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VIROLOGY
Virology 316 (2003) 75–83
www.elsevier.com/locate/yviro

The E1A proteins of all six human adenovirus subgroups target the p300/CBP acetyltransferases and the SAGA transcriptional regulatory complex

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Received 15 April 2003; returned to author for revision 30 May 2003; accepted 4 August 2003

Abstract

The N-terminal/conserved region 1 (CR1) portion of the human adenovirus (Ad) 5 E1A protein was previously shown to inhibit growth in the simple eukaryote *Saccharomyces cerevisiae.* We now demonstrate that the corresponding regions of the E1A proteins of Ad3,-4, -9,-12, and -40, which represent the remaining five Ad subgroups, also inhibit yeast growth. These results suggest that the E1A proteins of all six human Ad subgroups share a common cellular target(s) conserved in yeast. Growth inhibition induced by either full-length or the N-terminal/CR1 portion of Ad5 E1A was relieved by coexpression of the E1A binding portions of the mammalian p300, CBP, and pCAF acetyltransferases. Similarly, growth inhibition by the N-terminal/CR1 portions of the other Ad E1A proteins was suppressed by expression of the same regions of CBP or pCAF known to bind Ad5 E1A. The physical interaction of each of the different Ad E1A proteins with CBP, p300, and pCAF was confirmed in vitro. Furthermore, deletion of the gene encoding yGcn5, the yeast homolog of pCAF and a subunit of the SAGA transcriptional regulatory complex, restored growth in yeast expressing each of the different Ad E1A proteins. This indicates that the SAGA complex is a conserved target of all Ad E1A proteins. Our results demonstrate for the first time that the p300, CBP, and pCAF acetyltransferases are common targets for the E1A proteins of all six human Ad subgroups, highlighting the importance of these interactions for E1A function.
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Introduction

The proteins encoded by the human adenovirus (Ad) type 5 early region 1A (*E1A*) gene function as potent regulators of cell growth and gene expression (Bayley and Mymryk, 1994; Gallimore and Turnell, 2001; Frisch and Mymryk, 2002). Despite the intensive study of Ad5 E1A, relatively little is known about the E1A proteins of the other five human Ad subgroups. Comparison of the sequences of E1A from multiple Ad serotypes has identified four discrete regions with a high degree of sequence conservation that have been designated conserved regions (CR) 1, 2, 3, and 4 (Avvakumov et al., 2002). CR1 spans residues 42–72 of Ad5 E1A. In the currently known E1A protein sequences, CR1 is the most highly conserved, with an average identity of 59% with respect to Ad5 E1A, whereas the N-terminal portions share about 26% average identity with Ad5 E1A (Avvakumov et al., 2002).

The N-terminal/CR1 portion of Ad5 E1A is known to interact with several transcriptional coactivators, including the acetyltransferases pCAF (Reid et al., 1998), CREB Binding Protein (CBP), and related family member p300 (Eckner et al., 1994; Arany et al., 1995; Lundblad et al., 1995). Interaction with CBP and p300 appears to be required for E1A-induced progression from the G_1 phase into

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doi:10.1016/j.virol.2003.08.005

the S phase of the cell division cycle (Howe et al., 1990; Wang et al., 1991), E1A-mediated repression of enhancer-activated gene transcription (Jelsma et al., 1989; Rochette-Egly et al., 1990) and transformation in cooperation with activated ras (reviewed in Bayley and Mymryk, 1994). In addition to Ad5 E1A (subgroup C), Ad12 E1A (subgroup A) is known to bind p300 and CBP (Wang et al., 1993; Dorsman et al., 1997). It is not known if Ad12 E1A interacts with pCAF or whether any of the E1A proteins of the other Ad subgroups can interact with p300, CBP, or pCAF.

Genetic studies in the budding yeast *Saccharomyces cerevisiae* have demonstrated that one or more cellular targets of the N-terminal/CR1 portion of Ad5 E1A are conserved in this simple eukaryote. When introduced into many haploid strains of *S. cerevisiae,* this fragment of Ad5 E1A inhibits growth, leading to an accumulation of cells in the G_1 phase of the cell cycle (Miller et al., 1995). Growth inhibition by this fragment of E1A requires the yeast Spt-Ada-Gcn5-acetyltransferase (SAGA) complex, but is not directly related to transcriptional activation by E1A (Kulesza et al., 2002; Shuen et al., 2002). Indeed, the N-terminal/CR1 portion of E1A can physically interact with at least two components of the SAGA complex in yeast: yGcn5, the yeast homolog of pCAF (Shuen et al., 2002), and Tra1, the yeast homolog of TRRAP (Kulesza et al., 2002).

In this study, we show for the first time that the SAGA complex in yeast and the mammalian p300, CBP, and pCAF acetyltransferases are conserved targets for the E1A proteins of all six human subgroups. These observations highlight the importance of these interactions for E1A function.

