Effect of Herpes Simplex Virus Type 2 Infection on Mitochondrial Gene Expression

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SUMMARY

A cDNA clone derived from the mitochondrial cytochrome oxidase I gene has been used to show that the level of mitochondrially encoded RNA species declines during herpes simplex virus type 2 infection in a manner similar to that for RNA species derived from nuclear genes. In contrast to the situation for nuclear genes, however, no change in the transcription rate of the mitochondrial genome during infection was detected, indicating that post-transcriptional processes alone are responsible for the decline in the levels of mitochondrial RNA species during infection. Two stages in this post-transcriptional degradation have been defined, only one of which is dependent upon viral protein synthesis in the infected cell.

Initial infection of mammalian cells with herpes simplex virus (HSV) can result in a lytic cycle leading to cell death, in asymptomatic latent infections (Stevens, 1975), or possibly in cellular transformation (reviewed by Minson, 1984; Macnab, 1987). Such a variety of possible outcomes focuses attention on the cellular response to initial infection with HSV. During lytic infection a well characterized shut-off of cellular protein synthesis occurs (reviewed by Fenwick, 1984), which is mediated at a number of levels. Thus, the transcription of ribosomal RNA by RNA polymerase I (Wagner & Roizman, 1969) and of cellular and integrated viral genomes by RNA polymerase II (Stenberg & Pizer, 1982; Mayman & Nishioka, 1985; Latchman et al., 1987) is reduced in infected cells. Such transcriptional repression is significantly supplemented by post-transcriptional mechanisms, notably the disaggregation of polysomes synthesizing cellular proteins (Sydiskis & Roizman, 1967; Nishioka & Silverstein, 1978) and the degradation of cellular RNA species (Nishioka & Silverstein, 1977; Ingles, 1982).

In order to investigate further the extent of HSV-induced repression of cellular gene expression and its possible mechanisms, the effect of HSV infection on expression of the mammalian mitochondrial genome was studied. This 16 kb DNA molecule located within the mitochondria encodes a number of proteins important for mitochondrial metabolism (Bibb et al., 1981). It is transcribed as a single RNA molecule by an RNA polymerase distinct from the three nuclear RNA polymerases, the resulting transcript being processed, and the corresponding proteins synthesized, within the mitochondrion (Battey & Clayton, 1978).

BHK-21 clone 13 cells (Macpherson & Stoker, 1962) were either mock-infected or infected with HSV-2 strain 333 (Seth et al., 1974) at a multiplicity of 5 p.f.u. per cell and harvested 6 h after infection. Total cytoplasmic RNA was then isolated by the NP40 lysis method of Favoloro et al. (1980) and poly(A) RNA was prepared by chromatography on oligo(dT)-cellulose (Aviv & Leder, 1972). Aliquots of the RNA samples, equalized by solution hybridization with [3H]poly(rU) (Bishop et al., 1974), were denatured by incubation at 65 °C for 5 min in 20 mM-MOPS, 1 mM-EDTA, 5 mM-sodium acetate, 50% (v/v) formamide, 5% (w/v) formaldehyde, pH 7-2 (Murphy et al., 1983), adjusted to 1 x SSC (0·15 M-sodium chloride, 0·015 M-sodium citrate) and spotted onto a nitrocellulose membrane (Schleicher & Schüll). After prehybridization as described by Wahl et al. (1979) the filters were hybridized with a cDNA probe (pAG82) derived
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from the mitochondrial cytochrome oxidase subunit I gene (Scott et al., 1983; Murphy et al., 1983) which had been radiolabelled using the oligonucleotide priming technique of Feinberg & Vogelstein (1983).

