The product of varicella-zoster virus gene 62 autoregulates its own promoter

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Varicella-zoster virus (VZV) gene 62 encodes a protein with a predicted Mr of 140000 (140K) which has considerable amino acid identity with the major immediate early (IE) protein Vmw175 (ICP4) of herpes simplex virus type 1 (HSV-1). Vmw175 is an essential virus polypeptide with a pivotal role in the activation of early and late viral gene expression and also in the repression of IE gene expression. The VZV 140K protein has been shown to function as a strong transcriptional activator in transfection assays and largely complements for the loss of Vmw175 function in HSV-1. We report the results of cotransfection experiments which demonstrate that the 140K protein strongly represses expression from its own promoter, that of gene 62, thus establishing further functional similarity between it and Vmw175. However, whereas Vmw175 can substitute for the 140K protein in repression of the gene 62 promoter, the 140K protein does not repress the HSV-1 IE3 promoter in the reciprocal experiment. The integrity of a domain of Vmw175 (designated region 2), previously shown to be crucial for repression of the HSV-1 IE3 promoter, is also required for repression of the gene 62 promoter. Moreover, a similar requirement for the highly similar region 2 of the 140K protein for repression is demonstrated, suggesting that VZV 140K protein and HSV-1 Vmw175 autoregulate IE gene expression by a related mechanism.
Roizman, 1975; Preston et al., 1978; Preston, 1979; Watson & Clements, 1980). Since repression of the HSV-1 IE-3 promoter by Vmw175 has been reproduced in transfection assays (O'Hare & Hayward, 1985), it was of interest to determine whether the VZV 140K protein exhibited similar autoregulation of its own promoter. Therefore, plasmid pl40CAT was constructed in which the promoter–leader sequences of VZV gene 62 (which encodes the VZV 140K protein) drive expression of the chloramphenicol acetyltransferase (CAT) gene (Fig. 1a).

We have previously reported that gene 62 promoter activity is increased about 15-fold by cotransfection with a plasmid expressing Vmw65, the HSV-1 virion-associated IE promoter transactivator (McKee et al., 1990). The ability of the VZV 140K protein to repress the gene 62 promoter was therefore determined both in the absence and in the presence of stimulation by Vmw65. Plasmid p140SV, which contains the VZV gene 62 transcription unit (without its own promoter) linked to the simian virus 40 (SV40) early promoter and enhancer region, was used to express VZV 140K protein. The use of p140SV (rather than a plasmid which expresses VZV 140K protein from the gene 62 promoter) reduces difficulties in the interpretation of the results which would arise from promoter competition effects and through repression of the gene 62 promoter in the VZV 140K protein expression plasmid.

BHK cells, at a density of $10^6$ cells per 50 mm dish, were transfected (by calcium phosphate precipitation; Corsalo & Pearson, 1981) with $4 \mu g$ p140CAT in the presence or absence of pMC1 (which expresses Vmw65) and increasing amounts of p140SV. The total amount of plasmid DNA in each transfection was equalized to 14 $\mu g$ by the addition of pUC9. CAT assay extracts were prepared after 48 h and CAT activities determined as described by Gorman et al. (1982). The protein concentrations of the extracts were determined by the method of Bradford (1976) and the percentage conversion from substrate to product per microgram protein was calculated. The CAT activities of extracts prepared from cells transfected with test plasmids were expressed as a percentage of the control value (no test plasmid). The results, depicted graphically in Fig. 2(a), are from a typical repression experiment. Transfection of p140CAT alone into cells resulted in $4\%$ conversion per microgram protein (representing a total conversion of $36\%$ in the assay) and the inclusion of pMC1 in the transfection increased CAT activity 30-fold (determined using suitable extract dilutions). The results clearly show that both the basal and Vmw65-stimulated levels of CAT activity from p140CAT were repressed up to 50-fold by the addition of increasing amounts of p140SV. This repression is not due to promoter competition effects because transfection with equivalent molar amounts of pSVEB (which contains the SV40 promoter region present in p140SV but lacks coding sequences) resulted in levels of repression which were insignificant compared to those induced by p140SV (Fig. 2a). Similar results were obtained using Vero cells (data not shown).
Fig. 2. Graphs showing repression of expression from VZV gene 62 promoter-leader sequences (in pl40CAT) by VZV 140K protein and Vmw175 (and their mutant derivatives), and activation (rather than repression) of the HSV-1 IE3 promoter (in pIE3CAT) by the VZV 140K protein. BHK cells were transfected with 4 µg of pl40CAT (or pIE3CAT) and increasing amounts of test plasmids in the presence or absence of pMC1 (which expresses Vmw65). The test plasmids used express the following polypeptides: VZV 140K protein (p140SV); Vmw175 (p175); insertion mutant derivatives of the 140K protein and Vmw175, respectively (pC34 and pI13). The negative control plasmid, pSVEB, lacks coding sequences present in the test plasmids and is consequently approximately half the size. Hence the actual amounts used in transfections were 0.5, 1, 2 and 4 µg to maintain a molar equivalence of common sequences. (a) Repression of the gene 62 promoter by 140K protein in the absence and presence of pMC1 (●, p140SV; ▲, pSVEB; ■, p140SV + pMC1; △, pSVEB + pMC1). (b) Repression of the Vmw65 (pMC1)-stimulated gene 62 promoter by 140K protein, Vmw175 and their insertion mutant derivatives (●, p140SV; ○, p175; △, pSVEB; ■, pC34; ●, pI13). (c) Activation of the Vmw65 (pMC1)-stimulated IE3 promoter by 140K protein. Plasmid pIE3CAT (in which the HSV-1 IE3 promoter drives expression of the CAT gene) replaced pl40CAT in this experiment (●, p140; ○, p175; △, SVEB). The data shown in (a) and (b) are derived from a typical titration experiment. The degree of repression observed in titration experiments was variable and almost certainly reflects the variation in levels of expression from the gene 62 promoter noted in the text. However, these experiments have been repeated a minimum of three times [with the exception of titration in the absence of pMC1 in (a), which was performed twice] and in all cases the pattern of results was consistent with those above. The data in (c) are the result of a single titration experiment.