Results and discussion

Ad5 E1A-mediated inhibition of yeast growth can be suppressed by portions of CBP or p300 known to interact with Ad5 E1A

Expression of the Ad5 E1A protein suppresses growth in many haploid strains of *S. cerevisiae* (Handa et al., 1987; Wada et al., 1990; Miller et al., 1995). Inhibition of growth is often determined visually by comparing the size of yeast colonies expressing E1A with those of yeast transformed with a control expression vector. The N-terminal/CR1 and CR3 regions of Ad5 E1A can independently mediate growth inhibition in yeast (Shuen et al., 2002). It has been shown previously that expression of mammalian receptor for activated C kinase 1 (RACK1) can restore growth to yeast expressing Ad5 E1A, presumably via a direct physical interaction with the growth inhibitory N-terminal/CR1 domain (Sang et al., 2001). We reasoned that other mammalian proteins known to interact with this region of Ad5 E1A might similarly function to suppress growth inhibition. The coexpressed mammalian protein might compete with an endogenous yeast regulatory protein for interaction with E1A, thus freeing the yeast cellular protein to perform its normal function. To test this, we constructed a panel of vectors expressing portions of CBP (Fig. 1A), a well characterized mammalian protein targeted by Ad5 E1A. Yeast expressing full-length Ad5 E1A and a fragment of CBP spanning residues 1679–1891 grew rapidly as compared to yeast expressing only Ad5 E1A or Ad5 E1A and fragments spanning the remaining portions of CBP (Fig. 1B). Importantly, the region of CBP encompassed by residues 1679–1891 has been shown to physically interact with Ad5 E1A (Arany et al., 1995). Western blot analysis determined that restoration of growth was not related to decrease in the level of expression of the Ad5 E1A protein in response to CBP expression (Fig. 1C). The corresponding portion of the CBP related protein p300, which is also known to bind Ad5 E1A (Eckner et al., 1994), similarly suppressed growth inhibition by Ad5 E1A (Fig. 1D). Neither the CBP nor p300 fragment that restored growth to yeast expressing Ad5 E1A had any effect on growth inhibition by the unrelated Ad2 E1B 55-kDa protein, suggesting that their effects on growth are specific for yeast expressing E1A (data not shown).

Unfortunately, E1A mutants containing small deletions that impair p300 or CBP binding in mammalian cells do not efficiently suppress yeast growth (Miller et al., 1995). For this reason they could not be tested to further confirm that a direct interaction with CBP is required for restoration of yeast growth. However, our observation that only fragments of CBP or p300 known to bind Ad5 E1A can specifically suppress yeast growth inhibition mediated by Ad5 E1A suggests that growth restoration results from a physical interaction between E1A and CBP or p300. This raises the possibility that suppression of growth inhibition may be used under appropriate circumstances to determine if a physical interaction occurs.

The N-terminal/CR1 portions of the Ad3,-4,-5,-9,-12, and -40 E1A proteins can inhibit yeast growth and this can be suppressed by CBP

Expression of the N-terminal/CR1 portion of Ad5 E1A fused to the Gal4p DNA binding domain is sufficient to inhibit yeast growth (Miller et al., 1995; Shuen et al., 2002). We constructed vectors expressing the N-terminal/CR1 domain of one representative E1A protein from each of the six Ad subgroups and tested them for their ability to inhibit yeast growth (Fig. 2). Like Ad5 E1A (subgroup C), the N-terminal/CR1 portions of Ad9 (subgroup D), Ad4 (subgroup E), Ad3 (subgroup B), Ad12 (subgroup A), and Ad40 (subgroup F) inhibited yeast growth. The strongest growth inhibition, reflected by the smallest colony size, was reproducibly observed with the N-terminal/CR1 portion of Ad40 E1A, whose sequence is the least similar to that of Ad5 E1A. These results suggest that the N-terminal/CR1 portions of the E1A proteins of all six human Ad subgroups share a common cellular target(s) conserved in yeast.

