infection[13,14]. On the other hand, there is evidence that the E1A products stimulate transcription of the E1A transcription unit early in adenovirus infection[29,30]. It appears, therefore, that the E1A products can act as both negative and positive regulators of their own transcription units during lytic infection, and as positive regulators of all other viral transcription units (see refs 13–15). Note that the SV40 early gene product, the large-T antigen, also regulates its own synthesis, but by a more 'classical' repression mechanism that does not involve an interaction with the SV40 enhancer[31]. In addition, like the E1A products, the large-T antigen is a pleiotropic protein also activating transcription, as it has been reported recently that it stimulates the activity of the SV40 late promoter[32]. It is not known whether inhibition of cellular enhancers by the E1A products occurs during productive infection or during cell transformation by adenoviruses. As yet, the expression of only one defined gene has been shown clearly to be repressed by E1A products. The expression of a class I major histocompatibility complex (MHC) gene is repressed in rat cells transformed by the E1A transcription unit of the highly oncogenic adenovirus-12 (Ad-12), but not by that of the weakly oncogenic adenovirus-5 (ref. 19). Since the E1A products of Ad-12 also inhibit activation of transcription by SV40 and Py enhancers (our unpublished observations), we are now investigating the possibility that this class I MHC gene contains an enhancer whose activity can be repressed by the Ad-12 E1A products.

Gene expression in eukaryotic cells may be controlled by regulatory factors repressing enhancer activity. This has not yet been reported. However, the Py enhancer is not active in undifferentiated embryonal carcinoma cells, although mutations in the region containing the Py enhancer restore its activity in these cells[33–35]. It has been suggested that embryonal carcinoma cells express a protein with properties similar to those of the E1A products[36]. This protein may repress the activity of the Py enhancer. Thus, in addition to demonstrating that the adenovirus E1A products have both negative and positive regulatory functions, our study raises the possibility that cellular enhancers are regulated not only positively as suggested previously[1,2,4,6–8], but also negatively, which would significantly increase the combinatorial possibilities for the control of gene expression at the transcriptional level in eukaryotes.

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A bacterial repressor protein or a yeast transcriptional terminator can block upstream activation of a yeast gene

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A bacterial repressor protein blocks transcription of a gene in yeast when its operator is placed in the promoter between the upstream activator sequence and the transcription start point. Putative transcription terminators from yeast installed in the same region of the promoter have a similar effect.

PROMOTERS recognized by RNA polymerase II of the yeast *Saccharomyces cerevisiae* contain two essential elements: a stretch of DNA containing a sequence homologous to TATA, and an upstream activator sequence (UAS)[1]. For example, synthesis of *GAL1* mRNA, which begins 80 nucleotides downstream of the TATA region of the *GAL1* promoter, depends on a sequence called UAS_G located about 250 nucleotides upstream of the TATA region[24] (see Fig. 1). UAS_G function has been localized to a 75-nucleotide sequence[2], and, more recently, to a 15-base pair (bp) sequence with approximate 2-fold rotational symmetry (E. Giniger *et al.*, in preparation). The *GAL4* gene product is thought to bind to this sequence in the presence of galactose to activate transcription downstream. When it is placed in front of the TATA region of *CYC1*, UAS_G activates transcrip-

