The Adenovirus E1A Protein Targets the SAGA but Not the ADA Transcriptional Regulatory Complex through Multiple Independent Domains

Michael Shuen‡‡, Nikita Avvakumov‡¶, Paul G. Walfish‡‡, Chris J. Brandl‡¶, and Joe S. Myymyk‡‡¶

From the Departments of §Microbiology and Immunology, ¶Pharmacology and Toxicology, §§Biochemistry, and ¶¶Oncology, London Regional Cancer Centre, The University of Western Ontario, London, Ontario N6A 4L6 and *Samuel Lunenfeld Research Institute of Mount Sinai Hospital and Department of Medicine, Endocrinology Division, University of Toronto Medical School, Toronto, Ontario M5G 1X5, Canada

Expression of the adenovirus E1A protein in the simple eukaryote Saccharomyces cerevisiae inhibits growth. We tested four regions of E1A that alter growth and transcription in mammalian cells for their effects in yeast when expressed as fusions to the Gal4p DNA binding domain. Expression of the N-terminal/conserved region (CR) 1 or CR3, but not of the CR2 or the C-terminal portion of E1A, inhibited yeast growth. Growth inhibition was relieved by deletion of the genes encoding the yGen5p, Ngg1p, or Spt7p components of the SAGA transcriptional regulatory complex, but not the Ahc1p component of the related ADA complex, indicating that the N-terminal/CR1 and CR3 regions of E1A target the SAGA complex independently. Expression of the pCAF acetyltransferase, a mammalian homologue of yGen5p, also suppressed growth inhibition by either portion of E1A. Furthermore, the N-terminal 29 residues and the CR3 portion of E1A interacted independently with yGen5p and pCAF in vitro. Thus, two separate regions of E1A target the yGen5p component of the SAGA transcriptional activation complex. A subregion of the N-terminal/CR1 fragment spanning residues 30–69 within CR1 also inhibited yeast growth in a SAGA-dependent fashion. However, this region did not interact with yGen5p or pCAF, suggesting that it makes a third contact with another SAGA component. Our results provide a new model system to elucidate mechanisms by which E1A and the SAGA complex regulate transcription and growth.

The products of the human adenovirus type 5 E1A (garly region 1A) gene function as potent regulators of growth and gene expression (1, 2). There are two major E1A proteins of 289 and 243 amino acids that differ only by the presence of an internal sequence of 46 amino acids in the larger protein. Comparison of the E1A sequence of multiple adenovirus serotypes has identified four distinct regions of sequence conservation (3), designated conserved regions (CR) 1, 2, 3, and 4 (see Fig. 1A). CR3 coincides almost exactly with the region unique to the 289-amino acid protein.

E1A functions to reprogram cell growth and transcription by interacting with a variety of cellular proteins. The N-terminal/CR1 portion of E1A interacts with several transcriptional coactivators including the acetyltransferases pCAF (4), cAMP-response element-binding protein-binding protein, and related family member p300 (5–7), as well as the TATA-binding protein (8). CR3 interacts with various components of the general and specific transcriptional machinery, including the TATA-binding protein (8–11), several of the TATA-binding protein-associated factors (12, 13), ATF-2 (14, 15), and c-Jun (14, 16). Not surprisingly, CR3 of E1A is required for activation of gene transcription during adenovirus infection (1, 17, 18).

Genetic studies in the budding yeast Saccharomyces cerevisiae have demonstrated that one or more targets of E1A are conserved in this simple eukaryote. When introduced into many haploid strains of S. cerevisiae, E1A inhibits growth, leading to an accumulation of cells in the G2 phase of the cell cycle (19–21). Mutations within the N-terminal/CR1 and CR3 portions of E1A impair growth inhibition (19). The N-terminal/CR1 and CR3 domains are both important for the transcriptional activities of E1A in mammalian cells (1), suggesting that E1A may interact with conserved regulatory pathways in both types of organisms to alter transcription. Several additional lines of evidence support this hypothesis. First, yeast with impaired cyclic AMP signaling are insensitive to growth inhibition by E1A (19). In mammalian cells, E1A is known to act in synergy with cyclic AMP to activate gene expression (22, 23), suggesting a remarkable conservation of function between higher and lower eukaryotes. Second, SNF/SWI-dependent transcriptional activation is required for growth inhibition by the N-terminal/CR1 portion of E1A, suggesting that transcriptional activation by this fragment of E1A mediates toxicity (24).

