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Inactivation of pRB-Related Proteins p130 and p107 Mediated by the J Domain of Simian Virus 40 Large T Antigen

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Inactivation of the retinoblastoma tumor suppressor protein (pRB) contributes to tumorigenesis in a wide variety of cancers. In contrast, the role of the two pRB-related proteins, p130 and p107, in oncogenic transformation is unclear. The LXCXE domain of simian virus 40 large T antigen (TAg) specifically binds to pRB, p107, and p130. We have previously shown that the N terminus and the LXCXE domain of TAg cooperate to alter the phosphorylation state of p130 and p107. Here, we demonstrate that TAg promotes the degradation of p130 and that the N terminus of TAg is required for this activity. The N terminus of TAg has homology to the J domain of the DnaJ family of molecular chaperone proteins. Mutants with mutations in the J-domain homology region of TAg are defective for altering p130 and p107 phosphorylation and for p130 degradation. A heterologous J-domain from a human DnaJ protein can functionally substitute for the N terminus of TAg in the effect on p107 and p130 phosphorylation and p130 stability. We further demonstrate that the J-domain homology region of TAg confers a growth advantage to wild-type mouse embryo fibroblasts (MEFs) but is dispensable in the case of MEFs lacking both p130 and p107. This indicates that p107 and p130 have overlapping growth-suppressing activities whose inactivation is mediated by the J domain of TAg.

The retinoblastoma tumor suppressor gene (*Rb-1*) is mutated in all cases of retinoblastoma and is also frequently mutated in a variety of other cancers (55, 91, 92). Loss of the antiproliferative activity of the RB protein (pRB) is thought to play a critical role in oncogenic transformation. There are two other members of the RB family of proteins, p107 and p130. These proteins have a high degree of sequence homology to pRB (31, 38, 56, 62). pRB, p107, and p130 share a number of functional properties, including the ability to associate with and negatively regulate members of the E2F family of transcription factors (3, 6, 8, 36, 42, 78, 89) and the ability to induce a G_1 arrest in certain sensitive cell types (16, 43, 103). The presence of a p130-E2F complex has been proposed to define a quiescent, or G_0, state of a cell (80). pRB, p107, and p130 are phosphorylated in a cell cycle-dependent manner (2, 4, 10, 21, 61, 96). Phosphorylation in the mid/late G_1 phase inactivates the growth-suppressive properties of pRB and possibly also of p107 and p130 (15, 43, 103). pRB, p107, and p130 associate with cyclins (28, 30, 32, 38, 47, 54, 56, 78) and are likely to be phosphorylated by cyclin-dependent kinases (cdks). Despite all these similarities, no mutations in p107 or p130 have yet been described in human tumors, and the role of these two proteins in oncogenic transformation remains unclear.

pRB appears to be an essential protein in mouse embryonic development, as mice with homozygous deletions of the *Rb-1* gene die before birth (14, 46, 52). Moreover, mice heterozygous for *Rb-1* develop pituitary tumors with a penetrance of almost 100% (40, 45, 46). In contrast, mice lacking either p107 or p130 apparently develop normally (18, 53) and have not been reported to develop tumors. Therefore, it appears that loss of either p107 or p130 alone is not sufficient to induce oncogenic transformation. However, mice lacking both p130 and p107 die shortly after birth (18). The p107−/−;p130−/− double-knockout mice exhibit deregulated chondrocyte growth, suggesting an overlapping antiproliferative function for p107 and p130 (18).

Inactivation of pRB can occur by binding to the viral oncoproteins simian virus 40 (SV40) large T antigen (TAg), human papillomavirus E7, or adenovirus E1A. These proteins bind to pRB via the conserved LXCXE domain (where X is any amino acid) (22, 26, 93). Point mutations in the conserved residues or deletion of the LXCXE domain disrupts binding to pRB and renders each oncoprotein transformation defective (11, 27, 64, 67). The RB-related proteins p107 and p130 also associate with TAg through the LXCXE domain (25, 29, 56, 62). Since the genetics of binding of pRB, p107, and p130 to TAg are so similar, it has been difficult to assess their relative contribution to transformation. We have begun to address this issue by using primary mouse embryo fibroblasts (MEFs) genetically lacking the *Rb-1* gene. We and others have demonstrated that an intact LXCXE domain of TAg is required to transform MEFs derived from Rb knockout mice (13, 100). This result indicates that pRB is not the only cellular target of the LXCXE domain and suggests that p107 and p130 may also be essential targets in TAg-mediated transformation.

Transformation by TAg requires the LXCXE domain (residues 103 to 107) and at least two other functional domains. A C-terminal domain (residues 351 to 626) is required for binding to the p53 tumor suppressor protein, and mutants of TAg that are unable to associate with p53 are transformation defective (49, 83, 101). In addition, the N terminus of TAg (residues 1 to 82) is required for transformation (60, 63, 70, 86, 102). It is not known how this region contributes to transformation.

We have recently demonstrated that the N terminus of TAg (residues 1 to 82) is required to alter the phosphorylation state of p107 and p130 but not pRB (84). In addition to the N-terminal domain, the binding site for p130 and p107, the LXCXE motif, was required for this effect. Therefore, it appears that the N-terminal domain provides an activity, apart

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from binding, that is necessary to alter the phosphorylation state of the pRB-related proteins p107 and p130.

It has been suggested that the N terminus of TAg resembles the J domain of the DnaJ (or hsp40) family of molecular chaperones (7, 48). The J domain is an approximately 70-residue region present in all DnaJ proteins (reviewed in reference 79). DnaJ was originally isolated as an *Escherichia coli* host mutant that was unable to support bacteriophage lambda replication. Since then, DnaJ homologs have been identified in many organisms and found to participate in a wide variety of biological processes, including protein folding (references 41 and 50 and references therein), translocation into mitochondria (72) and the endoplasmic reticulum (75), and the cellular response to stress. The J domain of DnaJ proteins participates in binding to heat shock protein 70 (hsp70) (DnaK) and activating its ATPase activity (88, 90). Every DnaJ homolog contains the residues HPD near the middle of the J domain. Substitution mutants of the HPD motif of *E. coli* DnaJ are unable to support bacteriophage lambda replication and are defective in activating the ATPase activity of hsp70 in vitro (90). Each polyomavirus TAg, including SV40 TAg, contains the residues HPDKGG near the middle of the first exon (68). In addition to the sequence homology between TAg and DnaJ, the N terminus of TAg has been shown to specifically bind to hsc70, a member of the hsp70 family (76).

In this work, we sought to study in more detail the effect of TAg on the phosphorylation of p107 and p130 and to determine the functional consequences of this effect, if any. Specifically, we wanted to address whether the J-domain homology region of TAg was involved in the functional inactivation of p107 and p130.

MATERIALS AND METHODS

Plasmids. pCMV-HAp130 (89) and pCMVp107-HA (103) have been described previously. pSG5-T contains a cDNA for SV40 TAg cloned into the *Bam*HI site of pSG5 (Stratagene). pSG5-T and pSG5-K1 were described previously (100). PCR mutagenesis was used to create the substitution mutations H42Q and D44N in genomic SV40 (5). To create a cDNA for these two mutations, primers 5′-GCCGAATTCACCATGGATAAAGTTTTAAACAGAG AG-3′ and 5′-TGCTAGCATCCATAGGTTGGAATCTCAGTTGCATCCCA GAAGCC-3′ were used to amplify the relevant genomic SV40 DNAs to introduce *Eco*RI and *Pfl*MI sites and substituted into pSG5-T cut with the same enzymes. The plasmids pSG5-T L17K, A33R, Y34K, and del46–78 were created with the Bio-Rad Muta-gene kit with the following oligonucleotides: L17K (5′-GCTAATGGACCTTAAGGGTCTTGAAAGGA-3′), A33R (5′-CTCTGA TGAGAAAGCGCTATTTAAAAAAATG-3′), Y34K (5′-GATGAGAAAGGC AAAATTAAAAAAATGCA-3′), and del89–97 (5′-ATTCCAACCTATGGAA CTTTTAATGAGGAAAACC-3′), del46–74 was generated with T7 and 5′-TT CATCAGTTCCATAGGTTGGAATCTCAGTTGCATCCCAGAAGCCTCCT TTATCAGGATG-3′. The products were cut with *Eco*RI and *Pfl*MI and substituted into pSG5-T cut with the same enzymes.

