K. R. Tenore and R. B. Hanson [*Limnol. Oceanogr.* **25**, 553 (1980)].
8. The ^{15}N content was determined for two subsamples of plant material and a subsample of aged detritus from each of two replicate tests. All worms from a chamber on each occasion were ground together and a subsample taken for ^{15}N analysis. The ^{15}N analysis itself is reproducible to within ± 2 percent [H. Paerl and P. Bland, *Appl. Environ. Microbiol.* **43**, 218 (1982)].
9. Incorporation of ^{15}N [micrograms of ^{15}N per milligram of worm (dry weight) per day] = growth (per day) × nitrogen content [micrograms of nitrogen per milligram of worm (dry weight)] × ^{15}N content (micrograms of ^{15}N per microgram of nitrogen, in the worms).
10. *C. capitata* is a rapidly growing, opportunistic species which can grow 10 to 30 percent per day when fed seaweed detritus and 3 to 12 percent when fed marsh grass detritus (K. Tenore, unpublished data).
11. Three treatments with two observations per treatment, $P = .01$, $N = 6$, Kruskal-Wallis test for both marsh grass and seaweed detritus.
12. R. Harper, J. Fry, M. Learner, *Oikos* **36**, 211 (1981); L. Kofoed *J. Exp. Mar. Biol. Ecol.* **19**, 223 *(Berlin)* (1975); G. Lopez and J. Levinton, *Oecologia* **32**, 263 (1978); W. Odum, P. Kirk, J. Zieman, *Oikos* **32**, 363 (1979); D. Rice and K. Tenore, *Estuarine Coastal Shelf Sci.* **13**, 681 (1981); P. Wainwright and K. Mann, *Mar. Ecol. Prog. Ser.* **7**, 309 (1982).
13. M. Martin, J. Martin, J. Kukor, R. Merritt, *Oecologia (Berlin)* **46**, 360 (1980).
14. L. M. Cammen, *Mar. Biol.* **61**, 9 (1980); D. J. W. Moriarty, *Aust. J. Mar. Freshwater Res.* **32**, 245 (1981); J. Hobbie and C. Lee, in *Marine Benthic Dynamics*, K. Tenore and B. Coull, Eds. (Univ. of South Carolina Press, Columbia, 1980), p. 341. Benthic algae may also be a source of energy in shallow waters [M. Pace, S. Shimmel, W. Darley, *Estuarine Coast. Mar. Sci.* **9**, 121 (1979)].
15. We thank Drs. D. Menzel, J. Meyer, L. Pomeroy, and B. Wallace for helping improve early drafts, D. Lehsau for drafting the figures, and B. Christiansen for typing. This work was supported by NSF grant OCE82-00385 to K.R.T.
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Clustering of Leukocyte and Fibroblast Interferon Genes on Human Chromosome 9

Abstract. *At least ten leukocyte interferon genes and the single known fibroblast interferon gene have been localized on the pter→q12 region of human chromosome 9. Gene mapping was accomplished by blot hybridizations of cloned interferon complementary DNA to DNA from human-mouse cell hybrids with a translocation involving human chromosome 9. Supporting evidence suggests these genes are clustered.*

The interferons are a family of proteins that convey viral resistance in target cells, and also affect cell proliferation and the immune response (*1*). Three distinct forms of human interferon have been classified according to their biochemical and antigenic properties: IFN-α (leukocyte), IFN-β (fibroblast), and IFN-γ (immune) (*2*). Molecular cloning studies have led to the identification of more than ten distinct genes coding for IFN-α (*3*, *4*), and a single gene each for IFN-β (*5*) and IFN-γ (*6*). DNA sequence determinations indicate that the genes for IFN-α are closely related (80 to 95 percent nucleotide sequence homology), and the gene for IFN-β contains 40 to 50 percent sequence homology to the genes for IFN-α. The genes for IFN-α and IFN-β do not contain introns. By contrast, the IFN-γ has no significant DNA sequence homology with the IFN-α and IFN-β genes, and the coding region of the gene is interrupted by three introns (*6*).

