one can obtain insight into chromosome evolution. It is known that closely related genes, derived from a common ancestral gene, do not have to be located on the same chromosome. For example, the α- and β-globin genes are located on chromosomes 16 and 11, respectively (*17, 18*). However, it is estimated that α- and β-globin genes have diverged from this common ancestral gene approximately 500 million years ago (*19*). The γ-, δ-, and β-globin genes are closely linked on the short arm of human chromosome 11 (*20–22*). These genes have diverged much more recently (*19*). Other related genes, such as salivary and pancreatic amylase, are also closely linked on chromosome 1 in humans (*23, 24*). It is estimated that the *GH* and *CSH* genes diverged approximately 50 million to 60 million years ago (*8*). As shown here, they have retained chromosomal linkage. The prolactin and growth hormone genes diverged approximately 400 million years ago (*8*). With a probe for the human prolactin gene it would be possible to determine whether this gene is linked to *GH*, *CSH*, and *GHL*. Whether the genes are contiguous on the DNA of chromosome 17 is an issue that must be resolved by other methods, for example, by cloning of large DNA fragments.

DAVID OWERBACH
WILLIAM J. RUTTER
Department of Biochemistry and Biophysics, University of California, San Francisco 94143
JOSEPH A. MARTIAL
JOHN D. BAXTER
Endocrine Research Division, University of California
THOMAS B. SHOWS
Biochemical Genetics Section, Roswell Park Memorial Institute, New York State Department of Health, Buffalo

References and Notes

1. Chorionic somatomammotropin, generally symbolized CS, has been designated CSH in this report because the international system for human gene nomenclature has previously assigned the symbol *CS* to the human citrate synthetase gene; T. B. Shows *et al.*, *Cytogenet. Cell Genet.*, in press.
2. H. D. Niall, M. L. Hogan, R. Sauer, I. Y. Rosenblum, F. C. Greenwood, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 866 (1971).
3. J. Shine, P. H. Seeburg, J. A. Martial, J. D. Baxter, H. M. Goodman, *Nature (London)* **270**, 494 (1977).
4. J. A. Martial, R. A. Hallewell, J. D. Baxter, H. M. Goodman, *Science* **205**, 602 (1979).
5. J. C. Fiddes, P. H. Seeburg, F. M. DeNoto, R. A. Hallewell, J. D. Baxter, H. M. Goodman, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4294 (1978).
6. B. Shome and A. F. Parlow, *J. Clin. Endocrinol. Metab.* **45**, 1112 (1977).
7. T. A. Bewley, J. S. Dixon, C. H. Li, *Int. J. Pept. Protein Res.* **4**, 281 (1972).
8. J. A. Martial and N. E. Cooke, in *Central and Peripheral Regulation of Prolactin Function*, V. Scapagnini, Ed. (Raven, New York, in press).
9. T. B. Shows, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 348 (1972).
10. ———, in *Isozymes: Current Topics in Biological and Medical Research*, M. C. Rattazzi, J. G. Scandalios, G. S. Whitt, Eds. (Liss, New York, 1977).
11. D. Owerbach, G. Bell, W. J. Rutter, T. B. Shows, *Nature (London)*, in press.
12. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
13. J. M. Taylor, R. Illemensee, S. Summers, *Biochim. Biophys. Acta* **442**, 324 (1976).
14. H. M. Goodman, F. DeNoto, J. C. Fiddes, R. A. Hallewell, G. S. Page, S. Smith, E. Tischer, *Miami Winter Symp.*, in press.
15. A. Royal *et al.*, *Nature (London)* **279**, 125 (1979).
16. O. J. Miller, P. W. Allderdice, D. A. Miller, W. R. Breg, B. R. Migeon, *Science* **173**, 244 (1971).
17. A. Deisseroth, A. Nienhuis, P. Turner, R. Veelez, W. F. Anderson, F. Ruddle, J. Lawrence, R. Creagan, R. Kucherlapati, *Cell* **12**, 205 (1977).
18. A. Deisseroth, A. Nienhuis, J. Lawrence, R. Giles, P. Turner, F. H. Ruddle, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1456 (1978).
19. M. O. Dayhoff, *Atlas of Protein Sequence and Structures* (National Biomedical, Washington, D.C., 1978), vol. 5, p. 229.
20. A. F. Scott, J. A. Phillips, B. R. Migeon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4563 (1979).
21. J. Gusella, A. Varsanyi-Breiner, F. T. Kao, C. Jones, T. T. Puck, C. Keys, S. Orkin, D. Housman, *ibid.*, p. 5239.
22. A. J. Jeffreys, I. W. Craig, U. Francke, *Nature (London)* **281**, 606 (1979).
23. M. L. Rivas *et al.*, *Cytogenet. Cell Genet.* **16**, 347 (1976).
24. A. D. Merritt, E. W. Lovrien, M. L. Rivas, P. M. Conneally, *Am. J. Hum. Genet.* **25**, 523 (1973).
25. D. Denhardt, *Biochem. Biophys. Res. Commun.* **23**, 641 (1966).
26. G. M. Wahl, M. Stern, G. Stark, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3683 (1979).
27. T. B. Shows, L. Scrafford-Wolff, J. A. Brown, M. H. Meisler, *Somat. Cell Genet.* **5**, 147 (1979).
28. G. A. Koch and T. B. Shows, *Cytogenet. Cell Genet.*, in press.
29. S. L. Naylor, R. L. Eddy, L. L. Haley, M. G. Byers, D. Shaver, J. A. Brown, T. B. Shows, in preparation.
30. ———, *Cytogenet. Cell Genet.*, in press.
31. M. J. Champion, J. A. Brown, T. B. Shows, *ibid.* **22**, 498 (1978).
32. T. B. Shows and J. A. Brown, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2125 (1975).
33. ———, L. L. Haley, M. G. Byers, R. L. Eddy, E. S. Cooper, A. P. Goggin, *Cytogenet. Cell Genet.* **21**, 99 (1978).
34. ISCN, "An international system for human cytogenetic nomenclature," *ibid.*, p. 313.
35. S. L. Naylor, R. J. Klebe, T. B. Shows, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 6159 (1978).
36. We thank G. Bell and R. Pictet for helpful discussions; N. Cooke for providing growth hormone probe; L. Haley, R. Eddy, and M. Byers for technical assistance; and C. Young and L. Spector for assistance in preparing the manuscript. This work was supported by NIH grants GM 20454 and HD 05196 to T.B.S. and NIH grant AM 21344 to W.J.R.