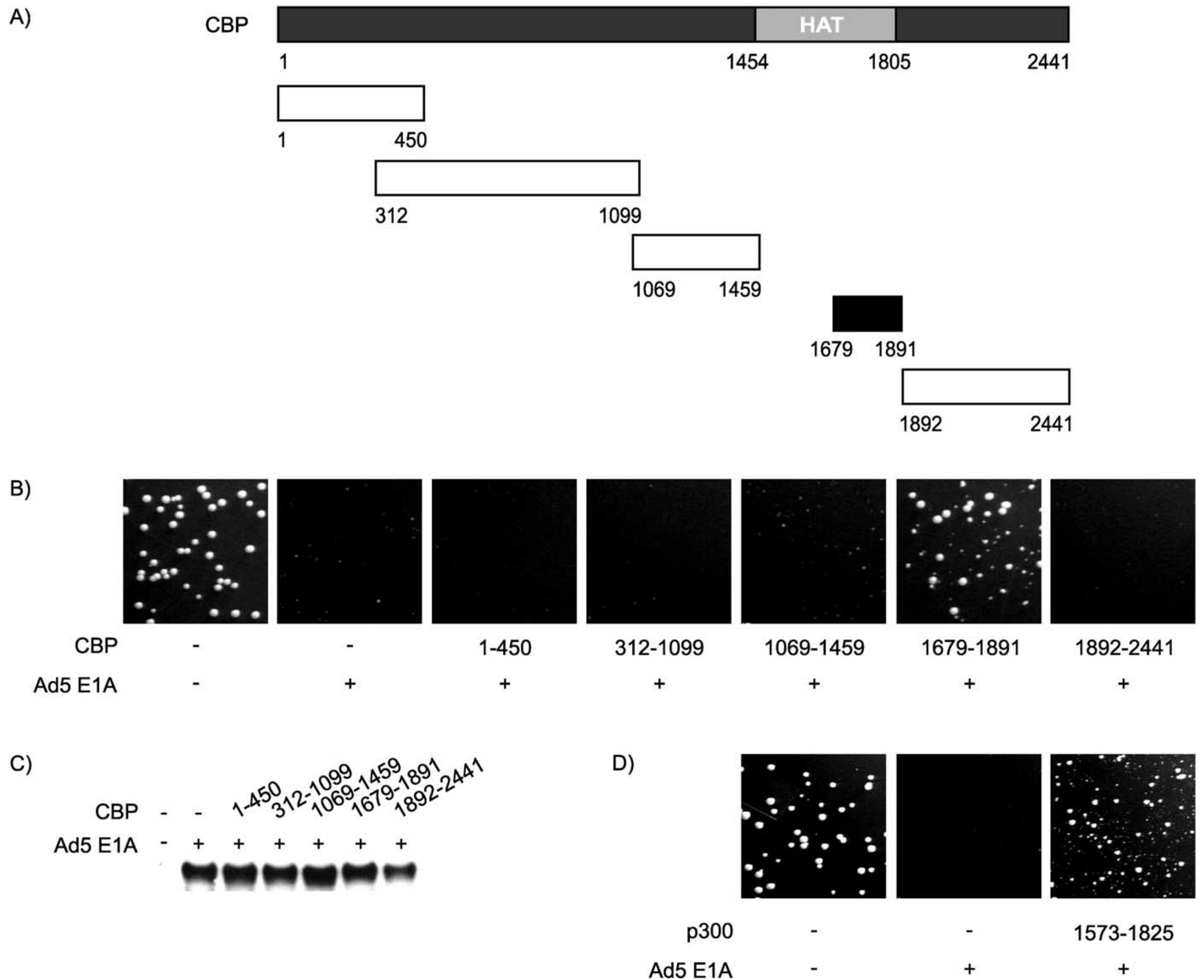

Fig. 1. Effect of CBP and p300 expression on Ad5 E1A-mediated growth inhibition. (A) Map of CBP and the regions that were coexpressed with E1A. The region spanning residues 1679–1891 has previously been shown to bind Ad5 E1A. (B) Yeast strain w303-1a was transformed with a vector expressing the full-length Ad5 289 residue E1A protein and vectors expressing the indicated portions of CBP. Transformed yeast were grown for approximately 72 h at 30°C and photographed. (C) Western blot analysis of the level of E1A protein expression in yeast coexpressing CBP fragments. Cell extracts were prepared as described under Materials and methods. (D) Yeast were transformed as described in B, except that a vector expressing the portion of p300 spanning residues 1573–1825 was used in place of a vector expressing a portion of CBP.

The results presented in Fig. 1 show that expression of the E1A binding domain of CBP can restore growth to yeast expressing full-length Ad5 E1A, likely as a result of a direct interaction between these two proteins. We tested the effect of coexpressing this fragment of CBP on growth inhibition mediated by the N-terminal/CR1 portions of each of the different Ad E1A proteins (Fig. 2). As expected, coexpression of residues 1679–1891 of CBP efficiently suppressed growth inhibition by the N-terminal/CR1 portions of Ad5 and Ad12 E1A, which are known to interact with this region of CBP (Arany et al., 1995; O'Connor et al., 1999; Lipinski et al., 1999). Interestingly, this fragment of CBP also suppressed growth inhibition mediated by the N-terminal/CR1 portions of the other E1A proteins (Fig. 2). This suggests that the E1A proteins of each of the six human Ad subgroups physically interact with this portion of CBP.

The N-terminal/CR1 portions of Ad3,-4,-5,-9,-12, and -40 interact with CBP and p300 in vitro

The ability of the various Ad E1A proteins to interact with CBP and p300 was further assessed in vitro. We expressed the N-terminal/CR1 portions of the Ad3,-4,-5,-9,-12 and -40 E1A proteins as fusions to glutathione *S*-transferase (GST) and tested their ability to interact with in vitro transcribed and translated fragments of CBP or p300 (Fig. 3). An interaction was observed between a fragment of CBP spanning residues 1679–1891 and the N-terminal/CR1

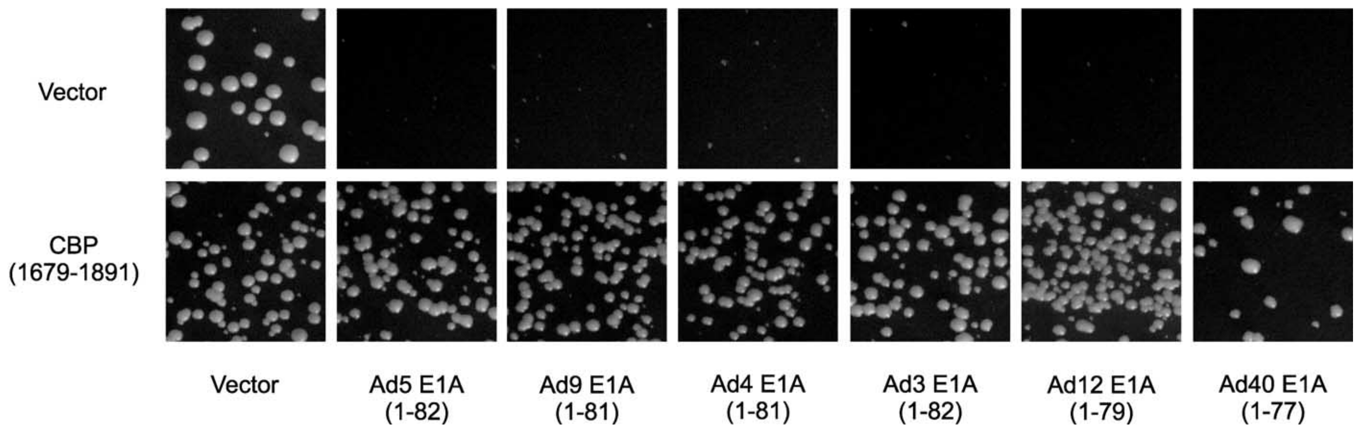

Fig. 2. Effect of CBP expression on growth inhibition mediated by the N-terminal/CR1 portions of representative E1A proteins from each of the six human adenovirus subgroups. Yeast strain w303-1a was transformed with vectors expressing the N-terminal/CR1 portions of the E1A proteins of Ad3,-4,-5,-9,-12 and -40 with or without a vector expressing a fragment of CBP spanning residues 1679–1891. Transformed yeast were grown for approximately 72 h at 30°C and photographed. The amino-acid residues present in each test protein fragment are indicated in brackets.

