Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 8173–8177, December 1985
Immunology

Regulation of expression of the human interferon γ gene

(DNase I hypersensitivity/lymphokines/gene expression/repetitive DNA/acquired immunodeficiency syndrome-related virus)

KENNETH J. HARDY, B. MATIJA PETERLIN, ROBERT E. ATCHISON, AND JOHN D. STOBO*

Howard Hughes Medical Institute and Department of Medicine, University of California School of Medicine, San Francisco, CA 94143

Communicated by H. Sherwood Lawrence, July 29, 1985

ABSTRACT **DNA fragments isolated from a genomic clone of human γ interferon (*IFN-γ*) as well as IFN-γ cDNA were used to map potential regulatory regions of the *IFN-γ* gene by DNase I-hypersensitivity analyses. In nuclei from the human T-cell line Jurkat, which can be induced to express the *IFN-γ* gene, we observed a strongly hypersensitive site in the first intervening sequence that localized to the only intracistronic repeat element in the gene. DNase I mapping of Jurkat cells was compared to that of several other cell types, including B cells, macrophages, and epithelial cells. The presence of strong intronic hypersensitivity was found only in cells capable of expressing the *IFN-γ* gene. No hypersensitivity was found in the 3′ regions of the gene. Further, no hypersensitivity was observed when purified genomic DNA from Jurkat was analyzed, suggesting that DNA–protein interactions, and not simply DNA sequence alone, were responsible for DNase I hypersensitivity. The sequence AAGTGTAATTTTTTGAGTTTCTTTT, which is directly in the intronic hypersensitive area of *IFN-γ*, is 83% homologous to a nearly identical sequence in the 5′ flanking region of the interleukin 2 gene. In interleukin 2, the homologous sequence is about 300 base pairs upstream of that gene's promoter in an area of potential regulatory importance.**

Activation of normal resting T cells requires two signals. One signal involves recognition of antigen in conjunction with major histocompatibility complex gene products by the T-cell antigen receptor. Antibodies with specificity for this receptor and its associated structures (i.e., T3) can substitute for this signal (1–3). Another signal is provided by accessory cells and/or their secreted products. Phorbol esters can substitute *in vitro* for this. Using such signals to activate the human T-cell line Jurkat, we have studied the events which culminate in the expression of two human lymphokines, interleukin 2 (IL-2) and γ interferon (IFN-γ) (1, 2). The results of these studies show that appearance of IFN-γ and IL-2 mRNA transcripts requires both signals and further suggest that these signals may effect regulation of lymphokine gene transcription.

In order to further investigate this possibility, we designed experiments to identify regions of the *IFN-γ* gene which are potential sites of transcriptional regulation. Such experiments take advantage of the unique conformation of regulatory regions of genes in intact nuclei, which often makes them unusually sensitive, hypersensitive (HS), to pancreatic DNase I digestion. We observe a strongly HS site in the first intervening sequence of the *IFN-γ* gene. Furthermore, strong DNase I HS in this intron is seen only in cells capable of synthesizing IFN-γ transcripts. It is intriguing that sequences strongly homologous to this area are found in the 5′ flanking region of the related lymphokine, IL-2.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Isolation of Cells and Nuclei. Cell lines Jurkat (wild type and subclone E6-1.11), RPMI 4265, HL-60, and HeLa were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and penicillin/streptomycin/glutamine at 37°C in a humidified, 95% room air/5% CO_2 incubator. Cells were collected by centrifugation, and pelleted cells were washed twice in Dulbecco's phosphate-buffered saline at 4°C and suspended to 1–3 × 10^7 cells/ml in lysis buffer: RSB (0.01 M NaCl/0.01 M Tris·HCl, pH 7.4/3 mM $MgCl_2$), 0.5% Triton X-100, 0.5 mM EGTA, and 0.5 mM phenylmethylsulfonyl fluoride ($PhMeSO_2F$). The lysis suspension was homogenized with 10 strokes in a Dounce homogenizer with a tight-fitting pestle, layered over equal volumes of RSB/0.1 mM EGTA/0.1 mM $PhMeSO_2F$/1.5 M sucrose, and centrifuged at 3000 × *g* for 30 min to pellet purified nuclei. Supernatant was decanted, and the pelleted nuclei were overlaid with 2.3 M sucrose/RSB/0.1 mM EGTA/0.1 mM $PhMeSO_2F$ and stored at −70°C.

