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The Amino-Terminal Transforming Region of Simian Virus 40 Large T and Small t Antigens Functions as a J Domain

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Simian virus 40 (SV40) encodes two proteins, large T antigen and small t antigen that contribute to virus-induced tumorigenesis. Both proteins act by targeting key cellular regulatory proteins and altering their function. Known targets of the 708-amino-acid large T antigen include the three members of the retinoblastoma protein family (pRb, p107, and p130), members of the CBP family of transcriptional adapter proteins (cap-binding protein [CBP], p300, and p400), and the tumor suppressor p53. Small t antigen alters the activity of phosphatase pp2A and transactivates the cyclin A promoter. The first 82 amino acids of large T antigen and small t antigen are identical, and genetic experiments suggest that an additional target(s) important for transformation interacts with these sequences. This region contains a motif similar to the J domain, a conserved sequence found in the DnaJ family of molecular chaperones. We show here that mutations within the J domain abrogate the ability of large T antigen to transform mammalian cells. To examine whether a purified 136-amino-acid fragment from the T antigen amino terminus acts as a DnaJ-like chaperone, we investigated whether this fragment stimulates the ATPase activity of two hsc70s and discovered that ATP hydrolysis is stimulated four- to ninefold. In addition, ATPase-defective mutants of full-length T antigen, as well as wild-type small t antigen, stimulated the ATPase activity of hsc70. T antigen derivatives were also able to release an unfolded polypeptide substrate from an hsc70, an activity common to DnaJ chaperones. Because the J domain of T antigen plays essential roles in viral DNA replication, transcriptional control, virion assembly, and tumorigenesis, we conclude that this region may chaperone the rearrangement of multiprotein complexes.

Simian virus 40 (SV40) encodes two proteins involved in tumorigenesis, the large and small tumor antigens. Large tumor antigen (T antigen) orchestrates many aspects of productive viral infection and is necessary and in many cases sufficient for tumorigenesis. T antigen is a 708-amino-acid multifunctional protein that elicits cellular transformation by acting on multiple targets, including members of the retinoblastoma tumor suppressor family (pRb, p107, and p130), members of the CBP family of transcriptional coactivators (CREB-binding protein [CBP], p300, and p400), and the tumor suppressor, p53. It is likely that additional T antigen targets important for transformation await discovery. T antigen sequences important for transformation map to two different regions of the molecule: the amino-terminal domain, which encompasses the first 125 amino acids, and a region located within the carboxy-terminal half of the molecule (Fig. 1). Major questions that remain to be answered are the following. How does T antigen act on each of the cellular targets? How does the concerted action of T antigen on these multiple targets lead to tumorigenesis?

Evidence that one or more independent transforming functions reside in the carboxy-terminal half of T antigen stems from the observation that carboxy-terminal fragments of T antigen have the ability to immortalize primary C57BL/6 mouse embryo fibroblasts (6). This activity maps to a bipartite region including amino acids 351 to 450 and 533 to 626 that correspond to sequences required for T antigen association with p53 (31). Recently, this region has been shown to be necessary for T antigen association with CBP, p300, and p400 (21, 35). It is not yet clear whether the CBP family proteins bind directly to T antigen or indirectly by associating with T antigen-bound p53.

The amino-terminal region of T antigen also carries independently acting transforming functions since it is capable of immortalizing primary cells, transforming established cell lines in culture, and inducing tumors in transgenic mice (8, 12, 13, 48). Three separate sequence motifs within the amino-terminal domain contribute to transformation. First, the sequence extending from amino acids 101 to 118 is similar to the cr2 motif found in adenovirus E1A proteins and papillomavirus E7 proteins (9, 10). This sequence is required for the association of T antigen with the three members of the retinoblastoma tumor suppressor family of proteins: pRb, p107, and p130 (17). Second, a region near the amino terminus of T antigen (amino acids 17 to 32) is similar to the cr1 motif found in adenovirus E1A proteins. Deletion of this sequence alters the transforming properties of T antigen, but a cellular target for this region has not been identified (41, 50). Finally, mutations within or near a hexapeptide (HPDKGG [amino acids 42 to 47]) conserved among the T antigens in all known polyomaviruses render T antigen transformation defective (39).

Recently, it has been noted that the first 82 amino acids of T antigen are similar to the J domain of the DnaJ family of molecular chaperones (7, 30). This similarity includes the HPDKGG motif (40) as well as the cr1-like sequence (Fig. 1). Since the amino-terminal 82 amino acids of large T antigen and small t antigen are identical, the J-domain homology region is included in small t antigen as well. One function of J-domain-containing chaperones is to interact with and mod-

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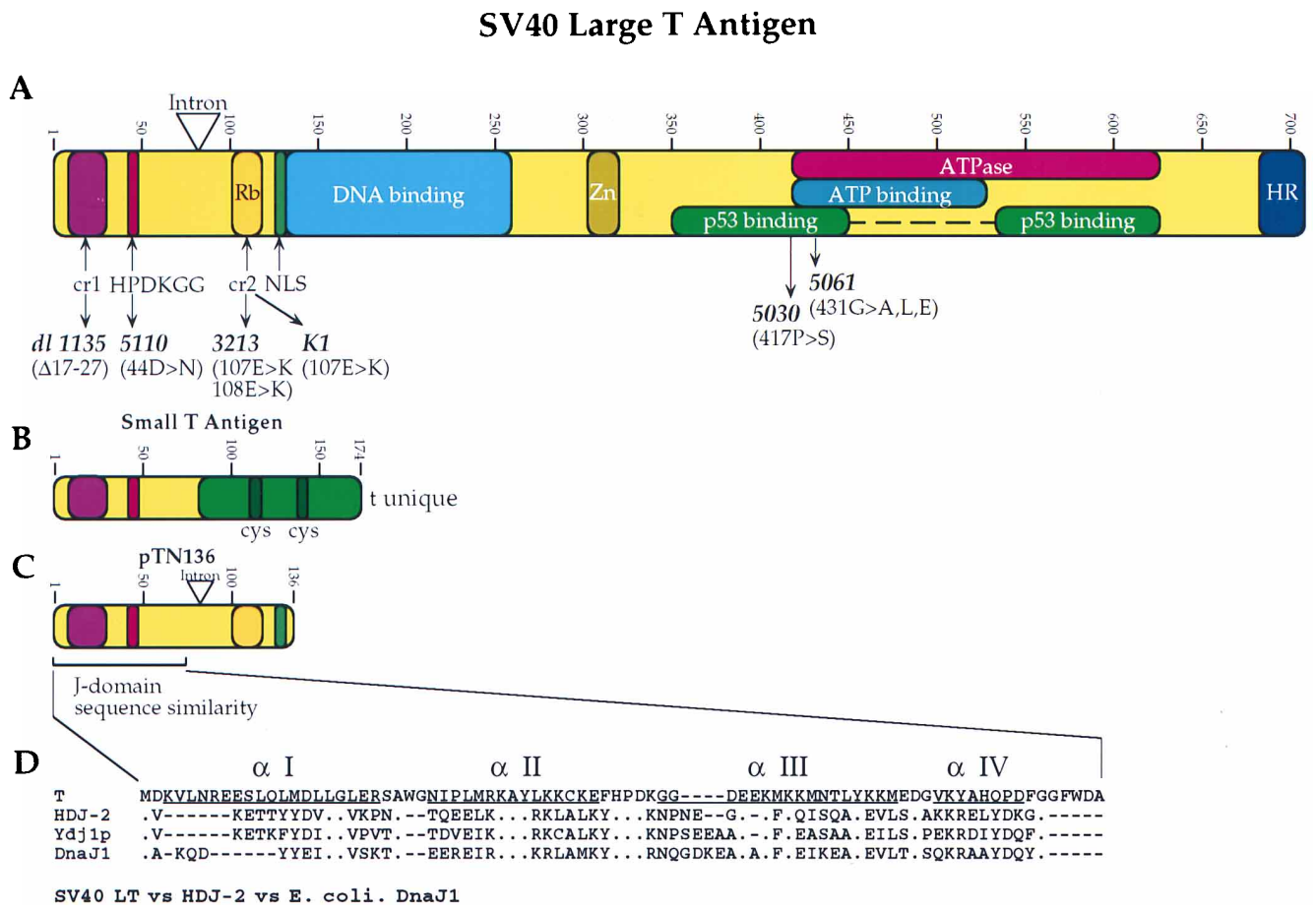

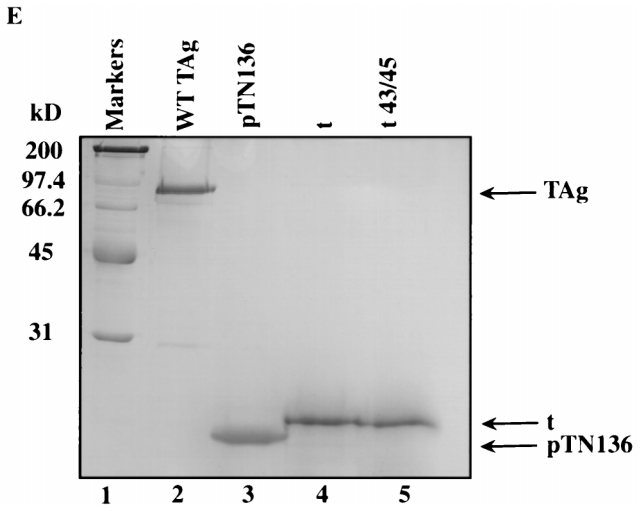

FIG. 1. Structure of SV40 large T antigen and small t antigen. (A) The domain structure of the 708-amino-acid large T antigen is diagramed, and the cr1, HPDKGG, and cr2 sequence motifs conserved among the large T antigens of all known polyomaviruses are indicated. NLS refers to the 7-amino-acid nuclear localization signal. The positions of the DNA binding domain, zinc finger motif, and sequences required for nucleotide binding, ATPase activity, and p53 association are shown. HR refers to the host range domain required for virion assembly. The positions and amino acid changes of each mutant used in this study are also shown. (B) Structure of the 174-amino-acid SV40 small t antigen. The first 82 amino acids are identical to those present in large T antigen, and the remaining 92 amino acids are unique to small t antigen. (C) Structure of pTN136. (D) Alignment of T antigen with the J domains of several DnaJ homologs. HDJ-2, human; YDJ1, *Saccharomyces cerevisiae*; DnaJ1, *E. coli*. The alignments were first reported by Cheetham et al. (7) and Kelley and Landry (30). Amino acids conserved among all four proteins are indicated by a dot below the T antigen sequence. Underscored sequences represent predicted helical segments of SV40 T antigen. (E) Wild-type and mutant large T antigens were purified from baculovirus-infected Sf9 cells by immunoaffinity chromatography. Small t antigens were purified as described in Materials and Methods. Each lane contains 4 μg of protein. Samples were separated on SDS–13% polyacrylamide gels and visualized by silver staining.