portions of all the E1A proteins, but not with GST (Fig. 3A). A similar result was obtained using a fragment of p300 spanning residues 1573–1825 (Fig. 3B). These in vitro results confirm the in vivo results shown in Fig. 2 and demonstrate that CBP and p300 are indeed common targets of each of the different E1A proteins we tested.

Previous characterization of the E1A-CBP interaction defined a transcriptional adapter motif (TRAM) located between residues 1811 and 1822 in CBP that is targeted not only by Ad5 and Ad12 E1A, but also by p53 and E2F-1 (O'Connor et al., 1999). Each of these proteins contains the consensus sequence FXE/DXXXL (where X is any amino acid) that was suggested to represent a TRAM binding motif. With the exception of Ad40 E1A, all the Ad E1A proteins tested contain an exact TRAM binding sequence,

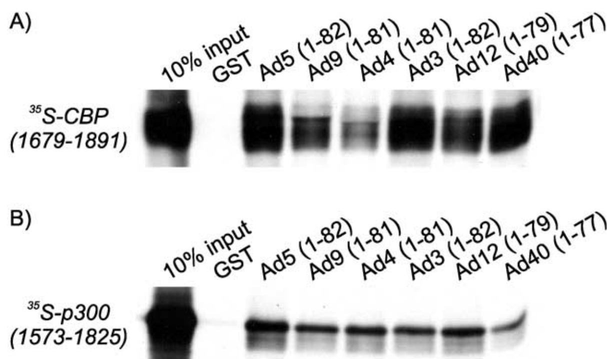

Fig. 3. Interaction of CBP with representative E1A proteins from each of the six human adenovirus subgroups. The N-terminal/CR1 fragments of Ad3,-4,-5,-9,-12 and -40 were prepared as recombinant fusions to GST and used in GST- pull-down assays with an in vitro transcribed and translated ^{35}S-labeled fragment of CBP spanning residues 1679–1891 as described under Materials and methods. Proteins were recovered with glutathione–Sepharose and analyzed by SDS-PAGE and radiography. One-tenth of the input of ^{35}S-labeled proteins used in the binding reactions is shown for comparison. The amino-acid residues present in each test protein fragment are indicated in brackets.

which is located at the extreme carboxyl edge of CR1. In Ad40 E1A, which is the most divergent of the E1A proteins with respect to Ad5 E1A that we tested, the closely related sequence FPERL-L (where the hyphen represents a gap) is presumably able to mediate interaction with the TRAM motif of CBP and the related p300 protein. Interestingly, Ad41 E1A, the only other member of subgroup F, contains the sequence FPNWM-L, which diverges even further from the consensus TRAM binding sequence.

Ad5 E1A-mediated inhibition of yeast growth can be suppressed by the region of pCAF known to interact with Ad5 E1A

Like CBP and p300, pCAF is known to interact with the N-terminal/CR1 portion of Ad5 E1A (Reid et al., 1998), raising the possibility that it might similarly function to suppress growth inhibition mediated by Ad5 E1A. We tested this by cotransforming yeast with vectors expressing full-length Ad5 E1A and vectors expressing either the amino (residues 1–352) or the carboxyl (residues 310–832) portions of pCAF. Yeast coexpressing full-length Ad5 E1A and residues 1–352 of pCAF continued to grow slowly, whereas expression of a fragment of pCAF spanning residues 310 to 832 restored growth to yeast expressing full-length Ad5 E1A (Fig. 4B). Importantly, residues 310–832 have been previously shown to interact with Ad5 E1A (Reid et al., 1998), indicating that the portion of pCAF that interacts with the growth inhibitory N-terminal/CR1 portion of E1A can function like CBP or p300 to suppress E1A function in yeast. We tested additional C-terminal truncations of pCAF (Fig. 4A) for suppression of Ad5 E1A-mediated growth inhibition. Suppression of growth inhibition became less efficient with progressive truncations, until eventually no detectable suppression was observed for a fragment of pCAF spanning residues 310–525 (Fig. 4B). These results

