci/ml) in 3 ml of Dulbecco modified Eagle medium minus methionine but supplemented with 0.5% dialyzed fetal bovine serum. For metabolic labeling with phosphate, subconfluent 100-mm-diameter dishes of cells were incubated for 3 h at 37°C in 3 ml of phosphate-free Dulbecco modified Eagle medium containing [^{32} P]phosphate (2 mCi/ml). Cell lysates were prepared as described earlier (41), except that when assay was for the phosphoamino acid content of proteins labeled in vivo, a modified lysis buffer containing 10 mM EDTA, 1 mM sodium vanadate, 20 mM sodium pyrophosphate, and no divalent cations was used to prevent phosphorylation and dephosphorylation during lysis. In control experiments in which parallel dishes of unlabeled cells were lysed in this buffer containing [γ - ^{32} P] ATP, phosphorylation during lysis was not detected.

Immunoprecipitation, protein kinase assays, and PI kinase assays. Polyclonal hamster antiserum directed against SV40 T antigens (29) was a gift of K. Rundell. Polyclonal rabbit antiserum that was prepared against polyomavirus ST and that recognizes all three polyomavirus T antigens (25) was used to immunoprecipitate MT or ST in all experiments. Immunoprecipitates were prepared as described elsewhere (25). Protein kinase and PI kinase assays were performed in vitro as described previously (16).

Gel electrophoresis and fluorography. 2D gel analysis was performed as described earlier (24). Gradients of pH 4.8 to 7.4 were obtained by mixing 400 μ l of pH 3.5 to 10 ampholine (LKB) with 150 μ l of pH 6 to 8 ampholine (LKB) per 10 ml of gel solution. Gels of methionine-labeled proteins were preincubated with En ^{3} Hance before exposure; gels of phosphate-labeled proteins were exposed with intensifying screens. All exposures were on Kodak XAR-5 film at -70°C.

RESULTS

Identification of MTAPs. Immunoprecipitates of MT from [^{35} S]methionine-labeled NIH 3T3 cells expressing wild-type MT (6) and from control cells were analyzed on 2D gels. Proteins with masses of 27, 29, 36, 51, 61, 63, and 85 kDa were present in immunoprecipitates from MT-expressing cells (Fig. 1a) but only in trace amounts in immunoprecipitates prepared from control cells with immune sera (Fig. 1b). To test the possibility that these proteins could be background proteins that were induced in cells expressing MT, we prepared immunoprecipitates from MT-expressing cells with preimmune and immune sera (Fig. 2). If the MTAPs represent MT-induced proteins nonspecifically associating with immunoprecipitates, the two immunoprecipitates should have the same amount of these proteins. However, if the proteins are specifically associating directly or indirectly with MT, they would be present at significantly higher levels in immunoprecipitates prepared with immune serum. Figure 2 shows that the latter possibility is true; all the MTAPs were specifically associating with MT. The number of spots present in the 85-kDa MTAP position varied from 1 (Fig. 2) to 3 in different experiments. Similarly, the number of spots in the 36-kDa MTAP position varied from 1 (Fig. 1) to 3, but in a manner independent of the 85-kDa spots (compare Fig. 1 and 2). The multiplicity of spots did not correlate with the antiserum used (compare Fig. 1 and 2 in which the same antiserum was used) but could reflect small differences in

labeling, in gels, or in modifications of the proteins. 2D gel analysis of Nonidet P-40 lysates both from cells expressing MT and from NIH 3T3 cells in parallel with MT immunoprecipitates revealed that only the 27- and 29-kDa MTAPs comigrated with predominant cellular polypeptides (data not shown).

Genetic evaluation of MTAPs. The relevance of MTAPs to transformation was examined by testing a series of transformation-defective MT mutants (Fig. 1 and Table 1). The relative transformation competence of these mutants, on the basis of growth in soft agar and focus formation, was in the following order: wild-type MT > *d11015* MT > *d123* MT > NG59 MT, which is totally defective (Table 1; 22). *c3* and *c14* MTs failed to induce NIH 3T3 cells to form colonies in soft agar but have not yet been assayed for focus formation. All but NG59 MT incorporated phosphate on tyrosine in vitro. However, only *d11015* and *d123* MTs were associated with wild-type levels of tyrosine kinase activity (4, 16); *c3* and *c14* MTs had less than one-tenth of the wild-type level of associated tyrosine kinase activity (data not shown). The amount of associated PI kinase activity for the various MTs is summarized in Table 1. Like *d123* MT, *c3* and *c14* MTs had less than one-tenth of the wild-type level of associated PI kinase activity.

