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The C-terminal 79 amino acids of the herpes simplex virus regulatory protein, Vmw65, efficiently activate transcription in yeast and mammalian cells in chimeric DNA-binding proteins

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Activation of herpes simplex virus immediate early gene expression normally requires the formation of a ternary complex between a virus *trans*-activator, Vmw65, a cellular octamer-binding protein, TRF and the *cis*-acting target sequence, the TAATGARAT motif. We report that the C-terminal 79 amino acids of Vmw65, which contain a potential acidic amphipathic helix, can activate transcription in both yeast and mammalian cells in the absence of TRF interaction when fused to the DNA-binding domain of the yeast transcription factor, GAL4. Together with our previous report which showed that the recruitment of TRF to the DNA by Vmw65 is insufficient for transcription activation, these results indicate that the octamer binding protein may not be directly involved in transcriptional induction mediated by Vmw65. The TRF – Vmw65 complex may therefore represent a novel class of transcription activator in which the protein domain responsible for sequence-specific DNA binding, present in TRF, and that necessary for induction of transcription, within Vmw65, are located on separate proteins. These results are discussed with reference to combinatorial transcriptional control and the role of octamer-binding proteins in other systems.

Key words: GAL4/herpes simplex virus/octamer binding protein/transcription regulation/Vmw65

Introduction

Accurate and efficient initiation of eukaryotic transcription is dependent on the ability of multiple different DNA-binding proteins to interact in a sequence-specific fashion with their cognate *cis*-acting DNA recognition sites (for review see Wasylyk, 1988). Regulation of gene expression may be achieved by a variety of mechanisms acting to modulate the binding, activity or cellular localization of such factors. In recent years evidence has accumulated to suggest that complex combinatorial protein – protein interactions play a major role in transcriptional control by, for example, enabling the same transcription factor to participate in regulation of genes that are not obviously coordinately expressed.

The induction of the herpes simplex virus (HSV) immediate-early genes (IE) provides a paradigm for such a combinatorial mechanism of transcriptional regulation and involves both a single viral *trans*-acting factor Vmw65 (Post *et al.*, 1981; Campbell *et al.*, 1984; Mackem and Roizman, 1982a), and a specific *cis*-acting sequence, the TAATGARAT motif (Mackem and Roizman, 1982b; Kristie and Roizman, 1984; Preston *et al.*, 1984; Whitton and Clements, 1984; O'Hare and Hayward, 1987).

However, since Vmw65 itself does not bind independently to DNA (Marsden *et al.*, 1987), we and others (Kristie and Roizman, 1987, 1988; McKnight *et al.*, 1987; O'Hare and Goding, 1988; Preston *et al.*, 1988) examined the possibility that the TAATGARAT elements might be recognized by a cellular host cell DNA-binding protein which mediated the regulatory response to Vmw65. Our results identified a factor, the TAATGARAT recognition factor (TRF), and demonstrated that TRF-binding was essential for IE induction (O'Hare and Goding, 1988). Moreover, we showed that TRF was similar or identical to the ubiquitous octamer-binding protein, variously called NF-A1 (Singh *et al.*, 1986), NFIII (Pruijn *et al.*, 1986; O'Neill and Kelly, 1988), OTF1 (Fletcher *et al.*, 1988), and OBP100 (Sturm *et al.*, 1987). By using HSV infected-cell extracts or reconstitution with purified Vmw65 we demonstrated the formation of a ternary complex involving the TAATGARAT element, Vmw65 and TRF and correlated induction of IE gene expression with formation of this complex (O'Hare *et al.*, 1988). Kristie and Roizman (1987) also detected a host factor, α-H1, which bound specifically to a TAATGARAT element and may be associated in a complex with Vmw65 (McKnight *et al.*, 1987) while Preston *et al.* (1988) demonstrated the presence of a host cell factor, HC3, and a Vmw65-containing DNA-binding complex in extracts from infected cells.

