Simian virus 40 (SV40) and other mammalian double-stranded DNA viruses adapt cellular processes to permit the synthesis of viral proteins, the replication of viral DNA, and the production of new virions. These adaptations result in increased metabolic, growth, and synthetic rates, which may induce stress in the cells. Such stress initiates cellular stress responses, which mediate signaling designed to limit cellular activity, while the cells attempt to recover. One consequence of these stress responses is the inhibition of translation (1, 11, 15), an event which would be deleterious to the course of the infection; thus, we hypothesized that viruses have means to adapt or counteract the effects of these stress responses. Indeed, our studies of the human cytomegalovirus have confirmed this (16).

In the present study, we specifically examine the effects of SV40 infection on signaling mediated by mammalian target of rapamycin (mTOR) (3, 25), a major controller of translational initiation (11, 23) and a target for inactivation by (i) cellular stress responses, such as hypoxia (1, 22), which causes mTOR to be maintained in its hypophosphorylated, inactive state, and (ii) the drug rapamycin (32), which blocks active mTOR from phosphorylation of its downstream effectors (8, 9, 11). mTOR is believed to be activated by a number of signaling pathways, including PI3K/Akt signaling (11). We and others have previously shown that viral proteins such as SV40 large T and small t antigens, polyomavirus middle T antigen, and the human cytomegalovirus major immediate early proteins can each activate PI3K/Akt signaling (5, 14, 31, 36, 37), thus potentially activating mTOR.

Activated mTOR increases translation by phosphorylating three major effectors (Fig. 1) (11). The phosphorylation of eIF4E-binding protein (4E-BP1) prevents it from binding eIF4E, the cap-binding protein. During active cap-dependent translation, eIF4E is a component of the eIF4F complex, which also contains an RNA helicase, eIF4A, the Mnk1 kinase, and the scaffolding protein eIF4G. As part of eIF4F, eIF4E is maintained in an active state, through phosphorylation by Mnk-1, and is able to mediate cap-dependent translation. Under stressful conditions (e.g., nutrient deprivation or hypoxia), mTOR becomes inactive (hypophosphorylated) and fails to phosphorylate 4E-BP1. This allows 4E-BP1 to bind eIF4E, dissociating it from the eIF4F complex, thus inhibiting cap-dependent translation (11). mTOR also phosphorylates and activates p70 S6 kinase (p70S6K), resulting in the phosphorylation of ribosomal protein S6; this correlates with increased ribosome biogenesis (11). Both p70S6K and 4E-BP1 can be converted from the phosphorylated state to the hypophosphorylated state by protein phosphatase 2A (PP2A), the major phosphatase to counteract mTOR kinase (19, 20). mTOR’s third substrate is eIF4G, the scaffolding protein of eIF4F; the functional consequences of eIF4G phosphorylation are unknown (11).
nonpermissive infections, increasing virus yields in lyrically in-
fected primate cells and enhancing the ability of large T anti-
gen to transform rodent and human cells (24). Small t antigen
is a 174-amino-acid protein which shares the N-terminal 82
residues with large T antigen. This shared region contains a
DnaJ domain which has been implicated in many functions of
these proteins (24, 29, 30). The mutations of residues 43 and 45
eliminate the DnaJ activity of small t antigen (21, 24, 29, 30).
The remaining 92 amino acids form the small t antigen's
unique carboxyl terminal domain, which binds to PP2A (24).

PP2A is a major protein phosphatase in eukaryotic cells
that counteracts the activity of several protein kinases (17).
The heterotrimeric complex contains a catalytic C subunit consis-
tutively bound to scaffold subunit A. The AC core dimer can
further complex with an array of regulatory B subunits which
modulate catalytic activity, substrate specificity, and subcellular
localization (27). Small t antigen can displace some B subunits
from the AC core dimer to form a tAC trimer (24). Small t
decreases PP2A phosphatase activity for most substrates; con-
sequently, these substrates may become hyperphosphorylated
by their cognate kinases.

