Interactions between Polyomavirus Medium T Antigen and Three Cellular Proteins of 88, 61, and 37 Kilodaltons

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Affinity-purified medium T antigen of wild-type polyomavirus and d8, a transforming mutant with a deletion in the medium T gene, is associated with three cellular proteins with apparent molecular weights of 88,000 (88K protein), 61,000 (61K protein), and 37,000 (37K protein). Medium T antigen encoded by the nontransforming hrt mutants fails to associate with these proteins, whereas medium T antigen of the nontransforming mutant dl1015 is able to do so. Medium T antigen of the nontransforming mutant dl23 binds to the 61K and 37K proteins; however, binding to the 88K protein is uncertain. The pattern of complex formation between these proteins and medium T antigen resembles that of pp60 src and medium T antigen. The binding of medium T antigen to the 88K, 61K, and 37K proteins, as well as to pp60 src, might represent a necessary but insufficient step in transformation. By mixing extracts from infected and uninfected cells, complex formation between medium T antigen and the 88K, 61K, and 37K proteins can be demonstrated in vitro. Pulse-chase experiments indicated that in vivo the association between medium T antigen and the 61K and 37K proteins is a slow process. The latter two proteins are probably bound to each other in uninfected cells. On two-dimensional gels of whole-cell extract, the 61K protein comigrated with a minor protein with an isoelectric point of 5.2. The 61K protein was neither phosphorylated nor glycosylated. Polyomavirus tumor serum precipitated the 61K and 37K proteins independently of medium T antigen. Therefore, the 61K protein or the 37K protein or both have the properties of a cellular tumor antigen.

Among the three tumor antigens encoded by polyoma virus, medium T antigen is the principle protein involved in transformation (34, 49, 50). It is required for transforming established cell lines (35, 37) and embryonic cells (36) in culture as well as for the induction of tumors in newborn rodents (1, 2, 7). Medium T antigen alone is sufficient for the transformation of embryonic chicken cells (25, 27) and the induction of tumors in chickens and newborn hamsters (2, 27). However, for transforming rat embryo fibroblasts, large T antigen, which plays a role in immortalization (37), is required in addition to medium T antigen (36). On the other hand, small T antigen complements medium T antigen in the induction of tumors in newborn rats (1).

Medium T antigen is associated with pp60 src, the cellular homolog of the transforming protein of Rous sarcoma virus, pp60 src (14-16). The tyrosine-specific protein kinase activity of the medium-T-bound pp60 src is strongly enhanced as compared with free pp60 src (5, 14). It has been proposed that the pp60 src kinase activity is regulated by phosphorylation at tyrosine such that phosphorylation results in inactivation of kinase activity (13, 14). Medium T antigen might block phosphorylation of tyrosine 527 and thereby prevent inactivation of the pp60 src kinase activity (11). However, an unphosphorylated tyrosine 527 is not the only reason for the enhanced kinase activity of medium-T-associated pp60 src, because the difference in activity between pp60 src with or without phosphate at tyrosine 527 is only 3-fold, whereas the difference in activity between free and medium-T-bound pp60 src is at least 20-fold (11). Free pp60 src becomes phosphorylated during the in vitro autophosphorylation reaction at tyrosine 416, whereas medium-T-bound pp60 src becomes phosphorylated on tyrosine 416 and at an additional tyrosine site in the amino-terminal half of the molecule (10, 54). It is unknown whether phosphorylation at that site effects kinase activity.

Studies with medium T antigen mutants shed some light on the role of pp60 src in transformation by polyomavirus. Medium T antigen of the host-range-nontransforming (hrt) mutants (3) forms a less stable complex with pp60 src than wild-type medium T antigen does, and the kinase activity of the associated pp60 src is not elevated (4). On the other hand, medium T antigen of the mutants dl1015 and dl23 forms a complex with pp60 src in which the tyrosine-specific protein kinase activity is activated (10, 24), yet these mutants do not transform (21, 32). Therefore, the interaction between medium T antigen and pp60 src might be a necessary but insufficient step in transformation.