It should be noted that repression of the gene 62 promoter by p140SV was most clearly observed when the levels of CAT activity in control transfections were high (about 50 to 200-fold above background). If the cells transfected poorly then the degree of repression was correspondingly reduced (as might be expected). Since repression was always most clearly observed with high level expression from the gene 62 promoter, most experiments included pMC1.

The results presented above clearly demonstrate that cotransfection of p140SV represses the gene 62 promoter by p140SV. Given the functional similarities of VZV 140K protein and Vmw175 in trans-activation transfection assays (Everett, 1984), it was of interest to determine whether the two proteins were functionally interchangeable in repression transfection assays. Therefore, p140CAT was cotransfected with increasing amounts of p175. The results show that Vmw175 represses the gene 62 promoter with an efficiency similar to that of the VZV 140K protein (Fig. 2b). The converse experiment to determine whether the 140K protein is able to repress the HSV-1 IE-3 promoter was performed in Vero cells, the cell type in which repression of the IE3 promoter by Vmw175 was first demonstrated (O'Hare & Hayward, 1985). (As noted above, the 140K protein efficiently represses the gene 62 promoter in Vero cells as well as BHK cells.) Plasmid pIE3CAT (in which the HSV-1 IE3 promoter drives expression of the CAT gene) replaced p140CAT as the reporter plasmid in these experiments. Fig. 2(c) shows the results of a single experiment in which pIE3CAT was cotransfected into cells with pMC1 and increasing amounts of test plasmids. The pMC1-stimulated level of expression from pIE3CAT was of the same order of magnitude as that from pMC1-stimulated p140CAT. IE3 promoter activity showed a small reduction in the presence of pSVEB, a maximum of twofold reduction being observed with the largest amount of plasmid. Titration of p175 led to a greater reduction in activity, fourfold in the presence of 8 µg p175. Although this was less efficient than in previous assays (Paterson & Everett, 1988), it does confirm repression of the IE3 promoter by Vmw175. In marked contrast, titration of p140SV resulted in an increase in IE3 promoter activity of two- to fourfold. In agreement with this, it was found that in BHK cells p140SV strongly trans-activated the basal level of expression from the IE3 promoter (in the absence of pMC1) approximately 40-fold (data not shown). Therefore, although both VZV
140K protein and Vmw175 repress their respective promoters, the mechanisms by which this occurs are not entirely interchangeable. This failure of the VZV 140K protein to repress the IE-3 promoter is consistent with the properties of a recombinant virus (HSV 140) in which the Vmw175-encoding sequences have been replaced by those of the VZV 140K protein in a transcription unit with the IE-3 regulatory sequences; infection by HSV 140 leads to high levels of VZV 140K protein expression (Disney & Everett, 1990).