Here we report that during SV40 lytic infection in monkey
cells, the phosphorylations of p70S6K, S6, and eIF4G are in-
creased early in infection (12 and 24 h postinfection [hpi]), but
at late times (48 hpi), the phosphorylations of mTOR, p70S6K,
and 4E-BP1 are dramatically decreased by a mechanism me-
diated by small t antigen and requiring small t antigen's PP2A
binding domain.

FIG. 1. The mTOR pathway. See the text for details. "p" indicates
protein phosphorylation.

RESULTS

Phosphorylation of p70S6K, S6, and 4E-BP1 is inhibited in
CV-1 cells late in an SV40 infection. To investigate the rela-
tionship between SV40 infection and cellular translational con-
trol, confluent CV-1 cells were serum starved for 24 to 48 h.
The serum-starved cells were treated with either 50 nM rapa-
mycin (dissolved in dimethyl sulfoxide [DMSO]) or an equal
volume of DMSO for 1 h prior to infection. The cells were
infected with purified, serum-free SV40 at a multiplicity of
infection of 4 PFU per cell, in the presence or absence of 50
nM rapamycin. Cell lysates were prepared at the indicated
hours post infection, and proteins of interest were analyzed by

Materials and Methods

Cells and viruses. CV-1 cells, an established African green monkey kidney
(AGMK) cell line, were propagated and maintained in Dulbecco's modified
Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 Units
penicillin, 100 μg/ml streptomycin, and 2 mM Gluta-Max (all reagents from
GIBCO-BRL, Gaithersburg, MD). Primary AGMK cells were purchased from
Diagnose Hybrids, Inc., Athens, Ohio, and grown in DMEM containing 10% fetal
bovine serum, 100 Units penicillin, 100 μg/ml streptomycin, and 2 mM
Gluta-Max. Rapamycin was added to culture medium at 50 nM. Wild-type SV40
strain 776, and small t antigen deletion mutant d888 (28) were propagated and
their titers were determined on CV-1 cells. Virus stocks were prepared by
infecting CV-1 cells at a multiplicity of infection (MOI) of 0.1. When cytopathic
effect was seen throughout the culture, the medium was harvested and cleared of

FIG. 2. SV40 infection affects mTOR signaling in CV-1 cells. (A) Serum-starved CV-1 cells were pretreated with 50 nM rapamycin or the same volume of DMSO (the rapamycin solvent) for 1 h at 37°C and then mock infected or infected with wild-type SV40 strain 776 at an MOI of 4 in the presence or absence of 50 nM rapamycin. Whole-cell lysates were harvested in RIPA buffer at 0 (Mock), 1, 2, 4, 8, 12, 24, and 48 hpi. Thirty micrograms of lysate protein was analyzed by Western analysis for large T and small t antigen expression and the levels of phosphorylated (P) and total p70S6K (Thr389), total ribosome protein S6 (Ser235/236), total eIF4G (Ser1108), and total mTOR (Ser2448). By use of a phospho-specific antibody, it can be seen that phosphorylation of each of these was inhibited by rapamycin, indicating that, despite serum starvation, mTOR kinase activity remained high in the CV-1 cells. This is not surprising, since CV-1 cells are established and immortalized and are not significantly growth arrested by serum starvation.

In infected cells, the phosphorylation of p70S6K increased moderately up to 8 hpi. In all cases, the phosphorylation was rapamycin sensitive. By 12 and 24 hpi, concomitant with detectable expression of small t antigen, phosphorylated p70S6K decreased and became undetectable regardless of the presence of rapamycin. Examination of total protein (p70S6K) (Fig. 2A) shows a moderate decrease in p70S6K by 48 hpi; however, this cannot account for the total loss of the phosphorylated forms at 24 and 48 hpi.

In agreement with the loss of active, phosphorylated p70S6K, the levels of P-S6 (Fig. 2A) decreased by 24 h postinfection and were very low by 48 h postinfection. Total levels of S6 (Fig. 2A) did not vary significantly over the course of the infection.