The J domains of HSJ1 and DNAJ2 were amplified by PCR from a ZAP library prepared from human 293 cells. The library was generously provided by William Kaelin. For HSJ1, 5′-CGGGATCCGCCACCATGGCATCCTACTA CGAG-3′ and 5′-CAGTTCCATAGGTTGGAATAGTTCCTGTCCCTGTCA G-3′ were used; for DNAJ2, 5′-CGGGATCCGCCACCATGGTGAAAGAA ACAACTTACTAC-3′ and 5′-CAGTTCCATAGGTTGGAATCTCTTTAAT TGCCTGTTC-3′ were used. The PCR products were cloned into the TA vector (Invitrogen), cut with *Eco*RI and *Pfl*M1, and cloned into pSG5-T cut with the same enzymes. The pSG5-HSJ1-T H-to-Q substitution and the pSG5-DNAJ2-T H-to-Q substitution were generated by site-directed mutagenesis with the Bio-Rad Muta-gene kit and 5′-GGCGCAAGGCTCTGCAGTGGCAACCAGACA AAAACC-3′ and 5′-TAGGAAACTGGCTCTTAAGTACCAACCAGATAAG AACC-3′. All of the newly created mutations in TAg were sequenced to confirm their authenticity.

Cell culture and transfection. Cotransfection of HA-p130 and various TAg constructs into U-2 OS cells was done as described previously (84). Wild-type and RB−/− MEFs were generated as described previously (100). p107−/−, p130−/−, and p107−/−;p130−/− MEFs were obtained from the laboratory of Nicholas Dyson. MEFs of passage 4 or less (passage 0 refers to the cells initially isolated from the mouse embryo) were transfected with TAg or TAg mutants as described previously (100). After selection in puromycin, the resulting colonies were pooled and expanded until they reached 90% confluence in three 100-mm plates, at which point they were considered established. Cells of no more than six passages after this stage were used for the transformation studies. The cells were always passaged at subconfluence. Growth in suspension assays and growth curves were performed as described previously (100).

Western blotting, immunoprecipitation, and pulse-chase analysis. Immunoprecipitations and Western blotting were performed as described previously (84). Pulse-chase experiments were performed as follows. At 24 h after removal of calcium phosphate precipitate from transfected U-2 OS cells, the cells were incubated in methionine-free medium for 1 h. The cells were subsequently pulse-labeled for 20 min with 0.5 mCi of [^{35}S]methionine in 4 ml of methionine-free medium per 100-mm plate. The cells were then washed twice with complete medium (10% serum) and chased in complete medium for the indicated times. At the end of the chase, the cells were harvested and immunoprecipitated. The immunoprecipitated material was resolved on a 5% polyacrylamide gel and subsequently transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was subjected to autoradiography and Western blotting.

Proteasome inhibitors. At 24 h after removal of the calcium phosphate precipitate, transfected U-2 OS cells were incubated for 12 h in the presence of 50 mM N-acetyl-Leu-Leu-norleucinal (LLnL), 50 mM E64, dimethyl sulfoxide (DMSO [1/1,000 dilution]), or 20 mM lactacystin. Lactacystin was obtained from E. J. Corey. The cells were harvested, immunoprecipitated and subjected to Western blotting as described elsewhere (84).

RESULTS

The J-domain homology region of SV40 TAg mediates a reduction in the levels of p130 protein, preferentially eliminating the hyperphosphorylated species. Figure 1A shows an alignment of the N termini of several polyomavirus TAgs including SV40 TAg and mouse polyomavirus TAg, with the J domains from several cellular DnaJ homologs, including *E. coli* DnaJ and two human homologs, DNAJ2 and HSJ1 (7, 65). This alignment reveals several residues that are conserved between the TAgs and the DnaJ homologs. In particular, the residues HPD (residues 42 to 44 of SV40 TAg) are invariant among all of the TAg and DnaJ proteins. In the TAgs, HPD is part of the larger conserved sequence HPDKGG (Fig. 1) (68). In addition, several other residues, including L17, A33, and Y34 (numbers correspond to SV40 TAg residues), are highly conserved. Many DnaJ homologs also contain a glycine- and phenylalanine (GF)-rich region immediately following the J domain (79). Both SV40 and polyomavirus TAgs contain several G and F residues immediately following the J-domain homology region. Figure 1B shows the position of the J domain and the adjacent LXCXE motif in the context of the full-length TAg protein. As indicated in the diagram, the J domain is located entirely within the first exon of TAg.

We have previously demonstrated that TAg alters the phosphorylation state of p130 and p107 and that this effect is mediated by the N terminus of TAg (84). We also observed that the levels of p130 frequently appeared to be lower in the presence of wild-type TAg. To test the possibility that conserved residues in the N-terminal J-domain homology region of TAg contributed to the effect of TAg on p130 levels and phosphorylation, we generated a series of point mutations and in-frame deletions in this sequence. These mutants were constructed as cDNAs, eliminating the expression of SV40 small t antigen, which shares the N-terminal 82 residues with TAg. Each of these TAg mutants was cotransfected with HA epitope-tagged human p130 into the human osteosarcoma cell line U-2 OS. Transfected p130 was immunoprecipitated and subjected to Western blotting with an antibody against the HA epitope tag.

In the absence of TAg, p130 migrates as a series of bands (Fig. 2A, lane 1) which we have shown previously is due to phosphorylation (84). When TAg was coexpressed, the level of p130 protein was greatly reduced (lane 2). Consistent with our earlier results, this effect was most apparent on the hyperphosphorylated species of p130. In the presence of wild-type TAg, little or no phosphorylated p130 could be detected. Expression

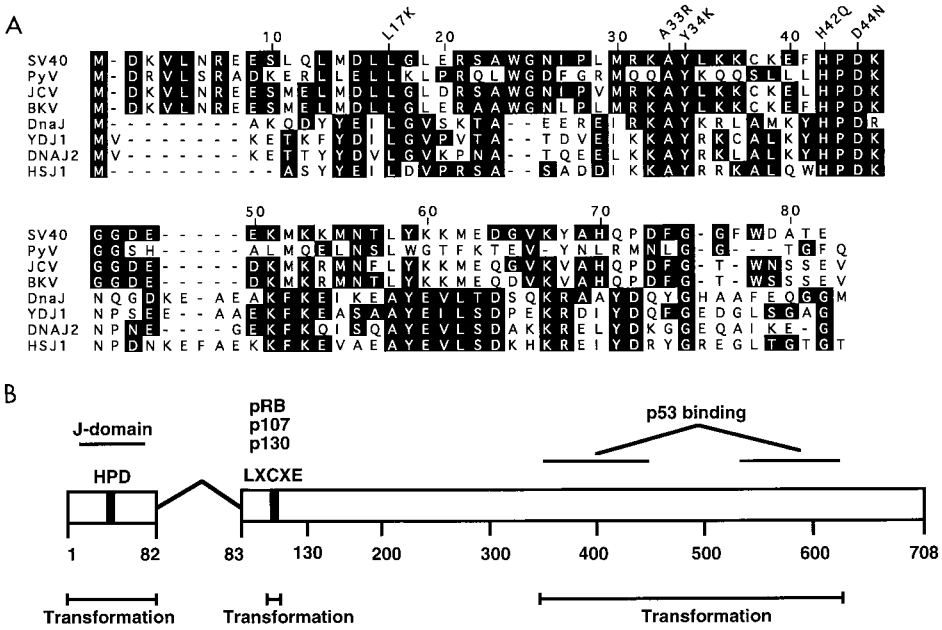

FIG. 1. The N terminus of TAg is homologous to the J domain of DnaJ proteins. (A) Alignment of the N-terminal region of polyomavirus TAgs with the J domain of cellular DnaJ proteins. Note the invariant HPD motif (residues 42 to 44 of SV40 TAg). In all the TAgs, the HPD forms part of the conserved sequence HPDKGG. Polyomavirus TAgs shown include SV40, mouse polyomavirus (PyV), and human JC virus (JCV) and BK virus (BKV) TAgs. J domains of DnaJ homologs from several species are shown, including *E. coli* (DnaJ), *S. cerevisiae* (YDJ1), and human (DNAJ2 and HSJ1). The numbers correspond to SV40 TAg sequences. The locations of point mutations of SV40 TAgs assayed in Fig. 2 are indicated. (B) Diagram of SV40 TAg, indicating the position of the J domain, including the HPD motif, and the LXCXE motif. The J domain is located entirely within the first exon of TAg (residues 1 to 82). Regions of TAg implicated in cellular transformation are indicated.