Subsequently we showed that the TAATGARAT motif itself was a bimodal *cis*-acting sequence with the GARAT domain, while not required for TRF binding *in vitro*, being essential for both complex formation and inducibility by Vmw65 (O'Hare *et al.*, 1988). On the basis of these results we proposed a model in which the mechanism of selective induction of IE genes involved Vmw65-mediated recruitment of TRF onto the TAATGARAT elements where TRF was qualitatively involved in promoting transcription. However, our recent work has shown by mutational analysis of Vmw65 that the ability of TRF to be recruited into the Vmw65 – TRF complex is in itself insufficient for transcription activation and that the C-terminal 79 amino acids of Vmw65, which are completely dispensable for complex formation, were vital for transcription activation (Greaves and O'Hare, 1989). Similar results on the requirement for the C-terminal region of Vmw65 for *trans*-activation were described by Triezenberg *et al.* (1988). Models to account for the role of TRF in IE induction included the possibility that the C-terminal region of Vmw65 induced a conformational change in complex bound TRF to expose a TRF activation domain. However, if the acidic region of Vmw65 was itself sufficient to activate transcription when bound to the DNA by means other than association with TRF, this would imply that the role of TRF in the complex would be to direct Vmw65 to the TAATGARAT elements rather than being mechanistically involved itself in the transcription response.

