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Tiny T Antigen: an Autonomous Polyomavirus T Antigen Amino-Terminal Domain

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Three mRNAs from the murine polyomavirus early region encode the three well-characterized tumor antigens. We report the existence of a fourth alternatively spliced mRNA which encodes a fourth tumor antigen, tiny T antigen, which comprises the amino-terminal domain common to all of the T antigens but is extended by six unique amino acid residues. The amount of tiny T antigen in infected cells is small because of its short half-life. Tiny T antigen stimulates the ATPase activity of Hsc70, most likely because of its DnaJ-like motif. The common amino-terminal domain may interface with chaperone complexes to assist the T antigens in carrying out their diverse functions of replication, transcription, and transformation in the appropriate cellular compartments.

The small, middle, and large T antigens expressed by the murine polyomavirus (muPy) are formed of a common amino-terminal sequence juxtaposed to unique carboxy-terminal sequences (33, 65). Such parsimony in protein structures reflects the pattern of viral gene expression, for the T antigens are encoded by a single primary transcript containing two splice acceptor and two splice donor sites (76) (Fig. 1), creating the potential of four separate mRNAs. Three of these RNAs encode the small, middle, and large T antigens. The fourth mRNA, heretofore undetected, should encode a protein with the T antigen common amino-terminal domain extended by six residues. Here, we report the detection of this mRNA and the partial characterization of the protein it encodes (tiny T antigen).

Genetic and biochemical evidence indicates the muPy T antigen common amino-terminal domain is required for cell transformation and promotes small and middle T antigen association with PP2A, c-Src, and perhaps other proteins (5, 26, 73). These cellular signal transducers strongly influence viral DNA replication, transcription, and translation; consequently, the T antigen common amino-terminal domain must contribute to virtually all aspects of the virus life cycle. Sequence homology between the T antigen amino-terminal domain and DnaJ proteins has been noted (36). DnaJ proteins modulate the ATPase activity of the hsp70 class of chaperones, and we observe that tiny T antigen expressed in *Escherichia coli* and purified to near homogeneity exhibits such a stimulatory activity in vitro. This activity within the T antigen amino-terminal domain very likely contributes to the functions of the T antigens by promoting their interactions with cellular chaperones.

MATERIALS AND METHODS

Cells and viruses. NIH 3T3, 3T6, and COS1 cells were obtained from the American Type Culture Collection. Polyomavirus transformed MOP-3T3 and MOP-3T6 cells were obtained from John Hassell (McMaster University, Ontario, Canada). Whole mouse embryo (WME) cells were prepared from outbred mice as described previously (24). All cells were grown in Dulbecco's modified Eagle's medium (low glucose) supplemented with 10% fetal calf serum.

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NIH 3T3 cells were transfected with DNA by calcium phosphate coprecipitation and glycerol shock as previously described (83) or with Lipofectamine (20 μl of Lipofectamine/60-mm-diameter plate) per directions by Gibco-BRL. MOP-3T3 and MOP-3T6 cells were transfected with DNA with Lipofectamine, and COS1 cells were transfected with Lipofectace (Gibco-BRL). 3T6 and WME cells were infected with the polyomavirus A3 strain at a multiplicity of approximately 10 PFU/cell as described previously (24). Cell extracts were made at 30 to 40 h by three freeze-thaw cycles in lysis buffer (20 mM Tris-Cl [pH 8], 25 mM NaCl, 0.1% Nonidet P-40) with protease inhibitors (1 mM EDTA, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 0.5 μg of leupeptin per ml, 2 μg of aprotinin per ml). The cell extracts were clarified by 10 min of centrifugation at 12,000 × *g* and stored in sodium dodecyl sulfate (SDS) sample buffer at −20°C for analysis.

NIH 3T3 cells harboring pcDNA3:tinyT or pPyST1 and pcDNA3 were prepared by transfection with these DNAs and selection with 500 μg of Geneticin (Gibco-BRL) per ml. After 2 to 3 weeks, groups of three or fewer visible foci were collected and pooled and expanded into cell lines.