of the K1 mutant of TAg (E107K) had no discernible effect on the level or phosphorylation state of p130 (lane 3), demonstrating the requirement for an intact LXCXE domain. In addition, altering the levels and phosphorylation pattern of p130 requires the N terminus of TAg, since the mutant T83–708, containing a deletion of the first exon (residues 1 to 82 [Fig. 1B]), was unable to affect p130 levels or phosphorylation (lane 11). This mutant is defective even though it contains an intact LXCXE motif and is able to bind to p130 (84).

TAgs containing the single amino acid substitution H42Q, D44N, or L17K were defective in altering the levels or phosphorylation of p130 (Fig. 2A, lanes 4 to 6). H42Q and D44N are point mutations of the invariant HPD motif of DnaJ homologs. The analogous mutations of the HPD domain of cellular J proteins commonly result in a defective protein (33, 88, 90). Substitution mutations A33R and Y34K, on the other hand, appeared to behave in a similar manner to wild-type TAg (lanes 7 and 8), even though these residues are conserved among the polyomavirus TAgs and many DnaJ homologs. An in-frame deletion of residues 46 to 74, immediately downstream of the HPDK motif, did not alter the levels or phosphorylation of p130 (lane 9). In contrast, TAg with a deletion of residues 89 to 97, located between the J-domain homology region and the LXCXE domain, behaved like wild-type TAg (lane 10). With the sole exception of the K1 LXCXE mutant, all mutants of TAg shown in Fig. 1 retained the ability to associate with p130 (data not shown). The expression of each TAg construct was confirmed by immunoprecipitation and Western blotting with a monoclonal antibody against TAg (Fig. 2A, bottom panel). We have also assayed another TAg, that of murine polyomavirus, for its effect on p130. We found that murine polyomavirus TAg, like SV40 TAg, was able to bind to p130 and significantly reduce the protein level, preferentially eliminating the hyperphosphorylated species (data not shown). The N terminus of murine polyomavirus shares very little homology with SV40 TAg apart from the J-domain homology (Fig. 1).

The data in Fig. 2A shows that several conserved residues of the J-domain homology region of TAg, most notably the HPD motif, are required to alter p130 levels and phosphorylation pattern. However, by mutating the N terminus of TAg, we can assay only for loss of function and cannot rule out the possibility that we are interfering with a function of the N terminus unrelated to its J-domain homology. To test more conclusively whether the effect on p130 levels and phosphorylation was a function of the J-domain homology region of TAg, we generated chimeric versions of TAg. The first exon of T (residues 1 to 82) was deleted, and the J domains from two different human DnaJ homologs, DNAJ2 (also known as HDJ-2 [9]) and HSJ1, was substituted. HSJ1 was isolated from a human brain expression library (7), and DNAJ2 was cloned from a human fibrosarcoma cell line (65). The amino-terminal 78 residues of HSJ1 or the amino-terminal 74 residues of DNAJ2 were fused to residues 83 to 708 of SV40 TAg. Coexpression of either the HSJ1-TAg chimera or the DNAJ2-TAg chimera with p130 resulted in reduced levels of p130, especially hyperphosphorylated p130 (Fig. 2B, lanes 4 and 6). To confirm that the effect of the chimeric proteins was specific for the J domain, mutant chimeric proteins with the H-to-Q mutation of the conserved histidine residue in the HPD motif were generated. Similar to the H42Q mutation of wild-type TAg, HSJ1-T HQ and DNAJ2-T HQ were unable to alter p130 levels or phosphorylation (Fig. 2B, lanes 5 and 7). Each of the four TAg chimeras retained the ability to associate with p130 (data not shown). This demonstrates that a heterologous J domain can functionally substitute for the N terminus of TAg in the effect on p130 levels and phosphorylation.

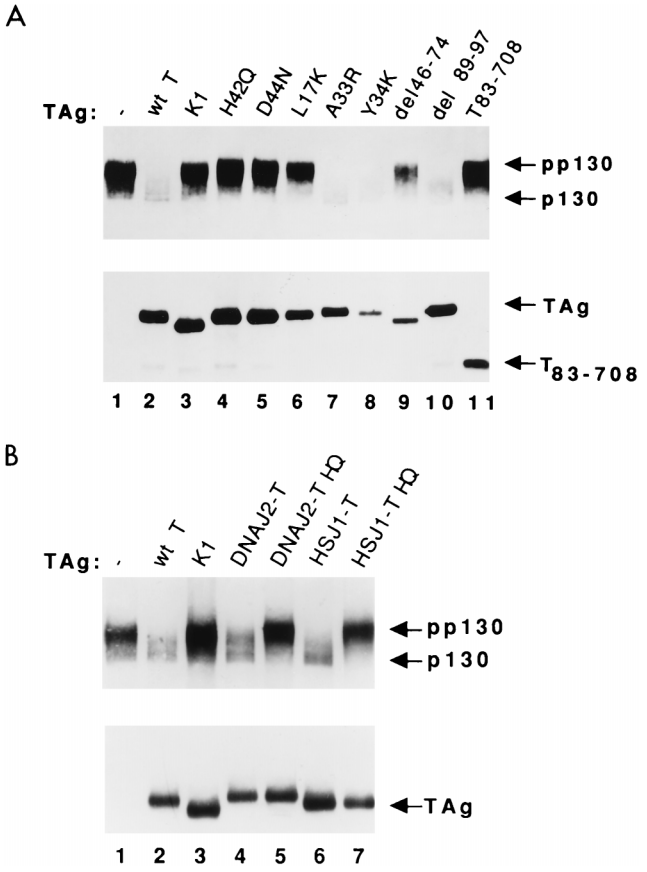

FIG. 2. The J domain of SV40 TAg mediates a reduction in the level of p130 protein, preferentially eliminating the hyperphosphorylated species. (A) A vector expressing HA epitope-tagged p130 was cotransfected with the indicated TAg cDNA constructs into U-2 OS cells. HA-p130 (top panel) was detected by immunoprecipitation followed by Western blotting with the 12CA5 anti-HA antibody. Expression of the various TAg constructs (bottom panel) was detected by immunoprecipitation followed by Western blotting with two monoclonal anti-TAg antibodies, PAb419 and PAb101. (B) The HA-p130 construct was cotransfected into U-2 OS cells with the indicated TAg constructs. The DNAJ2-T and HSJ1-T chimeric proteins are described in the text. HA-p130 (top panel) was detected as in panel A. Expression of the various TAg constructs (bottom panel) was detected by immunoprecipitation followed by Western blotting with the monoclonal anti-TAg antibody PAb101. wt, wild type.

TAg promotes the degradation of p130. The observation that TAg reproducibly appeared to reduce the level of p130 protein led us to ask whether TAg could promote the degradation of p130. A series of pulse-chase experiments were performed to test whether TAg had a specific effect on the half-life of p130. U-2 OS cells were transfected with HA-tagged p130, metabolically labeled for 20 min with [^{35}S]methionine, and chased with an excess of unlabeled methionine for up to 10 h. The cells were harvested at 0, 0.5, 2, 5, and 10 h after labeling and immunoprecipitated with either the anti-HA antibody or an anti-TAg antibody. The proteins were resolved in a sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred to PVDF membranes. The membranes were subjected to autoradiography and western blotting.

