Interferon Action in Heterokaryons (33763)

MARY A. GUGGENHEIM, R. M. FRIEDMAN, AND A. S. RABSON

Pathologic Anatomy Branch and Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Maryland 20014

If a mixture of human cells and nucleated chick erythrocytes is exposed to UV-inactivated Sendai virus, heterokaryons containing both chick and human nuclei are formed. In this heterokaryon the dense, pyknotic avian red cell nucleus enlarges, its chromatin is dispersed, and, in contrast to its former dormant state, it has active uptake of ^{3}H-thymidine and ^{3}H-uridine (1, 2). Moreover, as shown by our previous studies (3), this heterokaryon produces chick-specific interferon, an indication that the activated red cell nucleus can initiate the synthesis of a functional protein. Since the nonfused, inactive chicken erythrocyte neither produces nor responds to interferon, activation of this nucleus by fusion offers an unusual opportunity to study the mechanism of interferon action. The present communication describes the antiviral action of both human and chicken interferons on a human-chick heterokaryon. In addition we report some RNA turnover studies on these cells.

Materials and Methods. Human interferon was prepared from human lymphocytes by infecting them with Sendai virus. After dialysis, acid treatment, and centrifugation the interferon titer was 500 units/ml when assayed with Vesicular Stomatitis Virus on human cells (1 unit yields 50% inhibition of virus growth). Partially purified chick interferon (10,000 units/ml) was a gift of Dr. K. H. Fantes. The cell fusion system we have studied utilizes a continuous line of human diploid cells, AH-1, and a preparation of chick embryo erythrocytes. Details of the fusion method have been published (4).

Results. In order to determine if the heterokaryon does, indeed, respond to chick interferon it is mandatory to have fusion conditions that result in very close to 100% heterokaryon formation, for in a mixture of nonfused human cells and heterokaryons chick interferon inhibiton of viral growth in the fused human–chick cell could be masked by viral growth in the cells containing only human nuclei. In cell fusions in which approximately 50% of the cells contained activated RBC nuclei, there was a significant antiviral response only to human interferon; however, when conditions were such that virtually every cell contained one or more activated RBC nuclei, exposure of these cells to either human or chick interferon, or to a mixture of both, resulted in various degrees of viral inhibition (Table I). The results shown are typical of several experiments. In the control cells (AH-1 cells exposed to Sendai virus) there was a roughly tenfold inhibition of viral growth when human interferon was used, but partially purified chicken interferon had no effect. In the heterokaryons, the viral inhibitory effect of the same dose of human interferon was partially circumvented, showing only a threefold inhibition. Chick interferon did act on these cells causing a two- to threefold decrease in viral growth—a small, but nonetheless, significant and repeatable effect. Moreover, exposing the heterokaryons to both human and chicken interferon resulted in a synergistic effect with a more than tenfold inhibition. In two experiments in which an extraordinarily large proportion of RBC nuclei were fused (3–10 per AH-1 cell) an almost complete circumvention of the antiviral action of human interferon was found (Table II).

Since Sendai virus contains a potent hemolysin that quickly lyses the cytoplasm of the RBC in the cell fusion mixture, it is probably only the erythrocyte nucleus that fuses with the AH-1 cell. Thus, it appears that the activated chick nucleus contributes some factor(s) to the heterokaryon that (a) circumvents the viral inhibitory step induced by human interferon; and (b) renders the cell

TABLE I. Antiviral Action of Interferon on Heterokaryons.[a]

	Control ($\times 10^5$)	Human interferon (100 U/ml)	Chick interferon (100 U/ml)	Both interferons (each 100 U/ml)	Units
Control (AH-1 cells exposed to Sendai virus)	34	2.8	36	4.5	($\times 10^5$ pfu/ml)
		8.2	100.6	13	(% of control)
AH-1 – Chick RBC Heterokaryons	19	6.2	7.4	1.5	($\times 10^5$ pfu/ml)
		33	39	8.0	(% of control)