7 February 1980; revised 7 April 1980

Strain Dependence of the Antiproliferative Action of Interferon on Murine Erythroid Precursors

Abstract. *Electrophoretically pure mouse interferon inhibits erythropoietin-dependent proliferation of committed erythroid precursors (CFU-E) obtained either from adult mouse bone marrow or from 14-day fetal mouse livers. The degree of inhibition is significantly influenced by the genotype of the cell donor; about ten times as much interferon is required to inhibit proliferation of CFU-E from C57BL/6 than is needed for comparable inhibition of CFU-E from BALB/c or Swiss mice. These strain-dependent results point to the existence of genes that influence the degree of the inhibitory effect of interferon on cell multiplication.*

The committed erythroid precursors (CFU-E) of mammalian erythropoietic tissues are the target cells for erythropoietin [erythropoiesis-stimulating factor (ESF)] and depend strictly on the hormone for their proliferation and differentiation. When CFU-E are cloned in vitro in the presence of ESF (*1*), they give rise in 48 hours to erythroblastic colonies of 8 to 40 elements. This hormone-dependent system is sensitive to the antiproliferative effect of interferon preparation, as has bee observed in cultures of murine adult bone marrow (*2*) and fetal liver (*3*). However, because interferon represented at best only 0.1 percent of the protein in the preparations used for these studies, it was important to reexamine the effect of CFU-E of using electrophoretically pure mouse interferon, which has recently become available (*4*).

It was our aim to determine the dose-response curve relating concentration of electrophoretically pure interferon and the degree of inhibition of the growth of normal mouse femoral CFU-E. Our findings confirm the specific antiproliferative action of pure interferon on mouse CFU-E and support the new concept of a strain-to-strain variation in the sensitivity of mouse CFU-E to interferon. By use of a slight modification of the techniques of McLeod *et al.* (*5*), femoral bone marrow from male 8- to 10-week-old C57l-6, B10D2, Swiss, or BALB/c mice was cultured for the growth of erythropoietic colonies in plasma clots. Briefly, in any 1 ml of final mixture, 1×10^5 to 1.5×10^5 nucleated cells were suspended in supplemented Dulbecco's modified Eagle medium (DMEM) enriched with fetal calf serum, bovine serum albumin, antibiotics, and bovine citrated plasma. For each experiment, three or four culture samples containing a standard dose of 0.3 IRP unit of ESF were supplemented with a dose of pure interferon, graded from 5 to 500 antiviral units, delivered in 250-μl portions after dilution in DMEM. Controls were one sample without ESF or interferon and one sample with ESF only, both supplemented with 250 μl of diluted interferon buffer. Just after addi-

