JOURNAL OF VIROLOGY, Feb. 1987, p. 604-606
0022-538X/87/020604-03$02.00/0

Vol. 61, No. 2

A Mutant of Herpes Simplex Virus Type 1 Exhibits Increased Stability of Immediate-Early (*alpha*) mRNAs

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Received 15 July 1986/Accepted 15 October 1986

***vhs*1 is a herpes simplex virus type 1 mutant defective in the shutoff of both host and *alpha* polypeptide synthesis. In cycloheximide reversal experiments, *alpha* mRNAs were significantly more stable in *vhs*1-infected cells than in cells infected with wild-type virus, whether assayed by in vitro translation or Northern blotting.**

An early event during lytic herpes simplex virus type 1 (HSV-1) infections is the shutoff of most cellular protein synthesis (7, 8, 12, 14, 15, 18). This shutoff is initially caused by an unidentified component of the infecting virion which induces disaggregation of cellular polyribosomes and suppresses translation of preexisting host mRNAs (7, 15, 20–22). Another early event is the expression of the *alpha* or immediate-early viral genes. Synthesis of the *alpha* polypeptides peaks at early times and then declines (12, 13, 17, 18).

*vhs*1 is a mutant of HSV-1 strain KOS that is defective in the virion-associated host shutoff (*vhs*) function responsible for the initial suppression of host protein synthesis (18). Preliminary characterization of the mutant revealed that it was also defective in posttranscriptional shutoff of *alpha* polypeptide synthesis (18). This finding raises the possibility that the same viral gene product may be involved in both host and *alpha* shutoff (18). Recent studies indicate that, in Vero cells, host shutoff is accompanied by the degradation of cellular mRNAs (20). In this study, we demonstrated that a mutation carried by *vhs*1 dramatically increased the physical stabilities of at least three different *alpha* mRNAs.

The defect of *vhs*1 in *alpha* shutoff can be seen most dramatically in a cycloheximide reversal experiment (18). In an earlier study, Vero cells were infected with *vhs*1 or wild-type virus in the presence of 50 μg of cycloheximide per ml and maintained in the presence of the drug for 5 h to allow accumulation of the *alpha* mRNAs (18). At 5 h, the cycloheximide-containing medium was replaced with medium containing 5 μg of actinomycin D per ml. The cells were then incubated for various intervals before they were pulse-labeled with ^{14}C-amino acids to assay the rates of ongoing translation of *alpha* mRNAs that had been transcribed before the addition of actinomycin D. In wild-type virus infections, *alpha* polypeptide synthesis was observed immediately after removal of cycloheximde. However, 3 h after removal, ongoing *alpha* translation had fallen to barely detectable levels. In contrast, cells infected with *vhs*1 showed higher than normal levels of *alpha* polypeptide synthesis immediately after reversal of the cycloheximide block, and high levels of *alpha* translation continued for at least 10 h.

To examine this difference between the wild-type virus and *vhs*1, a cycloheximide reversal experiment was performed in which total cytoplasmic RNA was extracted from infected cells (2) either immediately after removal of the cycloheximide or after an additional 5 h of incubation in the presence of actinomycin D. The RNA was then translated in vitro with micrococcal nuclease-treated rabbit reticulocyte lysates (Promega Biotec, Madison, Wis.) according to procedures recommended by the manufacturer (Fig. 1). These lysates should be capable of translating any potentially translatable *alpha* mRNAs.

In wild-type infections, significant amounts of mRNA encoding the *alpha* polypeptides ICP0 and ICP27 were present immediately after removal of cycloheximide. However, these mRNAs had fallen to barely detectable levels by 5 h after removal. In contrast, cells infected with *vhs*1 had significantly higher amounts of translatable ICP0 and ICP27 mRNAs immediately after cycloheximide reversal, and mRNA levels remained high for at least 5 h. In these experiments, it was difficult to obtain high levels of in vitro ICP4 synthesis by using any of the mRNA samples as a template, perhaps because ICP4 mRNA is large (4.2 kilobases) and therefore is particularly susceptible to degradation. Nevertheless, it was apparent that by 5 h postreversal, significantly more translatable ICP4 mRNA was present in *vhs*1-infected cells than in cells infected with wild-type virus. At both times, there were reduced levels of translatable host mRNA in cells infected with wild-type virus, because *vhs*1 is defective in the virion function responsible for the degradation of cellular mRNAs (18, 20). Furthermore, while reduced in overall intensity, the pattern of host protein synthesis observed with RNA from wild-type virus infections was similar to that observed with RNA from *vhs*1-infected cells. This finding is consistent with earlier observations that the *vhs* gene product reduces synthesis of many cellular proteins to approximately equal extents (18).

