Analysis of Functional Domains of the Host Cell Factor Involved in VP16 Complex Formation*

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We present biochemical analyses of the regions of the host cell factor (HCF) involved in VP16 complex formation and in the association between the N- and C-terminal domains of HCF itself. We show that the kelch repeat region of HCF (residues 1–380) is sufficient for VP16 complex formation, but that residues C-terminal to the repeats (positions 381–450) interfere with this activity. However, these latter residues are required for the interaction between the N- and C-terminal regions of HCF. The extreme C-terminal region of HCF, corresponding to an area of strong conservation with a Caenorhabditis elegans homologue, is sufficient for interaction with the N-terminal region. These results are discussed with respect to possible differences in the roles of HCF in VP16 activity versus its normal cellular function.

A major factor in the control of transcription is the assembly of large multiprotein complexes and the operation of selectivity and regulation of the various protein/protein and protein/DNA interactions involved. Understanding the assembly of these multiprotein complexes is clearly essential for elucidating the general mechanisms that regulate gene expression. The POU domain class of proteins (1, 2) is an example of a large family of transcription factors whose members frequently function through the selective assembly of multicomponent complexes. Members of this class of proteins play an important role in a diverse range of regulatory processes, including the control of gene expression in development and in cellular differentiation pathways (for reviews, see Refs. 2–5). One member of the family, Oct-1, is ubiquitous and is present in most cells during all stages of the cell cycle. Recognition sites for the protein are widely distributed in vertebrate genomes and are present in genes that do not form any coordinate regulated group. Oct-1-binding sites are also closely related (and in certain cases, identical) to the recognition sites for other members of the family. However, Oct-1 selectively activates genes in response to developmental, cell cycle, or hormonal signals and is involved in expression of housekeeping genes (e.g. Refs. 6–13), and it has now been shown that different proteins can be recruited to complexes with Oct-1 when it is bound in different DNA contexts or in cell types that express different cofactors (14–17).

Oct-1 also plays an important role in the selective induction of the immediate-early transcription of herpes simplex virus by the virion activator VP16 (18), and analysis of this role has helped elucidate features that may be of broad applicability to transcriptional control mechanisms (reviewed in Refs. 19–21).

Transcription of herpes simplex virus immediate-early genes is activated by VP16 (22, 23) via specific cis-acting signals, the TAATGARAT motifs (24, 25). These motifs represent specialized binding sites for Oct-1 and are present upstream of each of the immediate-early genes. VP16 does not bind directly to DNA, but is recruited onto these sites by interaction with Oct-1, forming the transcription complex TRF-C (18, 26, 27). Results from several laboratories demonstrated that assembly of TRF-C requires an additional cellular protein, variously termed HCF,1 CFF, VCAF1, or C1 (26, 28–32). The factor was isolated and cloned by Wilson et al. (33), and the same gene was subsequently cloned by Kristie et al. (34).

Human HCF is translated as a 300-kDa precursor of 2035 amino acids (33, 34). The protein is processed by cleavage at a number of specific sites, the HCF repeats, which are located toward the middle of the precursor (between amino acids 1012 and 1435) to give rise to a range of polypeptides from 110 to 150 kDa. After cleavage, the majority of the N- and C-terminal regions of the protein remain stably associated.

Recent studies have shown that the N-terminal region contains critical determinants for association with VP16 and for complex formation on the TAATGARAT motifs (35–37). Residues 1–380 of HCF contain six related reiterations of a motif of 50 amino acids, termed the kelch repeat and originally defined in the Drosophila protein kelch (38). In vitro translated variants of HCF comprising this region promoted complex formation with VP16 proteins lacking the activation domain (35, 36). Previous results from our laboratory indicated that the N-terminal region of HCF (amino acids 2–450) interacted with VP16 and formed the DNA-binding complex with the POU domain of Oct-1 relatively poorly, whereas a fusion protein (HCF NC) of the N-terminal domain with the C terminus of HCF (amino acids 1496–2035) bound VP16 and promoted complex formation much more efficiently (37).

