Molecular and Cellular Biology, Apr. 1985, p. 768–779 Vol. 5, No. 4
0270-7306/85/040768-12$02.00/0
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Two Distinct Families of Human and Bovine Interferon-α Genes Are Coordinately Expressed and Encode Functional Polypeptides

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Received 4 October 1984/Accepted 14 January 1985

The classical human interferon-α (HuIFN-α) gene family is estimated to consist of 15 or more nonallelic members which encode proteins sharing greater than 77% amino acid sequence homology. Low-stringency hybridization with a HuIFN-α cDNA probe permitted the isolation of two distinct classes of bovine IFN-α genes. The first subfamily (class I) is more closely related to the known HuIFN-α genes than to the second subfamily (class II) of bovine IFN-α genes. Extensive analysis of the human genome has revealed a HuIFN-α gene subfamily corresponding to the class II bovine IFN-α genes. The class I human and bovine IFN-α genes encode mature IFN polypeptides of 165 to 166 amino acids, whereas the class II IFN-α genes encode 172 amino acid proteins. Expression in *Escherichia coli* of members of both gene subfamilies results in polypeptides having potent antiviral activity. In contrast to previous studies which found no evidence of class II IFN-α protein or mRNA expression, we demonstrate that the class I and class II IFN-α genes are coordinately induced in response to viral infection.

Interferons are secreted proteins produced by the cells of most vertebrates in response to viruses or other agents and are characterized by their ability to induce an antiviral state in a variety of target cells (reviewed in reference 53). Interferons have been shown to modulate the activity of T and B lymphocytes, natural killer cells, macrophages, and other cells involved in the immune response and to regulate the growth of tumor cells and other proliferating cell types. Several types of interferons have been differentiated on the basis of cellular origin and biochemical and antigenic properties. Interferon-α (IFN-α) and interferon-β (IFN-β) represent the major interferons synthesized by leukocytes and fibroblasts, respectively, after treatment with viruses, double-stranded RNA, or other inducers. IFN-γ is produced by T lymphocytes that have been stimulated by mitogens or specific antigens. Despite many similarities in their biological functions, however, these three types of interferon are distinguished by profound differences in structure and gene organization.

Human IFN-α (HuIFN-α) is encoded by a family of at least 15 related genes (15, 17, 23, 26, 35, 36, 55, 59, 65). The HuIFN-α genes which have been characterized lack introns, and most appear to be functional, although the extent of expression varies greatly (15, 21). The members of this family are related by greater than 85% nucleotide homology in their coding regions. HuIFN-β and HuIFN-γ are specified by unique genes (11, 16, 19, 56). The gene for HuIFN-β lacks introns (9, 25, 38) and encodes a protein possessing 29% amino acid homology with HuIFN-α1, suggesting that IFN-α and IFN-β genes have evolved from a common ancestor (56). By contrast, the HuIFN-γ coding region is divided by three introns (18) and exhibits extremely limited amino acid homology with HuIFN-α (13, 19). Interestingly, although only a single HuIFN-β gene has been unequivocally identified, bovine IFN-β (BoIFN-β) is encoded by a family of five or more homologous, yet distinct genes (25a). Moreover, the size of the IFN-β gene family varies considerably among different mammalian species (25a, 67).

In this paper we present evidence that the IFN-α gene family is more diverse than previously shown. DNA sequence analysis of IFN-α genes isolated from a bovine library has revealed one set of IFN-α genes similar to previously characterized HuIFN-α genes (class I) and a novel family of IFN-α genes with distinct structural characteristics (class II). A HuIFN-α gene representing a subfamily of homologous class II human genes also has been isolated. Members of both IFN-α gene classes are induced by viruses and encode polypeptides with antiviral activity.

MATERIALS AND METHODS

Hybridization conditions and probes. Hybridizations were performed in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–5× Denhardt solution (10)–0.1% sodium dodecyl sulfate–0.1% sodium pyrophosphate–50 μg of sonicated denatured salmon sperm DNA per ml–10% sodium dextran sulfate, containing either 20 or 50% formamide for nonstringent or stringent conditions, respectively. After incubation at 42°C, filters were washed at room temperature in 2× SSC–0.2% sodium dodecyl sulfate (nonstringent) or at 42°C in 0.2× SSC–0.1% sodium dodecyl sulfate (stringent). ^{32}P-labeled probes were prepared as described by Taylor et al. (57). For the analysis of class I and II genes in bovine and human DNA at high stringency (see Fig. 4), the following fragments were used (each contained the mature coding region of the corresponding IFN-α gene): class I bovine, the 540-base-pair (bp) *Xba*I-*Ava*II fragment of pBoIFN-α_I1; class II bovine, the 598-bp *Eco*RI-*Xmn*I fragment of pBoIFN-α_{II}1; class I human, the 565-bp *Eco*RI fragment of pHuIFN-α_I2/1 *Bgl*II hybrid (62); class II human, the 390-bp *Xba*I-*Acc*I fragment of pHuIFN-α_{II}1 (see Fig. 1 for a description of the plasmids).

Construction and screening of phage libraries. For the isolation of BoIFN-α genes, high-molecular-weight bovine DNA was prepared from frozen pancreas by the method of

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Blin and Stafford (3) and partially digested with *Sau*3A. DNA fragments of 15 to 20 kilobases (kb) were enriched by preparative sucrose gradient centrifugation (28), ligated with a twofold molar excess of isolated arms of *Bam*HI-digested bacteriophage λ Charon 30A vector (45), and packaged in vitro into phage particles (12). After amplification of the library (28), approximately 500,000 recombinant phage were screened by the method of Benton and Davis (2) under conditions of low stringency with a radioactive probe prepared from a 565-bp *Eco*RI fragment containing the mature coding region of the HuIFN-α2/1 *Bgl*II hybrid interferon gene (62). The HuIFN-α_{II}1 gene was similarly isolated from a human fetal liver-bacteriophage λ Charon 4A library constructed by Lawn et al. (24) (kindly supplied by Richard Lawn), with a probe prepared from the 501-bp *Xba*I-*Bgl*II fragment encoding the mature HuIFN-β protein (16).

DNA sequence analysis. DNA sequences were determined as described by Maxam and Gilbert (29) or by subcloning DNA fragments into M13 mp8 and mp9 vectors (32) and using the dideoxy-chain termination method (47).

Preparation and analysis of virally induced RNA. Peripheral blood lymphocytes (2×10^9) were suspended at 4×10^6 cells per ml in RPMI 1640 containing 5% heat-inactivated fetal calf serum. Cultures were incubated in T-175 flasks (Becton Dickinson Labware), and induced cultures were treated with 25 hemagglutinating units of Newcastle disease virus (NDV; gift of K. Zoon, National Institutes of Health) or Sendai virus (provided by A. Bollon, Wadley Institutes) per 10^6 cells. After 6 h of incubation with virus, the cultures were treated with 0.05% EDTA, cells were harvested by centrifugation and washed once with ice-cold medium, and polyadenylated [poly(A)$^+$] RNA was prepared as described by Ullrich et al. (60). Formaldehyde gel electrophoresis of RNA and Northern blot analysis were carried out as described by Maniatis et al. (27). The peripheral blood lymphocyte cDNA library was constructed as described by Goeddel et al. (17), except that double-stranded cDNA was ligated into the λgt10 vector (22).

Construction of bacterial expression plasmids for IFN-α genes. The construction of plasmids placing the expression of mature HuIFN-α_I1 and HuIFN-α_I2 under the control of the *Escherichia coli trp* promoter has been described previously (17, 62). Similar constructions were made which placed the N-terminal amino acid residue of mature BoIFN-α_I1, BoIFN-α_{II}1, and HuIFN-α_{II}1 directly adjacent to an initiator methionine codon, ribosome-binding site, and *trp* promoter (Fig. 1) and are briefly described as follows. The BoIFN-α_I1 coding region contains an Fnu4H site (GCTGC) within the codon for the first amino acid of the mature protein (cysteine). After Fnu4H digestion, filling in at the Fnu4H site with Klenow fragment of *E. coli* DNA polymerase I, and *Pst*I digestion, a 92-bp fragment extending from the restored cysteine residue to amino acid 31 was recovered. This fragment was joined to a 1,440-bp partial *Pst*I-*Bam*HI fragment containing amino acid residues 32 to 166 of BoIFN-α_I1 and inserted into the *trp* expression vector, pΔRIsrc (30), between a blunt terminus (containing a ribosome-binding site, followed by an ATG codon) and a *Bam*HI terminus ending within the tetracycline resistance gene of this pBR322 derivative. An ATG initiation codon was placed in front of the BoIFN-α_{II}1 mature coding region by an alternative strategy, similar to that described for the expression of HuIFN-β (16). The synthetic deoxyoligonucleotide, 5′-CATGTGTGACTTGTCT-3′, which encodes an ATG, followed by amino acids 1 to 4 of mature BoIFN-α_{II}1, was prepared by the phosphotriester method (6). This fragment