In this study, we show that residues 1–29, 30–69, or the CR3 portion of E1A were each sufficient to inhibit yeast growth when expressed as fusions to the Gal4p DNA binding domain (DBD). Growth inhibition by any portion of E1A required an intact yeast SAGA (Spt-Ada-Gen5-acetyltransferase) complex.
but was not related directly to transcriptional activation by E1A. We further show a physical interaction of \( \text{yGcn5p} \) and the CR3 portion of E1A.

### MATERIALS AND METHODS

#### Yeast Strains, Media, and Plasmid Constructions—Strains used in this study are shown in Table I. Yeast culture media were prepared using standard techniques (25). The yeast expression vector \( \text{pAS1U} \) (\( \text{URA3} \) marker) was constructed from \( \text{pAS1 (TRP1 marker)} \) (26) by subcloning the XhoI-Nael fragment of \( \text{pAS1} \) into the same sites of \( \text{pRS426} \) (27). Similarly, \( \text{pAS1L (LEU2 marker)} \) was constructed by subcloning the PvuII fragment of \( \text{pAS1} \) into the same site of \( \text{pK425} \) (27). The \( \text{N-terminal/CR1} \) domain of adenovirus type 5 E1A (amino acids 1–82) was expressed as a fusion with the Gal4p DBD (amino acids 1–147) by subcloning an \( \text{EcoRI-BamHI} \) fragment from \( \text{pMA424.82T} \) (19) into \( \text{pAS1U} \) and \( \text{pAS1L} \). The C-terminal domain of E1A (residues 187–289) was expressed as a fusion with the Gal4p DBD by subcloning an \( \text{EcoRI-BamHI} \) fragment from \( \text{pMA242.82T} \) into \( \text{pAS1U} \) and \( \text{pAS1L} \). The sequences encoding residues 99–128 (CR2), 129–204 (CR3), 1–29, 30–69, or 70–82 of adenovirus type 5 E1A were PCR-amplified and subcloned as \( \text{EcoRI-BamHI, EcoRI-SalI, or EcoRI-XhoI} \) fragments into \( \text{pAS1U} \) and \( \text{pAS1L} \). Plasmid \( \text{pADHVP16} \) (29), which expresses the herpes simplex virus VP16 transcriptional activation domain fused to the Gal4p DBD, was obtained from Dr. M. M. Smith (University of Virginia, Charlottesville, VA). Fragments of \( \text{pCAF} \) encoding residues 1–352 and 310–832 were generated by PCR with specific oligonucleotide primers and cloned into the vectors \( \text{pGJ-5 (OrgGene Technologies Inc., Rockville, MD)} \) or \( \text{pMAL-c2X (New England Biolabs, Mississauga, Ontario, Canada)} \). A yeast expression vector \( \text{pGuN} \), and the variant \( \text{pGuN-55} \), which expresses the adenovirus type 2 E1B 55-kDa protein (31), were obtained from Dr. M. M. Smith, Invitrogen Corporation.

#### Yeast Transformations—Yeast transformations were performed using standard techniques (25).

#### Growth Suppression Assay—Yeast transformations were performed using a modified lithium acetate procedure as described previously (32). Cells were plated onto appropriate synthetic complete omission plates and incubated at 30 °C. For assays of growth inhibition by E1A or E1B, plates were photographed 48 or 72 h post-transformation using a \( \text{Foto/Eclipse Fotodyne gel doc system (Fotodyne Inc., Hartland, WI)} \).  

#### \( \beta \)-Galactosidase Assays—Colonies of the yeast strain \( \text{Y190} \) transformed with plasmids expressing various portions of E1A fused to the Gal4p DBD were picked and used to inoculate 5 ml of glucose-supplemented synthetic complete medium lacking uracil and leucine. Cultures were grown overnight at 30 °C with agitation. Cells were collected by centrifugation at \( 4000 \times g \) for 5 min and washed twice with 5 ml of double distilled \( \text{H}_{2}\text{O} \). \( \beta \)-Galactosidase assays were performed as described previously (25). Enzymatic activity was calculated as \( (\text{A}_{420}/\text{A}_{600}) \times \text{culture volume (ml) } \times \text{reaction time (min)} \).