A similar loss of hyperphosphorylated forms of 4E-BP1 was noted over the infection time course. In these Western analyses, we used an antibody that recognizes total 4E-BP1; the several hyperphosphorylated forms are indicated by the slower migrating bands which disappear upon rapamycin treatment (Fig. 2A). Significant levels of hyperphosphorylated 4E-BP1 remained through 12 hpi; this phosphorylation is sensitive to rapamycin. Between 12 and 24 hpi, hyperphosphorylated forms of 4E-BP1 became undetectable in the infected cells regardless of the presence of rapamycin.

Unlike with p70S6K and 4E-BP1, the phosphorylation of eIF4G (Fig. 2A) did not decrease during the course of the infection in the absence of rapamycin, remaining as high at 48 hpi as it was in mock-infected cells. Rapamycin treatment lowered the levels of phosphorylated eIF4G but did not completely inhibit the phosphorylation. Resistance to rapamycin appears to increase after 12 hpi, and by 48 hpi, the phosphorylation of eIF4G was repeatedly found to be insensitive to rapamycin. The total levels of eIF4G (Fig. 2A) were stable throughout the infection time course.

The hypophosphorylated 4E-BP1 seen late in infection is predicted to bind eIF4E, the cap-binding protein, and inhibit cap-dependent translation by sequestering eIF4E away from its interaction with eIF4G in the eIF4F complex. When eIF4E is not in the eIF4F complex, its phosphorylation on Ser209 cannot be maintained (2, 10). Thus, we examined the phosphorylation of eIF4E over the infection time course (Fig. 2B). Despite the presence of hypophosphorylated eIF4E (Fig. 2A), there remains a significant level of phosphorylated eIF4E (P-4E) (Fig. 2B), suggesting that some eIF4E remains in the eIF4F complex at 24 h postinfection. However, by 48 h postinfection, no phosphorylated eIF4E is detected (Fig. 2B), in agreement with the extreme hypophosphorylation of 4E-BP1 at 48 h postinfection (Fig. 2A). Total levels of eIF4E remained constant during the course of the infection; therefore, the loss of phosphorylated eIF4E at 48 h is not due to a loss of total protein.

The loss of the phosphorylations of p70S6K and 4E-BP1 late in infection suggests that the activation of mTOR is lost late in infection. mTOR is activated via phosphorylation on Ser2448. By use of a phospho-specific antibody, it can be seen that phosphorylated mTOR (P-mTOR) does decrease in the infected cells by 24 hpi and is very low by 48 hpi (Fig. 2A). Total levels of mTOR do not change during the course of the infection (Fig. 2A).
Cellular translation is attenuated late in SV40 infection in CV-1 cells. The effects of SV40 infection on the phosphorylation levels of p70S6K, S6, 4E-BP1, and eIF4E suggest that translation, particularly cap-dependent translation, may be inhibited. To test this, serum-starved CV-1 cells were mock infected or SV40 infected for 24 and 48 h and then pulse labeled for 30 min with [35S]Met/Cys. Equal amounts of labeled extract were displayed by SDS-PAGE. Autoradiographs (Fig. 3) show that there was no apparent loss of incorporation of 35S-amino acids in infected cells at 24 hpi, in agreement with the observation that phosphorylated eIF4E remained at 24 hpi. However, at 48 hpi, there is a significant decrease in incorporation in infected cells compared to that in mock-infected cells (at least a 50% decrease in incorporated 35S), in agreement with the loss of phosphorylation of eIF4E at 48 h (Fig. 2B).

**SV40 small t antigen mediates the loss of phosphorylated 4E-BP1.** Large T and small t antigens are the major regulatory proteins of SV40; therefore, we asked whether either, or both, could mediate the loss of phosphorylated forms of p70S6K and 4E-BP1 seen late in infection. We focused these studies on the phosphorylation of 4E-BP1, since, as described above, hypophosphorylated 4E-BP1 binding to eIF4E is expected to have a significant inhibitory effect on cap-dependent translation.