RNA and PCR analyses and preparation of cDNAs. RNAs were extracted from cells, purified, and analyzed by Northern blotting and RNase protection as previously described (83) or by the Qiagen RNeasy protocol for reverse transcription-PCR (RT-PCR). First-strand cDNA synthesis was performed with the Gibco-BRL preamplification system primed with poly(dT), and typically 1/10 of such reaction mixtures was amplified for 30 to 40 cycles with the Gene Amp PCR reagents (Perkin-Elmer Cetus) with 1.5 mM $MgCl_2$. PCR products were analyzed by electrophoresis through 2% agarose gels stained with ethidium bromide or SYBR green (Molecular Probes).

Primers which pair to sequences spanning the splice junction of tiny T antigen mRNA were used to specifically detect tiny T antigen mRNAs (see Fig. 5). Primers capable of detecting mRNAs for all of the T antigens hybridize to contiguous exon sequences, except one primer which pairs just upstream of the ATG initiation codon (Fig. 5). Primers specific for glyceraldehyde-3-phosphate dehydrogenase RNAs were used to monitor the integrity of the RNAs.

The cDNA for tiny T antigen was isolated from RNAs isolated from NIH 3T3 cells transfected with pPyST1 (87) with the primers indicated in Fig. 3. This cDNA was amplified by PCR and cloned into the *Bam*HI site of pBluescript KS$^-$ (Stratagene). The sequence of the cloned product was determined and subsequently placed into pGST-2T (Pharmacia) and into pET-3d (68 [now known as pET-8c]) for expression in *E. coli*. A tiny T antigen mammalian expression vector was constructed by cloning the tiny T antigen cDNA into pcDNA3 (Invitrogen), yielding pcDNA3:tT1, to which was subsequently added the muPy origin of replication and enhancer (pcDNA3:tT1:ori), so as to make the plasmid competent for replication in MOP cells which express muPy large T antigen.

Expression of tiny T antigen, its purification, and production of antibodies. Tiny T antigen was expressed in *E. coli* from pGST-2T tiny T antigen and purified from cell extracts by affinity chromatography over glutathione Sepharose (Pharmacia), and then it was cleaved overnight with human thrombin (Sigma) in a mixture of 50 mM Tris (pH 8), 150 mM NaCl, and 2.5 mM CaCl at ambient temperature to release tiny T antigen containing two additional residues at its amino terminus. Tiny T antigen was dialyzed against 50 mM Tris (pH 8) and passed through Q Sepharose (Pharmacia), to which it did not bind. It was further purified by reverse-phase (C_{18}) liquid chromatography, where it eluted at approximately 60% acetonitrile with 0.1% trifluoroacetic acid in a 25 to 75% gradient of acetonitrile. This protein was greater than 95% pure, as measured by discontinuous SDS gel electrophoresis. For ATPase assays, the last step was

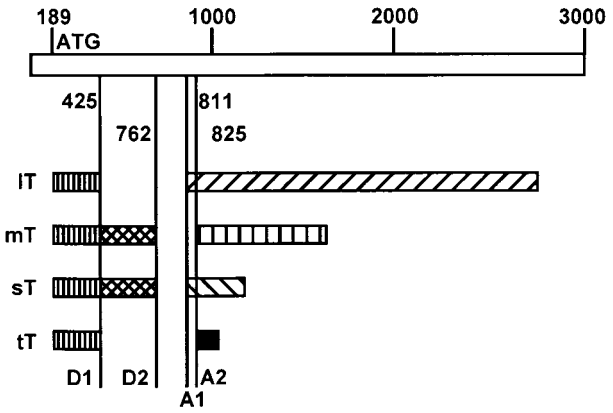

FIG. 1. Splice junctions and deduced protein products from the muPy early region. Numbers refer to the nucleotide position in muPy strain A3 (accession no. J02289 V01151). D1 and D2 refer to the donor splice position (5′ site), and A1 and A2 refer to the acceptor position (3′ site). lT, mT, sT, and tT refer to the large, middle, small and tiny T antigens, respectively.

replaced by chromatography over Affi-Blue (Bio-Rad). For preparation of antibodies, peak fractions from the reverse-phase column were lyophilized, resuspended in water, and injected into rabbits by the subcutaneous method of Clemons et al. (12). Transudate fluid and serum were harvested for polyclonal antibody.