ulate the activity of a specific member of the DnaK family (reviewed in references 4 and 27). In fact, the cytosolic mammalian DnaK homolog, hsc70, associates with T antigen through its amino-terminal domain (45). Recently, T antigen sequences important for association with hsc70 were mapped and found to include the HPDKGG motif and surrounding sequences (3).

Mutations within the J-domain region of T antigen affect diverse viral functions including DNA replication, transcriptional regulation, virion assembly, and tumorigenesis (39, 41, 47, 48). Recent reports suggest a role for the T antigen-hsc70 association in stimulating the degradation of the tumor suppressors p107 and p130 (26, 49). Furthermore, hybrid proteins in which the amino-terminal portion of T antigen is replaced with the J domain of the human HDJ-1 DnaJ homolog are proficient for two T antigen-specific activities: viral DNA replication and transformation (18). These data and the association of the J domain with hsc70 raise the possibility that T antigen functions as a DnaJ molecular chaperone.

We have been characterizing the individual roles played by T

antigen by studying the properties of a collection of mutants with altered amino acids within each of the known sequence motifs important for transformation. Mutant *dl*1135 synthesizes a T antigen lacking the cr1-like motif and fails to transform established cell lines and to immortalize primary cells (37, 41). On the other hand, *dl*1135 induces T-cell lymphomas in transgenic mice at the same efficiency as does wild-type T antigen (50). Even though *dl*1135 is defective for viral DNA replication in vivo, the purified mutant protein supports the replication of viral DNA in vitro nearly as well as the wild type does (11, 14). Mutant 5110 carries a single amino acid substitution of D44 to N within the conserved HPDKGG motif, has a reduced ability to transform established cell lines, and is defective for both association with hsc70 and viral DNA replication (3, 39). Finally, mutant 3213, containing two amino acid substitutions (E107K and E108K) within the cr2-like sequences, is defective for T antigen association with pRb. Preliminary experiments with these mutants and with a series of truncated T antigens have revealed that the contribution of T antigen to cellular transformation is complex. Not only does T antigen possess multiple independently acting transforming functions, but which activity or combination of activities is required for transformation depends on the cell type.

We have shown that the transformation of some cell lines and of some tissues in transgenic mice requires the coordinate action of multiple T antigen activities, some residing in the amino-terminal domain and some located in the carboxy-terminal half of the molecule (8, 48, 50, 56). A similar cooperative model for transformation has been proposed for the adenoviruses. In this study, we seek to understand better the role of the J-domain homology region of T antigen in transformation and how this activity cooperates with other T antigen-transforming functions.

MATERIALS AND METHODS

Cell lines. The C3H10T1/2 and REF52 fibroblastic cell lines established from mouse or rat embryos, respectively, were maintained in minimal essential medium supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified 5% CO_2 atmosphere as described previously (48). Sf9 insect cells were grown in Grace's medium containing 10% FBS with lactalbumin hydrolysate/yeastolate supplement and used for the production of baculoviruses. These cells were maintained in spinner flasks at 27°C.

Plasmids and baculoviruses. Plasmids that express the wild-type or mutant T antigens by using the simian virus 40 (SV40) early promoter were derived from pSV-B3 or a related vector (38). Most experiments were performed with pRSV-B-neo that expresses a G418 resistance gene, using the SV40 early promoter, and a cDNA or genomic version of the wild-type or mutant T antigens, using the Rous sarcoma virus (RSV) promoter. SV40 mutants *dl*1135 (Δ17–27), 3213 (E107K, E108K), K1 (E107K), 5110 (D44N), and 5061 (G431 to ALE) have been described previously (22, 39, 41). 5030 (P417S) is an ATPase-defective mutant (5). TN136 was generated by placing a stop codon immediately following codon 136 of a T antigen cDNA. The TN136/5110, TN136/3213, and TN136/K1 double mutants were generated by replacing the *Asp*718-*Bbs*I fragment in TN136 with the corresponding sequences from the appropriate mutant plasmid.

Recombinant baculoviruses expressing mutant T antigens were generated by standard methods as described previously (32). Baculovirus producing human pRB was kindly provided by Robert Weinberg (Massachusetts Institute of Technology), and the baculovirus expressing p107 was generously provided by Dan Peeper (Dana Farber Cancer Institute). The recombinant baculovirus expressing p130 (33) from amino acid 372 to the carboxy terminus was generated by inserting an ATG codon upstream of codon 372 of a p130 cDNA followed by standard procedures for baculovirus production.

Cellular transformation assays. REF52 or C3H10T1/2 cells were plated at a density of 5×10^5 cells per 10-cm dish approximately 24 h prior to transfection. The cells were fed with fresh medium containing 20% FBS 4 to 6 h prior to transfection. $CaPO_4$ DNA precipitates were applied directly to the medium (24). After incubation at 37°C for 4 h, the cells were washed twice with 5 ml of medium and treated with 5 ml of 15 to 20% glycerol for 1 min. The cell monolayer was then rinsed with 5 ml of HEPES-buffered saline and incubated at 37°C in minimal essential medium supplemented with 10% FBS.

To assay for dense focus formation, transfected REF52 or C3H10T1/2 cells were maintained for about 4 weeks with changes of medium every third or fourth day. The plates were scored visually for foci following staining with Giemsa in acetone or assessed visually under a microscope. Foci appeared at about 3 weeks posttransfection in dishes transfected with plasmids expressing wild-type T antigen. When dominant selection markers, such as resistance to the antibiotics G418 (Geneticin sulfate) or hygromycin, were used either singly or in combination, the transfected cells were split into medium containing the antibiotic(s) 48 h posttransfection and maintained in medium plus antibiotic(s) for 16 to 18 days, with twice-weekly changes of medium. Antibiotic-resistant colonies were clearly visible in 2 weeks. The transformation phenotypes were scored at about 3 weeks posttransfection and expressed as a ratio of transformed versus flat colonies. The antibiotic concentrations routinely used were 400 μg/ml (active), and 100 μg/ml for hygromycin.

Immunoprecipitation and immunoblotting. Exponentially growing cells were washed with phosphate-buffered saline and either lysed immediately or frozen at −70°C for later use. Pellets were lysed for 20 min on ice in lysis buffer containing 50 mM Tris-HCl (pH 8), 5 mM EDTA, 150 mM NaCl, and 0.5% Nonidet P-40. Clarified extracts containing equal amounts of protein were immunoprecipitated with the appropriate antibodies for 1 h at 4°C. Immunoprecipitates were collected with protein A-Sepharose and washed three times in SNNTE (5% sucrose, 1% Nonidet P-40, 0.5 M NaCl, 50 mM Tris HCl [pH 7.5], 5 mM EDTA) and once in NTE (50 mM NaCl, 1 mM Tris HCl [pH 7.5], 1 mM EDTA). The final pellet was resuspended in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer and analyzed by electrophoresis.

T antigen-specific monoclonal antibodies PAb416, which recognizes an epitope between amino acids 83 and 121, PAb419 and PAb108, which recognize epitopes within the first 82 amino acids, and KT3, which is specific for the carboxy-terminal 11 amino acids, have been described previously (25, 54). F5, a monoclonal antibody specific for the murine polyomavirus T antigens, was kindly provided by Carol Prives. Monoclonal antibodies PAb421, PAb246, and PAb240, and a polyclonal antiserum (Chemicon) were used to detect p53. Antibodies IF8 (anti-pRb) and SD9 (anti-p107) were purchased from Santa Cruz Biotechnology. A goat anti-rabbit horseradish peroxidase (HRP)-coupled immunoglobulin G (Sigma) or a goat anti-mouse HRP-coupled immunoglobulin G (Sigma) were used as secondary antibodies. For Western blotting the ECL protocol (Amersham) was used.

Purification of wild-type and mutant T antigens and small t antigen. Wild-type and variants of T antigen were purified from baculovirus-infected Sf9 or High Five cells by immunoaffinity chromatography essentially as described previously (20, 32, 46). T antigen-specific monoclonal antibody PAb416 or PAb419 was routinely used for this purpose. pdl1135 was purified with the KT3 antibody (14). Antibodies were purified from hybridoma supernatants or from mouse ascites fluid and then coupled to protein A-Sepharose or protein G-Sepharose (Pharmacia). Purified T antigen was dialyzed against 10 mM Tris (pH 8.0)–100 mM NaCl–1 mM EDTA–1 mM dithiothreitol (DTT)–50% glycerol and stored at −20°C. Following SDS-PAGE, the purity and integrity of each T antigen preparation were assessed by silver staining and Western blot analysis (Fig. 1E) and determined to be >90%.

