Efficient activation of transcription in yeast by the BPV1 E2 protein

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Received January 11, 1989; Revised and Accepted February 20, 1989

ABSTRACT

The full-length gene product encoded by the E2 open reading frame (ORF) of bovine papillomavirus type 1 (BPV1) is a transcriptional transactivator. It is believed to mediate its effect on the BPV1 long control region (LCR) by binding to motifs with the consensus sequence ACCN_6 GGT. The minimal functional cis active site, called the E2 response element (E2RE), in mammalian cells comprises two copies of this motif. Here we have shown that E2 can function in Saccharomyces cerevisiae by placing an E2RE upstream of a synthetic yeast assay promoter which consists of a TATA motif and an mRNA initiation site, spaced correctly. This E2RE-minimal promoter is only transcriptionally active in the presence of E2 protein and the resulting mRNA is initiated at the authentic start site. This is the first report of a mammalian viral transactivator functioning in yeast. The level of activation by E2 via the E2RE was the same as observed with the highly efficient authentic PGK promoter where the upstream activation sequence is composed of three distinct elements. Furthermore a single E2 motif which is insufficient in mammalian cells as an activation site was as efficiently utilized in yeast as the E2RE (2 motifs). Previous studies have shown that mammalian cellular activators can function in yeast and our data now extend this to viral-specific activators. Our data indicate however that while the mechanism of transactivation is broadly conserved there may be significant differences at the detailed level.

INTRODUCTION

The control of transcription by activator proteins has recently been shown to have been apparently largely conserved throughout eukaryotic evolution by the demonstration that the yeast transactivator GAL4 can function in mammalian, Drosophila sp. and plant cells (1–4). Conversely two related mammalian activator proteins, i.e. oestrogen and glucocorticoid receptor can function inSaccharomyces cerevisiae (5,6).

Similarly the transcription-associated Fos protein has been shown to have the ability to activate mRNA synthesis in yeast, but only as a LexA–Fos fusion using the DNA-binding function of LexA (7). These reports strongly suggest that the mechanism of transcriptional control is conserved from yeast to higher eukaryotes, for certain proteins. However it is not clear that all types of transactivators are conserved in this way.

To investigate this question further we chose to examine the possibility that the bovine papillomavirus type 1 (BPV1) transactivator, E2, could function in yeast. E2 protein binds to the DNA consensus sequence ACCN_6 GGT (8–10) and it appears that at least two of these motifs are necessary to form an E2 responsive enhancer element (E2RE; 8; M.P. Sowden, S.M. Harrison, R.A. Ashfield, A.J. Kingsman & S.M. Kingsman, manuscript in preparation). E2 can be divided into a DNA-binding domain, which consists of the C-terminal 101 amino acids (9) and an activation domain, which probably resides in the N-
Furthermore it has been proposed that the activation domain of E2 may require regions of negatively charged amino acids configured as amphiphilic \( \alpha \) helices as proposed for GAL4 (11, 12). However unlike GAL4, or other studied transactivators, the DNA-binding domain does not contain recognized protein-DNA interaction structures such as ‘helix-turn-helix’ (13) or ‘zinc fingers’ (14) or a DNA-binding associated ‘leucine zipper’ (15) and therefore it seems likely that E2 has a novel DNA-binding mechanism.

To study the action of E2 in yeast we chose to use a minimal assay promoter which is based on the phosphoglycerate kinase (PGK) TATA box and mRNA initiation site (RIS). This promoter was developed to eliminate all upstream promoter sequences other than those under examination. Here we report the effect of E2 expression on an E2 response element (E2RE), and variations thereof, fused to the minimal assay promoter.