To address this question, adenoviruses expressing either large T (Ad-LT) or small t antigen (Ad-st) were constructed and propagated. Serum-starved CV-1 cells were infected with purified, serum-free adenovirus stocks at an MOI of 5, and cellular extracts were prepared at 24 and 48 hpi. Figure 4 shows that both large T and small t antigens were expressed efficiently from the adenovirus vectors in CV-1 cells. 4E-BP1 shows at least three hyperphosphorylated bands in the 24- and 48-h mock-infected cells. Using the adenovirus infection control vector which expressed green fluorescent protein (Ad-GFP) we noted increased hyperphosphorylation, indicating that adenovirus infection alone can induce hyperphosphorylation of 4E-BP1. There was no difference in 4E-BP1 phosphorylation between Ad-GFP and Ad-LT at 24 h postinfection; however, at 48 h postinfection, Ad-LT did not induce as much hyperphosphorylated 4E-BP1 as Ad-GFP. This modest effect of the large T antigen was overshadowed by the results of infection with Ad-st, for which some hypophosphorylated 4E-BP1 was seen at 24 h, and this dramatically increased by 48 h. These data suggest that small t antigen mediates the loss of phosphorylated forms of 4E-BP1. This effect of small t antigen was also seen when both Ad-LT and Ad-st were coinfected for 24 or 48 h (data not shown); hence, under these experimental conditions, the effects of small t antigen are not altered by the presence of large T antigen. We were unable to test whether large T or small t antigen alters the phosphorylation of p70S6K, because Ad-GFP, the control adenovirus, induced a very strong phosphorylation of p70S6K (data not shown).

We also show in Fig. 4 that Ad-GFP, Ad-LT, and Ad-st infections had little effect on mTOR phosphorylation or total mTOR protein levels, suggesting that the loss of 4E-BP1 phosphorylation is not the result of inactivation of mTOR kinase activity. The maintenance of phosphorylated mTOR could be a result of the adenovirus infection, since we noted decreased phosphorylation of mTOR at late times in the SV40 infection (Fig. 2A). The data suggest that small t antigen-mediated de...
phosphorylation of 4E-BP1 can occur in the presence of activated mTOR.

To further confirm the role of small t antigen in the loss of phosphorylated 4E-BP1, serum-starved CV-1 cells were infected for 48 h with wild-type SV40 or small t antigen deletion mutant dl888 (28), which does not produce small t antigen, due to a deletion in the small t antigen unique coding region. Figure 5 shows that the wild-type infection caused the expected loss of hyperphosphorylated forms of 4E-BP1, while the levels of hyperphosphorylated 4E-BP1 were not altered by the small t antigen deletion mutant.

Presence of small t antigen causes eIF4E to associate with 4E-BP1 and not with eIF4G. As discussed for Fig. 1, the eIF4F complex consists of the cap-binding protein eIF4E; an RNA helicase, eIF4A; and a kinase, Mnk1; each bound to the scaffolding protein eIF4G. The binding of eIF4E to eIF4G can be disrupted when hypophosphorylated 4E-BP1 binds to eIF4E and removes it from the eIF4F complex, thus inhibiting cap-dependent translation. eIF4E can bind to the cap structure regardless of whether it is in association with eIF4G or 4E-BP1. Thus, the binding status of eIF4E in cellular extracts can be determined by using Sepharose beads coupled with cap analog, which binds eIF4E and its associated proteins. The associated proteins can be identified by Western analysis.