Electrophoretic analysis of tiny T antigen. T antigens were analyzed by the discontinuous gel procedure (59) as described in "Sigma Technical Bulletin no. MWM-100." After electrophoresis (usually 3.5 to 4 h at 75 V), proteins were transferred to nitrocellulose membranes (Schleicher & Schuell) in transfer buffer containing 20% methanol with cooling by a −80°C ice block (typically for 75 min, starting at 125 mA for a 1.5-mm-thick gel). The filter was allowed to air dry and then was incubated with 3% gelatin in TBSTw (10 mM Tris-Cl [pH 8.0], 0.5 M NaCl, 0.1% Tween 20) for 1 h at ambient temperature. The primary antibody was diluted into this solution (typically 1:100), the mixture was incubated for 30 to 60 min, and unbound antibody was removed by four 3- to 5-min washes in TBSTw. The secondary antibody, affinity-purified anti-rabbit immunoglobulin G conjugated to horseradish peroxidase (Amersham), was diluted 1:60,000 and incubated with the membrane for 30 min, and unbound antibody was removed as described above. Protein bands were visualized by enhanced chemiluminescence.

Immunoprecipitation of metabolically labeled T antigens. NIH 3T3 or 3T6 cells were infected with polyomavirus A3 strain, and at 20 to 24 h postinfection, cells were starved for 30 min in medium lacking methionine supplemented with 5% dialyzed fetal calf serum; Tran ^{35}S label (Amersham [typically 1 mCi/150-mm-diameter plate]) was then added for 2 to 4 h. Cells were washed with ice-cold phosphate-buffered saline (PBS) and lysed in a small volume of radioimmunoprecipitation assay (RIPA) lysis buffer (28) containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 0.5 μg of leupeptin per ml, 2 μg of aprotinin per ml, and 1 mM EDTA) while still attached to the plate. This and all subsequent procedures were conducted at 4°C. The lysate was clarified by centrifugation at 30,000 × *g* for 30 min, and to reduce background, the extract (200 μl) was incubated with normal rabbit serum (diluted 1:100) for 1 h with gentle mixing, followed by incubation for 1 h with protein G-coupled Sepharose (typically 20 μl of suspension) preequilibrated with RIPA buffer containing 5 mg of bovine serum albumin per ml. The nonspecific complexes were removed by two centrifugations, and the supernatant was mixed with primary antibody (diluted 1:100) directed against tiny T antigen and incubated for 1 h, followed by addition of protein G-Sepharose, again with gentle mixing. The immune complexes were washed three times with RIPA buffer and stored in SDS loading buffer until analyzed by gel electrophoresis.

Detection of tiny T antigen by immunofluorescence. NIH 3T3 cells were allowed to attach to coverslips coated with poly-L-lysine and transfected with pcDNA3:tT1 with Lipofectamine. Forty-eight hours after transfection, cells were washed three times with PBS and fixed with −80°C acetone for 30 min. Cells were washed four times with PBS, incubated overnight with monoclonal antibody KF4 (48), washed four times with PBS, blocked by incubation with 0.1% gelatin in PBS, incubated with a 1:100 dilution of anti-mouse cy5 (Jackson Laboratory) for 2 h, washed four times with PBS, and imaged with a Bio-Rad MRC600 confocal fluorescence microscope.

Half-life determination. MOP-3T3 cells were transfected with pcDNA3:tT1:ori as described above. Forty-eight hours after transfection, cells were treated with 5 μg of cycloheximide per ml for various times and lysed for Western analysis.

ATPase assay. Assays of Hsc70 ATPase were performed in 20-μl reaction mixtures (10) containing 40 mM HEPES (pH 7.2), 75 mM NaCl, 4.5 mM $MgCl_2$ and 1.5 mM $CaCl_2$, 50 μM ATP, and 1 to 2 μCi of α-^{35}S-ATP with 0.4 μg of bovine Hsc70 (Stressgene). Reaction mixtures were assembled on ice, and reactions were initiated by incubation at 37°C and stopped by pipetting of 1 μl in triplicate samples onto polyethyleneimine thin-layer chromatography plates. The ADP product was separated by chromatography in 1 M formic acid–1 M LiCl (63). ADP formation was quantitated by PhosphorImager analysis. Stimulation of ATPase activity was measured in the presence or absence of 0.4 μg of tiny T antigen purified as described above.