Small t antigen purification from insoluble bacterial fractions has been described previously (52). Briefly, protein was purified following solubilization with 10 M urea and renaturated in the presence of 2 mM DTT and 0.25 M $ZnSO_4$. Monomeric protein was separated from high-molecular-weight aggregates with Sephadex G-200. Further purification was accomplished by DEAE-cellulose and hydroxylapatite chromatography, and then chromatography on PAb419-Sepharose was performed to separate full-length 17-kDa protein from an internally initiated 14-kDa bacterial product.

T antigen binding to pRb, p107, and p130. Sf9 cells were coinfected with baculoviruses expressing T antigen and pRb, p107, or p130. Cell lysates were prepared as described above at about 43 h postinfection. Approximately 100 μg of each baculovirus lysate (1 mg for *dl*1135 lysates) was incubated with 1 μg of PAb416 for 45 min at 4°C to immunoprecipitate T antigen and associated proteins. For a positive control, extracts were also immunoprecipitated with pRb-, p107-, or p130-specific antibodies as appropriate.

The pellets were washed three times with SNNTE and once with NTE, resuspended in 15 μl of sample buffer, and resolved by SDS-PAGE (13% polyacrylamide). The proteins were transferred to polyvinylidene difluoride (PVDF) membrane and blocked in 1× phosphate-buffered saline–10% nonfat dried milk for 30 min. The blot was probed with IF8 to detect pRb, SD9 to detect p107, or an anti-p130 rabbit anti-peptide antibody (31a) for 2 h in blotting buffer (1 mM EDTA, 10 mM Tris, 100 mM NaCl)–1% nonfat dried milk. The blot was washed three times for 10 min each in rinse buffer (blotting buffer, 0.1% Tween 20). The blot was probed with an HRP-conjugated goat anti-rabbit antibody for 1 h and washed as above, and the pRb, p107, or p130 was detected by the ECL protocol.

ATPase assays and purification and analysis of Ssa1p and Ydj1p. Mammalian hsc70 was purchased from StressGen, Inc. Ssa1p was purified as described previously (2), dialyzed against buffer containing 50 mM Tris HCl (pH 7.4), 50 mM NaCl, 2 mM magnesium acetate, 0.8 mM DTT, and 5% glycerol, and stored. Ydjp1 was purified as described by Cyr et al. (15). ATPase assays were performed as described previously (15); the assay mixtures contained 1 μg of Ssa1p, 1 μg of the indicated protein, 50 μM ATP, and ~2 μCi of [α-^{32}P]ATP (Amersham). After 30 min at 30°C, 2-μl aliquots of the reaction mixtures were spotted onto cellulose-polyethyleneimine thin-layer chromatography plates (Fisher Scientific) and developed in 0.5 M LiCl–1 M formic acid. ATPase activity was apparent by the amount of [α-^{32}P]ADP liberated during the reaction. The results of the

ATPase assays were obtained on a Fuji PhosphorImager and quantified with the MacBas software program (version 2.2) from Fuji Photo Film Inc. Carboxymethyl lactalbumin (CMLA; Sigma) was iodinated and incubated with Ssa1p and various J-domain-containing proteins as described previously (15). All reaction mixtures contained 1 mM ATP in an ATP-regenerating system (2). Ssa1p-CMLA complexes were resolved by running 7.5 to 15% native polyacrylamide gels (supplemented with 1 mM ATP) at 4°C overnight.

RESULTS

We constructed a plasmid containing a truncated version of SV40 T antigen, TN136, that directs the synthesis of the first 136 amino acids of T antigen, including the amino-terminal transforming domain. Mutants *dl*1135, 5110, and 3213 (Fig. 1) were selected because they alter one of three conserved sequence motifs (cr1, HPDKGG, and cr2) located within the amino-terminal transforming domain. These mutants were engineered into full-length T antigen or placed in the context of the truncated TN136 protein. Expression in mammalian cells was driven either by the SV40 early promoter or by the RSV promoter as described in Materials and Methods. Baculovirus expression vectors were generated for each mutant in the context of a full-length T antigen or in the TN136 background and were purified from baculovirus-infected insect cells by immunoaffinity chromatography (20, 46).

Mutations affecting the J-domain homology region of T antigen are defective for cellular transformation. We examined the ability of each mutant to induce the morphological transformation of two established cell lines, C3H10T1/2 and REF52. The cells were transfected with vectors that express wild-type or mutant T antigens by using either the RSV long terminal repeat or SV40 early promoter, and they coexpress a marker for G418 resistance. Identical results were obtained when the cytomegalovirus immediate-early promoter was used to drive T antigen expression (data not shown). Following transfection, the cells were split to duplicate dishes. Some dishes were maintained for 6 weeks and scored for the presence of dense foci of transformed cells. The other set of dishes was treated with G418, and surviving G418-resistant colonies were then scored as possessing a normal or transformed morphology.

As previously reported (48), wild-type T antigen transformed both cell lines with equal efficiencies (Table 1). In the context of full-length T antigen, the three mutations each had a different effect on transformation. As reported, *dl*1135 did not transform REF52 cells (41). Furthermore, this mutant failed to transform the C3H10T1/2 line. We selected several G418-resistant cell lines generated from transfection with *dl*1135 and examined them for T antigen expression. Examples of these experiments are shown in Fig. 2A (lanes 9 and 10). The *dl*1135 T antigen was detected in both C3H10T1/2 and REF52 cells. Pulse-chase experiments and Western blot analyses demonstrated that pdl1135 was relatively unstable ($t_{1/2}$ = 2 h versus 12 h for the wild type) and accumulated to about 15% of the steady-state levels of wild-type T antigen in both cell types (data not shown).

Mutant 3213 was defective for the transformation of REF52 cells but did transform the C3H10T1/2 line at a reduced efficiency compared to the wild type. The K1 mutant consistently gave a higher frequency of transformation on C3H10T1/2 cells than did the double mutant 3213, but, like 3213, it failed to transform the REF52 line (Table 1). As shown in Fig. 2A (lanes 3 and 5 to 7), p3213 is expressed in both transformed C3H10T1/2 sublines as well as in morphologically normal REF52 cells. In both cell lines, p3213 migrated somewhat faster than wild-type T antigen through SDS-polyacrylamide gels. As previously reported, mutant 5110 showed a somewhat reduced efficiency of transformation on both cell types, but unlike *dl*1135, 5110-transformed lines that expressed the mutant T antigen could be readily isolated (Fig. 2A). All three mutant T antigens bound the cellular tumor suppressor p53 (Fig. 2A).

TABLE 1. Transformation of C3H10T1/2 and REF52 cells by T antigen mutants[a]

Mutant	Amino acid change(s)	Transformation[b]	
		C3H10T1/2	REF52
Expt A			
WT	1–708	78, 54, 67, 51	118, 153
*dl*1135	17–27 deleted	1, 0	0, 0
5110	D44N	55, 71	47, 32[c]
3213	E107K, E108K	7, 13	0, 0
K1	E107K	31, 28	0, 0
*dl*4000	40–708 deleted	0, 0, 0, 0	0, 0
Expt B			
WT	1–708	108, 83, 70	142, 98, 190
TN136	1–136	13, 18, 11	0, 0, 0
TN136/K1	1–136/E107K	0, 0, 0	0, 0, 0
TN136/3213	1–136/E107K, E108K	0, 0, 0	0, 0, 0
TN136/5110	1–136/D44N	0, 0, 0	0, 0, 0

[a] Subconfluent dishes of cells were transfected with 10 μg of DNA, with T antigen expression driven by the SV40 early promoter (experiment A), or 5 μg DNA, with T antigen expression driven by the RSV promoter (experiment B). *dl*4000 makes a short truncated T antigen with no known biological activity and is used here as a negative control (41)).

[b] The numbers represent dense foci per dish. Each number represents one dish.

[c] This result was first reported by Peden and Pipas (39).

We next examined whether the amino-terminal transforming region of T antigen, TN136, could transform these lines. We previously reported that *dl*1137, a mutant that synthesizes a truncated T antigen containing only the first 121 amino acids, transforms the C3H10T1/2 line with a frequency ranging from 10 to 20% of the wild-type frequency but fails to transform the REF52 line (48). The data in Table 1 shows that TN136 behaves identically. TN136 is relatively stable ($t_{1/2} \approx 6$ h) and accumulates to high levels in both C3H10T1/2 and REF52 cells as indicated by pulse-chase and Western blot experiments (data not shown). We then examined the effects of the 5110, K1 and 3213 mutations on the ability of TN136 to transform C3H10T1/2 and REF52 cells. When each of the three mutations was present in TN136, they were transformation defective (Table 1), even though each protein was expressed to levels comparable to that of wild-type and pTN136 levels (data not shown). We conclude that altering any of the three conserved sequence elements present within TN136 abolishes its ability to transform C3H10T1/2 and REF52 cells. However, in the context of a full-length T antigen, only *dl*1135 is defective for transformation of these lines.