0036-8075/80/0711-0292$00.50/0

tion of the bovine citrated plasma, each sample was pipetted in 0.1-ml portions into eight Disposo Tray microwells (Linbro). After 48 hours of incubation in a humidified atmosphere of 5 percent CO_2 in air, the plasma clots were collected on slides and stained with 3,3′-dimethoxybenzidine and hydrogen peroxide, then counterstained with hematoxylin, by the method of McLeod *et al.* (*5*). An average of five clots per sample was scored for erythroid colonies of at least eight erythroblasts.

During these dose-response assays it became apparent that the level of interferon-mediated inhibition was significantly influenced by the genotype of the bone marrow donor. In the presence of electrophoretically pure interferon graded at 5 antiviral units per milliliter, the number of colonies obtained from ESF-stimulated cultures of C57BL/6 marrow cells was hardly, or not at all, reduced in comparison with controls, whereas colony counts from BALB/c or Swiss cells consistently showed inhibition of 30 to 40 percent. Two experiments performed with B10D2 mice gave results identical to those of experiments performed with C57BL/6 mice. The difference between the two types of response subsisted with increasing interferon concentrations, but became less pronounced. In all instances the relationship between interferon concentration and residual number of colonies was linear on a semilog scale (Fig. 1).

An experiment with CFU-E from fetal liver instead of bone marrow confirmed the strain dependence of sensitivity to interferon. Fourteen-day BALB/c and C57BL/6 embryos were used as liver donors, since by the tenth day of gestation cells from whole mouse embryos become responsive to the inhibitory effect of interferon on cell multiplication (*6*). Liver cells from embryos of both strains were collected simultaneously and cultured in plasma clots, as was done for adult bone marrow. Electrophoretically pure interferon was used in amounts of 5, 50, and 100 units per 1-ml sample. Because fetal mouse livers reach their maximal erythropoietic activity around day 15 of gestation (*7*), the liver cell concentration was one-fifth that of adult bone marrow cells to allow for the high density of liver CFU-E at this period (Table 1).

These results confirm that the antiproliferative effect on CFU-E previously observed with crude or partially purified interferon preparations is due to interferon itself. They are in line with recent results (*4*, *8*, *9*) showing that pure interferon inhibits cell multiplication. This inhibition is observed after cell treatment with a very low dose of interferon, a concentration of 5 U/ml being sufficient to reduce by 30 to 40 percent the number of CFU-E derived from BALB/c and Swiss mice. However, adult as well as fetal CFU-E from two other inbred strains, C57BL/6 and B10D2, are much more resistant to inhibition by interferon, with approximately ten times as much interferon required for comparable inhibition of CFU-derived colony proliferation.

Table 1. Strain dependence of the inhibitory effect of pure mouse interferon on erythroid precursors from livers of 14-day embryos.

Interferon (U/ml)			
0	5	50	100
	C57BL/6		
502* (100)	487 (97)	429 (85)	176 (35)
	BALB/c		
385 (100)	191 (50)	96 (25)	74 (19)

*Each value is the number of erythroid colonies per 1×10^5 cells; percentage of the control value is given in parentheses.