As can be seen in the autoradiogram in Fig. 3A, lane 1, newly synthesized p130 was predominantly hypophosphorylated. However, within 30 min, a majority of the p130 was converted into the hyperphosphorylated form (lane 2), which decayed with a half-life of approximately 5 h. The Western blot (Fig. 3A, bottom panel) demonstrates that the steady-state level and phosphorylation state of p130 remained unchanged during the course of the experiment.

In parallel, we performed pulse-chase experiments with p130 in the presence of wild-type TAg (Fig. 3B), the K1 LXCXE mutation (E107K) (Fig. 3C), and the J-domain HPD mutant H42Q (Fig. 3D). Wild-type TAg had a profound effect on the appearance of p130. At 0 h, there was mostly incorporation of label into the hypophosphorylated forms, as was the case for p130 in the absence of TAg (Fig. 3B, lane 1). However, there was little evidence for the appearance of any hyperphosphorylated forms of p130 during the chase. Second, p130 was significantly less stable, as evidenced by the small amount of signal remaining after 5 or 10 h (Fig. 3B, lanes 4 and 5). The half-life of p130 in the presence of TAg was less than 1 h in this experiment.

In contrast, the K1 LXCXE mutant of TAg had no detectable effect on p130 stability or phosphorylation compared to p130 alone (Fig. 3C). The H42Q J domain mutation of TAg also did not interfere with the appearance of phosphorylated p130 (Fig. 3D) and did not increase the turnover of p130. In fact, p130 appeared to be somewhat stabilized in the presence of this mutant. The stabilities of the three species of TAg assayed in this experiment, wild-type TAg, K1, and H42Q, were indistinguishable for the duration of the chase (Fig. 3B, C, and D, respectively). It is evident from Fig. 3 that both an intact LXCXE domain and an intact HPD motif are required to affect p130 stability and phosphorylation. The failure of the H42Q mutant to induce p130 degradation and alter p130 phosphorylation indicates that association between TAg and p130 is necessary but not sufficient for these effects.

Wild-type TAg and the H42Q mutant of TAg were coprecipitated by p130, whereas the LXCXE mutant K1 was not. From the autoradiograms in Fig. 3B and D, it appears that an increasing amount of labeled TAg was coprecipitated with p130 over time. However, the Western blots show that the total amount of TAg coprecipitated at each time point does not vary significantly (Fig. 3B and D, bottom panel). Hence, we are observing an apparent delay between synthesis of new TAg and its incorporation into complexes with p130. It has been described previously that newly synthesized TAg does not complex with pRB (59), and it is likely that we are observing a similar effect in the case of TAg-p130 complexes.

The pulse-chase experiments in Fig. 3A to D suggested that the effect of TAg on p130 stability, as well as on phosphorylation, was mediated by the N-terminal J domain. To test this directly, we performed a pulse-chase experiment with the chimeric HSJ1-T and the corresponding H-to-Q mutation of its HPD motif (HSJ1-T HQ). As shown in Fig. 3E, the HSJ1-T chimera, like wild-type TAg, led to a rapid turnover of p130 and reduced the level of phosphorylated p130 (Fig. 3E, lanes 1 to 5). The H-to-Q mutant of the HSJ1-TAg chimera, like the H42Q mutant of TAg, did not induce degradation of p130 and did not alter the p130 phosphorylation pattern (lanes 6 to 10). Pulse-chase analysis showed that the stability of the HSJ1-T and HSJ1-T HQ proteins was indistinguishable from that of wild-type TAg (data not shown). These results demonstrate that a heterologous J domain can substitute for the N terminus of TAg in the effect on p130 stability as well as phosphorylation pattern.

We have previously demonstrated that the phosphorylation state of the RB-related protein p107 was affected by TAg in a manner similar to that of p130 (84). Given this observation, we wanted to determine whether the stability of p107 was also altered by TAg. We performed a pulse-chase experiment with HA-tagged p107 in the presence of wild-type TAg or the K1 LXCXE mutant (Fig. 3F). Consistent with our previous results,