To determine whether the small t antigen-mediated loss of phosphorylation of 4E-BP1 correlated with dissociation of eIF4E from eIF4G and the concomitant binding to 4E-BP1, we performed a cap-binding experiment using extracts from CV-1 cells infected with Ad-GFP, Ad-LT, Ad-st, or wild-type SV40. Figure 6A shows that equivalent amounts of eIF4E were recovered from all extracts by using the Sepharose beads coupled with cap analog. In the mock-infected cells, Ad-GFP (control)-infected cells, and the cells infected with Ad-LT, the eIF4E is exclusively associated with eIF4G indicating that it is translationally active as part of the eIF4F complex. However, in the cells infected with Ad-st or SV40 for 48 h, there was a significant reduction of eIF4E binding to eIF4G and a concomitant increase in binding to 4E-BP1. These data indicate that the small t antigen-induced loss of phosphorylation of 4E-BP1 (Fig. 4) results in the expected increase in binding of eIF4E to 4E-BP1 and decreased binding to eIF4G.

Figure 6B is an examination of the total proteins in the lysates used for the cap-binding experiment, showing that the total levels of eIF4E, 4E-BP1, and eIF4G do not differ between samples; thus, increases or decreases in protein concentrations cannot account for the results of the binding studies.

A PP2A binding mutant of small t antigen, but not a DnaJ domain mutant, failed to mediate the loss of phosphorylation of 4E-BP1. As described in the introduction, small t antigen has two characterized domains, an N-terminal DnaJ domain and a C-terminal PP2A binding domain. To determine which domain of small t antigen mediates the loss of phosphorylated 4E-BP1, we tested recombinant adenoviruses expressing two mutant small t antigens. P43L/K45N is a double mutation located in the DnaJ domain; this mutant is severely impaired in DnaJ function (21, 30). Figure 7 shows that the P43L/K45N mutant was fully competent for mediating the loss of phosphorylation of 4E-BP1.
The other small t antigen mutation, C103S, significantly reduces the ability of small t antigen to bind PP2A (18). This mutant showed a greatly diminished ability to mediate the loss of phosphorylated 4E-BP1 (Fig. 7), suggesting that the interaction of small t antigen with PP2A may be necessary to mediate the loss of phosphorylations of 4E-BP1 and, possibly, p70S6K (Fig. 2A).

SV40 infection of primary AGMK cells shows additional effects on mTOR effectors. CV-1 cells are an established and immortalized cell line which cannot be significantly growth arrested by serum starvation. Therefore, we felt that this inability to growth arrest the cells may mask phosphorylation of mTOR or its effectors mediated by SV40 infection. Therefore, we performed an infection time course in primary AGMK cells that had been serum-starved for 24 h and then mock infected or infected with purified, serum-free SV40 and maintained under serum-free conditions (Fig. 8). Western analysis was used to probe the cell lysates as described for Fig. 2. As seen for CV-1 cells, late in the infection (48 hpi), there was a marked decrease in the phosphorylations of mTOR, 4E-BP1, p70S6K, and S6 (P-S6). However, at earlier time points (12 and 24 hpi), we did detect additional effects of the viral infection. Levels of phosphorylated p70S6K were moderately increased, and this correlated with a significant increase in phosphorylated S6. Elevated levels of phosphorylated eIF4G in infected cells were detected at 12, 24, and even 48 hpi. Hyperphosphorylated forms of 4E-BP1 were detected in the serum-starved mock-infected samples; thus, it was not possible to determine whether the viral infection could cause the increased phosphorylation of 4E-BP1. In sum, the data suggest that SV40 infection can maintain or induce phosphorylations of p70S6K, S6, and eIF4G early in the infection (12 and 24 hpi) and then reduce the phosphorylation of mTOR, p70S6K, S6, and 4E-BP1 late in the infection (48 hpi).

