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Cloning and Expression of the Human Myeloid Cell Nuclear Differentiation Antigen: Regulation by Interferon α

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Abstract The human myeloid cell nuclear differentiation antigen (MNDA) is a protein of 406 amino acids that is expressed specifically in granulocytes, monocytes and earlier stage cells of these lineages. Degenerate oligonucleotides that could encode regions of MNDA amino acid sequence were used to amplify the MNDA cDNA sequence using the polymerase chain reaction. The amplified cDNA product was sequenced to confirm that it encoded the MNDA protein. It was then used as a probe to isolate five clones from a human bone marrow λgt10 cDNA library. A clone containing a 1,672 base pair cDNA insert was sequenced and found to encode the entire MNDA open reading frame, as well as 5′ and 3′ untranslated regions. The primary structure of the MNDA contains extensive regions of sequence similarity with the protein products of the interferon-inducible genes: 204 and interferon regulatory factor 2. In addition, a 12-base sequence matching the interferon-stimulated response element consensus sequence [GAAAN(N)GAAA] is located in the 5′ untranslated region of the MNDA cDNA. The 1.8 kb MNDA mRNA was detected only in cells that express the antigen and the level of MNDA mRNA was elevated in cells treated with either recombinant or natural interferon α. The MNDA mRNA was not induced by interferon α in cells that do not exhibit a constitutive level of the MNDA mRNA. The MNDA contains sequence motifs found in gene regulatory proteins. The expression and the primary structure of the MNDA indicates that it plays a role in the granulocyte/monocyte cell-specific response to interferon. © 1992 Wiley-Liss, Inc.

Key words: interferon-stimulated response element, polymerase chain reaction, nuclear protein, cDNA cloning, nucleotide sequence, Northern blots

The human myeloid cell nuclear differentiation antigen (MNDA) was discovered by immunoblot screening of nuclear antigens in the cell lines K562, KG-1, U937, and HL-60, which are used as a model of human myeloid cell differentiation (Goldberger et al., 1984, 1986). The hypothesis that nuclear proteins change during differentiation in concert with the regulation of stage- and lineage-specific gene expression provided the rationale for those early experiments. The MNDA was the only antigen detected in the HL-60 and U937 cell nuclei that was not in the less differentiated KG-1, KG-1a, and K562 cell nuclei (Goldberger et al., 1984). Later, the expression of the MNDA was found to be specific for the myeloid cell lineage (Goldberger et al., 1986). An analysis of acute leukemia cases confirmed the lineage- and stage-specific expression of the MNDA, and further suggested, that both the monoblast and the progranulocyte expressed the MNDA (Cousar and Briggs, 1990). Analysis of peripheral blood, bone marrow, and cases of leukemia demonstrated that once MNDA expression was initiated, it subsequently was maintained in all later stage cells, including the granulocytes and monocytes found in peripheral blood (Briggs et al., 1989). The expression of the MNDA in the granulocytic and monocytic lineages is consistent with their development from a common unique progenitor cell (Lubbert and Koeffler, 1988). The expression of the MNDA in HL-60 and U937 cells is also consistent with this conclusion, since both can be induced to differentiate to a mature granulocyte, or monocyte/macrophage cell, depending on the inducing agent (Laskin et al., 1990).

A number of other laboratories have identified nuclear proteins, based on their expression characteristics, which may play a specific role in

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myeloid cell differentiation (Chou et al., 1984; Epstein et al., 1987; Murao et al., 1985; Lord et al., 1990; Hromas et al., 1991). The patterns of expression of these proteins are different from the MNDA, and in most cases, sufficient molecular characterization has been completed to conclude that none are related to MNDA. However, each protein may play an important role in regulating differentiation and/or normal function of phagocytic cells. It is notable, as is also the case in lymphoid cell maturation, that very significant changes in cell phenotype are accompanied by few qualitative changes in nuclear proteins (Rabilloud et al., 1991).

Recently a monoclonal antibody directed against MNDA (Hudson et al., 1988) was used to affinity-purify the MNDA, from which 218 residues of sequence was determined (Burrus et al., 1992). The MNDA sequence showed a high level of similarity to the protein products of two interferon-inducible genes, 204 (Choubey et al., 1989) and the interferon response factor 2 (IRF2) (Itoh et al., 1989). The MNDA amino acid sequence and the similarity between regions of the MNDA and the mouse 204 gene protein product were used to design degenerate oligonucleotides for priming the polymerase chain reaction (PCR) amplification of an MNDA cDNA. First, one 954-base pair product was sequenced and found to encode 318 residues of MNDA sequence. Subsequently, the PCR product was used to select five clones from a normal human bone marrow cDNA library. One clone, containing a 1,672-base pair cDNA insert, provided the complete coding sequence of the MNDA gene and sequence from the 5′ and 3′ untranslated regions of the mRNA. Interferon α was found to elevate the level of the MNDA mRNA in MNDA-expressing cells.