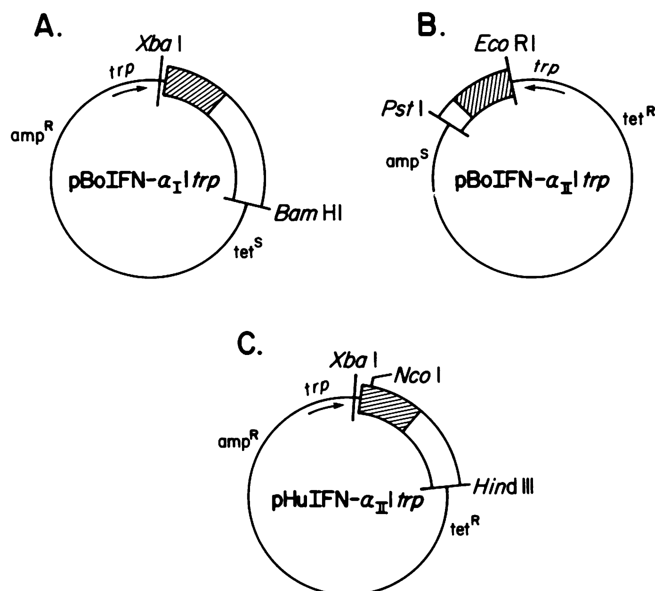

FIG. 1. Structure of plasmids directing the synthesis of mature IFN-α proteins in *E. coli*. The thick segment represents the IFN-α insert, with the mature coding region shaded. A, BoIFN-α_I1*trp*; B, BoIFN-α_{II}1*trp*; C, HuIFN-α_{II}1*trp*. Details of the construction of these plasmids are given in the text.

was phosphorylated with ATP and T4 polynucleotide kinase, annealed to the noncoding strand of a 319-bp *Hin*cII fragment containing amino acid residues S20 to 102 of BoIFN-α_{II}I, and enzymatically extended with Klenow fragment and dNTPs. After digestion with *Hgi*AI, the 181-bp blunt *Hgi*A fragment containing amino acids 1 to 60 was joined to a 508-bp *Hgi*A-*Pst*I fragment containing the carboxy-terminal portion of BoIFN-α_{II}1 and inserted into a *trp* expression vector, pHuIFN-γtrp69 (19), between a filled-in *Eco*RI site after the ribosome-binding site and a *Pst*I terminus ending within the ampicillin resistance gene of this plasmid. For the expression of HuIFN-α_{II}1, a synthetic DNA duplex containing an *Xba*I cohesive end, an ATG codon, and the codons for amino acids 1 to 7 of the mature protein and ending with an *Nco*I cohesive terminus was joined to the 1,290-bp *Nco*I-*Hin*dIII fragment containing the remainder of the HuIFN-α_{II}1 coding sequences and inserted into a *trp* expression vector, pHGH207-1 (8), between the *Xba*I and *Hin*dIII sites of this plasmid.

Detection of IFN-α antiviral activity in *E. coli*. Overnight cultures of *E. coli* K-12 294 transformed with the appropriate expression plasmids were grown, harvested, and extracted for antiviral assay as described by Goeddel et al. (16). Interferon activity was determined by cytopathic effect inhibition assays with either bovine kidney cells (MDBK) or human lung carcinoma cells (A549) obtained from the American Type Culture Collection, Rockville, Md. Vesicular stomatitis and encephalomyocarditis viruses were grown and used in the cytopathic effect inhibition assays as described previously (62). Values determined by this method are expressed as units relative to the NIH leukocyte standard G-023-901-527.

The antigenic identity of HuIFN-α_{II}1 was determined by previously published techniques (62). Antiserum prepared against HuIFN-β or Sendai-induced human leukocyte interferon was generously provided by Jan Vilcek, New York University, New York.

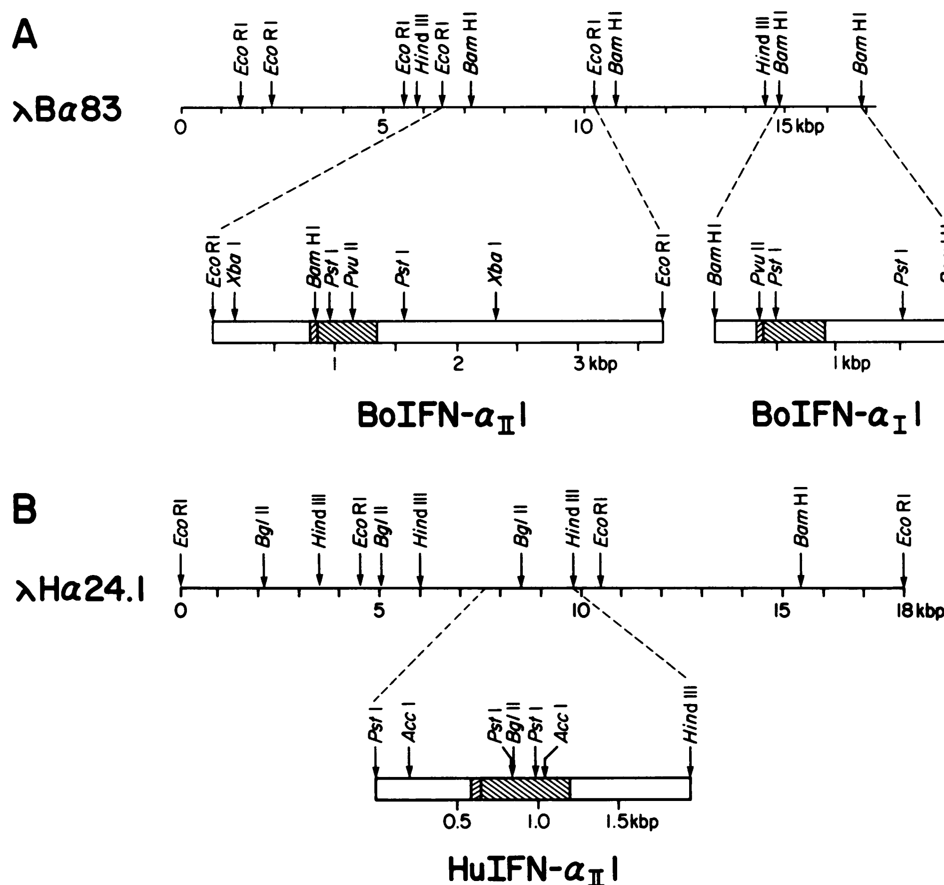

FIG. 2. Structures of phage recombinants containing the BoIFN-α_I1, BoIFN-α_{II}1, and HuIFN-α_{II}1 genes. (A) A restriction endonuclease map of the eucaryotic DNA insert of clone α83 which contains the linked BoIFN-α genes is shown. In the expanded scale the structures of the BoIFN-α_I1 and BoIFN-α_{II}1 genes are shown. The coding region is shown cross-hatched and is divided into the presequence (hatched left) and the mature coding regions (hatched right). (B) A similar restriction map of the HuIFN-α_{II}1 gene and flanking regions from the recombinant phage λ24.1 is shown.

RESULTS

Molecular cloning of two linked BoIFN-α genes. Southern blot analysis was used to establish hybridization conditions which would facilitate the detection of mammalian IFN-α genes by cross-species nucleotide homology with cloned HuIFN-α cDNA sequences. *Eco*RI-digested DNA from various mammalian species was electrophoresed through agarose gels, transferred to nitrocellulose paper, and hybridized with a radiolabeled HuIFN-α probe at several formamide concentrations to vary the stringency of annealing (see above). Under sufficiently nonstringent conditions, several hybridizing bands were observed in digests of bovine and other mammalian DNAs, suggesting that multigene IFN-α families similar to those found in the human genome are common to many animals and could be isolated by using this strategy.

Similar conditions were used to isolate the genes for BoIFN-α from a library of bovine genomic DNA constructed by inserting partial *Sau*3A-digested DNA fragments into a bacteriophage λ vector Charon 30A. In this manner 89 positive clones were recovered from a total of 500,000 clones screened, suggesting the presence of 15 to 20 IFN-α genes in the bovine genome. Six of the recombinant phage which were analyzed by restriction endonuclease mapping and blot hybridization appeared to contain nonoverlapping 15- to 20-kb segments of bovine DNA. One of these clones, λα83, consistently yielded two hybridizing fragments upon digestion with several restriction enzymes, suggesting either the presence of introns or the existence of two closely linked BoIFN-α genes. Restriction fragments containing sequences with homology to the HuIFN-α probe from both regions of clone λ83 as well as two other recombinant phage (clones λα35 and λα37) were subcloned into plasmid vectors for further characterization.

DNA sequence analysis revealed that the hybridizing regions found on these three phage each correspond to a complete BoIFN-α gene possessing significant homology with the class I HuIFN-α gene family. A restriction map of λα83 (Fig. 2A) shows the location and orientation of the two BoIFN-α genes within this phage recombinant. The nucleotide sequences of BoIFN-α_I1 and BoIFN-α_{II}1 (clone λα83) are compared in Fig. 3, whereas the sequences of BoIFN-α_I2 (clone λα35) and BoIFN-α_I3 (clone λα67) are very similar to BoIFN-α_I1 and will be presented elsewhere.