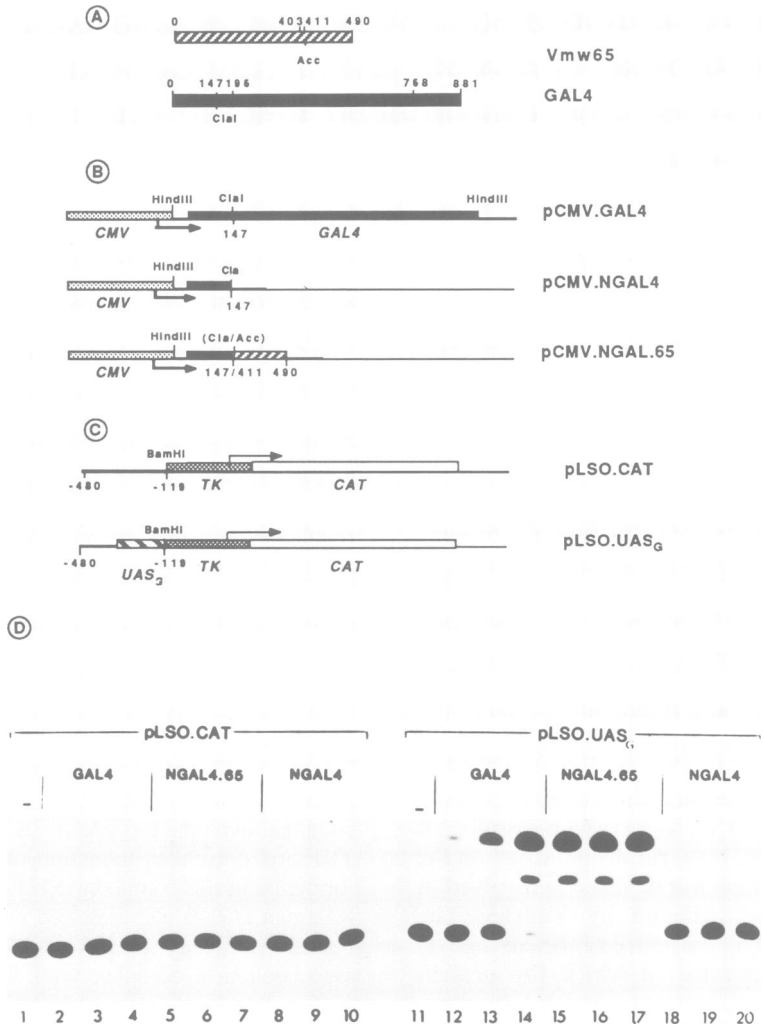

Fig. 1. A. Schematic showing coding regions of Vmw65 and GAL4. Numbers indicate amino acid positions flanking protein domains. the DNA-binding domain of GAL4 lies between amino acids 1−147 and the two activation domains between amino acids 147−196 and 768−881. The N-terminal 403 amino acids of Vmw65 are required for complex formation with TRF and the C-terminal region between amino acids 411 and 490 are required for transcription activation. **B.** Effector plasmids used in the mammalian expression experiments: all coding sequences are expressed from the CMV IE promoter. pCMV.NGAL4.65 expresses the 147 amino acid DNA-binding domain of GAL4 fused in frame to the C-terminal 79 amino acids of Vmw65. pCMV.GAL4 expresses the entire GAL4 coding sequences and pCMV.NGAL4 the N-terminal 147 amino acid DNA-binding domain of GAL4. **C.** Reporter plasmids used in the mammalian co-transfection experiments: pLSO.CAT contains the HSV thymidine kinase promoter, to −480 with a *Bam*HI linker placed at position −119, upstream of coding sequences for the CAT gene; pLSO.UAS$_G$ has a 159 base pair fragment containing the *GAL* UAS inserted at position −119 of the TK promoter. **D.** CAT assays showing that the GAL4−Vmw65 chimeric protein is a strong transcription activator in mammalian cells. 2 μg of each reporter plasmid, pLSO.CAT or pLSO.UAS$_G$ was transfected into COS cells either alone, lanes 1 and 11, or with 10 ng, 100 ng or 1000 ng, from left to right for each triplet, of the indicated activators. Carrier DNA, pUC19, was used to bring the total DNA in each transfection to 3 μg and CAT assays performed 60 h post-transfection.

In this paper we show that Vmw65 does indeed contain an activation domain and that a chimeric protein containing the C-terminal 79 amino acids of Vmw65 fused to the DNA-binding domain of the yeast transcription factor GAL4, efficiently activates transcription from promoters containing the *GAL* UAS in both yeast and mammalian cells. Thus the C-terminal region of Vmw65 can function as an activation domain in the absence of any interaction with the octamer-binding protein, TRF. These results are discussed with reference to the mechanism of Vmw65 action and the role of the octamer-binding protein in this and other systems.

Results and discussion

The Vmw65 activation region functions in the absence of TRF interaction

While the N-terminal 403 amino acids of Vmw65 are sufficient for complex formation with TRF, the C-terminal 79 amino acids are required for efficient transcription activation (Triezenberg *et al.*, 1988; Greaves and O'Hare, 1989). Protein secondary structure predictions indicate that the C-terminal domain contains three potential α-helices and is highly negatively charged. This is reminiscent of the yeast transcription activator GAL4, in which a distinct domain comprising a negatively charged amphipathic helix is required for transcription activation (Ma and Ptashne, 1987). A similar negatively charged domain is also required for transcription activation by another yeast transcription factor GCN4 (Hope and Struhl, 1986).

To test whether the C-terminal region of Vmw65 functioned as an activation domain, we reasoned that if these sequences were fused directly to a well characterized DNA-binding domain of a heterologous protein such as GAL4 then the chimeric protein should activate transcription from promoters bearing a GAL4 binding site. The regions of Vmw65 and GAL4 used in this study are indicated in Figure