MATERIALS AND METHODS

RNA and cDNA Preparation

Cells were exposed to immunoaffinity purified natural human interferon α (Interferon Sciences, Inc., New Brunswick, NJ), recombinant human interferon αA (Hoffmann-LaRoche, Inc., Nutley, NJ), for the times and concentrations specified. Total RNAs were isolated from proliferating cultures of K562, KG-1, KG-1a, U937, and HL-60 cells (Goldberger et al., 1986), from normal human bone marrow cells and from interferon-treated cells using the guanidinium, acidic phenol method described by Chomczynski and Sacchi (1987). PolyA+ mRNAs were selected by oligo(dT) cellulose chromatography, using an mRNA separator kit according to methods provided by the supplier (Clontech Laboratories, Inc., Palo Alto, CA). cDNAs were transcribed from U937 polyA+ and total RNAs with 200 units/μg RNA M-MLV reverse transcriptase (Gibco BRL, Gaithersburg, MD) primed with 200 ng random hexamers/μg RNA (Boehringer Manneheim, Indianapolis, IN) in the presence of 250 μM of each deoxynucleotide (dNTP). Second-strand synthesis was carried out by standard procedure (Sambrook et al., 1989), using RNase H and *E. coli* DNA polymerase I (Klenow, large fragment). At the termination of the reaction, the cDNAs were phenol-chloroform extracted and dialyzed to remove excess primers and products of less than 150 base pairs (bp) by centrifuging 3× with 2 ml sterile H_2O in Centricon 100™ microconcentrators (Amicon, Beverly, MA).

Polymerase Chain Reaction Amplification of cDNAs

Deoxyoligonucleotides were synthesized on an automated system (Applied Biosystems, Foster City, CA) with subsequent purification using Opec™ cartridges (Clontech). A Perkin-Elmer Cetus DNA Thermal Cycler was used to amplify specific cDNA fragments by the polymerase chain reaction (PCR). An MNDA-specific cDNA fragment of 954 bp was initially amplified from heterogeneous U937 cDNAs prepared as described above. cDNAs synthesized from 150 ng polyA+ RNA or 600 ng total RNA were denatured and annealed to 800 nM of each degenerate oligo pool described in Table I in a 25 μl volume of 1× buffer under mineral oil by heating at 95°C for 3 minutes followed by cooling to 47°C. A 25 μl mixture of the remaining components was added to give final concentrations of 5 units Vent DNA polymerase (New England Biolabs, Inc., Beverly, MA), 250 μM each dNTP, and 5 μg BSA in a buffer consisting of 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH 8.8), 2 mM $MgSO_4$, 0.1% Triton X-100. The 50-μl final reaction mixture was programmed for 30 cycles of 94°C for 1 minute, 47°C for 2 minutes, and 72°C for 2 minutes with a final extension at 72°C for 7 minutes. A 10 μl aliquot of the reaction mixture was immediately assessed for amplified products by gel electrophoresis and ethidium bromide visualization. Samples selected for further analysis were chloroform inverted, phenol-chloroform extracted and purified by Centricon 100 dialysis, as described in

TABLE I. Degenerate Oligonucleotides Used in PCR Cloning a Partial 954-Base Pair MNDA cDNA Product

Oligonucleotide sequence	Degeneracy	MNDA amino acid sequence	204 gene protein product amino acid sequence
NF[a] ATGGTGAATGAA TACAAGAARAAT	(2X)	MVNEYKKI	MVNEYKRI
S19 CCCRCTTGCTTGY TTGTAAAGYTGAG	(8X)	SQLYKQASG	SYLFSQARG
12–23 AARGGITTYGAR YTIATGGAYGAY TACCAYTTYAC	(256X)	KGFELMDDYHFT	RGLECINKHYFS

[a]*NF*, *NH*$_2$*-terminal* forward primer site; S19, peptide fragment 19 reverse primer site; 12–23, MNDA-specific degenerate oligonucleotide probe.

the previous section. Reamplification of the 954-bp product was performed in the same manner using circa 0.5 ng of the purified original product as template.

The PCR amplification of the 532-bp product for probing restriction enzyme digests was prepared using a high titer lysate of a purified λgt10 clone. Approximately 10^8 pfu (10 μl) of the liquid lysate was diluted to 100 μl with sterile H_2O and boiled for 5 minutes. Twenty-five microliters of this preparation was added to an equal volume of a 2× concentrated mixture of the PCR reaction components without enzyme. The mixture was heated at 95°C for 2 minutes and cooled to 50°C before the addition of the thermal polymerase. The primers were MNDA sequence-specific oligonucleotides encoding amino acids 207–215 (forward) and 394–401 (reverse). Amplification was carried out by programming 35 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 2 minutes, with a final 72°C, 7-minute extension. The product was assessed and purified as described for the 954-bp PCR product.

