the duplication time was less than 16 hours; growth rates have not yet been equally rapid in continuous culture, but the success of the growth apparatus already indicates that it may have broad applications for the culturing of rapidly growing suspensions of plant cells.

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References and Notes

1. G. Melchers and V. Engelmann, *Naturwissenschaften* **42**, 564 (1955); C. Wang and E. J. Staba, *J. Pharm. Sci.* **52**, 1058 (1963).
2. W. Tulecke, *Ann. N.Y. Acad. Sci.* **139**, 162 (1966).
3. P. S. S. Dawson, *Can. J. Microbiol.* **9**, 671 (1963).
4. Brooks Instrument Co., Hatfield, Pa.
5. Skinner V5 series, 10-watt; Skinner Precision Industries, New Britain, Conn.
6. Type TEC (R. W. Cramer Co., Ltd., Centerbrook, Conn.) or Eagle HP700 (E. W. Bliss Co., Davenport, Iowa).
7. KB17AY latching relay; Potter and Brumfield, Guelph, Ontario.
8. Series 31; Bristol Motors, Old Saybrook, Conn.
9. O. L. Gamborg, *Can. J. Biochem.* **44**, 791 (1966).
10. O. L. Gamborg, R. A. Miller, K. Ojima, *Exp. Cell Res.* **49**, 1 (1967).
11. R. B. Parker, *Appl. Microbiol.* **15**, 197 (1967).
12. Chicopee Mills, Inc., 1450 Broadway, New York.
13. P. Filner, *Biochim. Biophys. Acta* **118**, 299 (1966).
14. M. Mandels and A. Maguire, *U.S. Army Natick Laboratories 01760* (1966).
15. R. A. Miller, in preparation.
16. M. J. Johnson, *J. Biol. Chem.* **181**, 707 (1949).

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Interferon: Production by Chick Erythrocytes Activated by Cell Fusion

Abstract. *In the presence of Sendai virus inactivated with ultraviolet light, nucleated chick erythrocytes can be fused with several types of human cells to form heterokaryons. Although chick erythrocytes alone cannot be stimulated by Sendai virus to produce interferon, fusion with a human cell (AH-1) which itself may produce human interferon results in heterokaryons in which the erythrocyte genome is activated and chick interferon is produced. When nucleated chick erythrocytes are fused with another type of human cell (HeLa clone S-3) which does not produce human interferon when stimulated, no chick interferon is detectable, despite morphologic changes suggestive of activation of the erythrocyte nuclei.*

Much interest has developed in both spontaneously forming (*1*) and virus-induced cell fusion (*2*). The fused cells, which carry all or part of the genetic information of two separate cell types, have been used to study the genetic dominance of certain characteristics of tumor cells (*3*, *4*), to attempt to localize specific cell functions (for example, synthesis of thymidine kinase) to specific human chromosomes (*5*), to induce replication of noninfective "integrated" virus (*6*), and to examine mechanisms of genetic regulation (*7*, *8*). Harris and co-workers have demonstrated that the dormant nucleus of a chicken erythrocyte becomes activated when transferred into the cytoplasm of certain other cells. Their criteria for activation were morphologic changes and increased turnover of nuclear DNA and RNA as indicated by autoradiographic techniques (*7*).

We have examined the production of a specific cell protein, interferon, by heterokaryons containing nuclei from both human epithelial cells and chick erythrocytes. To do so, we took advantage of interferon's species specificity and the exquisite sensitivity of the interferon assay.

Sendai virus inactivated with ultraviolet light was used in our experiments to induce heterokaryons from circulating chicken erythrocytes and cells of human origin [HeLa clone S-3 and AH-1, a line derived from normal human epithelium (*9*)]. This technique has been described in detail by Harris (*7*).

Mixtures containing 5×10^6 human cells and 10^8 chicken erythrocytes (RBC's) per milliliter were exposed to 20,000 to 30,000 hemagglutinating units of inactivated Sendai virus. Chicken white cells (WBC) were removed from the RBC's by repeated centrifugation and removal of the top one-third of the cell pack. After fusion, the cell mixture was placed in a tissue-culture flask with medium 199 and fetal calf serum (20 percent) and incubated at 36.5°C for 24 hours. At this time the fluids were removed and dialyzed at *p*H 2 overnight against 20 to 40 volumes at 4°C. This treatment destroys all infectious Sendai virus. The fluids were then brought back to *p*H 7 before assay. Interferon activity was assayed by its effect on the single-cycle growth of Semliki Forest Virus (SFV) tested in either chicken (primary embryonic fibroblasts) or human (AH-1) cells. After exposure of the cells to the solutions containing interferon for 12 to 15 hours, the cell sheets were washed five times before addition of the challenge virus. Details of the assay have been described previously (*10*). In a few early experiments the heterokaryons were allowed to grow for 4 to 5 days after fusion, with daily medium changes, and then given a second virus stimulus (this time with SFV at 10 to 10^3 infectious virus particles per cell). Assays for interferon following this second stimulus were not quantitatively different from assays on the initial supernatant in which fusion took place.