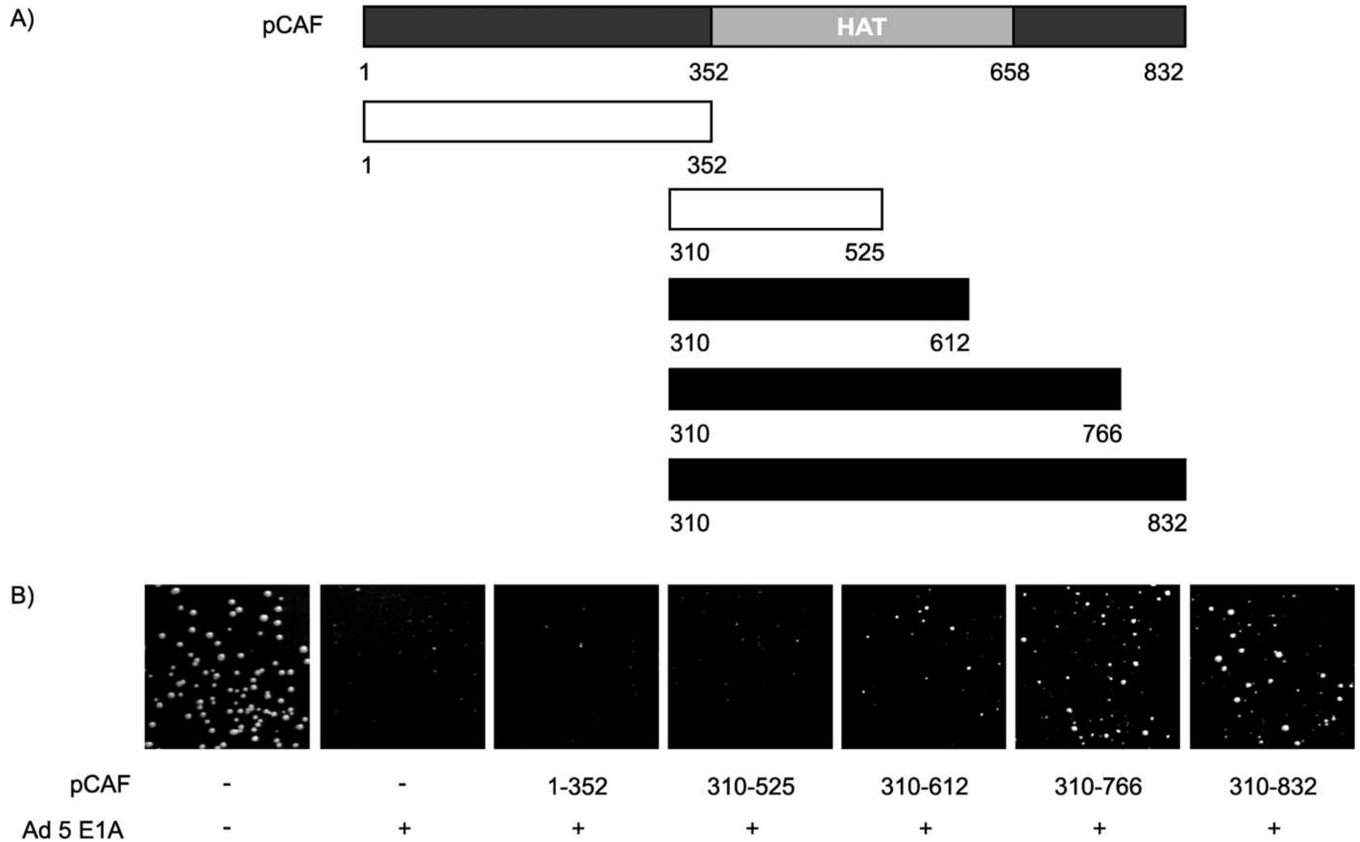

Fig. 4. Effect of pCAF expression on Ad5 E1A-mediated growth inhibition. (A) Map of pCAF and the regions that were coexpressed with Ad5 E1A. The region spanning residues 352–695 has previously been reported to bind to Ad5 E1A. (B) Yeast strain w303-1a was transformed with a vector expressing the full-length Ad5 289 residue E1A protein and vectors expressing the indicated portions of pCAF. Transformed yeast were grown for approximately 72 h at 30°C and photographed.

indicate that Ad5 E1A binds to the region of pCAF encompassed within residues 310–612.

Growth inhibition by the N-terminal/CR1 portions of the Ad3,-4,-5,-9,-12, and -40 E1A proteins can be suppressed by pCAF

The results presented in Fig. 4B and our previous demonstration that expression of a fragment of pCAF (residues 310–832) can restore growth to yeast expressing the N-terminal/CR1 portion of Ad5 E1A (Shuen et al., 2002) prompted us to determine the effect of this fragment of pCAF on growth inhibition mediated by each of the other Ad E1A proteins. Coexpression of this portion of pCAF efficiently suppressed growth inhibition by each of the N-terminal/CR1 portions of the other Ad E1A N-terminal/CR1 fragments, with the exception of Ad40 E1A (Fig. 5A). However, pCAF was able to weakly suppress growth inhibition mediated by the Ad40 E1A fragment after longer periods of growth (Fig. 5B). This result suggests that pCAF can interact with the N-terminal/CR1 regions of the E1A proteins of six Ad subgroups, although only weakly with Ad 40 E1A.

The N-terminal/CR1 domains of Ad3,-4,-5,-9,-12, and -40 interact with pCAF and yGcn5 in vitro

The ability of the various E1A proteins to interact with pCAF and its yeast homolog yGcn5 was further assessed in vitro. We expressed the N-terminal/CR1 portions of the Ad3,-4, -5,-9,-12 and -40 E1A proteins as fusions to glutathione *S*-transferase (GST) and tested their ability to interact with in vitro transcribed and translated fragments of pCAF or yGcn5 (Fig. 6). An interaction was observed between the C-terminal fragment of pCAF spanning residues 310–832 (Fig. 6A) or full-length yGcn5 (Fig. 6B) and the N-terminal/CR1 portions of all the E1A proteins, but not with GST. These in vitro binding results confirm that pCAF and yGcn5 can bind all the different Ad E1A proteins, although with a range of affinities. The in vitro interaction of Ad40 E1A with pCAF or yGcn5 was reproducibly the weakest of all the Ad E1A proteins tested, supporting our observation that pCAF could function as only a weak suppressor of growth inhibition mediated by the N-terminal/CR1 portion of Ad40 E1A (Fig. 5). Interestingly, the interaction with pCAF maps within the first 29 amino acids of Ad5 E1A (Shuen et al., 2002) that are only weakly conserved among the different