Structure of the BoIFN-α_I1 and BoIFN-α_{II}1 proteins. The predicted amino acid sequences of BoIFN-α_I1 and BoIFN-α_{II}1 are shown in Fig. 3. Each shares several important structural features with the class I HuIFN-α gene products. The four-amino acid sequence spanning the signal peptidase cleavage site of all class I HuIFN-α proteins, Ser-Leu-Gly/Cys, is also found in BoIFN-α_I1 and BoIFN-α_{II}1. The

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                                            BoIFN-αI1:   GAAAACAACGTTTGCGAAATGCTG   TCCTAACCCATTTGAAGAGTACAAACT GAAAAACAA
                                            BoIFN-αII1:  AAGTAATAGTTTAGACTGATGACAATTTCTTTGACCATATTCGGAATACATAAATGAAAATCAA
                                                         * ** ** **  * ****  ****** * ***   * ***  *   * ***    *

                                                                                                   ↓
AAACAAAAGTAGAAAGCAAGAGGGAACTTTCAGAAAATGGAAACCATGGACT  CCTATTTAA GACACAGACCTGAAGGAAGGTCTTCAGAGAACCTAGAAAGCAGGTTCACAGAGTC
AAAAGGAAGTACAAGTACACA GAAATGACTAGAAAATGAAAATTACTGTGTTCCCTATTTAATGGCCTTGCTTAGAAAGCATGGCATCAGAGAACCTACCTCAAGGTTCCACC AGAC
   ***    *  **** * * *  *****        *   ** ** ** **         * * *** ****   * * * * *                ********* *   **  *

                                     S1                                      S10                                    S20
                                     met ala pro ala trp ser leu leu leu ala leu leu leu leu ser cys asn ala ile cys
ACCCACCGCCCCAGGCCACAAGCATCTTCAAGGTCCCCG ATG GCC CCA GCC TGG TCC CTC CTC CTG GCT CTG CTG CTG CTC AGC TGC AAC GCC ATC TGC
GCTGTCTCAGCCAGCCCAGCAGCAGCCTCATCTTCCCC  ATG GCC TTC GTG CTC TCT CTA CTG ATG GCC CTG GTG CTG GTC AGC TAT GGC CCG GGA GGA
 * *** ****     *   **    * *   ***     *        phe val leu  *   *   * met  *      val     val     tyr gly pro gly gly

            1                                   10                                      20
ser leu gly cys his leu pro his ser his ser leu ala lys arg arg val leu thr leu leu arg gln leu arg arg val ser pro ser
TCT CTG GGC TGC CAC CTG CCT CAC TCC CAC AGC CTG GCC AAG AGG AGA GTC CTG ACA CTC CTG CGA CAA CTG AGG AGG GTC TCC CCT TCC
TCC CTG GGC TGT GAC TTG TCT CCG AAC CAC GTG CTG GTT GGC AGG CAG AAC CTC AGG CTC CTG GGC CAA ATG AGG AGA CTC TCC CCT CGC
  *           * asp *   ser pro asn     val     val gly     gln asn   * arg         gly     met       * leu         arg

        30                                      40                                      50
ser cys leu gln asp arg asn asp phe ala phe pro gln glu ala leu gly gly ser gln leu gln lys ala gln ala ile ser val leu
TCC TGC CTG CAG GAC AGA AAT GAC TTC GCA TTC CCC CAG GAG GCG CTG GGT GGC AGC CAG TTG CAG AAG GCT CAA GCC ATC TCT GTA CTC
TTC TGT CTG CAG GAC AGA AAA GAC TTT GCT TTC CCC CAG GAG ATG GTG GAG GTC AGC CAG TTC CAG GAG GCC CAG GCC ATT TCT GTG CTC
phe   *                 lys       *   *                 met val glu val         phe     glu   * gln       *       *

        60                                      70                                      80
his glu val thr gln his thr phe gln leu phe ser thr glu gly ser ala ala val trp asp glu ser leu leu asp arg leu arg thr
CAC GAG GTG ACC CAA CAC ACC TTC CAG CTT TTC AGC ACA GAG GGC TCG GCC GCT GTG TGG GAT GAG AGC CTC CTG GAC AGA CTC CGC ACT
CAT GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC CAC AAA GAG CGC TCC TCT GCT GCC TGG GAC ACT ACC CTC CTG GAG CAG CTC CTC ACT
  *     met leu   * gln ser     asn   *     his lys     arg   * ser     ala       * thr thr         glu gln     leu

        90                                      100                                     110
ala leu asp gln gln leu thr asp leu gln ala cys leu arg gln glu glu gly leu pro gly ala pro leu leu lys glu asp ser ser
GCA CTG GAT CAG CAG CTC ACT GAC CTG CAA GCC TGT CTG AGG CAG GAG GAG GGG CTG CCA GGG GCT CCC CTG CTC AAG GAG GAC TCC AGC
GGA CTC CAT CAG CAG CTG GAT GAC CTG GAT GCC TGT CTG GGC CTG TTG ACT GGA GAG GAA GAC TCT GCC CTG GGA AGG ACG GGC CCC ACA
gly   * his           * asp         asp             gly leu leu thr   * glu glu asp ser ala     gly arg thr gly pro thr

        120                                     130                                     140
leu ala val arg lys tyr phe his arg leu thr leu tyr leu gln glu lys arg his ser pro cys ala trp glu val val arg ala gln
CTG GCT GTG AGG AAA TAC TTC CAC AGA CTC ACT CTC TAT CTG CAA GAG AAG AGA CAC AGC CCT TGT GCC TGG GAG GTT GTC AGA GCA CAA
CTG GCC ATG AAG AGG TAC TTC CAG GGC ATC CAT GTC TAC CTG CAA GAG AAG GGA TAC AGC GAC TGT GCC TGG GAA ATC GTC AGA CTG GAA
      * met lys arg         gln gly ile his val   *       *         gly tyr     asp                 * ile         leu glu

        150                                     160                                     170
val met arg ala phe ser ser ser thr asn leu gln glu arg phe arg arg lys asp OP
GTC ATG AGA GCC TTC TCT TCC TCA ACA AAC TTG CAG GAG AGA TTC AGG AGA AAG GAC TGA CAC ACA CCT GGT TCA ACA CGGAAATGATTCTCACG
ATC ATG AGA TCC TTG TCT TCA TCA ACC AGC TTG CAA GAA AGG TTA AGA ATG ATG GAT GGA GAC CTG AAA TCA CCT TGA    CATGACTCTCACT
ile         ser leu       *       * ser       *   *   * leu   * met met   * gly asp leu lys ser pro OP  *****    *     *

GACCAACAGACCACACTTC          CTCCTGCGCTGCCATGTGGAA GACTC  ATTTCTGCTGTCATCAGGCACTGAACTGAATCAATTTGTTAAATGATTTCAG    GTATATTA
GACTAAGATGCCCCATCATCTTTGCACACTCATCTGTGGCCATTTCAAAAGACTCTGATTTCTGTTGTAGCCACAAAATTTATTGAATTACTTCAGCCAATACTTTGTCAGTAGTAAATGA
  *  * **  *  *************   * ** **    * **  *     **       *   ***  *** * ** *     * *  *****   **   *******   *  *

TGTGACATCATGATCTACT CTACAGGCACTACTCTGTCCCAG   ATACTCAAGCTAATCCATC   TACTTATTTATCTATTTGGTATTTATTTATCTAATT  TAATATTTATTTATC
ATATACATAAATTTTTTTGGCTGCAGGTGCATCAGTCCCGAAGTGAAGACTGCCCTTATTTTATTGTTTGCTTATTTATTTTGTTA TATTTATTCTTTTATTTCCTCATATTTATTTTTC
****    * *** * ****  *    ** ** ** ** **  *** *   *****  * **  **** *          * **  **         ** *  *  ** *         *

TATATATAAAGAATTAAATTATTTTGTTCATATAATT     ATGTATGTATAATTAATGGAAAAATATATTTTGTATTTAGTCAATTTATGAGTTTTCTTCATTCATTAAACCTTACTAT
CATATAAAATATTTTTGTTTACATTGTATTAAAATTTAACAAATACATTAACATTTT TATTTCATTATATTTGTAATTTGTTTTATTTATTAAATATTGTCAAGGTGAACTTCTTGAATT
*     *  ****  ***   **    **** * *  *****  **  ** * *  ** ***** *       ***     ** **        * ** * **   ****** ***   ****
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FIG. 3. Comparison of the BoIFN-α_{I}1 and BoIFN-α_{II}1 genes and polypeptides. The amino acid sequence of BoIFN-α_{I}1 is given above the nucleotide sequences. Amino acid residues differing in BoIFN-α_{II}1 are indicated below the sequence. The mature coding region of BoIFN-α_{II}1 is six amino acids longer than that of BoIFN-α_{I}1. Asterisks mark differences between the nucleotide sequences which do not result in a change of an amino acid residue. Potential polyadenylation signals (42) are underlined. The vertical arrow indicates the probable mRNA cap site by analogy to HuIFN-α sequences (35).

