

## CHROMOSOMAL LOCATION OF A HUMAN $\alpha$ INTERFERON GENE FAMILY\*

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Interferons comprise a family of vertebrate proteins, whose synthesis can be induced by a variety of agents, including viruses, double-stranded RNA, mitogens, and antigens. Interferon is released from the induced cell and can trigger a number of biological responses in target cells. Interferons can confer resistance to virus replication, inhibit cell growth, and activate certain cells of the immune system (1).

Human interferons have been divided into three major classes based on antigenic and biochemical criteria (1, 2):  $\alpha$  (produced mainly by peripheral blood leukocytes and established lymphoblastoid lines);  $\beta$  (synthesized primarily by cultured fibroblasts); and  $\gamma$  (produced by mitogen or antigen-stimulated T cells). Several human interferon genes have been cloned using recombinant DNA technology (3-6), and many of these cloned genes have been expressed in bacteria (7-9).

Although somatic cell genetic studies have been used in mapping human  $\beta$  interferon genes (10-14), human/rodent hybrid cells have not produced  $\alpha$  interferon under the induction conditions used. In this study, we used methods to detect specific  $\alpha$  interferon sequences in the DNA of somatic cell hybrids. Because these techniques allow direct characterization of the cellular genome, gene expression is not required for mapping. We have previously used this approach (15, 16) to map several other human and mouse structural genes, including immunoglobulin light and heavy chains. Our evidence indicates that an  $\alpha$  interferon gene cluster resides on human chromosome 9.

### Materials and Methods

*Materials and Methods.* Somatic cell hybrids were generated by fusing Chinese hamster E36 cells with human BD melanoma cells (BDXE36 hybrids) (Pravtcheva and Ruddle, unpublished results), temperature-sensitive Chinese hamster ovary cells and human leukocytes (141 BnpT hybrids), mouse A9 cells with GM17 human fibroblasts (AIM hybrids, ref. 13), or mouse A9 cells with GM589 human fibroblasts (BDA hybrids, ref. 13). Parental and hybrid cells were grown in monolayer culture in minimal essential medium or Dulbecco's modified essential

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medium with high glucose (both from Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) supplemented with 10% fetal bovine serum. Hybrids were karyotyped and tested for the presence of human marker isozymes, as previously described (13, 15).

DNA was extracted from pellets of HeLa, A9, E36, and hybrid cells, digested with restriction endonucleases, fractionated by agarose gel electrophoresis, and transferred to nitrocellulose filters according to Scangos et al. (17). Cloned  $\alpha_1$  interferon cDNA or genomic DNA in pBR322 (4, 7) was labeled with [ $^{32}$ P]deoxycytidine triphosphate to a specific activity of  $1 \times 10^8$  to  $2 \times 10^8$  dpm/ $\mu$ g by in vitro nick translation (18). The hybridization, washing, and autoradiography of the filters were performed as described by D'Eustachio et al. (16). Filters were incubated for 8 h with the radiolabeled probe at a concentration of 6–10 ng/ml in the presence of 10% dextran sulfate, using the method of Wahl et al. (19). Nonspecifically bound material was removed by washing the filters three times for 5 min at room temperature in 0.3 M NaCl, 0.03 M sodium citrate, and 0.1% sodium dodecyl sulfate, and two times for 30 min at 65° in 0.15 M NaCl, 0.015 M sodium citrate, and 0.1% sodium dodecyl sulfate. The filters were then dried and subjected to autoradiography.

## Results

Table I summarizes the karyotype and isozyme data obtained for the human/mouse and human/Chinese hamster hybrid cells used for mapping sequences related to human  $\alpha_1$  interferon. Fig. 1 illustrates the pattern of hybridization of the  $\alpha_1$  cDNA probe to DNA isolated from human HeLa cells, mouse A9 cells, and a series of mouse/human hybrid cells retaining every human chromosome except 19. All of the hybrids (tracks 3–7) contain mouse DNA sequences that cross hybridize to the human  $\alpha_1$  interferon sequence (as does the mouse parent line, track 2); however, they lack the characteristic human hybridization pattern (track 1) with distinct bands at 27 kb, 18 kb, 11 kb, 8 kb, 7 kb, 6.2 kb, 6 kb, 4.8, 3.4 kb, 2.3 kb, and 1.8 kb. BDA 17b17 retains chromosome 9 at low frequency (20%) and does not score positive for hybridization to the  $\alpha_1$  probe, although human  $\beta$  interferon can be produced by this line after poly(rI):poly(rC) superinduction (data not shown).

