MOLECULAR AND CELLULAR BIOLOGY, Oct. 1996, p. 5737–5743 Vol. 16, No. 10
0270-7306/96/$04.00+0

Adenovirus E1A Specifically Blocks SWI/SNF-Dependent Transcriptional Activation

MARY E. MILLER,[1] BRADLEY R. CAIRNS,[2] RANDY S. LEVINSON,[3] KEITH R. YAMAMOTO,[3] DANIEL A. ENGEL,[1] AND M. MITCHELL SMITH[1]*

Department of Microbiology and University of Virginia Cancer Center, School of Medicine, University of Virginia Charlottesville, Virginia 22908[1]; Department of Cell Biology, Stanford University School of Medicine, Stanford, California 94305[2]; and Department of Cellular and Molecular Pharmacology, University of California, San Francisco, California 94143-0450[3]

Received 9 May 1996/Returned for modification 20 June 1996/Accepted 11 July 1996

Expression of the adenovirus $E1A_{243}$ oncoprotein in *Saccharomyces cerevisiae* produces a slow-growth phenotype with accumulation of cells in the G_1 phase of the cell cycle. This effect is due to the N-terminal and CR1 domains of $E1A_{243}$, which in rodent cells are involved in triggering cellular transformation and also in binding to the cellular transcriptional coactivator p300. A genetic screen was undertaken to identify genes required for the function of $E1A_{243}$ in *S. cerevisiae*. This screen identified *SNF12*, a gene encoding the 73-kDa subunit of the SWI/SNF transcriptional regulatory complex. Mutation of genes encoding known members of the SWI/SNF complex also led to loss of E1A function, suggesting that the SWI/SNF complex is a target of $E1A_{243}$. Moreover, expression of E1A in wild-type cells specifically blocked transcriptional activation of the *INO1* and *SUC2* genes, whose activation pathways are distinct but have a common requirement for the SWI/SNF complex. These data demonstrate a specific functional interaction between E1A and the SWI/SNF complex and suggest that a similar interaction takes place in rodent and human cells.

The adenovirus E1A gene encodes potent oncoproteins that function as regulators of cellular and viral transcription. In the context of a normal adenovirus replicative cycle in permissive human hosts, the E1A proteins perform two main functions. On one hand, they activate the transcription of viral genes whose products play catalytic, regulatory, or structural roles during infection. In addition, they activate or repress the transcription of several cellular genes, some of which govern normal progress through the cell cycle. In doing so, the E1A proteins stimulate cellular DNA synthesis and cell division, thus creating a cellular environment that supports efficient viral replication.

The E1A proteins modify several important cellular processes that govern normal transcriptional regulation and cell cycle control. Transcriptional regulatory proteins targeted by E1A include the retinoblastoma (Rb) tumor suppressor protein and the transcriptional adaptor p300, which, like Rb, is involved in E1A-induced cellular transformation of rodent cells. E1A physically interacts with Rb, p300, and other key regulatory proteins, thereby altering their function and disrupting the normal cellular transcriptional program (4, 13).

We have developed genetic evidence that one or more targets of E1A are conserved between higher eukaryotic cells and the budding yeast *Saccharomyces cerevisiae* (41). Expression of E1A in *S. cerevisiae* results in a pronounced slow-growth phenotype with accumulation of cells in the G_1 phase of the cell cycle (41, 56). We have reported that domains of E1A that are necessary and sufficient for inhibition of yeast cell growth are located within the N-terminal and conserved-region 1 (CR1) domains of the protein (41). These regions are known to be important in the transforming and transcriptional regulatory functions of E1A and are also involved in binding to p300 (4, 13, 14, 57). The N-terminal domain alone (but not CR1) also can interact with the mammalian transcription factors YY1 and Dr1 (31, 38, 51, 62). Therefore, the regions of E1A that induce an abnormal growth phenotype in *S. cerevisiae* are similar or identical to two domains required for cellular transformation and binding to cellular transcription factors.

Additionally, we have observed similar requirements for cyclic AMP (cAMP) signaling in E1A function in yeast and mammalian cells (41). We and others previously identified a link between the function of the N-terminal and CR1 domains of E1A and the cAMP signaling system in mouse and human cells (2, 15, 16, 18, 27, 39). Activation of proto-oncogene c-*fos* transcription by E1A absolutely requires cAMP signaling, and an E1A response element within the c-*fos* transcriptional control region contains a binding site for cAMP-responsive transcription factors, including CREB. In addition, p300 and its close relative the CREB-binding protein can bind to CREB and mediate cAMP-dependent transcriptional activation. Consistent with these observations, we have reported that cAMP signaling is required for the growth-inhibitory activity of E1A in *S. cerevisiae*, since mutations in either *CDC35* (adenylate cyclase) or *CDC25* (GTP-GDP exchange factor for Ras proteins) lead to a complete loss of sensitivity to E1A (41).

The requirements for cAMP signaling and for the N-terminal and CR1 domains for E1A function in *S. cerevisiae* offer a striking parallel to the mammalian system and suggested that a genetic approach would be useful in the study of E1A function. Here, we report the results of a mutational analysis of the function of E1A in *S. cerevisiae*. These studies led to the identification and molecular cloning of a novel member of the yeast SWI/SNF transcriptional regulatory complex, *SNF12*. We demonstrate that disruption of normal growth by the N-terminal and CR1 domains of E1A in *S. cerevisiae* requires the function of the SWI/SNF complex. Moreover, expression of E1A in wild-type cells leads to a specific loss of SWI/SNF-dependent transcription. These results suggest that the SWI/

* Corresponding author. Mailing address: Department of Microbiology and University of Virginia Cancer Center, Box 441 Jordan Bldg., School of Medicine, University of Virginia, Charlottesville, VA 22908. Phone: (804) 924-2669. Fax: (804) 982-1071. Electronic mail address: mms7r@Virginia.EDU.

TABLE 1. Genotypes of yeast strains used in this study

Strain	Genotype	Source or reference
DBY747	*MAT***a** *his3-Δ1 leu2-3,112 trp1-289 ura3-52*	D. Botstein
MSY596	*MATα his3-Δ1 trp1-289 ura3-52 lys2*	41
MSY826	*MATα net1-1 his3-Δ1 trp1-289 ura3-52 lys2*	41
MSY849	*MATα net1-2 his3-Δ1 trp1-289 ura3-52 lys2*	This work
MSY757	*MATα snf2::URA3 his3-Δ1 trp1-289 ura3-52 lys2*	This work
CY296	*MAT***a** *gal4::LEU2 leu2-Δ1 his3-Δ200 trp1-Δ99 ura3-52 lys2*	C. Peterson
CY298	*MAT***a** *swi1Δ::LEU2 gal4::LEU2 leu2-Δ1 his3-Δ200 trp1-Δ99 ura3-52 lys2*	C. Peterson
CY306	*MAT***a** *sin1Δ::TRP1 swi1Δ::LEU2 gal4::LEU2 leu2-Δ1 his3-Δ200 trp1-Δ99 ura3-52 lys2*	C. Peterson

SNF complex is a target of the N-terminal and CR1 domains in mammalian cells and that the disruption of normal cell cycle control by E1A may be due in part to altered activity of the SWI/SNF complex.