first 23 amino acids of each BoIFN-α protein should therefore comprise a signal peptide and as expected contain a core of approximately 12 hydrophobic residues. Each mature BoIFN-α protein would contain four cysteine residues, at positions 1, 29, 99, and 139. Cysteine residues are present at identical positions in each of the class I HuIFN-α proteins. These have been shown to form two specific disulfide bridges in HuIFN-α_{I}2 (Cys1/Cys99 and Cys29/Cys139) (66) and, on the basis of IFN-α sensitivity to sulfhydryl reducing agents, would appear to be crucial for antiviral activity (34). Although amino acid homology among species is considerably less (50 to 63%; Table 1) than that observed among individual members of the class I HuIFN-α family (77 to 94%; 15), certain regions are nonetheless highly conserved between the bovine and human IFN-α proteins, suggesting a functionally important role. For example, in the region

TABLE 1. Pairwise comparison of homology in coding regions of BoIFN-α and HuIFN-α genes[a]

Interferon	BoIFN-α_I1	BoIFN-α_{II}1	HuIFN-α_I1	HuIFN-α_{II}1
BoIFN-α_I1		68.3	77.6	65.1
BoIFN-α_{II}1	53.4		69.8	77.4
HuIFN-α_I1	62.4	54.0		70.0
HuIFN-α_{II}1	50.3	63.1	57.7	

[a] Shown are the percent amino acid sequence homologies between pairs of interferon preproteins (lower left) and the percent nucleotide homology between the corresponding coding regions (upper right). The six additional C-terminal amino acid residues, or 18 added nucleotides, of the class II interferons (Fig. 3 and 5) are not included in comparisons with the class I interferons.

between amino acid residues 115 and 151, the class I HuIFN-α proteins are identical at 30 of 37 positions (15). BoIFN-α_I1 and BoIFN-α_{II}1 encode the same amino acids as do the class I HuIFN-α proteins at 28 and 23 of these positions, respectively. In this region, HuIFN-β also exhibits the greatest similarity with the class I HuIFN-α proteins; 17 of 37 amino acids are conserved (46%), a level much higher than 29% overall human IFN-α/IFN-β homology.

In other respects, BoIFN-α_I1 and BoIFN-α_{II}1 differ strikingly from one another. Although the functional class I HuIFN-α proteins are highly conserved (77 to 94% amino acid homology; 15), BoIFN-α_I1 and BoIFN-α_{II}1 are identical at only 53% of their amino acid residues (Table 1 and Fig. 3). BoIFN-α_I2 and BoIFN-α_I3, by contrast, share >90% amino acid homology with BoIFN-α_I1 (unpublished data). Remarkably, the BoIFN-α_I1 coding region is more related to each member of the class I HuIFN-α subfamily than to BoIFN-α_{II}1. BoIFN-α_I1 and HuIFN-α_I1, for example, are 62% homologous (Table 1). The BoIFN-α_{II}1 gene is also distinct in that it encodes a mature polypeptide of 172 amino acids, whereas BoIFN-α_I1, BoIFN-α_I2, and BoIFN-α_I3, like most class I HuIFN-α proteins, are 166 residues in length. The six additional amino acids present in BoIFN-α_{II}1 are found at its carboxy terminus (Fig. 3).

The BoIFN-α_I1 and BoIFN-α_{II}1 genes define two distinct classes of IFN-α genes. The above results demonstrate that two significantly different types of IFN-α genes are found in the bovine genome. To determine the approximate number of bovine genes related to BoIFN-α_I1 and BoIFN-α_{II}1, Southern blot analysis was carried out on bovine DNA with probes derived from the coding regions of each gene. Conditions were selected to minimize cross-hybridization between the two types of BoIFN-α genes. To permit accurate alignment of the resulting hybridization patterns, a single DNA blot was first hybridized with the BoIFN-α_I1 probe, then washed extensively to remove all of the bound radioactivity, and rehybridized with the BoIFN-α_{II}1 probe. The results obtained with bovine DNA digested individually with several different restriction endonucleases are shown in Fig. 4A. Each probe detects a distinct set of bovine DNA fragments; few fragments, if any, appear to be common to both probes. Since the restriction endonucleases chosen for this analysis are not expected to cut often within the coding regions of these genes, and none of the BoIFN-α and HuIFN-α genes characterized contains introns, it is likely that most of the hybridizing bands observed represent a complete BoIFN-α gene. These results thus demonstrate the existence of two distinct classes of BoIFN-α genes. The BoIFN-α_I1 gene represents the class I BoIFN-α gene subfamily consisting of 10 to 12 members. The class I BoIFN-α subfamily, defined by the BoIFN-α_{II}1 gene, consists of 15 to 20 members.

Identification of a human IFN-α gene (HuIFN-α_{II}1) and a corresponding HuIFN-α gene subfamily related to the class II BoIFN-α gene subfamily. A human genomic recombinant containing a novel HuIFN-α gene displaying greater homology to BoIFN-α_{II}1 than to the class I HuIFN-α gene subfamily was isolated serendipitously in the course of searching for additional HuIFN-β genes. Previous reports

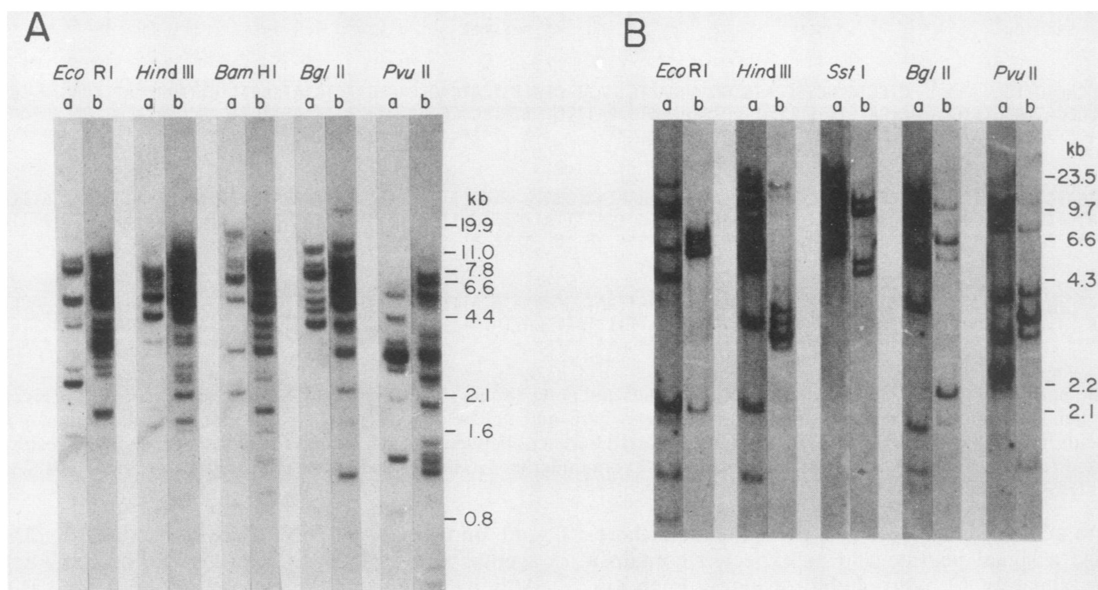

FIG. 4. Southern blot analysis of BoIFN-α and HuIFN-α gene families. High-molecular-weight bovine (A) or human (B) genomic DNAs (5 μg) were digested with restriction endonucleases as shown, electrophoresed through a 0.8% agarose gel, and subsequently transferred to nitrocellulose filter paper (52). Hybridizations were performed under stringent conditions (see the text) with probes derived from the following IFN-α coding regions: A, BoIFN-α_I1 (lane a) and BoIFN-α_{II}1 (lane b); B, HuIFN-α_I1 (lane a) and HuIFN-α_{II}1 (lane b). Molecular size standards correspond to *Eco*RI-digested bacteriophage λ Charon 30A and pBR322 DNAs and *Rsa*I-digested pBR322 DNA.

