MOLECULAR AND CELLULAR BIOLOGY, June 1995, p. 3227–3237 Vol. 15, No. 6
0270-7306/95/$04.00+0

Suppression of Mutations in Two *Saccharomyces cerevisiae* Genes by the Adenovirus E1A Protein

HELGE A. ZIELER,[1]* MARK WALBERG,[2] AND PAUL BERG[1]

Department of Biochemistry, Stanford University School of Medicine, Stanford, California,[1] and Simmons Cancer Center, UT Southwestern Medical School, Dallas, Texas[2]

Received 26 October 1994/Returned for modification 6 January 1995/Accepted 7 March 1995

The protein products of the adenoviral E1A gene are implicated in a variety of transcriptional and cell cycle events, involving interactions with several proteins present in human cells, including parts of the transcriptional machinery and negative regulators of cell division such as the Rb gene product and p107. To determine if there are functional homologs of E1A in *Saccharomyces cerevisiae*, we have developed a genetic screen for mutants that depend on E1A for growth. The screen is based on a colony color sectoring assay which allows the identification of mutants dependent on the maintenance and expression of an E1A-containing plasmid. Using this screen, we have isolated five mutants that depend on expression of the 12S or 13S cDNA of E1A for growth. A plasmid shuffle assay confirms that the plasmid-dependent phenotype is due to the presence of either the 12S or the 13S E1A cDNA and that both forms of E1A rescue growth of all mutants equally well. The five mutants fall into two classes that were named *web1* and *web2* (for "wants E1A badly"). Plasmid shuffle assays with mutant forms of E1A show that conserved region 1 (CR1) is required for rescue of the growth of the *web1* and *web2* E1A-dependent yeast mutants, while the N-terminal 22 amino acids are only partially required; conserved region 2 (CR2) and the C terminus are dispensable. The phenotypes of mutants in both the *web1* and the *web2* groups are due to a single gene defect, and the yeast genes that fully complement the mutant phenotypes of both groups were cloned. The *WEB1* gene sequence encodes a 1,273-amino-acid protein that is identical to SEC31, a protein involved in the budding of transport vesicles from the endoplasmic reticulum. The *WEB2* gene encodes a 1,522-amino-acid protein with homology to nucleic acid-dependent ATPases. Deletion of either *WEB1* or *WEB2* is lethal. Expression of E1A is not able to rescue the lethality of either the *web1* or the *web2* null allele, implying allele-specific mutations that lead to E1A dependence.

Early in an adenoviral infection, the gene products of the adenovirus E1A gene induce the mammalian host cell to enter S phase and to transcribe viral genes at high levels (reviewed in references 13 and 46). Most, or possibly all, of the effects of E1A proteins result from transcriptional changes in the host cell. The E1A proteins stimulate or repress transcription from a variety of viral and cellular promoters (50). E1A up-regulates cellular genes involved in nucleotide metabolism and DNA synthesis (84, 87), cell cycle progression (51, 70), and energy metabolism (32) and down-regulates genes responsible for maintaining the differentiated state of cells (6, 80).

Two major E1A proteins are translated from differentially spliced 12S and 13S mRNAs; these differ only by a 46-amino-acid domain, conserved region 3 (CR3), which is unique to the protein translated from the 13S mRNA (53). For simplicity, the proteins derived from the two different messages will be referred to as the 12S and 13S forms of E1A.

The 13S E1A protein appears to affect transcription by direct interaction of the CR3 domain with various components of the general transcriptional machinery such as the TATA-binding protein (7, 24), ATF-2, and c-Jun (39). The 12S form of E1A stimulates or represses transcription of a different set of promoters and does so in a more indirect fashion. Three domains common to the 12S and 13S forms of E1A mediate interactions with a variety of cellular proteins (13). These domains are conserved regions 1 and 2 (CR1 and CR2) and the N-terminal domain comprising the 25 amino-terminal residues. The ability of the 12S form of E1A to bind to these host proteins correlates strongly with its capacity to immortalize and transform cells (82).

CR2 mediates interaction with the retinoblastoma protein, Rb (12), and related proteins p107 (82) and p130 (22), which themselves interact with transcription factors, such as the E2F proteins (51, 67), and with cell cycle control proteins, such as cell cycle-dependent kinases and cyclins (18, 27). Some of these interactions also depend on the N-terminal portion of CR1 (4, 19, 29, 56, 82). In adenovirus-infected cells, E1A is found in association with E2F, p107, cyclin A, and cdk2 kinase in large complexes that have DNA binding activity (19). The amino acid motif in CR2, L-X-C-X-E, which mediates the interactions of E1A with Rb-related proteins, is found in other viral oncoproteins such as the large T antigen of simian virus 40 (SV40) and the E7 protein of human papillomavirus (10, 14).

Both the carboxy-terminal half of CR1 and the N-terminal domain are required for binding to p300 (4, 71, 82), a nuclear phosphoprotein with DNA binding activity that interacts with TATA-binding protein (1, 57). Interaction with p300 appears to be required for E1A-induced progression of G_1 cells into S phase (30, 76) and for E1A-mediated repression of enhancer-activated transcription of other genes (31, 59). p300 is highly homologous to the CREB binding protein (CBP), which is thought to be a transcriptional coactivator of CREB (2).

Despite characterization of several E1A-binding proteins, very little is known about how E1A stimulates or represses transcription. The mechanism by which these interactions alter cell cycle control also remains unclear. The fact that E1A interacts with a large number of other proteins suggests that a

* Corresponding author. Present address: Laboratory of Malaria Research, Building 4, Room 126, National Institutes of Health, Bethesda, MD 20892.

genetic approach might help to identify and clarify the interactions important to the regulatory functions of E1A.

It is becoming increasingly evident that many components of cell cycle control and transcriptional regulation are highly conserved or interchangeable between *Saccharomyces cerevisiae* and humans (8, 9, 36, 52). The adenovirus E3 promoter, which is strongly E1A dependent in mammalian cells, is actively transcribed in *S. cerevisiae* and appears to depend on the same upstream sites for transcriptional stimulation (33). Thus, there may be regulatory mechanisms common to *S. cerevisiae* and human cells related to E1A function. Accordingly, we sought to identify E1A-dependent mutants of *S. cerevisiae* whose phenotypes could be complemented or suppressed by E1A.

In this report, we describe isolation and characterization of yeast mutants that are dependent for growth on expression of either the 12S or the 13S E1A proteins. Mutations in CR1 and, to a lesser degree, in the N-terminal domain of E1A reduce its ability to complement these mutants. The genes whose mutations are suppressed by E1A have been cloned, sequenced, and shown to be essential. Suppression by E1A is specific to the particular isolated mutant alleles.

MATERIALS AND METHODS

Strains, media, and general growth conditions. The *S. cerevisiae* strains used in this study are derived from the strains YMW1 (*MAT*α *ade2-1 ade3*Δ*22 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*) and YMW2 (*MAT***a**, isogenic to YMW1). E1A-dependent mutants were generated by mutagenesis of wild-type strain YMW1 with ethyl methanesulfonate, as previously described (66). YMW2 was used for backcrosses. Gene disruptions were done with a diploid strain, YMW3, obtained by mating YMW1 and YMW2. Strains were grown and maintained either on the rich medium yeast extract-peptone-dextrose (YPD) or on the rich medium yeast extract-peptone-galactose (YPGal). The E1A-dependent mutant strains were maintained on YPGal or on selective media containing galactose as a carbon source in order to allow continued expression of E1A. All media were prepared as previously described (68). The pHs of all media were adjusted to 5.2 to 5.4. All strains were grown at 30°C unless specifically indicated.

Plasmids. Plasmids used in this study are summarized below and in Fig. 3A. Maps and sequences of all plasmids are available upon request. pMW29 (PUC18 *CEN4 ARS1 URA3 ADE3 GAL1* promoter-*GAL7* terminator), pMW20 (PUC18 *CEN4 ARS1 URA3 GAL1* promoter-*GAL7* terminator), and pMW20L (PUC18 *CEN4 ARS1 LEU2 GAL1* promoter-*GAL7* terminator) were constructed as centromeric plasmids with a *GAL1* promoter capable of directing the expression of cDNAs. The E1A-expressing derivatives of these plasmids (pMW29-E1A12S and pMW29-E1A13S, etc.) were constructed by cloning 12S and 13S E1A cDNAs (37) into the polylinker between the *GAL1* promoter and the *GAL7* terminator. The deletion mutants of 12S E1A diagrammed in Fig. 3A were cloned into the polylinker of pMW20L as *Eco*RI-to-*Bam*HI fragments.

Summary of the mutations in the mutant forms of E1A. The mutations in the mutant forms of E1A were as follows: 12S-PM2,3, double point mutant changing the second codon from Arg to Ala and the third amino acid from His to Asp; 12S-TR22, E1A coding region truncated after codon 22 by insertion of a stop codon; 12S-CTRL22, four-amino-acid insertion (Pro-Ile-Asp-Gly) between amino acids 22 and 23; 12S-Δ9-22, deletion of amino acids 9 to 22; 12S-Δ4-22, deletion of amino acids 4 to 22 and insertion of two amino acids (Ser-Ala) between codons 3 (His) and 23 (Leu); 12S-Δ23-85, deletion of amino acids 23 to 85; 12S-Δ38-67, previously described mutation (56), deletion of amino acids 38 to 67, and insertion of three amino acids (Ser-Ser-Arg) between codons 37 and 68; 12S-Δ86-106, deletion of amino acids 86 to 106; 12S-Δ86-120, deletion of amino acids 86 to 120; 12S-PM124, previously described mutation (47), point mutation of amino acid 124 from Cys to Gly; 12S-Δ91-138, deletion of amino acids 91 to 138; 12S-Δ121-126, deletion of amino acids 121 to 126; 12S-Δ121-138, deletion of amino acids 121 to 138; 12S-Δ141-176, deletion of amino acids 141 to 176; 12S-Δ177-219, deletion of amino acids 177 to 219 and insertion of four amino acids, Ser-Gly-Ile-Pro, between amino acids 176 and 220; 12S-PM2,3,124, triple point mutation of amino acids 2, 3, and 124; 12S-Δ4-22,121-138, double deletion of amino acids 9 to 22 and 121 to 138; 12S-Δ4-22,121-138, double deletion of amino acids 4 to 22 and 121 to 138; and 12S-TR220, E1A coding region truncated after codon 220 by the insertion of a stop codon.