**VP1 synthesis does not depend upon 4E-BP1 hyperphosphorylation.** The data presented suggest that 4E-BP1 becomes hypophosphorylated late in SV40 infection and can inhibit cap-dependent translation at a time in the infection when significant levels of virions structural proteins, e.g., VP1, need to be synthesized. Thus, we asked whether VP1 synthesis was affected by the hypophosphorylation of 4E-BP1. Figure 9 shows an infection time course done in the presence or absence of rapamycin. As discussed above, rapamycin inhibits mTOR kinase preventing hyperphosphorylation of 4E-BP1. Western
analysis shows that throughout the time course, in rapamycin treatment, 4E-BP1 was maintained in its hypophosphorylated state, which would inhibit cap-dependent translation. In the normal infection, hypophosphorylation of 4E-BP1 was noted at 24 hpi and greatly increased by 48 hpi, in agreement with the data in Fig. 2A and 8. VP1 accumulation, shown in a Coomassie-stained gel, is seen modestly at 24 hpi and abundantly at 48 hpi with little difference between normal and rapamycin-treated samples. Thus, VP1 translation and accumulation appear to be relatively resistant to 4E-BP1 hypophosphorylation. Possible explanations for this are discussed below.

**DISCUSSION**

Viruses depend on the host cell protein synthetic machinery for the production of viral proteins. Many viruses have evolved effective means to redirect the host translation machinery to favor viral protein synthesis (7, 26). The majority of control over cellular mRNA translation occurs during initiation. Our studies of AGMK cells demonstrate that during an SV40 infection, the phosphorylations of p70S6K, S6, and eIF4G are induced early in the infection (12 and 24 hpi). It is likely that the phosphorylation of 4E-BP1 can also be upregulated, but this cannot be measured in AGMK or CV-1 cells, since high levels of hyperphosphorylated 4E-BP1 are maintained in these cells even under serum-starved conditions.

The virally induced phosphorylation of these proteins is largely sensitive to rapamycin, suggesting that mTOR kinase is activated by the viral infection, most likely via activated Akt (Fig. 1). The activation of Akt by SV40 large T and small t antigens has previously been described (36, 37). Interestingly, late in the infection (by 48 hpi), the levels of phosphorylated mTOR, p70S6K, S6, and 4E-BP1 dramatically decrease in the infected cell. The only mTOR effector whose phosphorylation is not decreased is eIF4G, which remains highly phosphorylated at 48 hpi in both CV-1 and AGMK cells. In the rapamycin inhibition studies using CV-1 cells, we noted that at 48 hpi, the phosphorylation of eIF4G was rapamycin insensitive, suggesting that late in infection, an mTOR-independent mechanism is induced for the phosphorylation of eIF4G. We have previously reported mTOR-independent phosphorylation of eIF4G during human cytomegalovirus infection (16). At present, the effect of phosphorylation of eIF4G on translation or other cellular processes is unknown. However, the targeting of eIF4G for mTOR-independent phosphorylation by two very different DNA viruses suggests that eIF4G phosphorylation produces a cellular environment which favors viral protein synthesis or replication.

The data presented suggest that SV40 infection induces mTOR signaling early in the infection in order to ensure cap-dependent translation and ribosome biogenesis, situations which would aid the viral infection. In contrast, the dramatic dephosphorylations of mTOR, p70S6K, S6, and 4E-BP1 late in the infection suggest that the virus infection inhibits cap-dependent translation and ribosome biogenesis at late times. The inhibition of cap-dependent translation is supported by our finding that the hypophosphorylation of 4E-BP1, mediated by small t antigen, correlates with the removal of eIF4E from the eIF4F complex. Such inhibition late in the infection may not matter; it is possible that by the time the inhibition occurs, sufficient viral structural proteins have been made and capsid formation can occur regardless of the state of translation. However, this conclusion is not supported by the data in Fig. 9, which suggest that VP1 can accumulate in the presence of hypophosphorylated 4E-BP1 caused by the addition of rapamycin. Although the inhibitory effect of rapamycin on mTOR signaling is well established, its presence does not produce a global inhibitory effect on translation in mammalian cells (10); for example, reductions in total cap-dependent translation of no more than 30 to 35% have been reported. However, among specific mRNAs dependent on the cap for translation, there is a wide range in the degree of inhibition elicited by rapamycin treatment (10). Thus, it is possible that SV40 mRNAs are of the type that is more readily translated in the presence of rapamycin.