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HuIFN-αI1    TGAGAAACACGG    CTCTAAACTCATGTAAAGAGTGCATGAAGGAAAGCAAAAACAGAAATGGAAAGTGGCCCAGAAGCATTAAGAAAGTGGAAATCAG
HuIFN-αII1   T AGATTGTTGTCATCCTCTTAAGTCATAGGGAGAACACACAAATGAAAACAGTAAAAGAAACTGAAAGTACAG AGAAATGTTCAGAAAATGAAAACCA
              *   ***** *****    *  *    ****   ***  **  *    *  **  *     **      *****    ***  *     *  *   *  *

                                             ↓                                                                     S1
                                                                                                                  met ala
TATGTTCCCTATTTAAGGCATTTGCAGGAAGCAAGGCCTTCAGAGAACCTAGAGCCCAAGGTTCAGAG TCACCCATCTCAGCAAGCCCAGAAGTATCTGCAATATCTACG ATG GCC
TGTGTTTCCTATTAAAAGCCATGCATACAAGCAATGTCTTCAGAAAACCTAGGGTCCAAGGTTAAGCCATATCCCAGCTCAGTAAAGCCAGGAGCATCCTCATTTCCCA   ATG GCC
 *   *    *  *  ** ******    * *     *     * *      *  *** **    *    *  **    *  *   **  * ** * **

                             S10                                 S20         S23  1
ser pro phe ala leu leu met val leu val val leu ser cys lys ser ser cys ser leu gly cys asp leu pro glu thr his ser leu
TCG CCC TTT GCT TTA CTG ATG GTC CTG GTG GTG CTC AGC TGC AAG TCA AGC TGC TCT CTG GGC TGT GAT CTC CCT GAG ACC CAC AGC CTG
CTC CTG TTC CCT CTA CTG GCA GCC CTA GTG ATG ACC AGC TAT AGC CCT GTT GGA TCT CTG GGC TGT GAT CTG CCT CAG AAC CAT GGC CTA
leu leu   * pro *       ala ala   *     met thr  △  tyr ser pro val gly                     *     gln asn   * gly   *

10                                  20                                      30
asp asn arg arg thr leu met leu leu ala gln met ser arg ile ser pro ser ser cys leu met asp arg his asp phe gly phe pro
GAT AAC AGG AGG ACC TTG ATG CTC CTG GCA CAA ATG AGC AGA ATC TCT CCT TCC TCC TGT CTG ATG GAC AGA CAT GAC TTT GGA TTT CCC
CTT AGC AGG AAC ACC TTG GTG CTT CTG CAC CAA ATG AGG AGA ATC TCC CCT TTC TTG TGT CTC AAG GAC AGA AGA GAC TTC AGG TTC CCC
leu ser     asn         val   *     his         arg           *     phe leu       * lys         arg         * arg   *

40                                  50                                      60
gln glu glu phe asp gly asn gln phe gln lys ala pro ala ile ser val leu his glu leu ile gln gln ile phe asn leu phe thr
CAG GAG GAG TTT GAT GGC AAC CAG TTC CAG AAG GCT CCA GCC ATC TCT GTC CTC CAT GAG CTG ATC CAG CAG ATC TTC AAC CTC TTT ACC
CAG GAG ATG GTA AAA GGG AGC CAG TTG CAG AAG GCC CAT GTC ATG TCT GTC CTC CAT GAG ATG CTG CAG CAG ATC TTC AGC CTC TTC CAC
        met val lys   * ser     leu           * his val met                     met leu                 ser       · his

70                                  80                                      90
thr lys asp ser ser ala ala trp asp glu asp leu leu asp lys phe cys thr glu leu tyr gln gln leu asn asp leu glu ala cys
ACA AAA GAT TCA TCT GCT GCT TGG GAT GAG GAC CTC CTA GAC AAA TTC TGC ACC GAA CTC TAC CAG CAG CTG AAT GAC TTG GAA GCC TGT
ACA GAG CGC TCC TCT GCT GCC TGG AAC ATG ACC CTC CTA GAC CAA CTC CAC ACT GAA CTT CAT CAG CAA CTG CAA CAC CTG GAG ACC TGC
    glu arg   *           *     asn met thr                 gln leu his   *       * his       *     gln his *     * thr   *

100                                 110                                     120
val met gln glu glu arg val gly glu thr pro leu met asn ala asp ser ile leu ala val lys lys tyr phe arg arg ile thr leu
GTG ATG CAG GAG GAG AGG GTG GGA GAA ACT CCC CTG ATG AAT GCG GAC TCC ATC TTG GCT GTG AAG AAA TAC TTC CGA AGA ATC ACT CTC
TTG CTG CAG GTA GTG GGA GAA GGA GAA TCT GCT GGG GCA ATT AGC AGC CCT GCA CTG ACC TTG AGG AGG TAC TTC CAG GGA ATC CGT GTC
leu leu     val val gly glu         ser ala gly ala ile ser ser pro ala *   thr leu arg arg         gln gly     arg val

130                                 140                                     150
tyr leu thr glu lys lys tyr ser pro cys ala trp glu val val arg ala glu ile met arg ser leu ser leu ser thr asn leu gln
TAT CTG ACA GAG AAG AAA TAC AGC CCT TGT GCC TGG GAG GTT GTC AGA GCA GAA ATC ATG AGA TCC CTC TCT TTA TCA ACA AAC TTG CAA
TAC CTG AAA GAG AAG AAA TAC AGC GAC TGT GCC TGG GAA GTT GTC AGA ATG GAA ATC ATG AAA TCC TTG TTC TTA TCA ACA AAC ATG CAA
 *      lys                     asp             *               met             lys     * * phe                     met

160                 166
glu arg leu arg arg lys glu OC
GAA AGA TTA AGG AGG AAG GAA TAA TAT CTG GTC CAA CAT GAA AACAATTCTTATTGACTCATACACCAGGTCACGCTTTCATGAATTCTGTC      ATTTCAAA
GAA AGA CTG AGA AGT AAA GAT AGA GAC CTG GGC TCA TCT TGA AATGATTCTCATTGATTAATTTGCCATAATAACACTTGCACATGTGACTCTGGTCAATTCAAAA
        * *   * ser   * asp arg asp leu gly ser ser OP    **     *     * *  ***    **** ****  ***********  ******    **

GACTCTCACCCCTGCTATAACTATGACCATGCTGATAAACTGA
GACTCTTATTTCGGCTTTAATCACAGAATTGACTGAATTAGTTCTGCAAATACTTTGTCGGTATATTAAGCCAGTATATGTTAAAAAGACTTAGGTTCAGGGGCATCAGTCCCTAAGATG
     * *** *    *    ** ******  ***** ******************************************************************************

    TTTATCTATTTAAATATTTATT TAACTATTCATAAGATTTAAATTATTTTTGTTCATATAACGTCA TGTGCACCTTTACACTGTGGTTAGTGTAATAAAACATGTTCCTTATA
TTATTTATTTTTACTCATTTATTTATTCTTACATTTTATCATATTTATACTATTTATATTCTTATATAACAAATGTTTGCCTTTACATTGTATTAAGA TAACAAAACATGTTCAGCTTT
***** * * *  * * **         * *  **  *  *  *     * *     * *   *    ***** *  ***        *   ** *  **   *             **** *

TTTACTCAATCCATTATTTTGTGTTG   TTCATTAAACTTTTACTATAGGAACTTCCTGTATGTGTTTCATTCTTTAATATGAAATTCCTAGCCTGACTGTGCAACCTGATTAGAGAAT
CC ATTTGGTTAAATATTGTATTTTGTTATTTATTAAATTATTTTCAAACAAAACTTCTTGAAGTTATTTATTCGAAAACCAAAATCCAAACACTAGTTTTCTGAACCAAATCAAGGAAT
*** * *** ** *     * * *    ***  *       * *  *** * **  ** *  ** *  **  *    ****  ****  ******** ** ** ****     **  * **
                                                   △

AAAGGGTATATTTTATTTGCTTATCATTATTATATGTAAGA
GGACGGTAATATACACTTACCTATTCATTCATTCCATTTAC
** *    *** ** *  *  *  *** **** *** ****
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FIG. 5. Comparison of the nucleotide sequences of HuIFN-α_{II}1 with HuIFN-α_{I}1 and the proteins encoded by HuIFN-α_{II}1 with the members of the class I HuIFN-α_{I}1 gene family. Amino acid residues in HuIFN-α_{I}1 which are underlined represent those found in all of the class I HuIFN-α species reported to date (15, 50). Amino acid residues below the HuIFN-α_{II}1 DNA sequence indicate differences between HuIFN-α_{II}1 and HuIFN-α_{I}1. Asterisks mark changes in the nucleotide sequence which do not result in a change in the amino acid encoded. The polyadenylation signal (AATAAA) of HuIFN-α_{I}1 is overlined (15). The proposed polyadenylation signal (AATAAA) of HuIFN-α_{II}1 (see the text) is underlined. The vertical arrow indicates the mRNA cap site of HuIFN-α_{I}1 (35). The open triangles indicate the 5′ end and 3′ site of polyadenylic acid addition found for the longest HuIFN-α_{II}1 cDNA clone.

from two groups have suggested the existence of additional genes encoding HuIFN-β based upon the detection of differently sized poly(A)$^+$ RNA species derived from polyinosinic acid · polycytidylic acid-induced human diploid fibroblasts which direct IFN-β synthesis in *Xenopus* oocytes (49, 64). In an effort to identify these genes, a human genomic DNA library (24) was screened at low hybridization stringency with a probe prepared from a fragment spanning the mature

