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Action of Interferon in Enucleated Cells

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Interferon induces protection of enucleated BSC-1 cells against infectious vesicular stomatitis virus production if cells are treated before, but not after, enucleation.

It has been known for some time that the induction by interferon (IF) of an intracellular antiviral state may be inhibited if the cells are pretreated with actinomycin D (8). This suggests that the establishment of the antiviral state requires RNA transcription from a nuclear DNA template, but any inhibitory effects of actinomycin D on alternative metabolic processes, by which induction might be mediated, cannot be ruled out. One way to avoid this uncertainty is to enucleate cells physically, leaving the cytoplasm surrounded by a functional outer cell membrane. Recently, techniques for enucleating cells on a large scale have become available (6). Furthermore, it has been shown that such enucleated cells can support the growth of many cytoplasmic RNA viruses (2, 4; E. A. C. Follett, C. R. Pringle, and T. H. Pennington, J. Gen. Virol., in press) and will allow the production of vaccinia DNA (3, 5) and proteins (3). This implies that a considerable degree of cytoplasmic and cell surface structural integrity remains. Radke et al. (7) have demonstrated that IF will cause the inhibition of vaccinia DNA synthesis in chicken embryo fibroblasts if it is added before enucleation, but will not do so if added after enucleation. This strongly suggests that the nucleus is required for the establishment of the antiviral state. In the present communication, we come to the same conclusion by using an RNA virus that is able to grow in enucleated BSC-1 cells, and using a conventional assay of virus yield reduction to monitor the effect of IF.

The methods for preparing enucleated BSC-1 monkey cells on 3-cm plastic petri dishes, using cytochalasin B, have been described previously (1). Human IF, which is cross-specific for monkey cells, and mock IF, an appropriate control preparation, were a gift from P. I. Marcus to J. F. Williams in our Institute. Cells were treated for 6 h at 37 C with 2 ml (per dish) of IF or mock IF diluted fourfold in Eagle medium supplemented with 2% fetal calf serum. Cells were then washed with 2 ml of medium before infection with a plaque-purified, T particle-free stock of vesicular stomatitis virus cocal serotype. After 30 min of incubation at 37 C, the cells were washed and overlaid with 2 ml of growth medium (Eagle medium supplemented with 10% fetal calf serum), zero time samples were taken, and the remaining plates were incubated at 37 C for 12 h or, in one case, at 32 C for 18 h. Plates were frozen directly, and the total virus yield was assayed by plaque formation in BHK-21/C13 cells.

Table 1 shows the effect of enucleating cells before treatment with IF. IF is incapable of inducing a significant reduction in the yield of virus obtained from enucleated cells, whereas in the control cells and in the cytochalasin-treated, but not enucleated, cells there is a greater than 10^3-fold reduction in one experiment and a nearly 10^2-fold reduction in the other. Table 2 shows the effect of enucleating cells after treatment with IF. The inhibitory activity of IF remains at approximately the same level as in the control cells (though considerably lower than in the treated cells, for reasons unknown), demonstrating that, once the antiviral state has been established, enucleation itself does not seem to disturb it.

The yield of virus in enucleated cells is not as high as found previously (2). In the experiments shown in Table 1, this low level could be explained by the 6-h interval between recovery from enucleation and virus infection (during which IF or mock IF treatment was taking place), since it is known that the virus growth potential of enucleated cells falls with time (Follett et al., in press). This explanation cannot be true, however, for the experiment illustrated in Table 2, where cells were infected with virus immediately after the period of recovery from enucleation. Nevertheless, the increases in

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TABLE 1. *Effect of enucleation on subsequent treatment with interferon*

Expt	Cells[a]	Virus yields[b]		Log_{10} yield reduction[c]
		+ Mock IF	+ IF	
1[d]	Control	8.8×10^6	5.0×10^3	3.25
	Treated	1.8×10^7	9.8×10^3	3.27
	Enucleated	9.8×10^3	7.8×10^3	0.10
2	Control	3.6×10^6	4.0×10^4	1.95
	Treated	4.9×10^6	6.4×10^4	1.88
	Enucleated	2.9×10^4	1.5×10^4	0.29

[a] Cells to be enucleated received treatment with cytochalasin B and were centrifuged. Treated cells received treatment with cytochalasin B for the time required by the enucleation procedure, but were not centrifuged and thus were not enucleated. Control cells received neither cytochalasin B treatment nor centrifugation. Details may be found in reference 1. After a recovery period in fresh medium, cells were treated with IF or mock IF for 6 h at 37 C. IF titer, determined from the experiments reported here, was ~2.6 yield reduction units. Cells were infected at a multiplicity of 0.5 PFU/cell.

[b] Total yields, obtained after freeze-thawing, were measured by plaque assay on BHK-21/C13 cells. Expressed as PFU/0.2 ml.

[c] Yield reduction units = $\log_{10}$ (mock IF-treated yield/IF-treated yield).

[d] In the first experiment, infected cells were incubated for 12 h at 37 C and in the second they were treated for 18 h at 32 C.

virus yields in mock IF-treated cells when compared with zero time samples (data not shown) were always greater than 10^3-fold and were ample to demonstrate, by comparison, the presence or absence of the inhibitory effect of IF. In passing, it should be noted that the failure of IF to inhibit viral growth in enucleated cells indicates that the virus produced was not derived in large measure from the residual nucleated cells.

In conclusion, we have demonstrated that IF cannot induce the antiviral state in enucleated cells but that, once the antiviral state is established, physical enucleation does not abolish it. This is in agreement with the findings of Radke et al. (7) and extends their observations on a DNA virus (vaccinia) to include vesicular stomatitis virus (an RNA virus), which is capable of yielding progeny in enucleated monkey cells. Assuming that nuclear extrusion itself has not damaged irrevocably all of the putative cell surface receptors necessary for IF recognition and uptake, the most plausible explanation for both sets of observations is that the ability of interferon to induce the antiviral state depends on new mRNA transcripts from a nuclear DNA template.

TABLE 2. *Effect of enucleation on cells that have been treated previously with interferon*

Cells[a]	Virus yields		Log_{10} yield reduction
	+ Mock IF	+ IF	
Control	3.1×10^6	1.05×10^4	2.47
Treated	2.5×10^6	0.5×10^3	3.70
Enucleated ..	2.0×10^4	1.5×10^2	2.11

[a] Cells were treated with IF, or mock IF, for 6 h at 37 C. Then control, treated, and enucleated cells were prepared as described in the footnotes to Table 1. After the recovery period, cells were infected with virus and harvested after 12 h of incubation at 37 C.

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