To determine whether the decline in levels of translatable *alpha* mRNAs in wild-type virus infections was due to mRNA degradation, a cycloheximide reversal experiment was done in which RNA extracted at 0, 3, and 6 h after reversal was analyzed by Northern blotting (Fig. 2). RNA that had been treated with glyoxal and electrophoresed through 1.2% agarose gels (19) was electrophoretically transferred to GeneScreen Plus membranes (New England Nuclear Corp., Boston, Mass.) according to procedures recommended by the manufacturer. The filters were then probed with a nick-translated *Eco*RI fragment EK from the HSV-1 genome. To prepare the probe, the EK fragment was excised from plasmid pSG28 (10), purified (19), and labeled by using a nick translation kit (Bethesda Research Laboratories, Gaithersburg, Md.). Hybridization procedures have been described previously (11). The EK fragment hybridizes to

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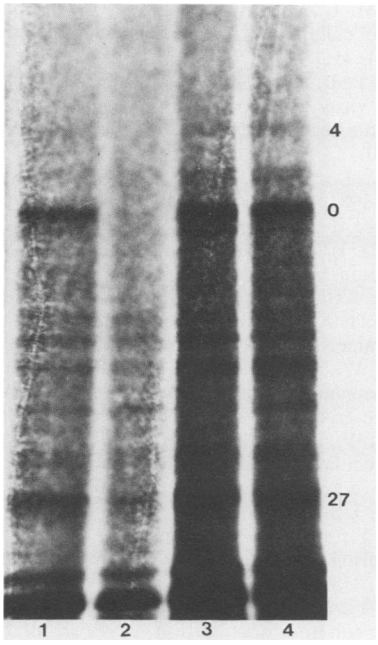

FIG. 1. In vitro translation of *alpha* mRNAs. Vero cells were infected with 40 PFU per cell of wild-type virus (lanes 1 and 2) or *vhs*1 (lanes 3 and 4) in the presence of 50 μg of cycloheximide per ml. After 5 h the cycloheximide-containing medium was replaced with medium containing 5 μg of actinomycin D per ml. Total cytoplasmic RNA was prepared either immediately (lanes 1 and 3) or after an additional 5 h of incubation (lanes 2 and 4). Samples of 375 μg were translated in vitro, and the products were analyzed by electrophoresis on 9.25% polyacrylamide gels and autoradiography as described previously (18). The mRNAs encoding the *alpha* polypeptides ICP4, ICP0, and ICP27 are indicated by their numbers.

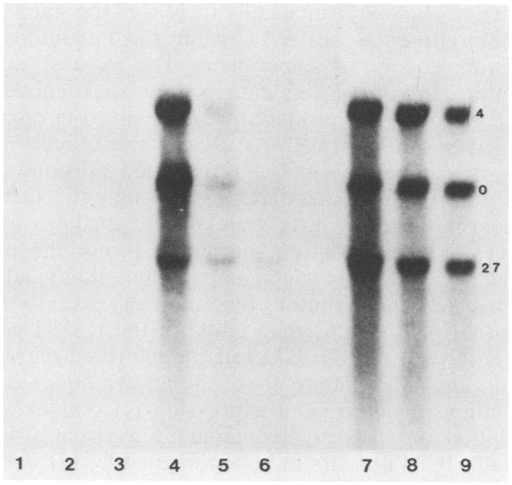

FIG. 2. Northern blot analysis of *alpha* mRNAs. Vero cells were mock infected (lanes 1 to 3) or infected with 30 PFU per cell of wild-type virus (lanes 4 to 6) or *vhs*1 (lanes 7 to 9), each in the presence of 50 μg of cycloheximide per ml. After 5 h, the cycloheximide-containing medium was replaced with medium containing 5 μg of actinomycin D per ml. Total cytoplasmic RNA was prepared immediately (lanes 1, 4, and 7) or after 3 h (lanes 2, 5, and 8) or 6 h (lanes 3, 6, and 9) of additional incubation. Samples of 5 μg were analyzed by electrophoresis and Northern blotting as described in the text by using nick-translated *Eco*RI fragment EK as a hybridization probe. The mRNAs encoding the *alpha* polypeptides ICP4, ICP0, and ICP27 are indicated by their numbers.

three *alpha* mRNAs which encode ICP4, ICP0, and ICP27 (1, 4, 23, 24). The levels of different *alpha* mRNAs were quantitated by densitometric scanning of the autoradiogram (Fig. 3).