A considerable decrease in the level of the cytochrome oxidase subunit I RNA in the infected cell sample was observed (Fig. 1 c); similar results were also obtained with another cDNA clone, derived from the mitochondrial cytochrome oxidase subunit II gene (data not shown). A decrease was also observed in the level of actin RNA in infected cells using the actin genomic clone pRT83 (a kind gift of Dr K. Willison), which hybridizes to both $\beta$ and $\gamma$ actin, as a hybridization probe (Fig. 1 b). This finding is in agreement with the results obtained by others in a variety of systems (Inglis, 1982; Mayman & Nishioka, 1985; Scheck & Bachenheimer, 1985) that the actin RNA, like the majority of those derived from nuclear genes, decreases in abundance upon infection. In contrast, no alteration was observed in the levels of the RNA homologous to the cDNA clone 126 which was isolated in our laboratory (Fig. 1 a). As we have previously shown that the abundance of this as yet uncharacterized RNA species is unaffected by HSV-2 infection (Kemp et al., 1986 a), this observation confirms that equal amounts of RNA were present in the two samples. These results indicate that, as with the RNA species derived from most nuclear genes, those derived from the mitochondrial genome decline in abundance upon infection with HSV-2.

In order to investigate this effect further, a time course study was carried out by spotting RNA samples prepared from cells at various intervals after infection onto nitrocellulose and hybridizing with the pAG82 and actin probes. In order to quantify the RNA levels precisely after hybridization and washing, the spots were cut out and counted for radioactivity. The results of this experiment (Fig. 2) reveal a progressive decline in both actin and cytochrome oxidase I RNA levels during infection. The time course of this decline was similar for both RNA species, beginning within 1 h of infection and producing a decline in RNA levels to below 50% of the pre-existing level within 2 h.

In view of the rapid onset of the decline in cytochrome oxidase RNA levels, we investigated whether this effect could be produced in the absence of viral protein synthesis. To do this, cells were either mock-infected or infected in the presence of 200 $\mu$g/ml cycloheximide [a concentration that effectively prevents all virus protein synthesis, but is not toxic to BHK cells; Kemp et al. (1986 b)] and the RNA was isolated and analysed as before. In this experiment (Table 1), a considerable decrease in the level of cytochrome oxidase I RNA was observed in the cells infected in the presence of cycloheximide, although this decline was somewhat less (58% compared to 76%) than that seen in normal infections. This suggests that at least a part of the decline in mitochondrial RNA levels in normal infection is brought about by a component of the virion, supplemented by processes requiring the synthesis of viral protein(s). A similar two-stage process, dependent initially upon a component of the virion and subsequently upon a newly made virus protein, has been shown to be responsible for the degradation of nuclear-encoded RNA species in a variety of infected cell types (Bastow et al., 1986; Fenwick & McMenamin, 1984; Scheck & Bachenheimer, 1985). This suggests that the degradation of mitochondrial and nuclear-encoded RNA species occurs by similar mechanisms.

It has been shown previously that the decline in the level of nuclear-encoded RNA species in HSV-2 infection is brought about by a combination of transcriptional (Mayman & Nishioka, 1985; Latchman et al., 1987) and post-transcriptional (Nishioka & Silverstein, 1977; Scheck & Bachenheimer, 1985) processes. An investigation into whether this dual mechanism was also responsible for the decline in mitochondrial RNA levels was carried out by measuring the transcription rate of the mitochondrial genome both before and after infection with HSV-2. As the normal methods of measuring transcription, which involve supplying radiolabelled ribonucleotides to isolated nuclei (see for example Patel et al., 1986), are unsuitable for a cytoplasmic genome, we made use of a modification of this technique (Gilroy et al., 1984) in which whole cells are permeabilized by incubation on ice for 5 min with 50 mM-KCl and allowed to continue transcription in the presence of radiolabelled precursors under conditions previously described for run-off assays using isolated nuclei (Patel et al., 1986). Although this technique is less efficient for nuclear genes than that involving isolation of nuclei, it readily allows
Fig. 1. Effect of HSV-2 infection on RNA levels. RNA samples prepared from mock-infected (M) or HSV-2 infected (I) cells were spotted onto nitrocellulose and hybridized with the following probes: (a) clone 126, (b) actin genomic clone pRT3, and (c) cytochrome oxidase I cDNA clone pAG82.

Fig. 2. Time course of actin and cytochrome oxidase RNA levels during HSV-2 infection. RNA samples were spotted onto nitrocellulose filters and, after hybridization with the actin probe (■) or the cytochrome oxidase probe (□), the spots were cut out and counted for radioactivity.