MATERIALS AND METHODS

Strains and media. Plasmids were maintained in the bacterial host *Escherichia coli* DH5α [*supE44 ΔlacU169* (ϕ80*lacZΔM15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*] by standard procedures (49). The relevant genotypes of the yeast strains used in this work are shown in Table 1. Strain MSY757 was derived from MSY596 by disruption of *SNF2* with a one-step disruption plasmid, pMSS14, provided by M. S. Santisteban. Transformants unable to grow on plates containing raffinose as the sole carbon source were analyzed by Southern blot hybridization to confirm the disruption of *SNF2*. Yeast strains were transformed with plasmid DNA by standard procedures (19).

YP, SC, and YNB media for yeast growth were prepared as described previously (48). YPD medium is YP medium with 2% glucose. SDC and SGC media are SC medium with 2% glucose and 2% galactose, respectively. SGEC medium is SC medium with 3% glycerol, 1% ethanol, and 0.05% glucose. Inositol starvation medium was prepared as described previously (36).

Selection of mutants. Mutagenesis of DBY747 was carried out essentially as described previously (48) with the following modifications. Ethyl methanesulfonate (Sigma) was added to 1.4% (vol/vol), resulting in 50% cell survival, and quenched with an equal volume of 10% (wt/vol) sodium thiosulfonate. The cells were washed twice with sterile water and resuspended in YPD medium. The culture was shaken for 2 h at 28°C and transformed with the E1A expression vector pMA424.82T (41). Transformants were plated on medium selecting for pMS424.82T, and mutants able to form colonies were isolated. A parallel transformation with the parental vector pMA424 indicated that approximately 48,000 viable mutagenized cells were subjected to selection.

Protein analysis. Western blot (immunoblot) analysis was carried out by the method of Harlow and Lane (21) with monoclonal antibody M29 (provided by E. Harlow) specific for the N terminus of E1A (20). The amount of Gal4-E1A$_{1–82}$ protein expressed in *net1-1* mutant cells, which were insensitive to E1A, was quantitatively the same as that produced in wild-type cells, showing strong growth inhibition. Thus, the suppression of E1A sensitivity in *net1* mutants is not due to decreased expression.

RNA analysis. RNA isolations (28) and Northern (RNA) blot analyses (49) were performed as described previously. A 772-bp *SUC2* probe was generated by PCR amplification of genomic DNA with primers P1 (5′-TGTGGTACGATGA AAAAGAT-3′) and P2 (5′-TAATGCACCGTAGGTTG-3′). The *INO1* probe was the 462-bp *Pvu*I-*Bgl*II fragment of pJH318 (23). The *ACT1* probe was the 1,100-bp *Xho*I-*Hin*dIII fragment from pMSS8. pMSS8 contains the *Xho*I-*Hin*dIII *ACT1* fragment of pRB147 (50) in plasmid pRS305 (52). The *PRC1* probe was generated by PCR amplification of genomic DNA with primers P3 (5′-ACTGT CGCCGCTGGTAAG-3′) and P4 (5′-CTTCATCCAATCACCCGC-3′). Relative mRNA levels were quantified with a PhosphorImager and ImageQuant version 3.3 software (Molecular Dynamics).

Additional probes were as follows: *SNF2*, the 1.1-kb fragment of pMSS14; *SNF5*, the 2.5-kb *Kpn*I fragment of pLY22, which is identical to pLY14 except that the *Bam*HI site instead of the *Hin*dIII site was used for cloning *SNF5* (35); *SNF6*, the 700-bp *Nco*I-*Bam*HI fragment of pEY111 (17); *SWI3*, the 2.6-kb *Pvu*II fragment of pLY23; and *NET1*, the 1.7-kb *Bam*HI-*Xba*I fragment of pUCA-SWP73.

RESULTS

Cellular mutants insensitive to E1A expression. To identify cellular genes whose products are required for E1A function, we selected mutant strains resistant to the growth-inhibitory effects of the N-terminal and CR1 domains, expressed as a Gal4-E1A fusion protein (Fig. 1A, Gal4-E1A$_{1–82}$). Gal4-E1A$_{1–82}$ was shown previously to be sufficient for triggering growth inhibition in *S. cerevisiae*. This effect is due solely to the E1A sequences within the fusion protein and not to the presence of the Gal4 DNA-binding domain (41). The initial selection was carried out in DBY747, in which expression of Gal4-E1A$_{1–82}$ is lethal (41).

The E1A-resistant strains carry mutations in genes whose normal function is to mediate the growth-inhibitory effect of E1A. We adopted a genetic complementation approach to identify the wild-type counterparts of such mutant alleles. However, the complementation strategy was complicated by the fact that introduction of the wild-type allele would restore growth inhibition in the presence of E1A, thus preventing recovery of a complementing clone. Therefore, to make complementation cloning feasible, it was first necessary to identify E1A-resistant mutants expressing a linked secondary phenotype. Accordingly, 227 E1A-resistant strains were

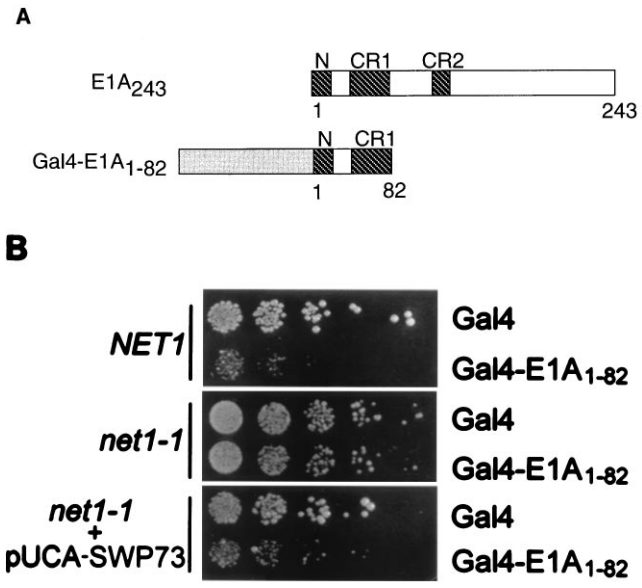

FIG. 1. (A) Structure of full-length E1A$_{243}$ and Gal4-E1A$_{1–82}$. The locations of three functional domains involved in mouse and human cellular transcriptional regulation and transformation are shown. Of these, only the N-terminal and CR1 domains are required for inhibition of yeast cell growth. The gray region of Gal4-E1A$_{1–82}$ represents amino acids 1 to 147 of yeast Gal4, which contain the DNA-binding domain. We showed previously that fusion of this region of Gal4 allows stable expression of the E1A$_{1–82}$ polypeptide (41). (B) Yeast strains MSY596 (*NET1*), MSY826 (*net1-1), or MSY826 (net1-1) carrying plasmid pUCA-SWP73 (NET1*) were transformed with control vector pMA424 (Gal4) or pMA424.82T (Gal4-E1A$_{1–82}$ [41]). Equal numbers of cells were serially diluted and assayed for growth at 28°C for 3 days (*NET1* and *net1-1* + pUCA-SWP73) or 5 days (*net1-1*). Because of these different incubation times, relative growth rates can be compared only between cells with and without E1A within a single strain in this figure.