Using human/Chinese hamster hybrid cells, sequences hybridizing to the human

TABLE I  
*Human Chromosomes Retained in Hybrid Cells*

Cell line	Chromosome composition	Hybridization to $\alpha$ interferon probe
AIM15aA1	1, 2, 5, (6), 7, 11, 12, 13, 14, 15, 17, 18, 20, 21, X	–
BDA14b25	1, 4, (6), 7, 12, 14, 18, X	–
BDA17b17	1, 2, 3, 4, 5, 6, (9), 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, X	–
BDA10a3	2, 3, 4, (5), 6, 8, 10, 11, 12, 13, 16, 18, 20, X	–
WAVR4dF94a	21	–
BDXE36-4a	2, 6, 9, 14, 20, 21, X	+
BDXE36-10	2, 6, (9), 10, 11, 12, 14, 17, 19, 20, 21, X	+
BDXE36-8b	2, 6, 11, 12, 14, 19, X	–
141BnpT-1	2, 9, 10, 11, 12, 19, X	+

Karyotype and isozyme analyses were performed on human/mouse or human/Chinese hamster hybrids as described (13, 15). A human chromosome was scored as present when it was observed in at least 20% of the metaphases scored or when a marker enzyme assigned to it was found in cell extracts. Numbers in parentheses indicate a discrepancy in the chromosome and isozyme data, reflecting a low retention frequency for the particular chromosome.

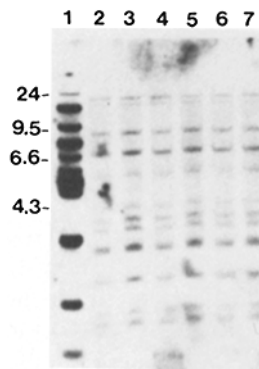


FIG. 1. Hybridization of Hind III restriction fragments to  $\alpha_1$  interferon probe. 30- $\mu$ g samples of DNA from human HeLa (track 1); mouse A9 (track 2) and human/mouse hybrid cells; A1M15aA1 (track 3); BDA10a3 (track 4); BDA14b25 (track 5); BDA17b17 (track 6); and WAVR4dF94a (track 7) were digested with Hind III restriction endonuclease, fractionated by agarose gel electrophoresis, and transferred to nitrocellulose filters as described in Materials and Methods. Fragments containing sequences related to human  $\alpha_1$  cDNA were detected by hybridization to  $^{32}$ P-labeled probe. The filter was then washed and autoradiographed.  $\lambda$  DNA digestion was run in parallel to monitor digestion and for molecular weight standardization (figures to left of autoradiogram, expressed as kb).

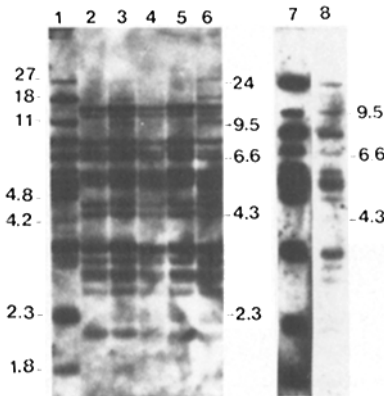


FIG. 2. Human  $\alpha_1$  interferon-related sequences in human, Chinese hamster, and human/Chinese hamster hybrid cell DNA. Hind III restriction fragments from HeLa (track 1); Chinese hamster E36 (track 2); and hybrid cells BDXE36-4a (track 3); BDXE36-8b (track 4); BDXE36-10 (track 5); and 141BnpT-1 (track 6) were fractionated as described in Materials and Methods and were scored for the presence of sequences hybridizing to a  $^{32}$ P-labeled  $\alpha_1$  interferon probe. Figures to the left of the first autoradiogram represent molecular weights (in kb) of fragments characteristic of human sequences hybridizing to the  $\alpha_1$  interferon probe, and those to the right represent size markers from Hind III digestion of bacteriophage  $\lambda$  DNA. Tracks 7 and 8 represent the hybridization pattern obtained under more stringent final wash conditions (0.015M NaCl, 0.0015 M sodium citrate, 0.5% sarkosyl) for HeLa (track 7) and hybrid 141 BnpT-1 (track 8) DNA. Figures to the right of these tracks represent molecular weights of Hind III-digested DNA fragments run in parallel.