Furthermore, a sequence homologue of HCF has been identified in the nematode Caenorhabditis elegans (37). The primary amino acid sequence of this protein resembles a natural NC fusion, having regions of high sequence identity to human HCF both within the kelch repeats of the N terminus and in the C-terminal domain. In addition, formation of a VP16 complex using C. elegans protein extracts has been reported (21).

In this study, we undertake further biochemical analysis of the regions of HCF involved in VP16 complex formation and examine regions of the protein that are required for the N- and C-terminal domains of HCF to associate in the complex. The results indicate that the kelch repeat region of HCF is indeed sufficient for complex formation, but that residues C-terminal to the repeats, which are necessary for the association between the N and C termini of HCF itself, may interfere with VP16 complex formation. The extreme C-terminal region of HCF, corresponding to the area of conservation with the C. elegans

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1 The abbreviations used are: HCF, host cell factor; PAGE, polyacrylamide gel electrophoresis; TRF-C, Tata rat recognition factor complex.
protein, is sufficient for interaction with the N-terminal kelch repeat domain.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—Plasmids for bacterial expression of VP16 and the POU domain (amino acids 270–444) from Oct-1 (39, 40) and for in vitro transcription and translation of VP16, HCF NC (amino acids 2–491 + 1496–2035), N450 (amino acids 2–450), and C2 (amino acids 1496–2035) have been described previously (37). Plasmids for the expression of other HCF mutants (see Figs. 1 and 8) were created as described below from these HCF plasmids, from pCGNHCFC (which contains full-length epitope-tagged HCF) (33), or from pSW127M (which contains two copies of the N-terminal domain of HCF) (37). All HCF expression plasmids were constructed in pGEM3Zf(+) (Promega).

Expression plasmids for HCF NCa to Nce, NCeΔtag, NCeΔe, NS58, and N491tag were created by cleaving pSW127M with AgeI/BamHI, purifying linear pGEM.HCF-(2–491), and inserting AgeI/BamHI fragments containing appropriate regions amplified by polymerase chain reaction. The DNAs coding for the C-terminal domains of NCa to Nce (including the C-terminal c-Myc tag and stop codon) were amplified from pSW127NC with the addition of 5’-AgeI and 3’-BamHI sites. DNA coding for CaΔtag (to terminate translation at HCF amino acid 2035 and at the DNA and the epitope tag) was amplified as for Co, but with the BamHI coding region added before the 3’-BamHI site. DNA coding for CaΔe was created by ligation of DNA coding for HCF amino acids 1786–2003, amplified from pSW127NC with the addition of 5’-AgeI and 3’-BamHI sites, to a SacII/BamHI oligonucleotide linker carrying c-Myc tag and stop codon (note that alanine was added between amino acid 2003 and the linker). DNA coding for HCF amino acids 491–538 was amplified by polymerase chain reaction (natural 5’-AgeI site and 3’-stop codon and BamHI added) from pCGNHCFC. DNA coding for the C-myc tag (and stop codon) was synthesized as an oligonucleotide linker with AgeI and BamHI overhangs.

The expression plasmid for HCF N491 was created by cleaving pSW127M with AgeI and BamHI, end filling, and ligating the blunt ends (note that isoleucine and leucine were added before the stop codon). The plasmid for HCF N380 was created by cloning DNA coding for HCF amino acids 2–380 (including the 5’-EcoRI site and N-terminal hemagglutinin tag), amplified by polymerase chain reaction from pSW127NC (3’-stop codon and BamHI added), into the EcoRI/BamHI sites of pGEM3Zf(+).

The plasmids for expression of HCF C1, C3, C4, C8, and C9 (see Fig. 8) were created by amplifying the required DNA by polymerase chain reaction targeting pSW127NC or pCGNHCFC (5’-EcoRI and 3’-SacII sites added), into a SacII/BamHI oligonucleotide linker carrying codons for the c-Myc tag and stop codon (note that alanine was added between amino acid 2003 and the linker). DNA coding for HCF amino acids 491–538 was amplified by polymerase chain reaction (natural 5’-AgeI site and 3’-stop codon and BamHI added) from pCGNHCFC. DNA coding for the C-myc tag (and stop codon) was synthesized as an oligonucleotide linker with AgeI and BamHI overhangs.