At least two regions in the medium-T-antigen polypeptide are important for both transformation and complex formation with pp60 src. One is a stretch of 22 uncharged and hydrophobic residues in the C-terminal half of the molecule responsible for anchoring medium T antigen in the plasma membrane (9, 33, 46) and the endoplasmic reticulum (18). Mutant medium T antigens lacking this region do not attach to membranes; they also do not form a complex with pp60 src and are nontransforming. Therefore, it appears that medium T antigen and pp60 src can only form a complex when both are inserted in the membrane. Interestingly, medium T antigen with the C-terminal hydrophobic domain of the vesicular stomatitis virus glycoprotein, replacing its own C terminus, can attach to membranes and activate tyrosine-specific protein kinase activity, but it is defective in transformation (48). This indicates that other properties of medium T antigen besides its ability to associate with membranes and to interact with pp60 src must be important for its transforming ability. Another important area of me-

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diurn T antigen is located in the amino-terminal region of the molecule. It makes contact with pp60<sup>src</sup>, as shown with N-terminal truncation mutants (47) and monoclonal antibodies directed against that region (17). From these studies, one can conclude that the attachment of medium T antigen to membranes is mediated by its C terminus, while its contact to pp60<sup>src</sup> is directed through the N terminus. Vice versa, pp60<sup>src</sup> is anchored to the membrane by its N terminus (8, 30) and probably binds medium T antigen through its C terminus.

We reasoned that medium T antigen might interact with other cellular proteins besides pp60<sup>src</sup>, e.g., src-related proteins with structural features in common with pp60<sup>src</sup>. By purification of medium T antigen under mild conditions, we found proteins with apparent molecular weights of 88,000 (88K protein), 61,000 (61K protein), and 37,000 (37K protein) specifically associated with medium T antigen (23, 26). Medium T antigen of transforming viruses but not that of the h<sup>rt</sup> mutants NG59 and SD15 was able to bind these proteins. The latter was associated instead with the 73K heat shock protein (52). In the present study, we show that the 88K, 61K, and 37K proteins bound to medium T antigen with the same specificity as pp60<sup>src</sup>. In vitro studies and pulse-chase experiments indicate that complex formation occurred in vitro and in vivo. Presumably, medium T antigen binds to a preformed complex of the 61K and 37K proteins. We demonstrate that the 61K or 37K protein or both have the properties of a cellular tumor antigen. 61K is a low-abundance protein, and its biochemical properties suggest that it is not a member of the tyrosine-specific protein family.

**MATERIALS AND METHODS**

Cell culture, virus infection, radiolabeling, and preparation of cell extracts. Unless otherwise stated, the procedures for growing, infecting, and radiolabeling 3T6 cells and for preparing cell extracts were carried out as previously described (26). Extracts were prepared in RIPA buffer (0.15 M NaCl, 10 mM sodium phosphate [pH 7.2], 1% deoxycholate, 1% Nonidet P-40 [NP]-40, 0.1% sodium dodecyl sulfate [SDS], 1 mM dithiothreitol, 50 mM leupeptin) or in NP-40 lysis buffer as indicated in the figure legends. Mostly the earlier experiments were carried out with RIPA extract. Later it was found that a larger quantity of the associated proteins was obtained in NP-40 buffer not containing SDS. Labeling of cells with <sup>3</sup>H]mannose and <sup>3</sup>H]glucosamine was carried out as described previously (42).

**Purification of medium T antigen.** Medium T antigen was purified with monoclonal peptide antibodies prepared against the internal peptide Glu-Glu-Glu-Glu-Tyr-Met-Pro-Met-Glu (Glu-Glu) and rabbit antiserum against the carboxy-terminal peptide Lys-Arg-Ser-Arg-His-Phe (Lys-Phe) (23, 52).

V8 protease digestion, one- and two-dimensional gel electrophoresis, and glycerol gradient analysis. Partial proteolytic mapping and one- and two-dimensional gel electrophoresis were carried out as previously described (12). Glycerol gradient analysis was performed as previously described (23).