Fusion of the erythrocyte nucleus

Table 1. Monolayers of either chick or human cells were exposed to dilutions (1 : 3) of the test medium or to control medium (199 and 20 percent serum) overnight at 37°C. Then the cells were washed five times, and a high multiplicity of SFV (approximately 10^3) was added and grown for one growth cycle (6 to 8 hours). Tenfold dilutions of these vials were assayed for plaque formation on fresh chick embryo fibroblasts. Results are given as average virus single growth cycle titers and growth as percentage of control. (Control, no exposure to interferon.) All assays were done in duplicate. In the columns labeled Interferon, + indicates its presence; — indicates its absence.

Group	Cells	Fluids titered on: Chick cells: Interferon	Chick cells: PFU/ml	Chick cells: Control (%)	Human cells: Interferon	Human cells: PFU/ml	Human cells: Control (%)
1	Chick RBC	—	27.5×10^7	98			
1	Control		28×10^7		—		
2	Chick RBC + HeLa	—	7.8×10^7	102		18.2×10^6	103
2	Control		7.5×10^7		—	17.5×10^6	
3	Chick WBC + HeLa	+	7.2×10^7	38		6×10^7	104
3	Control		19.2×10^7		+	5.5×10^7	
4	Chick RBC + AH-1	+	11.8×10^6	47		3.8×10^7	43
4	Control		25×10^7			9×10^7	

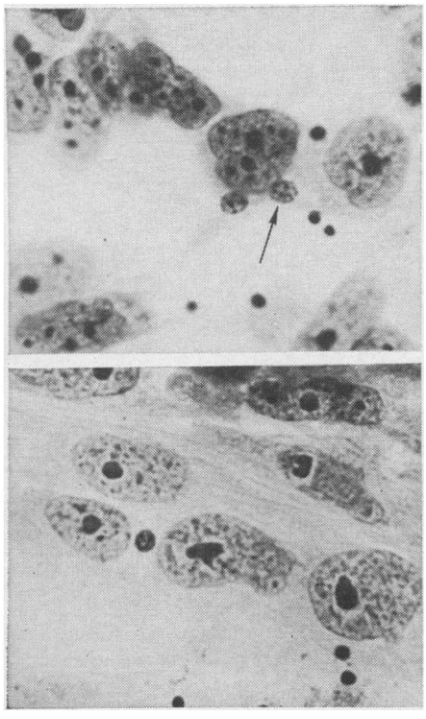

Fig. 1 (top). Chick RBC-HeLa cell heterokaryon 24 hours after virus-induced fusion. Arrow indicates chick nucleus in cytoplasm of HeLa cell. Nonfused erythrocyte nuclei are smaller and pyknotic (hematoxylin and eosin, ×690). (bottom) Chick RBC–AH-1 cell heterokaryon 24 hours after fusion (hematoxylin and eosin, × 690).

with both human cell types resulted in enlargement of the RBC nucleus and dispersion of the chromatin material (Fig. 1). These changes are similar to the process described by Harris (*8*). There were no apparent morphologic differences between the activated RBC nuclei in the two cell lines.

Table 1 summarizes the results of assays for interferon activity after fusion of chick erythrocytes with both lines

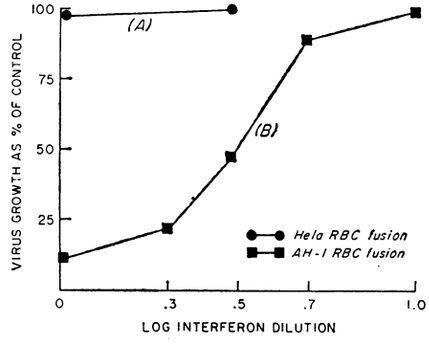

Fig. 2. Semilogarithmic curves of growth as a function of dose for chick interferon activity present in supernatants after fusion of chick erythrocytes with either HeLa cells (*A*) or AH-1 cells (*B*).

of human cells. Neither Sendai-treated erythrocytes alone (group 1) nor any combination of RBC's and HeLa cells (group 2) inhibited virus growth when tested in either the chicken or human assay system. In groups 2 and 4 great care was taken to remove all buffy-coat cells before fusion. In group 3 buffy-coat cells were intentionally included in the cell fusion mixture, and in the tissue culture flask there were RBC-HeLa heterokaryons and WBC-HeLa heterokaryons as well as single cells of each type. Virus growth was inhibited in chick cell-monolayers tested with fluids from this experiment, but, as before, there was no evidence of the production of human interferon. Thus neither interferon production by chicken buffy-coat cells nor the assay system used was blocked by contact with the HeLa cells or any product of the fusion mixture.