Gel Electrophoresis, Transfer, and Hybridization

DNA fragments generated by PCR or restriction enzyme digestion were separated by electrophoresis on 5% acrylamide, Tris-acetate EDTA mini-gels. Southern blots were prepared by equilibrating the gel in 20 volumes of water for 30 minutes prior to electro-transfer to activated nylon membranes (Zeta Probe, BioRad, Richmond, CA) in 0.05X TAE buffer for 15 minutes (Ishihara and Shikita, 1990). The membranes were denatured and neutralized after transfer and baked at 80°C for 30 minutes. Southern blots, probed with 5′ end-labeled oligonucleotides, were prehybridized and then hybridized at 45°C in 6X SSPE, 5X Denhardts, 0.2% SDS, and 100 μg/ml sheared, denatured salmon sperm DNA for 18–24 hours. The hybridized membranes were rinsed in 2X SSPE, 0.1% SDS for 15 minutes, 2X at room temperature, and then stringently washed at 50°C in the same solution for 15 minutes. Double-stranded PCR products labeled by the ramdon priming method were used as probes on Southern blots with prehybridization and hybridization at 63°C in 10% PEG, 1.5X SSPE, 2% SDS, 1% non-fat dry milk, and 25 μg/ml sheared, denatured *E. coli* DNA. After hybridization for 18–24 hours, the membranes were rinsed at room temperature as described above and stringently washed in 0.1X SSPE, 0.1% SDS for 20 minutes at 65°C.

RNAs were electrophoresed in 1% agarose, formaldehyde gels (Davis et al., 1986). Northern blots were prepared as described for Southern blots with the exception that the gel was soaked for an additional 30 minutes in 0.05X TAE buffer prior to electro-transfer (Ishihara and Shikita, 1990). A 5′ end-labeled human β-actin antisense oligo (27 mer, Clontech), used to standardize loading of polyA^+ samples derived from different sources, was hybridized to RNA blots using conditions identical to those used for oligo probes on Southern blots. The PCR 954-bp product labeled by the random priming method was hybridized to RNA blots at 42°C in 50% formamide, 0.25 M NaCl, 0.12 M Na_2HPO_4 (pH 7.4), and 7% SDS. Wash conditions were the same as described for PCR probes on Southern blots.

Synthetic oligonucleotides were 5′ end-labeled [γ^{32}P]ATP (> 7000 Ci/mmol, ICN) by T4 polynucleotide kinase. PCR products were labeled by incorporation of [α^{32}P]dATP (3,000 Ci/mmol, ICN) into transcribed strands using a commercial random priming kit (United States

Biochemical, Cleveland, Ohio). Probes purified by G-50 Sephadex exclusion routinely had specific activities of $> 10^9$ cpm/μg and were used at 10^6 cpm/ml in the hybridization solution.

Library Screening

A normal human bone marrow λgt10 cDNA library inserted at the EcoRI site (Clontech) was plated at a density of 1.2×10^4 pfu/100-mm plate. A total of 1.2×10^5 recombinants were screened by replicate lifts (Nytran filters, Amersham, Inc., Arlington Heights, IL) using the 954 PCR-amplified MNDA cDNA as a probe. Hybridization and washing conditions were the same as described for PCR probes on Southern blots.

M13 Subcloning

To obtain template for sequencing, both the 954-bp PCR product and the 1,672-bp λgt10 insert were subcloned into M13mp19. The PCR product was inserted into the HincII cloning site by blunt-end ligation as outlined by Huang and High (1990). A 10 μl ligation reaction containing 100 ng of DNA (70:1 molar ratio of insert:vector DNA) and 400 Units (6.0 Weiss Units) of T4 DNA ligase (New England Biolabs) was incubated for 18 hours at 23°C. The 1,672-bp MNDA cDNA insert in λgt10 was released by EcoRI digestion of purified phage DNA and re-ligated into the EcoRI site of M13. Conditions for the cohesive termini ligation were 200 ng DNA (3:1 molar ratio of insert:vector DNA) and 40 Units of T4 DNA ligase in a 10 μl reaction volume incubated at 15°C for 18 hours. Competent *E. coli* (DH5α) cells were transformed with the ligated DNA by a heat shock standard protocol (Sambrook et al., 1989) and plated with *E. coli* UT481 lawn cells. M13 transformants containing inserts were selected for MNDA sequence and orientation by probing duplicate lifts with sense and antisense oligonucleotides. Hybridization conditions were as described for oligonucleotide probes used on Southern blots.

Sequencing and Data Analysis

Purified single-stranded M13 template prepared by standard procedure (Sambrook et al., 1989) was sequenced by the chain-termination method of Sanger et al. (Sanger et al., 1977) using modified T_7 DNA polymerase (Sequenase Version 2.0, United States Biochemical, Cleveland, Ohio). Overlapping regions of sequence were obtained from both strands by using sequence-specific primers in addition to the M13 (−40 Universal) primer. Data were compiled and analyzed with the aid of the IntelliGenetics Suite Programs. The Swiss Protein (University of Geneva, Switzerland) and PIR (Protein Identification Resource of National Biomedical Research Foundation) data bases were searched for sequence similarities to the MNDA amino acid sequences using the IntelliGenetics Suite (release 5.4) (IntelliGenetics, Inc., Mountain View, CA).