**MATERIALS AND METHODS**

**Strains, media and growth conditions.**

Strains used were *Escherichia coli* MC1061 \([\Delta(lacIPOZYA) X74 \Delta(araABOIC-leu) 7697 galk hsdR hsdM rpsL]\) and *Saccharomyces cerevisiae* DBY745 \((\alpha adel-100 leu2-3 1eu2-112 ura3-52)\). *E. coli* cultures were grown on Luria broth (16) with 50 \( \mu \)g/ml ampicillin where necessary. Yeast cultures were grown in YEPD or defined minimal media with the necessary amino acid supplements at 30\( ^\circ \)C (17). Yeast transformations were performed following the procedure of Hinnen et al. (18). Transformed yeast strains were grown for DNA/RNA isolation or nuclear protein extract preparation by inoculation at a cell density of \( 5 \times 10^4 \) to \( 1 \times 10^5 \) cells/ml into defined minimal media and incubated, with agitation, at 30\( ^\circ \)C for 16 to 24h until the culture had reached a cell density of 4 to \( 8 \times 10^6 \) cells/ml.

**DNA manipulations.**

All DNA manipulations were carried out according to standard protocols (19). The minimal assay promoter plasmid, pKV560 is based on pMA1557 (20) which is a high copy number *E. coli*/yeast selectable shuttle plasmid with pBR322 and 2\( \mu \)m replication sequences. The promoter/gene cassette is flanked on its 5' side by 720 bp of *TRP1* DNA, the reporter gene is \( \alpha 2 \) interferon (IFN) and the transcription termination signals are derived from PGK (20). pKV560 was made from pMA1557 by the insertion of a synthetic BamH1-BglII fragment containing a minimal assay promoter sequence (see Fig. 1a) at the BamHI site in pMA1557 immediately 5' to the IFN coding sequence, thereby creating a unique BamHI site 5' to the minimal assay promoter for the insertion of activator sequences. The minimal assay promoter sequence consists of a TATA motif linked to an RIS, which was derived from the PGK promoter. The sequence surrounding these motifs was also drawn from authentic PGK sequence, however it has been reduced as much as possible (C.A.S., A. Chambers, A.J.K. & S.M.K., unpublished data). Constructions made by inserting various fragments at the BamHI site in pKV560 which were used in this study are as follows; pKV561 (insertion of the PGK UAS, nucleotides -538 to -402, 21); pKV565 (insertion of a BPV1 E2RE, nucleotides 7760 to 7797, 22); pKV566 (double E2RE insert) and pKV569, which has an insertion of a single E2 consensus motif, sequence:

\[
5' \text{GATCCACCGTCTTCGGTGCA} \ 3'.
\]

\[
\text{GTGGCAGAAGCCACGTCTAG}
\]

All of these constructions are represented diagrammatically in Fig. 1a.

Expression of the E2 ORF was achieved by placing it under the control of a hybrid
Figure 1 Plasmid constructions. (a) pKV560 and derivatives. pKV560 is a minimal assay promoter plasmid. The assay promoter contains a TATA motif and an RNA initiation site (RIS)—see sequence for details. Upstream of the assay promoter is a BamHI site into which has been inserted the PGK UAS (pKV561), an E2RE (pKV565), two E2REs (pKV566) and a single E2 consensus binding site (pKV569). (b) pKV483, a galactose-inducible E2 protein expression vector. B=BamHI, H=HindIII, R= EcoRI.
PGK/GAL1-10 promoter (23) in pKV483 (Fig. 1b). This plasmid was constructed by ligating a HindIII/Sall fragment from pUKC203 (a gift from Dr. M. Tuite, University of Kent, Canterbury) containing the 2 μm origin in YIPS, with a HindIII/Sall fragment from pKV49 which contains the PGK promoter with the GAL1-10 UAS in the place of the PGK UAS separated from the PGK translation and transcription stop signals by a BglII site (pKV482). The BPV1 E2 ORF (nucleotides 2537 to 4451) was inserted into this BglII site such that the ORF is under the control of the PGK/GAL promoter.

RNA, DNA isolation and analysis.

Sufficient yeast cell culture volumes were grown to permit isolation of DNA and RNA from all cultures. As a result for all mRNA assays a corresponding plasmid copy number DNA assay was performed. RNA isolation followed the method of Dobson et al. (24) and DNA was prepared using the method of Holm et al. (25). The mRNA levels were assayed by Northern blotting (26) using IFN and ribosomal specific nick translated probes (21, 27). The mRNA start sites were mapped using primer extension using an IFN mRNA homologous primer (28, 29). The plasmid copy numbers were determined by digestion of the DNA with EcoRI, followed by analysis by Southern blotting (30) using the same probes as used for the Northern blots.