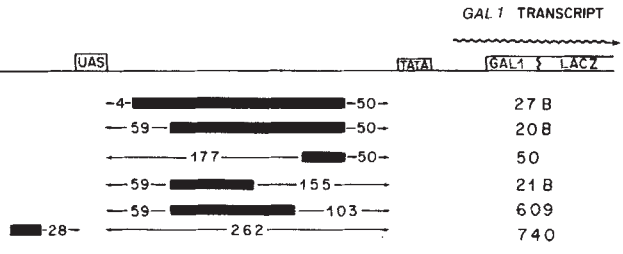

Fig. 1 Derivatives of the *GAL1* promoter. All carried a *Xho*I site into which *lexA* operators were inserted. Distance from the *Xho*I site to the TATA region and to the upstream activator sequence for various promoter derivatives is given in nucleotides. The UAS_G region contains two homologous 15-nucleotide regions of approximate 2-fold rotational symmetry separated by three nucleotides (one of which is sufficient for UAS_G function; E. Giniger *et al.*, in preparation). When defined in this way, there are 262 nucleotides between UAS_G and the first base of the TATA sequence. Numbering in *GAL1* derivatives is from the centre of the *Xho*I recognition sequence to the closest base in the upstream activator sequence, and to the first T of the *GAL1* TATA sequence. The mature *GAL1* transcript begins 85 nucleotides downstream of this T[4], and the *GAL1* coding sequence begins 60 nucleotides farther downstream[2,4]. The extent of promoter deleted in each derivative is shown by a dark bar. 36 nucleotides are deleted in 740. Plasmids containing sites for *lexA* operator insertion were made by combining members of two sets of deletions[2]. 609 and 740 were constructed as described in ref. 2. 20B and 27B are described in ref. 2. 50 and 21B were gifts of M. S. Lamphier.

tion from the hybrid promoter and renders this transcription inducible by galactose[5]. The distance between UAS_G and a TATA region is not critical, and the effect of UAS_G is still measurable even when it is located 500 nucleotides upstream of a TATA region (R. Yocum, personal communication). In these respects, UAS_G shares properties with the transcriptional enhancer sequences found in higher eukaryotes.

We describe two different ways to block *GAL1* promoter function by interposing two different kinds of genetic elements between its upstream activator sequence and its TATA region.

We constructed a strain of yeast that synthesizes lexA protein, a repressor protein from *Escherichia coli*. We also constructed variants of the yeast *GAL1* promoter that carried *lexA* operators at different positions within the promoter. We found that those *GAL1* promoters carrying a *lexA* operator between the upstream activator sequence and the TATA region were repressed by *lexA* protein.

We next installed sequences located downstream of the coding regions of the *CYC1* and *ADH1* genes between the *GAL1* upstream activator sequence and its TATA region. These sequences diminished *GAL1* transcription. A fragment of DNA containing a mutation interfering with proper *CYC1* termination and differing from the terminator fragment by a single base pair, was less able to decrease *GAL1* transcription.

LexA protein represses transcription

LexA protein is a repressor of many of the genes induced in *E. coli* as a consequence of DNA damage[6-9]. A plasmid directing the synthesis of LexA protein in yeast was constructed (Fig. 2). Use of anti-LexA antiserum allowed us to detect LexA protein in extracts of yeast carrying this plasmid. We estimate that the LexA protein comprises 0.005–0.025% of the total protein when yeast containing this plasmid are grown on galactose-containing medium. We inserted synthetic *lexA* operators into derivatives of the *GAL1* promoter (described in Fig. 1 legend) and chose a *lexA* operator sequence based on analysis of the sequence of many LexA protein-binding sites and on some of their interactions with the protein. Gel analysis of DNA–protein complexes[10] (K. Chapman and R.B., unpublished data) revealed that the synthetic 22-nucleotide operator bound LexA protein at least as tightly as any single naturally occurring operator thus far

Table 1 LexA protein repression of *GAL1* promoter with *lexA* operators

	Control plasmid	*lexA* plasmid
20B	5,000	5,000
+1 op	2,500	350
+2 op	2,500	250
27B	1,800	1,800
+1 op	1,000	150
50	5,000	5,000
+1 op	2,500	600
+2 op	2,400	500
21B	5,000	5,000
+1 op	2,500	400
+2 op	2,300	250
609	5,000	5,000
+1 op	3,100	400
740	5,000	5,000
+1 op	5,000	5,000