Sectoring assays and plasmid shuffle assays. Yeast strains containing null alleles in the *ADE2* and *ADE3* genes (*ade2 ade3*) appear white when grown on solid rich media (34). However, such double mutants carrying a plasmid that expresses *ADE3* give rise to uniformly red colonies because of the characteristic *ade2* phenotype (34). Loss of the *ADE3*-expressing plasmid and appearance of white sectors in a colony are normal occurrences for strains transformed with yeast centromeric plasmids that are grown on rich media in the absence of any selection (see Fig. 1). When the yeast strain is dependent on a gene on the plasmid for viability, no sectoring is observed (35). The sectoring assay is performed by streaking the appropriate yeast strains on YPGal medium and incubating the medium at 30°C for 5 to 6 days, by which time the red colony color due to the plasmid-borne *ADE3* gene is well developed.

The plasmid shuffle assay is designed to determine to what extent an incoming yeast plasmid transformed into a particular strain is able to relieve dependence on an existing essential plasmid already present in the strain (16). The plasmid shuffle is performed as follows: a nonsectoring, E1A-dependent mutant strain transformed with the E1A-expressing plasmid pMW29-E1A12S is transformed with a second plasmid (which lacks the *ADE3* color marker) and tested in a sectoring assay. The degree of sectoring reflects the degree to which the incoming plasmid supplies the activity essential for viability of the strain and allows loss of the *ADE3*-containing plasmid that confers the red colony color. The ability of pMW20L-E1A12S, but not of pMW20L, to restore sectoring of plasmid-dependent strains confirms the specific dependence on E1A for growth.

Arrest experiments and spot assays. Cultures of mutant yeast strains were inoculated at a density of 2×10^4 to 3×10^4 cells per ml and grown at 30°C. At a cell density of roughly 5×10^5 cells per ml, glucose was added to a final concentration of 2% in order to shut off E1A expression. Control cultures received galactose. Other cultures were arrested by adding KCl to a final concentration of 0.75 M and/or by changing the growth temperature from 30 to 38°C. Aliquots were removed periodically to determine cell densities and the cell cycle stage of the cells.

For the spot assays, yeast strains were streaked out on a fresh plate and incubated for 4 days at 30°C. Single colonies were scraped off the plates and suspended in 100 mM sorbitol, and cell concentrations were adjusted. Serial fivefold dilutions were made in a 96-well microtiter plate, and 3 μl of each dilution was spotted onto a plate. The plates were photographed after 2 to 3 days of growth at 30°C.

Libraries. A λ-YES yeast genomic library (17) was used for cloning of the *WEB1* and *WEB2* genes. Since the vector used in the construction of this library contains the yeast *GAL1* promoter, some of the *WEB2* clones isolated lack the putative *WEB2* promoter, have N-terminal truncations, and are expressed from the *GAL1* promoter in a galactose-dependent manner. *WEB1* was also cloned from a YCP50-based yeast genomic library (61).

Cloning of yeast genes complementing the *web1* and *web2* defects. The *WEB1* and *WEB2* genes were cloned by transforming the mutant strains with plasmid DNA from a yeast genomic library. Following 4 days of growth at 30°C, transformants were replica plated to selective media, which support the growth of wild-type yeast strains but not of the mutant strains (see Table 1 for phenotypes linked to E1A dependence). Plasmid DNA was isolated from transformed colonies resistant to the selective conditions as previously described (79). Restriction fragments from candidate inserts were cloned into pRS316, a yeast centromeric plasmid with the *URA3* selectable marker (69) and were assayed for their abilities to rescue the mutant phenotypes of the corresponding *web* strains.

Construction of *WEB1* and *WEB2* gene disruptions. *WEB1* and *WEB2* knockout plasmids pHZ 87 and pHZ 52 were constructed by cloning inverted fragments from the *WEB1* and *WEB2* genes into the yeast integrating plasmid YIPlac128 (21). These plasmids were linearized, transformed into diploid strain YMW3, and selected on minimal medium lacking leucine in a one-step gene replacement procedure (62). The *WEB1* disruption (strain YHZ12 *WEB1*/*web1*Δ::*LEU2*, isogenic to YMW3) results in the deletion of 78% of the *WEB1* coding region (3,037 bp deleted between *Hin*dIII upstream of the *WEB1* ATG and *Xba*I close to the 3′ end of the gene [see Fig. 5]) and insertion of the YIPlac128 plasmid sequences including a *LEU2* selectable marker. The *WEB2* disruption (strain YHZ11 *WEB2*/*web2*Δ::*LEU2*, isogenic to YMW3) results in the deletion of 36% of the *WEB2* coding region (2,476 bp deleted between *Eco*RV and *Eco*RI in the 3′ end of *WEB2*, including the C-terminal region showing homology to mammalian proteins [see Fig. 6]) and insertion of the YIplac128 plasmid sequences including a *LEU2* selectable marker. The correct disruptions were verified for both strains by Southern analysis.

Nucleotide sequence accession numbers. The sequence of the *WEB1* gene has been submitted to GenBank and is filed under accession number U15219. The sequence of *WEB2* can be found under GenBank accession number U00027 (gene H9986.16).

RESULTS

Expression of E1A in *S. cerevisiae*. Centromeric plasmids that express cDNAs encoding either the 12S form (pMW29-E1A12S) or the 13S form (pMW29-E1A13S) of adenovirus 5 E1A under the control of the galactose-inducible *GAL1* promoter were introduced into *S. cerevisiae* YMW1. Cells containing the plasmids produce E1A proteins in medium containing galactose but not in medium containing glucose (see Fig. 3B).

The plasmids mentioned above also contain the *ADE3* gene, resulting in a red colony color in *ade2 ade3* strains such as YMW1 and YMW2. When the YMW1 transformants are

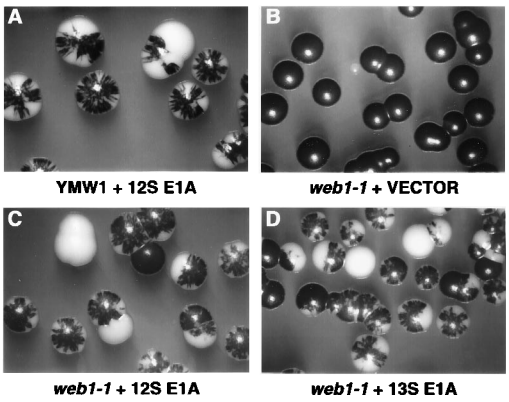

FIG. 1. Sectoring behavior of wild-type and mutant yeast strains. (A) Wild type strain (YMW1) transformed with the E1A-expressing plasmid pMW29-E1A12S; (B) sectoring of the *web1-1* mutant (YHZ1) transformed with control shuffle plasmid pMW20L; (C) sectoring of the *web1-1* mutant transformed with plasmid pMW20L-E1A12S, which expresses the 12S form of E1A; (D) sectoring of the *web1-1* mutant transformed with plasmid pMW20L-E1A13S expressing the 13S form of E1A. Transformants were streaked on YPGal and grown for 6 days at 30°C.

TABLE 1. Assignment of E1A-dependent mutants to complementation groups

Strain	*WEB* allele	Phenotypes
YHZ1	*web1-1*	Strongly plasmid-dependent on YPGal; temperature/osmotic defect, no growth at 37°C on YPGal + 0.75 M KCl; significantly lower plasmid dependence in minimal galactose medium; loss of E1A dependence on YPGal + 2 to 3% ethanol
YHZ2	*web1-2*	Same as YHZ1
YHZ3	*web1-3*	Same as YHZ1
YHZ4	*web2-1*	Less strongly plasmid dependent than the *web1* mutants on YPGal; temperature sensitive, no growth at 38°C; slightly reduced plasmid dependence in minimal galactose medium; slow-growth phenotype in YPGal, especially in liquid
YHZ5	*web2-2*	Less strongly plasmid dependent than the *web1* mutants on YPGal; slightly reduced plasmid dependence in minimal galactose medium

grown on rich medium containing galactose (YPGal) or glucose (YPD), the red colonies contain white sectors, reflecting the relatively high frequency of loss of the plasmid (Fig. 1A). A similar degree of colony sectoring is also seen with a plasmid that does not express E1A (pMW29 [not shown]). This indicates that the expression of either form of E1A does not appreciably alter the growth characteristics of the cells or the ability to segregate a centromeric plasmid. The sectored appearance of such yeast colonies permits rapid screening for mutants that are nonsectoring (unable to lose the plasmid) because they require a gene present on the plasmid (35, 74).

Yeast mutants are rescued by expression of either the 12S or the 13S E1A protein. To obtain mutants dependent on E1A, the YMW1 strain, transformed with pMW19-E1A12S or pMW19-E1A13S, was mutagenized with ethyl methanesulfonate and plated on rich medium containing galactose as a carbon source (YPGal). A total of 250,000 colonies were plated, 125,000 each for YMW1 transformed with either the 12S or the 13S form of E1A. Following 1 week of growth at 30°C, colonies that were nonsectoring (Fig. 1B) and galactose dependent were selected. A nonsectoring colony morphology and the inability to grow on YPD are diagnostic of cells that must retain and express the plasmid-borne E1A genes.

To confirm the dependence of these mutants on E1A, we used the plasmid shuffle assay, which relies on the ability of an incoming plasmid to provide a source of E1A and to relieve the requirement for the plasmid already present (16, 74). Each of the putative mutants detected in the initial screen was transformed with pMW20L-E1A12S, pMW20L-E1A13S, or pMW20L and then grown on YPGal. Mutants that sectored freely when transformed with the E1A-expressing shuffle plasmids but that remained all red when transformed with the control shuffle plasmid pMW20L were regarded as being dependent on E1A for growth (Fig. 1B, C, and D, shown only for *web1-1*).

Five mutants were recovered with this assay. Each of the five mutants is rescued to the same degree by the 12S and 13S forms of E1A (Fig. 1). Since the 12S form of E1A contains all but a 46-amino-acid domain present in the 13S form, all of the sequences necessary to complement the mutant phenotype must be present in 12S E1A. All subsequent experiments that addressed the ability of E1A to restore viability to the mutants were done with the 12S form.

The E1A-dependent mutants fall into two complementation groups. Each of the five mutants was backcrossed to YMW2 and sporulated, and E1A-dependent, nonsectoring, haploid segregants were recovered. Both mating types were recovered for each mutant, and the mutants were crossed together in all combinations. The resulting diploids were tested in a sectoring assay on YPGal and were either free-sectoring (wild-type phenotype) or nonsectoring (mutant phenotype). The sectoring behavior allowed assignment of each mutant to one of two complementation groups (Table 1). The groups were named *web1* and *web2* (for "wants E1A badly"). Three of the isolated mutants (mutants 5-1, 34, and 35) formed the *web1* group, and two others (mutants 53-1 and 46) constituted the *web2* group. After three backcrosses, the mutants were renamed in a manner consistent with other strain designations (Table 1).