These observations suggest that genes for IFN-α and the gene for IFN-β are more closely related evolutionarily than either is to the gene for IFN-γ [for gene mapping nomenclature, see (*7*)]. Because of these similar properties, it is of interest to determine whether the several leukocyte and the fibroblast interferon genes are clustered on the same chromosome. Clustering of the genes might be an important aspect of the control of their expression and of the evolutionary origin of the interferon genes. We had previously assigned the genes for IFN-α and IFN-β to chromosome 9 by using cloned interferon genes to examine human-mouse somatic cell hybrid DNA by the Southern hybridization technique (*8*). The chromosome 9 assignment of the gene for IFN-β has been confirmed (*9*, *10*). In addition, analysis of recombinant DNA clones established that several human IFN-α genes are located within several thousand nucleotides of one another (*4*). We now report the localization of leukocyte and fibroblast genes on the pter→q12 region of chromosome 9, suggesting that virtually all of the genes for IFN-α and perhaps the gene for IFN-β may be clustered.

Human fibroblasts (GM2836) with a reciprocal translocation [46,XY,t(9;17)(q12;p11)] involving chromosomes 9 and 17 (Fig. 1) were fused to mouse LM/TK$^-$ (thymidine kinase–deficient) cells, and cell hybrids were isolated (*11*). The translocation chromosomes 9/17 (9pter→9q12::17p11→17pter) and 17/9 (17qter→17p11::9q12→9qter) separate chromosome 9 into two regions with the breakpoint at 9q12 (see Fig. 1). The leukocyte and fibroblast interferon genes encoded on chromosome 9 can be localized on either of these two translocation chromosomes by using Southern blot analysis of DNA from somatic cell hybrids (*8*, *12*) individually retaining these rearranged chromosomes.

After cell fusion with polyethylene glycol (*13*), cell hybrids were isolated and cloned in the hypoxanthine-aminopterin-thymidine (HAT) selection medium (*11*) and examined for human chromosomes (*14*) and enzyme markers (*12*) assigned to each chromosome. The TK selectable marker located in the q21→q22 region of chromosome 17 [see (*15*)] was utilized with the HAT system to selectively retain one of the transloca-

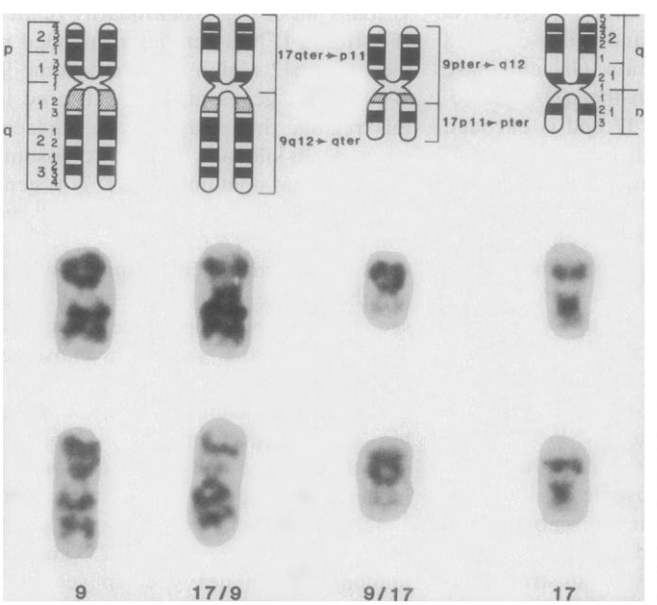

Fig. 1. The human fibroblast line GM2836, with a reciprocal translocation between chromosomes 9 and 17, was examined karyotypically by the Giemsa-trypsin procedure (*14*). A partial karyotype featuring the chromosomes involved is presented. All other chromosomes had a normal banding pattern. Nomenclature for the banding pattern follows the Paris Conference (*18*). The translocation has been designated [46, XY,t(9;17)(q12;p11)] and involves a 17/9 chromosome (17qter→17p11::9q12→9qter) and a 9/17 chromosome (9pter→9q12::17p11→17pter). These chromosomes are diagrammed (top) and examples from two different cells (bottom) are included for comparison. The fibroblasts (GM2836) were obtained from the Human Genetic Mutant Cell Repository, Camden, New Jersey.