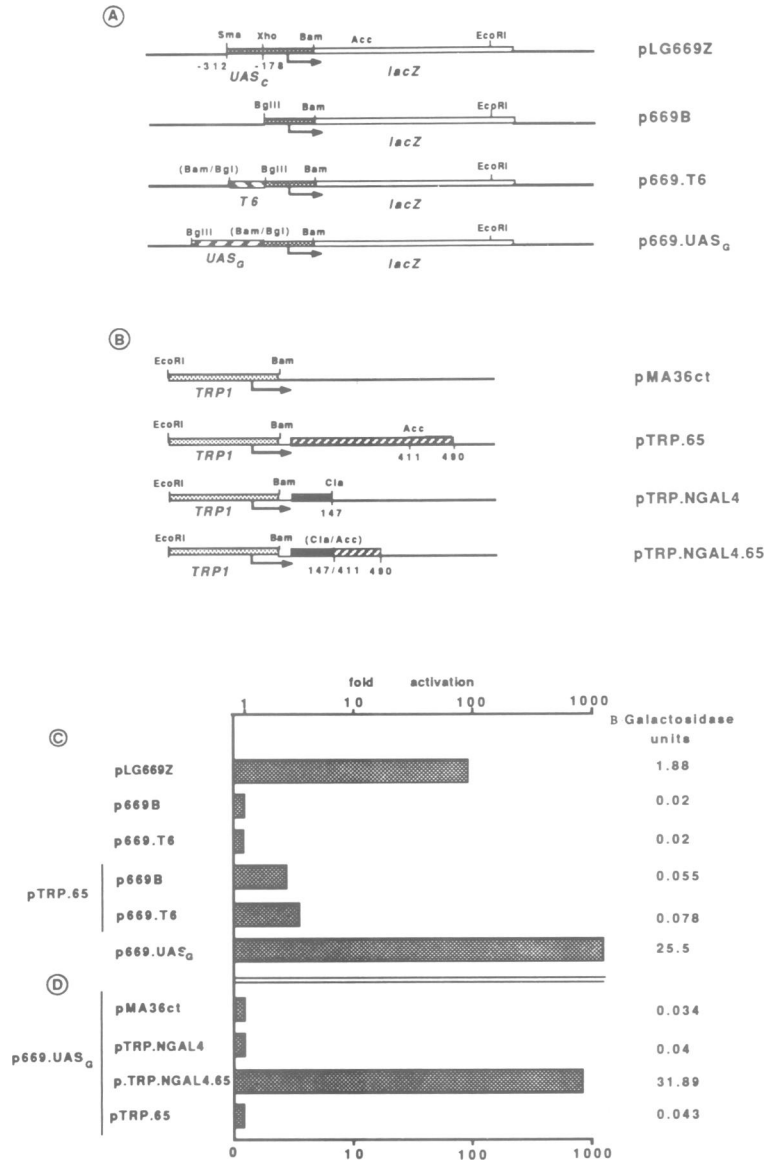

Fig. 2. Effector and reporter plasmids used for expression in yeast. **A.** pLG669Z contains the *CYC* promoter and UAS to position −312 fused to the coding sequences for the bacterial β-galactosidase gene; in p669B the *CYC* UAS has been deleted to the *Xho*I site at position −178 and a *Bgl*II linker inserted; p669.T6 has a 28 bp sequence containing the HSV IE 110 promoter proximal TAATGARAT motif inserted into the *Bgl*II site of p699B; p669.UAS$_G$ contains a 159 bp fragment containing the entire *GAL* UAS cloned into the *Bgl*II site of p669B. **B.** The yeast effector plasmids based on vector pMA36ct (see Materials and methods) which contains a unique *Bam*HI site downstream of the TRP1 promoter. pTRP.65 contains the entire Vmw65 coding sequences downstream of the TRP 1 promoter; pTRP.NGAL4 expresses the N-terminal 147 amino acid DNA-binding domain of GAL4 and pTRP.NGAL4.65 expresses the GAL4 DNA-binding domain fused to the C-terminal 79 amino acids of Vmw65. **C.** Analysis of TAATGARAT and Vmw65 function in yeast. The TAATGARAT element does not function as a UAS in yeast. The plasmids indicated were transformed into yeast strain DBY 745 either alone or together with pTRP.65 and assayed for β-galactosidase activity as described in Materials and methods. **D.** The GAL4−Vmw65 fusion protein efficiently activates transcription in yeast. Plasmid p669.UAS$_G$ was co-transformed together with different effectors into the GAL4 negative yeast strain 21 and assayed as above.

1A. We constructed the series of effector and reporter plasmids depicted in Figures 1B and C respectively. The effector plasmids are based on the vector pCMV.IL2 (Cullen, 1986) which contains the SV40 origin of replication and enables high levels of protein to be expressed from coding sequences placed downstream of the strong CMV IE promoter after transfection into COS cells. The reporter plasmids are based on the pLSO.CAT recombinant in which the HSV thymidine kinase promoter is placed upstream of the bacterial CAT coding sequences. A *Bam*HI site at position −119 enabled us to insert upstream of the TK sequences a 159 bp fragment containing the entire GAL4 upstream activating sequence (UAS$_G$) with its four binding sites for the GAL4 transcription factor.

Transfection of COS cells with either pLSO.CAT or pLSO.UAS$_G$ resulted in very low levels of constitutive CAT activity (Figure 1D, tracks 1,11). Low levels of CAT expression were also obtained if pLSO.CAT was co-transfected with any of the effector plasmids, pCMV.GAL4, pCMV.NGAL4 or pCMV.NGAL.65, expressing respectively, the entire GAL4 coding sequences, the N-terminal 147 amino acid DNA-binding domain of GAL4 or the C-terminal 79 amino acids of Vmw65 fused to the 147 amino acid DNA-binding domain of GAL4 (Figure 1D, track 2−10). In contrast, efficient activation (20- 200-fold in a dose-dependent fashion) of pLSO.UAS$_G$ was obtained by co-transfection with pCMV.GAL4 (Figure 1D, tracks 12−14), demonstrating that both the UAS$_G$ and GAL4