screened for those that were also temperature sensitive (Ts^-) for growth at the restrictive temperature of 37°C. Twenty-seven Ts^- Net^- mutants were confirmed after testing for retention of the Ts^- phenotype in the absence of E1A and checking for stability of the E1A-resistant phenotype by reintroduction of the Gal4-E1A$_{1-82}$ plasmid. Of these, 17 expressed detectable levels of Gal4-E1A$_{1-82}$ protein as determined by Western blot analysis with the anti-E1A monoclonal antibody M29 (provided by E. Harlow [data not shown]). To facilitate subsequent genetic manipulation, each isolate was backcrossed to the wild-type strain MSY596. An advantage of the MSY596 background is that despite being sensitive to the growth-inhibitory properties of E1A, MSY596 cells expressing E1A are able to grow well enough to provide material for RNA and protein analysis. This was not possible for DBY747. Tetrad analysis demonstrated that five of the mutants displayed tightly linked Ts^- and E1A-resistant phenotypes, and crosses with the wild type revealed that both of these phenotypes were recessive. The genes of interest were termed *NET* genes (for N-terminal E1A toxicity), on the basis of the function of the wild-type alleles (i.e., they confer sensitivity to E1A). They were placed in four complementation groups: two *NET1* alleles were recovered (*net1-1* and *net1-2*), as were one *NET2*, one *NET3*, and one *NET4* allele.

Cloning *NET1*. The *NET1* gene was cloned by screening a low-copy-number yeast genomic library (p366; provided by P. Hieter) for plasmids able to revert the Ts^- phenotype of *net1-1*. Of approximately 20,000 transformants, 6 grew well at 37°C, and three independent overlapping plasmids were identified. Sequence analysis revealed that the overlapping region of the plasmids included at least two genes: the *SEC12* gene, and a gene whose DNA sequence matched that of the GenBank locus SCPCPETIT (accession number X62430 [11a]). Subcloning and disruption of the region showed that this second gene was necessary for reversion of the Ts^- phenotype of *net1-1* (data not shown). This gene was independently identified in a parallel study involving a genetic screen based on expression of the glucocorticoid receptor in *S. cerevisiae* (6). Plasmid pUCA-SWP73, constructed for that study, which expresses only the open reading frame predicted by the SCPC PETIT GenBank locus, was found to be sufficient for reversion of the Ts^- phenotype of *net1-1* (data not shown).

We next tested whether the cloned gene would also cause reversion of the Net^- phenotype, as predicted if it plays a role in E1A function. Wild-type and *net1-1* strains were transformed with pUCA-SWP73, and transformants were assayed for growth in the presence and absence of Gal4-E1A$_{1-82}$ (Fig. 1). As expected, the wild type grew slowly in the presence of Gal4-E1A$_{1-82}$ compared with its growth in the presence of the Gal4 DNA-binding domain alone. Also, growth of the *net1-1* mutant was unaffected by expression of Gal4-E1A$_{1-82}$. In contrast, *net1-1* plus the test plasmid (pUCA-SWP73) grew very slowly when Gal4-E1A$_{1-82}$ was expressed, although it grew as well as the wild type in the presence of the Gal4 DNA-binding domain alone. Identical results were obtained with the *net1-2* allele (data not shown). Thus, the cloned gene was able to cause reversion of both the Ts^- and Net^- phenotypes of *net1-1*. The physical linkage of this gene to *SEC12* on the isolated plasmids led to the prediction that *net1-1* is genetically linked to *SEC12*. A cross between *net1-1 SEC12* and *NET1 sec12-4* strains produced only parental ditype tetrads in 22 asci analyzed, showing that the two genes are closely linked. Therefore, we conclude that the cloned gene is allelic with *NET1* and is identical to the gene defined by the SCPCPETIT GenBank sequence.

The SCPCPETIT DNA sequence was originally derived from a recombinant plasmid clone that complemented a *tpy1* mutant, which is unable to grow on medium with pyruvate as the sole carbon source (58). Tetrad analysis confirmed that *tpy1* (provided by C. Wills) was tightly linked to *SEC12*, consistent with *TPY1* being allelic to *NET1* and the SCPCPETIT GenBank locus. On the basis of the petite-like phenotype of *tpy1* mutants, and the physiology of mutant cells, *TPY1* was proposed to encode a protein involved in mitochondrial pyruvate transport (58). However, the results of the experiments described below suggest that the $Tpy1^-$ phenotype is more likely to be an indirect consequence of aberrant nuclear gene expression.

***NET1* is a component of the SWI/SNF complex.** The biochemical identity of the *NET1* gene product was provided through a parallel investigation of the yeast SWI/SNF transcriptional regulatory complex. The SWI/SNF complex functions in the transcriptional activation of several genes including *INO1*, *SUC2*, and *HO* (33, 35, 44, 61; for reviews, see references 7, 34, and 60). Genetic and in vitro studies indicate that it acts to reorganize chromatin to allow access of sequence-specific DNA-binding proteins to nucleosomal DNA (9, 24; for a review, see reference 46). Biochemically purified SWI/SNF complex is composed of at least 11 polypeptides, 6 of which have been previously identified as the products of the *SWI1*, *SWI3*, *SNF2*, *SNF5*, *SNF6*, and *SNF11* genes (5, 43, 54). Peptide sequencing of Swp73, the 73-kDa subunit of the complex, revealed that it was identical to the predicted gene product of the GenBank SCPCPETIT DNA sequence (6). Thus, *NET1* is a new SWI/SNF complex gene encoding the Swp73 protein.

