Proceedings of the National Academy of Sciences
Vol. 68, No. 2, pp. 299-302, February 1971

Interferon and Transcription of Early Virus-Specific RNA in Cells Infected with Simian Virus 40

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Communicated by John F. Enders, November 16, 1970

ABSTRACT Treatment with interferon reduced the content of *early* virus-specific RNA, as well as the content of an *early* viral protein (T antigen), in monkey cells acutely infected with simian virus 40 (SV40). This unexpected finding suggests either that the action of interferon involves inhibition of the transcription of *early* SV40 messenger RNA, or that the SV40 genome contains a "proto-*early*" gene whose product is required for the transcription of the remaining *early* genes.

Interferons are proteins produced by animal cells in response to infection by many different viruses, and after contact with several nonviral interferon inducers (1). A number of investigations have suggested that exposure to homologous (2) interferon induces cells to synthesize a new, intracellular, antiviral protein (3) which, in turn, inhibits the replication of a wide range of DNA and RNA viruses without affecting normal cellular synthetic activities (4). The capacity to promote the inhibition of viral functions without affecting host-cell macromolecular synthesis (4, 5) makes interferon an extremely useful tool for the analysis of virus–cell interactions, including those involving oncogenic viruses (6–8). However, the interpretation of the results of such analyses awaits an exact knowledge of the mechanism of action of interferon. In this regard, it has been demonstrated that interferon action involves an inhibition of the *early* (i.e., prereplicative) synthetic functions of both DNA and RNA viruses (7, 9). Furthermore, the results of *in vitro* studies of RNA viruses by Marcus and Salb (10) and Levy and Carter (11), and of studies of vaccinia virus infection in interferon-treated cells by Joklik and Merigan (12), suggest that the postulated interferon-induced antiviral protein inhibits the translation of viral messenger RNA (mRNA).

Interferon has already been utilized to examine viral gene function during and after the *in vitro* neoplastic transformation of cells by the oncogenic DNA virus, simian virus 40 (SV40). It was observed that in acutely infected cells the synthesis of SV40 T antigen (an *early* viral protein) was markedly inhibited by interferon (7). In contrast, the synthesis of this same virus-coded protein in SV40-transformed cells was not affected by the presence of interferon (8). An understanding of the basis for the fundamental difference in interferon-sensitivity of this *early* viral function in acutely infected and transformed cells will depend upon the determination of whether transcription or translation is being inhibited.

Abbreviations: SDS, sodium dodecyl sulfate; cRNA, RNA that hybridizes to a *complementary* DNA; VSV, vesicular stomatitis virus; AGMK, African green monkey kidney (cells).

SV40 offers several distinct advantages over the viruses utilized in earlier investigations of interferon action. It is a small DNA virus of relatively uncomplicated structure (13) which replicates in the cell nucleus and does not induce the early inhibition of cellular macromolecular synthesis (14) observed in cells infected with vaccinia (15) and with mengovirus (16). Furthermore, techniques are available to detect and quantitate both *early* viral mRNA (17) and an *early* viral protein, the SV40 T antigen (18), whose synthesis in acutely infected cells is markedly sensitive to interferon (7). Finally, when cytosine arabinoside is used to block DNA synthesis, the SV40 infection can be limited to the *early* viral functions. This is necessary if a distinction is to be made between inhibition of transcription and inhibition of translation, since a reduction in *early* viral protein synthesis, regardless of the mechanism by which it is induced, will result in decreased levels of *late* viral RNA and proteins. Accordingly, experiments were performed to determine simultaneously, in the presence of cytosine arabinoside, the effect of interferon on the transcription and translation of SV40 genetic information.

MATERIALS AND METHODS

Tissue cultures

Primary African green monkey kidney(AGMK), BSC-1 (19), and Vero (20) cells were propagated in Eagle's minimal essential medium supplemented with penicillin, streptomycin, and 2 mM glutamine, plus 10% fetal calf serum.