#### Glutathione S-transferase (GST) Pull Downs—GST-E1A fusion proteins were expressed in \( \text{Escherichia coli} \) BL21 bacteria and purified as per the protocol provided by the affinity resin manufacturer (Amersham Biosciences). In vitro-translated \( \text{[35S]} \) methionine-labeled full-length \( \text{yGcn5p} \) (obtained from Dr. S. Berger, The Wistar Institute, Philadelphia, PA) or the carboxyl portion of \( \text{pCAF} \) spanning amino acids 310–832 were prepared using the \( \text{TntT7} \) coupled transcription/translation system (Promega) according to the supplied protocol. To immobilize \( \text{GST-E1A fusion proteins} \), 10 \( \mu \)g of the appropriate \( \text{GST-E1A fusion protein} \) was incubated with 25 \( \mu \)l of glutathione-Sepharose beads in PD buffer (50 mM Tris/HCl, pH 7.4, 300 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 5 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 0.1% Triton X-100) in a total volume of 200 \( \mu \)l for 20 min at 4 °C. For GST pull-down assays, immobilized \( \text{GST-E1A fusion proteins} \) were incubated with 100,000 \( \text{cpm of in vitro-translated} \text{[35S]} \) methionine-labeled \( \text{yGcn5p} \) or \( \text{pCAF} \) in pull-down buffer (50 mM HEPES/KOH, pH 7.5, 150 mM KCl, 1 mM EDTA, 1 mM Na, 10 mM NaF, 5% glycerol, 0.1% Nonidet P-40, 2 \( \mu \)g/\( \mu l \) bovine serum albumin) containing Complete protease inhibitor mixture (Roche Diagnostics) in a total volume of 200 \( \mu l \) for 1 h at 4 °C with constant rotation. After extensive washes with pull-down buffer, interacting proteins were eluted by boiling for 2 min in 10 \( \mu l \) of SDS sample buffer, resolved on 10% SDS-polyacrylamide gels, and visualized by autoradiography.

#### Histone Acetyltransferase (HAT) Assays—GST, \( \text{GST-E1A} \) fusions, maltose-binding protein (MBP), and MBP-pCAF fusion proteins were expressed in and purified from the BL21 strain of \( \text{E. coli} \) as per protocols provided by the affinity resin manufacturers (Amersham Biosciences and New England Biolabs) and dialyzed against MBP column buffer. 5 \( \mu \)g of recombinant MBP-pCAF was mixed with 100 \( \mu \)g of the appropriate \( \text{GST-E1A fusion proteins} \) and incubated for 15 min on ice. Reaction mixtures were then washed with 10 \( \mu l \) of HAT buffer (50 mM HEPES/KOH, pH 8.0, 150 mM NaCl, 5 mM EDTA, 0.1% (v/v) Nonidet P-40, 1.0 \( \mu l \) of acetyl-[\( ^{3} \text{H} \)]CoA (ICN Biomedical Research Products, Costa Mesa, CA), and 15 \( \mu l \) of histone II-A (Sigma) or 15 \( \mu l \) of bovine serum albumin as a negative control. After 45 min at 30 °C, supernatant for each reaction was spotted onto circles of \( \text{p81 filter paper} \) (Whatman Nuclepore Canada, Toronto, Ontario, Canada). The air-dried filters were soaked in sodium carbonate/bicarbonate solution (50 mM \( \text{NaHCO}_{3} \), 50 mM \( \text{Na}_{2}\text{CO}_{3} \)) for 30 min at 37 °C, washed once in 30 ml of a 1:1:1 mixture of methanol:chloroform:acetone for 10 min, and washed twice more for 5 min. The filters were air-dried, and the amount of bound \( [^{3} \text{H}] \) acetyl was measured by liquid scintillation counting.