Yeast was transformed with two types of plasmids having two different selectable markers. One plasmid directed the synthesis of LexA protein and carried the *LEU2* marker. Another plasmid contained a derivative of the yeast *GAL1* promoter with a *lexA* operator site and carried the *URA3* marker. Transformed yeast were isolated and propagated on medium that selected for cells bearing both plasmids. The amount of transcription from the *GAL1* promoter was measured after cultures of doubly transformed cells had been grown on galactose-containing medium for at least five generations. The derivatives of the *GAL1* promoter in which *lexA* operators were installed are shown in Fig. 1. Synthetic *lexA* operators with the sequence

TCGAGTACTGTATGTACATACAGTAC

CATGACATACATGTATGTCATGAGCT

were inserted directly into *Xho*I-cut *GAL1* promoter derivatives (shown in Fig. 1) using standard plasmid construction techniques[19]. To construct a given doubly transformed strain, DNA from one of these plasmids and DNA from either pAAH5 (control plasmid) or pRB500 (which directed the synthesis of LexA protein) was used to transform strain DBY 745 to URA^+ and to LEU^+[20]. The amount of *GAL1* transcription in a given strain was estimated from indicator plates[28]. The numbers in this table and in the tables below refer to units of β-galactosidase measured in liquid assays as described in ref. 3. Each strain was assayed in triplicate on at least five different occasions. Normally the level of β-galactosidase in a given transformed strain varied by no more than 20% on the day of the assay. Assay-to-assay variation of the ratio of galactosidase activity from any two different strains was usually less than 10%. Measured units were averaged and rounded to the nearest 50 units. op, Operator.

Table 2 Effect of LexA protein on transcription of an operator containing *GAL1* promoter integrated into the chromosome

Control plasmid	2,000
lexA plasmid	200

Plasmid 118 (20B+2 lexA operators) was partially digested with *Eco*RI, ligated, and the ligation mixture cut with *Xba*I to linearize plasmids that retained the *Eco*RI fragment from 118 containing the 2μ origin. The ligation mixture was used to transform *E. coli* strain JM101[21], which lacks a functional *lacZ* gene. 427, a derivative of 118 that lacks the *Eco*RI fragment containing the replication origin, was isolated from a $lacZ^+$, Amp^R colony. 427 was cut in its *URA3* gene with *Apa*I and lithium acetate-treated DBY745 was transformed (see Table 1 legend) with the cut plasmid DNA. Six *URA* transformants arising from this construction were checked to see that the *URA* marker was not lost after growth for 40 generations on nonselective medium, and that *GAL1* promoter transcription in these strains was normally inducible if the yeast were grown on medium containing galactose. These six strains were made competent with lithium acetate, then transformed to LEU^+ with a plasmid directing the synthesis of LexA protein, pRB500, or with its parent plasmid, pAAH5. β-Galactosidase in these strains was assayed, in liquid and on indicator plates, as described in Table 1. There was no variation in *GAL1* transcription among the six strains when grown on galactose, glucose, and galactose with *GAL1* transcription repressed by LexA protein.