RESULTS

Our previously reported sequence analysis of the purified MNDA (Burrus et al., 1992) showed extended regions of similarity to the interferon-inducible mouse 204 gene protein product (Choubey et al., 1989). Two short regions (<10 residues) of MNDA amino acid sequence, highly similar to regions of the mouse 204 gene product, were used to design the degenerate oligonucleotide primers NF (NH_2-terminal) and S19 (Table I). The appropriate mouse coding sequence was used where codon selection required making the minimal number of base changes to encode the amino acids in the MNDA sequence. A third oligonucleotide, 12–23, was designed to encode amino acids 12 through 23 of the MNDA NH_2-terminal sequence, which has very little similarity to the mouse 204 gene (Table I). After the PCR amplification reaction, the 12–23 oligonucleotide was used as a probe to distinguish between either a putative product of the MNDA cDNA or a homolog of the mouse 204 gene. The 12–23 oligonucleotide mismatches the mouse 204 sequence at 10 nucleotide positions.

A 954-bp PCR product (Fig. 1) was obtained from the U937 cell cDNA templates prepared from total RNA and polyA$^+$ RNA. Initially, the use of Taq polymerase produced a number of variably sized products, but only a 954-bp product hybridized to the 12–23 oligonucleotide probe (data not shown). The use of Vent polymerase with the same primers and template provided a single 954-bp product that hybridized specifically with the 12–23 oligonucleotide probe (Fig. 1). The 954-bp product was subcloned into M13 for sequencing. The sequence of the 954-bp PCR product revealed an open reading frame encoding for amino acid sequence identical to that obtained from the purified MNDA (Burrus et al., 1992). The 954-bp PCR product was labeled and hybridized with polyA$^+$ RNA from MNDA-expressing U937 cells (determined previously by

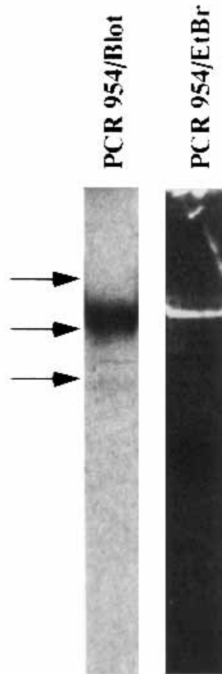

Fig. 1. Automated PCR amplification of a region of the MNDA cDNA. U937 cell cDNA, prepared from polyA$^+$ mRNA, was PCR-amplified using the NF and S19 oligonucleotides and Vent polymerase. The 954 product was electrophoretically transferred to a nylon membrane and probed with labeled 12–23 oligonucleotide. The 12–23 oligonucleotide hybridized specifically to the 954-bp product (blot). The 954-bp product was the major product amplified as determined by ethidium bromide (EtBr) staining. Markers (arrows) selected from ϕX174RF DNA, HaeIII fragments are 1352, 872, 603, and 310 bp.

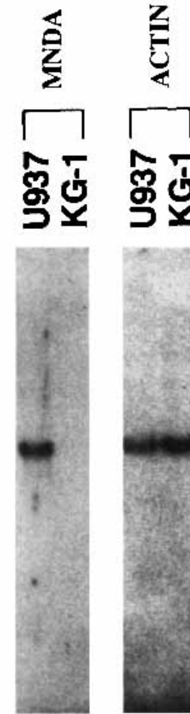

Fig. 2. Northern analysis of 2 μg of polyA$^+$ RNA from U937 cells and KG-1 cells. The labeled 954-bp PCR product was hybridized to the blot (left). After stripping the 954-bp PCR probe, the blot was re-hybridized with an end-labeled oligonucleotide complementary to human beta actin (right).

immunochemical analysis) and non-expressing KG-1 cells (Fig. 2). A 1.8-kb band of hybridization was observed with the 954-bp probe in U937 cells and no signal was observed in the KG-1 cell RNA (Fig. 2). After stripping the 954-bp probe from the blot, it was hybridized to an end-labeled oligonucleotide complementary to human β-actin (Fig. 2). The 1.9-kb β-actin mRNA was detected in the RNA from both cells, indicating that sample degradation or unequal sample loading does not account for the lack of MNDA mRNA signal in the KG-1 RNA (Fig. 2). A survey of total RNA from a number of human cell lines and bone marrow reveals that only cells exhibiting immunochemically active MNDA (HL-60, U937, and bone marrow), express the 1.8-kb mRNA (Fig. 3). Recently, THP-1 human monocytic cells were tested and found to express the MNDA mRNA as well (data not shown). These results demonstrated that the 954-bp product is a specific hybridization probe for the MNDA mRNA sequence.