#### RESULTS

The \( \text{N-terminal/CR1 or CR3 Portions of E1A Can Inhibit Growth Independently} \)—Expression of the adenovirus type 5 E1A protein suppresses growth when expressed in haploid strains of \( \text{S. cerevisiae} \) (19–21). Mutations within the \( \text{N-terminal/CR1} \) and \( \text{CR3} \) portions of E1A impair growth inhibition, although the \( \text{N-terminal/CR1} \) portion of E1A is sufficient on its own to block growth when fused to the Gal4p DBD (19–21, 33). To determine whether other portions of E1A could inhibit growth independently of the \( \text{N-terminal/CR1 region} \), we expressed the \( \text{CR2, CR3, and C-terminal regions of E1A as fusions} \) with the Gal4p DBD and tested them for their effect on yeast growth (Fig. 1). Like the \( \text{N-terminal/CR1 portion of E1A} \), expression of the \( \text{Gal4p DBD-CR3 chimera} \) inhibited growth. In contrast, the \( \text{Gal4p DBD or the Gal4p DBD fused to the CR2 or C-terminal regions of E1A did not affect growth} \) (Fig. 1B). These results demonstrate that CR3, like the \( \text{N-terminal/CR1} \)
portion of E1A, is sufficient to deregulate yeast growth independently.

The N-terminal/CR1 or CR3 Domains of E1A Can Activate Transcription in Yeast—Studies in the simple eukaryote \textit{S. cerevisiae} have shown that fusion of a strong transcriptional activation domain to the Gal4p DBD can inhibit yeast growth (29). We reasoned that growth inhibition by the N-terminal/CR1 and CR3 domains E1A could be produced by a similar effect, as both of these regions of E1A function as transcriptional activation domains when fused to a DNA binding domain in mammalian cells (34, 35). We tested the ability of the N-terminal/CR1, CR2, CR3, and C-terminal regions to function as activation domains in yeast when fused to the Gal4p DBD (Fig. 2). We transformed yeast strain Y190, which contains an integrated Gal4p-dependent \( \beta \)-galactosidase reporter gene, with expression vectors for the Gal4p DBD or the four Gal4p DBD-E1A fusions. The N-terminal/CR1 fragment of E1A strongly activated transcription in this system, and a lesser degree of transcriptional activation was observed for CR3. In contrast, neither the CR2 nor C-terminal regions activated \( \beta \)-galactosidase expression. Thus, the regions of E1A that inhibit growth in yeast (Fig. 1B) also function to activate transcription, raising the possibility that these two activities are related.

Growth Inhibition Mediated by E1A Requires the SAGA Transcriptional Activation Complex—Growth inhibition mediated by fusion of the herpes simplex virus VP16 transcriptional activation domain to the Gal4p DBD has been proposed to result from sequestration of limiting components of the transcription apparatus at genomic sites (29). This is supported by the observation that a Gal4p DBD-VP16 chimera does not inhibit growth in yeast strains disrupted for components of the SAGA yeast transcriptional activation complex (29, 36, 37). We tested the ability of the Gal4p DBD N-terminal/CR1 and CR3 chimeras to inhibit growth in yeast strains with disruptions of \textit{NGG1}, \textit{GCN5}, and \textit{SPT7} (\textit{ngg1}\Delta, \textit{gcn5}\Delta, and \textit{spt7}\Delta, respectively), which encode components of the SAGA complex (Fig. 3). As reported previously (36–38), Gal4p DBD-VP16 inhibited growth in wild-type yeast strains but not in strains disrupted for \textit{NGG1} or \textit{GCN5}. Interestingly, although Gal4p DBD fused to the N-terminal/CR1 or CR3 domains was toxic in matched wild-type strains, neither chimera inhibited growth in \textit{ngg1}\Delta, \textit{gcn5}\Delta, or \textit{spt7}\Delta strains. However, growth inhibition by the N-terminal/CR1 or CR3 regions was not affected by deletion of \textit{AHC1} (Fig. 4), which encodes a component specific to the yeast ADA transcriptional activation complex (39). These results demonstrate that growth inhibition mediated by either the N-terminal/CR1 or CR3 domains of E1A depends on the presence of an intact SAGA complex but not the related ADA complex. Mutation of SAGA components relieved growth suppression by E1A and VP16 specifically, as disruption of \textit{NGG1} had no effect on growth inhibition by the adenovirus E1B 55-kDa protein (data not shown).