were functional in this assay. As expected, transcription from pLSO.UAS_G was not induced by co-transfection with pCMV.NGAL4 (Figure 1D, tracks 18–20) since no transcription activation domain was present on the expressed protein. However, a striking activation of transcription (200-fold) was obtained when pLSO.UAS_G was co-transfected with pCMV.NGAL.65 (Figure 1D, tracks 15–17). The NGAL.65 chimera was even more efficient than the parent GAL4 protein as indicated in the dose response experiment where NGAL.65 induced expression of pLSO.UAS_G by 200-fold at the lowest dose tested (10 ng) while in parallel at this dose activation by GAL4 was 20-fold (Figure 1D, compare tracks 12 and 15). Thus the acidic C-terminus of Vmw65 confers an efficient activation function on a DNA-binding domain from a heterologous protein, strongly suggesting that Vmw65 performs an analogous function when complexed with TRF in the TAATGARAT-binding complex, that is, it is this domain of Vmw65 which functions to induce IE gene expression.

Intact Vmw65 and TAATGARAT elements do not function in yeast cells

Recent work from a number of laboratories has shown that, given the appropriate binding sites, transcription factors from yeast can function in mammalian cells and vice versa (Kakidani and Ptashne, 1988; Webster *et al.*, 1988; Metzger *et al.*, 1988; Struhl, 1988). The identification of a yeast TRF-like activity would enable a genetic approach to be applied to the study of the mechanism of transcription activation by the TRF–Vmw65 complex. Moreover, identification of a yeast TRF might also enable the existence of a possible cellular homologue to Vmw65 to be demonstrated. On the other hand, if no yeast TRF could be detected but the activation domain of Vmw65 was functional, yeast might then be used as a system for studying TRF–Vmw65 interactions after transformation with vectors expressing the gene for mammalian TRF and Vmw65 in the absence of any endogenous TRF.

Attempts to demonstrate a specific TRF-like activity using nuclear extracts of either *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe* have been negative. In addition, no specific binding activity was observed using either the immunoglobulin or the adenovirus NFIII-binding sites, while in control experiments yeast, API, ATF and CRF activities were readily detectable (data not shown). To explore further the possibility of a yeast TRF-like activity we next asked whether a TAATGARAT element could function as a UAS in a yeast expression vector containing the bacterial *lacZ* gene fused to the promoter sequences of the yeast *CYC1* gene. This construct together with the other yeast vectors used in this study are shown in Figures 2, A and B. Yeast strain DBY 745 was transformed with either pLG669Z containing the intact *CYC* UAS (UAS_C), p669B in which the UAS has been deleted or with p669.T6 in which a TAATGARAT element (T6), corresponding to that used in the pLSO.pUC vector in the mammalian experiments (O'Hare and Goding, 1988), was inserted at position −178 in place of the UAS_C. The results shown in Figure 2C demonstrate that while the UAS_C activated transcription efficiently, expression from both the p669B and p669.T6 vectors was barely detectable. Furthermore, the TAATGARAT sequence did not function as a Vmw65 inducible element in the yeast cells. Thus when p669.T6 was co-transformed with the yeast Vmw65 expression vector pTRP.65, no significant induction in β-galactosidase activity was observed (Figure 2C). (The low level of induction observed with p669.T6 and pTRP.65 was also observed with the control target p669.B, and is insignificant compared to that obtained with the *GAL4* UAS in yeast, see below.)

Functional activity of the Vmw65 carboxy terminus in yeast cells

It was possible that the absence of a functional response to Vmw65, rather than resulting from a lack of TRF activity, was because the C-terminal domain of Vmw65 that is required for efficient transcription activation in mammalian cells, was incompatible with the yeast transcription machinery. To test whether this was indeed the case, we co-transformed the GAL4-negative yeast strain 21 with p669.UAS_G containing the UAS_G upstream of the *CYC–lacZ* fusion and either pTRP.NGAL4 expressing the 147 amino-acid DNA-binding domain of GAL4 or pTRP.NGAL4.65 expressing the GAL4 DNA-binding domain fused in frame to the acidic C-terminal 79 amino acids of Vmw65 (Figure 2D). As expected the GAL4 DNA-binding domain did not activate transcription from the UAS_G and no activation was seen if pTRP.65 was used to express the intact Vmw65 protein. In contrast highly efficient activation (between 300- and 900-fold in different experiments) was obtained with the GAL–Vmw65 chimeric protein. These results indicate that the carboxy terminal region, in addition to functioning in mammalian cells, can also function extremely efficiently in yeast when linked to a heterologous DNA-binding domain.

