Early Virion-associated Suppression of Cellular Protein Synthesis by Herpes Simplex Virus is Accompanied by Inactivation of mRNA

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SUMMARY

Vero cells infected with herpes simplex virus in the presence of actinomycin or cycloheximide, or with u.v.-inactivated virus, suffered a rapid loss of functional mRNA as determined by translation in vitro. It is suggested that a virion-associated factor causes a structural change in the mRNA of the host cell.

Some strains of herpes simplex virus (HSV) carry in their virions a factor that causes, or initiates, the suppression of cellular protein synthesis, a process known as early, primary or virion-associated shut-off, to distinguish it from a distinct phenomenon known as delayed, secondary or expression-dependent shut-off which requires the synthesis of viral protein (Nishihoka & Silverstein, 1978; Fenwick & Walker, 1978; Fenwick & Clark, 1982a; Read & Frenkel, 1983). Delayed shut-off involves the degradation of cellular mRNA as identified by hybridization to a specific DNA probe but early shut-off does not (Nishihoka & Silverstein, 1978; Inglis, 1982). Inglis & Newton (1981) have shown by translation of extracted mRNA in vitro that cellular mRNA is inactivated after infection with HSV. We were interested to know whether inactivation occurs during early shut-off, while the mRNA remains in a hybridizable condition, or only later, as a result of delayed shut-off.

Infection of Vero cells with strain F of HSV type 1 [HSV-1(F)] or strain G of HSV type 2 [HSV-2(G)] (Ejercito et al., 1968), labelling of proteins and electrophoresis in SDS-polyacrylamide gradient gels were done as previously described (Fenwick et al., 1978). Extraction of total cytoplasmic RNA and its translation in a reticulocyte lysate were also described previously (Fenwick & Clark, 1982b). Preliminary tests were carried out to determine the concentration of RNA giving maximum incorporation of [35S]methionine. In subsequent experiments this concentration of RNA or twice this concentration were used in duplicate translations of each RNA sample. The results were the same in both cases.

HSV-2(G), which causes rapid early shut-off (Fenwick & Walker, 1978), was used to infect duplicate cultures of cells under conditions in which the production of viral proteins was prevented. One flask of each pair was washed and labelled with 14C-amino acids in the presence of 2 µg/ml actinomycin 2 to 2-5 h after infection. The second culture was lysed and the nuclei removed by centrifugation. Cytoplasmic RNA was extracted and translated in the presence of [35S]methionine. In subsequent experiments this concentration of RNA or twice this concentration were used in duplicate translations of each RNA sample. The results were the same in both cases.

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The suppression of cellular protein synthesis by HSV-2 is more efficient in the presence of actinomycin than with cycloheximide or with u.v.-inactivated virus (our unpublished observations). This is consistent with a shut-off mechanism involving inactivation of a pool of mRNA which is being relatively slowly replenished.
Mock  G  UV-G  Mock  G  Blank  Mock  G  UV-G  Mock  G
+ Act  + Act  + CX  + CX  + Act  + Act  + CX  + CX

Fig. 1. Autoradiograms of 7 to 20% acrylamide gradient gels showing electrophoretically separated polypeptides synthesized (a) in intact cells 2 to 2.5 h post-infection or (b) in vitro with cytoplasmic RNA extracted at 2 h post-infection. Cells were mock-infected or infected with HSV-2(G) in the presence of 2 μg/ml actinomycin (Act) or 50 μg/ml cycloheximide (CX) or with virus that had been irradiated with u.v. light (1.5 × 10⁻⁴ J/mm²) to virus suspended in 3 ml PBS/1% glucose in a 9 cm diam. Petri dish; UV-G). α-polypeptides ICP 4 and ICP 0 are indicated in lanes 5 and 6.

HSV-1(F) causes a slower early shut-off than does HSV-2(G) (Morse et al., 1978; Fenwick et al., 1979). This difference is illustrated in Fig. 2(a) and Fig. 2(b) shows that it is correlated with a partial inactivation of functional host mRNA by HSV-1(F), either u.v.-inactivated or in the presence of cycloheximide (lanes 2, 3), as compared to the more marked effect of HSV-2(G) (lanes 4, 5).

Early shut-off is accompanied by the disaggregation of polysomes (Sydiski & Roizman, 1966; Fenwick & Walker, 1978). Whether inactivation of mRNA precedes or follows dissociation of the polysomes is uncertain. A modification near the 5' end of the mRNA to prevent initiation of translation would not be enough to explain the breakdown of polysomes since the breakdown also occurs in the presence of cycloheximide, which stops the ribosomes running off the mRNA (Fenwick & Walker, 1978).

The HSV-1 mutant tsB7 (Knipe et al., 1981) causes early shut-off at 34 °C but not at 39 °C. We have reported that early shut-off caused by u.v.-inactivated tsB7 at 34 °C could be reversed by raising the temperature to 39 °C (Fenwick & Clark, 1982a). On the other hand, Read & Frenkel (1983), using mutant vhs4 which is also temperature-sensitive in its early shut-off function, found that if the temperature was raised to 39 °C after shut-off had occurred at 34 °C in the presence of actinomycin the suppression was not reversed. Our subsequent (unpublished) experiments with tsB7 have confirmed that after raising the temperature, the recovery of host protein synthesis (which was nearly complete within 90 min at 39 °C) was prevented by adding actinomycin, indicating that the recovery depended on the synthesis of new RNA and was not simply due to the reversal of temperature-sensitive interaction.

Nishioka & Silverstein (1978) showed that in Friend erythroleukaemia cells early shut-off by HSV-1(F) was not accompanied by the disappearance of hybridizable globin mRNA. Whether
this is also true of total Vero cell mRNA is not known. We can conclude, however, that the early virion-associated shut-off of cellular protein synthesis, which is accompanied by the disaggregation of polysomes, involves some alteration of host mRNA to the extent that it is no longer functional in a translation system in vitro.

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REFERENCES


Short communication


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