Since Swp73 was identified strictly through its physical association with the SWI/SNF complex, it was important to determine if it functions as a member of the complex. Accordingly, we tested *net1-1* and *net1-2* strains for their ability to support transcriptional activation of *SUC2* and *INO1*, two genes whose activation pathways are distinct but which have a common requirement for SWI/SNF function (44). Induction of *INO1* is triggered by low levels of inositol in the medium, while *SUC2* is induced by glucose starvation. Northern blot analysis was performed with RNA isolated from *net1-1* and *net1-2*, as well as from the isogenic wild type, under noninducing and inducing conditions (see Materials and Methods). As shown in Fig. 2, the magnitude of the induction of both *SUC2* and *INO1* mRNAs was significantly reduced in *net1* mutants compared with that in the wild-type. Deletion of *SNF2*, which is known to encode a functional component of the SWI/SNF complex (34), produced an identical loss of activation. These genetic and biochemical results demonstrate that Swp73 is a functional component of the SWI/SNF complex. Consequently, we have changed the name of the gene encoding Swp73 from *NET1* to *SNF12* to reflect the known function of the gene more appropriately.

Other *snf* and *swi* mutants are Net^-. The above results suggested that E1A activity might require the function of the SWI/SNF complex itself rather than a novel function of Swp73 distinct from its role in the SWI/SNF complex. To distinguish between these possibilities, we examined mutants with mutations in other SWI/SNF complex genes. If growth inhibition by E1A requires SWI/SNF function, mutants with mutations in other known SWI and SNF genes would be predicted to have Net^- phenotypes. As shown in Fig. 3, a *snf2*Δ strain was clearly Net^- when compared with the isogenic parent. Identical results were obtained with a strain carrying a null allele for another SWI/SNF component, *SWI1* (data not shown). Additionally, mutant alleles of *SWI3* and *SNF2* were identified from the original genetic screen for Net^- mutants (data not shown). Finally, several additional Net^- mutants isolated in our genetic

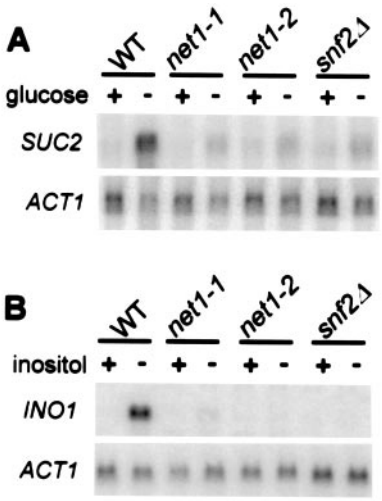

FIG. 2. Swp73 is a functional component of the SWI/SNF complex. Total cellular RNA from MSY596 (wild-type), MSY826 (*net1-1*), MSY849 (*net1-2*), and MSY757 (*snf2*Δ) was assayed by Northern blot hybridization with probes specific for *SUC2* or *INO1* (Materials and Methods). The filters were then stripped and probed with an *ACT1*-specific probe. (A) Cells from exponentially growing cultures were washed twice in sterile water and resuspended in YPD medium containing 2% (+) or 0.05% (−) glucose. RNA was isolated after incubation at 28°C for 2.5 h. (B) Cells were grown exponentially in medium containing 100 mM (+) or 10 mM (−) inositol and harvested for RNA isolation.

screen expressed a mutant Snf$^-$ phenotype. Of 64 strains assayed, 17 were unable to form colonies when raffinose was the sole carbon source (in the presence of antimycin A) or when inositol was absent from the medium. These data demonstrate that the function of E1A requires more than one member of the SWI/SNF complex and strongly suggest that SWI/SNF complex function itself is the target of E1A.

E1A blocks SWI/SNF-dependent gene expression. From the requirement for *SNF12*, *SNF2*, *SWI1*, and *SWI3* in the activity of E1A, we reasoned that E1A might act by either enhancing or inhibiting SWI/SNF-dependent transcriptional activation, contributing to the observed inhibition of cell growth. Therefore, an analysis of *SUC2* and *INO1* mRNA levels was undertaken to detect E1A-induced alterations in SWI/SNF-dependent transcriptional regulation. Indeed, when the Gal4-E1A$_{1–82}$ fusion protein was expressed in wild-type cells, normal induction of *SUC2* and *INO1* mRNAs was blocked (Fig. 4A and B). There was no effect of the Gal4 DNA-binding domain alone on either *SUC2* or *INO1* mRNA levels, and there was no effect of Gal4-E1A$_{1–82}$ at the level of actin mRNA. Also, longer exposures of the Northern blots revealed that there was no effect of E1A on uninduced (i.e., basal) *SUC2* or *INO1* mRNA levels (data not shown). These data demonstrate a specific effect of E1A on SWI/SNF-dependent transcriptional activation. A similar experiment was performed with a plasmid encoding full-length E1A$_{243}$ lacking the Gal4 sequence. Here, inducible expression of E1A$_{243}$ was under the control of the *GAL1* promoter. Figure 4C shows that expression of E1A$_{243}$ in wild-type cells resulted in an identical inhibition of *INO1* mRNA induction as was seen with Gal4-E1A$_{1–82}$. The ability of E1A$_{243}$ to inhibit induced transcription of the *INO1* gene demonstrates that the activity of Gal4-E1A$_{1–82}$ in blocking transcriptional activation is in fact due to E1A sequences and occurs independently of the Gal4 DNA-binding domain. These data are consistent with our earlier report that the Gal4 DNA-binding domain is not involved in growth inhibition by Gal4-E1A$_{1–82}$ (41).

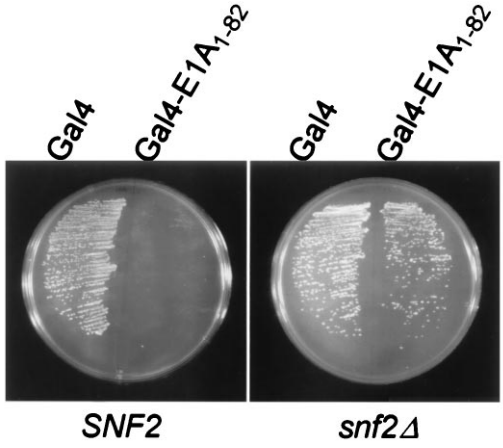

FIG. 3. Deletion of *SNF2* causes a Net$^-$ phenotype. Strains MSY596 (*SNF2*) or MSY757 (*snf2*::*URA3*) were transformed with pMA424 (Gal4) or pMA424.82T (Gal4-E1A$_{1–82}$). Transformants were streaked on SDC-HIS plates and assayed for growth.

