DNA SYNTHESIS IN ISOLATED NUCLEI AND ISOLATED MITOCHONDRIA OF HERPES SIMPLEX VIRUS INFECTED HeLa CELLS*

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1. Introduction

Cellular DNA synthesis in the nucleus of susceptible tissue culture cells ceases within several hours after infection with Herpes simplex virus (HSV) [1]. In contrast, there is even a stimulation of the DNA synthesis in mitochondria of infected cells during the initial period post infection (p.i.) when nuclear DNA synthesis is already significantly inhibited [2].

We are interested in possible nucleocytoplasmic interaction mechanisms. The purpose of these experiments was to examine whether the stimulation of mitochondrial DNA synthesis is an indirect consequence of a temporarily increased nucleotide pool p.i. or due to the direct action of an early viral product [1].

To investigate this problem we fractionated HSV-infected HeLa cells 2 hr p.i. into a nuclear and a mitochondrial fraction, determined the kinetics of the incorporation of adequate precursors in isolated nuclei and isolated mitochondria as well and characterized the radioactive DNA's by density centrifugation.

2. Materials and methods

Cultivation of HeLa cells, type of Herpes simplex virus used (Type 1) and the mode of infection have been described [2]. The multiplicity of infection in all experiments was between 1 and 3 infectious units/cell. Mock infection was carried out with used medium.

Nuclei as well as mitochondria were isolated 2–4 hr p.i. from the same cells. 3×10^8 HeLa cells were homogenized in a Dounce type glass homogenizer. Mitochondria were separated from nuclei and unbroken cells and purified on linear sucrose gradients as described by Nass et al. [3]. In this procedure homogenization of cells is followed by several centrifugations of the homogenate at 1000 *g* for 5 min to roughly separate the mitochondria containing supernatant from the heavier cell fractions which will be found in the pellet. Nuclei were isolated from this pellet according to Friedman et al. [4].

In vitro labelling of isolated nuclei with [^{3}H]thymidine triphosphate was carried out following the method of Kidwell et al. [5]. Isolated mitochondria were labelled *in vitro* with [^{3}H]thymidine as described before [6].

Herpes simplex virus DNA was separated from cellular DNA of nuclear origin by isopynic centrifugation in cesium chloride [1]. Mitochondrial DNA was extracted from isolated mitochondria following essentially the method described by Hirt [7, 2] and characterized by isopynic centrifugation in cesium chloride–ethidium bromide [8]. DNA and protein were quantitated and radioactivity was assayed as described before [1].

3. Results

As shown in fig. 1 nuclei from HSV-infected HeLa cells isolated early after infection exhibited a clearly lower incorporating activity than isolated nuclei from mock-infected cells. In contrast, isolated mitochondria from HSV-infected HeLa cells showed a DNA synthesizing activity comparable to that of mitochondria from mock-infected HeLa cells (fig. 2).

Fig. 3 demonstrates a typical radioactivity profile of DNA extracted from nuclei isolated 2–4 hr p.i. after an *in vitro*-labelling period of 1 hr when analyzed