Each mutant MT was defective in its ability to complex with MTAPs (Fig. 1). The partially transformation-defective mutants *d11015* and *d123* MTs complexed with the 27-, 29-, 36-, 51-, 61-, and 63-kDa MTAPs in amounts similar to those found in immunoprecipitates of wild-type MT (Fig. 1). However, the amount of the 85-kDa protein decreased 2-fold for *d11015* MT and 10- to 20-fold for *d123* MT. Because the decrease in the 85-kDa protein was only twofold for *d11015*, we decided to test independently generated cell lines. Three clonal and two polyclonal populations producing wild-type MT and one clonal and two polyclonal populations producing *d11015* MT were analyzed. Although on average there was 1.5-fold less 85-kDa protein associated with *d11015* MT than with wild-type MT, the ranges of values overlapped (data not shown). The transformation-defective *c3* and *c14* mutant MTs both gave significantly different patterns: in addition to the 85-kDa protein, the 61-, 51-, 29-, and 27-kDa proteins were also greatly reduced or absent, and smaller amounts of the 36- and 63-kDa MTAPs complexed with these mutants than with the wild type (Fig. 1e and figure legend). NG59 MT associated only with the 27- and 29-kDa MTAPs (data not shown). In addition, however, NG59 MT associated with the murine 70-kDa heat shock protein, as reported previously by Walter and colleagues (40).

We also wanted to determine whether any of the MTAPs associate with polyomavirus ST. ST may be viewed as a transformation-defective carboxy-terminal deletion mutant of MT with four additional amino acids appended to its carboxy terminus. Immunoprecipitates of ST from [^{35} S]methionine-labeled NIH 3T3 cells expressing only ST (6) were analyzed on 2D gels (Fig. 1f). Comparison of Fig. 1f with Fig. 1a and b reveals that the 36- and 63-kDa MTAPs, but not the other MTAPs, associated with ST. Furthermore, the ratio of the 63-kDa MTAP to the 36-kDa MTAP for ST was roughly the same as for MT.

In vivo phosphorylation of 85-kDa MTAP on tyrosine in cells expressing MT. We have shown previously that the 85-kDa MTAP is found in anti-phosphotyrosine immunoprecipitates from BALB/3T3 cells treated with platelet-derived growth factor and from cells expressing MT (15). However, it is possible that the 85-kDa protein is immunoprecipitated indirectly as part of a complex by this antiserum and does

FIG. 1. Proteins specifically coimmunoprecipitating with polyomavirus MTs and ST. Immunoprecipitates prepared from ^{35}S -labeled cells expressing wild-type (a), *dl1015* (c), *dl23* (d), and c3 (not shown) and c14 (e) MTs and ST (f) and from control cells (b) with polyclonal rabbit anti-T antigen serum (25) were analyzed in parallel by 2D gel electrophoresis, and radiolabeled proteins were visualized by fluorography and autoradiography. The pattern obtained for cells expressing c3 MT was indistinguishable from that obtained for c14 MT. Isoelectric focusing was from right (basic) to left (acidic). The pH values indicated were obtained by measuring the pH of slices from a parallel gel. Arrowheads indicate the positions of MTAPs, and the positions of actin (A) and tubulin (T) are indicated for reference. MT does not focus and therefore is not visible in these gels. The numbers indicate in thousands the molecular weights of MTAPs. The relative amount of MTs in wild type, *dl1015*, *dl23*, c3, and c14 cell lines, as estimated by densitometric scanning of lanes on an autoradiograph of a sodium dodecyl sulfate-polyacrylamide gel of the same immunoprecipitates, is 1:2:4:1:1 when normalized to cell number. To visualize the abundance of the various MTAPs relative to each other, the autoradiographs in the different panels were exposed to roughly equalize the 36- and 63-kDa MTAP spots. The actual ratio of the 63-kDa MTAP to MT, as determined by densitometric scanning of one-dimensional gels, is the same for wild-type, *dl1015*, and *dl23* MTs and approximately fivefold less for c3 and c14 MTs than for the wild type.