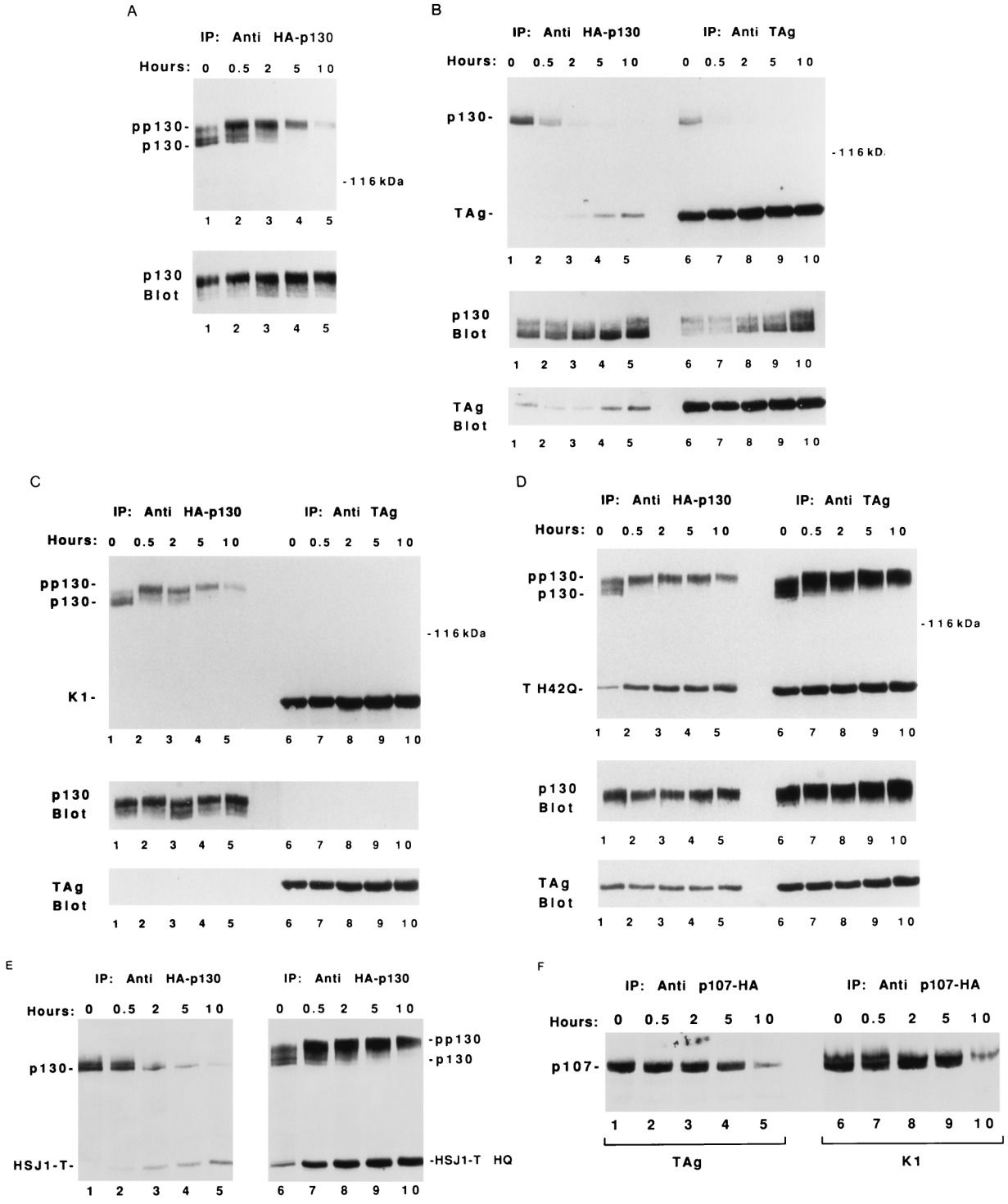

FIG. 3. TAg induces the rapid turnover of p130, and this effect requires an intact J domain. (A) U-2 OS cells transfected with HA-p130 were pulse-labeled for 20 min with [^{35}S]methionine and chased in complete medium for 0, 0.5, 2, 5, and 10 h. The cells were harvested at these time points and immunoprecipitated (IP) with the anti-HA antibody 12CA5. Immune complexes were resolved on a 5% polyacrylamide gel and transferred to a PVDF membrane. The membrane was then subjected to autoradiography (top panel) and Western blotting with the anti-HA antibody (bottom panel). (B) U-2 OS cells were transfected with HA-p130 and wild-type SV40 large TAg. Pulse-chase analysis was performed as in panel A. Lysates were immunoprecipitated with either the anti-HA antibody (lanes 1 to 5) or the anti-TAg antibodies PAb 419 and PAb 101 (lanes 6 to 10). Immune complexes were resolved by SDS-polyacrylamide gel electrophoresis (PAGE) on a 5% gel and transferred to a PVDF membrane. The membrane was then subjected to autoradiography (top panel) and Western blotting with the anti-HA antibody (middle panel) as well as Western blotting with anti-TAg antibodies (bottom panel). (C) U-2 OS cells were transfected with HA-p130 and the K1 LXCXE mutant of TAg. Pulse-chase analysis, immunoprecipitation, SDS-PAGE, autoradiography, and Western blotting were performed as in panel B. (D) U-2 OS cells were transfected with HA-p130 and the H42Q HPD mutant of TAg. Pulse-chase analysis, immunoprecipitation, SDS-PAGE, autoradiography, and Western blotting were performed as in panel B. (E) U-2 OS cells were transfected with HA-p130 and the HSJ1-T wild-type chimeric protein (lanes 1 to 5) or the HSJ1-HQ mutant chimeric protein (lanes 6 to 10). Pulse-chase analysis was performed as in panel A. Lysates were immunoprecipitated with the anti-HA antibody 12CA5, resolved by SDS-PAGE, transferred to a PVDF membrane, and subjected to autoradiography. (F) U-2 OS cells were transfected with p107-HA and wild-type TAg (lanes 1 to 5) or the LXCXE mutant K1 (lanes 6 to 10). Pulse-chase analysis was performed as in panel A. Lysates were immunoprecipitated with the anti-HA antibody 12CA5, subjected to SDS-PAGE, transferred to a PVDF membrane, and subjected to autoradiography.

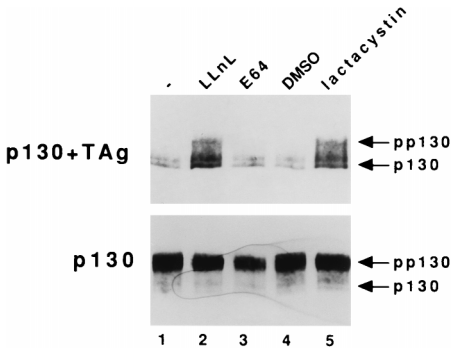

FIG. 4. Proteasome inhibitors reduce the effect of TAg on p130. U-2 OS cells were transfected with HA-p130 and wild-type TAg (top panel) or with p130 only (bottom panel). At 24 h after transfection, the cells were treated for 12 h with either no inhibitor (lane 1), 50 mM LLnL (lane 2), 50 mM E64 (lane 3), 0.1% DMSO (lane 4), or 20 mM lactacystin (lane 5). The cells were harvested, and the lysates were immunoprecipitated with the anti-HA antibody 12CA5. Immune complexes were resolved by SDS-PAGE in a 5% gel and transferred to a PVDF membrane. The membrane was subjected to Western blotting with the anti-HA antibody 12CA5.

only hypophosphorylated p107 could be detected in the presence of wild-type TAg (Fig. 3F, lanes 1 to 5), while in the presence of the LXCXE mutant K1, p107 was phosphorylated and migrated as a doublet (lanes 6 to 10). However, in contrast to p130, the half-life of p107 did not appear to be decreased by TAg.

It has been reported previously that pRB levels and phosphorylation are not affected by the presence of TAg (58, 59, 84). We have confirmed this result in our system by performing a pulse-chase experiment with HA-tagged pRB and wild-type TAg or the K1 mutant. Consistent with previously published results, TAg did not alter the stability or phosphorylation pattern of pRB and was able to associate only with the most hypophosphorylated species (data not shown).

Proteasome inhibition reduces the effect of TAg on p130. In light of the results of the pulse-chase experiments, indicating that TAg greatly increased the turnover of p130, we considered the possibility that TAg was targeting p130 for proteasome-mediated degradation. If so, specific inhibition of the proteasome should lead to increased levels of p130 in the presence of TAg. To address this, we contransfected HA-p130 and TAg as before and treated the transfected cells with proteasome inhibitors. The cells were incubated overnight either without inhibitor (Fig. 4, lane 1) or with the proteasome inhibitor LLnL (lane 2); with the lysosomal protease inhibitor E64, which does not inhibit the proteasome (lane 3); with DMSO (the solvent in which LLnL and E64 are dissolved) (lane 4); or with lactacystin, an inhibitor with high specificity for the proteasome (34) (lane 5). The proteasome inhibitors LLnL and lactacystin, but none of the controls, resulted in increased levels of p130 in the presence of TAg. This implicates the proteasome, directly or indirectly, in TAg-induced p130 degradation.

For comparison, we assayed the effect of these inhibitors on cells that were transfected with HA-p130 in the absence of TAg. In the absence of TAg, p130 was extensively phosphorylated and not significantly affected by the proteasome inhibitors (Fig. 4, bottom panel). This may be due to the relatively long half-life of p130 in the absence of TAg (Fig. 3A). With a half-life of approximately 5 h, it is not surprising that p130 did not accumulate significantly during the 12-h period when the inhibitors were present. The accumulation of p130 upon treatment with proteasome inhibitors only when TAg was coexpressed is consistent with the pulse-chase data indicating that p130 is rapidly degraded in the presence of TAg. We also observed what appeared to be some phosphorylated p130 in the presence of these inhibitors (Fig. 4, lanes 2 and 5); however, there was not a complete reversal of the effect of TAg. We did not observe any significant effect of these inhibitors on p130 in the presence of the K1 LXCXE mutant of TAg or the H42Q HPD mutant (data not shown).

The J domain of TAg mediates inactivation of p107 and p130 antiproliferative functions. The results shown in Fig. 2 and 3 demonstrate that the N-terminal J domain and the LXCXE motif of TAg cooperate to decrease the stability of p130 and to alter the phosphorylation pattern of both p107 and p130. As mentioned previously, the N terminus of TAg contributes to TAg-mediated transformation (60, 63, 70, 86, 102). This suggested the possibility that the requirement for the N terminus of TAg in transformation reflected a need to specifically inactivate p130 and possibly also p107.

To determine whether inactivation of p130 or p107 or both contributed to TAg-mediated transformation, we established primary MEFs with various TAg expression plasmids. Early-passage MEFs of the wild-type genotype or MEFs derived from mice carrying a homozygous deletion of the genes encoding pRB (Rb−/−), p107 (p107−/−), p130 (p130−/−), or both p107 and p130 (p107−/−;p130−/−) were used for transformation experiments. These MEFs were transfected with plasmids encoding either wild-type SV40 large TAg, the mutants K1 (E107K), H42Q, D44N, or the chimeric proteins HSJ1-T or HSJ1-T HQ, as well as with a puromycin resistance gene. Transfected cultures were selected in puromycin, and the resulting colonies were pooled and expanded. Each TAg construct tested was able to immortalize primary MEFs of each genotype, and no reproducible differences were observed in the time required by different TAg mutants to establish cell lines from MEFs of the different genotypes (data not shown). Thus, in the context of a full-length TAg, neither an intact J domain nor an intact LXCXE domain of TAg appeared to be necessary for immortalization of primary MEFs. This is consistent with an earlier observation that the first 127 residues of TAg, encompassing both the J domain and the LXCXE motif, were not required for immortalization of MEFs (86). However, there is evidence that the N-terminal region of TAg, including the J-domain region and the LXCXE, contain an immortalization or transformation activity that is independent of and redundant with the C-terminal immortalization activity associated with binding to p53 (1, 81, 82). We do not know whether the J domain contributes to this N-terminal immortalization activity. It has also been reported that in addition to the p53 binding region, both the N terminus and the LXCXE domain of TAg are required for efficient immortalization of primary MEFs (19). While our data strongly suggests that an intact J domain of TAg is not required for immortalization, more work is required to establish this firmly.