The N-terminal/CR1 or CR3 Domains of E1A Interact Independently with yGCN5p and pCAF—Our observation that growth inhibition by either the N-terminal/CR1 or CR3 domains of E1A requires an intact SAGA complex suggests that both these portions of E1A interact with components of the complex. The N-terminal/CR1 portion of E1A is known to interact with pCAF, a mammalian homologue of yGcn5p (4), and E1A has been shown to coprecipitate an HAT activity when expressed in yeast (40), suggesting that this region of E1A may also target yGcn5p. We expressed the N-terminal/CR1, CR2, CR3, and C-terminal portions of E1A as fusions to GST and tested their ability to interact with \textit{in vitro}-transcribed and -translated yGCN5p (Fig. 5A). An interaction was observed...
between yGcn5p and the N-terminal/CR1 and CR3 but not the CR2 or C-terminal portions of E1A. Otherwise identical experiments performed using in vitro-transcribed and -translated pCAF yielded similar results (Fig. 5B). These experiments show that the N-terminal/CR1 portion of E1A can interact not only with pCAF but with the homologous yGcn5p, as well. Importantly, this observation also suggests that CR3 can target these two related proteins independently of the N-terminal/CR1 portion of E1A.

Expression of pCAF Suppresses Growth Inhibition by E1A in Yeast—Based on the results presented above, growth inhibition by either the N-terminal/CR1 or CR3 portions of E1A likely results from an interaction with yGcn5p. We next tested whether expression of pCAF, a mammalian homologue of yGcn5p, would restore growth to yeast expressing these regions of E1A. We cotransformed yeast with vectors expressing either the N-terminal/CR1 or CR3 domains of E1A or to herpes simplex virus VP16. Transformed cells were allowed to grow ~48 h at 30 °C and photographed.

Fig. 2. Transcriptional activation by E1A domains fused to the Gal4p DBD. Yeast strain Y190 was transformed with expression vectors for the Gal4p DBD fusion proteins described for Fig. 1A or with a control vector expressing the Gal4p DBD alone. The ability of each portion of E1A to stimulate transcription from a Gal4-dependent β-galactosidase reporter gene was assayed in triplicate as described under “Materials and Methods.” Error bars indicate the S.E.

Fig. 3. Requirement for the SAGA transcriptional activation complex for growth inhibition by E1A. The indicated wild-type or mutant yeast strains were transformed with vectors expressing the Gal4p DBD or the Gal4p DBD fused to the N-terminal/CR1 or CR3 domain of E1A or herpes simplex virus VP16. Transformed cells were allowed to grow ~48 h at 30 °C and photographed. The effect of VP16 on growth of the Δsp7 strain could not be determined because of a lack of a suitable auxotrophic marker in this strain.

Fig. 4. The Ahc1p component of the ADA complex is not required for growth inhibition by E1A. Wild-type yeast, or an isogenic strain in which the gene encoding the Ahc1p component of the ADA transcriptional activation complex was disrupted, were transformed with vectors expressing the Gal4p DBD or the Gal4p DBD fused to the N-terminal/CR1 or CR3 domains of E1A or to herpes simplex virus VP16. Transformed cells were allowed to grow ~48 h at 30 °C and photographed.

Fig. 5. Interaction of yGcn5p and pCAF with E1A. Fragments of E1A depicted in Fig. 1A were prepared as fusions to GST and used in GST pull-down assays with 35S-labeled full-length yGcn5p or the C-terminal portion of pCAF as described under “Materials and Methods.” Proteins were recovered with glutathione-Sepharose and analyzed by SDS-PAGE and radiography. 1/10 of the input of 35S-labeled proteins used in the binding reactions is shown for comparison.
FIG. 6. Effect of pCAF expression on E1A-mediated growth inhibition. Yeast strain w303-1A was transformed with a vector expressing either the N-terminal/CR1 (A) or CR3 (B) portions of E1A and vectors expressing the indicated portion of pCAF. pCAF<sub>1</sub>, amino acids 1–352; pCAF<sub>2</sub>, amino acids 310–832; pCAF<sup>mut</sup>, amino acids 310–832 with tyrosine 616 and phenylalanine 617 mutated to alanines, which abolishes histone acetyltransferase activity. Transformed yeast were grown for 48–72 h at 30 °C and photographed.