FIG. 2. Immune serum requirement for coimmunoprecipitation of MTAPs from MT-expressing cells. Immunoprecipitates prepared from ^{35}S -labeled cells expressing wild-type MT with polyclonal rabbit preimmune or immune serum (25) were analyzed in parallel by 2D gel electrophoresis, and radiolabeled proteins were visualized by fluorography and autoradiography. Isoelectric focusing was from right (basic) to left (acidic). Arrowheads indicate the positions of MTAPs, and the numbers indicate in thousands the molecular weights of MTAPs. The preimmune and immune sera used were from the same animal.

not itself contain phosphotyrosine. The 85-kDa protein from the platelet-derived growth factor-treated cells was analyzed and found to contain phosphotyrosine, but it was difficult to obtain sufficient 85-kDa protein from MT-expressing cells to perform a similar analysis (15). To ascertain directly whether the 85-kDa MTAP is phosphorylated on tyrosine when associated with MT in vivo in cells not treated with platelet-derived growth factor, MT immunoprecipitates from MT-expressing cells labeled with phosphate were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the 85-kDa protein was excised and analyzed for phosphoamino acid content as described earlier (Fig. 3). The 85-kDa protein contained phosphate mainly in the form of phosphoserine; however, it also contained 15 to 20% of its phosphate as phosphotyrosine. MT from the same gel was also found to contain approximately 15% phosphotyrosine (data not shown). The 85-kDa MTAP was phosphorylated

primarily on tyrosine in vitro in MT immunoprecipitates (Fig. 3).

Coimmunoprecipitation of 36- and 63-kDa MTAPs with SV40 ST. Rundell and co-workers (30) showed that two proteins, estimated in their studies to be 32 and 56 kDa, associate with SV40 ST and that proteins of the same size coimmunoprecipitate with polyomavirus T antigens from cells expressing both ST and MT of polyomavirus. They did not determine whether the proteins associating with the T antigens of the two different viruses were identical or whether they associated only with polyomavirus ST, only with MT, or with both. To ascertain whether these 32- and 56-kDa proteins were equivalent to the 36- and 63-kDa MTAPs we observed, lysates from [^{35}S]methionine-labeled BALB/3T3 cells expressing only SV40 ST were immunoprecipitated with anti-SV40 T-antigen antiserum (29) and the immunoprecipitates were analyzed on 2D gels (Fig. 4). The

TABLE 1. Transformation competence of MTs and their ability to associate specifically with MTAPs and PI kinase activity

T antigen expressed in NIH 3T3 cell line	Transformation competence ^a	Association of cellular proteins with T antigen ^b				PI kinase activity ^c
		27 and 29	36 and 63	51 and 61	85	
MT						
Wild type	+	+	+	+	+	+
dl1015	+/-	+	+	+	+ ^d	+
dl23	+/-	+	+	+	-	-
c3 (39 kDa)	- ^e	-	+/-	-	-	-
c14 (43 kDa)	- ^e	-	+/-	-	-	-
NG59	-	+	-	-	-	-
ST	-	-	+	-	-	-
None ^f	-	-	-	-	-	-

^a The corresponding data for wild-type, c3, and c14 MTs and a control retrovirus construct are from transformation assays performed with the cell lines as described elsewhere (6, 22). +, Forms colonies in soft-agar assays and foci in monolayer cultures; -, forms neither soft-agar colonies nor foci in monolayer cultures; +/-, forms some foci but no soft-agar colonies.

^b -, Less than one-tenth of the wild-type level of MTAP; +/-, between one-tenth and one-half of the wild-type level of MTAP; +, greater than one-half of the wild-type level of MTAP. The numbers indicate, in thousands, the molecular weights of the associated proteins.

^c -, Less than one-tenth of the wild-type level of PI kinase activity; +, greater than one-half of the wild-type level of PI kinase activity. The data for wild-type, dl1015, dl23, and NG59 MTs are taken from Morgan et al. (22).

^d See Results.

^e c3 and c14 MTs were negative for inducing growth in soft agar but have not been tested for the ability to induce foci in monolayer culture.

^f Control retrovirus cell line.