Failure of T antigen J-domain mutants to complement for transformation. There is strong evidence that the transforming activities intrinsic to the J-homology region, cr2 motif, and p53-binding regions are distinct, independent T antigen functions, yet mutations in any one of these three regions render T antigen unable to transform the REF52 cell line and lead to a reduction in the transformation efficiency of C3H10T1/2 cells (48, 56). Therefore, we sought to determine if mutants defective in each of these activities could cooperate in *trans* to transform these lines. The results are shown in Tables 2 to 5. TN136 failed to complement either *dl*1135 or 3213, and 3213 was unable to complement *dl*1135. Experiments in which the

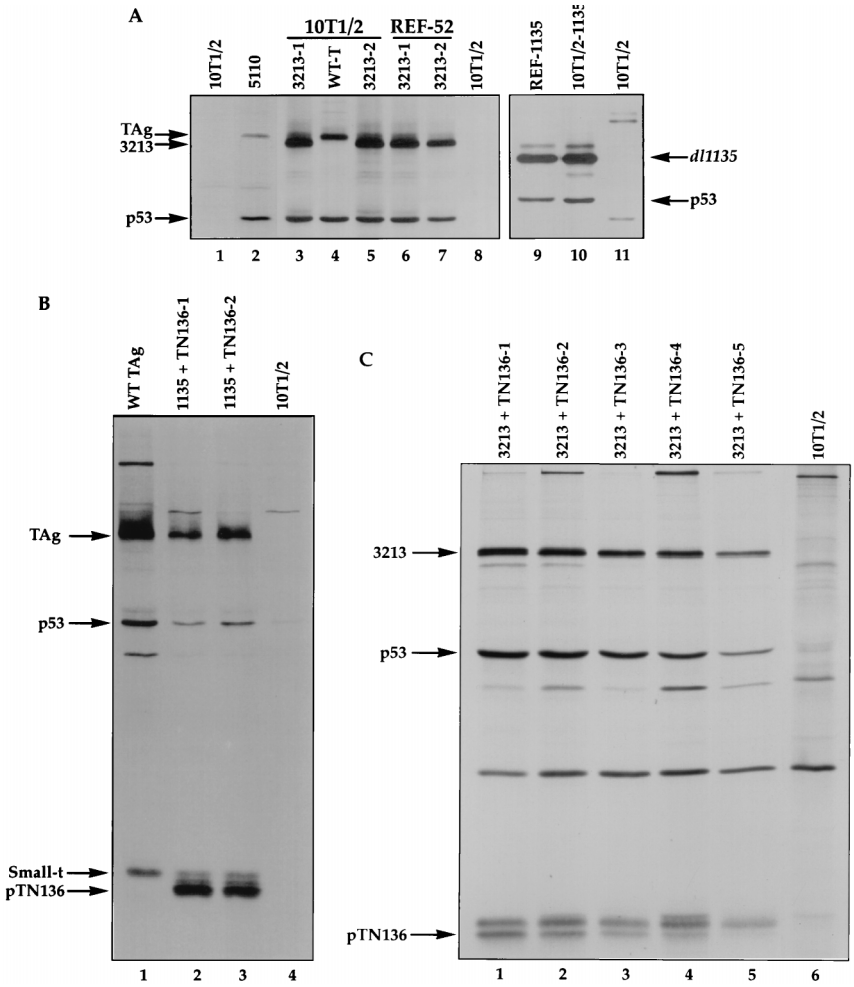

FIG. 2. Expression of mutant T antigens in C3H10T1/2 and REF52 cells. (A) T antigen expression in transfected cell lines. C3H10T1/2 (lanes 1 to 5, 8, 10, and 11) or REF52 (lanes 6 to 7 and 9) cells were metabolically labeled with [^{35}S]methionine for 2 h prior to the preparation of cellular extracts. T antigen was immunoprecipitated from the extracts with monoclonal antibody KT3 (*dl*1135), or PAb416 (5110 and 3213), resolved on SDS-polyacrylamide gels, and visualized by autoradiography. The figure is a composite of three different gels: lanes 1 and 2, lanes 3 to 8, and lanes 9 to 11). The lower signal for 5110p is typically seen under these labelling conditions. However, pulse-chase and immunoblot analyses indicate that 5110p is relatively stable and accumulates to near wild-type levels in transformed cells (data not shown). (B) Expression of pTN136 and pdl1135 in cotransfected cell lines. The coexpression of pdl1135 and pTN136 in C3H10T1/2 cells was detected by immunoprecipitating [^{35}S]methionine-labeled extracts with monoclonal antibody PAb416 followed by SDS-PAGE and autoradiography. Lane 1 shows cells transformed with wild-type T antigen, and lane 4 shows the parental C3H10T1/2 cell line. (C) Coexpression of pTN136 and p3213 in cotransfected cell lines. The coexpression of p3213 and pTN136 was detected as described for panel B. Lanes 2 to 5 contain extracts from morphologically normal REF52 cells cotransfected with both mutants. The band at about 30 kDa, between p53 and pTN136, nonspecifically immunoprecipitates with the antibody (see lane 6, untransformed 10T1/2 cells).

transfected cells were preselected by G418 resistance showed that all the expected mutant proteins were expressed (Fig. 2B and C). Some transformed colonies did appear on dishes cotransfected with TN136 and *dl*1135, suggesting a low level of complementation. However, upon further examination, we found that all of these clones contained wild-type T antigen, as evidenced by reactivity with monoclonal antibody PAb108 (see Materials and Methods). We conclude that the wild-type T antigen in these lines was generated by recombination between *dl*1135 and TN136. This failure to complement mutant versions of T antigen in *trans* suggests that the amino-terminal transforming functions of T antigen must exist in *cis* (on the same T antigen molecule) with one or more transforming activities intrinsic to the carboxy-terminal half of T antigen to achieve full transforming potential.

pTN136 expression does not lead to p53 stabilization or to elevated p53 levels. The E1A transforming proteins of the human adenoviruses are similar in many respects to the amino-

TABLE 2. Cotransfection assays for cellular transformation[a]

DNA 1 (Neo)	DNA 2 (Hyg)	No. of transformed/flat colonies of:			
		REF52		C3H10T1/2	
		Expt 1	Expt 2	Expt 1	Expt 2
WT	pHyg	67/78	42/27	56/41	34/28
*dl*1135	pHyg	0/16	0/29	0/63	0/60
TN136	pHyg	0/21	0/30	8/75	7/81
3213	pHyg	0/37	0/26	4/77	4/61
TN136	*dl*1135	3/48	1/53	11/68	13/60
3213	*dl*1135	0/25	0/21	6/70	4/69
pNeo	pHyg	0/18	0/21	0/55	0/60

[a] Plasmids (2 μg) containing a neomycin resistance gene (Neo) and expressing wild-type or mutant T antigens driven by the RSV promoter, and a second plasmid containing a hygromycin resistance (Hyg) gene alone or in combination with *dl*1135, were cotransfected into REF52 or C3H10T1/2 cells. Colonies resistant to both Neo and Hyg were scored as having a normal flat or dense transformed morphology. Experiments 1 and 2 were duplicate transfections.

TABLE 3. Transformation of 3213-expressing REF52 cells[a]

Mutant	No. of transformed foci in:	
	REF3213-1	REF3213-2
WT	103, 116	168, 120
*dl*1135	0, 0	0, 0
TN136	0, 0	0, 0
Mock	0, 0	0, 0

[a] Two REF52 sublines that stably express p3213 were transfected with 5 μg of the indicated plasmids. Expression of T antigen was driven by the RSV promoter. The transfected cells were maintained in drug-free medium, and the number of transformed foci was scored at 3 weeks.

terminal transforming region of T antigen. One consequence of E1A expression is the stabilization and increased steady-state levels of the cellular tumor suppressor p53 that results in p53-dependent apoptosis (16, 36). Although an increase in apoptosis in either C3H10T1/2 cells or REF52 cells expressing pdl1137 or pTN136 has not been observed, we examined p53 stability and levels in these cells. We found that the half-life of p53 in the C3H10T1/2 cell line (Fig. 3A) and in sublines expressing *dl*1137 (Fig. 3B) was ~20 min. Similar results were obtained in sublines expressing pTN136 (range from 15 to 28 min). Consistent with these findings, an immunoblot analysis revealed no increase in the steady-state levels of p53 in pTN136-expressing lines (Fig. 3D). On the other hand, expression of the E1A 243R protein in C3H10T1/2 cells did lead to increased p53 stability ($t_{1/2} \approx 1.2$ h) and steady-state levels, indicating that the normal p53 response pathway was functional in this cell line (Fig. 3C). Similar results were obtained in the REF52 cell line (data not shown).

The 3213 and K1 mutations alter the phosphorylation state of pTN136. We noticed that pTN136 migrated to multiple bands when electrophoresed through SDS-polyacrylamide gels (Fig. 4A). When pTN136 was expressed in a baculovirus vector system, multiple bands were also evident. This observation could be explained if pTN136 exists in multiply phosphorylated states. In contrast, when the 3213 (Fig. 4A) or K1 (not shown) mutations were placed within TN136, the proteins were apparent as a single band with a higher mobility than pTN136. To assess the amount of phosphate present in these proteins, the cells were metabolically labeled with [^{32}P]orthophosphate and cell extracts were prepared.

This result was confirmed by experiments in which pTN136 and its 3213 derivative were treated with phosphatase before electrophoresis (Fig. 4B and C). Treatment of pTN136 isolated from either transformed C3H10T1/2 cells or baculovirus-infected insect cells expressing pTN136 with potato acid phosphatase (PAP) resulted in the conversion of pTN136 to a single, rapidly migrating band. Phosphatase treatment had no effect on the migration of pTN136/3213. We conclude that the 3213 mutation results in a hypophosphorylation of pTN136. The K1 mutation had a similar effect on pTN136 phosphory-

TABLE 4. Transformation of 1135-expressing REF52 cells[a]

Mutant	No. of transformed/flat colonies of REF1135-1	
	Expt 1	Expt 2
WT	29/38	49/32
TN136	0/29	0/38
TN136/3213	0/36	0/23
*dl*1135	0/30	0/31
3213	0/32	0/35
RSV-neo	0/21	0/23

[a] A hygromycin-resistant REF52 subline stably expressing pdl1135 was transfected with 2 μg of the indicated plasmids. Colonies were selected for G418 resistance and scored as having a normal flat or dense transformed morphology. Experiments 1 and 2 were duplicate experiments.