The 954-bp product was used to screen a normal human bone marrow cDNA library in order to obtain clones with cDNA inserts representing the entire MNDA mRNA. Five clones were selected based on an intense signal from duplicate lifts. The largest EcoRI-released insert obtained from one clone was subcloned in both orientations into M13 and overlapping sequence was obtained from each strand (Fig. 4). The 1,672-bp MNDA cDNA contains a coding sequence for a 407 amino acid open reading frame and 202 bp of 5′ and 249 bp of 3′ untranslated regions (Fig. 5). All the MNDA amino acid sequence determined previously from isolated MNDA is identical to that encoded by the cDNA (Fig. 5). The sequence of the cDNA insert differs from the 954-bp PCR product (excluding primer sites) at one position (base 679 is a G in the PCR product), which did not alter amino acid coding. The 954-bp cDNA sequence originated from U937 cell RNA and the 1,672-bp cDNA insert originates from a normal bone marrow cell RNA. The one base difference between the two sequences could be due to variation between individuals or error associated with in vitro strand

synthesis. A restriction fragment analysis of all five of the originally isolated clones showed that they were derived from the same mRNA (Fig. 6). Purified λ DNA prepared from each clone was digested with both PstI and BamHI. A fragment of 532 bp was detected in all clones when hybridized to a 538-bp PCR product designed specifically to contain sequence within the restricted fragment (Fig. 6). A single fragment from the same region also was detected when the five clones were digested with MboI (data not shown).

Extended regions of sequence similarity (> 50% identical residues) exist between the MNDA and the protein product of the mouse 204 gene (Burrus et al., 1992) (Fig. 7). The longest region of similarity between MNDA and the 204 gene products extends over 200 amino acids (Fig. 7B). Although the 200-amino acid region is duplicated in the mouse 204 gene with a high level of conservation (Choubey et al., 1989), the 200-amino acid sequence motif is not duplicated in MNDA. The region between amino acids 67 and 179 (Fig. 7) of the MNDA shows no similarity to the 204 gene protein product. Within this region (residue 67 to 179) of the MNDA there are two highly basic regions. One basic, amphipathic, α-helical region (Fig. 5) shows similarity to the interferon regulatory factor 2, a protein that binds the interferon-stimulated response element (ISRE) in interferon-inducible genes (Fig. 7C). Also, a consensus ISRE element is located in the 5′ untranslated region of the MNDA mRNA (Fig. 5). The presence of this element is consistent with the observed similarity between the MNDA and the protein products of the two interferon-inducible genes. The detection of five S(T)PXX motifs in the MNDA indicates that the MNDA is a DNA-binding gene-regulatory protein (Suzuki, 1989). Eleven consensus phosphorylation sites were also located in the MNDA.

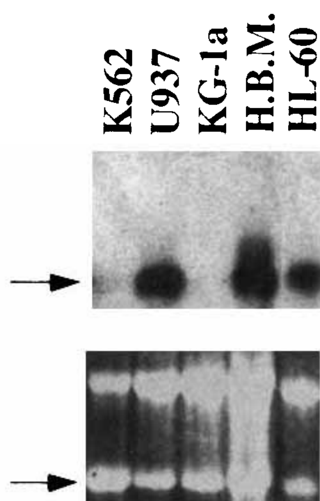

Fig. 3. Northern analysis of 20 μg of total RNA from myeloid cells. The labeled 954-bp PCR product was hybridized to the blot. A 1.8-kb band of hybridization (top arrow) was observed in the RNA derived from the MNDA expressing U937, HL-60, and bone marrow cells. The ethidium bromide staining of the 18S rRNA (bottom arrow) shows intact total RNA in all lanes.

U937 cells were exposed to recombinant human interferon α and harvested at various times (Fig. 8A). The level of MNDA mRNA was elevated at 6 hours after initiating the treatment. Concentrations of interferon α from 50–1,000 units/ml showed the same degree of mRNA elevation (data not shown). Natural human interferon α was also effective in elevating the level of MNDA mRNA in U937 cells (Fig. 8B). KG-1a cells which contain no detectable MNDA mRNA by Northern blot analysis (Fig. 3) could not be induced to express MNDA mRNA with interferon α (data not shown).

DISCUSSION

The characterization of isolated MNDA provided more than half the amino acid sequence (Burrus et al., 1992). That information was obtained from NH_2-terminal sequencing of the isolated protein and 12 peptide fragments. The isolated protein and some of the overlapping peptides were immunochemically active, thereby ensuring that the sequence originated from MNDA. The open reading frame of the 1,672-bp cDNA insert encodes the identical amio acid sequence obtained from the isolated MNDA and its peptides. In addition, a single 1.8 kb mRNA was specifically detected only in cells and tissue

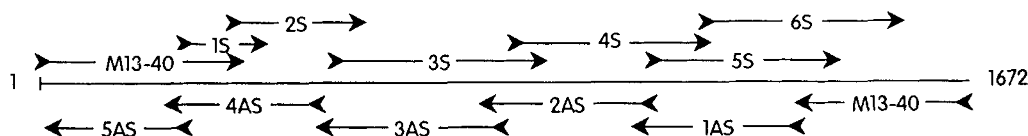

Fig. 4. Sequencing strategy for the 1672-bp MNDA cDNA in M13 subclones. Sense oligonucleotide primed sequences (S), antisense oligonucleotide primed sequences (AS), and M13 universal primed sequence (M13-40).