RESULTS

Detection and analysis of mRNAs encoding tiny T antigen. The muPy large, middle, and small tumor antigens (T antigens) have in common their first 79 amino acid residues and then differ as a result of alternative splicing of the early region primary transcript (33, 65, 75, 76). The mRNAs for small T antigen and large T antigen share a common 3′ splice site (A1 in Fig. 1) but have different 5′ splice sites (D1 and D2 in Fig. 1), analogous to the situation for simian virus 40 (SV40) and most other papovaviruses. The mRNA for middle T antigen has the same 5′ splice site as that for small T antigen (D2) but utilizes a different 3′ splice site (A2) 14 nucleotides (nt) downstream of the 3′ splice site used by small T antigen and large T antigen (A1), thereby altering the reading frame. A potential fourth mRNA having the same 5′ splice site as large T antigen (D1) joined to the 3′ splice site of middle T antigen (A2) was not detected in previous analyses, perhaps because the methods used were insufficiently sensitive.

We detected a fourth muPy T antigen RNA in Northern blots of the RNAs of NIH 3T3 cells transfected by plasmid pPyST1, which contains the cDNA for small T antigen (87). Two RNAs hybridized to a polyomavirus early region probe, one of 2.7 knt the expected size for small T antigen mRNA, and another mRNA of 2.3 knt (Fig. 2). The cDNA for small T antigen present in the plasmid pPyST1 lacks the small T antigen splice sites; however, it contains the 5′ splice site used for large T antigen (D1 [Fig. 1]), the 3′ splice acceptor site used for middle T antigen (A2), and several possible lariat branch sites between nt 720 and 760. We speculated that the 2.3-knt mRNA might encode a fourth T antigen (tiny T antigen), whose existence was first conjectured by Treisman et al. (76). A cDNA of this mRNA was prepared, cloned, and sequenced. The sequence at the putative splice junction (TTCCAG/AAC GGC) corresponded to that expected between D1 and A2 described above (Fig. 3).

We searched for tiny T antigen mRNA in muPy virus-infected 3T6 cells and WME cells and in NIH 3T3 cells transfected with small T antigen or tiny T-antigen cDNA by utilizing

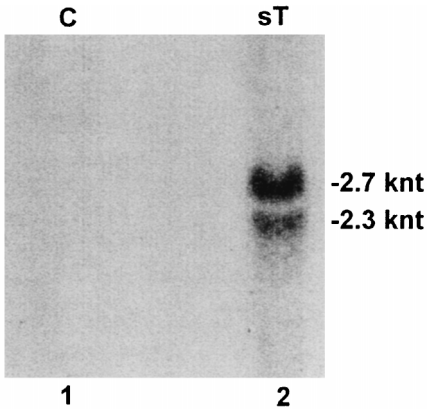

FIG. 2. Northern blot analysis of 3T3 cell RNA. Lanes: 1, RNA from control (C) cells; 2, RNA from cells transfected with pPysT1 (sT).

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                *189
ccggatccgctagcaccATGGATAGAGTTCTGAGCAGAG
                M  D  R  V  L  S  R  A

CTGACAAAGAAAGGCTGCTAGAACTTCTAAAACTTCCCA
  D  K  E  R  L  L  E  L  L  K  L  P  R

GACAACTATGGGGGGATTTTGGAAGAATGCAGCAGGCAT
  Q  L  W  G  D  F  G  R  M  Q  Q  A  Y

ATAAGCAGCAGTCACTGCTACTGCACCCAGACAAAGGTG
  K  Q  Q  S  L  L  L  H  P  D  K  G  G

GAAGCCATGCCTTAATGCAGGAATTGAACAGTCTCTGGG
  S  H  A  L  M  Q  E  L  N  S  L  W  G

GAACATTTAAAACTGAAGTATACAATCTGAGAATGAATC
  T  F  K  T  E  V  Y  N  L  R  M  N  L
              425* *825
TAGGAGGAACCGGCTTCCAG|AACGGCGGAGCGAGGAAC
  G  G  T  G  F  Q    N  G  G  A  R  N

TGAGGAGAGCGGCCACAGTCCACTACACGATGACTACTG
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GTCATTCAGCgctagcggatccgg
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FIG. 3. Nucleotide sequence of cloned muPy sequence and deduced amino acid sequence for tiny T antigen. MuPy sequences are capitalized, while non-muPy sequences in the PCR primers are not. Numbers refer to the nucleotide position in muPy strain A3 (accession no. J02289 V01151). The 5′ forward primer sequence is ccggatccgctaggcaccATGGATAGAGTTCTGAGCAGA (nt 189 to 209), and the 3′ reverse primer sequence is ccggatccgctagcGCTGAATG ACCAGTAGTCATC (nt 872 to 891). The vertical bar indicates the position of the splice junction.