**RESULTS**

Association of the 88K, 61K, and 37K proteins with medium T antigen of nontransforming mutants d11015 and d213. Previous experiments revealed that medium T antigen encoded by the nontransforming h<sup>rt</sup> mutants is unable to form a complex with the 88K, 61K, and 37K cellular proteins (26). It was of interest to study a different class of nontransforming mutants of medium T antigen with deletions in the C-terminal half of the polypeptide. Two such mutants, d11015 and d213, encode proteins missing amino acids 302 to 335 and 338 to 347, respectively (20–22, 32). They bind pp60<sup>src</sup>, and activate its kinase activity (10, 24). To examine whether the d11015 and d213 medium T antigens also bind the 88K, 61K, and 37K proteins, the antigens were purified by affinity chromatography from infected 3T6 cells by using two antipeptide antibodies (23). The extract was first incubated with antibodies against an internal peptide of medium T antigen, Glu-Glu-Glu-Glu-Tyr-Met-Pro-Met-Glu (corresponding to amino acids 311 to 319). Bound medium T antigen was released from the immune complex with a large excess of this peptide. It was incubated with antibodies against the C-terminal peptide, Lys-Arg-Ser-Arg-His-Phe, and again released with a large excess of the peptide. The purified proteins, together with copurified cellular proteins, were then analyzed on SDS-polyacrylamide gels. Medium T antigen of d11015 was associated with all three cellular proteins (Fig. 1, lane e). Medium T antigen of d213 was lost during purification because the first antiserum was directed against a region in the protein that was deleted by the mutation (lane g). As expected, no medium-T-antigen-associated proteins were detected in this case. When d213 extract was incubated with the first antipeptide serum and the supernatant, containing the unbound d213 medium T antigen, was incubated with polyomavirus tumor serum,

![FIG. 1. Purification of medium T antigen (mT) and associated proteins from cells infected with wild-type and nontransforming mutants. Infected 3T6 cells (2 × 10<sup>5</sup>) were labeled on a 5-cm plate from 24 to 27 h after infection with 90 μCi of [<sup>35</sup>S]methionine in methionine-free medium and lysed in 0.4 ml of NP-40 lysis buffer (1% NP-40, 50 mM Tris [pH 8.0], 1 mM dithiothreitol, 10 mg of aprotinin per liter). Extract (200 μl) was incubated with 20 μl of Glu-Glu-Sepharose (0.7 mg of immunoglobulin G per ml) for 2 h at 4°C. After three washes with NP-40 lysis buffer, proteins were eluted with 60 μl of NP-40 lysis buffer containing 2% NP-40 and 10 μg of Glu-Glu-peptide. The Sepharose was washed with 60 μl of Tris buffer without NP-40 and peptide, and the wash was combined with the first eluate. This material was incubated with 20 μl of Lys-Phe-Sepharose (2.5 mg of immunoglobulin G per ml) for 2 h at 4°C and washed three times with NP-40 lysis buffer. Proteins were solubilized in gel electrophoresis sample buffer containing 100 mM Tris hydrochloride, pH 6.8, 4% SDS, 40% 2-mercaptoethanol, 20 mM dithiothreitol, 10% glycerol, and 0.05% bromophenol blue. Samples were boiled for 5 min, and 10-μl samples were run on SDS-polyacrylamide gels (10% acrylamide). Lanes, a, mock; b, wild type (WT); c, NG59; d, SD15; e, d11015; f, d18; g, d213; h, supernatant from first purification step of d213 precipitated with tumor serum. HSP, Heat shock protein.](http://jvi.asm.org/)
then medium T antigen and the 61K and 37K proteins were precipitated (lane h). Because of a high background, it is unclear whether the 88K protein was also associated with dl23 medium T antigen.

Figure 1 also shows the purification of medium T antigen encoded by the wild type (lane b), NG59 (lane c), SD15 (lane d), and dl8 (lane f). These results confirm the previous notion that medium T antigen of the wild type and dl8 associates with the 88K, 61K, and 37K proteins, whereas that of the hrt mutants NG59 and SD15 binds to the 73K heat shock protein, previously called 72K protein (52). A protein migrating between dl23 and dl8 medium T antigen was found in all preparations and, on the basis of its size, is probably actin. Several proteins migrating ahead of the 37K protein could be medium-T-derived breakdown products, since their molecular weights varied with the intensity and size of medium T antigen. It is worth mentioning that some 73K heat shock protein was found in all medium T preparations, although it was predominantly associated with medium T antigen of hrt mutants.