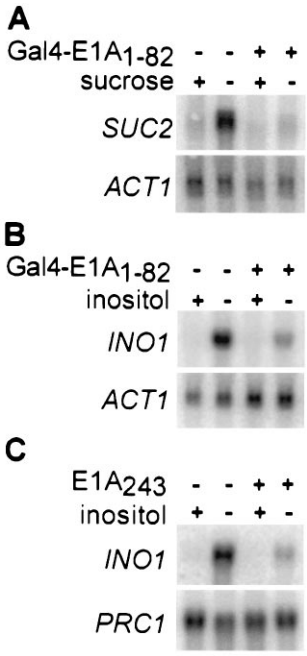

FIG. 4. Expression of E1A blocks SWI/SNF-dependent transcription. (A and B) MSY596 (wild-type) cells were transformed with the control Gal4 expression vector pMA424 (−), or the Gal4-E1A$_{1–82}$ expression vector pMA424.82T (+). The cells were assayed for *SUC2* (A) or *INO1* (B) mRNAs as described in the legend to Fig. 2. (C) MSY596 (wild-type) cells were transformed with a galactose-inducible E1A$_{243}$ expression vector or with the control vector pRS316. Transformants were grown in YNB medium with 2% glycerol, 1% ethanol, 0.05% glucose, and either 100 mM (−) or 10 mM (−) inositol. During early log phase, galactose was added to a final concentration of 2%, and the cultures were allowed to continue to grow for two doublings. Total cellular RNA was isolated and assayed by Northern blot hybridization with an *INO1*-specific probe. Following this, the filter was stripped and probed with a *PRC1*-specific probe, which served as an internal control.

E1A acts at the level of the SWI/SNF complex. Two types of experiments were performed to examine the genetic level at which E1A acts to interfere with SWI/SNF-dependent transcription. First, Northern blot analysis of steady-state mRNAs for five cloned SWI/SNF complex components (*SNF2*, *SNF5*,

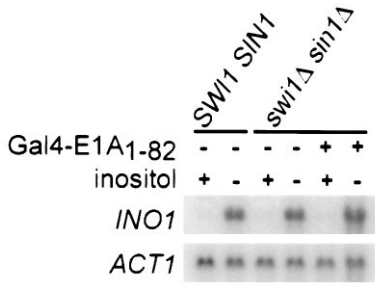

FIG. 5. A *sin1* mutation suppresses the Gal4-E1A$_{1-82}$ block of SWI/SNF-dependent transcription. Strain CY306 (*swi1*Δ*sin1*Δ) was transformed with either the control Gal4 expression vector pMA424 (lanes *swi1*Δ*sin1*Δ, −) or the Gal4-E1A$_{1-82}$ expression vector pMA424.82T (lanes *swi1*Δ*sin1*Δ, +). Transformants were grown in medium containing 100 mM (+) or 0 mM (−) inositol and assayed for *INO1* and *ACT1* mRNAs as described in the legend to Fig. 2. Also shown are *INO1* mRNA levels from isogenic wild-type cells (strain CY296) grown in the presence and absence of inositol and lacking E1A. As expected, no induction of *INO1* mRNA was observed in *swi1*Δ single mutants (strain CY298 [not shown]).

SNF6, *SWI3*, and *SNF12*) was performed with RNA isolated from control cells or cells expressing Gal4-E1A$_{1-82}$ (see Materials and Methods). No differences were detected, demonstrating that E1A does not act by altering the transcription of SWI/SNF components (data not shown).

Second, the effect of E1A on the transcription of *INO1* was examined by using cells carrying a null allele of *SIN1*. Induction of *INO1* is triggered by low levels of inositol in the medium, and this induction normally requires the SWI/SNF complex. Deletion of *SIN1*, which encodes a chromatin-associated nonhistone protein, relieves this requirement such that efficient induction can be triggered in cells lacking functional SWI/SNF (37, 45). To determine if E1A is able to block SWI/SNF-independent transcriptional activation of *INO1*, Gal4-E1A$_{1-82}$ was expressed in cells in which both the *SIN1* and *SWI1* genes were deleted. Data in Fig. 5 show that a wild-type level of induction occurred in the *swi1*Δ*sin1*Δ strain, consistent with an earlier report (45). Despite this high level of activation, expression of Gal4-E1A$_{1-82}$ had no effect on *INO1* mRNA levels. The single *sin1*Δ mutant was very sensitive to growth inhibition by Gal4-E1A$_{1-82}$ (data not shown), demonstrating that deletion of *SIN1* itself does not abrogate the effect of E1A. Therefore, since the transcriptional activation pathway for *INO1* is intact in the double mutant, the target of E1A is neither a specific component of this activation pathway nor a specific component of the general transcription machinery. This and the finding that E1A expression blocks the induction of both *INO1* and *SUC2*, whose activation pathways are distinct but have a common requirement for SWI/SNF, lead us to conclude that E1A acts at the level of the SWI/SNF complex.

DISCUSSION

A remarkable number of biological functions are now known to be conserved across evolutionary kingdoms. These include mechanisms of transcription (10, 29, 30), cell division cycle control (3, 22, 40), and signal transduction (47, 55). The genetic analysis of mammalian and viral genes in *S. cerevisiae* has been a valuable approach for understanding these functions, often providing new insights into the molecular biology of both mammalian and yeast cells. This strategy has recently been initiated for the adenovirus E1A gene with interesting genetic results (41, 56, 63). Starting with a Net$^-$ strain background, Zieler et al. (63) screened for yeast cellular mutants that became dependent on continued E1A expression for viability. This screen identified two genes, *WEB1*/*SEC31*, encoding a protein involved in vesicular budding from the endoplasmic reticulum, and *WEB2*, encoding a putative nucleic acid-dependent ATPase. Although the functional significance of these genetic interactions is not yet clear, both genes are essential and clearly important for yeast cell physiology.

The genetic experiments reported here were designed to investigate the function of E1A in *S. cerevisiae* and the basis of the Net phenotype. This approach was based on our previous findings that E1A inhibits yeast cell growth and that this inhibition depends both on the N-terminal and CR1 domains of E1A and on an intact cellular cAMP signaling pathway (41). These features of E1A function in *S. cerevisiae* are strikingly similar to those in mammalian cells, in which components of the cAMP signaling pathway are required for transcriptional activation and repression by the N-terminal and CR1 domains (1, 2, 16, 18, 26, 27, 39). These parallels suggested that further genetic analysis in the yeast system might lead to clues regarding both gene regulation in yeast cells and E1A biology in mammalian cells. As a result of these studies, we have identified a new genetic interaction between E1A and the SWI/SNF transcriptional regulatory complex, an interaction of potential importance for E1A function in mammalian cells, and we have cloned the *SNF12* gene, a novel component of the yeast SWI/SNF complex.

The results of both biochemical and genetic experiments show that *SNF12* encodes a subunit of the SWI/SNF complex. First,the Snf12 protein copurifies with the SWI/SNF complex, demonstrating a physical association (6). Second, *snf12* mutants exhibit a defect in normal SWI/SNF-dependent transcription, showing that Snf12p is also a functional member of the complex. *SNF12* is an unusual member of the SWI/SNF gene family. Unlike most other known genes of the SWI/SNF complex, the loss-of-function *snf12* alleles reported here, as well as the *snf12* null allele (6), produce cells that are Ts$^-$ lethal, indicating that an otherwise normal cellular function becomes defective at elevated temperature in the absence of Snf12p function. This phenotype suggests that *SNF12* participates in an additional process distinct from its documented role in the SWI/SNF complex, and it will be important to determine the full spectrum of *SNF12* functions. For E1A, we conclude that the SWI/SNF activity of *SNF12* is responsible for the sensitivity, since deletion of other SWI/SNF subunit genes, such as *SWI1*, *SNF2*, and *SWI3*, yields mutants that are also E1A resistant but are not Ts$^-$.