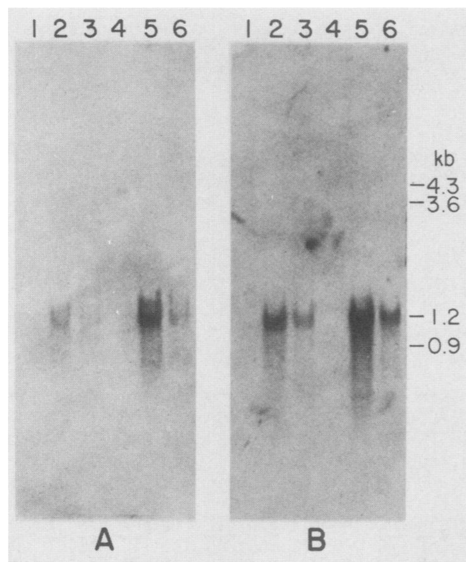

FIG. 6. Analysis of virally induced class I and class II IFN-α transcripts. Five-microgram samples of poly(A)$^+$ RNA were analyzed in each lane. Hybridizations with the class I (IFN-α_{I}2) probe (A) and the class II (IFN-α_{II}1) probe (B) are shown. Lanes 1, 2, and 3 are samples from donor 1, and lanes 4, 5, and 6 are samples from donor 2. Lanes: 1 and 4, uninduced controls; 2 and 5, induction by Sendai virus; 3 and 6, induction with NDV. The molecular size markers are derived from an *Eco*RI-plus-*Pst*I digestion of pLeIF A25 (3.6 and 0.9 kb; 15) and an *Eco*RI-plus-*Hin*dIII digestion of pHuIFN-α_{II}*trp* (4.3 and 1.2 kb; Fig. 1). The 0.9-kb pLeIF A25 fragment contains the IFN-α_{I}2 coding region, and the 1.2-kb pHuIFN-α_{II}*trp*1 fragment contains the IFN-α_{II}1 coding region.

coding region of the HuIFN-β gene. Of seven positive clones recovered by this screening procedure, four proved to contain the HuIFN-β gene by restriction mapping, Southern analysis, and hybridization at high stringency with the HuIFN-β probe. The remaining three recombinants appeared to represent overlapping segments of the human genome containing a distantly related gene which only hybridized to the HuIFN-β probe at low stringency. A 4.1-kb *Hin*dIII fragment containing the hybridizing region from one of these clones, λ24.1, was subcloned into pBR322 for further characterization by restriction endonuclease mapping (Fig. 2B) and nucleotide sequencing.

DNA sequence analysis of this fragment (Fig. 5) reveals an interferon gene exhibiting substantially more nucleotide homology in its coding region with the HuIFN-α genes (for example, 70% with HuIFN-α_{I}1; Table 1) than with the HuIFN-β genes (48%). Similarly, although the HuIFN-α_{I}1 protein and the gene product of $\lambda\alpha$24.1 each share only 30% amino acid homology with HuIFN-β, they are approximately 58% homologous with one another (Table 1). We conclude that the gene contained on $\lambda\alpha$24.1 encodes an IFN-α rather than an IFN-β.The sequence of this protein, however, is surprisingly dissimilar to the other HuIFN-α gene products.

Significantly, when the sequence of this unusual HuIFN-α is compared with the two types of BoIFN-α proteins (Table 1), it is found to be more homologous to BoIFN-α_{II}1 (63.1%) than to HuIFN-α_{I}1 (57.7%) and the other class I HuIFN-α proteins (data not shown). Furthermore, the novel HuIFN-α, like BoIFN-α_{II}1, contains six additional amino acids at its carboxy terminus, three of which are identical in each protein (Fig. 3 and 5). These similarities are reflected at the level of nucleotide homology as well (Table 1). Taken together, these observations strongly suggest that this novel HuIFN-α and BoIFN-α_{II}1 represent homologous gene products, distinct from the class I IFN-α subfamily which includes the previously sequenced functional HuIFN-α genes, as well as BoIFN-α_{I}1, BoIFN-α_{I}2, and BoIFN-α_{I}3. Accordingly, we have named this gene HuIFN-α_{II}1 to distinguish it from the class I HuIFN-α genes.

The HuIFN-α_{II}1 sequence contains a potential glycosylation sequence, Asn-Met-Thr, at positions 78 to 80. Interestingly, a similar sequence is found at the same position in HuIFN-β which is known to be modified in vivo by carbohydrate addition (20). Although glycosylation sites are absent from the other known HuIFN-α and BoIFN-α gene products, they have been recently found for a cloned mouse IFN-α gene (50).

The similarity between the HuIFN-α_{II}1 and BoIFN-α_{II}1 genes suggested that HuIFN-α_{II}1 might define a new subfamily of IFN-α genes in the human genome. To examine this possibility, blot hybridization analysis was performed with human genomic DNA with probes derived from the HuIFN-α_{I}1 and HuIFN-α_{II}1 coding region under stringent conditions which do not permit cross-hybridization of the two genes. The results of such an experiment (Fig. 4B) indicate that the HuIFN-α_{I}1 and HuIFN-α_{II}1 probes define distinct gene subfamilies. The HuIFN-α_{II}1 probe demonstrates the presence of six to seven class I genes in the human genome.

Expression of class II IFN-α genes is inducible by virus. The HuIFN-α_{II}1 protein or related class II IFN-α gene products have not been identified in interferon preparations from virally induced cell lines (1, 68), nor have the corresponding DNA sequences been found in cDNA libraries prepared from lymphoblastoid cell lines (15) or peripheral blood lymphocytes (36, 65) induced by viruses. To determine whether class II HuIFN-α genes are transcribed in response to virus infection, RNA from the peripheral blood lymphocytes of two donors induced with either Sendai or NDV was analyzed by blot hybridization. After a 6-h incubation with virus, poly(A)$^+$ RNA was isolated from the cultures, electrophoresed on formaldehyde gels, transferred to nitrocellulose filters, and hybridized with either a class I (HuIFN-α_{I}2) or class II (HuIFN-α_{II}1) probe. Transcription of both the class I (Fig. 6A) and class II (Fig. 6B) IFN-α genes is induced by both Sendai virus (lanes 2 and 5) and NDV (lanes 3 and 6) and is not detectable in uninduced cultures from either donor (lanes 1 and 4). To compare the levels of expression of the class I and class II genes, filters hybridized with each probe were exposed to film until DNA markers containing the coding regions of either HuIFN-α_{I}2 (Fig. 6A) or HuIFN-α_{II}1 (Fig. 6B) gave signals of equal intensity (data not shown). From this analysis it appears that the class II genes are transcribed at a level comparable to the class I genes (Fig. 6). In addition, it appears that Sendai virus induces severalfold more class I and II interferon message than does NDV, consistent with the recent report of Hiscott et al. (21). This suggests that the class I and class II genes are regulated similarly in response to viral infection.

To confirm the conclusion that the IFN-α_{II}1 gene is expressed, a complementary DNA library was constructed from poly(A)$^+$ RNA isolated from Sendai virus-induced peripheral blood lymphocytes (see above). A HuIFN-α_{II}1 coding region probe was used to screen 10,000 plaques under stringent hybridization conditions. Two HuIFN-α_{II}1 clones were recovered. DNA sequence showed that the longer of the two cDNA clones extended from the polyadenylic acid of the mRNA to within the sequence encoding the signal

peptide of HuIFN-α_{II}1. The corresponding sequence within the HuIFN-α_{II}1 gene is indicated in Fig. 5.

Class II IFN α-genes encode proteins with antiviral activity. To determine whether class II IFN-α genes encode active proteins and to compare their host range with those of class IFN-α polypeptides, bacterial vectors were constructed for the expression of BoIFN-α_I1, BoIFN-α_{II}1, and HuIFN-α_{II}1 genes. The resulting plasmids join a *trp* operon promoter, ribosome-binding site, and initiator methionine codon to the first amino acid residue to each mature IFN-α coding region (Fig. 1). Extracts prepared from *E. coli* strains transformed with each of the three plasmids and grown under conditions leading to depletion of tryptophan from the growth media contain significant amounts of antiviral activity as measured by cytopathic effect inhibition assays (Table 2). The relative activities of each IFN-α were compared with those of two class I HuIFN-α proteins, HuIFN-α_I2 (17, 55) and HuIFN-α_I1 (36, 62), on a bovine (MDBK) cell line challenged with vesicular stomatitis virus and on a human lung carcinoma (A549) cell line challenged with encephalomyocarditis virus. Although BoIFN-α_I1 antiviral activity is extremely specific for MDBK cells (Table 2), HuIFN-α_{II}1 and BoIFN-α_{II}1, like HuIFN-α_I2, show approximately equal activity on each cell type. The class II IFN-α proteins therefore appear to have specificities which overlap those of several class I IFN-α gene products in these as well as other cell lines (54, 62).

To further characterize the antiviral activity associated with HuIFN-α_{II}1, we examined the ability of antisera prepared against IFN-α and IFN-β to neutralize HuIFN-α_{II}1 activity. Anti-HuIFN-β did not significantly affect the activity of HuIFN-α_{II}1, whereas an antiserum prepared against Sendai virus-induced interferon from human leukocyte cultures did neutralize HuIFN-α_{II}1 antiviral activity (Table 3). Since the latter induction protocol has been shown to result primarily in the production of HuIFN-α rather than HuIFN-β, these results confirm the assignment of HuIFN-α_{II}1 to the IFN-α family, made on the basis of protein homology.