**Immunoprecipitation of 61K and 37K proteins from uninfected cell extract.** It is an established fact that antisera from polyomavirus tumor-bearing animals recognize the virus-encoded large, medium, and small T antigens, whereas cellular proteins are usually not recognized because the animals are immunotolerant against their own proteins. When screening a large number of individual rat polyomavirus tumor sera for the ability to precipitate the polyomavirus T antigens, we found one serum which precipitated an unusually large amount of the 61K and 37K proteins (data not shown). We then tested whether this particular serum (called 8079) might precipitate these proteins directly, i.e., independently of their association with medium T antigen. A small amount of the 61K and 37K proteins was precipitated with serum 8079 from an extract of uninfected 3T6 cells (Fig. 2, lane b). As expected, more of these proteins, in addition to large, medium, and small T antigens, was precipitated from infected cell extract (lane c). Medium T antigen and the associated 61K and 37K proteins precipitated with medium-T-specific antipeptide serum (lane a). There was a high background of nonspecific proteins in lanes b and c because serum 8079 did not have a high absorbance at 280 nm and therefore had to be used in relatively large quantity and because the 61K protein is a very minor cellular protein (see below). Because of the high background, we were unable to determine whether serum 8079 also precipitated the 88K protein from the uninfected cell extract. The finding that the 61K protein from uninfected cells is identical with the medium-T-associated 61K protein was confirmed by peptide mapping with V8 protease. The 61K protein from both sources produced the same proteolytic fragments V1 through V4 (Fig. 3). In less-pure preparations of the 61K protein, such as that obtained by immunoprecipitation with serum 8079, a protein migrating more slowly than the 61K protein was repeatedly observed on 15% gels used for V8 peptide mapping (Fig. 3, lane d). This protein gave rise to fragments migrating between V3 and V4. We also analyzed the 37K protein by V8 protease digestion and found that its map is distinctly different from that of the 61K protein. This indicates that the 61K and 37K proteins are not related (data not shown).

**Complex formation in vitro and in vivo.** Complexes between medium T antigen and other proteins are relevant only if they are produced in vivo. If, on the other hand, complex formation occurs only in the extract after cell lysis, it would be a less interesting phenomenon. To check the latter possibility, extract from [35S]methionine-labeled uninfected cells was mixed with extract from uninfected cells and medium T antigen was subsequently purified from the mixture by affinity chromatography. Medium T antigen indeed associated in vitro with the 88K, 61K, and 37K proteins (Fig. 4, lane a). As a control, the purification was carried out with labeled uninfected extract alone, i.e., in the absence of unlabeled medium T antigen. In this case, the associated proteins were not seen. Lane c shows medium T antigen with its associated proteins as purified from [35S]methionine-labeled infected cell extract. Except for the labeled medium T antigen and a strong band below medium T, probably the major polyomavirus structural protein VP1, this pattern is similar to that shown in lane a. To approach the question of whether complex formation takes place in vivo, pulse-chase experiments were carried out (Fig. 5). dl8-infected cells were pulse-labeled for 15 min with [35S]methionine (lane a) and chased for 15, 30, 60, 90, and 180 min (lanes b, c, d, e, and f, respectively). The results show that during a 15-min pulse, little if any 61K and 37K protein became associated with medium T antigen. With increasing chase time, the amount increased and reached a maximum after 180 min. The pattern observed after a 180-min chase was similar to that of a continuous 12-h label (lane g). This result indicates that complex formation also took place in vivo. Alternatively, one could argue that the cellular proteins have to be modified in vivo in a time-dependent fashion before they can associate with medium T antigen after cell lysis. At present, we cannot exclude the latter possibility. It seems unlikely, however, since the 61K protein is neither phosphorylated nor glycosylated (see below). It is difficult to determine the proportion of complex formation in vivo, occurring during a 3-h labeling period, and in vitro during cell lysis. The pulse-chase experiments indicate that the contribution by complex formation in vitro is low, because if it were high, one would not expect to find a strong increase in the amount of complex during the chase.
Instead, the amounts after the pulse and the chase would be similar. A pulse-chase experiment was also carried out with cells infected with the nontransforming hrt mutant NG59 encoding medium T antigen that forms a complex with the 73K heat shock protein (52). No significant increase in the amount of medium-T-associated 73K heat shock protein took place during the chase (Fig. 5B). Therefore, it is possible that in this case most, if not all, complex formation occurred in vitro.

Destruction of complex between medium T antigen and 61K and 37K proteins complex by SDS. We repeatedly observed that the yield of the medium-T-associated proteins was dependent on the purification conditions. For example, the 88K protein was not observed when cell extracts were prepared in RIPA buffer containing 0.1% SDS, and the yields of the 61K and 37K proteins were decreased during purification in RIPA buffer as compared with purification in NP-40-containing buffer. To test directly the SDS sensitivity of the complex between medium T antigen and the 61K or 37K protein, affinity-purified complex (Fig. 6, lane a) was boiled in 1% SDS and then reprecipitated with polyomavirus tumor serum 8079 after the SDS concentration was reduced to 0.1% (Fig. 6, lane b). Alternatively, the purified complex was reprecipitated without SDS (lane c). Clearly, the complex between medium T antigen and both proteins was completely destroyed by SDS. The experiment also demonstrates that serum 8079, which precipitated free 61K and 37K proteins from uninfected cell extract (Fig. 2), was unable to recognize SDS-denatured 61K or 37K protein. On the other hand, denatured medium T antigen was efficiently precipitated.