There was a clear difference between the half-lives of *alpha* mRNAs in mutant and wild-type virus infections. In cells infected with either *vhs*1 or wild-type virus, significant levels of ICP4, ICP0, and ICP27 mRNAs were present immediately after reversal of the cycloheximide block. In cells infected with wild-type virus, the amounts of ICP4 and ICP0 mRNAs fell to approximately 10% of the initial levels by 3 h postreversal and to undetectable levels by 6 h postreversal. The decline in the amount of ICP27 mRNA followed a similar pattern, although the decrease was not quite as rapid. In contrast, in cells infected with *vhs*1, the amounts of the three *alpha* mRNAs at 3 h postreversal ranged from 65 to 90% of the initial values, and at 6 h, amounts ranged from 42 to 55%.

In this experiment, similar amounts of hybridizable *alpha* mRNA were present at 0 h postreversal in wild-type and *vhs*1 virus infections. In contrast, in the first experiment (Fig. 1), significantly more translatable *alpha* mRNA was present in *vhs*1-infected cells at 0 h postreversal than in cells infected with wild-type virus. The reason for this discrepancy is unclear. One possibility is that in the wild-type virus infections, some of the *alpha* mRNAs that were detected by Northern blotting had been modified so that they were no longer capable of normal activity as measured by in vitro translation.

These experiments demonstrated that one or more muta-

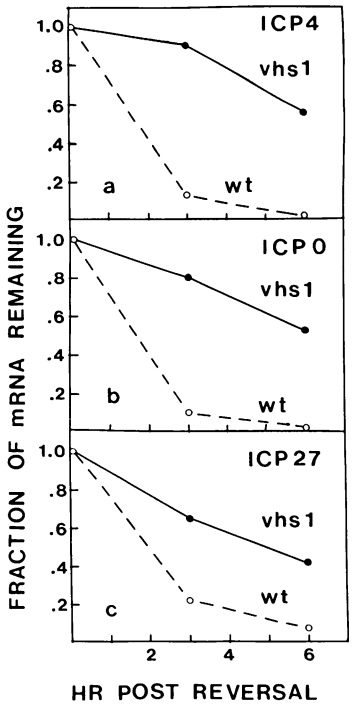

FIG. 3. Quantitation of *alpha* mRNAs. The autoradiogram shown in Fig. 2 was scanned, and the areas under the peaks were integrated. For each virus, the amount of mRNA present at 0, 3, or 6 h is expressed as a fraction of the amount present at 0 h postreversal. The results are shown for mRNAs encoding ICP4 (a), ICP0 (b), and ICP27 (c).

tions carried by *vhs*1 dramatically increased the stabilities of three different *alpha* mRNAs under the conditions of a cycloheximide reversal experiment. The most straightforward interpretation of the data is that *vhs*1 encodes an altered form of a gene product that is involved in regulating the half-lives of *alpha* mRNAs. Another possibility is that *vhs*1 carries mutations in the transcribed portions of the genes for ICP4, ICP0, and ICP27 and that the increased stabilities of these mRNAs were due to changes in their sequences. That this latter alternative is not the case is indicated by recent data which map the mutation(s) affecting the host and *alpha* shutoff functions of *vhs*1 to *Eco*RI fragment A (0.49 to 0.63 map units) of the HSV-1 genome (C. R. Krikorian and G. S. Read, unpublished data). This fragment does not contain any of the known *alpha* genes.

Regulation of *alpha* gene expression is complex. *Alpha* transcription is positively regulated by a virion structural protein (3). ICP4 appears to be autoregulatory (6), probably at the level of transcription (5, 16), and ICP4 transcription is negatively regulated in a direct or indirect way by the *beta* polypeptide ICP8 (9). In addition to these forms of transcriptional control, the data presented in this paper suggest that HSV-1 encodes a function that negatively regulates the stabilities of *alpha* mRNAs.

We thank Myron Levine for providing pSG28, Charlie Krikorian for helpful discussions, and Cecilia Doyle for excellent technical assistance.