**Electrophoretic Mobility Shift Assays**—Binding reactions were performed in 20 μl containing −1 ng of 32P-labeled oligonucleotide probe containing the sequence ATGCTAAGGATAT from the herpes simplex virus-1 IE1 promoter, 25 mM HEPES (pH 7.9), 50 mM NaCl, 10% glycerol, 200 μg/ml bovine serum albumin, 0.05% Nonidet P-40, 1 mM dithiothreitol, and 1 mM EDTA. Typically, purified POU domain (0.25–0.5 ng), VP16 (0.5–2 ng), and up to 3 μl of TnT lysate were used in each reaction as indicated in the figure legends. Minor adjustments were made based on the specific activity of the purified preparations. Formation of the complex between HCF N- and C-terminal regions via the recruitment of the HCF C terminus into the complex was more readily detected using higher amounts of VP16 and the programmed lysate, but this N-C complex could be assayed under standard conditions (see Figs. 7 and 9). Complexes were resolved and analyzed as described previously (37) and quantitated using a PhosphorImager.

**RESULTS**

We and others have previously shown that assembly of TRF-C can be recapitulated in vitro using Oct-1, or its POU DNA-binding domain, purified VP16, and HCF supplied after in vitro transcription and translation of the gene. To pursue dissection of important determinants within HCF, plasmids were constructed to allow expression and radiolabeling of deletion mutants of HCF using in vitro transcription and translation reactions in the presence of [35S]methionine (“TnT reactions”). Fig. 1A shows a representation of full-length HCF (with domains of interest indicated), together with the composition of the HCF variants examined in this work. The regions of strong homology between the human and C. elegans HCF proteins are indicated by the darkly hatched areas in HCF NC. Products from in vitro expression were analyzed by SDS-PAGE (Fig. 1B), with lane numbers corresponding to the schematic in Fig. 1A. Each of the mutants was expressed as a single protein species with relative migration as expected.

**The C-terminal Region of HCF Is Not Critical for Efficient Complex Formation**—We previously demonstrated that a HCF variant consisting of a fusion between the N- and C-terminal
regions promoted VP16 complex formation much more efficiently than the N-terminal region alone (37). To examine this further, the amino-terminal end of the "C" domain in HCF NC was progressively deleted in the series of mutants HCF NCa to NCe (Fig. 1a). The region of this domain that is homologous to the C. elegans protein remains intact in NC, NCa, and NCb; is partially deleted in NCc and NCd; and is completely removed in NCe. The abilities of these mutant proteins and HCF N450 to form VP16-dependent complexes were compared in gel retardation assays. Equal amounts of the HCF mutants (at each of two doses) were incubated with intact purified VP16 and POU domain together with a 32P-labeled oligonucleotide probe containing a TAATGARAT motif. Complexes were separated by electrophoresis on nondenaturing gels and visualized by autoradiography (Fig. 2).

Various controls were included in the experiments. The independent binding of the POU domain to the site is shown in Fig. 2 (lane 1). Under the conditions of these experiments, the POU domain and VP16 did not form a complex in the absence of HCF (lane 2). When unprogrammed lysate was included in the reaction, an additional band was seen representing a non-specific complex (lane 3, asterisk). Variable amounts of this complex were seen throughout these experiments, and although the explanation for this is unknown, it was not related to complex formation and was not investigated further. Additional controls with all the HCF mutants showed that the induced complexes were not observed when VP16 was omitted from the reactions or when a DNA probe containing an octamer site without a 3'-GARAT motif was used (data not shown).