DISCUSSION

Two distinct classes of IFN-α genes. Previous studies have described a family of approximately 15 nonallelic human IFN-α genes, which are related by at least 85% nucleotide homology in their coding regions (15, 23, 26, 35, 59, 65). For clarity, these genes are referred to as the HuIFN-α_I subfamily. We have described here the isolation of a phage recombinant containing two closely linked BoIFN-α genes, BoIFN-α_I1 and BoIFN-α_{II}1, by low-stringency hybridization with a HuIFN-α cDNA probe. Each gene encodes a functional interferon polypeptide as determined by its ability to program the expression of antiviral activity in *E. coli*. Despite the close linkage of these two loci as well as evidence suggesting that each gene is functional in vivo, the nucleotide sequences of BoIFN-α_I1 and BoIFN-α_{II}1 are strikingly different. By using conditions which do not permit cross-hybridization of the BoIFN-α_I1 and BoIFN-α_{II}1 genes, Southern analysis of bovine genomic DNA with probes derived from each gene demonstrates that BoIFN-α_I1 and BoIFN-α_{II}1 define two distinct classes of IFN-α genes. The class represented by BoIFN-α_I1 contains approximately 10 to 12 members, and DNA sequence analysis has shown that the products of two of these genes, BoIFN-α_I2 and BoIFN-α_I3, are 95% homologous to BoIFN-α_I1. Remarkably, BoIFN-α_I1, BoIFN-α_I2, and BoIFN-α_I3 display greater homology in their coding and flanking regions to the members of the class I HuIFN-α_I gene subfamily than to the BoIFN-α_{II}1 gene (Table 1).

In an effort to determine whether IFN-α genes similar to BoIFN-α_{II}1 exist in the human genome, we identified such a gene, HuIFN-α_{II}1, among a collection of clones initially isolated by screening a human genomic library with a

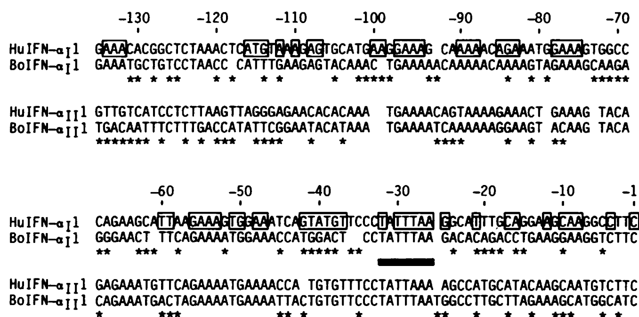

FIG. 7. Comparison of the 5'-flanking regions of the class I and class II IFN-α genes. A region corresponding to -1 and -135 bp upstream from the mRNA cap site (see arrows in Fig. 3 and 5) of HuIFN-α_I1 (35) is compared for the class I and class II IFN-α genes. The sequences are aligned to maximize homology. Asterisks indicate nucleotide mismatches between each pair of class I or class II genes. Boxed sequences include the nucleotides conserved in other characterized class I HuIFN-α genes (43). The Goldberg-Hogness sequences (M. Goldberg, Ph.D. thesis, Stanford University, Stanford, Calif., 1979) observed in genes transcribed by RNA polymerase II are underlined.

TABLE 2. IFN-α activity in extracts of *E. coli*[a]

E. coli 294 transformed by:	IFN-α activity (U/liter of culture)		
	A549-EMC	MDBK-VSV	A549/MDBK ratio
pBoIFN-α_I1	1.7×10^4	5.7×10^7	0.0003
pBoIFN-α_{II}1	1.6×10^6	1.8×10^6	0.89
pHuIFN-α_I1	2.5×10^5	1.9×10^6	0.13
pHuIFN-α_I2	1.4×10^8	1.3×10^8	1.1
pHuIFN-α_{II}1	5.0×10^5	4.7×10^5	1.1

[a] Interferon antiviral activities on human and bovine cells. *E. coli* K-12 (strain 294) cultures containing the appropriate expression plasmids were grown, and lysates were prepared for interferon bioassay as described by Goeddel et al. (16). Interferon antiviral activity in the lysates was determined in a cytopathic effect inhibition assay with MDBK (bovine kidney) cells and vesicular stomatitis virus (VSV) or A549 (human lung carcinoma) cells challenged with encephalomyocarditis virus (EMC). Results are expressed as the amount of antiviral activity per liter of bacterial culture grown to an optical density of 1.0 at 550 nm.

TABLE 3. HuIFN-α_{II}1 activity is neutralized by antibody to HuIFN-α[a]

Interferon	Interferon activity (U/ml)		
	Control	+Anti-IFN-α	+Anti-IFN-β
HuIFN-α_I2	64	<4 (>16)	32 (2)
HuIFN-α_{II}1	384	<4 (>96)	192 (2)
Natural HuIFN-α	128	<4 (>32)	ND
Natural HuIFN-β	64	ND	<4 (>16)