The genetic relationship between E1A and the SWI/SNF complex is illustrated in Fig. 6. In the absence of E1A expression, the SWI/SNF complex is required to overcome the repressive effects of histones and other Sin and Spt chromatin proteins and to facilitate the access of transcription factors to the promoter (Fig. 6A) (59). In principal, E1A could inhibit transcription either at the level of the SWI/SNF complex or by blocking one or more downstream steps. However, the genetic results place the function of E1A at the level of the SWI/SNF complex (Fig. 6B), since a *sin1*Δ mutation bypasses the effect of E1A and restores transcriptional activation (Fig. 6C). Thus, mutation or deletion of SWI/SNF genes eliminates both the function of the SWI/SNF complex and the sensitivity to the growth-inhibitory properties of E1A. Consistent with this, the growth properties of the *snf12-1* (*net1-1*) strain MSY826 are similar to those of the wild-type congenic strain MSY596 expressing E1A. The identity of the SWI/SNF-dependent genes required for normal cell growth on rich glucose medium is not known, but it will be of interest to determine the identity of those genes, and understand the mechanism of their activation.

We note that whereas the simple model of Fig. 6 is sufficient

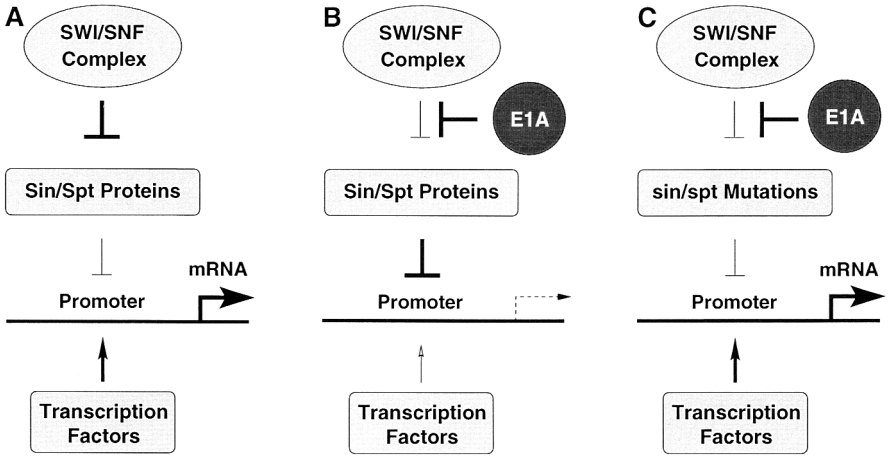

FIG. 6. Genetic interactions of E1A with the SWI/SNF complex. A model for the function of the SWI/SNF complex and its interactions with adenovirus E1A is illustrated in schematic form for three different cell states. Positive and negative functions for transcription are represented by the arrowheads and perpendicular bars, respectively. (A) Wild-type cell, no E1A. The repressive effects of Sin and Spt chromatin proteins on promoter activity are blocked by the function of the SWI/SNF complex, allowing strong activation by transcription factors and the production of mRNA. (B) Wild-type cell, with E1A. Expression of E1A specifically blocks the activity of the SWI/SNF complex, resulting in strong expression by the Sin and Spt chromatin proteins and turning off of transcription. (C) *sin* mutant, with E1A. Expression of E1A still blocks the activity of the SWI/SNF complex. However, gene repression by the Sin and Spt chromatin proteins is alleviated directly by mutation, bypassing the requirement for SWI/SNF and the repressing effect of E1A and resulting in strong transcriptional activation.

to explain the inhibition of SWI/SNF-dependent transcription by E1A, it is not sufficient to explain the isolation of the *net* mutants in the original selection with strain DBY747. In the original selection, the *swi*/*snf* mutations did not just make cells insensitive to E1A but, rather, caused growth rather than death in the presence of E1A expression. This result suggests that the active SWI/SNF complex and E1A act together in DBY747 to cause a synthetic cell death. Mutations in SWI/SNF complex genes then eliminate both the synthetic cell death and further sensitivity to E1A expression. This is a recessive phenotype of DBY747, since the diploid made by mating DBY747 and MSY596 exhibits just the E1A-insensitive phenotype of MSY596. We presume that the more complicated DBY747 phenotype was lost from the background during the multiple backcrosses to MSY596 carried out to isolate the *net* alleles.

At present we do not know if E1A blocks SWI/SNF function directly or indirectly. E1A might physically interact with the complex directly, or, alternatively, it might interact with a pathway that regulates SWI/SNF activity. Interestingly, E1A provides the first genetic link between SWI/SNF complex function and the cAMP signaling pathway (41). Thus, we anticipate that the cAMP signaling pathway either will be involved by controlling transcription of those SWI/SNF-regulated genes critical for growth inhibition by E1A or else will serve to regulate SWI/SNF complex function directly. Experiments are in progress to investigate the interaction of these two regulatory pathways more thoroughly.

Identification of the yeast SWI/SNF complex as a functional target of E1A has clear implications for the activity of E1A in mammalian cells, in which more than one type of SWI/SNF complex may exist (11, 25, 32, 42). In *S. cerevisiae*, E1A expression results in a block to SWI/SNF-dependent transcriptional activation in a manner that depends on sequences within the N-terminal and CR1 domains (41). Interestingly, these domains also mediate transcriptional regulation in mammalian cells, including both activation and repression (4, 13). We propose that some of these effects are due to an inhibition of mammalian SWI/SNF function by E1A. Since the N-terminal and CR1 domains are also known to bind to p300 (4, 13), we speculate that p300 is involved in normal SWI/SNF-dependent transcriptional regulation. Consistent with this, a p300-related protein, p270, has recently been shown to exist in a complex along with hBRG1, a human homolog of yeast *SNF2* (8). A connection between E1A and mammalian SWI/SNF is also suggested by the fact that Rb, itself a target of E1A, can physically interact with hBRG1 and hBRM, a second human *SNF2* homolog (12, 53). Therefore, E1A may have evolved to alter mammalian SWI/SNF function in more than one way. In *S. cerevisiae*, E1A may bind directly to a specific member of the SWI/SNF complex, a homolog of p300, or a distinct protein. Identification of the direct target of E1A in *S. cerevisiae* will allow further genetic analysis of this system, which plays important regulatory roles in both yeast and mammalian cells.