In the erythrocyte–AH-1 fusion (group 4) there was evidence that both chicken and human interferon were present in the supernatant. Although the interferon titer was low, the expected relation of dose and response was found (Fig. 2). The fluid from this experiment was tested in an assay with primary mouse fibroblasts, and no interference with virus growth was found. Also, the chick interferon was stable to incubation at 60°C for 30 minutes and was not sedimented by centrifugation at 200,000*g* for 30 minutes. Incubation of the active fluid with trypsin resulted in partial (approximately 50 percent) loss of activity. High concentrations of serum are known to interfere with complete inactivation of interferon by trypsin. The low titers of interferon prevented us from testing sensitivity to trypsin at lower concentrations of serum. Production of interferon is blocked by actinomycin D (*11*). When AH-1–RBC heterokaryons were given a second virus stimulus 1 week after cell fusion and simultaneously exposed to 1 μg of actinomycin per milliliter, no interferon activity against either human or chick cells was found. Thus, although the titers of interferon activity were low, the AH-1–RBC heterokaryons did produce detectable activity that was not dialyzable, stable to heat (60°) and *p*H 2, not precipitated by ultracentrifugation, sensitive to trypsin, and inactive when tested on mouse cells. Furthermore, the activity was not a nonspecific product of the AH-1 cells since neither AH-1 cells alone treated with inactive Sendai virus nor a mixture of AH-1 cells and chicken RBC's (mixed but not fused with virus) produced the interferon-like substance. It seems likely that the stimulus for interferon production by the heterokaryons was the ultraviolet-inactivated Sendai, for the AH-1 line is free of mycoplasma and neither electron microscopy nor prolonged passage in tissue culture has shown any evidence of latent viruses.

These experiments demonstrate the synthesis of a specific cell protein in a heterokaryon after virus-induced cell fusion. Nuclear activation induced by heterokaryon formation had previously been demonstrated by Harris, who found increased uptake of H^3-uridine and H^3-thymidine by fused erythrocyte nuclei (*7*). It was of interest to determine whether the complex sequence of steps from activation of DNA to production of active protein could be completed by these cells. Since production of interferon specific to chicken cells must be directed by the erythrocyte DNA, it would seem that this sequence can take place. As chicken erythrocytes alone do not produce interferon, fusion with a permissive cell, such as AH-1, seems to result in an activation or "unmasking" of at least this portion of the previously dormant chicken genome.

The results of fusion of erythrocytes with HeLa cells are provocative. There may be present in this clone of HeLa cells a repressor that prevents the production of interferon. This repressor might block interferon production directed by both the HeLa cell and chicken erythrocyte nucleus.

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References and Notes

1. G. Barski, S. Sorieul, Fr. Cornefert, *J. Nat. Cancer Inst.* **26**, 1269 (1961).
2. H. Harris and J. F. Watkins, *Nature* **205**, 640 (1965).
3. V. Defendi, B. Ephrussi, H. Koprowski, M. C. Yoshida, *Proc. Nat. Acad. Sci. U.S.* **57**, 299 (1967).
4. B. Finch and B. Ephrussi, *ibid.*, p. 615.
5. M. Weiss and H. Green, *ibid.* **58**, 1104 (1967).
6. H. Koprowski, F. C. Jensen, Z. Steplewski, *ibid.*, p. 127.
7. H. Harris, J. F. Watkins, C. E. Ford, G. I. Schoefle, *J. Cell Sci.* **1**, 1 (1966).
8. H. Harris, *ibid.* **2**, 23 (1967).
9. Supplied by the Media Section, Laboratory Aids Branch, courtesy of George Gardner.
10. R. M. Friedman and H. L. Cooper, *Proc. Soc. Exp. Biol. Med.* **125**, 901 (1967).
11. D. C. Burke, *Interferons* (North-Holland, Amsterdam, 1966), pp. 69–75.
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