As reported previously (37), complex formation was much more efficient with HCF NC (Fig. 2, lanes 4 and 5) than with HCF N450 (lanes 20 and 21, arrow). Mutants NCa to NCe promoted complex formation relatively efficiently (lanes 6–11). Note the progressively increasing mobility of the HCF-induced complexes, with the exception of NCc, which, although 50 residues smaller than NCh, migrated marginally more slowly. Deletion of the 52 residues between amino acids 1878 and 1930 (NCd) resulted in significantly less complex formation (lanes 12 and 13). All of these constructs promoted complex formation much more efficiently than the construct with the N-terminal domain alone, N450. The construct with the shortest section of additional residues from the C terminus was NCe, which contained only the 31 extreme C-terminal amino acids (plus an epitope tag), yet this construct promoted complex formation significantly better than N450 (lanes 14 and 15). To examine if these C-terminal residues were critical for the more efficient complex formation, we deleted them from NCa to yield NCaΔe (Fig. 1). These results show that although there was some reduction upon deletion of the extreme C terminus, NCaΔe still promoted complex formation much more efficiently than N450 (lanes 16 and 17).

To ensure that the C-terminal epitope tag was not influencing complex formation, we removed it from NCe to yield NCeΔtag. Somewhat surprisingly, there was a relatively minor (3–4-fold) but reproducible reduction in complex formation (Fig. 2, lanes 18 and 19). Nevertheless, neither the "e" region itself (residues 2004–2035) nor the epitope tag appeared to account for the more efficient complex formation promoted by NCe compared with N450.

HCF N450 Forms a Complex Less Well than Either Longer or Shorter Regions of the N Terminus—One additional difference between NCe and N450 was that NCe, as for the other NC mutants, contained residues 2–491 of the N terminus, rather than residues 2–450 of the original construct used to examine the independent N-terminal region.

Therefore, we next constructed plasmids to express different lengths of the N terminus of HCF (see Fig. 1). HCF N491, the N-terminal region that is present in the NC mutants, was expressed both with and without a c-Myc tag at its C terminus (Fig. 1a, constructs 10 and 11). HCF N538 has the same number of amino acids as HCF NCe and was created to examine whether the length of the HCF molecule, rather than its sequence, had any influence on complex formation (Fig. 1a, construct 12). HCF N380 contains the kelch repeats only (Fig. 1a, construct 13). The ability of these HCF variants to promote complex formation was assayed as before, using equal amounts of HCF at each of two doses (Fig. 3).

Although marginally less efficient than HCF NCe, HCF N491 and N491tag both formed complexes much more efficiently than HCF N450 (Fig. 3, compare lanes 5–8 with lanes 3 and 4). HCF N538 appeared to form a complex at least as well as HCF NCe, whereas HCF N380 also promoted complex formation much more efficiently than HCF N450.
We further compared the efficiency of these N-terminal variants of HCF, this time using equal amounts of each in vitro translated variant (Fig. 4a) with increasing amounts of VP16. The results again show that although HCF N450 is comparatively inefficient, HCF N380 promotes complex formation at least as well as HCF N491. Together, the data indicate that HCF N380 is sufficient to promote complex formation, in agreement with previous results (35, 36), but that the presence of residues 381–450 is detrimental for complex formation. Complex formation is recovered when HCF is extended to amino acid 491 and is increased further with extension to amino acid 538 or with the addition of regions of the C terminus (NC to NCe).

Complexes Formed with HCF N450 or N491 Are Less Stable than Others—The dissociation kinetics of the complexes formed with the different HCF mutants were compared. In this case, to measure starting complex formation with HCF N450, a higher dose of VP16 was used (corresponding to the top dose in Fig. 4, ~10 ng/assay). Bulb binding reactions of HCF mutants, the POU domain, VP16, and radiolabeled DNA were prepared and allowed to bind to equilibrium. An excess of unlabeled DNA probe was then added; aliquots of the mixture were loaded onto running gels at various times thereafter; and complexes were analyzed as before. Amounts of complexes remaining at each time point were quantified and are expressed graphically as percentages of the amount observed immediately before the addition of competitor DNA for each variant (Fig. 5).

Complexes formed with HCF N450, N491, or N491tag were significantly less stable than those formed with either a longer or a shorter section of the N terminus (N380 or N538). It is noteworthy that although HCF N491 promoted complex formation relatively well (Figs. 3 and 4), the complex was as unstable as that formed with HCF N450.