Growth phenotypes of the E1A-dependent mutants. The sectoring behavior of the two groups of mutants differs reproducibly, with the *web1* mutants sectoring less than the *web2* mutants. This probably reflects a more complete growth defect resulting from the *web1* mutations compared with that of the *web2* mutations. Figure 2 shows the relative levels of E1A

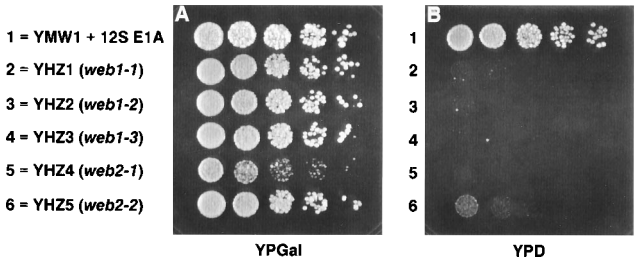

FIG. 2. Galactose dependence of the five E1A-dependent mutants. A wild-type strain transformed with plasmid pMW29-E1A12S expressing the 12S form of E1A (YMW1 + 12S E1A) and five E1A-dependent mutants expressing the 12S form of E1A from the same plasmid (YHZ1 to -5) were serially diluted and spotted in a horizontal series of spots onto plates of rich medium containing galactose (YPGal) (A) or glucose (YPD) (B). Each spot represents fivefold-more cells than the spot to the right of it. The leftmost spot contains about 7,500 cells. The plates were photographed after 2 days (YPD plate) or 2.5 days (YPGal plate) of growth at 30°C.

dependence of the five mutants compared with that of the wild-type YMW1 strain. All six strains grow similarly on YP Gal during 2.5 days, with the exception of the *web2-1* strain, which has a slower growth rate. It is apparent that the five mutant strains grow very poorly compared with the wild type after 2 days at 30°C on YPD. The *web2-2* strain is slightly less galactose dependent than the other four mutants, consistent with the higher sectoring rate of *web2* mutants on galactose. The galactose dependence of the *web2-1* strain resembles that of the *web1* mutants.

To establish that the need for galactose was linked to the induction of E1A and not to the utilization of galactose as a carbon source, each of the five mutants was transformed with a plasmid that expresses a fusion protein consisting of the *GAL4* DNA binding domain and ER.VP16, a transcriptional activation domain dependent on steroid hormones for activity. This plasmid, GAL4.ER.VP16, can induce the *GAL1* promoter in glucose medium in the presence of 3×10^{-7} M β-estradiol (40). Growth of the E1A-dependent mutants on YPD was normal only in the presence of the hormone. Without β-estradiol, each of the mutants containing the GAL4.ER.VP16 construct was as defective for growth on YPD as the untransformed mutants (data not shown). This result suggests that suppression of the *web1* and *web2* growth defects by E1A occurs in various rich media and does not depend directly on the utilization of galactose as a carbon source.

The phenotypes of *web1* and *web2* mutants are due to single gene defects. Tetrad analysis was performed on the sporulation products of diploid strains heterozygous for the *web1* or the *web2* mutations. Tetrads dissected on YPD plates resulted in a clear pattern of 2 wild type:2 dead spores for all five mutants (data not shown). Therefore, the inability of the mutants to grow in the presence of glucose, i.e., in the absence of E1A expression, segregates as a single mutation in both groups of mutants.

Dissections on YPGal of the *web1* heterozygous diploids (YHZ6, -7, and -8) gave rise to two wild-type colonies per tetrad (white or sectoring) as well as zero, one, or two nonsectoring segregants. The nonsectoring segregants represent spores that received the *web1* allele as well as the E1A-expressing plasmid. The segregation of E1A dependence is consistent with a single gene defect whose lethality can be rescued by cosegregation of the E1A-containing plasmid and activation of the *GAL1* promoter driving E1A (not shown).

Tetrads obtained from the *web2* heterozygous diploids (YHZ9 and -10) typically gave rise to four viable spores on galactose (not shown). Two of these were phenotypically wild type, while the other two grew extremely slowly. Cosegregation of the E1A-expressing plasmid into one of the slowly growing spores resulted in a more rapid growth rate and an all-red colony. As in the case of *web1*, the *web2* mutations segregate as a single gene defect. However, the *web2* mutations do not cause complete loss of viability on galactose-containing medium.

Table 1 summarizes several other phenotypes that are a consequence of the *web1* and *web2* mutations. These phenotypes were found after three backcrosses in all haploid segregants that exhibited the nonsectoring colony morphology. The three *web1* mutants have a temperature and osmotic sensitivity in addition to their E1A dependence. The *web2-1* mutant strain is temperature sensitive at 38°C. Since these mutant phenotypes are due to the mutations that cause E1A dependence, they were used to clone the *WEB1* and *WEB2* genes (see below).

The plasmid dependence of both groups of mutants is more pronounced on YPGal than on synthetic medium. This may reflect an E1A requirement at higher growth rates, because growth on rich medium at 16°C was also accompanied by a higher rate of sectoring. When each of the five mutants is plated on medium containing glucose and grown for several days, colonies that have lost the E1A-expressing plasmid arise. Nevertheless, cells from these colonies generally retain the temperature or osmotic sensitivities of the mutant strains and are heterogeneous in their growth rates. Thus, the E1A dependence of these mutants is not observed in all media and under all growth conditions. This is especially true of the *web1* mutants which grow independently of E1A on rich medium containing 2 to 3% ethanol (Table 1).

TABLE 2. Summary of arrest phenotypes of *web* mutants

Mutant	Arrest phenotypes
web1-1, *-2*, *-3*	Complete arrest within two cell divisions following the addition of glucose; complete and immediate arrest upon addition of KCl and raising the temperature to 38°C; arrest at all stages of the cell cycle
web2-1	Incomplete arrest following the addition of glucose; complete arrest within one cell division after raising the temperature to 38°C; arrest both in glucose and at high temperature with >90% of all cells enlarged with large buds
web2-2	Incomplete arrest following the addition of glucose; no arrest at high temperatures; arrest in the presence of glucose with >90% of all cells enlarged with large buds

***web2* mutants arrest on glucose in a cell cycle-dependent fashion as cells with large buds.** To explore the physiological consequences of the *web1* and *web2* mutations, representatives of both groups were grown logarithmically in liquid culture under permissive conditions. Glucose was added to the cultures at a low cell density, in order to shut off E1A expression. Identical cultures were shifted to the nonpermissive temperature, or the KCl concentration was raised to 0.75 M and the incubation was continued at 38°C. The results of these experiments are summarized in Table 2.

The *web1* mutants (YHZ1, -2, and -3) arrest within two cell divisions following the addition of glucose to a YPGal culture grown at 30°C. Following the addition of KCl and a shift to 38°C, the cultures arrested without completing another division. The arrest in response to osmotic and temperature stress was observed irrespective of whether glucose or galactose was the carbon source. Each of the *web1* mutants arrested at various stages of the cell cycle, with no signs of a specific cell cycle arrest.

The *web2* mutants (YHZ4 and -5) did not completely arrest upon the addition of glucose, reflecting the lower plasmid dependence observed during growth on YPGal plates. However, when the temperature was shifted to 38°C, the cells of the *web2-1* strain arrested within one cell division. More than 90% of the arrested cells appeared as greatly enlarged cells with large buds. A single nucleus was observed in the vicinity of the neck of the bud when the arrested cells were stained with 4′,6-diamidino-2-phenylindole (DAPI; not shown). The same morphology was observed in *web2-1* and *web2-2* cultures arrested by the addition of glucose.

Conserved region 1 is necessary but not sufficient for the growth rescue of *web1* and *web2* mutants. Considering the wealth of information about E1A's activities in mammalian cells (13, 45), it was important to determine which domains of E1A were responsible for the ability to support the growth of