DNase I Digestion. Frozen nuclei were thawed at 4°C and suspended at 1.0 mg of nucleic acid/ml in digestion buffer (RSB/0.1 mM EGTA/0.1 M $PhMeSO_2F$) at 4°C. Nuclei were digested either for various times or for 3 min with various DNase I concentrations. Supplemental calcium and magnesium were added as described (4). A series of nuclear digests from undigested (lane 1 in Figs. 2 *A* and *B* and 3 *A–F*) to completely digested was established for every cell type. Digests were prescreened by ethidium bromide/agarose gel electrophoresis and comparably digested samples were treated with restriction endonuclease and electrophoresed under identical conditions as described (5). In each figure showing DNase I mapping, the far right lane shows the least detectable (or undetectable) hybridization signal. Digestions were stopped by the addition of 0.10 vol of 10% (wt/vol) Sarcosyl/0.5 M Tris·HCl (pH 8.0)/0.2 M EDTA/10 mg of Pronase per ml (Calbiochem). These samples were digested at 37°C overnight and then were adjusted to 2.5 M ammonium acetate (with 10 M stock) by gently pipeting with a large bore pipet. DNA was precipitated with 1 vol of room temperature isopropanol by slow tumble mixing, which solubilized ammonium salts and digested proteins. DNA was quantitatively recovered by centrifugation in a table top centrifuge at room temperature. DNA pellets were washed once with 2.5 M ammonium acetate in 80% (vol/vol) ethanol again by gently pipeting with a large-bore pipet. The samples were then centrifuged, were washed twice in 80% (vol/vol) ethanol, and were hydrated in sterile 1 mM EDTA in 0.01 M Tris·HCl (pH 7.4) overnight. DNA purified by this rapid, organic solvent-free technique is essentially free of proteins and hybridizable RNA and is virtually indistinguishable from organically extracted DNA.

Abbreviations: IFN-γ, γ interferon; IL-2, interleukin 2; HS, hypersensitive(ity); bp, base pair(s); kb, kilobase(s).

*Present address: Department of Medicine, The Johns Hopkins Hospital, Baltimore, MD 21205.

Molecular Probes and Fragment Isolation. *Escherichia coli* containing the plasmid pB9KB-1 (6) was the generous gift of Patrick Gray (Genentech, South San Francisco, CA). The plasmid was amplified and plasmid DNA was purified by CsCl as described (7). Fragments of the *IFN-γ* gene for use as molecular probes were isolated from the plasmid by using combinations of appropriate restriction enzyme digestions, agarose gel electrophoresis, and DEAE adsorption as described (7). Fragments were nick-translated just prior to hybridization to specific activities of 1–9 × 10^8 cpm/μg of DNA.

Digestion with Restriction Endonucleases, Electrophoresis, and Blotting. Purified genomic DNA samples were digested with either *Bam*HI, or *Pvu* II in medium core buffer (7) at 37°C overnight. Digests containing identical amounts of DNA (30 μg) were stopped by addition of 0.2 vol of 0.25% bromphenol blue/0.25% xylene cyanol/15% (wt/vol) Ficoll/0.6% $NaDodSO_4$/60 mM EDTA, heated 15 min at 65°C, then electrophoresed through 1.0% agarose in 40 mM Tris (pH 7.8)/20 mM sodium acetate/2 mM EDTA. Gels were depurinated 1 hr in 0.1 M HCl, denatured 1 hr in 0.5 M NaOH containing 1.5 M NaCl, and neutralized for 1 hr at 4°C with 2 vol of 2.5 M ammonium acetate. DNA was transferred (blotted) to Genetran (Plasco, Woburn, MA) filters by diffusion in 1.0 M ammonium acetate at 4°C overnight. Blots were rinsed in 6× SSPE [1× SSPE = 0.18 M NaCl, 10 mM sodium phosphate (pH 7.4), and 1 mM EDTA], air dried, and baked 2 hr at 80°C prior to hybridization.