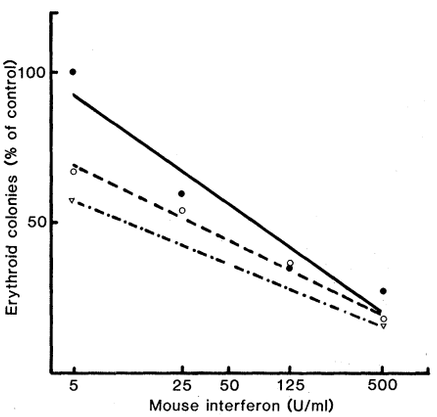

Fig. 1. Strain dependence of the inhibitory effect of pure mouse interferon on bone marrow erythroid colony formation. Each regression line is calculated from the results of seven separate experiments. (●) C57BL/6, slope −36, with a standard deviation from the regression line of 8.3 percent. The values for the Swiss and BALB/c strains are (○) Swiss, −25, 2.5 percent, and (△) BALB/c, −20.7, 1.6 percent. Electrophoretically pure mouse interferon derived from C-243 cells induced with Newcastle disease virus was obtained as described previously (*4*). The specific activity of the preparation used was 2×10^9 NIH reference units per milligram of protein, in good agreement with our previously published value. Analysis by polyacrylamide gel electrophoresis on slab gel showed only the two interferon bands previously described. All experiments were carried out with a single interferon preparation with a titer of 64,000 units per 0.2 ml, which was distributed into 0.1-ml portions and stored frozen at −70°C. When used, the portion was thawed and further diluted in DMEM. Sheep plasma erythropoietin (step 3, lot 3019-6, 15 IRP units per milligram; Connaught Medical Laboratories) was used.

These results suggest that sensitivity to the inhibitory effect of interferon on cell multiplication is genetically controlled, in the sense that multiplication of the same type of cells is more or less inhibited by interferon depending on the genotype of the cell. Strain dependence indicates that there are genes influencing the extent of the growth inhibitory effect of interferon. Genetic analysis will provide clues to linkage of these genes and possibly lead to their identification.

The immunomodulatory effect of interferon is also influenced by the genotype of the mouse, less interferon being required to inhibit the expression of delayed hypersensitivity to sheep red blood cells in BALB/c mice than in C57BL/6 mice (*10*). This difference may well be a reflection of the differential effect on cell multiplication described in this report, since the most likely explanation—admittedly hypothetical—for the inhibitory effect on the expression of delayed hypersensitivity is inhibition of replication of antigen-stimulated cells.

In addition to being theoretically interesting, our results may have practical implications for the current clinical trials with interferon in neoplastic diseases. Indeed, if there is a comparable genetic difference in sensitivity to the inhibitory effect of interferon on cell multiplication in man, interferon could have different effects on the same type of tumor in different patients.

ODETTE GALLIEN-LARTIGUE
DANIÈLE CARREZ
EDWARD DE MAEYER
JAQUELINE DE MAEYER-GUIGNARD
Institut Curie, Bâtiment 110, Université de Paris-Sud, 91405 Orsay, France

References and Notes

1. J. R. Stephenson, A. A. Axelrad, D. L. McLeod, M. M. Shreeve, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1542 (1971); N. N. Iscove, F. Sieber, K. H. Winterhalter, *J. Cell. Physiol.* **83**, 309 (1974).
2. E. van t'Hull, A. Schellekens, B. Lowenberg, M. J. De Vries, *Cancer Res.* **38**, 911 (1978); J. A. Ortega, A. Ma, N. A. Shore, P. P. Dukes, T. C. Merigan, *Exp. Hematol.* **7**, 145 (1979).
3. K. A. Smith, T. N. Frederickson, L. E. Mobraaten, E. De Maeyer, *Exp. Hematol.* **5**, 333 (1977).
4. J. De Maeyer-Guignard, M. G. Tovey, I. Gresser, E. De Maeyer, *Nature (London)* **271**, 622 (1978).
5. D. L. McLeod, M. M. Shreeve, A. A. Axelrad, *Blood* **44**, 517 (1974); *Nature (London)* **261**, 492 (1976).
6. K. Drasner, C. J. Epstein, L. B. Epstein, *Proc. Soc. Exp. Biol. Med.* **160**, 46 (1979).
7. R. G. Tarbutt, R. J. Cole, *J. Embryol. Exp. Morphol.* **24**, 429 (1970).
8. I. Gresser, J. De Maeyer-Guignard, M. G. Tovey, E. De Maeyer, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5308 (1979).
9. E. Knight, *Nature (London)* **262**, 302 (1976).
10. E. De Maeyer and J. De Maeyer-Guignard, *Ann. N.Y. Acad. Sci.*, in press; in *Interferon 1979*, I. Gresser, Ed. (Academic Press, London, 1979).

11. Supported by Institut National de la Santé et de la Recherche Médicale grant ATP 40-76-72 and Délégation Générale à la Recherche Scientifique et Technique grant 79-7-205/206.

7 December 1979; revised 7 February 1980