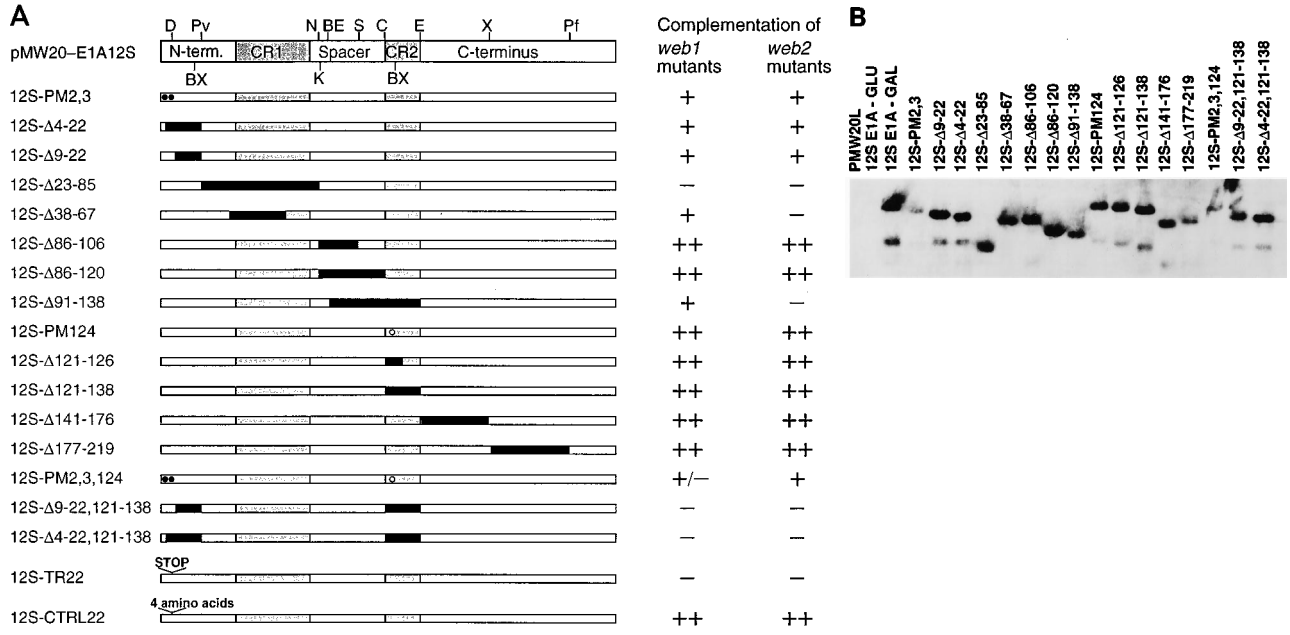

FIG. 3. Mutant forms of E1A. (A) Schematic diagram of the mutant forms of E1A used in the plasmid shuffle. The diagram at the top represents the 12S E1A gene with restriction sites used in the construction of the mutants (D, *Dsa*I; BX, *Bst*XI; Pv, *Pvu*II; N, *Nae*I; K, *Kas*I; BE, *Bsp*EI; S, *Sty*I; C, *Cla*I; E, *Eco*O109I; X, *Xba*I; Pf, *Pfl*MI). Domains of the E1A 12S protein that are encoded by the various parts of the gene are denoted by shading and captions within the box. The diagrams below the E1A gene represent mutant forms of E1A; black regions are sequences deleted in the mutants, and small circles within the box denote point mutations. STOP and 4 amino acids, insertions in the gene. The adjacent table summarizes the abilities of various mutant E1As to complement the *web1* or *web2* mutants. Activities were assigned to the E1A mutants on the basis of their ability to rescue sectoring in a plasmid shuffle assay and their ability to sustain growth of E1A-dependent yeast mutants in a spot assay. ++, wild-type activity; +, some activity; +/−, low but detectable activity; −, no activity. (B) Western blot (immunoblot) of protein extracts from a wild-type yeast strain (YMW1) expressing the various forms of E1A. Protein extracts were electrophoresed on an SDS–10% polyacrylamide–urea gel, transferred to nylon membrane, and probed with the E1A-specific M73 antibody.

the *web1* and *web2* mutants. Accordingly, a series of point mutations and in-frame deletions were made in the E1A coding sequence (Fig. 3A), and these modified E1A genes were incorporated into plasmids under the transcriptional control of the *GAL1* promoter. These plasmids resemble the shuffle plasmids pMW20L-E1A12S and pMW20L-E1A13S discussed earlier.

Following introduction into YMW1 and induction with galactose, each of the constructs was shown to be expressed as a protein of the predicted size (Fig. 3B). Protein extracts from YMW1 expressing the different mutant forms of E1A were electrophoresed on sodium dodecyl sulfate (SDS)–polyacrylamide–7.5 M urea gels and probed with the M73 monoclonal antibody that recognizes an epitope at the extreme C terminus of E1A (3, 26). All of the mutant forms of E1A were expressed to approximately the same level, with the exception of the point mutants 12S-PM2,3 and 12S-PM2,3,124, whose expression varied from 10 to 50% of that of the wild-type E1A protein (Fig. 3B).

The plasmid shuffle assay was used to determine the abilities of the modified forms of E1A to rescue sectoring of the *web1* and *web2* groups. The plasmid pMW20L, which lacks an E1A insert, and the plasmid 12S-TR22 served as negative controls. The plasmids pMW20L-E1A12S and 12S-CTRL22 provided positive controls (Fig. 3A). The abilities of the various constructs to provide E1A activity in the sectoring assay are summarized in Fig. 3A. Three different levels of E1A activity were discernible: wild-type activity, partial activity, and no activity. The activity of a particular E1A mutant as judged by its sectoring behavior was very similar with individual members of the *web1* and *web2* groups of mutants. Mutations in the regions that have been implicated as sites of interactions with mammalian proteins are discussed in detail below, with a focus in particular on the N terminus, CR1, and CR2 (Fig. 3A).

N terminus. Three N-terminal mutations (12S-PM2,3, 12S-Δ4-22, and 12S-Δ9-22) reduce the ability of E1A to restore sectoring in the *web* mutants without abolishing it completely. This effect is more pronounced in the deletion mutants than in the point mutant.

CR1. An extended deletion (12S-Δ23-85) which removes CR1 as well as a part of the N terminus completely abolishes E1A activity in *web1* as well as *web2* mutants. A smaller deletion that disrupts only the N-terminal half of CR1 (12S-Δ38-67) impairs E1A function almost as much as the larger deletion (12S-Δ23-85), but it affects the *web2* mutants more than the *web1* mutants.

CR2. E1A constructs with point mutations and deletions that affect only CR2 (12S-PM124, 12S-Δ121-126, and 12S-Δ121-138) have wild-type activity in the sectoring assay. However, a large deletion that removes part of the CR1-CR2 spacer region and all of CR2 (12S-Δ91-138) has severe effects on E1A activity in the *web2* group and only moderate effects in the *web1* group.

Deletions of other parts of the protein such as the CR1-CR2 spacer or sequences beyond CR2 (mutants 12S-Δ86-106, 12S-Δ86-120, 12S-Δ141-176, and 12S-Δ177-219) have little effect on E1A's ability to suppress the growth defect of the *web1* and *web2* mutants. However, a mutation that deletes the extreme C terminus (12S-TR220) reduces the amount of sectoring in all five mutants. Since this truncation removes both the nuclear localization signal and the epitope of the M73 monoclonal antibody (3, 41), the reason for its lower activity is uncertain. In summary, the results with the mutants described so far suggest that CR1, and to a lesser degree the N terminus, is necessary

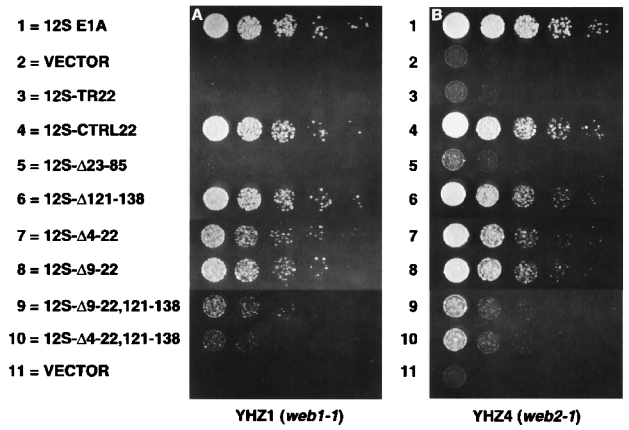

FIG. 4. Spot assays showing the relative activities of mutant forms of E1A. The *web1-1* strain (YHZ1) (A) and the *web2-1* strain (YHZ4) (B) transformed with a single plasmid expressing a mutant form of E1A were spotted in serial dilutions onto YPGal plates and incubated at 30°C. Each horizontal series of spots represents a single transformant, with each spot having received five times as many cells as the spot immediately to the right of it. Equivalent numbers of cells are present in every vertical column. The leftmost spot in each row received about 7,500 cells.

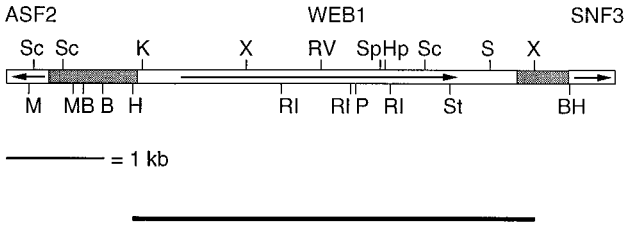

FIG. 5. Characterization of the *WEB1* gene. The top part of the diagram is a schematic representation of the 6-kb insert of the RC16-1 library clone that complements all of the mutant phenotypes of the *web1-1* mutant (YHZ1). Various restriction sites are indicated (Sc, *Sac*I; M, *Mlu*I; B, *Bgl*II; H, *Hin*dIII; K, *Kpn*I; X, *Xho*I; RI, *Eco*RI; RV, *Eco*RV; P, *Pml*I; Sp, *Spe*I; Hp, *Hpa*I; St, *Stu*I; S, *Sal*I; BH, *Bam*HI). The coding regions of *WEB1* and two adjacent genes are unshaded. Shaded regions denote sequences between the open reading frames. The black bar below shows the smallest subfragment of the RC16-1 insert which fully complements the *web1* mutant strains.

for E1A to suppress the growth defect of the *web1* and *web2* mutants. The remaining sequences appear to contribute little to E1A's function in suppressing *web* mutations.

To test the supposition that an intact CR1 domain is sufficient for E1A to suppress the *web1* and *web2* growth defect, several double mutants were constructed and tested in the same way. These include two double deletion mutants (12S-Δ4-22,121-138 and 12S-Δ9-22,121-138) and a double point mutant (12S-PM2,3,124) that combines the mutations of 12S-PM2,3 and 12S-PM124. Each of the three double mutant proteins was much less active in the sectoring assay than expected on the basis of the activities of the corresponding single mutants. Indeed, the activity of the two mutants with double deletions was almost as low as the activity of the mutant with only the large CR1 deletion (12S-Δ23-85), even though the CR1 domain was left completely intact. Expression of the complete CR1 domain is not sufficient, therefore, for suppressing the *web* mutants. In mammalian cells, similar double mutations also substantially reduce the activity of E1A and in this way resemble deletions that remove all of CR1 (30, 76).

Spot assays confirm the relative activities of mutant forms of E1A. The abilities of mutant forms of E1A to support growth of the five *web* mutants were tested in a second assay. After the five *web* mutants were transformed with the shuffle plasmids containing the modified E1A genes, the cells were cured of the wild-type E1A-expressing plasmid by being grown on minimal medium at room temperature. Under these conditions, the *web* mutants are able to grow in the absence of E1A, possibly because of a reduced dependence on E1A in media and at temperatures that fail to support rapid growth (Table 1). The single transformants were then spotted in serial dilutions onto YPGal plates and incubated at 30°C for 2 days. Under these conditions, the mutants are strongly dependent on E1A and, as a consequence, the reduced activity of the modified E1As results in lower growth rates. Figure 4 shows the results of the spot assay of the *web1-1* and *web2-1* strains transformed with the indicated forms of E1A. As anticipated, there were striking differences in the growth rates of the various transformants. The E1A activity measured in this way paralleled the results of the sectoring assay. An apparent exception to this seems to be the two N-terminal mutants of E1A (12S-Δ4-22 and 12S-Δ9-22), which appear to have almost wild-type activity in the spot assay when tested with the *web2-1* mutant. However, in other experiments the difference between these constructs and the wild-type 12S E1A was more evident, and therefore we conclude that there is a small but significant difference between the activities of the N-terminal mutants and the wild-type activity of pMW20L-E1A12S or 12S-Δ121-138.