Nucleic Acid Hybridizations. Blots were incubated 1–3 hr with 1 M NaCl containing 1% $NaDodSO_4$ at 65°C. ^{32}P-labeled probes (50 ng per filter) were denatured in 0.3 M NaOH, adjusted to 2.5 M ammonium acetate (with 10 M stock), and added to fully solubilized 65°C hybridization solution containing 10% (wt/vol) dextran sulfate, 1 M NaCl, 1% $NaDodSO_4$, and salmon sperm DNA (100 μg/ml). Blots were hybridized 12–24 hr at 65°C in special hybridization chambers (Hoefer, San Francisco) that enabled all steps, including prehybridization incubation, hybridization, and all wash steps to be efficiently performed in sequential fashion at 65°C within a perfusible, closed chamber.

Analysis of RNA Transcripts. mRNA from intact cells was isolated using guanidinium hydrochloride essentially as described (8). RNA was assayed by hybridization analysis (7) using IFN-γ, IL-2, and actin cDNA probes. Prehybridization, hybridization, and wash conditions were identical to those described above for Southern analyses. Jurkat cells were always screened for IL-2 by a biological assay (9) and for IFN-γ and IL-2 transcripts prior to DNase I digestion, to assure inducibility of these genes (data not shown).

Computer Sequence Analysis. An IBM PC with the Beckman Microgenie Sequence Analysis program was used to search recent primate, mammal, animal, prokaryotic, and viral data banks for homology to sequences in the *Hin*dIII/*Sac* I intronic region of the human *IFN-γ* gene. Fifty-base-pair (bp) entries of published *IFN-γ* sequences (6) were screened individually against all data banks. Program stringency was high enough to find only strong homologies—i.e., regions generally greater than 40 nucleotides in length with at least 75% homology or regions at least 15 nucleotides in length having even stronger homology.

RESULTS

The genomic sequence and detailed restriction map of human *IFN-γ* have been reported (6) and are shown in Fig. 1. Also shown is the restriction map of the genomic subclone pB9KB-1 and the appropriate fragments used as probes for DNase I HS mapping. Two restriction sites not previously reported, *Mst* II and *Sac* I, are also included in the genomic map.

To determine whether DNase I HS exists in the *IFN-γ* gene, we first used IFN-γ cDNA to probe *Bam*HI-digested DNA from Jurkat nuclei that had been digested with various amounts of DNase I. The results in Fig. 2*A* show the appearance of a 4.6-kilobase (kb) band as DNase I digestion proceeds. In this particular experiment, digestion was purposely incomplete to demonstrate the appearance of a strong 4.6-kb band at a time early in the digestion when no obvious loss of hybridization signal to the 8.6-kb fragment was observed. Several smaller fragments were seen only on long exposure.

Genomic fragments shown in Fig. 1 were used to more precisely map this site using the indirect end labeling approach described (10). Two fragments, *Sac* I/*Pvu* II and *Pvu* II/*Eco*RI, can be used as independent DNase I probes of 5′ and 3′ regions of the *IFN-γ* gene, respectively. Fig. 2*B* shows the results of hybridizing the *Sac* I/*Pvu* II probe to *Pvu* II digested DNA from Jurkat nuclei which were digested with DNase to completion. Again, a strongly DNase I HS site is observed early in digestion. It maps 1.6 kb on the 5′ side of the third intron *Pvu* II site or approximately 150 bp on the 3′ side of the intronic *Hin*dIII site. HS, thus, maps near the center of the repetitive fragment in the first intervening sequence. The map in Fig. 2*C* shows that the HS site defined with the cDNA probe localizes to the identical area. Using