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Table 1. Presence of IFN-α and IFN-β genes in translocation cell hybrids. NSL hybrids were isolated from the fusion of human GM2836 containing a [46,XY,t(9;17)(q12;p11)] translocation, and mouse LM/TK$^-$. The ICL control hybrid was from the fusion of GM1006, a karyotypically normal fibroblast line, and mouse LM/TK$^-$. Leukocyte and fibroblast interferon gene sequences were detected (*8*) in the following way: IFN-α probes were prepared from gel-isolated fragments of two IFN-α complementary DNA plasmids, pLEIF A and pLEIF D (*3*), and IFN-β probes were prepared from the complementary DNA clone pFIF 3 (*17*). DNA was labeled with ^{32}P to specific activities of about 10^8 cpm/μg and hybridized to nitrocellulose filters for 12 hours at 42°C in 50 percent formamide, fivefold strength standard saline citrate, 50 m*M* sodium phosphate (*p*H 6.5), fivefold strength Denhardt's reagent, 10 percent dextran sulfate 500 (Pharmacia), and sonicated salmon sperm DNA (200 μg/ml); filters were washed in 0.1 strength standard saline citrate at 60°C (*8*). Chromosomes (*14*), enzyme markers (*12*), and interferon (*8*) were tested on the same cell passage.

Hybrid	Interferon genes		Chromosomes				Enzyme markers		
	α	β	9	17/9	9/17	17	ACO1	AK1	GALK
ICL-11	+	+	+	−	−	+	+	+	+
NSL-5	−	−	−	+	−	−	−	+	+
NSL-9	−	−	−	+	−	+	−	+	+
NSL-15	−	−	−	−	−	+	−	−	+
NSL-16	+	+	−	+	+	+	+	+	+

tion chromosomes in hybrids, in this case the 17/9 chromosome (Fig. 1), which encodes the TK gene. Thus, in these cell hybrids, human TK and either the normal 17 or the 17/9 translocation, or both, are always retained; in control hybrids the normal 17 is retained (Table 1).

Of the 16 cell hybrids isolated and screened for informative combinations of chromosomes 9, 17, 9/17, and 17/9 (Table 1), four informative cell hybrids were identified and examined for IFN-α and IFN-β genes (Table 1). Assays for enzyme markers encoded by genes on chromosomes 9 and 17 have been described (*12*). Aconitase 1 (ACO1) is located in the pter→p13 region of chromosome 9 and is encoded on the 17/9 translocation. Adenylate kinase 1 (AK1) is located in the 9q34 band and is encoded on the 17/9 translocation. Galactokinase (GALK) is located in the q21→q22 region of chromosome 17 and is encoded on the 17/9 translocation (*15*). The control hybrid, ICL-11, made with a karyotypically normal human fibroblast, GM1006, retained an intact chromosome 9 (and therefore the genes for both IFN-α and IFN-β), the chromosome 9 short-arm marker ACO1, and the 9 long-arm marker AK1. NSL-15 retained chromosome 17 and had GALK but neither part of chromosome 9 nor the genes for IFN-α and IFN-β. NSL-5 and NSL-9 retained only the 17/9 translocation and expressed the chromosome 9 long-arm marker AK1 and the chromosome 17 marker GALK but did not have genes for IFN-α or IFN-β. This demonstrates that the leukocyte genes for IFN-α and the fibroblast gene for IFN-β are not located in the q12→qter region of chromosome 9. NSL-16 retained the 9/17 translocation in addition to the 17/9 translocation. Both IFN-α and IFN-β genes were detected, as well as ACO1, AK1, and GALK. Since the 17/9 translocation was eliminated as the site for IFN-α and IFN-β genes in NSL-5 and NSL-9 hybrids, NSL-16 demonstrates that genes for IFN-α and IFN-β are encoded in the pter→q12 region of chromosome 9 represented by the 9/17 translocation in NSL-16.

Although association of fibroblast interferon genes with chromosomes 2 and 5 has been suggested [see (*10*)], there is currently no evidence at the nucleic acid hybridization level to involve chromosomes other than human chromosome 9. Thus, our genetic evidence indicates that virtually all of the evolutionarily related IFN-α and IFN-β genes are encoded in the pter→q12 region of chromosome 9. While these genes may be clustered in this region, they may not be adjacent since a 36-kilobase pair chromosome fragment that contained the IFN-β gene but no IFN-α genes has been described (*16*).