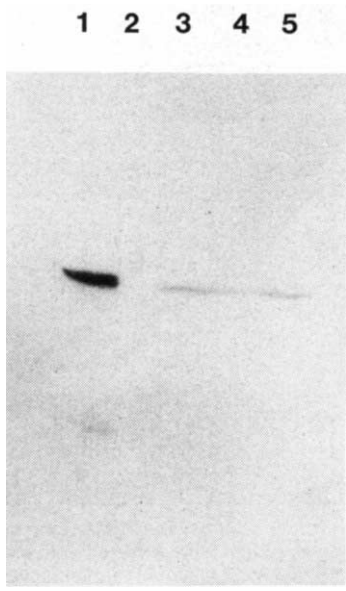

Fig. 2 *E. coli* LexA protein manufactured in yeast. Lane 1, 1 μg of purified LexA protein[9]. About 10% of this LexA protein preparation consisted of the amino- and carboxy-terminal autolytic fragments[23]. The carboxy-terminal fragment is visible on the gel. Lane 2, extract from DBY745/pAAH5. Lane 3, extract of DBY 745/pRB500. Lane 4, extract of DBY 745/pRB500. Lane 5, extract of DBY 745/pAAH5, spiked with 50 ng purified LexA protein.
Methods: pRB500, a plasmid which directs the synthesis of LexA protein, was made by inserting a 1,000-nucleotide *Hin*dIII-*Hin*dIII piece from pRB480 (unpublished data), which contained DNA from 62 bases upstream to ~200 nucleotides downstream of *lexA*, into the *Hin*dIII site of pAAH5. pAAH5 is a plasmid which carries the *LEU2* gene, a 2 μ origin of replication, and several hundred nucleotides of DNA from around the *ADH1* (*ADC1*) promoter. The *ADH1* fragment ends 11 nucleotides upstream of the ATG that would begin the *ADH1* structural gene. At this site a *Hin*dIII linker has been inserted. pAAH5 was constructed by Gustav Ammerer. Yeast strain DBY745 (*α adel-100 leu2-3 leu2-112 ura3-52*) was transformed with pAAH5 or pRB500, using the lithium acetate technique (M. Rose, personal communication). To produce each extract, yeast were grown in glucose minimal medium without leucine[18] to a density of 2×10^7 ml^{-1}. 2 ml of this culture were concentrated by centrifugation, resuspended in 0.2 ml Laemmli sample buffer[24], and lysed by grinding with glass beads[25]. 0.05 ml of each extract was run on a 12% polyacrylamide/SDS gel[24]. Proteins in the gel were transferred electrophoretically to a nitrocellulose membrane; the membrane was probed with anti-LexA protein antibody followed by ^{125}I-protein A from *Staphylococcus aureus*[13]. The membrane was washed and exposed to XAR-5 film for 24 h. In other experiments, protein gels of lysates of yeast that carry this plasmid were stained with Coomassie brilliant blue and revealed a faint new band whose mobility was the same as that of pure LexA protein (not shown). LexA protein was also observed in these strains after cellular proteins were labelled with ^{35}S-methionine and immunoprecipitated with anti-LexA antiserum (not shown). From immunoprecipitation experiments and from stained gels, we estimated that LexA protein constituted between 0.02% and 0.1% of the total soluble protein in cells transformed with this plasmid grown in glucose. As the *ADH1* promoter is about five times less active when cells are grown in galactose as when cells are grown in glucose (refs 26, 27 and our unpublished results), we suspect that there is one-fifth this amount of LexA protein in galactose-grown yeast.

examined[7,9], with an apparent dissociation constant (K_d) of $<10^{-9}$ M. The *GAL1* promoter derivatives were carried on plasmids that supplied a replicator and a selectable marker. The *GAL1* structural gene was fused in each case to a *lacZ* gene so that the β-galactosidase level in a plasmid-carrying cell was a measure of the *GAL1* transcription.

Derivatives of the *GAL1* promoter containing *lexA* operators between the upstream activator sequence and the TATA region (derivatives 27B, 20B, 50, 21B and 609; Fig. 1) were repressed 4–10-fold by LexA protein (Table 1). All *GAL1* promoter derivatives containing two *lexA* operators were repressed more efficiently than corresponding derivatives that contained only a single operator. No repression was observed when the operator was installed upstream of the UAS_G (in *GAL1*, derivative 740; see Fig. 1). In the absence of LexA protein, *lexA* operators at certain sites in the *GAL1* promoter slightly diminished transcription (Table 1). This effect may be related to the slight homology between the *lexA* operator and sequences implicated in yeast transcription termination (unpublished data).

We sought to confirm that the apparent *lexA*-mediated repression of operator-containing *GAL1* promoters was not caused by variation in plasmid copy number. Accordingly, we constructed nonreplicating derivatives of two *lexA*-repressible *GAL1* plasmids and directed their integration into a yeast chromosome[29]. We examined six different integrants of each plasmid and found that repression by LexA protein was as efficient, but no more efficient, than the repression observed when the operator-containing *GAL1* promoter was present in many copies on a plasmid. Repression of one such integrated promoter is shown in Table 2.