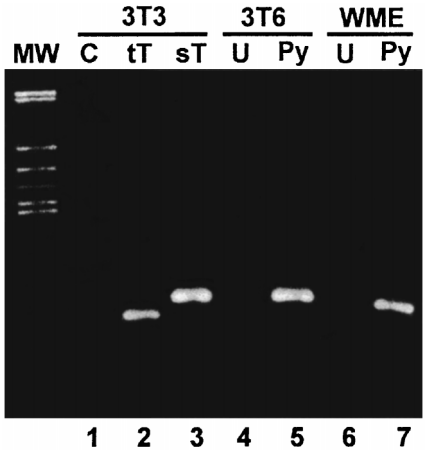

FIG. 5. RT-PCR analysis for tiny T antigen mRNA. cDNAs were synthesized with poly(dT) primer and amplified with the tiny T antigen-specific junction primer TCGCTCCGCCGTTCTGGAA (muPY nt 420 to 425 and 825 to 837) and a primer immediately upstream of the T antigen initiation codon, CATTT CAGCCTCACCACCATC (nt 168 to 188), except in lane 2, where the primer GGATAGAGTTCTGAGCAGAGCT (nt 191 to 212) was used, which is complementary to sequences within the T antigen coding region, since the cloned tiny T antigen lacks polyomavirus sequences upstream of the initiation codon (Fig. 1 and 3). Lanes: 1, 4, and 6, control (C), uninfected (U) cells; 2 and 3, NIH 3T3 cells transfected with tiny T (tT) or small T (sT) antigen cDNA respectively; 5 and 7, muPy (Py)-infected 3T6 or WME cells, respectively. MW, molecular weight markers.

RNase protection assays and by RT-PCR analysis. These methods are capable of distinguishing among the four T antigen mRNAs, of which those for large T antigen and tiny T antigen differ by only 14 nt. In RNase protection analyses, no protected bands were observed with RNA from uninfected cells (Fig. 4, lane 1), while two bands were observed with RNA from cells transfected with pPyST1 (Fig. 4, lane 2) and from infected cells (Fig. 4, lane 3). The upper band of 304 bp was the size expected for tiny T antigen mRNA, while the lower band of 237 bp was the size of the common first exon of small, middle, and large T antigen mRNAs. For RT-PCR analysis, primers were designed which spanned the splice junction asymmetrically and which were unique to each splice junction. Using a tiny T antigen-specific primer, we observed products of the expected sizes, either 247 or 271 bp, in NIH 3T3 cells expressing either tiny T antigen or small T antigen (Fig. 5, lanes 2 and 3) and in polyomavirus-infected 3T6 or WME cells (lanes 5 and 7), but not in untransfected or uninfected cells (lanes 1, 4, and 6). By using primers which span the tiny T antigen intron, all four T antigen mRNAs can be detected simultaneously in muPy virus-infected cells by RT-PCR. The PCR products for tiny T antigen and large T antigen differ by only 14 bp and could be resolved, whereas the larger products for small T antigen and middle T antigen were not well resolved. The large T antigen product was the most prevalent, followed by the middle T antigen and small T antigen products, while the tiny T antigen product typically gave only a faint band (data not shown).

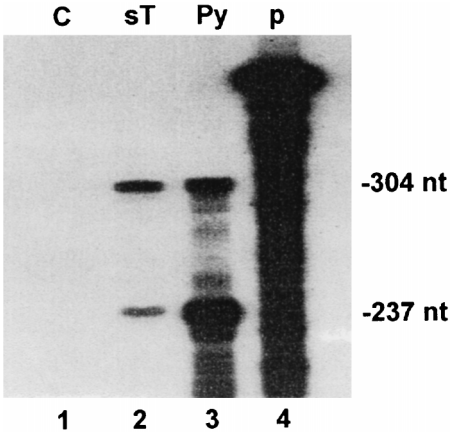

FIG. 4. RNase protection assay with cellular RNA from cells transfected with small T antigen cDNA or infected with muPy. The antisense probe from the tiny T antigen cDNA includes muPy nt 189 to 425 joined to nt 825 to 891 (Fig. 3) and additional vector sequences which cannot be protected by viral mRNAs. Lanes: 1, control cells; 2, 3T3 cells transfected with pPysT1 (sT); 3, muPy (Py)-infected 3T6 cells; 4, undigested probe (P).