Table 1. The effect of cycloheximide on the ability of HSV-2 to reduce cytochrome oxidase RNA levels*

<table>
<thead>
<tr>
<th></th>
<th>Mock-infected</th>
<th>HSV-2-infected†</th>
<th>Mock-infected‡</th>
<th>HSV-2-infected‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome oxidase</td>
<td>11374</td>
<td>2736</td>
<td>10512</td>
<td>4375</td>
</tr>
<tr>
<td>Percentage decrease</td>
<td>75.9</td>
<td>58.3</td>
<td>58.3</td>
<td>58.3</td>
</tr>
</tbody>
</table>

* Figures (average of two determinations) are c.p.m. obtained on hybridization of RNA samples with the cytochrome oxidase probe.
† Normal infection.
‡ Infection or mock infection in the presence of cycloheximide.

measurements of both nuclear and mitochondrial transcription rates in a single experiment. Total cellular RNA containing the labelled RNA products produced by the infected or mock-infected cells under run-off conditions was isolated, contaminating DNA was degraded by incubation with DNase I, and the RNA was used to probe replicate dot blots onto which the cytochrome oxidase cDNA clone pAG82 had been spotted. The relative reactivity of the two labelled samples with this clone provides a measure of the change in the rate of transcription of the whole mitochondrial genome, which is transcribed as one large RNA species. The results of this experiment (Fig. 3) indicated that HSV-2 infection did not reduce the mitochondrial transcription rate; indeed, it was marginally increased in the infected cells. In contrast, the transcription rate of the actin gene declined to about 50% of the control value, in agreement with the previously observed effect of infection on the transcription of actin and other nuclear genes (Mayman & Nishioka, 1985; Latchman et al., 1987).
We have recently shown that the immediate early protein ICP22 is responsible for this decline in the transcription of most cellular genes in HSV-2 infection, and have proposed that this protein may interact directly with the nuclear transcriptional apparatus to produce this effect (L. M. Kemp & D. S. Latchman, unpublished data). ICP22 is a phosphorylated nuclear protein (Fenwick et al., 1980; Hay & Hay, 1980; Wilcox et al., 1980) and it is possible that rapid transport of this protein into the nucleus immediately after synthesis results in its exclusion from the mitochondria and, thus, allows the continued transcription of the mitochondrial genome. Alternatively, the simpler nature of the mitochondrial transcription apparatus, and in particular of the mitochondrial RNA polymerase, may preclude an interaction with any ICP22 entering the mitochondrial compartment. If this proves to be the case, comparative studies of the nuclear and mitochondrial transcription systems and their interaction with ICP22 should throw some light on the manner in which this protein represses the transcription of nuclear-encoded genes.

Whatever the precise mechanism by which the mitochondrial genome escapes transcriptional repression, it is clear that the observed decline in mitochondrial RNA levels must be brought about entirely by increased degradation of mitochondrial RNA in the infected cell. The virus proteins that mediate increased degradation of nuclear-encoded RNA species, notably the gene product defined by Vhs mutants defective in the early shut-off of host cell biosynthesis (Read & Frenkel, 1983; Strom & Frenkel, 1987), may, therefore, be capable of entering the mitochondrion and degrading the RNA species localized within it.

In conclusion, it is clear, from the experiments presented in this communication, that lytic infection with HSV-2 causes a rapid degradation of mitochondrial mRNA. Such degradation parallels the decreased efficiency of calcium uptake by mitochondria isolated from HSV-infected cells (Lund & Ziola, 1985), although it is interesting to note that mitochondrial DNA synthesis is stimulated by HSV infection (Radsak & Albring, 1974). Further study of the mechanisms of the effect of HSV on mitochondrial gene expression, in particular the similarities and differences from the effect on nuclear gene expression, should throw light on the interaction of HSV with permissive cell types. Similarly, the observation that repression of this vital component of the cellular machinery begins very early in infection, prior to commitment to the lytic cycle, suggests that studies of this effect during transformation with defective virus (Duff & Rapp, 1971) or during the establishment of artificial latent infection in vitro (Shiraki & Rapp, 1986) may help to explain how one virus can produce the very different states of lysis, latency and transformation.

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REFERENCES


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