Protein–DNA binding assay.

Nuclear protein extracts were prepared largely according to Stanway et al. (31) but with the following modifications; after spheroplasting the cells were resuspended in homogenization buffer (Tris 10mM, pH 8; MgCl₂ 1.5mM; KCl 15mM; EDTA 0.1 mM; PMSF 1mM; 2-mercaptoethanol 1mM), chilled on ice and dounced 15 times. The homogenate was spun at 12000 g at 4°C for 10 minutes and the resultant pellet was resuspended in 0.6M NaCl protein extraction buffer.

Protein–DNA binding reactions were performed as described by Stanway et al. (31) except that the NaCl concentration was 200mM. Retardation gels were 0.5×TBE, 5% polyacrylamide, which were electrophoresed at 180V for ca. 1.5h.
Figure 2 Expression of E2 in yeast induces transcription of a reporter gene via E2 consensus binding sites.
(a) Northern blot comparing IFN mRNA levels directed by pKV560, pMA91-8 (native PGK promoter, 29) and pKVs 569, 565 & 566 with and without E2 expression. R = ribosomal mRNA, IFN = IFN mRNA.
(b) Southern blot analysis of total genomic and plasmid DNAs to estimate plasmid copy number. DNA was digested with EcoRI prior to electrophoresis. R = ribosomal, i.e. genomic DNA (n.b. there are 100-140 repeats of the ribosomal cistron, 21). IFN = IFN DNA, i.e. plasmid DNA.
(c) Northern blot demonstrating that as a single transformant pKV565 does not direct elevated levels of IFN mRNA when grown on galactose compared with the mRNA levels from glucose-grown cells. pMA91-8 (native PGK promoter) on glucose and galactose shows the level of transcription pKV565 is capable of in the presence of E2. R = ribosomal mRNA; IFN = IFN mRNA.
RESULTS

Experimental strategy: use of a yeast minimal promoter.

In order to investigate whether BPV E2 might be able to activate transcription in *S. cerevisiae* we decided to use as simple a promoter as possible. We have created an inactive yeast assay promoter on a high copy number shuttle plasmid (pKV560) which allows the analysis of potential activator sequences in isolation. The inactive assay promoter, which is derived from the *PGK* sequence, consists of a TATA motif and a recognized RNA start site spaced as closely as possible while still maintaining the accuracy of initiation when activated (Fig. 1a; C.A.S., A. Chambers, A.J.K. & S.M.K., unpublished data). When fused to an activator sequence, e.g. the *PGK* UAS, the assay promoter is stimulated to a level equivalent to the complete *PGK* promoter as determined by assaying for α2 interferon (IFN) mRNA levels (Fig. 1a; C.A.S., A. Chambers, A.J.K. & S.M.K., unpublished data).

A bovine papillomavirus type 1 (BPV1)E2 responsive enhancer element (E2RE) defined by Harrison *et al.* (22; nucleotides 7760 to 7797), which contains two copies of the E2 recognition motif (ACCGGGT) was inserted into pKV560 as a single or double insert (Fig. 1a, pKV565 and pKV566, respectively). Similarly a single synthetic E2 motif was inserted into pKV560 (Fig. 1a, pKV569). A galactose-inducible E2 expression vector (pKV483) was constructed by insertion of the E2 ORF (nucleotides 2537 to 4451) immediately 3' to a *PGK/GAL1*−10 UAS hybrid promoter (23) carried in a yeast *E. coli* high copy number vector (Fig. 1b). Using these plasmids three doubly transformed yeast strains were created by transformation using the strain DBY745 as a recipient, i.e. pKV569/pKV483, pKV565/pKV483 and pKV566/pKV483. By growing these strains either on glucose (no E2 expressed) or galactose (E2 expressed) the effect of E2 on the yeast transcriptional machinery was assessed.