Detection of tiny T antigen protein. Previous analyses of the expression of muPy T antigens in infected or transformed cells did not detect tiny T antigen, probably because the electrophoretic separation and detection systems were not optimized for a small basic protein, and because tiny T antigen is unstable. We were able to detect tiny T antigen expression in transiently transfected MOP-3T3 and MOP-3T6 cells and COS1 monkey cells following transfection with plasmids capable of replicating in these cell lines (e.g., Fig. 6, lane 2). In NIH 3T3 cells transiently transfected with tiny T antigen cDNA, tiny T antigen was found to be located diffusely in the cytoplasm and in a punctate manner in the nucleus (Fig. 7). The stability of tiny T antigen in MOP-3T3 cells was estimated by blocking de novo protein synthesis with cycloheximide. Immunoreactive tiny T antigen decayed with a half-life of approximately 20 min, in contrast to the much more stable small, middle, and large T antigens, whose half-lives exceeded several hours (Fig. 8). This helps explain why it is difficult to detect tiny T antigen in NIH 3T3 cell lines stably transformed with tiny T antigen cDNA or small T antigen cDNA (Fig. 6, lanes 3 and 4) or in infected 3T6

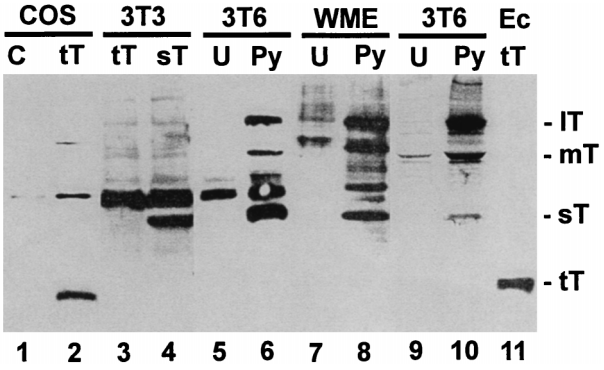

FIG. 6. Expression of tiny T antigen protein. Lanes: 1, 5, and 7, mock-transfected (control [C]) or uninfected (U) cells; 2, COS cells transiently transfected with tiny T antigen (tT) cDNA; 3 and 4, NIH 3T3 cells stably transfected with tiny T antigen or small T antigen (sT) cDNAs, respectively; 6 and 8, 3T6 or WME cells infected with muPy (Py) virus; 9 and 10, immunoprecipitated proteins from uninfected (lane 9) or muPy-infected (lane 10) 3T6 cells metabolically labeled with ^{35}S; 11, tiny T antigen produced in *E. coli*. The bars labeled lT, mT, sT, and tT are the expected sizes for the large, middle, small, and tiny T antigens, respectively.

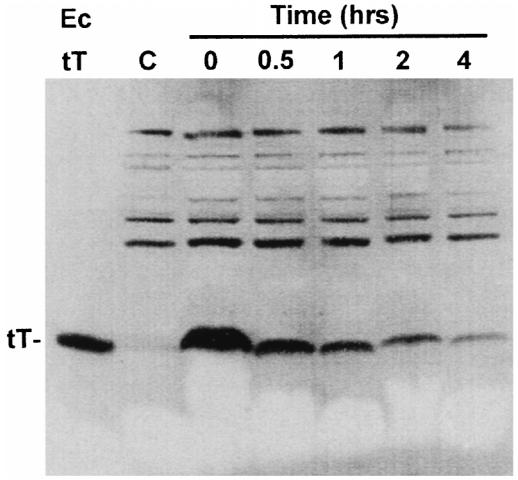

FIG. 8. Stability of tiny T antigen (tT) in MOP-3T3 cells. Times after addition of cycloheximide are indicated. Tiny T antigen produced in *E. coli* (Ec tT) serves as a marker. C indicates mock-transfected control cells.

or WME cells (Fig. 6, lanes 6 and 8, respectively), all of which are known to express tiny T antigen mRNA (Fig. 5).