Viruses

SV40 strain 777 (21) was propagated in BSC-1 cells at low multiplicities of infection. Cells and medium were harvested when all cells exhibited typical SV40 cytopathic effects, frozen and thawed three times, and clarified by centrifugation at 1000 $\times g$ for 30 min. The clarified virus, which titered $10^{9.3}$ plaque-forming units per ml in AGMK cells, was stored in aliquots at −70°C. The Indiana strain of vesicular stomatitis virus (VSV) was propagated in chick embryo tissue culture.

Interferons

Human leukocyte interferon was prepared by the method of Strandler and Cantell (22) from fresh human leukocytes challenged with UV-inactivated Newcastle disease [*A*] or Sendai [*B*] virus. The preparations employed were the kind gift of C. Chany [*A*], and N. B. Finter and R. A. Bucknall [*B*]. Monkey cell interferon was produced by challenging monolayer cultures of primary AGMK cells with rubella virus and harvest-

ing the supernatant fluid 48 hr later (23). Mouse serum interferon, stimulated *in vivo* by the intravenous inoculation of Newcastle disease virus (24), was the kind gift of S. Baron. Interferon preparations were held at pH 2 in the cold to inactivate inducing virus and then neutralized and clarified by centrifugation prior to use. Control preparations consisted of supernatant fluids from sham-infected cultures (or serum from uninoculated mice) treated in parallel with the interferon preparations.

Plan of experiments

Confluent monolayer cultures of primary AGMK or Vero cells in large glass roller bottles were treated with interferon or appropriate control preparations for 18 hr at 37°C. Cultures were then washed and infected with SV40 at a multiplicity of 20–50 plaque-forming units per cell in the presence of cytosine arabinoside at a concentration (20 μg/ml) that was observed to inhibit both total- and SV40-DNA synthesis by more than 99%. [5-^{3}H]Uridine was added (final concentration: 50 μCi/ml) 6 hr after infection and the RNA was extracted with hot phenol–sodium dodecyl sulfate (SDS) 12–18 hr later. The details of labeling, harvesting, and RNA extraction have already been described (25). Aliquots of the harvested cells from each culture were counted and assayed for the T antigen of SV40 by an indirect immunofluorescence technique (18).

Interferon preparations were assayed in the cells used in each experiment, employing a VSV plaque-reduction technique. One unit of interferon is defined as that quantity contained in each ml of a solution that produces a 50% inhibition of VSV plaque formation under the conditions employed. The activity of the human leukocyte interferons in AGMK and Vero cells was observed to be significantly lower (20–50-fold) than that of the same preparations assayed in human foreskin fibroblasts or human embryonic kidney cells.

The extracted RNA was quantitated by an orcinol method (26) and its radioactivity determined as acid-precipitable (10% trichloroacetic acid) cpm. SV40-specific RNA (SV40c-RNA) was measured by RNA–DNA hybridization, using a modification (25) of the procedure of Gillespie and Spiegelman (17). The SV40 DNA employed was extracted by papain digestion, followed by SDS-phenol extraction (27) from purified virus and demonstrated to be free of any homology with monkey cell RNA (Oxman, M. N., A. S. Levine, C. S. Crumpacker, M. J. Levin, P. H. Henry, and A. M. Lewis Jr.; manuscript in preparation). Nitrocellulose filters (13 mm) containing 1.0 μg of DNA were used. Hybridization was performed in 2 × SSC + 0.05% SDS (SSC = 0.15 M NaCl + 0.015 M Na Citrate) at 60°C for 18 hr in a final volume of 0.25 ml. Control filters containing *Escherichia coli* DNA were included in each hybridization vial. After hybridization, the filters were washed, treated with RNase, and washed again before scintillation counting. Host cell-specific RNA (host cell cRNA) was measured by RNA–DNA hybridization in free solution with monkey cell DNA by the procedure of Nygaard and Hall (28). *E. coli* and monkey cell DNA were prepared by the method of Marmur (29).