The *WEB1* gene complements all of the phenotypes of the three mutants in the *web1* group. To better understand the genetic defects leading to E1A dependence, we sought to identify the genes whose defects are suppressed by E1A expression. Accordingly, the *web1-1* strain (YHZ1) was transformed separately with two yeast genomic libraries in centromeric plasmids, and transformants were selected for their abilities to grow at 37°C on rich medium (YPD or YPGal) containing 0.75 M KCl and to sector freely on YPGal medium. Three complementing plasmid clones whose inserts overlapped, as determined by cross-hybridization, were obtained (not shown). The shortest of these, RC16-1, had an insert of approximately 6 kb (Fig. 5). Various subfragments of RC16-1 were cloned into pRS316 (69) and analyzed for their abilities to rescue the plasmid dependence and osmotic sensitivity of the three mutants in the *web1* group. A 4.1-kb *Hin*dIII-*Xho*I subfragment of the RC16-1 insert was the smallest fragment that fully restored the wild-type phenotype of the *web1-1* mutant (Fig. 5).

The sequence of the RC16-1 insert revealed an open reading frame of 3,819 bp encoding a 1,273-amino-acid protein. No strong similarity was found to any protein in the database, nor was there any significant homology to E1A. The gene was named *WEB1* and was mapped to the right arm of chromosome 4 between *SNF3* and *ASF2*, on the basis of sequence overlaps between our clone and the sequences of *SNF3* and *ASF2* in the GenBank database. The protein encoded by *WEB1* contains nine WD-40 repeats (11, 72), which are 40-amino-acid-long repeats containing a 15-amino-acid core motif. WD-40 repeats were originally identified in the β-subunits of heterotrimeric G proteins and have since been found in a variety of proteins with different functions and cellular localizations. The WD-40 motifs are clustered in the N-terminal half of the WEB1 protein. A region rich in prolines (20% proline content over 380 amino acids) occurs in the C-terminal half.

The mutant alleles from the three strains in the *web1* group were isolated by colony hybridization. The three mutations in the *WEB1* gene were mapped to an N-terminal part of the gene, and the mutant sequence was determined. Although isolated independently, the three mutants of the *web1* group

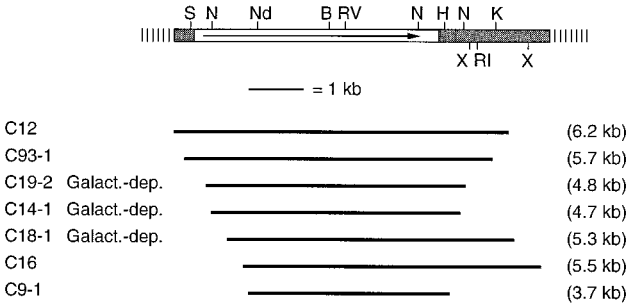

FIG. 6. Characterization of the *WEB2* gene. Diagram of overlapping genomic fragments from library clones complementing the mutants in the *web2* group (YHZ4 and -5). The diagram at the top of the figure shows a region on chromosome 8 with its restriction sites (S, *Sca*I; N, *Nco*I; Nd, *Nde*I; B, *Bgl*I; RV, *Eco*RV; H, *Hpa*I; X, *Xho*I; RI, *Eco*RI; K, *Kas*I). The open reading frame encoding the *WEB2* protein is unshaded and is surrounded by noncoding sequences (shaded). The black bars below show overlapping inserts from seven plasmids, isolated from a yeast genomic library, that complement the *web2* mutations. Some of the inserts were dependent on transcription from the *GAL1* promoter present on the λ-YES library plasmid and are indicated by Galact.-dep.

(YHZ1, -2, and -3) all contain the same mutation, a single base change that replaces Asp-189 with Asn. This mutation affects one of the conserved residues in the fourth WD-40 repeat.

A fortuitous circumstance allowed us to compare the sequence of *WEB1* with the unpublished sequence of *SEC31* isolated by Nina Salama and Randy Schekman (63). The two genes are identical in sequence and map to the same chromosomal position. *SEC31* encodes a 150-kDa protein that forms a complex with the SEC13 gene product and provides an essential cytoplasmic activity for the budding of transport vesicles from the endoplasmic reticulum in vitro (55, 64). Thus, it appears that expression of the 12S E1A gene in *S. cerevisiae* is able to suppress a lethal mutation in *WEB1*/*SEC31.*

The *WEB2* gene shows homology to mammalian proteins. Fourteen overlapping plasmid clones were isolated from a centromeric plasmid-based yeast genomic library that reproducibly complemented the temperature sensitivity and nonsectoring phenotype of both mutants in the *web2* group. Several of these are diagrammed in Fig. 6. The map position of the putative gene was determined by hybridizing a fragment from clone C93-1 to dot blots of phage lambda clones containing yeast genomic DNA inserts (58). The locus was mapped to the right arm of chromosome 8 next to *DBF3*/*PRP8*. The sequence of this region, obtained from Mark Vaudin (from cosmid clone 9986, GenBank accession number U00027, gene H9986.16), established that the locus spanned by the 14 overlapping clones corresponds to a 4,569-bp open reading frame that can be translated into a protein of 1,522 amino acids. Because several of the 14 complementing clones are truncated at the N terminus, we presume that the N-terminal portion of this protein is dispensable for complementation of the *web2* mutants. In some cases, expression of the complementing activity is dependent on the *GAL1* promoter of the λ-YES library plasmid. All clones contain at least three quarters of the coding region, including the entire C terminus (Fig. 6). None of the complementing clones had C-terminal truncations.

Tight linkage exists between the *web2* mutations and the cloned *WEB2* gene (not shown), indicating that mutations in this gene are directly responsible for the E1A-dependent phenotype. In addition, the alleles of *WEB2* cloned from the two *web2* mutant strains are defective, as they are unable to complement either the *WEB2* deletion or the phenotypes of the *web2* mutant strains (75).

The protein encoded by the *WEB2* gene has no homology to the E1A proteins, but it does show limited homology to several mammalian and yeast nucleic acid binding proteins. WEB2 is most strongly homologous to SμBP2, a human protein that binds specifically to a single-stranded guanine-rich sequence related to the immunoglobulin μ-chain switch region (20). SμBP2 has homologs in mice (43) and hamsters (unpublished data; GenBank accession number L15625) which are more than 75% identical to the human sequence. All three proteins contain motifs found in helicases and nucleic acid-dependent ATPases (23). The homology of WEB2 to these three proteins is localized to the C terminus of WEB2 and extends over 400 amino acids, with an average of 25% sequence identity including several of the helicase- and DNA-dependent ATPase motifs.

Disruptions of *WEB1* and *WEB2* are lethal and not suppressible by E1A. Disruptions of the *WEB1* and *WEB2* genes in diploid strains were made by the one-step gene replacement procedure (62). The partial gene deletions were accompanied by insertion of the *LEU2* selectable marker in both strains. The strains were sporulated and tetrads were dissected on YPD and YPGal. A 2:0 segregation of two viable and two dead spores was observed for YHZ11 (*web2*Δ/*WEB2*) and YHZ12 (*web1*Δ/*WEB1*) on both media. There were no Leu^+ segregants recovered amongst several hundred spores obtained from the heterozygous *WEB1* and *WEB2* strains (Fig. 7A, shown only for *WEB1*). These results clearly indicate that both *WEB1* and *WEB2* are essential genes.

YHZ11 and YHZ12 were transformed with centromeric plasmids expressing E1A, *WEB1*, or *WEB2* to test whether the chromosomal deletions could be rescued by expression of either of the three genes. After sporulation and dissection, the colonies were replicated onto synthetic media lacking uracil or leucine in order to monitor segregation of the markers. YHZ12 (*WEB1*/*web1*Δ) bearing pHZ60 (*URA3 WEB1*) gave rise to spores that were Leu^+ and therefore had received the knockout allele of *WEB1*; all of these spores were also Ura^+. Therefore, a plasmid-borne *WEB1* gene rescues the lethal *WEB1* chromosomal disruption (Fig. 7B). By contrast, neither the E1A-expressing plasmid pMW29-E1A12S (*URA3* p*GAL1*-E1A12S) nor the *WEB2*-expressing plasmid C93-1 (*URA3 WEB2*) rescued the lethality of a *WEB1* knockout (Fig. 7C, middle panel, shown only for pMW29-E1A12S). However, pMW29-E1A12S segregated normally into the wild-type spores (Fig. 7C, bottom panel). E1A expression is therefore unable to rescue a deletion of the *WEB1* gene. Similarly, the *WEB2* knockout allele (strain YHZ11) could be rescued by cosegregation with a plasmid expressing the wild-type *WEB2* gene but not by plasmids that expressed *WEB1* or E1A (not shown).

These results make it highly unlikely that E1A can provide all of the functions performed by the *WEB1* and *WEB2* genes. While it is still possible that E1A is able to perform one of several functions normally performed by the wild-type *WEB1* or *WEB2* protein, it seems more likely that the suppressive effect of E1A is indirect. E1A could activate other pathways which bypass the requirement for a particular function or activity of *WEB1* and *WEB2*, or it could interact directly with the mutant forms of *WEB1* and *WEB2* to restore their stability or activity. Further work is needed to distinguish among these possibilities.