Association of medium T antigen with preformed 61K-37K complex. In a previous report, we demonstrated by sedimentation analysis that the medium-T-61K complex was distinct from the medium-T-pp60c-src complex (23). We could not clearly identify the 37K protein in the same complex with medium T antigen and 61K protein because of a background of other proteins with similar molecular weights. By using improved methods, we now demonstrate that medium T antigen and the 61K and 37K proteins are linked in the same complex. Extract of polyomavirus wild-type-infected cells was sedimented on a 5 to 20% glycerol gradient, and each fraction was subjected to affinity purification of medium T antigen with two antipeptide sera in sequence instead of only one precipitation as in the previous experiment. It can be seen that medium T antigen, the 61K, and 37K proteins comigrated in the same fractions (Fig. 7). To answer the question of whether the 61K and 37K proteins form a
complex in the absence of medium T antigen, extract from uninfected cells was sedimented on a 5 to 20% glycerol gradient and each fraction was immunoprecipitated with the polyomavirus tumor serum 8079, which has been shown to recognize the 61K and 37K proteins in uninfected cell extracts. The 61K and 37K proteins indeed comigrated, indicating that they formed a complex (Fig. 8). The background of proteins was high because, as mentioned above, 8079 is not a strong serum. If the 61K and 37K proteins form a complex, then the question arises which of the two proteins is recognized by the serum 8079 and which one makes contact with medium T antigen (see Discussion).

Is the 61K protein related to pp60csrc? At the moment we have no clue as to the function of the medium-T-associated 88K, 61K, and 37K proteins. We found previously that the 61K protein does not become phosphorylated in vitro during an autophosphorylation reaction with immunoprecipitates of medium T antigen (23). Furthermore, the 61K protein purified from 32P-labeled d8-infected cells showed no detectable incorporation of phosphate (23). The latter finding was confirmed under a variety of experimental conditions, including different concentrations of vanadate, an inhibitor of phosphatases, in the labeling medium and in the lysis buffer, as well as short and long labeling times. Under no condition was the 61K protein phosphorylated. This result argues against the 61K protein being src related. We also examined whether the 61K or 37K protein is a glycoprotein by labeling d8-infected cells with glucosamine and mannose. However, no incorporation of these sugars into either protein was detected (data not shown).

Abundance of the 61K protein. It was of interest to measure the abundance of the 61K protein in 3T6 cells. The 61K protein was purified from [35S]methionine-labeled 3T6 cells, mixed with unlabeled whole-cell extract, and analyzed on a two-dimensional polyacrylamide gel. Unlabeled proteins were visualized with silver stain, and the location of the radioactive 61K protein was determined by fluorography. The 61K protein was superimposed on a very minor stained protein which had an isoelectric point of 5.2 (Fig. 9). The experiment does not prove that this stained protein is identical with the 61K protein, but it indicates that the 61K protein is a minor cellular protein, no more abundant than the comigrating stained protein. We studied the subcellular distribution of the 61K protein by cell fractionation experiments. Unlabeled nuclear, cytoplasmic, and membrane frac-

FIG. 6. SDS sensitivity of complex between medium T antigen (mT) and the 61K and 37K proteins. d8-infected 3T6 cells were labeled from 26 to 29 h postinfection with 600 μCi of [35S]methionine per 10-cm dish in methionine-free medium. Extract was prepared in 1 ml of lysis buffer (pH 8). It was incubated with 200 μl of anti-Glu-Glu-protein A-Sepharose for 90 min at 4°C, and proteins were released from the immune complex with Glu-Glu-peptide in 200 μl of release buffer as described in the legend to Fig. 1. A sample of the released material was diluted with 1 volume of electrophoresis sample buffer and analyzed directly on a 7.5% acrylamide gel (lane a). Another sample (30 μl) was adjusted to a final concentration of 1% SDS with a 10% SDS stock and boiled for 3 min. After a 10-fold dilution with release buffer without peptide (to lower SDS to 0.1%), serum 8079 (10 μl) was added. Precipitation was carried out with protein A-Sepharose. The precipitate is shown in lane b. A third sample (30 μl) was diluted 10-fold with release buffer without peptide and precipitated with serum 8079 (lane c).