Activation of Hsc70 ATPase activity by tiny T antigen. The sequence homology to the DnaJ domain within tiny T antigen (36) suggests that tiny T antigen might interact with and stimulate the ATPase activity of hsp70 family members, as do other DnaJ family members. We tested this directly and found that tiny T antigen is capable of stimulating the ATPase activity of bovine Hsc70, typically about two- to fourfold (Fig. 9), while denatured bovine serum albumin was unable to stimulate Hsc70 (data not shown). The extent of stimulation is variable, but it is typical of that seen with other cochaperones, including DnaJ and DnaK (40).

DISCUSSION

The sequence conservation of the T antigen common amino-terminal domains among the various members of the polyomaviruses (53) points to specific elements that are likely to be important for function. Most recognizable is the homology to the *E. coli* DnaJ protein (36). Proteins with DnaJ motifs serve as cochaperones in numerous cellular processes, including protein synthesis, translocation, degradation, assembly and disassembly of multimeric complexes, DNA replication, and signal transduction (see references 17, 19, 30, 56, and 64 for recent reviews). Chaperones bind to exposed hydrophobic surfaces of proteins, preventing their aggregation and facilitating productive folding (23, 29, 51). Multiple cycles of binding and release, dependent on ATP hydrolysis by hsp/Hsc70 are necessary to achieve folding (71). DnaJ cochaperones provide specificity in binding and increase the rate of cycling of exposed hydrophobic domains on and off the chaperone assembly (7, 10, 35, 71, 78, 88). Our evidence suggests a similar function for the muPy T antigen amino-terminal domain, for tiny T antigen interacts with and stimulates the ATPase activity of a mammalian Hsc70 protein. This property may help explain the importance of the amino-terminal domain in the disparate functions of the individual T antigens.

DnaJ proteins help regulate cellular signaling pathways, including those involving $pp60^{Src}$ tyrosine kinases, the transcrip-

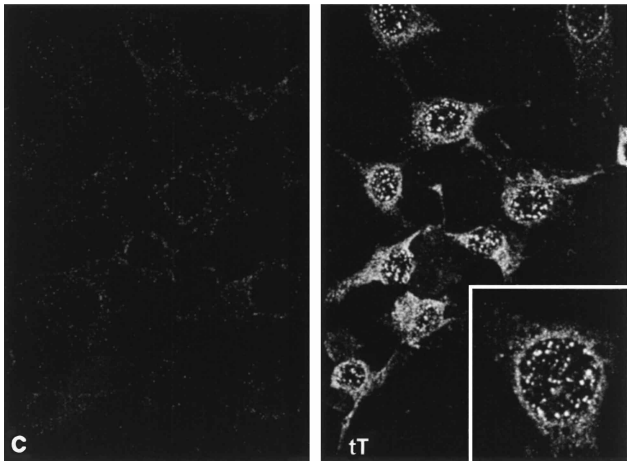

FIG. 7. Localization of tiny T antigen by immunofluorescence. (Left panel) Control cells (C). (Right panel) Cells transfected with pcDNA3:tinyT (tT).

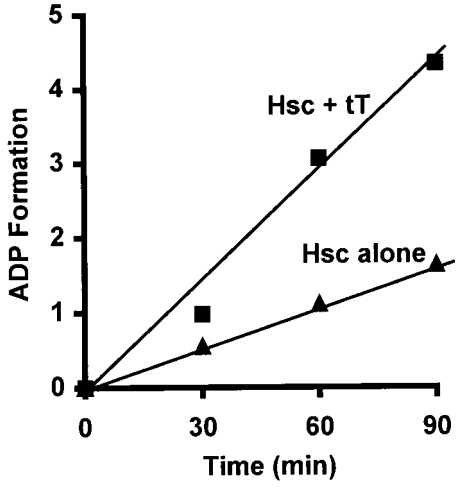

FIG. 9. Tiny T antigen (tT) stimulation of bovine Hsc70 ATPase activity. The kinetics of ADP formation by Hsc70 is given as the percentage of total ATP in the presence or absence of tiny T antigen at the indicated times.