DISCUSSION

The isolation of yeast mutants dependent on expression of the adenoviral oncogene E1A was motivated by the quite sim-

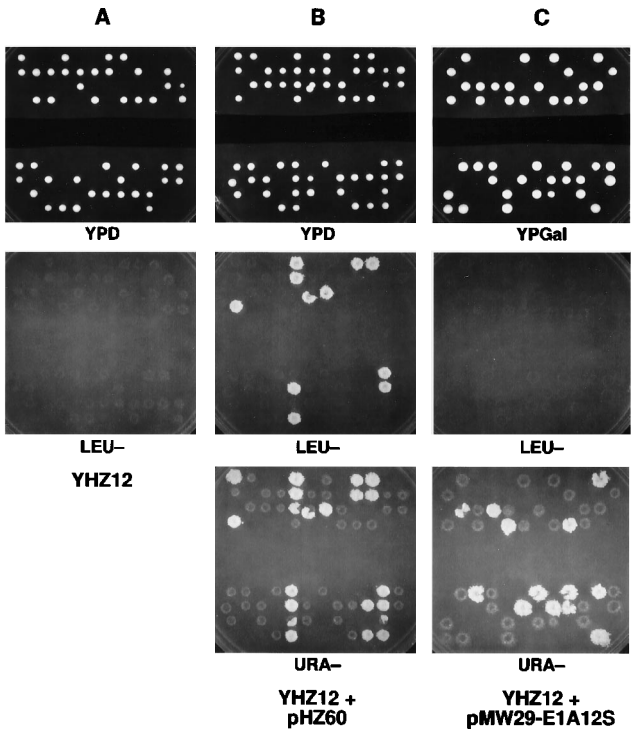

FIG. 7. Disruptions of *WEB1* and *WEB2* are lethal and are not complemented by E1A. (A) Deletion of the *WEB1* gene is lethal. The YHZ12 strain heterozygous for the *WEB1* deletion was sporulated, and tetrads were dissected on YPD (top plate). When colonies had grown, they were replica plated onto leucine dropout plates (LEU$^-$; bottom plate). No Leu$^+$ colonies were found, indicating lethality of the disruption. (B) Deletion of the *WEB1* gene is rescued by *WEB1* on a centromeric plasmid. YHZ12 transformed with pHZ 60 (*CEN ARS URA3 WEB1*) was sporulated, and tetrads were dissected on YPD (top plate). When colonies had grown, they were replica plated onto leucine dropout plates (LEU$^-$; middle plate) and uracil dropout plates (URA$^-$; bottom plate). All colonies that are Leu$^+$ are also Ura$^+$. (C) Deletion of the *WEB1* gene is not rescued by E1A expression. YHZ12 transformed with pMW29-E1A12S (*CEN4 ARS1 URA3* p*GAL1*-E1A12S) was sporulated, and tetrads were dissected on YPD (top plate). When colonies had grown, they were replica plated onto leucine dropout plates (LEU$^-$; middle plate) and uracil dropout plates (URA$^-$; bottom plate). No Leu$^+$ colonies were found, yet the *URA3*-containing plasmid expressing E1A segregated at a normal frequency into the viable spores (URA$^-$; bottom plate).

ple premise that the machinery for basic cell processes, e.g., transcription and cell cycle control, is mediated by sets of proteins that are highly conserved in all eukaryotes (8, 9, 36). E1A is a nuclear oncoprotein that affects both transcription and cell cycle in mammalian cells, and we sought to determine if it is capable of performing an essential function in a distantly related organism such as *S. cerevisiae*. Moreover, by studying E1A function in *S. cerevisiae*, a set of powerful genetic tools is available to explore the nature of this function.

The aim of our mutant screen was to identify lethal mutations in *S. cerevisiae* that are suppressible by E1A. This approach makes no assumptions about the nature of E1A functions. However, the screen allows only the isolation of mutations in genes whose function is essential and not redundantly encoded in the yeast genome. E1A may function by directly replacing some or all of the functions performed by the mutated yeast protein, or it may act as an indirect suppressor of particular mutations. The system that we developed to isolate such mutants includes a colony color sectoring assay that allows rapid screening for plasmid-dependent mutants by their nonsectoring colony morphology.

General characteristics of the E1A-dependent *web* mutants. We isolated five yeast mutants which can be rescued by expression of the 12S form of E1A. Although screens for dependence on either the 12S or the 13S E1A proteins were done, mutants specifically dependent on the 13S form of E1A were not observed. The dependence of these mutants specifically on E1A expression, and not on anything else on the plasmid, was demonstrated in plasmid shuffle experiments.

The five mutants fall into two complementation groups, named *web1* and *web2*. The E1A-dependent phenotype of each group stems from mutations in only a single gene. Mutants in the *web1* group are more tightly E1A dependent, but the arrest upon glucose repression of E1A expression does not occur at a specific stage in the cell cycle. By contrast, shutoff of E1A expression causes the *web2* mutants to specifically arrest as enlarged cells with large buds. This morphology is observed when the *web2* strains are grown in the absence of E1A or at the nonpermissive temperature (*web2-1* only).

A puzzling feature of the E1A requirement is its variability under different growth conditions. Thus, the E1A dependence is strongest on rich medium at 30°C and is reduced to various degrees on minimal medium, at lower temperatures, or on specific media. Loss of the E1A dependence experienced under such growth conditions is generally not a permanent change; when regrown under conditions of strong E1A dependence, the cells that have lost the E1A-expressing plasmid show a growth defect (Fig. 4). It is likely that the dependence on E1A is most pronounced at maximal growth rates.

Features of the *web1* mutants and the *WEB1* gene. One route to understanding the functional relationship between E1A and *WEB1* is to determine the molecular nature of *WEB1*. Accordingly, the *WEB1* gene was cloned and sequenced. *WEB1* does not contain any strong homology to any other known protein, but it has noteworthy features in two separate regions of the protein. The N-terminal half of *WEB1* contains 9 WD-40 repeats which were originally identified in the β-subunits of heterotrimeric G proteins (11, 72). WD-40 repeats are thought to be involved in interactions with other proteins, although the structure formed by the repeats has not been defined (60). The C terminus of *WEB1* contains a region rich in prolines. The three mutant alleles of *WEB1* have been isolated and sequenced, and although the three *web1* mutants were independently isolated, they all show the same mutation of Asp-189 to Asn in the fourth WD-40 repeat of *WEB1*. We speculate that the WD-40 repeat affected by mutation in the *web1* mutants may mediate the interaction between *WEB1* and another yeast protein.

An unexpected finding was that the *WEB1* gene is identical to *SEC31*, whose protein product associates with the SEC13 protein to form a large complex essential for the budding of transport vesicles from the endoplasmic reticulum (5, 55, 64). A C-terminal mutation in *WEB1*/*SEC31* has been isolated by the Schekman laboratory, and this mutation results in a temperature-sensitive strain because of a transport defect (63). The temperature sensitivity caused by this mutation is not suppressed by E1A (86).

The suppression by E1A of a mutation in *WEB1*/*SEC31* is not easily explained by any of the known mammalian functions of E1A. In adenoviral infections, the E1A proteins are found exclusively in the nucleus (41, 65, 81), and all their functions described to date are consistent with a nuclear localization. However, immunofluorescence studies with antibodies specific to *WEB1*/*SEC31* show punctate cytoplasmic staining with an absence of nuclear staining (63). While it is formally possible that E1A can suppress mutations in *WEB1* by actively participating in the transport process, this idea is contradicted by

previous studies which have shown that the 13S form of E1A localizes predominantly to the nucleus of a yeast cell while the 12S form is found throughout the cell (73). Since both forms are able to rescue viability of the *web1* and *web2* mutants, we consider it likely that the relevant function of E1A occurs in the nucleus. We cannot rule out, however, the possibility that a fraction of the E1A protein is found in the vicinity of the endoplasmic reticulum and acts there to affect the activity of vesicle transport complexes.

Suppression of a mutation in a transport protein may also be indirect and linked to transcriptional or other changes caused by E1A. It is known that E1A stimulates the expression of heat shock proteins in mammalian cells and *S. cerevisiae* (25, 49), and this may be a mechanism by which E1A could suppress mutations in different yeast genes. Mutations in the transport machinery are also known to affect transcription of genes involved in cell growth, such as ribosomal protein genes (42). A partial transport block caused by the *web1* mutations may cause transcriptional changes in the cell that are incompatible with cell growth but can be counteracted by E1A. However, E1A does not seem to act by stimulating the expression of *WEB1*. Expression of E1A affects neither *WEB1* mRNA levels nor the in vivo enzyme activity of plasmid constructs that fuse the *WEB1* coding region to β-galactosidase (85).

Features of the *web2* mutants and the *WEB2* gene. Shutoff of E1A expression in the *web2* mutants causes them to specifically arrest as enlarged cells with large buds, suggesting that turning off expression of E1A (or shifting the *web2-1* mutant to the nonpermissive temperature) affects passage through the cell cycle. The large-bud cell morphology of *web2* mutants in response to shutoff of E1A expression resembles that of cells arrested because of mutations in proteins required for passage through S phase (54). Fluorescence-activated cell sorter analysis indicates that the cells arrested in the large-bud state have a 2N DNA content, consistent with an arrest in G_2/M (75). Thus, the cell cycle block caused by the *web2* mutations is suppressed by E1A, indicating that E1A is able to rescue a cell cycle defect in the *web2* mutants, and seems to act at a stage of the yeast cell cycle which is known to be influenced by E1A in mammalian cells.

The protein encoded by the *WEB2* gene contains homology to mammalian DNA-binding proteins and therefore may be a DNA-binding protein involved in transcription or DNA replication. Although *WEB2* is unlikely to be a yeast homolog of these mammalian genes, it is possible that the sequence homology reflects a functional similarity. The mutations found in the *web2-1* and *web2-2* mutants have not yet been mapped and sequenced, but several lines of evidence indicate that the cloned *WEB2* gene corresponds to the gene that is defective in the *web2* strains. First, in tetrad analyses tight linkage is observed between the *WEB2* gene and the mutation causing E1A dependence. Second, the mutant alleles cloned from genomic DNA of the *web2* mutants are unable to complement either the *WEB2* deletion or the mutant phenotypes of the *web2* mutants. Current experiments are aimed at characterizing in greater detail the function of *WEB2* and the nature of the suppression of the *web2* mutations by E1A. As in the case of *WEB1*, *WEB2* mRNA levels are not induced by E1A (75).

Although there is strong evidence that defects in the cloned *WEB1* and *WEB2* genes cause the mutants' E1A-dependent phenotype, our results indicate that E1A is probably not replacing the function of *WEB1* or *WEB2*. Neither gene shows any homology to E1A, nor is E1A able to rescue the viability of the null alleles. When expressed in primary baby rat kidney cells (BRK cells), neither *WEB1* nor *WEB2* shows any of the immortalization or transforming activities characteristic of E1A (48). As a result we have essentially ruled out the possibility that the two genes are functional homologs of E1A. It is likely that E1A acts as a suppressor of specific point mutations in *WEB1* and *WEB2*. E1A may associate with or stabilize the mutant *web1* and *web2* gene products, may stimulate expression of genes capable of suppressing the *web* mutations, or may activate parallel pathways that can obviate the need for particular functions of the WEB proteins.

Domains of E1A required for suppression of the *web* defects. Keeping in mind the various transcriptional and proliferative activities of E1A in mammalian cells that have been mapped to specific regions within the protein, we sought to identify the sequences of the 12S form of E1A that are required for suppression of the *web* mutations. This was done by making specific structural alterations in E1A and determining the abilities of the modified forms to suppress the *web1* and *web2* mutant defects. CR1 appears to be essential, the N terminus is somewhat required, and CR2 is completely dispensable for suppression of both *web1* and *web2* growth defects. However, mutations affecting both the N terminus and CR2 abolish E1A activity to the same extent as CR1 deletions. There are only two deletion clones that show a significant difference between the two groups of *web* mutants. Both 12S-Δ38-67 and 12S-Δ91-138 show more severe effects in *web2* mutants than in *web1* mutants.