Table 3 Putative transcriptional terminators, installed between the *GAL1* upstream activator sequence and its TATA region, decrease *GAL1* transcription

20B	5,000
+*cycl-512*	2,500
+*cycl-512-F*	800
+*ADH1* terminator	50
+82-nucleotide polylinker	5,000
+234-nucleotide pBR322 *Hae*III fragment	5,000

Plasmids carrying *cycl-512* and *cycl-512-F Rsa*I. pAAH5 was cut with *Hin*dIII and *Sph*I. Fragments containing putative transcriptional terminators, the 182-nucleotide *Eco*RV-*Rsa* fragment, and the 324-nucleotide *Hin*dIII-*Sph*I fragment, were isolated and inserted into the *Stu*I site of pRB486 (unpublished construction). The unique *Stu*I site in this plasmid arises from the juxtaposition of two *Xho*I linkers. Derivatives of pRB486 that carried each mutation were cut with *Xho*I and the *Xho*I-ended terminator fragments inserted into the *Xho* site of 20B. Compared with the wild-type promoter, the *GAL1* promoter derivative carried on 20B has 153 nucleotides missing. Derivatives of 20B that carried these terminator fragments in the wild-type orientation were used to transform DBY745. A similar strategy was used to insert into 20B an 82-nucleotide *Hin*dIII-*Hae*III polylinker fragment from plasmid 701 (unpublished), a derivative of puc19[21]. 20B-G is a derivative of 20B that contains, in its *Xho*I site, a 234-nucleotide *Hae*III fragment from pRB322 (ref. 22). This plasmid was constructed by Jun Ma. Yeast bearing these plasmids were grown on galactose and assayed on indicator plates and in liquid as described in Table 1 legend.

Upstream terminators decrease transcription

Sequences downstream of certain yeast genes have been identified tentatively as sites where transcription terminates; it is possible, although less likely, that these sequences are signals which cause a longer transcript to be processed[11,12]. Putative transcription terminators are found downstream of the coding sequences of the *CYC1* and *ADH1* (*ADC1*) genes (see Fig. 3 legend). We inserted fragments of DNA carrying these putative terminators or mutant derivatives of them between the *GAL1* upstream activator sequence and its TATA region. We placed the terminator fragments in a deletion derivative of the *GAL1* promoter to ensure that the distance between the upstream activator sequence and the TATA region in the terminator-containing construction was approximately that found in the wild-type promoter. One fragment was taken from the 3′ end of a *CYC1* gene (the allele *cyc1-512-F*) in which the correct messenger RNA (mRNA) is formed. Another fragment differs by a single base pair and was taken from a mutant (*cyc1-512*) in which the correct 3′ end of the mRNA is only formed inefficiently, presumably because termination is rendered defective by the changed base pair (refs 11, 12 and K. Zaret, M. Hampsey and F. Sherman, personal communication). In addi-

tional experiments, we used a fragment that carried the putative transcriptional terminator from the 3′ end of the *ADH1* gene[12].

The fragments derived from the two *CYC1* alleles differed in their ability to depress *GAL1* transcription. The fragment carrying the putative transcriptional terminator (*cyc1-512-F*) decreased *GAL1* transcription by a factor of six (Table 3). In contrast, the fragment derived from the termination-deficient mutant (*cyc1-512*) decreased transcription by a factor of two. The fragment derived from *ADH1* showed a larger effect. Installation of this piece of DNA decreased *GAL1* transcription by 100-fold. Control pieces of DNA from pBR322 and pUC19, installed in the same deletion, had no effect on *GAL1* transcription (Table 3). If the *CYC1* terminator is inserted between the *CYC1* upstream activator sequence and its TATA region, the ability of a fragment to depress transcription is correlated with its ability to function as a transcriptional terminator (B. Osborne and L. Guarante, personal communication).