Each of the cell lines obtained expressed TAg. The different mutants of TAg were expressed at similar levels in MEFs of each genotype (Fig. 5, bottom panel for wild-type MEFs and data not shown for the rest). The expression of the three endogenous RB-related proteins was assessed in wild-type MEFs stably expressing the different TAgs (Fig. 5). Immunoprecipitations of p130 and p107 followed by Western blotting revealed that wild-type TAg as well as the HSJ1-T chimera significantly reduced the levels of phosphorylated p130 and p107 (Fig. 5, lanes 1 and 5). In the case of p130, the overall protein level also appeared to be decreased in the presence of wild-type TAg and HSJ1-T. The LXCXE mutant K1 and the J-domain mutants of TAg did not alter the levels or phosphor-

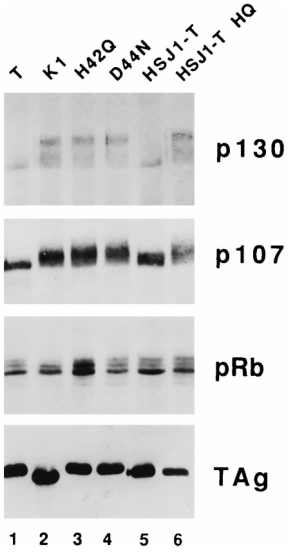

FIG. 5. Expression and phosphorylation pattern of endogenous pRB-family proteins in MEFs stably expressing various forms of TAg. The endogenous RB-family proteins were assayed by immunoprecipitation followed by Western blotting in MEFs expressing various TAg constructs described in the text. A 2.5-mg portion of lysate was immunoprecipitated and subjected to Western blotting with the C-20 anti-p130 antibody (Santa Cruz Biotechnology); 0.8 mg of lysate was immunoprecipitated and subjected to Western blotting with the C-18 anti-p107 antibody (Santa Cruz Biotechnology); 1.5 mg of lysate was immunoprecipitated and subjected to Western blotting with the anti-pRB antibody 245 (Pharmingen). The bottom panel shows the expression of the various TAgs. A 0.5-mg portion of lysate was immunoprecipitated and subjected to Western blotting with the anti-TAg antibody PAb101.

ylation state of p130 or p107 (lanes 2 to 4 and 6). These results with endogenous p107 and p130 are very consistent with what we observed in the transient-transfection assay in Fig. 2. We have reported earlier that expression of TAg had no detectable effect on pRB phosphorylation or stability (59, 84, 100). We confirmed and extended this observation here by immunoprecipitation and Western blot analysis of pRB from these cell lines. Figure 5 demonstrates that the pRB levels and phosphorylation were similar in each of the cell lines tested.

A mouse fibroblast cell line, such as BALB/c 3T3 cells, will grow to a certain density in tissue culture, and then cell division stops as the cells become arrested by "contact inhibition." The cell density reached is proportional to the amount of serum in the medium (44). However, 3T3 cells transformed by SV40 have a greatly reduced serum requirement (44, 74) and can grow to very high densities. The properties of cells transformed by SV40 typically include growth to a high density, reduced serum requirement, anchorage independence, the ability to induce tumors in susceptible animals, and growth on monolayers of normal cells (reviewed in reference 87).

One property of transformed cells is the ability to grow in an anchorage-independent manner, as assayed by their ability to form colonies in soft agarose. MEFs expressing wild-type TAg, H42Q, D44N, the HSJ1-T chimera, and the HSJ1-T HQ mutant chimera were each able to form colonies in soft agarose (data not shown). We were unable to detect any significant differences in the percentage of cells forming colonies or the size of each colony. In contrast, and consistent with previous results (11, 100), MEFs expressing the LXCXE mutant K1 did not form colonies in soft agarose. These results indicate that an intact J domain of TAg is not essential to confer the ability to grow in an anchorage-independent manner. This is in contrast to the LXCXE motif of TAg, which was needed for efficient anchorage-independent growth in MEFs of all genotypes studied (data not shown).

While an intact J domain was not required for growth in soft agarose, we considered that it may contribute to other properties of SV40-transformed cells, such as the ability to grow to high densities and the ability to grow in a low concentration of serum. To address this, the growth rate of MEFs immortalized with wild-type TAg and various TAg mutants was tested. Cells were seeded and fed every 3 days with medium containing 10% serum. At 9 days after seeding, duplicate plates of each cell line were counted. Figure 6A shows that wild-type MEFs expressing either wild-type TAg or the HSJ1-T chimera grew to a higher cell density than did MEFs expressing either the LXCXE mutant K1 or the J-domain mutants of TAg. This was also true for MEFs of the p130−/−, p107−/−, and Rb−/− genotypes. In contrast, p107−/−;p130−/− double-knockout MEFs expressing J-domain mutants of TAg grew to a similar density to those expressing wild-type TAg or the chimeric HSJ1-TAg. That is, the p107−/−;p130−/− MEFs expressing J-domain mutants reached a much higher percentage of the density reached by the MEFs expressing wild-type TAg and HSJ1-T. This suggested that the J domain of TAg was required to confer a growth advantage to wild-type MEFs or MEFs lacking any one member of the RB family but was dispensable in MEFs lacking both p107 and p130.

To study this growth advantage further, we performed a more detailed analysis of the growth of wild-type and p107−/−;p130−/− MEFs expressing the various TAg constructs. Cells were seeded at low density and fed every 3 days with medium containing 10% serum. Triplicate plates of cells were counted every 2 days. Wild-type MEFs expressing wild-type TAg or HSJ1-T continued to grow for the duration of the experiment (Fig. 6B). Wild-type MEFs expressing either the LXCXE mutant K1 or the J-domain mutants of TAg, on the other hand, grew to a density of 2 to 4 million cells per plate, and then their numbers did not increase further. In contrast to wild-type MEFs, p107−/−;p130−/− double-knockout MEFs expressing J-domain mutants of TAg grew to an extent similar to that of those expressing wild-type TAg and HSJ1-T and showed signs of reduced growth rate only at very high densities. The LXCXE domain was required to confer a growth advantage to p107−/−;p130−/− cells, since double-knockout MEFs expressing the K1 mutant of TAg were unable to grow to a high density (Fig. 6B). The failure of the K1 mutant to confer a growth advantage to p107−/−;p130−/− cells is presumably due to the antiproliferative activity of pRB or possibly other as yet uncharacterized members of the RB family.

We also assayed the ability of these cells to grow in a low concentration of serum. Cells were seeded at a low density, and 1 day later the concentration in serum was lowered to 1%. Triplicate plates of cells were counted every 2 days. In the case of wild-type MEFs, the cell lines expressing either wild-type TAg or HSJ1-T grew better than did MEFs expressing any of the other TAg mutants tested (Fig. 6C). In the case of p107−/−;p130−/− double-knockout MEFs, the cell lines expressing J-domain mutants of TAg grew to a similar extent to the cell lines expressing wild-type TAg or HSJ1-T. Hence, an intact J domain of TAg conferred a growth advantage to wild-type MEFs at low concentrations of serum but was dispensable for the growth of p107−/−;p130−/− MEFs in at low concentrations. As was the case for MEFs in 10% serum, the LXCXE motif was required to confer a growth advantage to both wild-type and p107−/−;p130−/− cells.