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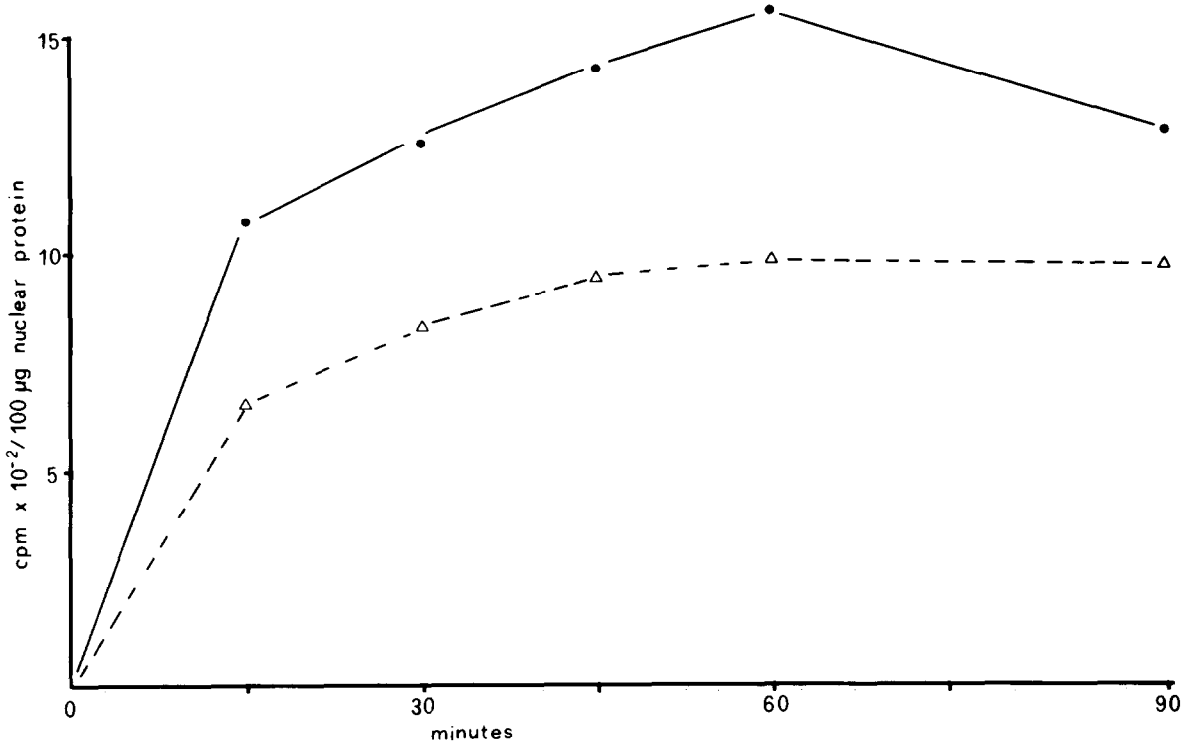

Fig. 1. Incorporation of [^{3}H]thymidine triphosphate by isolated nuclei of Herpes simplex virus infected HeLa cells (△- - -△- - -△) and mock-infected control cultures (●—●—●). Nuclei were isolated 2–4 hr p.i. Infected nuclei exhibited a lower incorporating activity than those from mock-infected cells.

by cesium chloride density centrifugation. DNA samples originating from control nuclei were found in one single band at a density of approx. 1.7 g/cm^3. The radioactivity profile resulting when DNA from HSV-infected nuclei was analyzed on cesium chloride gradients exhibited several peaks (fig. 3). The lightest band showed a density corresponding to cellular DNA of nuclear origin. The heavier bands with a density ranging from 1.710–1.720 g/cm^3 presumably represent DNA related to viral DNA. It remains to be elucidated why several heavier peaks were repeatedly obtained instead of one for this 'virus-like' DNA.

As indicated in table 1 specific radioactivity of cellular DNA of nuclear origin was significantly lower in nuclei from HSV-infected HeLa cells.

When DNA of *in vitro* labelled isolated mitochondria from HSV-infected and mock-infected HeLa cells was submitted to cesium chloride–ethidium bromide density centrifugation no significant differences could

Table 1
Specific radioactivity of cellular DNA from isolated nuclei of uninfected and Herpes simplex virus infected HeLa cells after *in vitro* labelling for 1 hr.