Discussion

Our experiments provide genetic evidence that a bacterial repressor protein manufactured in the yeast cytoplasm can enter the yeast nucleus, recognize its operator and repress transcription from a yeast promoter. Immunofluorescence experiments (performed in collaboration with P. Silver) show that LexA protein is localized to the nucleus in a minority of cells in a population that produces it (data not shown). In most members of the population, fluorescence is uniformly spread throughout the cytoplasm and we conclude that LexA protein has passively entered the nucleus. This behaviour is in contrast to the strictly nuclear localization of regulatory proteins native to yeast, which are thought to have a distinctive stretch of amino acids causing them to enter and remain in the nucleus (refs 13, 14, R. Moreland and L. Hereford, personal communication). Uniform cellular distribution of LexA protein should be sufficient to repress operator-containing *GAL1* promoters. If LexA protein constitutes 0.025% of the total protein in cells grown on galactose, and if it is evenly distributed throughout the cell, there should be about 750 LexA molecules in the nucleus. As the volumes of *E. coli* and a yeast nucleus are about the same, nuclear LexA protein concentration is probably equivalent to the concentration found in a single *E. coli*, which contains 100–1,000 molecules of LexA protein (unpublished data). We have shown here that the affinity of the synthetic operator for LexA protein is at least as high as that found for naturally occurring *lexA* operators. Therefore, 100–1,000 molecules of LexA per nucleus should be sufficient to occupy the operator[15].

Consider two possible mechanisms by which LexA protein might repress *GAL1* transcription. First, LexA protein could repress *GAL1* transcription because its binding site is close to or overlaps the binding site of some protein necessary for transcription. Although some sites of operator insertion are located far from either the upstream activator sequence or the TATA region, all sites are at least moderately close (within 59 nucleotides) to one region or the other. Therefore, we cannot exclude this possibility. Note, however, that LexA protein has no effect at a site 28 nucleotides upstream of the upstream activator sequence, so proximity of an operator to the upstream activator sequence is not sufficient to produce a repressible promoter. Second, the observed repression may be caused by LexA protein repressing *GAL1* transcription by blocking transmission of some stimulus (for example, RNA polymerase or some component of the transcription apparatus) from the upstream activator sequence to the TATA region. These two mechanisms are not mutually exclusive; depending on the location of the *lexA* operators within the *GAL1* promoter, one or both of them may exist.

Our results with transcription terminators reported here might be regarded as evidence in support of the second proposed mechanism. Transcription between the upstream activator sequence and the TATA region, however, has never been observed. It is possible that RNA polymerase makes an unstable transcript between the upstream activator sequence and the observed transcription start point, or that RNA polymerase or some other component of the transcription apparatus moves through this region in some other manner sensitive to the effects of transcription terminators. But we cannot exclude the possibility that a protein binds to the functional terminator but not to the mutant terminator, and that the bound protein interferes with transcription in the same way as LexA protein.

There is evidence that other yeast genes can be repressed by negative regulatory factors bound between the upstream activator sequence and the TATA region. For example, it is likely that *MATA1* transcription is repressed in diploid yeast by binding of a negative regulatory factor (which is dependent on the expression of *MATA1* and *MATα2*) to a site between the *MATA1* upstream activator sequence and its TATA region[16]. *STE6* transcription is repressed by the *MATα2* product, which binds to a specific site in the *STE6* promoter, probably located between the upstream activator sequence and the TATA region (A. Johnson, K. Wilson and I. Herskowitz, personal communication). The repression of *MATA1* and *STE6* is at least 50 times greater than the 10-fold repression we observe for *lexA* operator-containing derivatives of the *GAL1* promoter. We do not understand why these yeast promoters are repressed so efficiently relative to the *lexA* operator-containing *GAL1* promoter.

Further studies may use well-characterized prokaryotic regulatory proteins to control expression of other yeast genes and genes in higher eukaryotes. It will be of interest to see whether repressor proteins can block the activation of genes by enhancer sequences[17] found in higher eukaryotes. Repressor proteins may also be useful in studying the mechanism of negative regulation in yeast in those cases where negative control is exerted at a distance, such as the repression of silent genes of the yeast mating type locus by the *MAR/SIR* gene products[18].

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