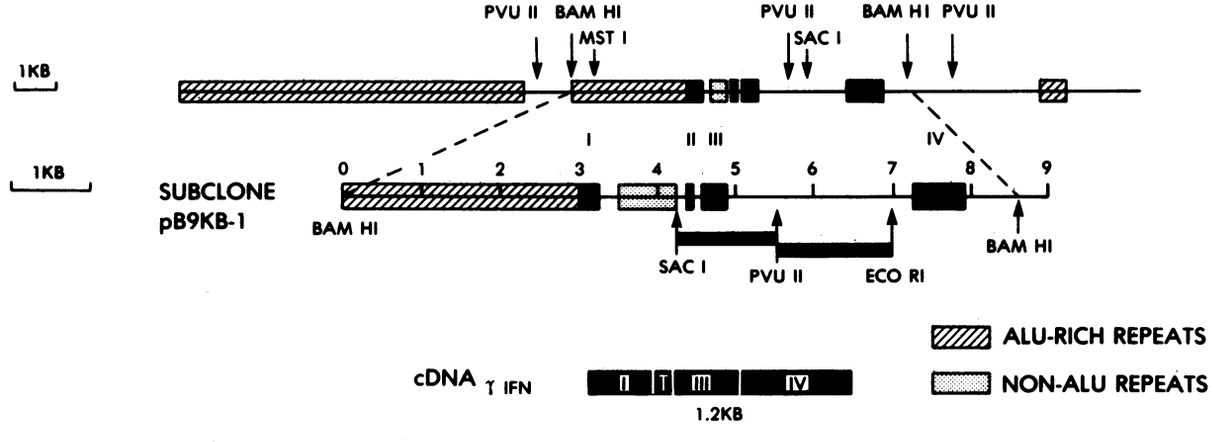

FIG. 1. Abbreviated restriction map of the human *IFN-γ* gene. Upper level shows genomic arrangement as described (6), and insert depicts subclone pB9KB-1 used to generate DNase I probes. Restriction fragments *Sac* I/*Pvu* II and *Pvu* II/*Eco*RI were used to map 5′ and 3′ areas of the gene, respectively. Repetitive elements are shown as hatched (*Alu*-rich), or stippled (non-*Alu*) boxes.

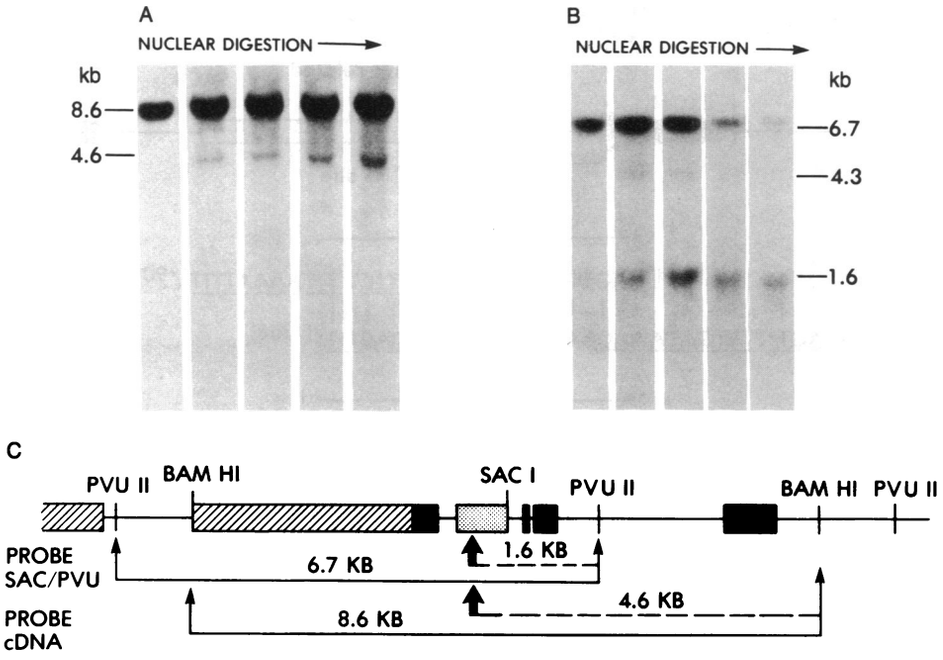

FIG. 2. Localization of DNase I hypersensitivity in the human *IFN-γ* gene. Jurkat nuclei were DNase I digested (increasing amounts of digestion are shown from left to right), and purified DNA was digested with restriction endonuclease, electrophoresed, blotted, and hybridized to appropriate probes. (*A*) DNase I profile of *Bam*HI-digested DNA hybridized to IFN-γ cDNA probe. (*B*) DNase I profile of *Pvu* II-digested DNA hybridized to the *Sac* I/*Pvu* II probe. (*C*) Localization of HS site (stippled box) by mapping from appropriate 3′ restriction site.

the *Pvu* II/*Eco*RI fragment as a probe (Fig. 1), no HS sites are found to the 3′ side of the internal *Pvu* II site (data not shown).