Exp. no.	Specific radioactivity of cellular DNA of uninfected HeLa cell nuclei (cpm/µg DNA)	Specific radioactivity of cellular DNA of Herpes simplex virus infected HeLa cell nuclei (cpm/µg DNA)
1	40	26
2	59	31

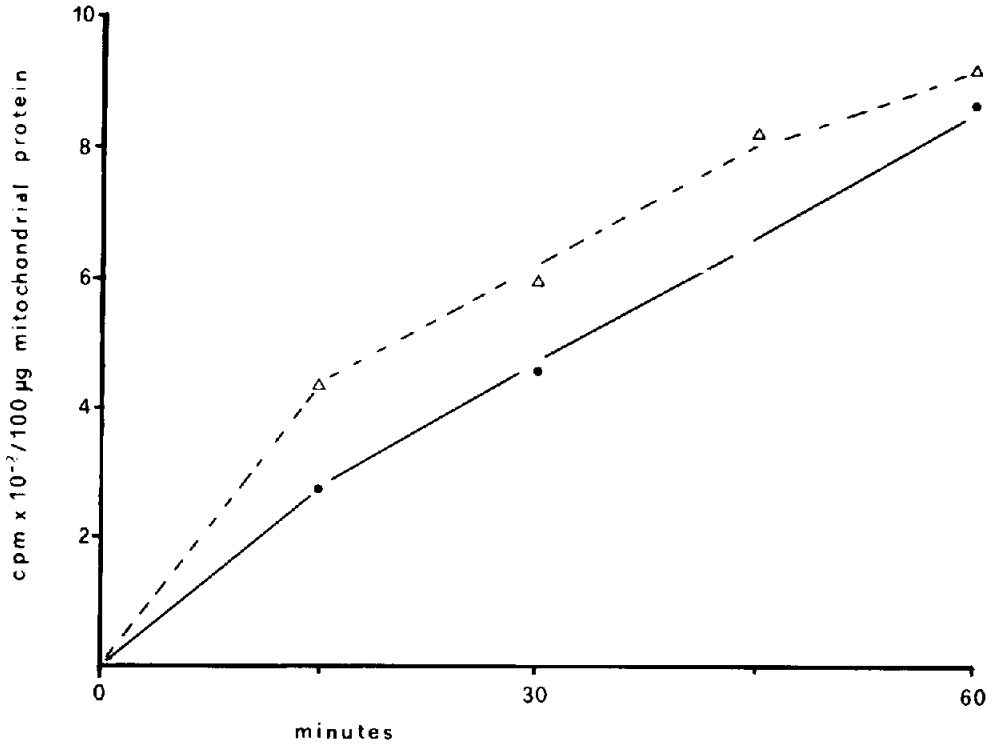

Fig. 2. Incorporation of [^{3}H]thymidine by isolated mitochondria from Herpes simplex virus infected HeLa cells (△- - -△- - -△) and mock-infected control cultures (●—●—●). Mitochondria were isolated in the time interval 2–4 hr p.i. Incorporating activity of mitochondria from infected cells did not differ significantly from that of mitochondria from mock-infected cells.

be observed (fig. 4). Attempts to determine the specific radioactivity of the covalently closed circular double stranded DNA represented by the heavier peak in fig. 4 were unsuccessful because the yield of mitochondrial DNA from the amount of cells used in the experimental schedule was too little.

4. Discussion

Isolated nuclei as well as isolated mitochondria are able to continue macromolecular synthesis for a limited time interval *in vitro* as indicated by the incorporation of adequate precursors into macromolecules [9–11]. Nuclear systems from virus-infected cells have also been used as a tool to approach the problem of DNA synthesis in mammalian cells [12–16].

Applying these experimental procedures to our question we find that a stimulation of mitochondrial DNA synthesis as observed in intact cells [2] cannot be verified with isolated mitochondria from HSV-infected cells. This result suggests that there is no direct action of an early viral or virus-induced product on mitochondrial DNA-synthesis at a time p.i. where viral DNA synthesis in isolated nuclei of the same cells is progressing in a perhaps altered way and cellular DNA synthesis of nuclear origin is clearly inhibited. However, it cannot be excluded that viral products are removed from the mitochondria during the isolation procedure. It seems more probable on the other hand, that mitochondrial DNA synthesis is insensitive to viral inhibitors [1] and that stimulation of mitochondrial DNA synthesis during the initial period p.i. [2] is an indirect consequence of viral activity in infected cells [2].