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CGGCTGAGAAATGAAAGCTAACAAGGAAAAATGGCACTTGTTAGAGCCACTCTCAGCCCTT   61
TACAAGATTAAAATAGTCTGCAGTTTAATCTCTCCAAAGCTTTACGGACAGTGATTCTGT  121
CCTAAACAAGACAGTGACTCCAGGATTTCTGAAGACTATTGTGGAAGAAGCATCCATTAA  181
GGCCAAGCTATAACATCAGAA ATG GTG AAT GAA TAC AAG AAA ATT CTT TTG CTG AAA GGA  241
                      Met Val Asn Glu Tyr Lys Lys Ile Leu Leu Leu Lys Gly
TTT GAG CTC ATG GAT GAT TAT CAT TTT ACA TCA ATT AAG TCC TTA CTG GCC TAT GAT TTA  301
Phe Glu Leu Met Asp Asp Tyr His Phe Thr Ser Ile Lys Ser Leu Leu Ala Tyr Asp Leu
GGA CTA ACT ACA AAA ATG CAA GAG GAA TAC AAC AGA ATT AAG ATT ACA GAT TTG ATG GAA  361
Gly Leu Thr Thr Lys Met Gln Glu Glu Tyr Asn Arg Ile Lys Ile Thr Asp Leu Met Glu
AAA AAG TTC CAA GGC GTT GCC TGT CTA GAC AAA CTA ATA GAA CTT GCC AAA GAT ATG CCA  421
Lys Lys Phe Gln Gly Val Ala Cys Leu Asp Lys Leu Ile Glu Leu Ala Lys Asp Met Pro
TCA CTT AAA AAC CTT GTT AAC AAT CTT CGA AAA GAG AAG TCA AAA GTT GCT AAG AAA ATT  481
Ser Leu Lys Asn Leu Val Asn Asn Leu Arg Lys Glu Lys Ser Lys Val Ala Lys Lys Ile
AAA ACA CAA GAA AAA GCT CCA GTG AAA AAA ATA AAC CAG GAA GAA GTG GGT CTT GCG GCA  541
Lys Thr Gln Glu Lys Ala Pro Val Lys Lys Ile Asn Gln Glu Glu Val Gly Leu Ala Ala
CCT GCA CCC ACC GCA AGA AAC AAA CTG ACA TCG GAA GCA AGA GGG AGG ATT CCT GTA GCT  601
Pro Ala Pro Thr Ala Arg Asn Lys Leu Thr Ser Glu Ala Arg Gly Arg Ile Pro Val Ala
CAG AAA AGA AAA ACT CCA AAC AAA GAA AAG ACT GAA GCC AAA AGG AAT AAG GTG TCC CAA  661
Gln Lys Arg Lys Thr Pro Asn Lys Glu Lys Thr Glu Ala Lys Arg Asn Lys Val Ser Gln
GAG CAG AGT AAG CCC CCA GGT CCC TCA GGA GCC AGC ACA TCT GCA GCT GTG GAT CAT CCC  721
Glu Gln Ser Lys Pro Pro Gly Pro Ser Gly Ala Ser Thr Ser Ala Ala Val Asp His Pro
CCA CTA CCC CAG ACC TCA TCA TCA ACT CCA TCC AAC ACT TCG TTT ACT CCG AAT CAG GAA  781
Pro Leu Pro Gln Thr Ser Ser Ser Thr Pro Ser Asn Thr Ser Phe Thr Pro Asn Gln Glu
ACC CAG GCC CAA CGG CAG GTG GAT GCA AGA AGA AAT GTT CCC CAA AAC GAC CCA GTG ACA  841
Thr Gln Ala Gln Arg Gln Val Asp Ala Arg Arg Asn Val Pro Gln Asn Asp Pro Val Thr
GTG GTG GTA CTG AAA GCA ACA GCG CCA TTT AAA TAC GAG TCC CCA GAA AAT GGG AAA AGC  901
Val Val Val Leu Lys Ala Thr Ala Pro Phe Lys Tyr Glu Ser Pro Glu Asn Gly Lys Ser
ACA ATG TTT CAT GCT ACA GTG GCC AGT AAG ACT CAA TAT TTC CAT GTG AAA GTC TTC GAC  961
Thr Met Phe His Ala Thr Val Ala Ser Lys Thr Gln Tyr Phe His Val Lys Val Phe Asp
ATC AAC TTG AAA GAG AAA TTT GTA AGG AAG AAG GTC ATT ACC ATA TCT GAT TAC TCT GAA 1021
Ile Asn Leu Lys Glu Lys Phe Val Arg Lys Lys Val Ile Thr Ile Ser Asp Tyr Ser Glu
TGT AAA GGA GTA ATG GAA ATA AAG GAA GCA TCA TCT GTG TCT GAC TTT AAT CAA AAT TTT 1081
Cys Lys Gly Val Met Glu Ile Lys Glu Ala Ser Ser Val Ser Asp Phe Asn Gln Asn Phe
GAG GTC CCA AAC AGA ATT ATC GAA ATA GCA AAT AAA ACT CCC AAG ATC AGT CAA CTT TAC 1141
Glu Val Pro Asn Arg Ile Ile Glu Ile Ala Asn Lys Thr Pro Lys Ile Ser Gln Leu Tyr
AAG CAA GCA TCT GGA ACA ATG GTG TAT GGG TTG TTT ATG TTA CAA AAG AAA AGC GTA CAC 1201
Lys Gln Ala Ser Gly Thr Met Val Tyr Gly Leu Phe Met Leu Gln Lys Lys Ser Val His
AAG AAG AAC ACA ATT TAT GAA ATA CAG GAT AAT ACA GGA TCC ATG GAT GTA GTG GGG AGT 1261
Lys Lys Asn Thr Ile Tyr Glu Ile Gln Asp Asn Thr Gly Ser Met Asp Val Val Gly Ser
GGA AAA TGG CAC AAT ATC AAG TGT GAG AAA GGA GAT AAA CTT CGA CTC TTC TGC CTT CAA 1321
Gly Lys Trp His Asn Ile Lys Cys Glu Lys Gly Asp Lys Leu Arg Leu Phe Cys Leu Gln
CTG AGA ACA GTT GAC CGC AAG CTG AAA CTG GTG TGT GGA AGT CAC AGC TTC ATC AAG GTC 1381
Leu Arg Thr Val Asp Arg Lys Leu Lys Leu Val Cys Gly Ser His Ser Phe Ile Lys Val
ATC AAG GCC AAG AAA AAC AAG GAA GGA CCA ATG AAT GTT AAT TGAAATATGAAAGCTGAA 1441
Ile Lys Ala Lys Lys Asn Lys Glu Gly Pro Met Asn Val Asn
ATGCAACAAACAACTTCCGCTTAAAACAATTAAGTTGTTAATAACTGTGATTTTGTAAAT 1501
TTCAGTAATTCATTTAAATGATGTTTCAGTAGATATATTCTAGCATATTAAGAGCTTTTA 1561
TAACTGAGTTATAGATTAGTTTGCTTTCTGGAATAAAATTTTCTTCTTATACTCTTCCTT 1621
TTTTTTAGATATTACATTTTGCTTTTATGACATTCACGAGGCAAAAAAACCG         1672
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Fig. 5. The 1672-base pair cDNA insert and the translated MNDA. The single solid underlined region matches the consensus ISRE sequence. The double underlined sequence is a basic amphipathic α-helical region with greater than 50% similarity to the IRF2. The S(T)PXX motifs are indicated (▲). The broken underlining denotes the coding regions that match the NH_2-terminal sequence data obtained from the isolated MNDA (Burrus et al., 1992). (GenBank accession number M81750.)

