Inhibition of Histone H2B Gene Transcription and of Cellular Growth by a Truncated Viral trans-Activator Protein

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The herpes simplex virus virion protein Vmw65 trans activates the viral immediate-early genes and some octamer-containing cellular genes, including that encoding histone H2B. We found, however, that a truncated form of this virion protein repressed H2B gene transcription and also dominantly inhibited induction of the gene by intact Vmw65. A cell line expressing this truncated protein expressed reduced levels of H2B and grew more slowly than the parental cell line or a similar line expressing the intact protein.

The herpes simplex virus virion protein Vmw65 (VP16) is one of the most potent known activators of transcription (13). Although lacking DNA-binding activity, this protein forms a complex with the cellular octamer-binding protein OTF-1, which binds to the octamer sequence motif found in several cellular genes (for a review, see reference 1) and to the related TAATGARAT element in the herpes simplex virus immediate-early genes (9, 11). Such binding results in the trans activation of the viral immediate-early genes and of some but not all octamer-containing cellular genes both during viral infection and after transfection of Vmw65 (6, 8, 9, 11).

To further characterize the interaction of Vmw65 with octamer-containing cellular genes, we have studied its effect on the histone H2B gene. The promoter of this gene contains an octamer motif with the consensus sequence ATGCAAT, which is essential for its cell cycle-dependent pattern of expression (7, 14). We previously demonstrated (8) that transfection of the gene encoding Vmw65 into cells resulted in increased expression of this gene, indicating that it is inducible by Vmw65.

To investigate the concentration dependence of this effect, we introduced different amounts of plasmid MVmw65 (encoding Vmw65 [15]) into BHK-21 cells by calcium phosphate-mediated transfection (3) and measured the effect on H2B gene transcription in a nuclear run-on assay (10). In these experiments, the total amount of DNA applied to each plate was equalized with plasmid vector (pAT153) DNA.

The effect of Vmw65 on H2B gene transcription exhibited a precise concentration dependence, increasing amounts of Vmw65 producing increased transcription up to a peak level, after which transcription declined with increasing amounts of Vmw65 (Fig. 1). No effect of Vmw65 on the transcription of the histone H4 gene, which does not contain an octamer motif (5), was observed. A similar concentration dependence has also been observed for the induction of herpes simplex virus immediate-early gene transcription by Vmw65, which is also decreased at high levels of Vmw65 (4).

To determine the regions of Vmw65 that were necessary for the alterations in H2B transcription, we used a construct (MVmw65 del) which encodes a truncated form of Vmw65 that lacks the C-terminal acidic tail of the molecule necessary for the activation of transcription but which can still interact with octamer-binding proteins (15). When BHK-21 cells were transfected with this construct, no increase in the transcription of the H2B gene was observed whatever the amount of DNA transfected (Fig. 2). Interestingly, however, at high levels of transfected DNA, H2B gene transcription actually decreased below the control level, paralleling the decline in H2B gene inducibility observed at high levels of intact Vmw65. As before, no effect was observed on the transcription of the H4 gene, indicating that these effects are specific to the octamer-containing H2B gene. This finding indicates that although the acidic activation domain of Vmw65 is required for induction of H2B gene transcription, it is not involved in mediating the decline in inducibility that occurs at high levels of Vmw65.

The ability of truncated Vmw65 to repress the H2B gene suggested that it might also be able to dominantly interfere with the induction of H2B by intact Vmw65 even when present at only relatively low levels, below those required for repression of transcription. To investigate this possibility, different amounts of MVmw65 del DNA were transfected with a constant amount of MVmw65 sufficient to cause induction of H2B transcription, and the effect on gene transcription was observed. As before, the amount of DNA applied to each plate was equalized with plasmid vector DNA.