Experiments applying cycloheximide to this virus–host cell system could help to confirm this hypothesis since nuclear and viral macromolecular synthesis is

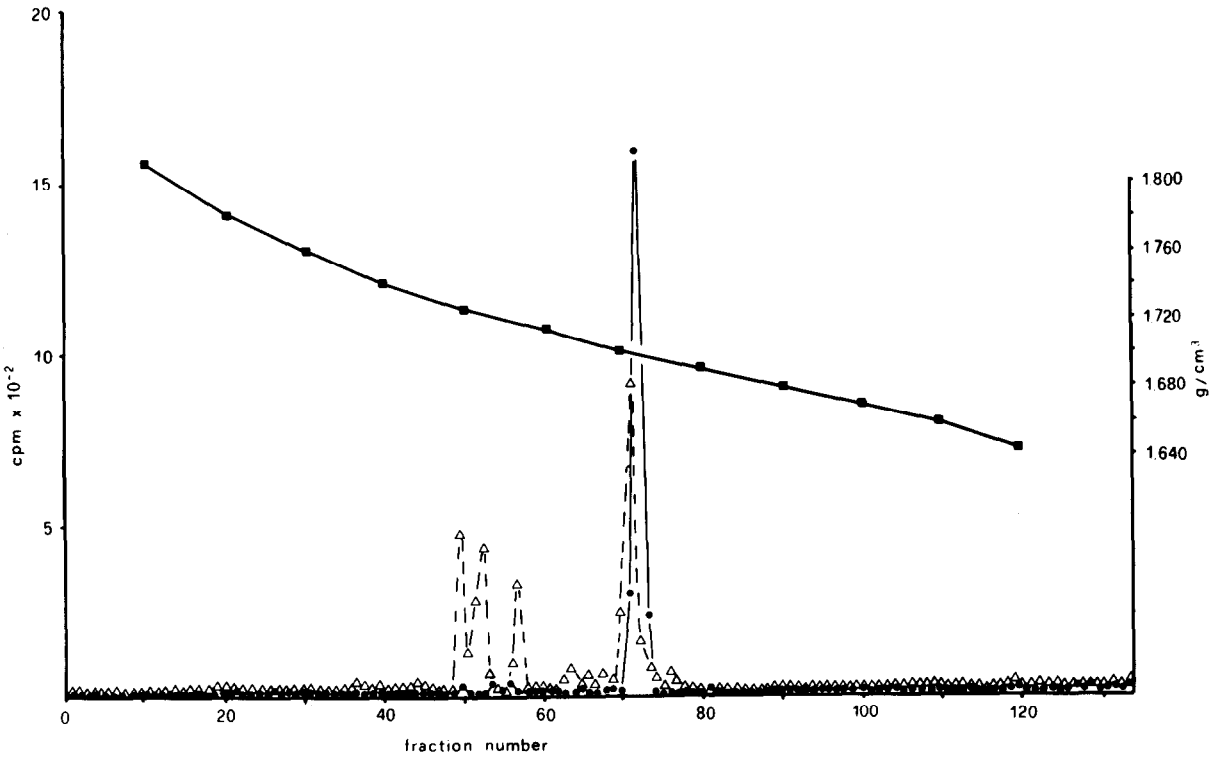

Fig. 3. Isopynic centrifugation in cesium chloride of DNA extracted from isolated nuclei of Herpes simplex virus infected HeLa cells after *in vitro* labelling for 1 hr (△- - -△- - -△) and DNA extracted from isolated nuclei of mock-infected cells labelled under identical conditions (●—●—●). DNA of infected nuclei could be recovered from several bands the lightest of which corresponded to the single band that could be observed in gradients which had been loaded with DNA from mock-infected nuclei.

known to be sensitive to this compound whereas mitochondrial macromolecular synthesis seems to be much less sensitive [17].