[a] Cells were maintained for 4 days after fusion in diploid growth medium with 10% fetal bovine serum. On day 4 the cells were washed and incubated either with control medium or interferon as indicated. After 8 hr at 37° the cells were washed $\times 5$, 10^8 pfu of Vesicular Stomatitis Virus (VSV) adsorbed for 1 hr, and the cells were again washed $\times 5$. The VSV was grown for 14–16 hr at 37° (one growth cycle), frozen, and titered in duplicate by plaque assay on chick embryo cells. Results are given as actual plaque forming units (pfu) of VSV and as percentage of control. (Control cells were never exposed to interferon.)

sensitive to the inhibitory effect of chick interferon. Carver *et al.* (5) showed that hybrid mouse–hamster cells were sensitive to hamster interferon whereas the parent hamster cell line was quite insensitive. In this system too, therefore, genetic information (present in the hamster cell) seems to be expressed better in the heterokaryon than in the parent cell.

Recent publications (6, 7) have suggested that the primary effect of interferon is to alter ribosomes so that they no longer are able to combine efficiently with viral RNA to form functional polysomes. As it is generally accepted, on the basis of inhibitor studies, that interferon action requires the production by the cell of a specific protein, Marcus and Salb (6) postulated that it is this protein which alters the ribosomes. We have, therefore, performed some experiments which give an estimation of the synthetic capacity for ribosomal RNA of the heterokaryon. The AH-1 ribosomal RNA was labeled with ^{3}H-uridine prior to fusion or exposure to Sendai virus, and the cells were incubated for several days in the presence of cold uridine so that the label was primarily in the stable ribosomal RNA fraction. At this time some of the ^{3}H-labeled AH-1 cells were fused with chick erythrocytes and other AH-1 cells simply exposed to Sendai virus. Four days after fusion the three groups of cells (normal AH-1, AH-1 exposed to Sendai virus, and heterokaryons) were exposed to $^{32}P_i$ for 24 hr. The ribosomal RNA was then extracted, and fractionated by sucrose gradient analysis as previously described (8). Ten-drop fractions were collected and assayed for ^{3}H, ^{32}P, and OD 260 mμ. (Fig. 1). The ^{3}H and ^{32}P specific activities of the 28 S RNA peak give an indication of the relative turnover rates of ribosomal RNA. The ^{3}H specific activity reflects the "dilution" of the human ribosomal pool by RNA synthesized after fusion. As Fig. 1 shows, the turnover of ribosomal RNA is markedly depressed in the heterokaryons and in the cells exposed to virus as compared to normal AH-1 cells.

TABLE II. Antiviral Action of Human Interferon on Heterokaryons Containing a Very High Proportion of RBC Nuclei.

	Control ($\times 10^5$ pfu/ml)	Human interferon (10 U/ml)
Control (AH-1 cells exposed to Sendai virus)	24	7.0×10^5 pfu/ml
		29% of control
AH-1 – RBC Heterokaryons	32.5	30×10^5 pfu/ml
		92% of control

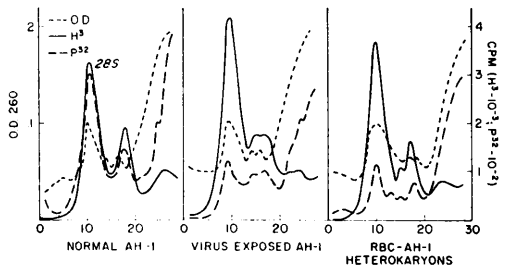

FIG. 1. The RNA was extracted with phenol-SDS and layered over a 6–30% sucrose gradient. After centrifugation fractions were collected, aliquots put on paper and acid washed, and assayed for ^{32}P and ^{3}H in a Packard Tri-Carb scintillation counter; absorbance at 260 mμ was measured in a Beckman spectrophotometer; ^{3}H reflects ^{3}H-uridine to which the AH-1 cells had been exposed prior to fusion; $^{32}P_2$ was added to all cells 5 days after fusion. Control AH-1 cells (I) and AH-1 cells exposed to Sendai virus (II) were treated exactly as the heterokaryons (III) except that either the RBC or both the virus and RBC were omitted at the time of fusion.