Conclusions

The results presented in this paper show clearly that the C-terminal 79 amino acids of Vmw65 can act as a potent transcription activator, in both yeast and mammalian cells, when linked to a heterologous DNA-binding domain. While this manuscript was in preparation recent work from Sadowski *et al.* (1988) has also demonstrated that the C-terminal region of Vmw65 could act as a transcription activator in mammalian cells although no results were presented on the ability of the GAL4–Vmw65 chimera to activate transcription efficiently in yeast or on the presence or absence of a yeast TRF-like activity. It is likely that these C-terminal amino acids perform the same function when present in the intact Vmw65 protein, implying that the role of Vmw65 in *trans*-activation is to provide the TRF–Vmw65 complex with an efficient transcription activation domain.

Our previous work (Greaves and O'Hare, 1989) on regions of the COOH terminus of Vmw65 which are involved in *trans*-activation, consistent with the earlier results of Triezenberg *et al.* (1988), indicates that amino acids located within the region ~430–450 are essential for functional activity. Secondary structure predictions indicate that this region would encompass an α helix which from helical wheel analysis would be organized with acidic and hydrophobic amino acids aligned on opposite faces (Figure 3). Clearly this feature of Vmw65 resembles that of the GAL4 and GCN4, yeast transcription factors which contain activation domains organized as acidic amphipathic helices (Hope *et al.*, 1988; Ma and Ptashne, 1987; Giniger and Ptashne, 1987). Although Vmw65 may therefore function

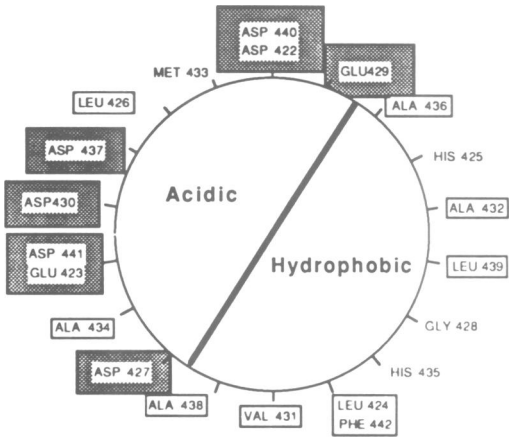

Fig. 3. Helical wheel analysis with an incremental angle of 100° of the putative α-helix between amino acids 422 and 442 of Vmw65. Numbers indicate positions of amino acids with acidic residues in shaded boxes and hydrophobic residues in open boxes. The acidic and hydrophobic faces of the helix are indicated. In this depiction the C-terminal residues project out of the plane of the paper.

in a qualitatively similar manner to GAL4 and GCN4 the precise mechanism of action for any of these proteins remains unclear, especially since the primary amino acid sequence of the various activation domains identified share no apparent homology. It is likely, however, that activation involves multiple protein−protein interactions with the acidic amphipathic helices perhaps providing a surface for intimate ionic contacts with core elements of transcriptional machinery such as the TATA factor or subunits of RNA polymerase. That is not to say that acidic amphipathic helices are the only type of structure able to perform an activation function; a second region within GAL4, for example (Ma and Ptashne, 1987), is not notably acidic and is unlikely to form an amphipathic helix, while in contrast, the activation domains of Sp1 are highly glutamine rich (Courey and Tjian, 1988). However, in the case of Vmw65 it is evident that the highly charged amphipathic helix within the activation domain is almost certainly responsible for the ability of the Vmw65−TRF complex to activate transcription.