While there are several possible ways of explaining the observed pattern of required and cooperating sequences, we find it striking that the sequences within E1A that seem to mediate suppression of the *web* mutants are very similar to sequences that are known to interact with mammalian proteins. As a result, we consider it possible that the manner by which E1A suppresses the phenotypes caused by *web* mutations in *S. cerevisiae* is related to how E1A affects transcription and cell cycle processes in mammalian cells.

The observation that CR1 is necessary but not sufficient for the suppression of *web* mutations may seem contrary to the relatively specific interaction of mammalian proteins with short stretches of sequence within E1A. Yet various activities of E1A in mammalian cells, such as the induction of DNA synthesis in BRK cells, have similarly cooperative sequence requirements. Mutants that lack CR1 or double mutants in both the N terminus and CR2 are unable to induce S phase (30, 38, 44, 76). This reflects binding of distinct sets of cellular proteins with the N terminus and with CR2; binding of both sets of proteins may be required for efficient induction of DNA synthesis. CR1 is also required, since it participates in interactions with CR2-binding proteins as well as with proteins that bind to the N terminus (4, 15, 82). Recent results also suggest that the binding of mammalian proteins to the different domains of E1A is cooperative, explaining why some activities are apparently dependent on E1A's interaction with more than one mammalian protein (4, 77, 78). Our results do not distinguish between one or multiple cellular binding partners of E1A in *S. cerevisiae*, and investigations to identify yeast proteins that interact with E1A are under way.

The full-length and various N-terminal fragments of SV40 large T antigen were also expressed in the *web* mutants to test for the ability of T antigen to replace E1A in the sectoring assay. Interestingly, expression of an N-terminal domain of SV40 large T antigen suppresses the *web2-1* mutation and partially suppresses the *web2-2* mutation (28). This fragment contains 147 amino acids, including the Rb binding site and sequences that are thought to be similar in function to the p300-binding parts of E1A (83). Larger fragments of SV40 large T antigen that contain the same sequences and that are expressed to similar levels in *S. cerevisiae* do not complement

the *web2* mutants in the same manner. As a result, there may be similarities between the rescue of the cell cycle defects of the *web2* mutations by E1A and SV40 large T antigen and the cell cycle activities of these two viral oncoproteins in mammalian cells. The *web1* mutants are not rescued by any of the SV40 large T antigen constructs (28).

Conclusion. The screen for E1A-dependent yeast mutants described in this study was successful in isolating two essential yeast genes whose partial loss of function can be suppressed by E1A. Biochemical studies and additional genetic analysis are needed to identify the direct targets of E1A in *S. cerevisiae*. The genetic tools of *S. cerevisiae* and the battery of mutant forms of E1A should then be valuable for dissecting in detail the function of such targets and their interaction with E1A. Further investigation may unravel the mechanism by which E1A rescues growth of the *web* mutants and may allow the use of *S. cerevisiae* as a model system to study the function of the 12S form of E1A.

ACKNOWLEDGMENTS

We thank Betty Moran for providing the 12S E1A mutants 928 and Δ38-67, for helpful discussions, and for critical reading of the manuscript, Sandy Ramer for providing the λ-YES phage yeast genomic library, Nina Salama and Randy Schekman for discussions about their unpublished observations, John McCusker and Steve Elledge for numerous helpful suggestions and discussions in the course of this work, Mark Vaudin for providing the sequence of cosmid clone 9986 before publication, Paolo Sassone-Corsi for clones containing the 12S and 13S E1A cDNAs, and Jim Alwine for a cDNA clone of SV40 large T antigen.

This work was supported by grant GM13235 from the National Institute of General Medical Sciences. Helge Zieler was supported by NIH training grant GM07599. Mark Walberg was a Howard Hughes Medical Institute Physician Postdoctorate Fellow.

REFERENCES

1. **Abraham, S. E., S. Lobo, P. Yaciuk, H. G. Wang, and E. Moran.** 1993. p300, and p300-associated proteins, are components of TATA-binding protein (TBP) complexes. Oncogene **8:**1639–1647.
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. **Arsenault, H., and J. M. Weber.** 1993. Mapping of the mAb73 epitope on Ad2 E1A proteins with random peptide libraries and deletion mutants. FEMS Microbiol. Lett. **114:**37–40.
4. **Barbeau, D., R. Charbonneau, S. G. Whalen, S. T. Bayley, and P. E. Branton.** 1994. Functional interactions within adenovirus E1A protein complexes. Oncogene **9:**359–373.
5. **Barlowe, C., L. Orci, T. Yeung, M. Hosobuchi, S. Hamamoto, N. Salama, M. F. Rexach, M. Ravazzola, M. Amherdt, and R. Schekman.** 1994. COPII: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. Cell **77:**895–907.
6. **Boulukos, K. E., and E. B. Ziff.** 1993. Adenovirus 5 E1A proteins disrupt the neuronal phenotype and growth factor responsiveness of PC12 cells by a conserved region 1-dependent mechanism. Oncogene **8:**237–248.
7. **Boyer, T. G., and A. J. Berk.** 1993. Functional interaction of adenovirus E1A with holo-TFIID. Genes Dev. **7:**1810–1823.
8. **Buratowski, S., S. Hahn, P. A. Sharp, and L. Guarente.** 1988. Function of a yeast TATA element-binding protein in a mammalian transcription system. Nature (London) **334:**37–42.
9. **Conaway, R. C., and J. W. Conaway.** 1993. General initiation factors for RNA polymerase II. Annu. Rev. Biochem. **62:**161–190.
10. **DeCaprio, J. A., J. W. Ludlow, J. Figge, J. Y. Shew, C. M. Huang, W. H. Lee, E. Marsilio, E. Paucha, and D. M. Livingston.** 1988. SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. Cell **54:**275–283.
11. **Duronio, R. J., J. I. Gordon, and M. S. Boguski.** 1992. Comparative analysis of the beta transducin family with identification of several new members including PWP1, a nonessential gene of Saccharomyces cerevisiae that is divergently transcribed from NMT1. Proteins **13:**41–56.
12. **Dyson, N., P. Guida, C. McCall, and E. Harlow.** 1992. Adenovirus E1A makes two distinct contacts with the retinoblastoma protein. J. Virol. **66:** 4606–4611.
13. **Dyson, N., and E. Harlow.** 1992. Adenovirus E1A targets key regulators of cell proliferation. Cancer Surv. **12:**161–195.
14. **Dyson, N., P. M. Howley, K. Munger, and E. Harlow.** 1989. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science **243:**934–937.
15. **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.
16. **Elledge, S. J., and R. W. Davis.** 1988. A family of versatile centromeric vectors designed for use in the sectoring-shuffle mutagenesis assay in Saccharomyces cerevisiae. Gene **70:**303–312.
17. **Elledge, S. J., J. T. Mulligan, S. W. Ramer, M. Spottswood, and R. W. Davis.** 1991. Lambda YES: a multifunctional cDNA expression vector for the isolation of genes by complementation of yeast and Escherichia coli mutations. Proc. Natl. Acad. Sci. USA **88:**1731–1735.
18. **Faha, B., E. Harlow, and E. Lees.** 1993. The adenovirus E1A-associated kinase consists of cyclin E-p33cdk2 and cyclin A-p33cdk2. J. Virol. **67:**2456–2465.
19. **Fattaey, A. R., E. Harlow, and K. Helin.** 1993. Independent regions of adenovirus E1A are required for binding to and dissociation of E2F-protein complexes. Mol. Cell. Biol. **13:**7267–7277.
20. **Fukita, Y., T. R. Mizuta, M. Shirozu, K. Ozawa, A. Shimizu, and T. Honjo.** 1993. The human S mu bp-2, a DNA-binding protein specific to the single-stranded guanine-rich sequence related to the immunoglobulin mu chain switch region. J. Biol. Chem. **268:**17463–17470.
21. **Gietz, R. D., and A. Sugino.** 1988. New yeast-Escherichia coli shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene **74:**527–534.
22. **Giordano, A., C. McCall, P. Whyte, and B. Franza, Jr.** 1991. Human cyclin A and the retinoblastoma protein interact with similar but distinguishable sequences in the adenovirus E1A gene product. Oncogene **6:**481–485.
23. **Gorbalenya, A. E., E. V. Koonin, A. P. Donchenko, and V. M. Blinov.** 1989. Two related superfamilies of putative helicases involved in replication, recombination, repair and expression of DNA and RNA genomes. Nucleic Acids Res. **17:**4713–4730.
24. **Green, M., P. M. Loewenstein, R. Pusztai, and J. S. Symington.** 1988. An adenovirus E1A protein domain activates transcription in vivo and in vitro in the absence of protein synthesis. Cell **53:**921–926.
25. **Handa, H., T. Toda, M. Tajima, T. Wada, H. Iida, and T. Fukasawa.** 1987. Expression of the human adenovirus E1a product in yeast. Gene **58:**127–136.
26. **Harlow, E., B. 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.
27. **Herrmann, C. H., L. K. Su, and E. Harlow.** 1991. Adenovirus E1A is associated with a serine/threonine protein kinase. J. Virol. **65:**5848–5859.
28. **Higgins, M., and H. A. Zieler.** 1994. Unpublished data.
29. **Howe, J. A., and S. T. Bayley.** 1992. Effects of Ad5 E1A mutant viruses on the cell cycle in relation to the binding of cellular proteins including the retinoblastoma protein and cyclin A. Virology **186:**15–24.
30. **Howe, J. A., J. S. Mymryk, C. Egan, P. E. Branton, and S. T. Bayley.** 1990. Retinoblastoma growth suppressor and a 300-kDa protein appear to regulate cellular DNA synthesis. Proc. Natl. Acad. Sci. USA **87:**5883–5887.
31. **Jelsma, T. N., J. A. Howe, J. S. Mymryk, C. M. Evelegh, N. F. Cunniff, and S. T. Bayley.** 1989. Sequences in E1A proteins of human adenovirus 5 required for cell transformation, repression of a transcriptional enhancer, and induction of proliferating cell nuclear antigen. Virology **171:**120–130.
32. **Kaddurah-Daouk, R., J. W. Lillie, G. H. Daouk, M. R. Green, R. Kingston, and P. Schimmel.** 1990. Induction of a cellular enzyme for energy metabolism by transforming domains of adenovirus E1a. Mol. Cell. Biol. **10:**1476–1483.
33. **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.
34. **Koshland, D., J. C. Kent, and L. H. Hartwell.** 1985. Genetic analysis of the mitotic transmission of minichromosomes. Cell **40:**393–403.
35. **Kranz, J. E., and C. Holm.** 1990. Cloning by function: an alternative approach for identifying yeast homologs of genes from other organisms. Proc. Natl. Acad. Sci. USA **87:**6629–6633.
36. **Lee, M. G., and P. Nurse.** 1987. Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2. Nature (London) **327:**31–35.
37. **Leff, T., R. Elkaim, C. R. Goding, P. Jalinot, P. Sassone-Corsi, M. Perricaudet, C. Kedinger, and P. Chambon.** 1984. Individual products of the adenovirus 12S and 13S EIa mRNAs stimulate viral EIIa and EIII expression at the transcriptional level. Proc. Natl. Acad. Sci. USA **81:**4381–4385.
38. **Lillie, J. W., P. M. Loewenstein, M. R. Green, and M. Green.** 1987. Functional domains of adenovirus type 5 E1a proteins. Cell **50:**1091–1100.
39. **Liu, F., and M. R. Green.** 1994. Promoter targeting by adenovirus E1a through interaction with different cellular DNA-binding domains. Nature (London) **368:**520–525.
40. **Louvion, J. F., B. Havaux-Copf, and D. Picard.** 1993. Fusion of GAL4-VP16 to a steroid-binding domain provides a tool for gratuitous induction of galactose-responsive genes in yeast. Gene **131:**129–134.
41. **Lyons, R. H., B. Q. Ferguson, and M. Rosenberg.** 1987. Pentapeptide nuclear