tional activator p53, and steroid hormone receptors (4, 8, 37, 56, 69), and the T antigens intercede in these pathways by associating with effector proteins such as pp60^{Src}, c-Yes, c-Fyn, Shc, Grb2, PP2A, the 14.3-3 proteins, pRb, p107, p130, p300/CBP, p53, TEF-1, TBP, AP1, and AP2 (3, 6, 11, 16, 21, 22, 27, 32, 38, 47, 49, 74, 80). The amino-terminal domain is also important for transactivation and cell transformation (9, 14, 15, 20, 39, 41, 43, 45, 54, 55, 67, 70, 73, 74, 84, 86). Thus, it is readily understandable how a DnaJ domain activity within the primary structure of the T antigens might be utilized: the DnaJ domain could help assemble or disassemble T antigen complexes, perhaps in a fashion analogous to the *cis*-acting DnaJ domain in the auxilin protein (77).

Chaperones also help sort proteins into various cellular compartments. In this regard, hsp70 is required to help transport SV40 large T antigen into the nucleus (34, 62, 82) through its association with hsp70, which requires the amino-terminal domain (44, 57, 58). Cellular chaperones may assist the other T antigens to their cellular destinations, for example, by shepherding middle T antigen to its location in various cellular membranes. Previously, middle T antigen association with hsp70 has been detected (49, 79). Genetic analyses indicate that DNA replication in vivo relies in some measure upon the common amino-terminal domain of large T antigen (45, 50). Failure to replicate may derive from defects in large T antigen's function as an initiator of replication, which requires proper folding and oligomerization and association with cellular replication proteins such as DNA polymerase α-primase (13, 81).

While the sequence homology between the amino-terminal domains of the SV40 and muPy T antigens suggests conservation of function, there are, nevertheless, significant differences in the biological activities of these T antigens. Functions present in the SV40 amino-terminal domain necessary for full transformation of primary rat cells are lacking from the amino-terminal domain of muPy large T antigen (42). Also notable is the observation that deletion of the amino-terminal domain of the muPy large T antigen (up to residue 260, which occurs in the second exon) only marginally impairs viral DNA replication in growing mouse cells (25), whereas, as mentioned above, the amino-terminal domain of SV40 large T antigen may be absolutely required for replication. Because the muPy large T antigen functions in DNA replication in a fashion analogous to the SV40 large T antigen, one might conjecture that another domain of the muPy T antigen provides the function specified by the SV40 large T antigen amino-terminal domain.

There is substantial evidence for the involvement of the amino-terminal domain of middle T antigen in its transforming function (15, 26, 73), and this can be rationalized by the requirement of the extreme amino-terminal sequences for association with protein phosphate 2A and pp60^{Src} (5, 26). Surprisingly, mutations within the highly conserved HPDK element of the DnaJ motif have little effect upon this association or upon the transformation of NIH 3T3 cells as measured by focus formation (5, 26). However, the effect of mutating the HPDK element upon cellular transformation as measured by anchorage independence, a more stringent assay, is not known. In contrast, mutation of the HPDK motif in SV40 T antigens is required for transformation and for transactivation of the cyclin A promoter, providing strong evidence for an essential function of the DnaJ element (54). The muPy amino-terminal sequences are most homologous with the SV40 and the consensus DnaJ domains up through the HPDK sequence, whereupon they diverge. This suggests that the muPy amino-terminal domain may differ in structure from that of the canonical DnaJ domain, whose structure has been solved by nuclear magnetic resonance (31, 52, 72). Such differences in structure are likely to dictate the chaperone "partners" with which this region interacts (60).

That muPy encodes an autonomous amino-terminal domain in the form of tiny T antigen raises the question as to whether it has a separate function in productive infection and transformation of cells. One cannot doubt that such an autonomous DnaJ domain might have a function, because for instance, only part of the *E. coli* DnaJ protein is required to complement λ replication (78). It is possible that tiny T antigen is responsible for some of the activities ascribed to small T antigen in cell transformation and tumorigenesis (1, 2, 18, 46), since it is expressed in cells from the small T antigen cDNA used to define these properties. However, the short half-life of tiny T antigen and its failure to accumulate in cells suggest that it is a "disposable" domain: its capacity to compete for binding of the cellular partners of the small, middle, and large T antigens might even reduce the vigor of the viral replication cycle. Reports of short-lived SV40 and bovine polyomavirus T antigens containing the common amino-terminal domain have also been made, but their functions have not been defined (61, 66, 85).

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