FIG. 3. Phosphoamino acid analysis of 85-kDa MTAP. MT immunoprecipitates were prepared from MT-expressing cells labeled with [^{32} P]phosphate and from parallel unlabeled cells with polyclonal rabbit anti-polyomavirus T antigen serum (25). The immunoprecipitates of unlabeled proteins were incubated with [γ - ^{32} P]ATP as described in Materials and Methods, and then all immunoprecipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The 85-kDa MTAP labeled with phosphate in vitro or in vivo was excised from the gel with the aid of an autoradiograph and processed and analyzed for phosphoamino acid content as described previously (24), except that after electrophoresis (left to right) at pH 3.5 the phosphoamino acids were separated in dimension 2 by chromatography (from bottom to top) (13). The mobilities of standards are indicated as follows: P-ser, phosphoserine; P-thr, phosphothreonine; P-tyr, phosphotyrosine.

32- and 56-kDa proteins specifically coimmunoprecipitated with the SV40 ST and comigrated on 2D gels with the 36- and 63-kDa MTAPs (Fig. 4), a result indicating that the proteins are the same and therefore that they bind both to ST and to MT of polyomavirus.

DISCUSSION

In this report we describe a 2D gel analysis of the proteins that can associate either directly or indirectly with MT of polyomavirus. We have identified and analyzed two proteins that were heretofore unknown. We have demonstrated that the 36- and 63-kDa proteins reported in the polyomavirus literature associate with both ST and MT and that these proteins are probably identical to the 32- and 56-kDa SV40 ST-associated proteins described previously by Rundell and co-workers (30). We have demonstrated that the 85-kDa protein is phosphorylated on tyrosine in the complex isolated from cells expressing MT and that it is the only MTAP whose association with MT changes consistently in parallel with MT-associated PI kinase activity. All the MTAPs can be coimmunoprecipitated with our polyclonal rabbit anti-polyomavirus T antigen sera raised against ST purified from bacteria (25) or with rat anti-T ascites (33) (Fig. 1 and data not shown). Whereas the association of MT with these proteins could have occurred during or after extraction, the results of genetic analysis suggest that the interactions may be functionally important. However, the exact functional significance of these proteins remains to be determined. The relative levels of MT and the MTAPs were determined by densitometric scanning of proteins labeled metabolically for 6 h; therefore, our quantitative estimates of the levels of the various MTAPs are subject to potential differences in stabilities, as well as relative methionine content.

Our results extend, and to some extent clarify, previous reports (12, 23, 30, 33, 34). In addition to pp60^{c-src} and pp62^{c-yes}, cellular proteins of 32 to 36 kDa (23, 30, 33–35) and of 61 to 63 kDa (12, 30, 33–35) have been reported to coimmunoprecipitate with polyomavirus T antigens. These proteins were found in anti-tumor ascites immunoprecipitates of T antigens from cells infected with a mutant virus which produces ST and large T antigen but no MT, but these

FIG. 4. Comigration of 36- and 63-kDa polyomavirus MTAPs on 2D gels with SV40 ST-associated proteins. BALB/3T3 cells expressing SV40 ST (1) (A) and NIH 3T3 cells expressing polyomavirus MT (14) (B) were labeled for 5 h with [^{35} S]methionine and lysed, and T antigen (imm) or control immunoprecipitates were prepared with polyclonal antisera (see Materials and Methods) and analyzed on parallel 2D gels. The positions of actin (A) and tubulin (T) are indicated for reference. The 36-kDa MTAP resolves into several spots in these gels.