**Direct Interaction between VP16 and HCF Mutants Reflects Complex Formation Activity**—HCF has been shown to interact directly with VP16 in the absence of the other components of TRF-C (28, 30, 36, 37, 41). Direct interaction between VP16 and the HCF mutants was assayed by HCF co-immunoprecipitation using antibodies directed against VP16. Equal amounts of HCF mutant proteins were incubated with 35S-labeled or unlabeled VP16 expressed in vitro. Unlabeled VP16 was used in the co-precipitation assays since the test HCF species might be masked by comigrating radiolabeled VP16. Monoclonal antibody against VP16 (LP1) was added; immune complexes were recovered and washed; and the proteins were analyzed by SDS-PAGE. Fig. 6a shows the equalized input amounts of HCF mutants and VP16. A control demonstrated that VP16 was recovered only in the presence of antibody (Fig. 6b, lanes 1 and 2). For each HCF variant, the first lane shows the background control, precipitated in the absence of VP16, and should be compared with the third lane showing precipitation in the presence of unlabeled VP16. (Precipitation of VP16 is demonstrated for each test variant using radiolabeled VP16 in the middle lane of each panel.)

Quantitation of the amounts of mutants recovered was performed by analysis on a PhosphorImager. Specific co-precipitation above background was observed for each of the variants HCF NC, NCe, N538, N491, and N380. In contrast, the amount of HCF N450 recovered in the co-immunoprecipitation was not significantly above background recovery by the antibody alone (cf. Fig. 6b, lanes 15 and 17). Therefore, as for the complex formation assay, HCF N450 interacts with VP16 less strongly than the other HCF mutants.

**Requirements for the Interaction between HCF N and C Termi**—In vivo, after HCF is cleaved at the HCF repeats, the majority of N- and C-terminal fragments remain stably associated (33, 34). The relevance of this association is not known,
although it is reasonable to propose that this interaction between the N- and C-terminal regions may be important for the cellular function of HCF.

When separate in vitro lysates programmed with HCF N450 or with the C-terminal domain of HCF were mixed, we were unable to detect an interaction between the domains (37). However, when the proteins were generated simultaneously by co-translation, a proportion of the molecules associated. Using the co-translated N- and C-terminal regions of HCF in an electrophoretic mobility shift assay with the POU domain and VP16, we observed a complex that included both termini of HCF (37). We therefore next wished to pursue requirements for this interaction. We constructed plasmids to facilitate expression of different lengths of the N or C terminus and analyzed the interaction of these mutants in the VP16

To first examine the regions within the N terminus, in vitro lysates were programmed with one of the different N-terminal constructs HCF N538, N491, N491tag, N450, and N380 together with Fig. 7, lanes 2–6) or without (lanes 7–11) the C-terminal construct HCF C2, i.e. the C-terminal region that is within HCF NC. The lysates were incubated with the POU domain, VP16, and radiolabeled probe, and complexes were analyzed as before. We note that higher levels of VP16 were generally required to detect the N/C-terminal domain interactions, producing an artificially high level of the independent N complex for HCF N450. Restricting the level of VP16 to reflect the true comparison for the independent N complex resulted in a weaker, but readily detected N complex (Fig. 7).

When assayed alone, the C terminus of HCF was unable to promote any complex formation (Fig. 7, lane 1). A series of complexes (N complexes; lanes 2–6) that corresponded to those seen with the N-terminal regions alone (cf. lanes 7–11) was observed with the lysates containing co-translated products. However, an additional complex of slower mobility (labeled N+C), not present in the C-alone or N-alone programmed samples, was clearly observed for HCF N538, N491, N491tag, and N450. Differences in the mobility of these complexes were not as marked as those containing the N terminus only, but the