These results demonstrate that the J domain of TAg confers a growth advantage to wild-type MEFs and MEFs lacking pRB or p107 or p130 but is dispensable for p107−/−;p130−/−

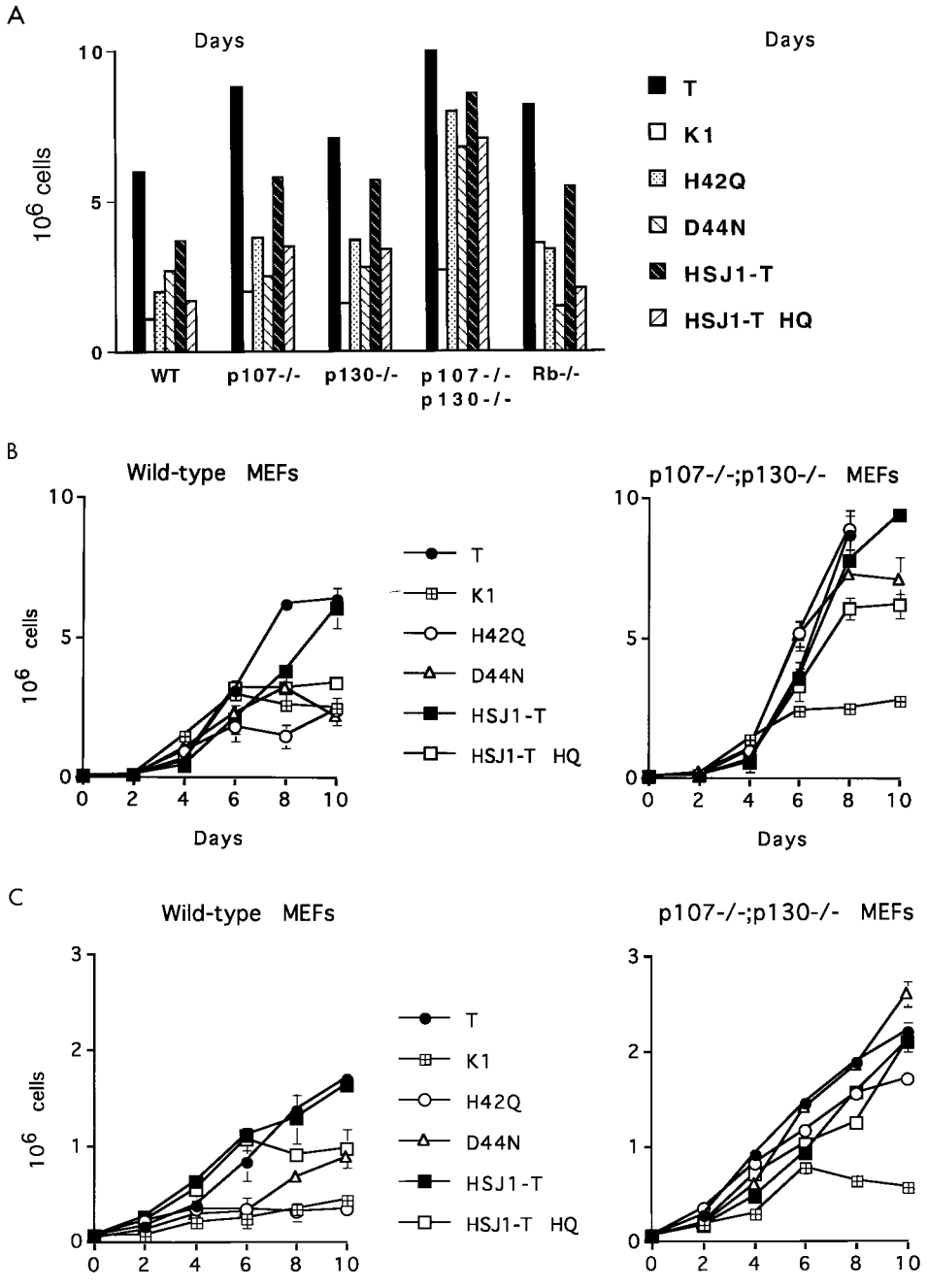

FIG. 6. The J domain of TAg confers a growth advantage to MEFs. (A) A total of 5×10^4 cells of the genotypes indicated, expressing either wild-type (T) or mutant TAgs, were plated in 60-mm dishes containing medium with 10% serum. At 9 days later, two replica plates were trypsinized and the cells in each plate were counted. The resulting average cell count is shown for each cell line. The TAg construct expressed by each cell line is indicated on the right. (B) Growth at high serum concentrations. A total of 5×10^4 cells of wild-type (left) and p107−/−;p130−/− double-knockout genotype (right) MEFs expressing either wild-type or mutant TAgs were plated in 60-mm dishes containing 10% serum. On the indicated days, three replica plates were trypsinized and the cells in each plate were counted. The resulting mean cell count and standard deviation are shown for each time point. The TAg construct expressed by each cell line is indicated on the graph. It was not possible to obtain a cell count at 10 days for p107−/−;p130−/− MEFs expressing wild-type TAg, H42Q, or HSJ1-T because the cells had become so dense that they started to detach from the plate. (C) Growth at low serum concentrations. A total of 5×10^4 cells of wild-type (left panel) and p107−/−;p130−/− double-knockout genotype (right panel) MEFs expressing either wild-type or mutant TAgs were plated in 60-mm dishes containing 10% serum. One day later, the medium was changed to 1% serum. On the indicated days, three replica plates were trypsinized and the cells in each plate were counted. The resulting mean and standard deviation are shown for each time point. The TAg construct expressed by each cell line is indicated on the graph.

double-knockout MEFs. Specifically, an intact J-domain is required for cells to grow to a high density and to grow in low concentrations of serum.

DISCUSSION

The initial observation that the LXCXE domain of TAg was required to transform RB−/− MEFs (100) suggested that the interaction of TAg with the pRB-related proteins p107 and p130 may play a role in transformation. The finding that the N terminus of TAg cooperated with the LXCXE domain to reduce the abundance of phosphorylated p107 and p130 (84) turned our attention to the potential J-domain homology contained in this region. In this report, we describe a novel mechanism for inactivation of the growth suppressors p130 and p107. The inactivation of these proteins is mediated by a J domain, which is a conserved motif present in DnaJ molecular chaperone proteins and polyomavirus T antigens. Furthermore, the overlapping antiproliferative activities of p130 and p107 are demonstrated. To our knowledge, this is the first example of a J domain contributing to the inactivation and degradation of proteins involved in growth suppression.

The J domain of TAg induces the rapid turnover of p130 and reduces the levels of phosphorylated p130 and p107. The effect of TAg on p107 and p130 phosphorylation and p130 stability could not be separated genetically in our assays, and we believe that they are both manifestations of the same TAg activity. Based on the requirement for a J domain, we can speculate that the mechanism of these effects may involve an associated hsp70. An association has been demonstrated between the N-terminal region of TAg and hsc70, a constitutively expressed form of mammalian hsp70 (76), and recent work has demonstrated that the HPD motif is critical to this interaction (5). The J domain of DnaJ proteins has been shown to activate the intrinsic ATPase activity of associated hsp70 (88, 90). ATP hydrolysis may contribute to the ability of certain DnaJ homologs to modulate the folding of multimeric protein complexes. In TAg, activation of the associated hsc70 ATPase could alter the folding of the associated p107 and p130 and interfere with complex formation with E2F or cyclin/cdks. Furthermore, the J-domain–hsc70 complex could, directly or indirectly, target p130 for proteasome-mediated degradation.

From the point of view of the RB-related proteins, at least two distinct mechanisms could be proposed to account for the effect of TAg on p107 and p130 phosphorylation and p130 stability. The first is that TAg prevents or significantly delays the phosphorylation of p130 and p107 and that the hypophosphorylated species of p130 is inherently unstable and turns over rapidly. The second possibility is that p130 and p107 become phosphorylated but the phosphorylated species is very rapidly degraded in the presence of TAg. These possibilities are not mutually exclusive.