Efficient transcription directed by an E2 response element (and derivatives) in yeast.

The effect of E2 expression (from pKV483) on IFN mRNA levels directed by pKV569 (one motif), pKV565 (two motifs) and pKV566 (four motifs) was examined by Northern blot analysis (Fig. 2a) from cultures grown on glucose or galactose. On glucose the *PGK/GAL1*−10 hybrid promoter is repressed and it is induced about 1000 fold by galactose (23). Consequently E2 protein is only produced by these transformed strains when grown on galactose (data not shown). From the IFN mRNA levels in Figure 2a it is apparent that in the absence of E2 pKV569, pKV565 and pKV566 are not transactivated as they are indistinguishable from pKV560. However in the presence of E2 transactivation occurs, and approximately equally so, for all three of the test promoters. Furthermore these elevated levels of IFN mRNA are equivalent to those directed by the native *PGK* promoter (pMA91−8; 32) indicating that this E2-induction of transcription is very efficient.

This Northern blot was also probed for ribosomal RNA levels to show that an equal amount of RNA was loaded onto each track (Fig. 2a). The reporter gene plasmid copy number of each culture was determined by Southern blot analysis by comparison with the ribosomal DNA cistron repeat and was found not to vary significantly (Fig 2b); i.e. in the presence of E2, in each case, the plasmid copy number was not higher than in the absence of E2. Furthermore the plasmid copy number in the presence of E2 is roughly equivalent for pKV569 and pKV565, whilst pKV566 has a slightly higher copy number. No activation of transcription directed by pKV565 was observed in the absence of pKV483 (i.e. DBY745 transformed only with pKV565) in galactose-grown cells compared with the level from glucose-grown cells demonstrating that pKV565 is not simply a galactose inducible promoter (Fig 2c). Therefore these observations confirm that E2 can activate
transcription in yeast, that the induction is efficient and that there is no difference in this efficiency when one, two or four E2 consensus motifs are used as the response sequence. E2 activated transcription is initiated accurately.

The 5' end of the mRNA synthesised by pKV565, in the presence and absence of E2, was mapped by primer extension and compared with an extension reaction from pKV561, which is the PGK UAS in the minimal assay promoter. It has already been demonstrated that pKV561 uses the authentic mRNA start site to initiate transcription (C.A.S., A. Chambers, A.J.K. & S.M.K., unpublished data). A comparison of the extension products from pKV561 and pKV565 in the presence of E2 clearly shows that their initiation profiles are similar, i.e. starting on the two A residues at positions -19 and -18 (Figs. 1a & 3); although there does appear to be a difference in the ratio between these two bases. Therefore it is evident that E2 induction of pKV565 leads to accurate as well as efficient transcription.

E2 protein synthesised in yeast binds specifically to its consensus sequence.

To confirm that this E2 transactivation event most probably occurs via protein-DNA binding at the consensus motifs, yeast nuclear protein extracts were made from untransformed DBY745 and DBY745/pKV483 grown either on glucose or galactose. These
Figure 4  E2 produced in yeast binds specifically to an E2RE. Retardation gel analysis of binding reactions using a \(^{32}P\)-labelled DNA fragment containing two E2 motifs and a yeast nuclear protein extract from cells expressing E2. Lanes 3, 4 & 5 show the effect of competition with unlabelled DNA also containing two E2 motifs (pSP46E2RE cleaved with Sau3A); whilst lanes 7, 8 & 9 show the effect of non specific DNA competition (pSP46 (21) cleaved with Sau3A). Lane 1: fragment only; all other lanes: fragment plus \(5\mu\)g nuclear protein extract, plus 100ng sonicated calf thymus DNA. Lanes 3, 4 & 5 plus 25, 50 & 100ng pSP46E2RE, respectively. Lanes 7, 8 & 9 plus 25, 50 & 100ng pSP46, respectively. F? indicates the free radiolabelled fragment, the other arrows indicate the specifically retarded complexes.