Regions of HCF Involved in VP16 Complex Formation

**Fig. 6.** Analysis of direct interactions between HCF mutants and VP16. VP16, expressed in vitro with or without [35S]methionine, was incubated with equal amounts of different HCF mutants together with monoclonal antibody directed against VP16 (LP1). Immune complexes were recovered by the addition of Protein A-Sepharose; proteins were separated on SDS-7.5% polyacrylamide gels; and 35S was visualized using a PhosphorImager. *a,* shown are proteins from 10% of the reaction inputs. *b,* in control assays, 35S-labeled VP16 was incubated without (lane 1) or with LP1 (lane 2), and VP16 immunoprecipitation was dependent upon the addition of the antibody. Results for each HCF variant are in panels of three lanes. The first lane represents background precipitation of the HCF species in the absence of VP16. This should be compared with the third lane of each panel, which shows HCF precipitation in the presence of unlabeled VP16. The middle lane of each panel shows results using radiolabeled VP16 and serves to confirm VP16 immunoprecipitation. VP16 binding above background could be observed for each of the HCF variants, with the exception of N450.

**Fig. 7.** Amino acids 381–450 are necessary for the HCF N terminus to interact with the C terminus, allowing formation of a VP16-induced complex containing both termini. Lysates were programmed with each of the HCF N-terminal variants together with (lanes 2–6) or without (lanes 7–11) HCF C2 (residues 1496–2035). Equal amounts of the lysates were incubated with 0.5 ng of POU domain and 2 ng of VP16 per reaction. The control assay for the C2 region in the absence of any N-terminal constructs is shown in lane 1. Complexes were analyzed as described for Fig. 2. The nonspecific complex is marked with an asterisk. N marks the positions of complexes formed with only the N-terminal mutants, whereas the positions of complexes formed specifically upon the addition of HCF C2 is labeled N+C. Such complexes were observed for each of the N-terminal constructs, with the exception of N380.
It was difficult to determine whether the N-terminal region (amino acids 1440–2035) to HCF C5 (amino acids 1786–2035). As the size of the C-terminal constructs decreased, the mobility of the additional N-terminal end (HCF C1–C7) or carboxyl-terminal end (HCF C8 and C9). We note that all these mutants appeared to be somewhat more susceptible to degradation than the N-terminal products (compare with Fig. 1). In vitro lysates were programmed with HCF N450 together with one of the different C-terminal deletion variants, and the co-translated products were incubated with the POU domain, VP16, and radiolabeled DNA (Fig. 9).

The complex formed by HCF N450 without the C terminus was observed in all test lanes (Fig. 9, labeled N). The addition of HCF C2 resulted in an additional complex (labeled N+C) not observed for either C2 alone or N450 alone (cf. lanes 1–3). As expected, if the different HCF C-terminal products were being recruited, the mobility of the additional N+C complex increased as the size of the C-terminal constructs decreased (lanes 3–7). The N+C complex was observed with HCF C1 (amino acids 1440–2035) to HCF C5 (amino acids 1786–2035). It was difficult to determine whether the N+C complex was formed with the next C-terminal variant C6 (amino acids 1827–2035) since although a novel band was not observed, it might nonetheless have been formed, but not been readily separable from the independent N complex. We were unable to obtain evidence for an N+C complex with C6 or C7 by altering resolubilization conditions. Finally, we tested variants of C2 from which the extreme C terminus had been deleted (C8 and C9). The results show that deletion of the C-terminal 32 residues from C2 abrogates formation of the N+C complex (cf. lanes 4 and 10). These results allow the identification of a minimal domain that is required for the C terminus of HCF to interact with the N terminus and to form a VP16-POU complex containing both termini. This domain spans from between residues 1786 and 1827 at its N terminus and 2035 at its C terminus and corresponds closely to the region of strong homology within the C terminus between the human and C. elegans HCF proteins.

**FIG. 8.** a, summary of HCF C-terminal mutants. The hatched area indicated within HCF C1 is >60% identical to a region of the C terminus of the C. elegans homologue. b, SDS-PAGE analysis of the HCF mutants expressed and radiolabeled in vitro (2 μl of each programmed TnT lysate).