RESULTS

The effect of pretreatment with interferon on the *early* SV40-specific RNA and SV40 T antigen content of monkey cells acutely infected with SV40 is summarized in Table 1. Human and monkey interferon reduced SV40 T antigen formation in both AGMK and Vero cells. In addition, virus-specific RNA, as measured by either the proportion of input RNA hybridizing with SV40 DNA or by the total SV40cRNA content of the cells, was markedly reduced in cultures pretreated with interferon ($P < 0.001$). Furthermore, the extent of this inhibition of SV40cRNA was similar to the degree of inhibition of SV40 T antigen formation.

In contrast, pretreatment with interferon had little or no effect upon either the proportion of input RNA that hybridized with host cell DNA or the total yield of [^{3}H]RNA. The small reduction in the total [^{3}H]RNA extracted from the interferon-treated cultures in experiments 1 and 3 was the result of a smaller yield of cells at the time of harvesting due either to random variation or to a toxic effect of the preparations of interferon employed. Neither the total [^{3}H]RNA per cell nor the specific activity of the extracted RNA was different in interferon-treated and control cultures.

The results obtained in cultures pretreated with mouse serum interferon are instructive (Expt. 4). This heterologous interferon is inactive in primate cells and, as expected, produced no inhibition of SV40 T antigen or VSV plaque formation in the Vero cells. It was, however, somewhat cytotoxic at the concentration employed. The cells exhibited granularity and cytoplasmic vacuolization, and the total yield of [^{3}H]-RNA was reduced by 33%. No significant decrease, however, in the proportion of the input [^{3}H]RNA hybridizing with SV40 DNA, or in the virus–host ratio of hybridizable RNA was recorded.

DISCUSSION

The reduction of *early* virus-specific RNA observed in the interferon-treated cells was unexpected. If the action of interferon involves only inhibition of the translation of virus-specific RNA, as studies with RNA viruses have suggested (10, 11), the observed inhibition of SV40 T antigen formation should not be accompanied by any alteration in the amount of *early* SV40cRNA present. Four possible explanations for these findings are:

(*1*) The reduction of SV40cRNA is not the result of the action of interferon but that of some other component present in the interferon preparations employed. Since "pure" interferon is not available, this possibility cannot be conclusively eliminated. However, the absence of any significant inhibition of host cell cRNA synthesis, and the species-specificity of the effect, suggest that the observed reduction in SV40cRNA is attributable to the action of interferon itself.

(*2*) The reduction of SV40cRNA in the interferon-treated cultures may be a secondary effect of interferon action. For example, inhibition of virus polysome formation, a postulated mechanism of interferon action (10–12), might result in the more rapid degradation of free SV40cRNA molecules. However, Joklik and Merigan (12) observed no instability of free vaccinia messenger RNA in interferon treated cells.

(*3*) The SV40 genome may contain an *early* gene ("proto-*early*") which can be transcribed by a cellular DNA-dependent RNA polymerase. The product of such a gene, which might be a virus-specific DNA-dependent RNA polymerase (30), or a sigma-like factor that alters the specificity of a host-cell RNA polymerase (31), would be essential for the transcription of the remaining *early* genes. If a mechanism of this sort were operative, interferon-induced inhibition of the translation of the

TABLE 1. *The effect of interferon on early SV40-specific RNA and SV40 T antigen synthesis in acutely infected monkey cells*