FIG. 7. Sedimentation analysis of medium-T-antigen-61K protein-37K protein complex. [35S]Methionine-labeled extract from wild-type-infected 3T6 cells was prepared in RIPA buffer and sedimented on a 5 to 20% glycerol gradient. Each fraction was subjected to purification with two peptide antibodies as described in the legend to Fig. 1. Samples were analyzed on a 10% gel. Molecular weight markers (shown on the bottom) were run in a parallel gradient. HSP, Heat shock protein.

FIG. 8. Sedimentation analysis of 61K and 37K proteins from uninfected cells. The extract was prepared and sedimented as described in the legend to Fig. 7. Each fraction was precipitated with serum 8079. The samples were analyzed on a 10% gel. T, Immunoprecipitate of unfraccionated extract with serum 8079; M, molecular weight markers as described in the legend to Fig. 5.
speculate that medium T antigen transforms by activation of a cellular proto-oncogene (15). We propose that the associations between medium T antigen and the 88K, 61K, and 37K proteins might be protein-protein interactions in the medium-T-antigen-pp60c-src complex because these proteins behave like pp60c-src in regard to complex formation with medium T antigen of transforming and nontransforming mutants. Considering that a much larger fraction of medium T antigen is bound to the 88K, 61K, and 37K proteins than to pp60c-src, it is conceivable that these proteins could be more important for transformation than pp60c-src is. One has to keep in mind that there is no direct evidence that the increased tyrosine-specific protein kinase activity of the medium-T-antigen-associated pp60c-src, as measured in vitro, plays a role in vivo. In this regard, it is interesting that the level of phosphotyrosine in polyomavirus-transformed cells, in contrast to Rous sarcoma virus-transformed cells, is not increased over the level in untransformed cells (43).

At present, we know little about the stoichiometry and spatial arrangement of the individual components in the medium-T-antigen complexes. Sedimentation studies indicate that medium T antigen and the 61K and 37K proteins are linked together in the same complex, but it is unclear whether medium T antigen makes contact with one or both proteins. The 88K protein has not been found under these conditions. Since it is loosely attached, it might be dissociated during the glycerol gradient analysis which is carried out in RIPA buffer. Previous experiments have shown that the bulk of the medium-T-antigen-61K protein complex migrates more slowly on glycerol gradients than the medium-T-antigen-pp60c-src complex (23). Other experiments indicate that the medium-T-antigen-pp60c-src complex also contains some 61K protein (26). It is possible that medium T antigen interacts with a multiprotein complex that includes pp60c-src, the 88K, 61K, 37K proteins, and the cy-yes protein. During cell lysis and centrifugation, this complex might disintegrate into several different substructures. Such a multiprotein complex could play a role in the transduction of growth-controlling signals through the plasma membrane or from the plasma membrane to the nucleus. It is conceivable that the 88K, 61K, and 37K proteins are involved in growth regulation of normal cells and, as such, could be proto-oncogenes. Like the simian virus 40 large-T-antigen-associated 53K protein (29), these proteins might belong to a class of potentially transforming proteins whose genes are not picked up by RNA tumor viruses and are not active in transfection experiments with tumor DNA on NIH 3T3 cells. It is interesting that the simian virus 40 small-T-antigen-associated cellular proteins of 56,000 and 32,000 daltons (6, 38, 39, 53) are identical to the medium-T-antigen-bound 61K and 37K proteins (G. Walter and K. Rundell, manuscript in preparation).

It was important to assess that the association of medium T antigen with cellular proteins occurs in vivo. The pulse-chase experiments indicate that this was the case for the complexity of medium T antigen and the 61K and 37K proteins. In this context, it is interesting that immune sera from polyomavirus tumor-bearing rats precipitated the 61K or 37K proteins or both independently of medium T antigen. Hence, one or both proteins could be cellular tumor antigens like the simian virus 40 large-T-associated 53K protein (31). Since the tumors were induced by polyomavirus-transformed rat cells, one would have expected that the animals (rats) were tolerant against rat proteins. One possible explanation for our finding is that the conformation and antigenicity of either the 61K or 37K protein or both was changed by
complex formation with medium T antigen. As a result these proteins might be recognized by the immune system as foreign. This would imply, however, that the complex of medium T antigen and the 61K and 37K proteins exists in the tumor. Alternatively, we cannot exclude the possibility that the 61K and 37K proteins are precipitated by tumor antisera because they share a determinant with medium T antigen and not because they are by themselves immunogenic.

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LITERATURE CITED