There are several notable examples of proteins that become phosphorylated prior to rapid degradation by the ubiquitin-proteasome pathway (reviewed in reference 23). Examples include IκB (12, 24), cyclin E (17, 95), and the *Saccharomyces cerevisiae* G_1 cyclin CLN2 (20, 94, 98). Interestingly, the phosphorylation and degradation of CLN2, as well as the degradation of certain other proteins, are impaired in yeast strains with deletion of YDJ1, an *S. cerevisiae* DnaJ homolog (51, 99). Regulating protein turnover appears to be an important means of regulating cell proliferation. It will be interesting to determine whether cellular DnaJ proteins normally participate in the regulation of protein stability.

To our knowledge, this is the first example of a DnaJ activity that leads to the inactivation of a growth suppressor. However, TAg is not the only viral oncoprotein that induces the degradation of a cellular protein. The degradation of the tumor suppressor protein p53 by the E6 oncoprotein of human papillomaviruses (77) has been extensively studied. While it is clear that E6 mediates the degradation of p53 via the ubiquitin-proteasome pathway, there has been no evidence to date that a DnaJ family member participates in this process.

TABLE 1. Summary of LXCXE and HPD domain functions

TAg function	Requirement for:	
	LXCXE	HPD
Association with pRB, p107, and p130	Yes	No
Alteration of p130 and p107 phosphorylation	Yes	Yes
Decrease of p130 half-life	Yes	Yes
Growth in soft agarose	Yes	No
Growth to a high density/growth at low serum levels	Yes	Yes[a]

[a] Not required in p107−/−;p130−/− MEFs.

The J domain of TAg confers a growth advantage to MEFs that may contribute to transformation. The N terminus is one of the most highly conserved regions among polyomavirus TAgs (Fig. 1) (68), yet the function of this region has long remained a mystery. For SV40 TAg, there is considerable evidence that this region contributes to transformation (60, 63, 70, 86, 102). Considering the requirement for the N terminus of TAg in transformation, we wanted to determine whether the effects of TAg on p107 and p130 contributed to TAg-mediated transformation.

We found that MEFs expressing J-domain mutants of TAg exhibited some properties of transformed cells but not others. MEFs expressing J-domain mutants of TAg were capable of anchorage-independent growth but were not able to grow to a high density or to grow efficiently in low serum concentrations. We conclude that the J domain confers a growth advantage to MEFs which may contribute some of the properties associated with the transformed phenotype.

Anchorage-independent growth is usually correlated with the ability to overcome density arrest and to grow independently of growth factors (66). Nevertheless, there are examples of cells that are able to grow in soft agarose but show density arrest and dependence on growth factors for proliferation (37, 73). Some of the TAg-mediated properties of transformed cells have been separated genetically. For example, it has been demonstrated that the N-terminal 127 residues of TAg are dispensable for immortalization of MEFs and for tumorigenicity in nude mice but are required for growth to a high density and for anchorage-independent growth (86). Furthermore, it has been demonstrated that anchorage-independent growth, growth to a high density, and growth at low serum concentrations require an intact LXCXE domain (11, 100). Our results are consistent with these observations. As a further genetic dissection of the properties of TAg, our data indicates that an intact HPD motif, in addition to an intact LXCXE motif, is required for growth to a high density and growth at low serum concentrations. However, the HPD motif is dispensable for anchorage-independent growth. This distinction supports the notion that growth in suspension and reduced serum dependence are independent manifestations of the transformed phenotype in fibroblasts. The known functions of the LXCXE and HPD domains are summarized in Table 1.

Given the high homology of the N-terminal J-domain regions of TAgs from many viruses (Fig. 1) (68), one might predict that many or all of these TAgs would have similar

effects on the RB family of proteins. There is some evidence that this is indeed the case. It has been reported that cells expressing the TAg of BK virus, a polyomavirus that infects humans, have lower levels and faster-migrating forms of RB-family proteins than control cells do (39). This is very similar to the effect of SV40 TAg on the RB-related proteins (84) and most probably occurs by a similar if not identical mechanism. Furthermore, we have found that murine polyomavirus TAg was able to bind to p130 and reduce the level of phosphorylated p130. Interestingly, murine polyomavirus TAg does not transform cells on its own but is required for transformation by polyomavirus middle TAg and for the ability of transformed cells to grow in low serum concentrations (35, 71). It will be interesting to determine whether the J domains of BK virus and murine polyomavirus TAgs contribute to their effects on cell growth.

The growth advantage conferred by the J domain of TAg reflects p107 and p130 inactivation. An intact J domain of TAg is required to confer a growth advantage to wild-type MEFs or to MEFs that lack either p107, p130, or pRB. In contrast, an intact J domain of TAg is not necessary for p107−/−;p130−/− double-knockout MEFs to grow to a high density or at low serum concentrations. These results suggest that the requirement for an intact J domain reflects the necessity for TAg to inactivate both p107 and p130. Thus, p107 and p130 appear to have redundant antiproliferative activities. p107 and p130 have been previously shown to have overlapping functions in mouse development (18). It is noteworthy that chondrocytes in the long-bone epiphyses of p107−/−;p130−/− mice exhibited increased rates of proliferation and reached a higher density than in normal mice or in mice lacking either p107 or p130 (18).

The basis for the requirement of the J domain of TAg for inactivation of p107 and p130 is not yet understood. It is possible that phosphorylated p107 and p130 have a specific antiproliferative activity. This activity could involve the binding and modulation of members of the E2F family of transcription factors, binding of cyclins A and E, or other biochemical activities of these proteins that have yet to be defined. In the case of p130, the destabilization that accompanies the effect on phosphorylation could be essential for its inactivation. It is also possible that TAg will be unable to bind all of the endogenous p107 and p130 when they are expressed at normal levels. However, J-domain mutants of TAg do associate with p107 and p130, and we cannot rule out that these mutants of TAg are perturbing the function of p107 and p130 in some way that we are not assaying for.

In contrast to the J-domain mutants of TAg, the LXCXE mutant K1 (E107K) was unable to confer a growth advantage to MEFs of any of the genotypes tested. In the p107−/−; p130−/− MEFs, pRB itself is the only RB-family protein present, with the possible exception of as yet unidentified RB-related proteins. The failure of the K1 LXCXE mutant of TAg to confer a growth advantage to p107−/−;p130−/− double-knockout MEFs supports the role of pRB as a negative regulator of cell growth. Taken together, our data suggests a requirement for inactivation of all three RB-family proteins, p107, p130, and pRB, in TAg-mediated growth to a high density and growth at low serum concentrations.

In our assays for growth to a high density and growth at low serum concentrations, the integrity of the J domain appears to be the only requirement of the N terminus of TAg, and inactivation of p107 and p130 seems to be the only biochemical activity of the J domain relevant to conferring a growth advantage. However, we cannot rule out that the J domain also modulates the interaction of TAg with other cellular proteins. The N terminus of TAg contains an activity that complements the transforming function of the p300-binding region of the adenovirus E1A oncoprotein (97). Based on this observation, it has been suggested that the N terminus of TAg may interact with the E1A-associated protein p300. Recent work, however, indicates that p300 binds to TAg via residues that overlap with the p53-binding domain of TAg (57). Nevertheless, it is possible that the N-terminal J domain in some way affects bound p300 or other TAg-associated proteins. The N terminus of TAg has also been implicated in overcoming a p53-induced growth arrest (70), but it is presently unclear whether this involves an activity of the J domain.

The contribution of the N terminus of TAg to the formation of tumors in vivo has not been extensively studied. An exception to this is work done with the N-terminal deletion mutant *dl*1135 (del 17–27) (69). When wild-type TAg and *dl*1135 were targeted to specific cell types in transgenic mice, the *dl*1135 mutant induced only a subset of the malignancies induced by the wild-type protein (85). This indicates that the activity of the J domain of TAg may be relevant to in vivo tumorigenesis.

SV40 TAg manipulates a variety of basic cellular processes to promote cell growth. It appears that TAg has used a common cellular motif, the J domain of DnaJ molecular chaperone proteins, as one of its means of regulating cell proliferation. This represents a novel mechanism for the inactivation growth suppressor proteins. It remains to be seen whether DnaJ proteins participate in other cases of virally induced tumorigenesis or normal control of cellular proliferation.

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The first two authors contributed equally to this work.

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