Fig. 5. Effect of pCAF expression on growth inhibition mediated by the N-terminal/CR1 portions of representative E1A proteins from each of the six human adenovirus subgroups. Yeast strain w303-1a was transformed with vectors expressing the N-terminal/CR1 portions of the E1A proteins of Ad3,-4,-5,-9,-12 and -40 with or without a vector expressing a fragment of pCAF spanning residues 310–832. Transformed yeast were grown for approximately 72 h at 30°C and photographed. (B) Yeast strain w303-1a was transformed with a vector expressing the N-terminal/CR1 portion of Ad40 E1A with or without a vector expressing the C-terminal portion of pCAF. Yeast were photographed after 7 days of growth at 30°C.

Ad E1A proteins (Avvakumov et al., 2002). Further study will be required to refine the pCAF interaction region in E1A and additional comparisons between the different Ad E1A proteins should aid these investigations.

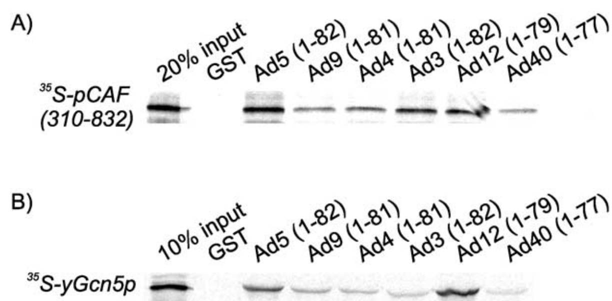

Fig. 6. Interaction of pCAF and yGCN5 with representative E1A proteins from each of the six human adenovirus subgroups. The N-terminal/CR1 fragments of Ad3,-4,-5,-9,-12 and -40 were prepared as recombinant fusions to GST and tested for interaction with an ^{35}S-labeled fragment of pCAF spanning residues 310–832 (A) or full-length yGcn5p (B) as described in Fig. 3 and under Materials and methods. The amino-acid residues present in each test protein fragment are indicated in brackets.

Our in vitro results confirm that pCAF and its homolog yGcn5 are indeed common targets of each of the different E1A proteins we tested. However, the ability of the E1A proteins to interact with yGcn5 is unlikely to be the sole determinant of growth inhibition, as Ad40 E1A displayed the weakest binding to yGcn5 (Fig. 6B), but was reproducibly the most effective inhibitor of yeast growth (Figs. 2 and 5A). In addition to yGcn5, Ad5 E1A has been shown to bind the Tra1 component of the yeast SAGA complex (Kulesza et al., 2002). This interaction likely requires the N-terminal/CR1 portion of Ad5 E1A, as residues 12–54 are required for the interaction of E1A with TRAPP, the mammalian homolog of Tra1 (Deleu et al., 2001). Further studies will be necessary to determine if each of the different Ad E1A proteins also bind Tra1 and TRAPP.

Growth inhibition by the N-terminal/CR1 portions of the E1A proteins of all six Ad subgroups requires the SAGA transcriptional activation complex

The data presented in Fig. 6B show that each of the Ad E1A proteins is able to interact with the yGcn5 component

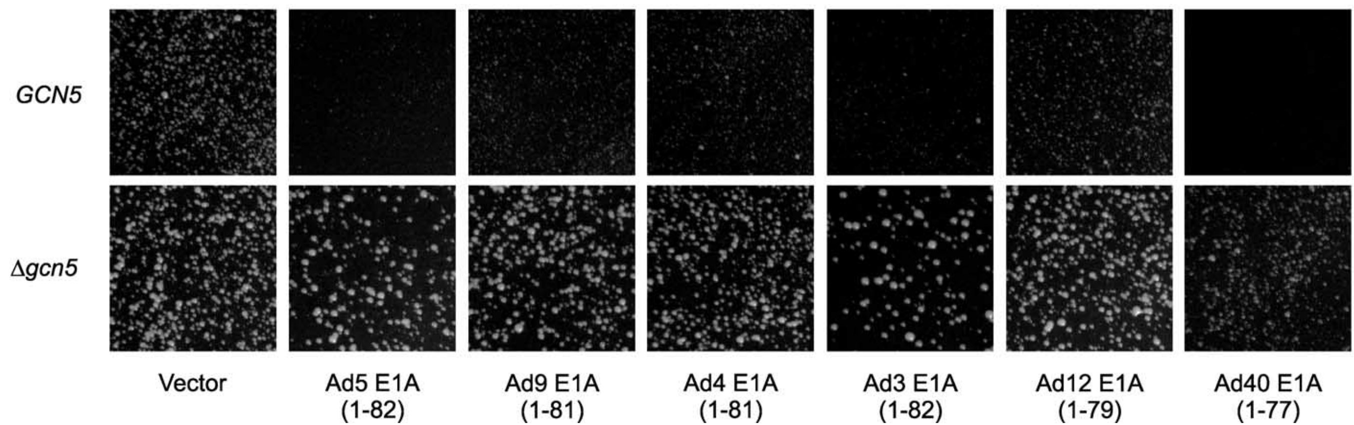

Fig. 7. Requirement for the SAGA transcriptional activation complex for growth inhibition by representative E1A proteins from each of the six human adenovirus subgroups. Wild-type yeast strain FY86 (top) and otherwise isogenic yeast strain FY1370 in which the gene encoding yGcn5 was disrupted (Δ*gcn5;* bottom) were transformed with vectors expressing the indicated N-terminal/CR1 portions of the E1A proteins of Ad3,-4,-5,-9,-12 or -40. Transformed cells were allowed to grow approximately 48 h at 30°C and photographed.