The addition of MVmw65 del had a strongly inhibitory effect on the ability of MVmw65 to induce H2B gene transcription, which was observable even at a ratio of 2.5:1 between the intact and truncated templates, whereas a ratio of 1:2 was able to effectively prevent gene induction (Fig. 3). Hence, the deleted form of Vmw65 is able to effectively prevent the induction of cellular gene transcription by Vmw65 even when present at relatively low levels, paralleling its similar ability to prevent the induction of the viral immediate-early genes by Vmw65 (15). No parallel has been described however, in the viral system for the other aspect of this phenomenon, namely, the ability of high levels of the truncated protein to actually repress cellular gene transcription in the absence of the intact trans activator. Both of these phenomena are likely due to the ability of the truncated protein to interact with cellular octamer-binding protein coupled with its inability to activate transcription.

Having established the existence of these effects, it was of particular interest to determine whether they had any effect on the growth of cells expressing wild-type or truncated Vmw65. We therefore prepared stable cell lines expressing each of these proteins. To do this, L cells were transfected

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with plasmid pSV2neo and either MVmw65 or MVmw65 del. Cells that had taken up the plasmid DNA and were expressing the bacterial neo gene product were selected by their ability to grow in the presence of the antibiotic G418 at a concentration of 0.2 mg/ml and cloned by limiting dilution.

Transcription of the H2B and H4 genes in the clonal cell lines was measured by nuclear run-on assay (10). No alteration in transcription of either histone gene was observed in

FIG. 1. Effect of transfecting MVmw65 (encoding intact Vmw65) on transcription of the histone H2B gene (○) and the histone H4 gene (●). The amount of DNA transfected is plotted against the counts per minute binding to the indicated gene in two replicate nuclear run-on assays. The amount of DNA added to each plate was equalized with plasmid DNA (pAT153).

FIG. 2. Effect of transfecting MVmw65 del (encoding truncated Vmw65 lacking the 61 C-terminal amino acids) on transcription of the histone H2B gene (○) and the histone H4 gene (●). Values plotted were obtained as described in the legend to Fig. 1.

FIG. 3. Effect of cotransfecting MVmw65 del on the induction of the histone H2B gene by Vmw65. The amount of MVmw65 del cotransfected with 5 μg of MVmw65 is plotted against the counts per minute binding to the histone H2B gene in two replicate nuclear run-on assays.

the cell line expressing full-size Vmw65, although transcription of the Vmw65 gene was readily detectable (Table 1). In contrast, the cell line expressing truncated Vmw65 exhibited a clear decrease in H2B gene transcription, although H4 transcription was unaffected. Hence, the truncated Vmw65 protein was being expressed in the cell line at a level sufficient to repress H2B transcription.

To determine whether such repression had any effect on the growth of these cells, we compared their growth rates with that of the parental L-cell line. In three replicate experiments (Fig. 4), the cell line expressing full-size Vmw65 grew as well as the parental line. However, the cell line expressing the truncated protein grew less well, with a doubling time of approximately twice that of the other cell lines. This difference was consistently observed in three replicate experiments carried out in both the presence and absence of the antibiotic G418, indicating that it is not mediated by differences in the levels of expression of the neo gene in the different cell lines. In addition to its ability to repress H2B gene transcription, the truncated form of Vmw65 is also able therefore to inhibit cellular growth.

As discussed above, Vmw65 is an extremely potent trans activator of transcription (13). The experiments presented here indicate, however, that a truncated form of this protein

<table>
<thead>
<tr>
<th>Gene</th>
<th>Binding (cpm) a in given cell line</th>
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<tr>
<td></td>
<td>L cells expressing intact Vmw65</td>
</tr>
<tr>
<td>Histone H2B</td>
<td>55 (7)</td>
</tr>
<tr>
<td>Histone H4</td>
<td>35 (5)</td>
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<tr>
<td>Vmw65</td>
<td>4 (-)</td>
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 a Values (averages of two determinations whose range is given in parentheses) indicate counts per minute binding to the indicated clone in nuclear run-on assays, using nuclei prepared from each cell line.

 b No variation between replicates.
FIG. 4. Growth curves of untransfected L cells (○) and L cells expressing intact (■) or truncated (▲) Vmw65. One million cells of each type were seeded on day zero of the experiment. Values are means of three replicate experiments carried out in the presence or absence of G418.

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LITERATURE CITED