that express the immunochemically active MNDA (Goldberger et al., 1984; 1986). As discussed above, a number of laboratories have identified nuclear proteins other than MNDA that are expressed in a stage- or lineage-specific manner in human myelopoiesis. Recently, it was shown that the expression of some of the genes for the homeobox proteins also appears restricted to stages and lineages of differentiation in hematopoiesis (Shen et al., 1989; Lowney et al., 1991; Magli et al., 1991; Deguchi and Kehrl, 1991). Some of the homeobox proteins may play a role in the production of specialized myeloid cells. In contrast, the primary structure of the MNDA and its unique pattern of expression in hematopoiesis distinguishes it from all other nuclear proteins previously described that have been proposed to play a role in stage-specific and lineage-specific differentiation of myeloid cells.

In some cases, a nuclear protein expressed in a cell- or tissue-specific manner mediates a special response to an external stimulus (Evans, 1988). The primary structure of the MNDA, the presence of the ISRE in the 5′ untranslated

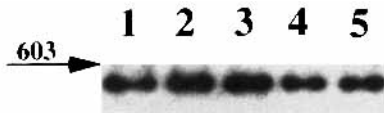

Fig. 6. Restriction enzyme digest of 5 unique cDNA inserts from λgt10 recombinant clones. The purified clones were cut with PstI (nucleotide 706) and BamHI (nucleotide 1238), electrophoretically separated, blotted, and hybridized to a random primed labeled PCR-amplified cDNA probe (nucleotide 865 to 1405).