Expt. no.	Cells	Interferon*	SV40 T antigen†	Total extracted [^{3}H]RNA (cpm × 10^6)	% of input RNA hybridized with:‡ SV40 DNA (cpm)	% of input RNA hybridized with:‡ Host cell DNA (cpm)	% Inhibition Host cell cRNA§	% Inhibition SV40 cRNA§	% Inhibition SV40 T antigen
1	AGMK	Control	80	49	0.0056 (0.004)	0.23 (0.004)	—	—	—
		Human leukocyte interferon *A*¶ (2 units/ml)	27	38	0.0026 (0.0001)**	0.26 (0.008)††	0	54	66
2	Vero	Control	74	113	0.0039 (0.0013)	0.17 (0.02)	—	—	—
		Human leukocyte interferon *B*¶ (10 units/ml)	3	116	0.0007 (0.0002)‡‡	0.16 (0.05)§§	6	82	96
3	Vero	Control	93	71	0.0078 (<0.0001)	0.25 (0.002)	—	—	—
		AGMK interferon (20 units/ml)	4	64	0.0006 (<0.0001)‡‡	0.25 (0.005)	0	92	96
4	Vero	Control	94	90	0.0061 (0.0030)	0.82 (0.13)	—	—	—
		AGMK interferon (50 units/ml)	1	93	0.0005 (<0.0001)‡‡	0.89 (0.01)§§	0	92	99
		Human leukocyte interferon *B* (40 units/ml)	2	80	0.0004 (<0.0001)‡‡	0.91 (0.04)§§	0	94	98
		Mouse serum interferon (inactive in Vero cells)	96	63	0.0059 (0.0025)§§	0.97 (0.06)¶¶	0	3	0

* Interferon concentration employed, as determined by VSV plaque reduction assay in the cells used for each experiment, is given in parentheses.

† Given as % of cells unequivocally exhibiting SV40 T antigen (18); details have been described previously (7).

‡ Expts. 1 and 3: each point represents a single roller bottle culture; hybridizations with each RNA preparation were performed in duplicate. Expts. 2 and 4: each point represents the average of the results of two roller bottle cultures handled separately; hybridizations with each RNA preparation were performed in triplicate. *E. coli* DNA blanks (1.0 μg) were included in each hybridization vial and the cpm bound (<3 × 10^{-4} % of input) subtracted from that bound to the SV40 DNA filters. Figures in parentheses are standard deviations. Probabilities are calculated using the *t* test for small samples and a table of the distribution of *t* for certain probability levels (34).

§ $\frac{\%\text{ input hybridized (control)} - \%\text{ input hybridized (interferon treated)}}{\%\text{ input hybridized (control)}} \times 100.$

¶ See *Materials, Interferons* for definition of letters.

** $P < 0.01$.

†† $P > 0.02$.

‡‡ $P < 0.001$.

§§ $P > 0.3$.

¶¶ $P > 0.05$.

SV40cRNA from this proto-*early* gene would prevent the transcription of SV40cRNA from the remaining *early* genes, resulting in a marked reduction in total *early* SV40cRNA content. The findings of Joklik and Merigan (12) that interferon did not inhibit the synthesis of the *early* RNA of vaccinia virus appear to contrast with our results. However, the vaccinia virion, itself, contains a DNA-dependent RNA polymerase (32). The presence of this enzyme may permit the transcription of *early* vaccinia RNA without the need for the prior synthesis of new viral proteins, such as we propose may be required in the case of SV40.

(4) The mechanism of action of interferon, at least in SV40-infected monkey cells, may involve inhibition of the transcription of *early* viral messenger RNA. The experiments with vaccinia virus reported by Ohno and Nozima (33) provide some support for this possibility. These authors observed that interferon treatment reduced the synthesis of the rapidly labeled RNA that regularly occurs after vaccinia infection of untreated chick embryo cells. However, the characterization of this RNA as virus-specific was incomplete.

If none of the first three possibilities proposed prove tenable, it may be necessary to accept this mechanism, and abandon the unitary concept that interferon inhibits all viruses, DNA and RNA alike, at the level of translation.

We thank Drs. C. Chany, N. B. Finter, R. A. Bucknall, and S. Baron for their kind gifts of interferon, and Drs. P. H. Henry, H. B. Levy, W. P. Rowe, and J. F. Enders for their advice and encouragement.

This work was supported by Research grant E-576 from the American Cancer Society and, in part, by PHS grant AI-01992 from the National Institute of Allergy and Infectious Disease.

Dr. Levin held an USPHS postdoctoral traineeship (5-701-AI-00350-03).

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