(Fig. 6). The observation that pCAF expression suppresses growth inhibition mediated by either the N-terminal/CR1 or CR3 portions of E1A supports our observation that pCAF interacts independently with each of these regions (see Fig. 3 and Fig. 5). Interestingly, expression of the carboxyl portion of pCAF containing two point mutations that abolish HAT activity was still able to restore rapid growth to yeast expressing E1A. As suppression of E1A induced growth inhibition was independent of the enzymatic activity of pCAF, it likely does not result from a complementation of yGcn5p activity by mammalian pCAF. Instead, the expression of pCAF may compete with endogenous yGcn5p for interaction with E1A, effectively preventing binding to yGcn5p.

**DISCUSSION**

We report here that either the N-terminal/CR1 or CR3 domains of E1A are sufficient to inhibit yeast growth when expressed as fusions with the Gal4p DBD. In contrast, neither the CR2 nor C-terminal domains of E1A affected yeast growth when fused to the Gal4p DBD (Fig. 1B). These results agree with previous reports demonstrating that the N-terminal/CR1 domain can inhibit yeast growth (19) and with the observation that deletions within both the N-terminal/CR1 or CR3 domains impair growth inhibition (19, 21). Our results suggest that the CR3 domain, like the N-terminal/CR1 domain, is targeting a cellular function(s) conserved in yeast and raises the possibility that genetic analysis of CR3 function in yeast will provide useful insight into the mechanisms by which this region of E1A functions in higher eukaryotic cells.

In mammalian cells, both the N-terminal/CR1 and CR3 domains are equally potent activators of gene expression when fused to the Gal4p DBD (35, 42). When tested in yeast, these two regions also functioned to activate transcription when fused to the Gal4p DBD (Fig. 2). This raised the possibility that their ability to inhibit growth results from activation of transcription at inappropriate genomic sites. However, this is not entirely consistent with our observation that although the N-terminal/CR1 and CR3 domains were similarly proficient at inhibiting growth (Fig 1B), transcriptional activation by the CR3 domain was much weaker than that observed for the N-terminal/CR1 domain (Fig. 2). In addition, although residues 1–29 or 30–69 of E1A are sufficient for growth inhibition (Fig. 9B), neither is capable of activating transcription (Fig. 9C). Thus, growth inhibition is clearly not related to the ability of E1A to activate transcription and likely results from trapping of limiting cellular factors.

Previous studies in yeast using the strong herpes simplex virus transcriptional activator VP16 showed that a Gal4p DBD-VP16 chimera does not inhibit growth in yeast strains disrupted for components of the SAGA yeast transcriptional activation complex (36–38). As observed for VP16, neither the N-terminal/CR1 or CR3 portions of E1A inhibited growth in yeast strains in which the genes encoding the Gcn5p, Ngg1p, or Spt7p components of the SAGA transcriptional activation complex were disrupted. However, both portions of E1A inhibited growth in matched wild-type strains (Fig. 3) or in yeast in which the gene encoding the Abc1p component of the ADA transcriptional regulatory complex was disrupted (Fig. 4).
These results demonstrate that growth inhibition mediated by the N-terminal/CR1 and CR3 portions of E1A requires a functional SAGA complex and that growth inhibition is independent of the related ADA complex, which shares the yGcn5p, Ada2p, and Ngg1p proteins in common with SAGA (39). The requirement for the SAGA complex appears specific for growth inhibition by E1A, as growth inhibition by the unrelated adenovirus E1B 55-kDa protein was not affected by disruption of the SAGA complex (data not shown).