of the SAGA complex in vitro. We and others have previously shown that growth inhibition mediated by the N-terminal/CR1 portion of Ad5 E1A requires a functional SAGA yeast transcriptional activation complex (Kulesza et al., 2002; Shuen et al., 2002). To determine if the SAGA complex is required for growth inhibition mediated by each of the N-terminal/CR1 portions of the other Ad E1A proteins, we analyzed their ability to inhibit growth in a yeast strain containing a disruption of *GCN5* (Δ*gcn5*). As expected, the N-terminal/CR1 portion of Ad5 E1A was unable to inhibit growth in the Δ*gcn5* strain. Interestingly, none of the other Ad E1A proteins inhibited growth in a Δ*gcn5* strain although each suppressed growth in an isogenic wild-type strain (Fig. 7). These in vivo results demonstrate that the SAGA complex is indeed a target of the E1A proteins of the six Ad subgroups.

The exact mechanism by which the E1A proteins inhibit yeast growth in a SAGA-dependent fashion are not known. Clearly, growth inhibition does not simply result from blocking the ability of the SAGA complex to modulate transcription as it is dispensable for yeast viability. However, growth inhibition by E1A requires a functional complex, as disruption of any of the nonessential components abrogates growth inhibition by Ad5 E1A (Kulesza et al., 2002). One possible mechanism could be that E1A is actively directing the enzymatic activity of this complex to inappropriate targets. Alternatively, the interaction of E1A with SAGA may sequester the Tra1 component, which is essential for yeast viability, preventing it from carrying out its critical functions. This second explanation is less likely as E1A can bind Tra1 directly, but the observed effects of E1A on growth require the entire intact SAGA complex (Kulesza et al., 2002). Perhaps additional interactions between E1A and other SAGA components such as yGcn5 effectively stabilize the interaction leading to an effective sequestration of the essential Tra1 protein.

As to the reasons why the various E1A proteins target CBP, p300, and pCAF in mammalian cells, one potential explanation is that they do so simply to disrupt the normal functions of these acetyltransferases. These proteins typically function as coactivators for numerous tissue-specific transcription factors, and disruption of their activities by Ad5 E1A represses most differentiation-specific gene expression while maintaining housekeeping and epithelial-specific gene expression. This is thought to convert the infected cell into a default phenotype more suitable for the viral life cycle (Frisch and Mymryk, 2002). Whether similar alterations in transcription are achieved by the E1A proteins of other adenovirus serotypes remains to be determined, but seems likely given their common abilities to target CBP, p300, and pCAF.

It is also possible that instead of, or in addition to, disrupting the ability of CBP, p300, or pCAF to function as coactivators of cellular transcription factors, the E1A proteins may retarget their enzymatic activities to the regulatory regions of other genes. This is particularly evident for Ad12 E1A, which strongly activates the viral E2 transcription unit in an N-terminal/CR1-dependent fashion (Brockmann and Esche, 2003). Ad12 E1A recruits CBP-dependent acetyltransferase activity to the E2 promoter by simultaneously interacting with the cAMP-response element binding protein (CREB) and CBP (Fax et al., 2000b). In addition, Ad12 E1A binding stimulates the acetyltransferase activity of CBP, leading to a further enhancement of transcription from this viral gene (Fax et al., 2000a).

Importantly, Ad5 E1A has also been shown to recruit p300 to acetylate pRb, hindering the phosphorylation-dependent regulation of pRb activity by cyclin-dependent kinases (Chan et al., 2001). Interestingly, all of the human or simian adenovirus E1A proteins that have been sequenced to date contain a consensus pRb binding motif (Avvakumov et al., 2002) and could similarly function to enhance acetylation of pRb because we show here that they also bind p300 and CBP. Additional proteins associated with E1A

could similarly be subjected to covalent modification in this fashion, but this remains to been determined. Binding to CBP, p300, and pCAF will also likely contribute to the loss of cell cycle control and transformation by all the E1A proteins, as mutants of Ad5 E1A that fail to associate with these proteins are unable to oncogenically transform rodent cells (Bayley and Mymryk, 1994; Gallimore and Turnell, 2001; Frisch and Mymryk, 2002).

In conclusion, we demonstrate that the E1A proteins of each of the six human adenovirus subgroups have the remarkably conserved capacity to bind to the p300, CBP, and pCAF acetyltransferases in vivo and in vitro despite considerable sequence divergence. In addition, each of the E1A proteins can functionally interact with the SAGA transcriptional regulatory complex in vivo. Our observation that these cellular targets are common to all the different E1A proteins highlights the importance of these interactions for E1A's effects on the infected or transformed cell and provide a genetic system to further explore their consequences.