This work was supported by Public Health Service grant AI21501 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Anderson, K. P., R. H. Costa, L. E. Holland, and E. K. Wagner.** 1980. Characterization of herpes simplex virus type 1 RNA present in the absence of de novo protein synthesis. J. Virol. **34**:9–27.
2. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell **12**:721–732.
3. **Campbell, M. E. M., J. W. Palfreyman, and C. M. Preston.** 1984. Identification of herpes simplex virus DNA sequences which encode a trans-acting polypeptide responsible for stimulation of immediate early transcription. J. Mol. Biol. **180**:1–19.
4. **Clements, J. B., J. McLauchlan, and D. J. McGeoch.** 1979. Orientation of herpes simplex virus type 1 immediate early mRNAs. Nucleic Acids Res. **7**:77–91.
5. **DeLuca, N. A., and P. A. Schaffer.** 1985. Activation of immediate-early, early, and late promoters by temperature-sensitive and wild-type forms of herpes simplex virus type 1 protein ICP4. Mol. Cell. Biol. **5**:1997–2008.
6. **Dixon, R. A. F., and P. A. Schaffer.** 1980. Fine-structure mapping and functional analysis of temperature-sensitive mutants in the gene encoding the herpes simplex virus type 1 immediate-early protein VP175. J. Virol. **36**:189–203.
7. **Fenwick, M. L., and J. Clark.** 1982. Early and delayed shut-off of host protein synthesis in cells infected with herpes simplex virus. J. Gen. Virol. **61**:121–125.
8. **Fenwick, M. L., and M. J. Walker.** 1978. Suppression of the synthesis of cellular macromolecules by herpes simplex virus. J. Gen. Virol. **41**:37–51.
9. **Godowski, P. J., and D. M. Knipe.** 1986. Transcriptional control of herpes virus gene expression: gene functions required for positive and negative regulation. Proc. Natl. Acad. Sci. USA **83**:256–260.
10. **Goldin, A. L., R. M. Sandri-Goldin, M. Levine, and J. C. Glorioso.** 1981. Cloning of herpes simplex virus sequences representing the whole genome. J. Virol. **38**:50–58.
11. **Graham, D. E., D. Medina, and G. H. Smith.** 1984. Increased concentration of indigenous proviral mouse mammary tumor virus long terminal repeat-containing transcript is associated with neoplastic transformation of mammary epithelium in C3H/Sm mice. J. Virol. **49**:819–827.
12. **Honess, R. W., and B. Roizman.** 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. **14**:8–19.
13. **Honess, R. W., and B. Roizman.** 1975. Regulation of herpesvirus macromolecular synthesis: sequential transition of polypeptide synthesis requires functional viral polypeptides. Proc. Natl. Acad. Sci. USA **72**:1276–1280.
14. **Nishioka, Y., and S. Silverstein.** 1977. Degradation of cellular mRNA during infection by herpes simplex virus. Proc. Natl. Acad. Sci. USA **74**:2370–2374.
15. **Nishioka, Y., and S. Silverstein.** 1978. Requirement of protein synthesis for the degradation of host mRNA in Friend erythroleukemia cells infected with herpes simplex virus type 1. J. Virol. **27**:619–627.
16. **O'Hare, P., and G. S. Hayward.** 1985. Three *trans*-acting regulatory proteins of herpes simplex virus modulate immediate-early gene expression in a pathway involving positive and negative feedback regulation. J. Virol. **56**:723–733.
17. **Pereira, L., M. H. Wolff, M. L. Fenwick, and B. Roizman.** 1977. Regulation of herpes virus macromolecular synthesis. V. Properties of *alpha* polypeptides made in HSV-1 and HSV-2 infected cells. Virology **77**:733–749.
18. **Read, G. S., and N. Frenkel.** 1983. Herpes simplex virus mutants defective in the virion-associated shutoff of host polypeptide synthesis and exhibiting abnormal synthesis of α (immediate early) viral polypeptides. J. Virol. **46**:498–512.
19. **Read, G. S., and W. C. Summers.** 1982. *In vitro* transcription of the thymidine kinase gene of herpes simplex virus. Proc. Natl. Acad. Sci. USA **79**:5215–5219.
20. **Schek, N., and S. L. Bachenheimer.** 1985. Degradation of cellular mRNAs induced by a virion-associated factor during herpes simplex virus infection of Vero cells. J. Virol. **55**: 601–610.
21. **Sydiskis, R. J., and B. Roizman.** 1966. Polysomes and protein synthesis in cells infected with a DNA virus. Science **153**:76–78.
22. **Sydiskis, R. J., and B. Roizman.** 1967. The disaggregation of host polysomes in productive and abortive infection with herpes simplex virus. Virology **32**:678–686.
23. **Watson, R. J., C. M. Preston, and J. B. Clements.** 1979. Separation and characterization of herpes simplex virus type 1 immediate-early mRNAs. J. Virol. **31**:42–52.
24. **Watson, R. J., M. Sullivan, and G. F. Vande Woude.** 1981. Structures of two spliced herpes simplex virus type 1 immediate-early mRNA's which map at the junctions of the unique and reiterated regions of the virus DNA S component. J. Virol. **37**: 431–444.