TABLE 5. Transformation of 1135-expressing C3H10T1/2 cells[a]

Mutant	Amino acid changes	No. of transformed/ flat colonies	
		Expt 1	Expt 2
WT	1–708	68/41	53/59
TN136	1–136	12/99	8/68
TN136/K1	1–136/E107K	0/145	0/131
TN136/3213	1–136/E107K, E108K	0/126	0/111
*dl*1135	17–27 deleted	0/136	0/81
3213	E107K, E108K	6/85	10/63
RSV-neo		0/76	0/88

[a] C3H10T1/2 cells expressing pdl1135 were transfected with 2 μg of a plasmid expressing the indicated mutant driven by the RSV promoter and a G418-resistance marker driven by the SV40 promoter. Following G418 selection, colonies were scored as having a normal flat or a dense transformed morphology. Experiments 1 and 2 were duplicate experiments.

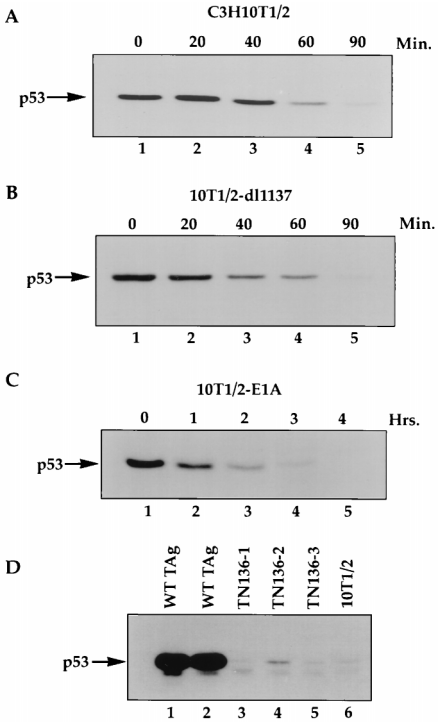

FIG. 3. Stability and steady-state levels of p53 in pTN136-expressing C3H10T1/2 cells. (A) p53 stability in normal C3H10T1/2 cells. The cells were labelled with [^{35}S]methionine for 1 h and chased in medium containing an excess of unlabeled methionine for the indicated times, and extracts were prepared as described in Materials and Methods. The samples were then immunoprecipitated with PAb421 and resolved by SDS-PAGE, and the gels were scanned with an AMBIS radioanalytic imaging system. (B) p53 stability in *dl*1137-transformed C3H10T1/2 cells. The stability of p53 in a pdl1137-expressing subline was determined as described for panel A. (C) p53 stability in C3H10T1/2 cells expressing the adenovirus E1A region. The stability of p53 in a C3H10T1/2 subline expressing the adenovirus E1A 243R protein was determined as described for panel A. (D) Steady-state levels of p53 in C3H10T1/2 cells expressing pTN136. Lysates containing 1 mg of total cellular protein were immunoprecipitated with a rabbit anti-p53 polyclonal serum, blotted onto a PVDF membrane, and probed with an monoclonal antibody cocktail containing PAb421, PAb246, and PAb240.

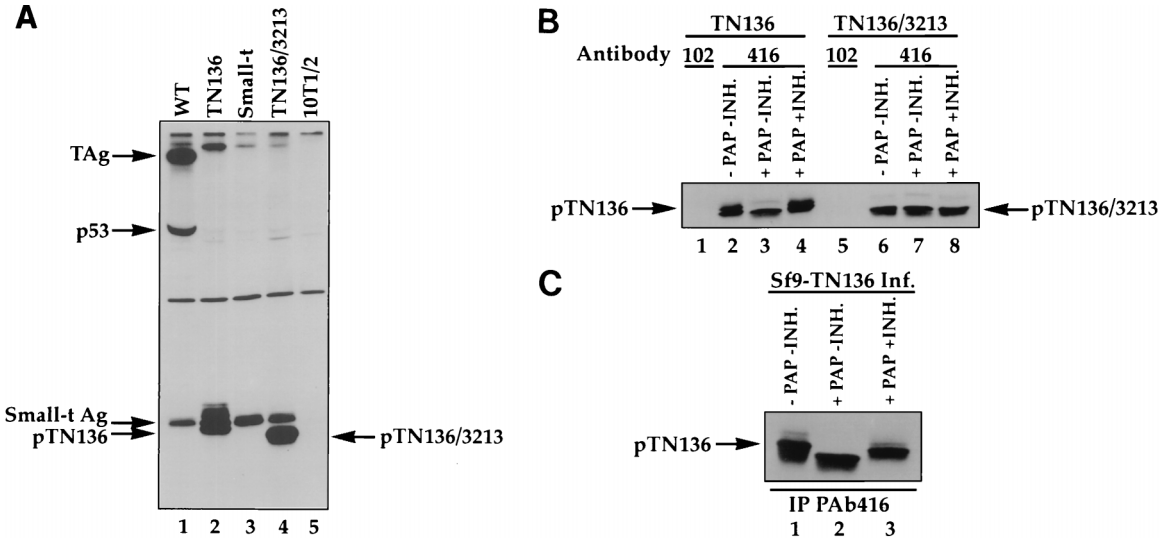

FIG. 4. Phosphorylation of pTN136. (A) Immunoprecipitation of pTN136 and pTN136/3213 from C3H10T1/2 cells. C3H10T1/2 cells (lane 5) or sublines expressing wild-type (WT) T antigen (lane 1), pTN136 (lane 2), SV40 small t antigen (lane 3), or pTN136/3213 (lane 4) were labeled with [^{35}S]methionine. T antigen (TAg) was immunoprecipitated from labeled cell extracts with PAb419, resolved by SDS-PAGE, and visualized by autoradiography. The band between p53 and pTN136 is a nonspecific contaminant in the immunoprecipitate (Fig. 3). (B) Phosphatase treatment of pTN136 expressed in C3H10T1/2 cells. C3H10T1/2 cells expressing pTN136 (lanes 1 to 4) or pTN136/3213 (lanes 5 to 8) were metabolically labeled with [^{35}S]methionine, and extracts were prepared and immunoprecipitated with PAb416. The immunoprecipitates were recovered with protein A-Sepharose beads and washed as described in Materials and Methods. The Sepharose beads were finally washed in PAP reaction buffer and then treated with PAP as described in the text. The control reactions were identical except that phosphatase inhibitors were added along with PAP. After treatment, the beads were washed again and analyzed by SDS-PAGE. PAb102 recognizes an epitope in the carboxy terminus of T antigen which is not present in pTN136. This antibody was used as a negative control for the immunoprecipitations (lanes 1 and 5). (C) Phosphatase treatment of pTN136 expressed from a baculovirus vector. pTN136 expressed in baculovirus-infected Sf9 cells was analyzed as described for panel B.

lation (data not shown). Some of the effects of the 3213 mutation may be due to the absence of phosphorylation of key amino acids.

Interaction of pTN136 with members of the retinoblastoma family of tumor suppressors. We next examined the intrinsic ability of each mutant T antigen to associate with pRb, p107, and p130. Insect cells were coinfected with baculovirus vectors expressing a T antigen mutant and a member of the retinoblastoma family. T antigen was immunoprecipitated from infected cell extracts and analyzed by SDS-PAGE. The separated proteins were then transferred to PVDF membranes, and the retinoblastoma family member present in complex with T antigen was detected by immunoblotting.

As displayed in Fig. 5A, pTN136 bound pRb as well as or better than wild-type T antigen did. Mutant 3213 failed to associate with pRb, while the *dl*1135 and 5110 mutations did not affect T antigen interaction with pRb (data not shown). Both full-length wild-type T antigen and pTN136 also associated with p107 and p130 (Fig. 5). However, in this case the 3213 mutation, in the context of full-length T antigen, reduced but did not eliminate the association of the protein with p107 and p130. A significant amount of p107 and p130 remained complexed to p3213. In contrast, when the K1 mutation was placed in the context of pTN136, the association with p107 and p130 was completely eliminated. We conclude that the interaction of p107 and p130 with the cr2 motif is influenced by amino acids within the carboxy-terminal two-thirds of T antigen.

The amino-terminal transforming domain of T antigen activates the ATPase activity of hsc70. The amino-terminal domain of T antigen harbors a region containing significant similarity to the J domain, a motif found at the amino terminus in members of the DnaJ family of molecular chaperones (reviewed in references 4 and 27) (Fig. 1). In all cases thus far examined, the ATPase activity of DnaK molecular chaperones is greatly stimulated upon interaction with their cognate DnaJ chaperone. Since pTN136 contains a region of sequence similarity to DnaJ proteins and associates with the mammalian DnaK homolog, hsc70 (45), we tested the effect of pTN136 on hsc70 ATPase activity. Figure 6A shows that the addition of pTN136 resulted in a fivefold increase in the ATPase activity of hsc70. We next tested the effect of full-length wild-type T antigen on the hsc70 ATPase activity. Figure 6B shows that full-length T antigen also activated the hsc70 ATPase. Full-length wild-type T antigen had a higher rate of ATP hydrolysis (8.2 pmol of ATP hydrolyzed per min) than did hsc70 (2.2 pmol/min), while a mixture of the two showed a synergistic effect (14.5 pmol/min). The level of hsc70 ATPase activation increased with increasing levels of pTN136 (Fig. 6C). We also examined a variety of irrelevant proteins, including bovine serum albumin and a monoclonal antibody, for their abilities to activate the hsc70 ATPase, and they all failed to function as a DnaJ (data not shown).