If the activation domain of the Vmw65−TRF complex is provided by Vmw65 then presumably the major role of TRF in the complex is to direct Vmw65 to the appropriate *cis*-acting elements. If this is the case then it raises a number of important questions about the role of TRF in the uninfected cell. It is not clear, for example, whether TRF itself possesses an activation domain. If so we then need to ask why the TAATGARAT motifs do not function as constitutive promoter elements in transfected cells in the absence of Vmw65. Recent experiments using an *in vitro* transcription system showed that trancription from an octamer-containing promoter was increased by the addition of purified TRF (NF-A1) to TRF-depleted nuclear extracts (LeBowitz *et al.*, 1988). Although this might be taken as evidence that TRF itself contains an activation domain, these experiments cannot exclude the possibility that TRF function is dependent on interaction with a putative cellular homologue of Vmw65.

In general, combinatorial mechanisms of transcription control are likely to play a key role in regulation of eukaryotic gene expression. For example, the association of the yeast PRTF factor with the product of the *Mat α1* gene results in the formation of a complex required for activation of a set of cell-type specific genes (Bender and Sprague, 1987). Furthermore, the data recently presented by Shaw *et al.* (1989) on the serum responsive transcription activation of the c-*fos* gene appear strikingly similar to those we have obtained for activation of HSV IE gene expression by Vmw65. Thus, it appears that serum activation of *fos* transcription correlates with the formation of a complex between the DNA-binding serum response factor (SRF), and a non-DNA-binding protein, p62, and that while sequences within the serum response element (SRE) alone are necessary and sufficient for binding of SRF, additional sequences located outside the SRE are required for complex formation. Our results, indicating that the role of Vmw65 in the complex with the octamer-binding protein is to provide an efficient transcription activation domain, may be relevant to understanding the function of p62 in its association with SRF.

In conclusion it would appear therefore that the Vmw65−TRF complex represents a novel class of transcription activator in that the protein domain responsible for sequence-specific contact with DNA and that necessary for induction of transcription are located on separate proteins, in contrast to other factors such as GAL4 or GCN4 where both activities reside within a single polypeptide. Since efficient expression of HSV IE genes requires interaction between TRF and Vmw65, it is possible that the progress of infection will be affected by levels of TRF available for complex formation in a virus infected cell. Therefore, the requirement for TRF interaction for IE induction may provide a means to couple IE induction to levels of competent TRF and thus to particular metabolic states of the cell or particular cell types. This possibility is being investigated.

Materials and methods

Strains and media

Strains used were *E.coli* K12 Mc1061 [ara D138 Δ (*ara, leu*)7697, Δ*lacx*74, *galU*$^-$, *galK*$^-$, *hsr*, *hsm*$^+$, *strA*] Casadaban and Cohen (1980); *S.cerevisiae* strain DBY 745 (α, *ade1-100, leu2-3, leu2-112, ura3*-52) West *et al.* (1984) and *S.cerevisiae strain 21 (a, gal4-2, ura3-52, leu2-3, leu2-112, ade1*, MEL1) Johnston and Hopper (1982). Yeast cultures were grown at 30°C in either YEPD (1% yeast extract, 2% glucose, 1% peptone) or in minimal medium (0.67% yeast nitrogen base) with glucose (1%) and amino acids (0.002%) added as required.

Yeast transformations

Yeast transformations were performed following the procedure of Hinnen *et al.* (1978).

β-galactosidase assays

Yeast cultures were assayed for β-galactosidase according to Harshman *et al.* (1988). The protein concentrations of individual cell suspensions was determined by the method of Bradford (1976).

Yeast vector constructions

The vector pLG669Z has been described elsewhere (Guarente and Ptashne, 1981) and contains the *CYC* promoter truncated to position −312 upstream of a *CYC−lacZ* fusion gene. In p669B the *CYC* upstream sequences have been further deleted to the *Xho*I site at position −178, removing the UAS_c, and a *Bgl*II linker inserted. p669.T6 was generated from p669B by cloning a 28 bp fragment containing the promoter proximal TAATGARAT element T6, (O'Hare and Goding, 1988) from the HSV IE 110 promoter into the unique *Bgl*II site. p669.UAS_G has the *GAL1-10* UAS inserted into the *Bgl*II site of p669B. This 159 bp fragment containing the UAS_G was derived form a plasmid pMA1200-1 (a gift from S.Kingsman) and carries all four GAL4 DNA-binding sites between the *Rsa*I and AluI restriction sites.