**FIG. 9.** Amino acids 1786–2035 are necessary and sufficient for the HCF C terminus to interact with the N terminus, allowing formation of a VP16-induced complex containing both termini. Lysates were programmed with HCF N450 alone (lane 2) or together with each of the C-terminal variants (lanes 3–11). The control assay for the unprogrammed lysate is shown in lane 1. Lysates were incubated with the POU domain (0.5 ng), VP16 (2.0 ng), and probe and analyzed as described for Fig. 2. The nonspecific complex is marked with an asterisk. The position of the complex formed only with HCF N450 is marked N, whereas the positions of complexes containing both HCF N450 and the various C-terminal mutants are marked N+C.
regions 382–450 are removed (33), it is possible that the inhibition of complex formation by this region could be relevant and that these amino acids may act in some way to negatively regulate the function of the N terminus, the role of the alternative splice being to release the inhibition. However, extension of the N terminus to amino acid 538 or the addition of sections of the C terminus, even in the presence of residues 381–450, allows stronger and more stable interactions with VP16 and more efficient complex formation, and we assume that, in this context, the inhibitory effect of residues 381–450 is masked.

The relative inefficiency of complex formation with HCF N450 compared with HCF N380 was not observed in previous analysis that compared similar variants (36). However, this earlier work used versions of VP16 that in addition to being glutathione S-transferase fusions, also lacked the C-terminal acidic activation domain. We have shown that deletion of the acidic domain of VP16 promotes more efficient complex formation with HCF N450 (37), allowing a possible explanation for why the difference between HCF N380 and N450 was not previously observed and indicating that the detrimental effect of HCF residues 381–450 may be targeted toward the acidic domain of VP16.

Although the first 380 residues of HCF are sufficient for the interaction with VP16, they are not sufficient for the interaction with the HCF C terminus (this work and Ref. 21). This latter interaction requires additional residues between positions 381 and 450, i.e. the precise region that appears to be detrimental to VP16 binding. Therefore, notwithstanding the observation that extension to residue 538 restored complex formation, it remains possible that there is some form of interplay between the interaction of the N- and C-terminal regions of HCF, which requires amino acids 381–450, and the interaction of VP16 and HCF, which is inhibited by residues 381–450. Also, although residues 381–450 are required for the interaction of the N-terminal region of HCF with its C terminus, we do not know if this region is sufficient, and it is possible that the C terminus makes additional contacts with the kelch repeats. If this is so, our data on interactions of the co-translated products indicate that during complex formation, the kelch repeat region of HCF makes simultaneous contacts with VP16 on the one hand and with the C terminus of HCF on the other.

Another functional determinant within HCF was identified using a hamster cell line with a mutation within the kelch repeats conferring a temperature-sensitive cell cycle defect (42). Cells at the nonpermissive temperature were transfected with plasmids to express different lengths of HCF and were examined for rescue of the cell cycle defect (36). The kelch repeat region was necessary to rescue the defect, but was not sufficient. N-terminal residues extending to position 902 were required. Therefore, although there are similarities between the HCF interaction with VP16 and the HCF interaction with at least one of its cellular targets in that both were sensitive to the same mutation conferring temperature sensitivity, there are clear differences since the kelch repeat region is sufficient for the interaction with VP16, but is insufficient to complement the temperature-sensitive defect in the cellular protein.

Although the kelch repeats may be sufficient for the VP16 complex, both our results and previous analysis (36, 37) indicate that the VP16-kelch complex retains the HCF C-terminal domain in the DNA-binding complex with Oct-1. This HCF C-terminal domain contains a nuclear localization signal, and current results indicate that this nuclear localization signal motif may be an important determinant for VP16 nuclear import (43). However, the present information on the kelch repeat domain itself yields few clues as to any particular function it would serve intracellularly, over and above promoting VP16 recruitment to the complex with Oct-1. We previously postulated (37) that a conformational change took place upon the HCF/VP16 interaction, masking an otherwise inhibitory effect of the acidic domain on recruitment to DNA. This remains a useful rationale, but does not explain why the kelch repeat domain specifically serves this purpose. It may be that cellular functions of the kelch repeats in, for example, protein interactions or compartmentalization and cell type differences in these properties provide part of the explanation. Our current results should help both in the biochemical characterization and re- construction of the complex and in the elucidation of cellular aspects of VP16 function.

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