T antigen functions as a DnaJ chaperone for the yeast DnaK homolog Ssa1p. Next, the ability of T antigen to activate the ATPase activity of Ssa1p, a yeast cytosolic hsc70 homolog, was assayed. The ATPase activity of Ssa1p is elevated 8- to 10-fold by its DnaJ partner, Ydj1p, while peptides stimulate the ATPase activity of Ssa1p 2-fold or less (15, 57). Ydj1p and ATP are also able to promote the release of a permanently unfolded polypeptide, CMLA, from Ssa1p in vitro (15). Figure 7A shows that pTN136 activated the ATPase activity of Ssa1p 9.4-fold. Treatment of pTN136 with thermolysin, which completely hydrolyzed the protein (data not shown), eliminated its ability to stimulate the ATPase activity of Ssa1p (Fig. 7A).

To demonstrate that this activation by pTN136 was specific, we measured the ability of pTN136 to stimulate the hydrolysis of ATP by Ssa1p in the presence of a monoclonal antibody directed against pTN136, PAb419. A monoclonal antibody specific for the murine polyomavirus large T antigen, F5, was

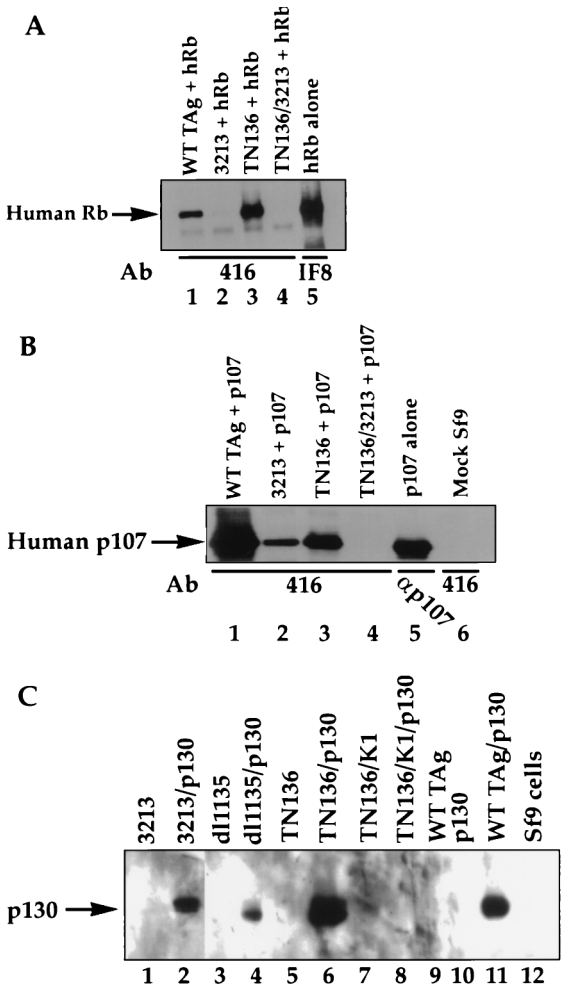

FIG. 5. Interaction of pTN136 with pRb, p107, and p130. Lysates were derived from Sf9 cells which were coinfected with baculoviruses expressing either pRB, p107, p130 and various T antigens as indicated. (A) Extracts containing equal amounts of total protein were immunoprecipitated with PAb416 (anti-T) or IF8 (anti-RB; Santa-Cruz Biotechnology), resolved by SDS-PAGE (7.5% polyacrylamide), and transferred to a PVDF membrane. The blot was probed with IF8 to detect pRB. (B) Lysates from Sf9 cells coinfected with baculoviruses expressing T antigen and p107 were processed as described in Materials and Methods. A rabbit polyclonal p107 antibody (Santa Cruz Biotechnology) was used to probe the Western blot. (C) Extracts containing 100 μg of total protein (1 mg for *dl*1135 reactions) were immunoprecipitated with PAb416, resolved by SDS-PAGE (13% polyacrylamide), and transferred to a PVDF membrane. The blot was probed with anti-p130 rabbit polyclonal serum.

also tested. The PAb419 epitope maps near the amino terminus of T antigen and is destroyed by the deletion present in *dl*1135 (9), while the F5 antibody reacts poorly with SV40 T antigen (data not shown). We discovered that while F5 marginally stimulated pTN136 activation of the ATPase activity of Ssa1p, PAb419 inhibited this activity ~twofold (Fig. 7B).

We also tested the ability of two ATPase-defective T antigen mutants, 5030 and 5061, to activate Ssa1p. Both 5030 and 5061 stimulated the Ssa1p ATPase activity five- to sixfold and exhibited little endogenous ATPase activity when assayed alone (Fig. 7C). Finally, because the J-domain sequence homology lies within the first 82 amino acids of large T antigen and because these sequences are also present in small t antigen, we tested the ability of purified small t antigen to stimulate the ATPase activity of Ssa1p. Figure 7C shows that small t antigen stimulated the Ssa1p ATPase activity approximately fourfold. Importantly, purified small t antigen containing mutations in the conserved proline and lysine in the J domain (at positions 43 and 45 [Fig. 1]) failed to stimulate the ATPase activity of Ssa1p, demonstrating a specific requirement for the J domain. Similarly, pTN136/5110 showed a reduced ability to stimulate Ssa1p ATPase activity (data not shown). We conclude that both large T antigen and small t antigen contain a J domain that is able to interact functionally with Ssa1p.

To assay further for the productive interaction of Ssa1p with its cognate DnaJ chaperone, we determined that Ydj1p promotes the release of a permanently unfolded polypeptide, CMLA, from Ssa1p in the presence of ATP (Fig. 7D, lane 4). The Ydj1p-catalyzed release of CMLA from Ssa1p was previously used to indicate the formation of a productive DnaJ-DnaK chaperone pair (15). T antigen was also able to effect this release (lane 4), as was the ATPase-defective variant, p5030, and the T antigen J domain alone, pTN136 (lanes 5 and 6). T antigen- and Ydj1p-mediated release occurred at nearly identical levels in these experiments. Thus, the J domain of T antigen acts in vitro as a DnaJ-like chaperone for the yeast hsc70, Ssa1p.

DISCUSSION

SV40 T antigen is a regulatory protein that controls four fundamental biological processes: (i) DNA replication, (ii) transcriptional regulation, (iii) virion assembly, and (iv) tumorigenesis. In this study, we have shown that SV40 large T antigen has the properties of a DnaJ molecular chaperone and that J-domain function is required for cellular transformation. Previous studies have demonstrated that the J domain is also important for viral DNA replication (39, 41), transcriptional control (42, 48), and virion assembly (47). One common theme that connects these disparate processes is the assembly, disassembly, and rearrangement of multiprotein complexes, a function that DnaJ family members orchestrate along with DnaK homologs.

T antigen functions as a DnaJ molecular chaperone. The two major early proteins encoded by SV40, large T antigen and small t antigen, have identical sequences through their first 82 amino acids. Recently, a significant sequence similarity between the amino terminus of polyomavirus T antigens and the J domain of DnaJ molecular chaperones was noted (30). One function of the J domain is to mediate the physical association of the DnaJ protein with its DnaK partner (reviewed in references 4 and 27). Previous reports showing that (i) T antigen is associated with a DnaK homolog, hsc70, in SV40-transformed or -infected cells, (ii) ATP stimulates the dissociation of the T antigen-hsc70 complex, and (iii) the interaction with hsc70 is mediated by the amino-terminal region of T antigen suggested that T antigen possesses a functional J domain (45). Furthermore, the T antigen sequences important for its association with hsc70 correspond to those known to govern the interaction of the *Escherichia coli* DnaJ and DnaK proteins (3).

In this study, we have shown that T antigen possesses two hallmarks of a DnaJ chaperone, the ability to elevate the ATPase activity of a DnaK homolog and the ability to catalyze the release of a polypeptide substrate from the DnaK (15, 34). pTN136, a truncated form of T antigen that contains an amino-terminal T antigen sequence sufficient for transformation, also elevates hsc70 ATPase activity and dissociates a permanently unfolded polypeptide from hsc70. The ability of pTN136 to stimulate hsc70 ATPase activity is destroyed by prior treatment with protease and is partially inhibited by PAb419, a specific monoclonal antibody that binds to the amino terminus of T