The data thus far localize a strongly HS site to the first intervening sequence of the *IFN-γ* gene in Jurkat cells. To determine if this correlated with *IFN-γ* expression, similar analyses were undertaken on a number of cells which differ in their ability to express *IFN-γ*. Wild-type Jurkat cells are a T-cell line readily inducible by two signals (phorbol esters and phytohemagglutinin) to express *IFN-γ* (1, 2). E6-1.11 cells, a spontaneous variant subcloned from Jurkat cells, have lost the ability to produce detectable IFN-γ or IL-2 transcripts (data not shown). Non-T cells neither express nor can be induced to express *IFN-γ* (11, 12). RPMI 4265 is an Epstein–Barr virus-transformed B-cell line, and HL-60 is a promyelocytic cell line, neither of which synthesizes IFN-γ transcripts (data not shown). HeLa cells are of epithelial origin and, thus, serve as a nonlymphoid cell control. Fig. 3 demonstrates striking differences in the availability of the intron 1 DNase HS site when Jurkat cells are compared to all other cell types. In Jurkat cells, we consistently found that the HS site is attacked earlier and much stronger during the course of the DNase I digestion. Fig. 3*F* shows the absence of HS when protein-free DNA is digested by DNase I. Thus, DNA sequence alone does not account for the HS observed.

In both Figs. 2*B* and 3, additional weak HS is detected as one or two faint bands that map 4.0 and 4.3 kb to the 5′ side of the intronic *Pvu* II site. These weak sites, which are seen in all cell lines, show no correlation with *IFN-γ* gene expressibility, and serve as internal references of HS strength. Because they are seen in samples not digested with exogenous DNase I, it is likely they result from endogenous nuclease activity and/or shear forces developed during nuclear isolation.

Computer scanning for stringent homology between sequences in the intronic HS region of *IFN-γ* and other genes revealed an unreported homology within the *IL-2* gene. The sequence AAGTGAATTTTTTGAGTTTCTTTT, which is 114 bp to the 3′ side of the intron 1 *Hin*dIII site in *IFN-γ* (directly adjacent to the area of strong HS), is 83% homologous to the sequence AAGAAAATTTTCTGAGTTACTTTT, located in the 5′ regulatory region of the human *IL-2* gene, ≈300 bp upstream of the promoter (Fig. 4). It was shown that in human lymphocytes, this exact region in the *IL-2* gene is also strongly HS (U. Seibenlist, personal communications).

Further scanning of sequences in the direct area of *IFN-γ* HS for homology to over 3000 animal, bacterial, and viral genes demonstrated that only three genes, human *IL-2*

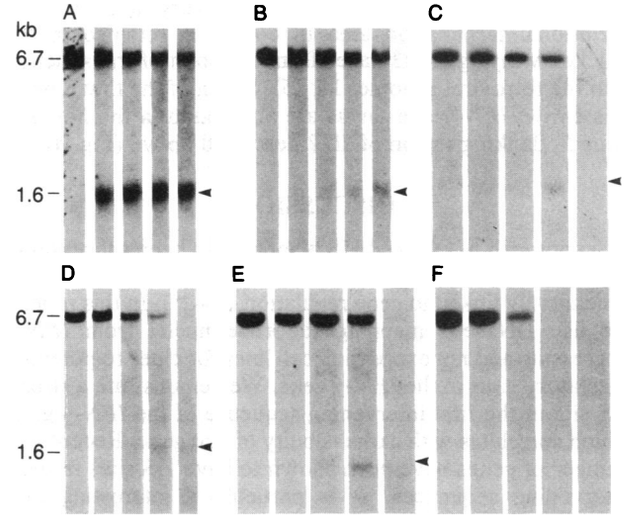

FIG. 3. Intronic HS in T-cell, B-cell, monocyte, and nonleukocyte cell lines. DNase I digestion as shown proceeds toward completion from left to right. Purified DNA from all cell lines was digested with *Pvu* II, electrophoresed, transferred to filters, and hybridized to the *IFN-γ* genomic probe, *Sac* I/*Pvu* II. (*A*) Jurkat cells (T cell). (*B*) Jurkat E6-1.11 cells (a non-IFN-γ expressing T-cell variant). (*C*) RPMI 4265 cells (B cell). (*D*) HL-60 cells (monocyte). (*E*) HeLa cells (nonleukocyte). (*F*) Purified Jurkat cell DNA.