extracts were tested using the gel retardation technique for the ability to bind to an E2RE-containing fragment (i.e. the 38bp fragment composed of two E2 motifs described in the experimental strategy). The only extract to demonstrate a discrete retarded complexes was the galactose-grown DBY745/pKV483 extract (data not shown). This demonstrates that the retarded species shown in Fig. 4 are dependent on E2 expression. These retardation events were shown to be E2RE specific by addition of increasing amounts of unlabelled competitor DNAs, i.e. pSP46 (21) or pSP46E2RE (Fig. 4). Therefore E2 produced in yeast binds efficiently to its target sequence. It appears highly likely that the two more intense and slower migrating complexes are the result of occupancy of one and two E2 binding sites; whilst the third and fastest migrating complex is derived from partially proteolysed E2 binding as observed by Dostatni et al. (33).

DISCUSSION
We have demonstrated that the BPV1 E2 protein, a mammalian viral-specific transactivator, can also function in yeast. We have achieved this by the creation of a synthetic promoter consisting of multiples of the E2 consensus binding site fused to a minimal assay promoter. These hybrid promoters are only transcriptionally active in the presence of E2, which mediates its effect, almost certainly, by binding to the E2 consensus motifs.

Recently two steroid hormone receptors and a LexA?Fos fusion have been shown to
be able to activate transcription in yeast apparently via their DNA-binding sites (5–7). Both of the hormone receptor proteins have been shown to possess DNA-binding zinc-finger motifs (34, 36) whilst LexA requires a helix-turn-helix structure for DNA recognition (13). E2 however, has neither of these motifs in its DNA-binding domain (9). Our data therefore indicate that the conservation of protein-DNA interactions between yeast and mammalian systems extends to a third type of structural interaction, exemplified by E2.

It is evident from the results presented here that a single E2 interaction site can function as efficiently as an E2RE (two motifs) or as a double E2RE (four motifs) and that the transactivation achieved is approximately as effective as the level directed by the PGK UAS. It is important to note that in mammalian cells an induction of transcription by E2 appears to require at least two E2 motifs (8, 22). This contrast between yeast and higher eukaryotes may indicate a significant difference; i.e. yeast may not require multiple enhancer motifs to constitute a cis active site as is the case for most higher eukaryotes. This seems unlikely in view of the complexity of the PGK UAS (see below). An alternative explanation is that the proximity of the E2 motif to the other promoter elements in the synthetic assay promoter may increase its efficiency.

The efficiency of transcription conferred on the minimal assay promoter by a single E2 motif, in the presence of E2, is equivalent to the level of activation directed by the PGK UAS. This is remarkable because the PGK UAS requires three different motifs, (one of them present as three repeats) to achieve its characteristic highly efficient activation of transcription (31, 36; C.A.S., A. Chambers, A.J.K. & S.M.K., unpublished data). This suggests that E2 is a very potent activator and that it can either simply bypass some of the functions provided by the PGK UAS or that it is a multifunctional protein. Interestingly the viral activator of herpes simplex virus VP16 has recently also been shown to be a very powerful activator in comparison with cellular transcription factors in mammalian cells (37).

In summary we have extended the description of transcriptional activators which can function in evolutionarily distant cell types to include a highly efficient mammalian viral-specific transactivator, BPV1 E2, functioning in yeast. This implies broad conservation of the transcriptional machinery from lower to higher eukaryotes, however it appears that there may be differences between yeast and mammalian cells because a single E2 motif is functional in the former but not in the latter. Furthermore it is important to note that a single E2 motif can compensate for a complex yeast UAS without any loss in transcriptional efficiency.

ACKNOWLEDGEMENTS
We would like to thank the SERC and GLAXO plc for supporting this research and S.M. Harrison and A. Chambers for helpful discussions. M.P.S. has an SERC Biotechnology Directorate postgraduate award. C.A.S. acknowledges the award of the Florey research fellowship by Lady Margaret Hall, Oxford. We are grateful to Elaine Byles for excellent technical assistance.

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REFERENCES


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