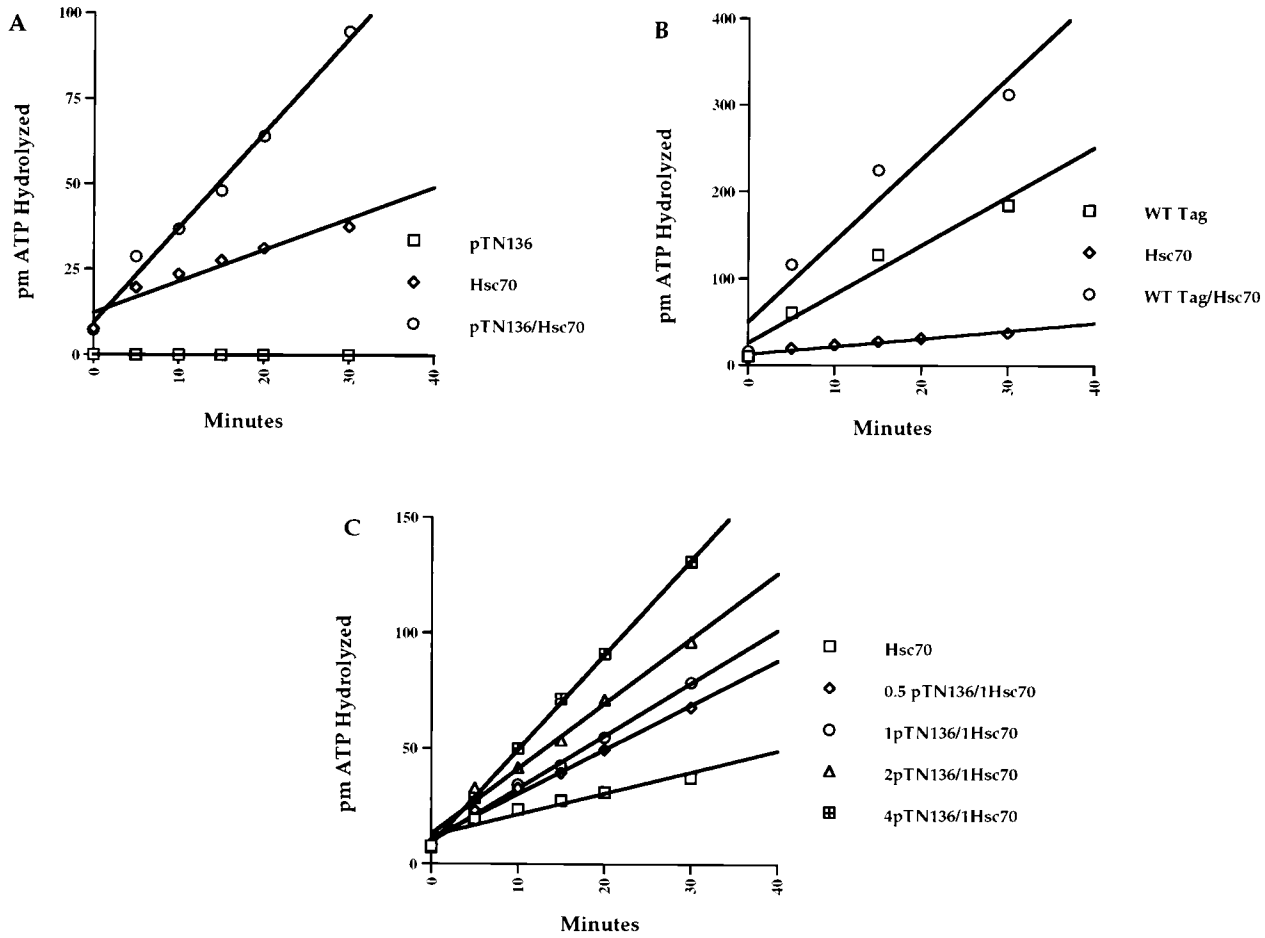

FIG. 6. Stimulation of hsc70 ATPase activity by pTN136. Each protein was incubated with [α-^{32}P]ATP at 37°C. The final concentration of ATP was 10 μM. At various times, aliquots were removed from the reaction mixture and analyzed by polyethyleneimine-cellulose chromatography. The amount of ATP hydrolyzed was determined by AMBIS analytical imaging. The protein concentrations were as follows: wild-type T antigen, 15 pmol; pTN136, 60 pmol; hsc70, 30 pmol. The proteins used in each assay are indicated. In panel C, 30 pmol of hsc70 was used with either 15, 30, 60, or 120 pmol of pTN136. (A) Stimulation of hsc70 ATPase by pTN136. (B) Stimulation of hsc70 ATPase by wild-type T antigen. (C) Effect of increasing pTN136 levels on hsc70 ATPase activity.

antigen. Because the J-domain sequence similarity is contained within sequences common to large T antigen and small t antigen, we also showed that small t antigen elevates the ATPase activity of hsc70.

The three-dimensional nuclear magnetic resonance spectroscopy solution structures available for the *E. coli* DnaJ and human HDJ-1 J domains (28, 43, 51) both show a finger-like structure formed by two α-helical segments (α-2 and α-3) inked by a loop containing the invariant sequence HPD (Fig. 8). Two additional α-helical segments (α-1 and α-4) are joined to the finger structure via nonconserved loops. Secondary-structure prediction programs predict a similar structure for the first 76 amino acids of T antigen, with the conserved HPDKGG motif forming a loop between two α-helical segments (Fig. 1D). Mutations of these amino acids in yeast DnaJ homologs and in T antigen results in a temperature-sensitive phenotype and loss of T antigen association with hsc70, respectively (3, 19, 23). A mutation in the conserved histidine also inactivates *E. coli* DnaJ (53). Furthermore, hybrid proteins in which the J domain of DnaJ is replaced with the corresponding sequences from SV40 T antigen are functional in that they restore *E. coli* growth at the nonpermissive temperature and bacteriophage λ replication (29). Hybrid proteins in which the J-domain region of T antigen is replaced with the corresponding sequences from HDJ-1 are replication and transformation competent (18). Taken together, this evidence indicates that the amino termini of both large T antigen and small t antigen form a J domain that is structurally and functionally similar to the J domains of DnaJ and HDJ-1.

J-domain action is required for SV40 transformation. The amino-terminal transforming region of T antigen consists of the J-domain and the cr2 motif, which governs T antigen association with the pRb-family. Mutations in either cr1 (*dl*1135) or the HPD loop in the J domain (5110) abolished the ability of T antigen to transform cells. A mutation in the cr2 motif (3213) also inactivated the transforming potential of the amino-terminal domain. We conclude that the J domain acts in concert with cr2 to effect transformation. Since the J domain directs the association of T antigen with hsc70 (3), these results suggest that a ternary complex including T antigen, hsc70, and pRb, p107, or p130, that bind via cr2, is necessary for transformation and is consistent with recent reports that both the J-domain region and cr2 are needed to induce the degradation of p107 and p130 (49).

The *dl*1135 mutation removes a portion of the predicted α-1 helix from the J domain of T antigen (Fig. 1) and is defective for the transformation of both C3H10T1/2 and REF52 cells. In contrast, the HPD loop and cr2 motif are not absolutely re-