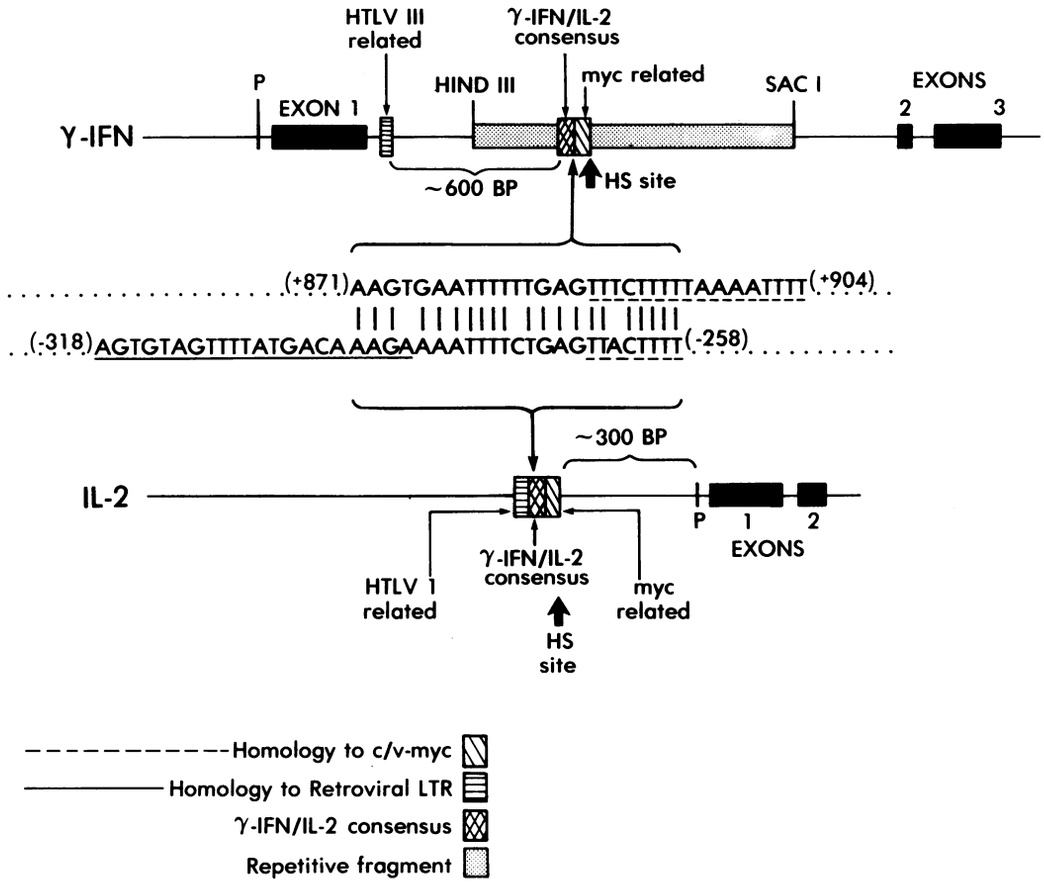

FIG. 4. Schematic localization and comparison of homologous sequences in the intronic HS area of *IFN-γ* and the 5′ flanking region of *IL-2*. P, promoter region.

(described above), chicken c-*myc*, and chicken v-*myc*, shared strong homology. The *IFN-γ* hypersensitive region sequence TTCTTTTAAAATTT, which is the short 3′ extension of the sequence described above (Fig. 4), is 100% homologous to a sequence in the chicken c-*myc* and v-*myc* genes (13). This same sequence, found very near the transcriptional termination area in c-*myc*, is retained in the chicken v-*myc* gene 13 bases from the point of transduction into the retroviral genome (14, 15). Seven of the first 8 bp of this c-*myc*/*IFN-γ* consensus are also found in the homologous 5′ flanking region of *IL-2* described above (Fig. 4).

DISCUSSION

The approach of DNase I HS mapping has been successfully used by many investigators to localize genomic regions functionally linked to gene regulation (16–25). In this report, we use DNase I mapping to probe nuclei from *IFN-γ* expressing and nonexpressing cell lines for clues to potential regulatory sites in the *IFN-γ* gene. We demonstrate a strong HS site in the first intervening sequence of the *IFN-γ* gene which correlates with expressibility of that gene. Recently, a number of genes have been shown to have HS sites in their intervening sequences. It is particularly interesting that several such genes, including those encoding the immunoglobulins μ or κ (21–24), *IFN-γ*, and *HLA-DRα* (unpublished results), have immunologic function. *IL-2* itself was shown to have several HS sites, at least one of which is found in the first intronic region (U. Seibenlist, personal communications). Interestingly, sequences homologous to known human and viral enhancers were recently demonstrated in the second intron of murine interleukin 3 (26).