Materials and methods

Yeast strains, media, and plasmid construction

Yeast strain w303-1A was provided by Dr. Michael Christman (Boston University, Boston, MA, USA) and used for all the growth-related experiments except where indicated otherwise. FY86 (*wt*) and the otherwise isogenic strain FY1370 that contains a disruption of yGCN5 ($\Delta gcn5$) were provided by Dr. Chris Brandl (University of Western Ontario, London, ON, Canada). Yeast culture media were prepared using standard techniques (Adams et al., 1998). Yeast expression vectors pRS423 (Christianson et al., 1992) and pRS423-13S E1A (Zhang et al., 2001), which expresses the full-length 289 residue Ad5 E1A protein under the control of the GAL1 promoter, were described previously. Yeast expression vectors pAS1U, pAS1U-Ad5 1–82, pGuN, and pGuN-55 have been described previously (Liang et al., 1995; Shuen et al., 2002). The sequences encoding the N-terminal/CR1 portions of the E1A proteins of Ad3,-4,-9,-12 and -40 were PCR amplified with specific oligonucleotide primers from DNA templates provided by Drs. A. Turnell and P. Gallimore (Cancer Research UK Institute for Cancer Studies, University of Birmingham, Birmingham, UK), E. Blair (University of Leeds, Leeds, UK), R. Javier (Baylor College of Medicine, Houston, TX, USA), R. Padmanabhan (Georgetown University, Washington, DC, USA), and J.C. D'Halluin (INSERM U524, Lille Cedex, France) and subcloned as *Eco*RI-*Sal*I fragments into pAS1U and pGEX4T-1 (Amersham Pharmacia Biotech Inc., Baie d'Urfé, Québec, Canada). For experiments in which the Ad E1A proteins were coexpressed with CBP, p300, or pCAF, the coding region for the N-terminal/CR1 portions of Ad3,-4,-5,-9,-12 and -40 were subcloned into the yeast vector pRS426-GAL. This plasmid resembles pAS1U except that it contains the *GAL1* rather than the *ADH1* regulatory region controlling expression of the Gal4 DBD. Derivatives of pJG4-5 (OriGene Technologies Inc., Rockville, MD) expressing fragments of pCAF, CBP, or p300 were generated by PCR or the use of convenient restriction digest sites where possible. Selected portions of pCAF and CBP were subcloned into pCDNA3.1 (Invitrogen Life Technologies, Carlsbad, CA, USA) or pCITE-2a (Novagen, Madison, WI, USA) for in vitro transcription and translation experiments. T7-yGCN5 (obtained from Dr. P. Walfish, Mount Sinai Hospital, Toronto, ON, Canada) was used for in vitro transcription and translation of full-length yGcn5.

Growth suppression assay

Yeast transformations were performed using a modified lithium acetate procedure as described previously (Gietz et al., 1995). Cells were plated onto appropriate synthetic complete (SC) omission plates and incubated at 30°C. For assays of growth inhibition by E1A or E1B, plates were photographed approximately 48 or 72 h posttransformation using a Foto/Eclipse Fotodyne gel doc system (Fotodyne Inc., Hartland, WI, USA) unless otherwise noted in the figure legends.

Yeast cell extracts and Western blot analysis

Yeast colonies were picked from the SC omission plates and used to inoculate 5 ml of glucose-supplemented SC liquid media. Cultures were allowed to grow at 30°C until they reached an OD_{600} of 1.0. Yeast cells were collected by centrifugation, washed, resuspended in 5 ml of galactose-supplemented SC media, and allowed to grow for an additional 4 h at 30°C. Cultures were then cooled to 4°C, harvested by centrifugation, and washed with 1 ml of cold extraction buffer [200 mM Tris (pH 8.0), 150 mM ammonium sulfate, 10% glycerol, 1 mM EDTA, 2 mM DTT, 1X complete protease inhibitor cocktail (Roche Diagnostics, Laval, Québec, Canada)]. Cell pellets were resuspended in 100 μl of extraction buffer and vortexed for 1 min in the presence of 200 μl of glass beads followed by incubation on ice for 1 min. This was repeated 5 times, after which supernatants were collected by centrifugation for 10 min at 4°C. Twenty micrograms of total protein from each sample were resolved on 10% sodium dodecyl sulfate–polyacrylamide gels (SDS–PAGE), and E1A proteins were detected by Western blot analysis with the M73 antibody (Harlow et al., 1985).

Glutathione S-transferase (GST) pull-downs

GST-E1A fusion proteins were expressed in *Escherichia coli* strain BL21 Codon Plus and purified using the protocol provided by affinity resin manufacturer (Amersham Pharmacia Biotech Inc., Baie d'Urfé, Québec, Canada). In vitro translated ^{35}S-labeled methionine full-length yGcn5p, the carboxyl portion of pCAF spanning amino acids 310–832,

or a fragment of CBP spanning amino acids 1679–1891 were prepared using the TNT T7 coupled transcription/translation system (Promega Corp., Madison, WI, USA) according to the supplied protocol. GST pull-downs were performed as described previously (Shuen et al., 2002).

Acknowledgments

We thank Jay Loftus for technical assistance. We thank Drs. M.M. Smith, F. Winston, C. Brandl, A. Turnell, P. Gallimore, E. Blair, R. Javier, R. Padmanabhan, P. Walfish, S. Berger, A. Tartakoff, and J.-C. D'Halluin for their generous gifts of plasmids and strains. This work was supported by a grant from the Canadian Institutes of Health Research (MOP 14631) awarded to J.S.M. J.S.M. is a Scholar of the Canadian Institutes of Health Research. J.T. holds a Pharmaceutical Manufacturer's Association of Canada Scholarship. M.S. holds a joint Canadian Institutes of Health Research/London Regional Cancer Centre studentship. N.A. holds a National Science and Engineering Research Council of Canada Studentship.

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