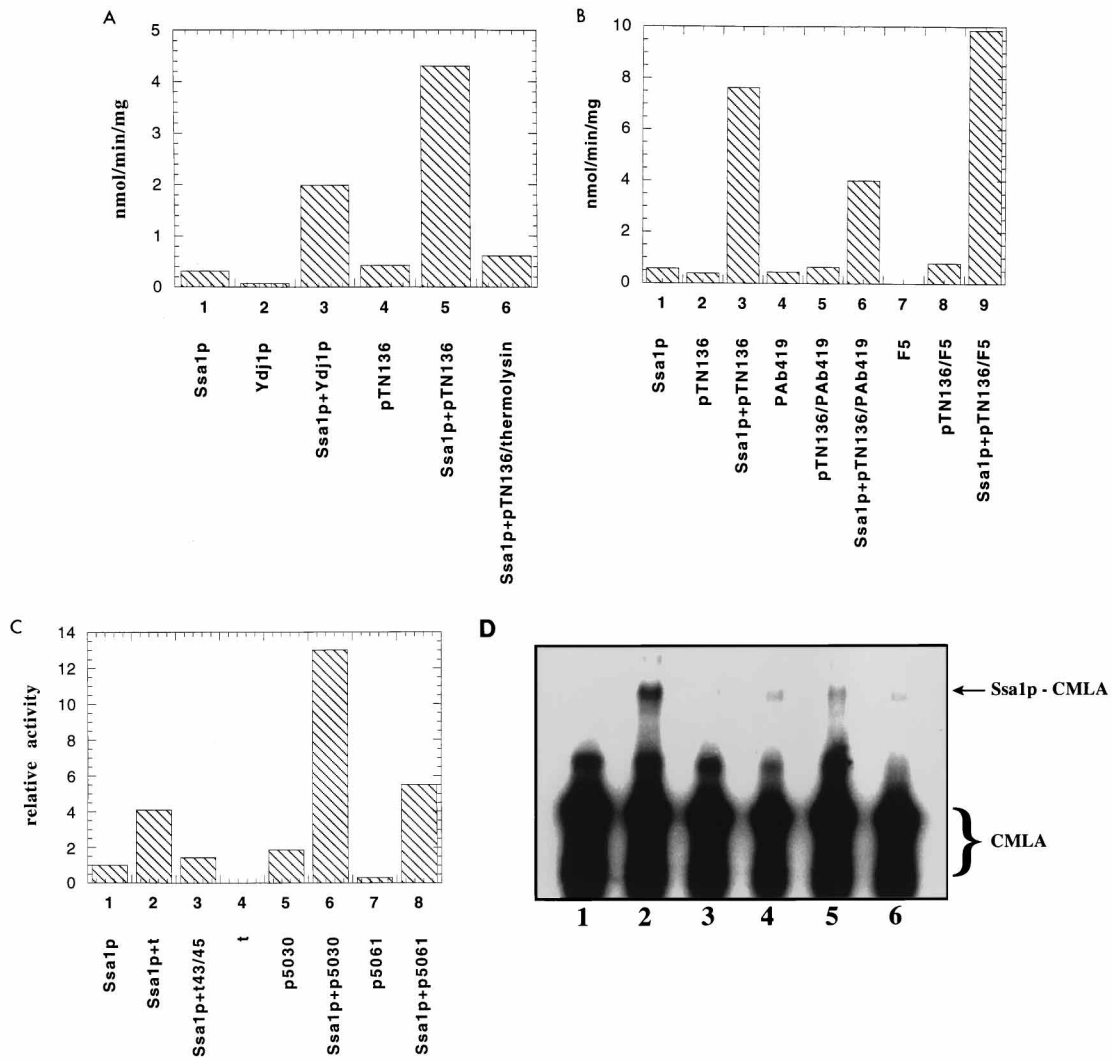

FIG. 7. Large T antigen and small t antigen stimulate the ATPase activity of a yeast hsc70. (A) A yeast DnaJ homolog, Ydj1p, and pTN136 stimulate the ATPase activity of Ssa1p. ATPase assays in the absence or presence of the yeast hsp70 Ssa1p and the indicated proteins were assembled and analyzed as described in Materials and Methods. Where indicated, pTN136 was treated with thermolysin, and the protease was inactivated before the reaction. Protease inactivation was confirmed by incubation with purified Ssa1p. Protease-treated pTN136 lacked endogenous ATPase activity (data not shown). Activity is expressed in nanomoles of ATP hydrolyzed per minute per milligram of Ssa1p. (B) An antibody directed against pTN136 inhibits the ability of pTN136 to stimulate fully the ATPase activity of Ssa1p. pTN136 was preincubated with the indicated antibody for 45 min at ~20°C before Ssa1p was added to the reaction mixture. PAb419 reacts with the amino-terminal 30 amino acids of pTN136, while F5 is an antibody directed against murine polyomavirus large T antigen. (C) Small t antigen (t) and ATPase-defective full-length derivatives of T antigen (p5030 and p5061) also stimulate the ATPase activity of Ssa1p, while mutations in the HPD conserved motif in small t antigen prevent the ability of the protein to stimulate the ATPase activity of Ssa1p. Assays were performed as described above, and relative ATPase activities were normalized to that of Ssa1p in the absence of any additional factors. (D) The J domain of T antigen, like Ydj1p, catalyzes the release of a permanently denatured polypeptide from Ssa1p. Reactions containing radioiodinated CMLA either alone (lane 1) or in the presence of 0.6 μM Ssa1p (lane 2), 2.6 μM Ydj1p (lane 3), 1.28 μM T antigen (lane 4), 2.6 μM p5030 (lane 5), or 5.2 μM TN136 (lane 6) were assembled and analyzed as described in Materials and Methods.

quired for transformation in the context of a full-length T antigen; however, mutations in these sequences render TN136 transformation defective (Table 1). This indicates that the presence of the carboxy-terminal region of T antigen can compensate for mutations in both the J domain and cr2. One possible explanation for this observation is that hsc70 and one or more members of the pRb family contact the carboxy-terminal region of T antigen as well as cr2. In fact, although p107 and p130 bind p3213, this binding was abolished when the 3213 mutation was placed in TN136 (Fig. 5). The 3213 mutation also abolished T antigen binding to pRb in both full-length T antigen and TN136. We are currently exploring the possibility that p107 and p130 directly contact the carboxy-terminal region of T antigen.

Interaction of the J domain with multiple T antigen transforming functions. We examined the ability of mutants affecting individual transforming functions to complement one another in *trans*. We found that *dl*1135, which deletes a portion of α-1 of the J domain, fails to complement 3213. This is consistent with the hypothesis that the J-domain activity is essential for T antigen to act on pRb, p107, or p130 and that at least in C3H10T1/2 and REF52 cells, the J domain cannot be supplied in *trans*. Since the J domain directs T antigen association with hsc70, we hypothesize that a ternary complex involving T an-

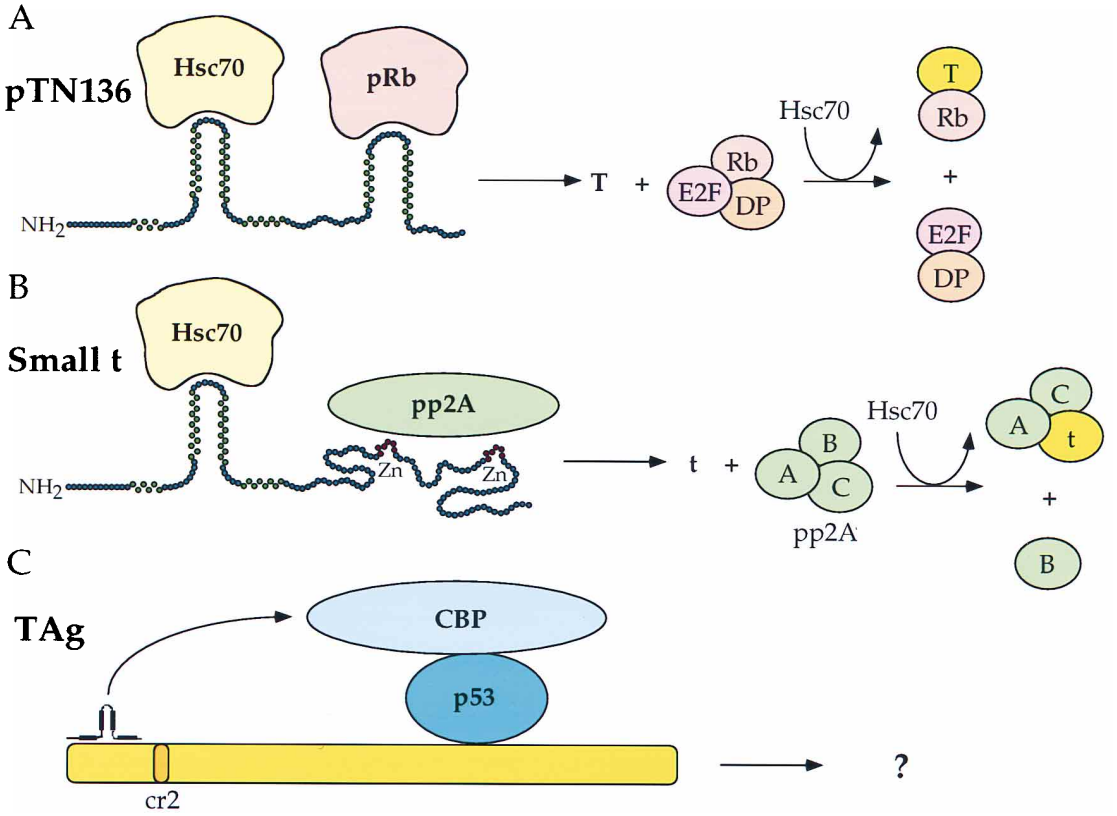

FIG. 8. Possible role for the T antigen J domain in transformation. (A) The amino-terminal transforming region of large T antigen consists of two elements: the J domain, which directs T antigen association with hsc70, and cr2, which is required for T antigen binding to members of the retinoblastoma (Rb) family. The action of T antigen on pRb results in the release of the heterodimeric transcription factor E2F-DP and the formation of a T antigen-pRb complex. We hypothesize that the role of the J domain is to facilitate the release of E2F-DP from pRb and/or to stimulate the formation of the T antigen-pRb complex. (B) Small t antigen contains a J domain adjacent to a zinc binding domain that directs the association of small t antigen with the trimeric phosphatase pp2A. The B regulatory subunit of pp2A is released and is replaced by small t antigen on the trimer. (C) The J domain acts on one or more transforming functions contained in the carboxy-terminal region of T antigen. Possible targets include p53 and/or members of the CBP family. The nature of the molecular rearrangements involved in this action is not known.

tigen, hsc70, and a member of the pRb family is required for cellular transformation.

The failure of *dl*1135 to complement TN136 suggests that α-1 must act in *cis* with some activity localized to the carboxy-terminal region of T antigen to induce transformation. T antigen association with two important classes of cellular proteins, the tumor suppressor p53 and members of the CBP family of transcriptional adapters, CBP, p300, and p400, is directed via the carboxy-terminal region of T antigen (1, 35). At least one report suggests that the amino-terminal domain of T antigen can alter p53 function (44). More recently, it was reported that while the major binding site for the CBP family lies within the carboxy-terminal p53 binding domain, sequences near cr2 are at least partially involved (21). These data suggest that the J domain might be directing hsc70 to CBP, p300, or p400 via a complex with T antigen and that this action must take place in *cis* to effect cellular transformation.

That the J domain acts on the CBP family or some other target of the carboxy-terminal region of T antigen during cellular transformation is consistent with the results of experiments demonstrating the ability of T antigen to cooperate with the adenovirus E1A protein. E1A mutants defective for transformation because they cannot associate with p300 can be complemented in *trans* by wild-type T antigen, while the T antigen mutant *dl*1135 is unable to complement these E1A mutants (55). Thus, T antigen can bypass the requirement for p300-E1A interaction, and the deletion of amino acids within the cr1-like motif of T antigen destroys this activity. It has recently been shown that T antigen association with p300 requires the carboxy-terminal half of T antigen; therefore, the cr1 motif is not required for p300 binding (35). Our results suggest that the J domain may act on p300 or other members of the CBP family to effect T antigen action.

Rearrangement of multiprotein complexes: a role for molecular chaperones in viral infection and neoplasia? We hypothesize that the J domain directs the association of hsc70 or some other cellular DnaK-family chaperone to multiprotein complexes that are targets for T antigen action. This hypothesis is illustrated in Fig. 8. For example, one well-characterized action during T antigen transformation is the release of the heterodimeric transcription factor E2F/DP from a complex with pRb, p107, or p130. Since the J domain is directly adjacent to cr2, it may be positioned to direct hsc70 to this complex. Similarly, the J domain present in small t antigen is positioned to direct hsc70 to the trimeric enzyme phosphatase pp2A (Fig. 8B). The consequence of this interaction is to disassemble the enzymatic complex. Furthermore, our data show that the J domain must act on one or more activities that map to the carboxy-terminal region of T antigen, sequences known to be critical for its association with p53 and members of the CBP family (Fig. 8C).

Since SV40 T antigen co-opts cellular systems to effect virus growth and transformation, it is likely that DnaJ-DnaK interactions are important for tumorigenicity of nonviral etiology.

Therefore, the disruption of DnaJ-DnaK interactions may represent a new target for anticancer drug therapies. Finally, the ability of SV40 to utilize a chaperone machine for infection is not unique. The initiation of λ DNA replication involves the assembly of a multiprotein complex including the *E. coli* DnaK and DnaJ proteins that activate the DnaB helicase (58). Since J-domain mutations render T antigen defective for viral DNA replication, we speculate that T antigen-hsc70 interactions may function in a similar manner during SV40 DNA replication.

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