ACKNOWLEDGMENTS

We thank C. Peterson for *sin1* mutant strains, M. S. Santisteban for the *SNF2* disruption plasmid, and M. S. Santisteban, E. Moran, C. Peterson, and M. Carlson for helpful discussions during the course of this work.

This work was supported by NIH grants CA60675 (D.A.E.), GM36659 (R. Kornberg), CA20535 (K.R.Y.), and GM28920 (M.M.S.).

REFERENCES

1. **Arany, Z., D. Newsome, E. Oldread, D. M. Livingston, and R. Eckner.** 1995. A family of transcriptional adapter proteins targeted by the E1A oncoprotein. Nature (London) **374:**81–84.
2. **Arany, Z., W. R. Sellers, D. M. Livingston, and R. Eckner.** 1994. E1A-associated p300 and CREB-associated CBP belong to a conserved family of coactivators. Cell **77:**799–800.
3. **Bartlett, R., and P. Nurse.** 1990. Yeast as a model system for understanding the control of DNA replication in eukaryotes. Bioessays **12:**457–463.
4. **Bayley, S. T., and J. S. Mymryk.** 1994. Adenovirus E1A proteins and transformation. Int. J. Oncol. **5**425–444.
5. **Cairns, B. R., Y. J. Kim, M. H. Sayre, B. C. Laurent, and R. D. Kornberg.** 1994. A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. Proc. Natl. Acad. Sci. USA **91:**1950–1954.
6. **Cairns, B. R., R. S. Levinson, K. R. Yamamoto, and R. D. Kornberg.** Essential role of Swp73p in function of the yeast Swi/Snf complex. Genes Dev., in press.
7. **Carlson, M., and B. C. Laurent.** 1994. The SNF/SWI family of global transcriptional activators. Curr. Opin. Cell Biol. **6:**396–402.
8. **Cheney, W., P. Dallas, V. Bowrin, V. Srinivas, and E. Moran.** Personal communication.

9. **Côté, J., J. Quinn, J. L. Workman, and C. L. Peterson.** 1994. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science **265:**53–60.
10. **Coulombe, B., M. Killeen, P. Liljelund, B. Honda, H. Xiao, C. J. Ingles, and J. Greenblatt.** 1992. Identification of three mammalian proteins that bind to the yeast TATA box protein TFIID. Gene Expr. **2:**99–110.
11. **Dingwall, C., S. Kandels-Lewis, and B. Seraphin.** 1995. A family of Ran binding proteins that includes nucleoporins. Proc. Natl. Acad. Sci. USA **92:**7525–7529.

11a. **Donald, K. A. G., J. Hill, and D. E. Griffiths.** Unpublished data.