Two observations support the notion that the intronic HS site noted in our study is strongly associated with regulation of *IFN-γ* gene expression. First, this site is less sensitive to DNase I digestion in all cells that do not express IFN-γ, yet strongly HS in Jurkat cells that can express the gene. Second, we now report strong homology between sequences in the intronic HS area of *IFN-γ* and the 5′ regulatory region of human *IL-2*, a related lymphokine gene. The evolutionary significance and functional role of these sequences as potential coordinate regulatory elements are still unclear.

It is of interest that in the *IL-2* gene the 5′ sequences, which are homologous to intronic HS sequences in *IFN-γ*, are contiguous with and partially overlapped by the 20-bp sequence known to share strong homology (85%) with the type I human T-cell leukemia (HTLV-I) viral long terminal repeat (27) (Fig. 4). Similarly, the sequence AAATGACTGAATATCGACTTGCT, which is known to be 82% homologous to a regulatory region (a long terminal repeat) in the acquired immunodeficiency syndrome (AIDS) retroviral genome (28), is found in the first intron of the *IFN-γ* gene, only 600 bases upstream of the HS site. This unusual combination of retroviral long terminal repeat homologies and shared lymphokine gene sequences residing in or near strongly HS regions of two potentially coregulated genes, *IL-2* and *IFN-γ*, is intriguing. Furthermore, T-cell activation is known to result in increased c-*myc* transcription (29). How this relates to the presence of c-*myc*/v-*myc* homologies in these same regions is still unanswered.

It is noteworthy that the intronic HS site of the human *IFN-γ* gene localizes to the only restriction fragment in the entire intracistronic region of that gene, which is known to contain repetitive sequences. More detailed computer analysis of sequences throughout this fragment is most revealing.

Regions 5′ from the HS area showed no homology to any genes in our banks. Regions within 50 bp of the immediate HS area showed homology to only the three genes described in the results. As one computer "walks" 50 bp to the 3′ side of the immediate area of HS, a family of genes, all human α interferons, are the only genes recognized (unpublished results). More to the 5′ side of this area one encounters a multitude of human, primate, bovine, rodent, and avian genes, having in common the alternating purine/pyrimidine oligonucleotide, CACACACACACACACA. This sequence is also found in several highly repetitive animal *alu*-like families that do not share homology with known human *alu* sequences. Some stretches of alternating purine/primidine have been demonstrated *in vitro* to form Z-DNA (30), which has been implicated in both structure and genetic regulation of eukaryotic chromatin (31).

In this report we describe striking sequence homology in potential regulatory regions of at least two genes, *IFN-γ* and *IL-2*, which often can be coordinately expressed (1, 2). It is possible that other eukaryotic genes share potential regulatory regions as well. Certainly this has been shown to be the case in some prokaryotic transcriptional systems (32). One might envision several immunologically related genes, coregulated by such sites. The implications of a single regulatory factor effecting multiple elements of an immune response are provocative. Accordingly, one might foresee immunologic dysfunction at multiple levels as a result of altered regulation of a single genomic locus.

Finally, it should be emphasized that strong intronic DNase I HS is seen in nonstimulated Jurkat cells when no IFN-γ transcripts are detectable. Two signal activations of Jurkat cells result in the appearance of IFN-γ transcripts without any detectable change in the character of the intronic HS site (unpublished results). One might, thus, expect to find a different site functionally correlated with stimulation of Jurkat cells. In fact, preliminary data in our laboratory suggest such a site appears ≈200 bp upstream of the *IFN-γ* promoter after phytohemagglutinin/phorbol ester stimulation. It is too early to speculate on the exact function of the *IFN-γ* HS sites during the activation of that gene by T-cell stimulation, but it is likely they represent specific areas of important genetic regulation. An elucidation of their precise structure and function remains as an exciting challenge for the future.

We thank Dr. Patrick Gray of Genentech, Inc., for providing us with the plasmid pB9KB-1. We also thank Dr. Bernard Manger for assistance with IL-2 assays, and Dr. Arthur Weiss for the Jurkat cell subclone, E6-1.11. Finally, we wish to thank Dr. Dan Cashman for assistance with computer sequence analyses. This work was supported by the Howard Hughes Medical Institute, Public Health Service Grant AI14104, and Arthritis and Metabolic Diseases Training Grant AM07304. B.M.P. is an Assistant Investigator of the Howard Hughes Medical Institute. J.D.S. is an Investigator of the Howard Hughes Medical Institute.