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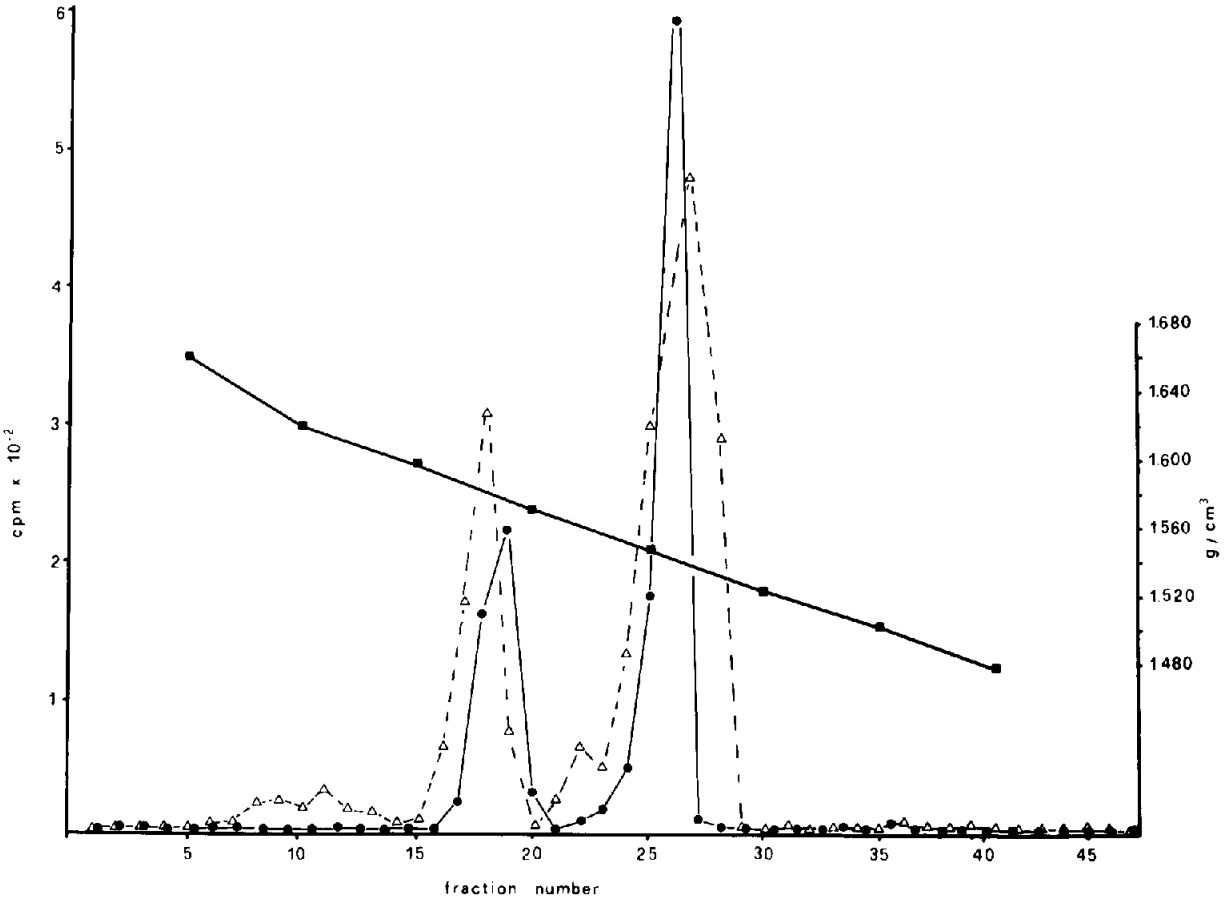

Fig. 4. Isopycnic centrifugation in cesium chloride-ethidium bromide of DNA extracted from isolated mitochondria of Herpes simplex virus infected HeLa cells after *in vitro* labelling for 1 hr (△- - -△- - -△) and DNA extracted from isolated mitochondria of mock-infected cells labelled under identical conditions (●—●—●). There was essentially no difference in the radioactivity profile between gradients loaded with DNA of mitochondria from infected cells and those loaded with DNA of mitochondria from mock-infected cells.

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