The integrity of homology region 2 of Vmw175 (amino acid residues 315 to 484; Fig. 1b) has been shown to be of crucial importance for repression of the IE-3 promoter (Paterson & Everett, 1988; DeLuca & Schaffer, 1988; Shepard et al., 1989). To determine whether region 2 of Vmw175 was also involved in the repression of the gene 62 promoter, plasmid p113 (Paterson & Everett, 1988) was cotransfected in increasing amounts with p140CAT. Plasmid p113 contains an in-frame insertion of four amino acids in the proline codon at position 324 of Vmw175 (region 2; see Fig. 1b). The effect of the mutation is essentially to eliminate repression of the IE-3 promoter by Vmw175 (Paterson & Everett, 1988). The same effect was observed in cotransfections of p140CAT and p113 (Fig. 2b). Therefore the same region of Vmw175 is involved in the repression of both the gene 62 and IE-3 promoters.

Plasmid pC34 encodes a mutant form of the VZV 140K protein which is analogous to the protein expressed by p113. It was derived from p140 (McKee et al., 1990) by the method of Paterson & Everett (1988) and contains a mutation resulting in the insertion of four amino acids into the glycine codon at position 471 of the 140K protein, which lies within region 2 (amino acid residues 467 to 641; see Fig. 1b). Like p113, pC34 was unable to repress p140CAT (Fig. 2b). The interpretation of this result is potentially complicated because pC34 uses the gene 62 promoter instead of the SV40 early promoter in p140SV. This raises the possibility that the lack of repression by pC34 is not due to the mutation in region 2 of the VZV 140K protein but that autoregulation of C34 polypeptide expression from pC34 results in insufficient protein to repress the gene 62 promoter in p140CAT as well. However, it is difficult to envisage how the C34 polypeptide might repress its own expression from pC34 without also reducing gene 62 promoter activity in p140CAT. In addition, in trans-activation transfection assays the C34 mutation, like that of p113 (Paterson & Everett, 1988), eliminates the ability of plasmid pC34 to trans-activate the HSV-1 glycoprotein D (gD) promoter (whose activity is increased approximately 100-fold by the 140K protein; results not shown). Therefore it is most likely that, as with the mutations in region 2 of Vmw175 (Paterson & Everett, 1988; DeLuca & Schaffer, 1988; Shepard et al., 1989), the C34 mutation results in an inactive polypeptide. The experiment therefore demonstrates that the same similar regions of Vmw175 and the VZV 140K protein are involved in the repression of their respective promoters.

The mechanism of repression of the IE-3 promoter by Vmw175 requires a functional Vmw175 DNA-binding domain (Paterson & Everett, 1988; Shepard et al., 1989) and the presence of a cognate DNA-binding site at the cap site (Roberts et al., 1988). This binding site includes a consensus sequence (ATCGTC) common to many, but not all, sequences with which Vmw175 can associate (Muller, 1987; Faber & Wilcox, 1986; Michael et al., 1988). We attempted to determine whether analogous sequences in the region of the gene 62 cap site were required for repression by the VZV 140K protein by constructing deletion variants of p140CAT in which promoter sequences upstream of the TATA box remained intact but leader or cap site–leader sequences were deleted. It was found, however, that the intrinsic activities of both deleted promoters were consistently reduced by at least 10-fold relative to p140CAT, which implies that sequences in the gene 62 leader region contribute to the efficiency of its expression. The activity of both promoters was increased 15- to 30-fold when pMC1 was present in transfections (as with the intact promoter) but promoter activity was still at least 10-fold less than that of the pMC1-activated gene 62 promoter. As noted above, low levels of promoter activity result in relatively poor levels of repression and therefore it was not possible to draw unequivocal conclusions from the results obtained with these constructs.

In conclusion, we have shown that the functional similarity of Vmw175 of HSV-1 and the VZV 140K protein extends to the repression of their respective promoters and that Vmw175 is also able to repress the VZV gene 62 promoter. Since the C34 mutation, which inactivates repression by the VZV 140K protein, is located within a region similar to that of the DNA-binding domain of Vmw175, we speculate that the mechanism of repression used by the VZV 140K protein might involve sequence-specific DNA binding. However, our attempts to identify a 140K protein DNA recognition element by deletion of cap site or leader sequences from p140CAT were inconclusive. We have also carried out preliminary studies to investigate the DNA-binding properties of the 140K protein by using nuclear extracts from cells infected with HSV140 (which contain 140K protein in abundance) in gel retardation DNA-binding assays with probes encompassing the entire gene 62 promoter region in p140CAT. Although we have been able to show that the VZV 140K protein binds to DNA, as yet we have been unable to define any specific VZV 140K protein recognition sequence in the
gene 62 promoter region. Further elucidation of the properties of VZV 140K protein, and the comparison of its functions and mechanisms of action with those of Vmw175, will be facilitated by the availability of more purified preparations of the protein. These studies are in progress.

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