On the other hand, the inhibition of cap-dependent translation may promote the success of the viral infection by favoring translation using internal ribosome entry sites (IRESs) in order to produce the large amounts of virion structural proteins (VP1, VP2, and VP3) needed for virion formation. The 16S and 19S SV40 late mRNAs are splice variants which are polycistronic; the 16S mRNA encodes the agnoprotein and the major virion structural protein VP1; the 19S mRNA encodes the agnoprotein and the minor virion structural proteins VP2 and VP3, as well as VP1. Although no studies have been done to identify IRESs in the SV40 late mRNAs, utilization of the IRESs could explain how these polycistronic mRNAs can produce sufficient amounts of virion structural proteins. SV40 VP1 must be produced in large amounts late in infection in order to efficiently produce virions. The translational start codon (AUG) for VP1 in both the 16S and 19S mRNAs is the last utilized AUG. In the best situation, the 16S late mRNA, the VP1 AUG is the third AUG in the message (4). Thus, the utilization of an IRES to facilitate translational initiation at the VP1 AUG would be a reasonable mechanism. For the 19S mRNA, a strong case can be made for IRES-directed utilization of the AUG for VP3, since this AUG is downstream of the VP2 AUG and there is no translational stop codon prior to the VP3 AUG. If IRESs are utilized, then the inhibition of cap-dependent translation late in infection would enhance the translation of virion structural proteins at a time when they are most needed for virion formation.

An additional reason to believe that the cessation of cap-dependent translation may be significant for the success of the viral infection is our finding that the decrease in the phosphorylation of 4E-BP1, and probably that of p70S6K, is a specific function of small t antigen. Many viable mutants of small t antigen have been studied (28, 33). Although the mutants are viable for lytic growth in monkey cells, the rate of growth, virus yield, and plaque size are generally reduced in infections by these deletion mutants. These phenotypes are compatible with the model of IRES-dependent late protein synthesis; specifically, the loss of small t antigen would result in cap-dependent translation being maintained, and IRES utilization would not be enhanced, thus hindering the accumulation of virion structural proteins.

Our data suggest that small t antigen’s ability to interact with PP2A is needed to induce hypophosphorylation of 4E-BP1. PP2A is suspected to be the phosphatase which acts on 4E-BP1 and p70S6K (19, 20). The small t antigen-PP2A interaction...
usually results in inhibition of PP2A function (24), however our data suggest that PP2A's phosphatase activity may be specifically activated and targeted to dephosphorylate 4E-BP1 in the presence of small t antigen. This is not unprecedented; increased PP2A activity in the presence of small t antigen has previously been reported for the dephosphorylation of histone H1 (35); in addition, recent data suggest that small t antigen may mediate the transfer of PP2A to the androgen receptor, thus directing phosphatase activity to this protein (34). It is possible that small t antigen-PP2A directs the dephosphorylation and inactivation of mTOR, resulting in decreased phosphorylations of 4E-BP1 and p70S6K. However, the data in Fig. 4 indicated that small t antigen expressed from the adenovirus vector had little effect on mTOR phosphorylation, while there was significant loss of 4E-BP1 phosphorylation. This suggests that small t antigen can mediate the hypophosphorylation of 4E-BP1 in the presence of activated mTOR. Thus, our data suggest two potential mechanisms: (i) the interaction of small t antigen with PP2A results in the known inhibition of PP2A, but this inhibition allows the activation of a second phosphatase which actively dephosphorylates 4E-BP1.

ACKNOWLEDGMENTS

We thank Kathy Randell for reagents and helpful discussion and all the members of the Alwine laboratory for support and critical evaluation of the experiments and data.

This work was supported by Public Health Service grant R01 CA28379-25 awarded to J.C.A. by the National Cancer Institute.

REFERENCES


Downloaded from http://jvi.asm.org/ on September 9, 2017 by guest