12. **Dunaief, J. L., B. E. Strober, S. Guha, P. A. Khavari, K. Alin, J. Luban, M. Begemann, G. R. Crabtree, and S. P. Goff.** 1994. The retinoblastoma protein and BRG1 form a complex and cooperate to induce cell cycle arrest. Cell **79:**119–130.
13. **Dyson, N., and E. Harlow.** 1992. Adenovirus E1A targets key regulators of cell proliferation. Cancer Surv. **12:**161–195.
14. **Egan, C., T. N. Jelsma, J. A. Howe, S. T. Bayley, B. Ferguson, and P. E. Branton.** 1988. Mapping of cellular protein-binding sites on the products of early region 1A of human adenovirus type 5. Mol. Cell. Biol. **8:**3955–3959.
15. **Engel, D. A., S. Hardy, and T. Shenk.** 1988. cAMP acts in synergy with E1A protein to activate transcription of the adenovirus early genes E4 and E1A. Genes Dev. **2:**1517–1528.
16. **Engel, D. A., U. Muller, R. W. Gedrich, J. S. Eubanks, and T. Shenk.** 1991. Induction of c-fos mRNA and AP-1 DNA-binding activity by cAMP in cooperation with either the adenovirus 243- or the adenovirus 289-amino acid E1A protein. Proc. Natl. Acad. Sci. USA **88:**3957–3961.
17. **Estruch, F., and M. Carlson.** 1990. *SNF6* encodes a nuclear protein that is required for expression of many genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**2544–2553.
18. **Gedrich, R. W., S. T. Bayley, and D. A. Engel.** 1992. Induction of AP-1 DNA-binding activity and c-*fos* mRNA by the adenovirus 243R E1A protein and cyclic AMP requires domains necessary for transformation. J. Virol. **66:**5849–5859.
19. **Gietz, D., A. St. Jean, R. A. Woods, and R. H. Schiestl.** 1992. Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. **20:**1425.
20. **Harlow, E., B. R. Franza, Jr., and C. Schley.** 1985. Monoclonal antibodies specific for adenovirus early region 1A proteins: extensive heterogeneity in early region 1A products. J. Virol. **55:**533–546.
21. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. p. 421–510. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Hatakeyama, M., J. A. Brill, G. R. Fink, and R. A. Weinberg.** 1994. Collaboration of G1 cyclins in the functional inactivation of the retinoblastoma protein. Genes Dev. **8:**1759–1771.
23. **Hirsch, J. P., and S. A. Henry.** 1986. Expression of the *Saccharomyces cerevisiae* inositol-1-phosphate synthase (*INO1*) gene is regulated by factors that affect phospholipid synthesis. Mol. Cell. Biol. **6:**3320–3328.
24. **Hirschhorn, J. N., S. A. Brown, C. D. Clark, and F. Winston.** 1992. Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. Genes Dev. **6:**2288–2298.
25. **Imbalzano, A. N., H. Kwon, M. R. Green, and R. E. Kingston.** 1994. Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature (London) **370:**481–485.
26. **Janaswami, P. M., D. V. Kalvakolanu, Y. Zhang, and G. C. Sen.** 1992. Transcriptional repression of interleukin-6 gene by adenovirual E1A proteins. J. Biol. Chem. **267:**24886–24891.
27. **Kalvakolanu, D. V., J. Liu, R. W. Hanson, M. L. Harter, and G. C. Sen.** 1992. Adenovirus E1A represses the cyclic AMP-induced transcription of the gene for phosphoenolpyruvate carboxykinase (GTP) in hepatoma cells. J. Biol. Chem. **267:**2530–2536.
28. **Keleher, C. A., M. J. Redd, J. Schultz, M. Carlson, and A. D. Johnson.** 1992. Ssn6-Tup1 is a general repressor of transcription in yeast. Cell **68:**709–719.
29. **Kelleher, R. J., III, P. M. Flanagan, D. I. Chasman, A. S. Ponticelli, K. Struhl, and R. D. Kornberg.** 1992. Yeast and human TFIIDs are interchangeable for the response to acidic transcription activators in vitro. Genes. Dev. **6:**296–303.
30. **Kornuc, M., R. Altman, D. Harrich, J. Garcia, J. Chao, P. Kayne, and R. Gaynor.** 1988. Adenovirus transcriptional regulatory regions are conserved in mammalian cells and *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:**3717–3725.
31. **Kraus, V. B., J. A. Inostroza, K. Yeung, D. Reinberg, and J. R. Nevins.** 1994. Interaction of the Dr1 inhibitory factor with the TATA binding protein is disrupted by adenovirus E1A. Proc. Natl. Acad. Sci. USA **91:**6279–6282.
32. **Kwon, H., A. N. Imbalzano, P. A. Khavari, R. E. Kingston, and M. R. Green.** 1994. Nucleosome disruption and enhancement of activator binding by a human SW1/SNF complex. Nature (London) **370:**477–481.
33. **Laurent, B. C., and M. Carlson.** 1992. Yeast SNF2/SWI2, SNF5, and SNF6 proteins function coordinately with the gene-specific transcriptional activators GAL4 and Bicoid. Genes Dev. **6:**1707–1715.
34. **Laurent, B. C., I. Treich, and M. Carlson.** 1993. Role of yeast SNF and SWI proteins in transcriptional activation. Cold Spring Harbor Symp. Quant. Biol. **58:**257–263.
35. **Laurent, B. C., M. A. Treitel, and M. Carlson.** 1990. The SNF5 protein of *Saccharomyces cerevisiae* is a glutamine- and proline-rich transcriptional activator that affects expression of a broad spectrum of genes. Mol. Cell. Biol. **10:**5616–5625.
36. **Lawrence, C. W.** 1991. Classical mutagenesis techniques. Methods Enzymol. **194:**273–281.
37. **Lefebvre, L., and M. Smith.** 1993. Mutational and functional analysis of dominant *SPT2* (*SIN1*) suppressor alleles in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**5393–5407.
38. **Lewis, B. A., G. Tullis, E. Seto, N. Horikoshi, R. Weinmann, and T. Shenk.** 1995. Adenovirus E1A proteins interact with the cellular YY1 transcription factor. J. Virol. **69:**1628–1636.
39. **Lundblad, J. R., R. P. S. Kwok, M. E. Laurance, M. L. Harter, and R. H. Goodman.** 1995. Adenovirual E1A-associated protein p300 as a functional homolog of the transcriptional coactivator CBP. Nature (London) **374:**85–88.
40. **Malhotra, P., C. F. Manohar, S. Swaminathan, R. Toyama, R. Dhar, R. Reichel, and B. Thimmappaya.** 1993. E2F site activates transcription in fission yeast Schizosaccharomyces pombe and binds to a 30-kDa transcription factor. J. Biol. Chem. **268:**20392–20401.
41. **Miller, M. E., D. A. Engel, and M. M. Smith.** 1995. Cyclic AMP signaling is required for function of the N-terminal and CR1 domains of adenovirus E1A in *Saccharomyces cerevisiae*. Oncogene **11:**1623–1630.
42. **Muchardt, C., C. Sardet, B. Bourachot, C. Onufryk, and M. Yaniv.** 1995. A human protein with homology to Saccharomyces cerevisiae SNF5 interacts with the potential helicase hbrm. Nucleic Acids Res. **23:**1127–1132.
43. **Peterson, C. L., A. Dingwall, and M. P. Scott.** 1994. Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement. Proc. Natl. Acad. Sci. USA **91:**2905–2908.
44. **Peterson, C. L., and I. Herskowitz.** 1992. Characterization of the yeast SWI1, SWI2, and SWI3 genes, which encode a global activator of transcription. Cell **68:**573–583.
45. **Peterson, C. L., W. Kruger, and I. Herskowitz.** 1991. A functional interaction between the C-terminal domain of RNA polymerase II and the negative regulator SIN1. Cell **64:**1135–1143.
46. **Peterson, C. L., and J. W. Tamkun.** 1995. The SWI-SNF complex: a chromatin remodeling machine? Trends Biochem. Sci. **20:**143–146.
47. **Powers, S.** 1992. Genetic analysis of ras homologs in yeasts. Semin. Cancer Biol. **3:**209–218.
48. **Rose, M. D., F. Winston, and P. Hieter.** 1990. Methods in yeast genetics: a laboratory course manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
49. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
50. **Shortle, D., P. Novick, and D. Botstein.** 1984. Construction and genetic characterization of temperature-sensitive mutant alleles of the yeast actin gene. Proc. Natl. Acad. Sci. USA **81:**4889–4893.
51. **Shrivastava, A., S. Saleque, G. V. Kalpana, S. Artandi, S. P. Goff, and K. Calame.** 1993. Inhibition of transcriptional regulator Yin-Yang-1 by association with c-Myc. Science **262:**1889–1892.
52. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
53. **Strober, B. E., J. L. Dunaief, S. Guha, and S. P. Goff.** 1996. Functional interactions between the hBRM/hBRG1 transcriptional activators and the pRB family of proteins. Mol. Cell. Biol. **16:**1576–1583.
54. **Treich, I., B. R. Cairns, T. E. Brewster, and M. Carlson.** 1995. SNF11, a new component of the yeast SNF-SWI complex that interacts with a conserved region of SNF2. Mol. Cell. Biol. **15:**4240–4248.
55. **Vincent, A. C., and K. Struhl.** 1992. ACR1, a yeast ATF/CREB repressor. Mol. Cell. Biol. **12:**5394–5405.
56. **Wada, T., Y. Nogi, H. Handa, and T. Fukasawa.** 1990. Strain-specific lethal effect of the adenovirus E1A protein on Saccharomyces cerevisiae. Biochem. Biophys. Res. Commun. **170:**470–476.
57. **Whyte, P., N. M. Williamson, and E. Harlow.** 1989. Cellular targets for transformation by the adenovirus E1A proteins. Cell **56:**67–75.
58. **Wills, C., T. Martin, and T. Melham.** 1986. Effect of gluconeogenesis of mutants blocking two mitochondrial transport systems in the yeast *Saccharomyces cerevisiae*. Arch. Biochem. Biophys. **246:**306–320.
59. **Winston, F., and M. Carlson.** 1992. Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet. **8:**387–391.
60. **Wolffe, A. P.** 1994. Transcriptional activation. Switched-on chromatin. Curr. Biol. **4:**525–528.
61. **Yoshinaga, S. K., C. L. Peterson, I. Herskowitz, and R. K. Yamamoto.** 1992. Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. Science **258:**1598–1604.
62. **Zhou, Q., R. W. Gedrich, and D. A. Engel.** 1995. Transcriptional repression of the c-fos gene by YY1 is mediated by a direct interaction with ATF/CREB. J. Virol. **69:**4323–4330.
63. **Zieler, H. A., M. Walberg, and P. Berg.** 1995. Suppression of mutations in two *Saccharomyces cerevisiae* genes by the adenovirus E1A protein. Mol. Cell. Biol. **15:**3227–3237.