localization signal in adenovirus E1a. Mol. Cell. Biol. **7:**2451–2456.
42. **Mizuta, K., and J. R. Warner.** 1994. Continued functioning of the secretory pathway is essential for ribosome synthesis. Mol. Cell. Biol. **14:**2493–2502.
43. **Mizuta, T. R., Y. Fukita, T. Miyoshi, A. Shimizu, and T. Honjo.** 1993. Isolation of cDNA encoding a binding protein specific to 5′-phosphorylated single-stranded DNA with G-rich sequences. Nucleic Acids Res. **21:**1761–1766.
44. **Moran, B., and B. Zerler.** 1988. Interactions between cell growth-regulating domains in the products of the adenovirus E1A oncogene. Mol. Cell. Biol. **8:**1756–1764.
45. **Moran, E.** 1993. DNA tumor virus transforming proteins and the cell cycle. Curr. Opin. Genet. Dev. **3:**63–70.
46. **Moran, E.** 1993. Interaction of adenoviral proteins with pRB and p53. FASEB J. **7:**880–885.
47. **Moran, E., B. Zerler, T. M. Harrison, and M. B. Mathews.** 1986. Identification of separate domains in the adenovirus E1A gene for immortalization activity and the activation of virus early genes. Mol. Cell. Biol. **6:**3470–3480.
48. **Moran, E., and H. A. Zieler.** 1994. Unpublished data.
49. **Nevins, J. R.** 1982. Induction of the synthesis of a 70,000 dalton mammalian heat shock protein by the adenovirus E1A gene product. Cell **29:**913–919.
50. **Nevins, J. R.** 1986. Control of cellular and viral transcription during adenovirus infection. Crit. Rev. Biochem. **19:**307–322.
51. **Nevins, J. R.** 1992. E2F: a link between the Rb tumor suppressor protein and viral oncoproteins. Science **258:**424–429.
52. **Norbury, C., and P. Nurse.** 1992. Animal cell cycles and their control. Annu. Rev. Biochem. **61:**441–470.
53. **Perricaudet, M., G. Akusjarvi, A. Virtanen, and U. Pettersson.** 1979. Structure of two spliced mRNAs from the transforming region of human subgroup C adenoviruses. Nature (London) **281:**694–696.
54. **Pringle, J. R., and L. H. Hartwell.** 1981. The *Saccharomyces cerevisiae* cell cycle, p. 97–142. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*, vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
55. **Pryer, N. K., N. R. Salama, R. Schekman, and C. A. Kaiser.** 1993. Cytosolic Sec13p complex is required for vesicle formation from the endoplasmic reticulum in vitro. J. Cell Biol. **120:**865–875.
56. **Raychaudhuri, P., S. Bagchi, S. H. Devoto, V. B. Kraus, E. Moran, and J. R. Nevins.** 1991. Domains of the adenovirus E1A protein required for oncogenic activity are also required for dissociation of E2F transcription factor complexes. Genes Dev. **5:**1200–1211.
57. **Rikitake, Y., and E. Moran.** 1992. DNA-binding properties of the E1A-associated 300-kilodalton protein. Mol. Cell. Biol. **12:**2826–2836.
58. **Riles, L., J. E. Dutchik, A. Baktha, B. K. McCauley, E. C. Thayer, M. P. Leckie, V. V. Braden, J. E. Depke, and M. V. Olson.** 1993. Physical maps of the six smallest chromosomes of Saccharomyces cerevisiae at a resolution of 2.6 kilobase pairs. Genetics **134:**81–150.
59. **Rochette-Egly, C., C. Fromental, and P. Chambon.** 1990. General repression of enhanson activity by the adenovirus-2 E1A proteins. Genes Dev. **4:**137–150.
60. **Ron, D., C. H. Chen, J. Caldwell, L. Jamieson, E. Orr, and D. Mochly-Rosen.** 1994. Cloning of an intracellular receptor for protein kinase C: a homolog of the beta subunit of G proteins. Proc. Natl. Acad. Sci. USA **91:**839–843.
61. **Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink.** 1987. A Saccharomyces cerevisiae genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60:**237–243.
62. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101:**202–211.
63. **Salama, N. R., and R. W. Schekman.** 1994. Personal communication.
64. **Salama, N. R., T. Yeung, and R. W. Schekman.** 1993. The Sec13p complex and reconstitution of vesicle budding from the ER with purified cytosolic proteins. EMBO J. **12:**4073–4082.
65. **Schmitt, R. C., M. L. Fahnestock, and J. B. Lewis.** 1987. Differential nuclear localization of the major adenovirus type 2 E1a proteins. J. Virol. **61:**247–255.
66. **Schnell, R., L. D'Ari, M. Foss, D. Goodman, and J. Rine.** 1989. Genetic and molecular characterization of suppressors of SIR4 mutations in Saccharomyces cerevisiae. Genetics **122:**29–46.
67. **Schwarz, J. K., S. H. Devoto, E. J. Smith, S. P. Chellappan, L. Jakoi, and J. R. Nevins.** 1993. Interactions of the p107 and Rb proteins with E2F during the cell proliferation response. EMBO J. **12:**1013–1020.
68. **Sherman, F., G. R. Fink, and C. W. Lawrence.** 1979. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
69. **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.
70. **Spitkovsky, D., P. Steiner, J. Lukas, E. Lees, M. Pagano, A. Schulze, S. Joswig, D. Picard, M. Tommasino, and M. Eilers.** 1994. Modulation of cyclin gene expression by adenovirus E1A in a cell line with E1A-dependent conditional proliferation. J. Virol. **68:**2206–2214.
71. **Stein, R. W., M. Corrigan, P. Yaciuk, J. Whelan, and E. Moran.** 1990. Analysis of E1A-mediated growth regulation functions: binding of the 300-kilodalton cellular product correlates with E1A enhancer repression function and DNA synthesis-inducing activity. J. Virol. **64:**4421–4427.
72. **van der Voorn, L., and H. L. Ploegh.** 1992. The WD-40 repeat. FEBS Lett. **307:**131–134.
73. **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.
74. **Walberg, M. W., and P. Berg.** 1994. Unpublished data.
75. **Walberg, M. W., and Z. X. Xuan.** 1994. Unpublished data.
76. **Wang, H. G., G. Draetta, and E. Moran.** 1991. E1A induces phosphorylation of the retinoblastoma protein independently of direct physical association between the E1A and retinoblastoma products. Mol. Cell. Biol. **11:**4253–4265.
77. **Wang, H. G., Y. Rikitake, M. C. Carter, P. Yaciuk, S. E. Abraham, B. Zerler, and E. Moran.** 1993. Identification of specific adenovirus E1A N-terminal residues critical to the binding of cellular proteins and to the control of cell growth. J. Virol. **67:**476–488.
78. **Wang, H. G., P. Yaciuk, R. P. Ricciardi, M. Green, K. Yokoyama, and E. Moran.** 1993. The E1A products of oncogenic adenovirus serotype 12 include amino-terminally modified forms able to bind the retinoblastoma protein but not p300. J. Virol. **67:**4804–4813.
79. **Ward, A. C.** 1990. Single-step purification of shuttle vectors from yeast for high frequency back-transformation into E. coli. Nucleic Acids Res. **18:**5319.
80. **Webster, K. A., G. E. Muscat, and L. Kedes.** 1988. Adenovirus E1A products suppress myogenic differentiation and inhibit transcription from muscle-specific promoters. Nature (London) **332:**553–557.
81. **White, E., D. Spector, and W. Welch.** 1988. Differential distribution of the adenovirus E1A proteins and colocalization of E1A with the 70-kilodalton cellular heat shock protein in infected cells. J. Virol. **62:**4153–4166.
82. **Whyte, P., N. M. Williamson, and E. Harlow.** 1989. Cellular targets for transformation by the adenovirus E1A proteins. Cell **56:**67–75.
83. **Yaciuk, P., M. C. Carter, J. M. Pipas, and E. Moran.** 1991. Simian virus 40 large-T antigen expresses a biological activity complementary to the p300-associated transforming function of the adenovirus E1A gene products. Mol. Cell. Biol. **11:**2116–2124.
84. **Yoder, S. S., B. L. Robberson, E. J. Leys, A. G. Hook, M. Al-Ubaidi, C. Y. Yeung, R. E. Kellems, and S. M. Berget.** 1983. Control of cellular gene expression during adenovirus infection: induction and shut-off of dihydrofolate reductase gene expression by adenovirus type 2. Mol. Cell. Biol. **3:**819–828.
85. **Zieler, H. A.** 1994. Unpublished data.
86. **Zieler, H. A., and N. R. Salama.** 1994. Unpublished data.
87. **Zimmerman, J., Jr., K. Raska, Jr., and W. A. Strohl.** 1970. The response of BHK21 cells to infection with type 12 adenovirus. IV. Activation of DNA-synthesizing apparatus. Virology **42:**1147–1150.