Several human gene families whose members have a high degree of DNA sequence and polypeptide homology are clustered on the same chromosome. These are, for example, the β-globin genes on chromosome 11p12, the α-globin genes on chromosome 16p, the growth hormone and chorionic somatomammotropin genes on chromosome 17q22→24, the histocompatibility complex on chromosome 6p21→23, and the immunoglobulin heavy chain genes on chromosome 14 [see (*15*)]. Those members of the large human IFN-α gene family that share DNA sequence homology greater than 75 percent and that readily cross-hybridize are also clustered, most likely on the short arm of chromosome 9. Several of these IFN-α genes are found in the same or on overlapping human genomic DNA recombinant clones (*4*). In addition, we have found that the related gene for IFN-β is also localized on this same region of chromosome 9. Distantly related genes for IFN-α or IFN-β, or pseudogenes not easily detectable by DNA cross-hybridization, could be located elsewhere in the human genome.

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References and Notes

1. A. Isaaca and J. Lindenmann, *Proc. R. Soc. London Ser. B* **177**, 258 (1957); D. H. Metz, *Cell* **6**, 429 (1975); W. E. Stewart, *The Interferon System* (Springer-Verlag, Vienna, Austria, 1979).
2. Interferon nomenclature, *Nature (London)* **286**, 110 (1980).
3. D. V. Goeddel *et al.*, *ibid.* **290**, 20 (1981); C. Weissmann, *Interferon* **3**, 101 (1981).
4. R. M. Lawn, J. Adelman, T. J. Dull, M. Gross, D. Goeddel, A. Ullrich, *Science* **212**, 1159 (1981); S. Nagata, C. Brack, A. Henco, A. Schambock, C. Weissmann, *J. Interferon Res.* **1**, 333 (1981); C. Brack, S. Nagata, N. Mantei, C. Weissmann, *Gene* **15**, 378 (1981); A. Ullrich, P. Gray, D. V. Goeddel, T. J. Dull, *J. Mol. Biol.* **156**, 467 (1982).
5. T. Taniguchi, M. Sakai, Y. Fujii-Kuriyama, M. Muramatsu, S. Kobayashi, T. Sudo, *Proc. Jpn. Acad. Ser. B* **55**, 464 (1979).
6. P. W. Gray *et. al.*, *Nature (London)* **295**, 503 (1982).
7. In accordance with human gene nomenclature and for gene mapping, the human genes for IFN-α, IFN-β, and IFN-γ are designated *IFA*, *IFB*, and *IFG*, respectively [T. B. Shows *et al.*, *Cytogenet. Cell Genet.* **25**, 96 (1979)].
8. D. Owerbach, W. J. Rutter, T. B. Shows, P. Gray, D. V. Goeddel, R. M. Lawn, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2123 (1981).
9. A. Meager, H. Graves, D. C. Binke, D. M. Swallow, *Nature (London)* **280**, 493 (1979).
10. P. M. Pitha, D. L. Slate, N. B. K. Raj, F. Ruddle, *Mol. Cell. Biol.* **2**, 564 (1982).
11. T. B. Shows, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 348 (1972).
12. ———, A. Y. Sakaguchi, S. L. Naylor, *Adv. Hum. Genet.* **12**, 341 (1982).
13. R. Davidson and P. Gerald, *Somat. Cell Genet.* **2**, 165 (1976).
14. T. B. Shows, J. A. Brown, L. L. Haley, M. G. Byers, R. L. Eddy, E. S. Cooper, A. P. Goggin, *Cytogenet. Cell Genet.* **21**, 99 (1978).
15. T. B. Shows and P. McAlpine, *ibid.*, in press.
16. G. Gross, U. Mayr, W. Bruns, F. Grosveld, H.-H. M. Dahl, J. Collins, *Nucl. Acids Res.* **9**, 2594 (1981).
17. D. V. Goeddel, M. Shepard, E. Yelverton, D. Leung, R. Crea, A. Sloma, S. Pestka, *ibid.* **8**, 4057 (1980).
18. Paris Conference on Standardization in Human Genetics, in *Birth Defects* (Original Article Series), (The National Foundation, March of Dimes, New York, 1971), vol. 8, No. 7.
19. We thank L. L. Haley, R. L. Eddy, M. Byers, W. M. Henry, and C. Young for assistance. Supported in part by NIH grants GM 20454 and HD 05196 and by Genentech, Inc.

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