There is some new ribosomal RNA being made, as indicated by ^{32}P uptake, but it is impossible to say whether this represents chicken or human ribosomal RNA or both.

Discussion. The results show that chick interferon was active in chick–human heterokaryons whereas it was inactive in unfused human cells (AH-1) and that these heterokaryons were less sensitive to human interferon than were AH-1 cells without chick nuclei. Both chick and human interferons together were more active in the heterokaryon than was either interferon alone. The question arises as to whether these results are consistent with the hypothesis that the site of interferon action is the ribosome (6, 7).

A simple model for interferon action would place the site of species specificity in the nucleus, e.g.,

Chick interferon + chick nucleus → "viral inhibitory protein" → altered ribosome.
Human interferon + human nucleus → "viral inhibitory protein" → altered ribosome.

According to this model, once the specific interferon has acted upon its target nucleus, the resulting protein no longer carries any species specific information. However, the fact that the presence of the chick nucleus circumvents the action of human interferon is inconsistent with such a model unless one further postulates that the chick nucleus has an inhibitory effect on the human interferon–human nucleus interaction.

An alternative model would extend the species specificity to the level of ribosomes, e.g.,

Chick interferon + chick nucleus → chick "viral inhibitory protein" → altered chick ribosome,
Human interferon + human nucleus → human "viral inhibitory protein" → altered human ribosome.

Such a model would be consistent with the partial circumvention of interferon action and also consistent with the additive effect of exposing the cells to both interferons simultaneously. However, this model requires the synthesis, in significant amounts, of chick-specific ribosomes. It is not possible, at this time, to make a definitive statement regarding the presence of chick ribosomes in the heterokaryons. To do so requires a technique for differentiating chick ribosomes from human ribosomes—if indeed there are significant differences between the two. It is clear, however, from our results shown in Fig. 1 that the rate of ribosomal RNA synthesis in the chick–human fibroblast heterokaryons is very low and the probability that large amounts of chick specific ribosomes are present is, therefore, small. It is also theoretically possible that the chick nucleus can initiate the synthesis of specific ribosomal proteins that combine with human RNA to form a "hybrid" ribosome. Such a molecule might interact with a chick specific antiviral protein. At the present state of knowledge of ribosomal structure this possibility cannot be tested experimentally.

Harris (personal communication) approached the question of ribosome production by the activated chick nucleus with another technique and concluded that during the first few days following activation of the RBC nucleus by fusion, there is no 28S RNA produced by this nucleus. This immediate post-

fusion period covers the time during which the interferon studies described above were carried out. If, indeed, there are few or no chick ribosomes produced by the heterokaryon during the early postfusion period, we would suggest that the effects of specific interferons on these cells can be better explained by a model that does not require ribosomes to be the site of interferon action.

Summary. Chick interferon was active in chick red blood cell–human fibroblast heterokaryons, whereas it was inactive in the human fibroblasts without fused chick nuclei. The heterokaryons were much less sensitive to human interferon than were the human fibroblasts without chick nuclei. Both human and chick interferons together were more active in heterokaryons than was either interferon alone. Ribosomal turnover studies in heterokaryons and in the human cells without chick nuclei suggest the possibility that the site of interferon action may not be on the ribosomes.

We thank Mrs. Dorothy Hughes and Mrs. Sandra Tyrrell for technical assistance. M. A. G. supported by PHS fellowship from NCI, F2-CA-35, 040-01.

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Received Nov. 25, 1968. P.S.E.B.M., 1969, Vol. 130.