1. Weiss, A., Wiskocil, R. L. & Stobo, J. D. (1984) *J. Immunol.* **133,** 123–128.
2. Weiss, A., Imboden, J., Wiskocil, R. & Stobo, J. (1984) *J. Clin. Immunol.* **4,** 165–173.
3. Weiss, A., Imboden, J., Schoback, D. & Stobo, J. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4169–4173.
4. Siebenlist, U., Hennighausen, L., Battey, J. & Leder, P. (1984) *Cell* **37,** 381–391.
5. Becker, P., Renkawitz, R. & Schutz, G. (1984) *EMBO J.* **3,** 2015–2020.
6. Gray, P. W. & Goeddel, D. V. (1982) *Nature (London)* **298,** 859–863.
7. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
8. Cheley, S. & Anderson, R. (1984) *Anal. Biochem.* **137,** 15–19.
9. Gillis, S., Ferm, M. M., Ou, W. & Smith, K. A. (1978) *J. Immunol.* **120,** 2027–2032.
10. Wu, C. (1980) *Nature (London)* **286,** 854–860.
11. Kirchner, H. & Marcucci, F. (1984) in *Interferons and the Immune System*, eds. Vilcek, J. & De Maeyer, E. (Elsevier, Amsterdam), pp. 7–34.
12. Epstein, L. (1984) in *Interferons and the Immune System*, eds. Vilcek, J. & De Maeyer, E. (Elsevier, Amsterdam), pp. 185–219.
13. Watson, D. K., Reddy, E. P., Duesberg, P. H. & Papas, T. S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2146–2150.
14. Reddy, E. P., Reynolds, R. K., Watson, D. K., Schultz, R. A., Lantenberger, J. & Papas, T. S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2500–2504.
15. Alitalo, K., Bishop, J. M., Smith, D. H., Chen, E. Y., Colby, W. W. & Levinson, A. D. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 100–104.
16. Gindes, G. D., Whitters, M. J. & Pohlman, J. K. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3954–3958.
17. Fritton, H. P., Igo-Kemenes, T., Nowock, J., Strech-Jurk, V., Theisen, M. & Sippel, A. E. (1984) *Nature (London)* **311,** 163–165.
18. Burch, J. B. & Weintraub, H. (1983) *Cell* **33,** 65–76.
19. Groudine, M., Kohwi-Shigematsu, T., Gerlinas, R., Stamatoyannopoulos, G. & Papayannopoulou, T. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7551–7555.
20. Mercola, M., Wang, X.-F., Olsen, J. & Calame, K. (1983) *Science* **221,** 663–668.
21. Picard, D. & Schaffner, W. (1984) *Nature (London)* **307,** 80–82.
22. Mills, F. C., Fisher, L. M., Kuroda, R., Ford, A. M. & Gould, H. J. (1983) *Nature (London)* **306,** 809–812.
23. Parslow, T. G. & Granner, D. K. (1982) *Nature (London)* **299,** 449–451.
24. Banerji, J., Olsen, W. & Schaftner, W. (1983) *Cell* **33,** 729–740.
25. Gillies, S. D., Morrison, L., Oi, V. T. & Tonegawa, S. (1983) *Cell* **33,** 717–728.
26. Miyatake, S., Yokota, T., Lee, F. & Arai, K.-I. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 316–320.
27. Holbrook, N. J., Lieber, M. & Crabtree, G. R. (1984) *Nucleic Acids Res.* **12,** 5005–5013.
28. Starich, B., Ratner, L., Josephs, S. F., Okamoto, T., Gallo, R. C. & Wong-Staal, F. (1984) *Science* **227,** 538–540.
29. Kelly, K., Cochran, B. H., Stiles, C. D. & Leder, P. (1983) *Cell* **35,** 603–610.
30. Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G. & Rich, A. (1979) *Nature (London)* **282,** 680–686.
31. Nordheim, A., Pardue, M. L., Lafer, E. M., Moller, A., Stollar, B. D. & Rich, A. (1981) *Nature (London)* **294,** 417–422.
32. Lewis, B. (1983) *Genes* (Wiley, New York), pp. 246–251.