The N-terminal/CR1 portion of E1A has been shown to interact directly with a C-terminal portion of pCAF (43). This suggested that E1A might target yGcn5p, the yeast homologue of pCAF. We confirmed that the N-terminal/CR1 portion of E1A bound pCAF using an in vitro GST pull-down approach (Fig. 5) and showed that it also binds to the related yGcn5p protein. Importantly, we observed that CR3 also bound both Gcn5p and pCAF in vitro (Fig. 5), a novel finding. We also found that expression of the E1A binding portion of pCAF restored growth to yeast expressing either the N-terminal/CR1 or CR3 portions of E1A. Importantly, the ability of this portion of pCAF to restore growth to yeast expressing E1A was independent of its intrinsic HAT activity (Fig. 6). This observation suggests that the ability of pCAF to suppress growth inhibition results from its ability to interact directly with either the N-terminal/CR1 or CR3 portions of E1A. Importantly, the ability of this portion of pCAF to restore growth to yeast expressing E1A was independent of its intrinsic HAT activity (Fig. 6). This observation suggests that the ability of pCAF to suppress growth inhibition results from its ability to interact directly with either the N-terminal/CR1 or CR3 portions of E1A. Thus, expression of pCAF may compete with yGcn5p for interaction with E1A, effectively sequestering it from endogenous yGcn5p. Taken together, these results clearly show that two independent portions of E1A bind to yGcn5p and pCAF. As a consequence of this interaction, either the N-terminal/CR1 or CR3 portions of E1A inhibited the acetyltransferase activity of pCAF (Fig. 7). Although we did not detect any interaction of the CR2 portion of E1A with pCAF, this region also reduced the HAT activity of pCAF in vitro. This reduction was reproducible but not as pronounced as that observed with the N-terminal/CR1 or CR3 portions of E1A. A similar CR2-dependent reduction in the in vitro HAT activity of p300 has been reported (44), despite the fact that this region is not involved in p300 interaction, suggesting that it may have some general effect on this assay.

Using small portions of E1A, we determined that the N-terminal 29 residues of E1A were sufficient for interaction with...
Gcn5p and pCAF (Fig. 8B). A previous report indicated that a deletion spanning residues 55–60 reduces, but does not ablate, the association of E1A with pCAF (43). Reduced binding may indicate that the deleted region stabilizes the interaction with pCAF, which is supported by the observation that deletion of residues 38–67 reduces the association of E1A with HAT activity in yeast (40). Importantly, residues 1–29 were sufficient for growth inhibition (Fig. 9B), showing that a direct interaction between this portion of E1A and yGcn5p is sufficient to inhibit growth. However, residues 1–29 were not able to activate transcription, suggesting that whereas binding to yGcn5p may be necessary for transcriptional activation, it is not sufficient on its own. Thus, the recruitment of the yGcn5p or pCAF acetyltransferases must function in cooperation with additional E1A interacting factors to activate transcription.

Intriguingly, residues 30–69 of E1A were also sufficient for growth inhibition, although they were not able to bind yGcn5p or pCAF (Fig. 8B) or activate transcription (Fig. 9C). Given that disruption of multiple components of the SAGA complex relieves growth inhibition by the entire N-terminal/CR1 por-
tion of E1A, it seems likely that residues 30–69 of E1A are targeting a second distinct component of the SAGA complex. This is further supported by our observation that disruption of GCC5 blocks growth inhibition by either residues 1–29 or 30–69 of E1A (Fig. 9B). Interestingly, an N-terminal portion of E1A spanning residues 12–54 interacts with TRRAP (45), the mammalian homologue of the Tra1p component of the SAGA complex, and the 243-amino acid E1A protein coprecipitates with fragments of Tra1p expressed as GST fusion from yeast extracts (33). These results suggest that residues 30–69 of E1A may inhibit growth by targeting Tra1p.

In conclusion, our results demonstrate that two separate domains of E1A that function as transcriptional activators in mammalian cells retain this function in S. cerevisiae, underscoring the conservation of basic mechanisms of transcriptional regulation between yeast and higher eukaryotes. We have also shown that three independent domains of E1A have evolved to target the SAGA complex in vivo, highlighting the important role of this complex in gene regulation. This provides a new genetic system to further elucidate mechanisms by which E1A and the SAGA complex regulate transcription and growth.

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