[a] Interferon was prepared and assayed as described in the legend to Table 2. Anti-IFN-α was prepared by immunization of a rabbit with natural HuIFN-α purified as described by Cantell et al. (5). Anti-IFN-β was prepared by immunization of a calf with natural HuIFN-β purified by the method of Van Damme and Billiau (61). Numbers within parentheses give the fold reduction in antiviral activity after treatment with antisera. ND, Not determined.

```
                               CAGGT             A   AA
                                 \/              \/  \/
                                                                                                                        Val
IFN-αM        CC TTG AGG AGG TAC TTC CAG GGA ATC CGA GTC TAC CTG AAA GAG AAG AAA TAC AGT GAC TGT GCT TGG GAG GTT GTC AGA GTG GAA -TC
IFN-αII1    ACC TTG AGG AGG TAC TTC CAG GGA ATC CGT GTC TAC CTG AAA GAG AAG AAA TAC AGC GAC TGT GCC TGG GAA GTT GTC AGA ATG GAA ATC
                                                 *                                   *           *       *               *       *
            Thr Leu Arg Arg Tyr Phe Gln Gly Ile Arg Val Tyr Leu Lys Glu Lys Lys Tyr Ser Asp Cys Ala Trp Glu Val Val Arg Met Glu Ile
                120                                     130                                     140

                        Phe Ser Ser         Asp Leu                                 Glu                 Ala
IFN-αM      ATG AAA TCC TTC TCT TCA TCA ACA GAC TTG CAA G-- -GA CTG AGA AGT AAG GAT GAA GAC CTG GGG TCT GCT TTA GTCTTTCTTATTTCTTCC
IFN-αII1    ATG AAA TCC TTG TTC TTA TCA ACA AAC ATG CAA GAA AGA CTG AGA AGT AAA GAT AGA GAC CTG GGC TCA TCT TGA AATGATTCTCATTGATTAA
                          *  **  *          *   *        ** *               *       **              *   *  *    *  ***** ** **  ** **
            Met Lys Ser Leu Phe Leu Ser Thr Asn Met Gln Glu Arg Leu Arg Ser Lys Asp Arg Asp Leu Gly Ser Ser OP
                150                                     160                                     170
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FIG. 8. The HuIFN-αM pseudogene is closely related to HuIFN-α_{II}1. The DNA sequence reported by Ullrich et al. (59) is compared with a 3′ portion of the HuIFN-α_{II}1 nucleotide sequence corresponding to the region from amino acid 119 (Fig. 5) through the stop codon and then extending into the 3′-untranslated region of HuIFN-α_{II}1. Sequences written above the HuIFN-αM sequence represent insertions with respect to HuIFN-α_{II}1. Amino acid residues encoded by the HuIFN-αM pseudogene are given where they differ from HuIFN-α_{II}1. Dashes indicate deletions in the HuIFN-αM sequence which were introduced to maximize alignment with HuIFN-α_{II}1.

HuIFN-β cDNA probe. Comparison of DNA homologies, however, as well as antigenicity studies on the HuIFN-α_{II}1 protein expressed in *E. coli*, firmly establishes that this gene encodes an IFN-α rather than an IFN-β protein. HuIFN-α_{II}1 is significantly more homologous to BoIFN-α_{II}1 than to the class I HuIFN-α and BoIFN-α genes and, like BoIFN-α_{II}1, encodes a mature polypeptide of 172 amino acid residues. These results demonstrate the existence of two homologous, but distinct, classes of IFN-α genes in the human and bovine genomes.

Southern blot analysis of human DNA suggests that the class II HuIFN-α gene subfamily may contain as many as six or seven different members. As yet, none of the HuIFN-α genes isolated from cDNA and genomic libraries whose sequences have been reported contains an intact member of the class II HuIFN-α subfamily. Brack et al. (4) described the isolation of several clones containing sequences which hybridize weakly to class I IFN-α probes. Although all of the clones of this type that were sequenced have turned out to contain IFN-α pseudogenes (65), it is possible that several are more closely related to class II than class I IFN-α genes. Recent work in our laboratory indicates that several class II HuIFN-α members are pseudogenes (unpublished data).

The availability of the HuIFN-α_{II}1 gene sequence makes possible the identification of a previously characterized clone which contains a fragment of a human gene resembling a class II IFN-α. Ullrich et al. (59) determined the sequence of a portion of chromosome 9, cloned into Charon 4A, which included two closely related class I HuIFN-α genes. A 154-bp segment which possessed some homology to the other genes was found at one end of the insert of this recombinant. It was suggested that this pseudogene fragment, which contains multiple translational termination codons in all three reading frames, might have arisen from a gene intermediate in structure to HuIFN-α_I and HuIFN-β. The homology between the HuIFN-α_{II}1 gene and the sequence reported by Ullrich et al. (59), called HuIFN-αM, is shown in Fig. 8. It is apparent from this comparison that the HuIFN-αM pseudogene is a member of the class II HuIFN-α subfamily. Starting at the 5′ end of the fragment which corresponds to amino acid position 119 of mature IFN-α (Fig. 5) and extending to the stop codon of HuIFN-α_{II}1, greater than 85% nucleotide homology between HuIFN-αM and HuIFN-α_{II}1 is observed (Fig. 8). Beyond the termination codon of HuIFN-α_{II}1, the homology with HuIFN-αM abruptly disappears. By comparison, only 70% nucleotide homology exists between HuIFN-α_I1 and HuIFN-αM (data not shown). The isolation of a human IFN-α pseudogene similar to IFN-α_{II}M has also been reported by Revel (44).

The members of the class I HuIFN-α subfamily, as well as HuIFN-β, have been localized to chromosome 9 (40), whereas the gene for HuIFN-γ is found on chromosome 12 (37). Recent experiments indicate that most, if not all, members of the class II HuIFN-α subfamily are also located on chromosome 9 (unpublished data). Many of the class I HuIFN-α genes have been shown to reside within 5 to 10 kb of one another (4, 23, 59). Our results show that the BoIFN-α_I1 and BoIFN-α_{II}1 genes are similarly linked, demonstrating a common chromosomal location for class I and II BoIFN-α genes and suggesting that members of both classes may be interspersed along the chromosome.

Transcriptional control of class II IFN-α genes. Differential regulation of IFN-α mRNA levels has been suggested by the greater than 10-fold range in frequency with which individual HuIFN-α mRNA sequences are found in virally induced cell cultures (15, 21). In our efforts to compare transcriptional regulation of class I and II IFN-α genes, we used NDV or Sendai virus to induce interferon synthesis in human peripheral blood lymphocytes. Comparable levels of class I and class II mRNA were readily observed in poly(A)$^+$ RNA prepared from each culture after 6 h of viral induction (Fig. 6). In addition, Sendai virus appeared to induce severalfold more IFN-α transcripts of class II, as well as class I types, indicating similar transcriptional activation of either class of IFN-α gene by viral induction. In light of the observation that class II cDNA clones were not identified in libraries prepared from Sendai virus-induced cultures of human leukocytes (36, 65) or a human myeloblastoid cell line (15), this is the first evidence for the viral induction of class II IFN-α synthesis. This result has been further confirmed by the isolation of a complementary DNA clone which includes the 3′ end of the IFN-α_{II}1 mRNA and most of its coding region (Fig. 5).

Weidle and Weissmann (63) have shown that NDV induction of HuIFN-α_I sequences transfected into mouse L cells results from an increase in transcription initiation rather than from a reduction in mRNA turnover rates. The region responsible for viral induction of transcription has been localized by deletion analysis to a stretch of 117 bp preceding the RNA cap site of HuIFN-α_I1 (43). Since sequences required for virally induced transcription of class I and II IFN-α genes are likely to reside in this region, we compared the corresponding regions of a HuIFN-α and BoIFN-α gene of each type as shown in Fig. 7. This comparison is of

particular interest since the genes appear to be activated coordinately but are only 57% homologous in this region (Fig. 7). Blocks of sequence which are highly conserved are candidates for essential regulatory elements. One prominent feature within this purine-rich 117-bp region is the repetition of the sequence GAAA at positions −97, −78, −56, and −49 among the expressed, highly homologous class I IFN-α genes (21, 43). This sequence is found at similar positions in each class I and II HuIFN-α and BoIFN-α gene (Fig. 7), except at −78 of BoIFN-α_{II}1, where ACAA appears instead. Further studies will be required to determine the role of such sequences in the viral induction of the class I and class II IFN-α genes.

Evolution of IFN-α gene families. Previous estimates of the divergence time of the class I HuIFN-α genes have been based upon calculations which assume that divergence time is proportional to the accumulation of nucleotide differences in the coding region (41). Estimates based on changes at silent sites (33) or replacement sites (55, 65) suggest that the earliest class I HuIFN-α gene duplication could have occurred as recently as 25 to 30 million years (MY) ago, well after the separation of present-day mammalian species (7). As a consequence of such recent duplication events, it has been proposed that the IFN-α multigene family of humans is distinct in organization from that of other mammals (33). This conclusion must be reconciled, however, with the observation that many mammalian species possess multigene IFN-α families (unpublished data; 67). Alternatively, it has been suggested that the existence of multiple IFN-α genes preceded the divergence of mammals and that the apparent divergence among IFN-α members within a species is less than that among species as a result of unequal crossing over or gene conversion which serves to homogenize the genes (14, 50, 65).

The available evidence suggests that such mechanisms have operated on the class I HuIFN-α genes. For example, in an unexpectedly high number of instances, two or more class I HuIFN-α genes differ from the remaining class I genes at a given amino acid or nucleotide position by an identical change (14, 65). In addition, two class I HuIFN-α genes have been characterized which do not differ in their coding regions while exhibiting completely different restriction maps in their 5′ and 3′ regions (unpublished data; 58). Similar segmental gene conversion events between nonallelic duplicated genes have been observed for γ-globin genes (51), α-globin genes (48), mouse γ2a immunoglobulin genes (39), and mouse H-$2k^b$ histocompatibility genes (31).

An important point suggested by comparisons of the class I and class II IFN-α genes is that despite close similarities in the sequences of these genes, their common and possibly interspersed chromosomal location, and the apparently frequent gene conversion events which have occurred among members of the class I HuIFN-α subfamily, the class I and class II IFN-α genes have retained distinct identities within the human, bovine, and perhaps many other mammalian genomes. Table 4 shows the percent nucleotide divergence at replacement and silent sites within the coding regions of the class I and II BoIFN-α and HuIFN-α genes calculated by the method of Perler et al. (41). The extent of divergence reported here between human and bovine class I genes (Table 4) is similar to that between murine IFN-α_I1 and HuIFN-α_I1 (22.5% at replacement sites and 48.9% at silent sites) reported by Shaw et al. (50). If the mammalian species diverged 85 MY ago (46), then a unit evolutionary period (the time required for 1% change in replacement sites) of 2.81 and 2.47 MY can be estimated for the class I and class II IFN-α genes of the human and bovine genomes, respectively. This rate of sequence change suggests that class I and class II IFN-α genes diverged 116 to 132 MY ago. Most mammals should therefore maintain the two distinct IFN-α gene families that we have observed in the bovine and human genomes. Avian species, however, which diverged from mammals about 250 to 300 MY ago (67) may be expected to have a single IFN-α family. It will be of considerable interest to confirm the divergence time of the two IFN-α families by examining IFN-α genes from more distantly related vertebrates and to compare both the regulation of these two classes of IFN-α genes and the biological and physical properties of the polypeptides encoded by them.

TABLE 4. Percentage of corrected nucleotide divergence of class I and II IFN-α coding regions[a]

Nucleotide sequence	% of corrected divergence	
	Replacement sites	Silent sites
HuIFN-α_I1/BoIFN-α_I1	22.14	47.13
HuIFN-α_{II}1/BoIFN-α_{II}1	22.62	38.13
HuIFN-α_I1/HuIFN-α_{II}1	30.27	69.19
BoIFN-α_I1/BoIFN-α_{II}1	34.47	60.01

[a] The percentage of corrected divergence for each pair of nucleotide sequences was calculated by the method of Perler et al. (41). Nucleotide sequences were aligned as shown in Fig. 2 and 4. The last 18 nucleotides of the class II IFN-α genes (encoding amino acids 167 to 172) were compared with one another but were excluded in class I versus class II comparisons.

ACKNOWLEDGMENTS

We thank Jane Baldridge, Philip Hollingshead, and Wendy Berger for technical assistance and Colin Watanabe for programming the method of Perler et al. (41) for calculating nucleotide divergence on the VAX 11/780 computer. We also thank Jeanne Arch and Alane Gray for help in preparing the manuscript, the Genentech Organic Chemistry Department for deoxyoligonucleotide synthesis, and the Genentech Bioassay Group for performing interferon assays.

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