$\alpha_1$  interferon probe could be clearly observed in cellular DNA when chromosome 9 was retained (Fig. 2). The human and hamster hybridization patterns could be distinguished readily (tracks 1 and 2); several human-specific bands (27 kb, 18 kb, 11 kb, 4.8 kb) could be observed in BDXE36-4a (track 3) and 141 BnpT-1 (track 6). Hybrid BDXE36-10 gave very weak hybridization (evident on longer exposures and in other gels) in these regions, whereas BDXE36-8b (track 4), lacking chromosome 9,

did not exhibit human-specific bands. Some human-specific bands (for example, those at 4.2 kb, 3.8 kb, 2.3 kb, and 1.8 kb) were not observed under our standard hybridization conditions; for weak bands, such as the 3.8 kb band, the dilution of human sequences against a hamster background may make detection impossible. Under more stringent washing conditions (0.015 M NaCl, 0.0015 M sodium citrate, 0.5% sarkosyl, three washes at 50°C), where most hamster sequences related to  $\alpha_1$  interferon do not hybridize, all of the human (HeLa) bands >2.3 kb were clearly present in hybrid 141 BnpT-1 (tracks 7 and 8). Very weak hybridization was observed for the 2.3 kb band. Chromosome 19 retention was not related to the presence of  $\alpha_1$  sequences. Subclones were made for BDXE36-4a and BDXE36-10, and karyotype, isozyme, and DNA hybridizations were performed; segregation of chromosome 9 below a frequency of 20–30% resulted in a failure to detect  $\alpha_1$  interferon-related sequences by our method (data not shown).

The hybridization of human  $\alpha_1$  interferon DNA sequences to mouse and Chinese hamster genomic DNA results in distinctly different band patterns (track 2 of Figs. 1 and 2). The molecular weights of the major mouse bands are estimated to be 21 kb, 18 kb, 9.4 kb, 7.2 kb, 5.8 kb, 4.1 kb, 3.8 kb, 3.5 kb, 3.1 kb, 2.6 kb, 2.2 kb, and 2.1 kb; those of the hamster are 14 kb, 12 kb, 8 kb, 7 kb, 6 kb, 5.4 kb, 4.6 kb, 4.4 kb, 3.4 kb, 3.1 kb, 2.8 kb, 2.6 kb, and 2.1 kb. Using appropriate cell hybrids, it should be possible to map these rodent  $\alpha$  interferon-related sequences.

### Discussion

Our analysis of a series of human/rodent somatic cell hybrids indicates that a cluster of human  $\alpha$  interferon genes resides on human chromosome 9. Similar results were recently reported by Owerbach et al. (20) using human/mouse hybrid cells; they localized at least eight of the  $\alpha$  interferon genes to chromosome 9. The  $\alpha$  interferon genes that have been cloned in *Escherichia coli* appear to lack introns, as does the  $\beta$  interferon gene also mapped to chromosome 9 (20).

The human  $\alpha$  interferon genes on chromosome 9 are probably closely linked because genomic fragments containing several  $\alpha$  sequences have been isolated (4, 21). Another class of  $\alpha$  interferons, whose mRNA sequences do not hybridize to an  $\alpha_1$ -related probe, has recently been described (22), increasing the complexity of the  $\alpha$  interferon system. It is not known whether this class of  $\alpha$  interferon sequences also maps to chromosome 9.

The  $\beta$  interferon gene(s) have been mapped to several chromosomes (10–14, 20); recent studies by Weissenbach et al. (23) and Sehgal and his colleagues (24, 25) have provided evidence for the existence of multiple  $\beta$  interferon mRNA species. In the former study, two cDNA sequences for  $\beta$  interferon were cloned and found not to cross hybridize. These results suggest that the  $\beta$  interferons may also constitute a multigene family, with its members possibly mapping to different chromosomes.  $\gamma$  interferon has not yet been purified or mapped to a particular chromosome.

Because the patterns of hybridization of the human  $\alpha_1$  interferon probe to mouse and Chinese hamster restriction fragments can be distinguished, we are also trying to map the mouse  $\alpha$ -like sequences. In a panel of mouse/Chinese hamster hybrid cells, whose chromosome constitution allows positive gene mapping to every mouse chromosome except 11 (because it is not retained in any hybrid examined), we failed to observe any mouse-specific hybridization (D'Eustachio and Ruddle, unpublished

data). Because the mouse interferons can be divided into  $\alpha$ ,  $\beta$ , and  $\gamma$  classes, it will be interesting to see whether murine interferon genes are organized as their counterparts are in the human genome.

### Summary

To determine the chromosomal location of the human  $\alpha$  interferon genes, we scored a series of human/rodent somatic cell hybrids for the presence of DNA sequences hybridizing to an  $\alpha_1$  interferon DNA probe. The presence of human chromosome 9 in a hybrid correlated with the presence of a family of  $\alpha$  interferon genes.

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