Vector pMA36ct was made from the high copy number plasmid pMA36c (Dobson *et al.*, 1983) by inserting terminator sequences from the yeast PGK promoter, isolated as a 290 bp *Bam*HI−*Sal*I fragment from the yeast vector pDT86 (S.Kingsman). Coding sequences inserted into the unique *Bam*HI site of pMA36ct are expressed from the yeast TRP1 promoter. pTRP.65 contains the entire coding sequences from Vmw65, subcloned as a 1.7 kb *Bam*HI−*Bgl*II fragment from pRG10 (R.Greaves, unpublished) and

pTRP.NGAL4 expresses the N-terminal 147 amino acid DNA-binding domain of GAL4. The *GAL4* gene was subcloned as a 2.9 kb *Hin*dIII fragment from pEK26 (Delta Biotechnology) into the unique *Hin*dIII site of a pUC-based vector, pUC.poly3c (C.Goding, unpublished) containing a novel synthetic polylinker to give pGAL4. Digestion of pGAL4 with *Cla*I removed coding sequences of GAL4 C-terminal to amino acid 147 and part of the polylinker. The 5′ *Hin*dIII site, immediately adjacent to the initiation codon, was then converted to a *Bam*HI site by digestion with *Hin*dIII, repairing the ends with klenow and addition of a *Bam*HI linker to give plasmid pNGAL4B (C.Goding, unpublished). This 5′ *Bam*HI site together with a 3′ *Bgl*II site present in the synthetic polylinker were used to generate a fragment containing the N-terminal 147 amino acid coding sequences of *GAL4* which was inserted into the unique *Bam*HI site of pMA36ct downstream of the TRP1 promoter.

pTRP.NGAL4.65 expresses the GAL4 DNA-binding domain fused in frame to the C-terminal 79 amino acids of Vmw65. The C-terminus of Vmw65 was subcloned as a 469 bp *Acc*I−*Bgl*II fragment into the unique *Cla*I and *Bgl*II sites of pNGAL4B to create pGAL4.65B. A *Bam*HI−*Bgl*II fragment containing the GAL4−Vmw65 chimeric sequences was then subcloned into the *Bam*HI site of pMA36ct.

Mammalian vector constructions

The GAL4 expression vector pCMV.GAL4 was produced by the ligation of a 2.9 kb *Hin*dIII fragment from plasmid pGAL4 (see yeast vectors) into the *Hin*dIII site of pCMV.IL2 (Cullen, 1986) downstream of the strong CMV IE promoter. The vectors pCMV.NGAL4 and pCMV.NGAL.65, expressing the N-terminal 147 amino acids DNA-binding domain GAL4 or the GAL4−Vmw65 chimera respectively, were made by the ligation of either a 450 bp *Hin*dIII−*Bgl*II fragment derived from plasmid pNGAL4 or a 920 bp *Hin*dIII−*Bgl*II fragment from pNGAL4.65 between the *Hin*dIII and *Bgl*II sites of pCMV.IL2.

The reporter plasmid, pLSO.CAT is derived from a linker-scanning mutant of the HSV thymidine kinase (TK) promoter, LS 119−109 (kindly provided by S.McKnight), and contains the TK promoter to position −480 with a *Bam*HI linker placed at between positions −119 and −109. A *Bam*HI−*Sal*I cassette containing the coding sequences for the bacterial chloramphenicol acetyl transferase gene is inserted downstream of the TK promoter at position +60. pLSO.UAS$_G$ is derived from pLSO.CAT by the insertion of the 159 bp *GAL1-10* UAS from plasmid pMA1200-1(S.Kingsman) into the *Bam*HI site at position −119.

Mammalian cell culture and transient expression assays

COS cells were grown in Dulbecoo's modified Eagle's essential medium containing 10% newborn calf serum. Transfections were performed by the method of Chen and Okayama (1987) using *N.N*-bis(2-hydroxyethyl)-2-aminoethane sulphonic acid (BES) buffered saline (BBS). COS cells were seeded overnight at 4 × 10^5 cells/3cm well and fresh medium added 2 h prior to transfection. Cells remained in BBS overnight (18 h), the medium was then replaced and the transfected cells harvested 24 h later. Transient expression assays were otherwise as previously described (O'Hare and Hayward 1987). The levels of acetylated chloramphenicol were quantified by excision of the spots from the TLC plates followed by liquid scintillation counting in an LKB 216 counter.

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