proteins were not found in similar immunoprecipitates from NG59-infected cells (19). However, it was still possible that the tumor ascites sera recognized these proteins directly and that the expression of these proteins was induced by the wild-type T antigens but not by NG59 T antigens. Walter and co-workers (12, 17), using antisera specific for MT, showed that proteins of 37, 61, and 88 kDa associate with wild-type MT but not with a transformation-defective mutant NG59 MT. In addition, a 32-kDa protein has been described that specifically coimmunoprecipitates with ST from cells expressing only ST (23). We have shown that the 36- and 63-kDa MTAPs are identical to two proteins previously described and shown to be present in cells in the absence of T antigens by Rundell (29). These two proteins are probably identical to all the non-tyrosine kinase 32- to 36- and 61- to 63-kDa cellular proteins identified to date in immunoprecipitates of ST or MT. The 27-, 29-, and 51-kDa proteins appear to represent MTAPs not previously identified from ^{35}S -labeled cells; however, the data are consistent with the 51-kDa protein being a proteolytic breakdown product of a 61-kDa MTAP. Although the small amount of the 27- and 29-kDa MTAPs in control immunoprecipitates is reminiscent of sticky proteins like hsp70, there does appear to be a specific association with MT above the level nonspecifically bound to control immunoprecipitates, and this specific association is affected by mutations in the MT (Fig. 1). The more basic of the 61-kDa MTAPs migrated similarly, if not identically, to pp60^{c-src}. The more-acidic 61-kDa proteins, which migrated slightly slower in the sodium dodecyl sulfate-polyacrylamide gel electrophoresis dimension of 2D gels, are not immunoprecipitated by GD11 (26), an anti-pp60^{c-src} serum, and may represent pp62^{c-yes} or modified forms of pp60^{c-src} that react less well with the serum (data not shown). The 85-kDa MTAP is likely the same as an 80-kDa protein observed by Dilworth (10) in MT immunoprecipitates which incorporates phosphate on tyrosine in kinase assays in vitro.

Our data suggest that MT can form several different stable complexes and indicate possible MT domains that are important for binding the various MTAPs. The first complex contains MT and the 36- or 63-kDa MTAPs or both, but not the other MTAPs. This complex can be seen most clearly for the c3 and c14 mutant MTs. The 36- and 63-kDa MTAPs may form a complex with the same MT molecule or form separate individual complexes. Since ST binds the 36- and 63-kDa MTAPs, the 191-amino-acid region common to ST and MT is sufficient for association. Further assignment in this region is not yet possible. A second complex consists of MT and pp60^{c-src} and may contain the 36- and 63-kDa MTAPs. This complex is clearly seen in the case of *d123* MT. The 85-kDa protein appears to be associated with the MT-pp60^{c-src} complex, because it could be immunoprecipitated by anti-pp60^{c-src} but only when MT was present (8, 15; our unpublished results). It is likely that the 85-kDa protein interacts with pp60^{c-src} rather than MT, because it is known that activated pp60^{c-src} will bind the 85-kDa protein (D. Kaplan, H. Piwnicka-Worms, and T. M. Roberts, unpublished data). It appears that another complex consists of MT and the 27- and 29-kDa MTAPs, because NG59 MT bound only these two MTAPs. This result implies that the binding site(s) of the 27- and 29-kDa proteins is distinct from those of the other MTAPs. It appears that sequences in both the common and the unique portions of MT are necessary for the binding of the 51-kDa and the acidic 61-kDa MTAPs, because these MTAPs are absent from NG59 MT and ST immunoprecipitates.

The genetic data suggest that the 85-kDa MTAP plays an important role in MT-mediated transformation. Four of five transformation-defective MT mutants were clearly deficient in association with the 85-kDa MTAP. The inability of *d123* MT to associate efficiently with the 85-kDa MTAP may explain why this and other MT mutants (21) capable of associating with and activating pp60^{c-src} are not fully transformation competent. Association with the 85-kDa MTAP is therefore a better indicator of MT transformation competence than is association with and activation of pp60^{c-src}. However, the fact that *d1015* MT associated with near-wild-type levels of the 85-kDa MTAP suggests there may be an additional yet undiscovered basis for its defect in transformation. The 85-kDa MTAP is closely correlated with PI kinase activity. The relative amounts for wild-type, *d1015*, and *d123* MTs (Fig. 1) correlate with the relative levels of PI kinase activity which we have shown to be associated with immunoprecipitates of these proteins (15, 22), whereas the abundance of other MTAPs is unchanged. The PI kinase activities associated with the c3, c14, and NG59 MTs also parallel the amount of 85-kDa protein; all three are more than 10-fold less than wild-type levels (Table 1). The possible connection between the 85-kDa MTAP and PI kinase has been noted recently (8, 15); that connection is strengthened, however, by the fact that the other MTAPs were unchanged for *d123* MT. We are currently raising monoclonal antibodies to the 85-kDa protein and the other MTAPs to determine their roles in cellular transformation by MT.

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