A.

```
MNDA       1 MVNEYKkIlLLkGfElmddyhFtsiKSLLAyDLgLttkmQEeYnrIkItdlMEkKFqgvac
             |||||| | || | |      |   ||||| || |    || |  | |   || ||
204        1 MVNEYKrIvLLrGlEcinkhyFslfKSLLArDLnLerdnQEqYttIqIanmMEeKFpadsg

MNDA      62 LdKLIE  67
             | ||||
204       62 LgKLIE  67
```

B.

```
MNDA     179 SSS  tpSNtsftpNQetQaQrQvdaRrnV
             |||   ||     ||  | | |   |  |
204      216 SSSsasSNipsakNQksQpQnQnipRgaV

MNDA     207 pqndPvTVvVLkATaPFkYESPEngkstMfHATVAsktQYFHVKVFdINLKEKFvrKkvIt
                 | || || || || |||||     | |||||   |||||||| |||||||  |  |
204      245 lhsePlTVmVLtATdPFeYESPEhevknMlHATVAtvsQYFHVKVFnINLKEKFtkKnfIi

MNDA     268 ISdYsEcKGvmEIkEaSSV  sdfnQnfEVPNrIIeiANktPKIsqlyKqaSGtmvYGlFm
             || | | ||  || | |||      |  |||| ||  ||  |||    |  ||   || |
204      306 ISnYfEsKGilEInEtSSVleaapdQmiEVPNsIIrnANasPKIcdiqKgtSGavfYGvFt

MNDA     327 LqKKsVhkKNTIYEIqDntGSmdVVGSGKWHNIkCekGDKLrLFClqLrTvDRklKLVCGs
             | || |  ||||||| |  ||  |||||||||| |  |||| |||  | | ||  |||||
204      367 LhKKtVnrKNTIYEIkDgsGSieVVGSGKWHNInCkeGDKLhLFCfhLkTiDRqpKLVCGe

MNDA     388 HSFIKviK  395
             |||||  |
204      428 HSFIKisK  435
```

C.

```
MNDA      87 SKvaKKiKTqekapVKkInQEeV
             ||  || ||     || | || |
IRF2     119 SKkgKKpKTekedkVKhIkQEpV
```

Fig. 7. Regions of amino acid sequence similarity (>50% identical residues) between the MNDA and the mouse 204 gene protein product (**A** and **B**) and the human IRF-2 (**C**) determined using the genalign module of the IntelliGenetics Suite.

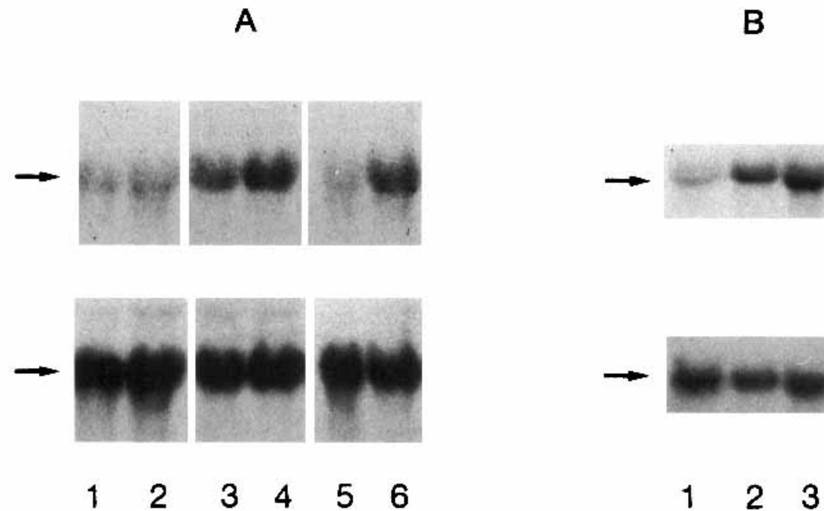

Fig. 8. Effect of human interferon α on MNDA mRNA levels. (**A**) Total RNA was isolated from U937 cells exposed to 2,000 units/ml of recombinant human interferon αA for 4 hours (lane 2), 6 hours (lane 4), and 12 hours (lane 6). RNA was also prepared from untreated cells at 4 hours (lane 1), 6 hours (lane 3), and 12 hours (lane 5). The RNA blot was probed for the 1.8 kb MNDA mRNA (arrow, upper panels) and for the 1.9 kb βactin mRNA (arrow, lower panels). (**B**) Total RNAs isolated from untreated U937 cells (lane 1) or exposed to natural human interferon α for 6 hours at 100 units/ml (lane 2) or 50 units/ml (lane 3).

region, and the ability of interferon α to elevate the mRNA level provide evidence to support the possibility that the cell lineage- and stage-specific expression of the MNDA is associated with the granulocyte/monocyte response to interferon α. The primary structure of the MNDA contains a basic amphipathic α-helical region with similarity to the IRF-2 (Fig. 7C). The highly basic region in the IRF-2 is also observed in the same location in the NH_2-terminal half of the IRF-1 protein. This site has been implicated in the sequence-specific DNA binding to the ISRE (Miyamoto et al., 1988; Li-Yuan et al., 1990). Proteins that exhibit this sequence in the NH_2-terminal end and bind the same DNA sequences have been proposed to constitute a new class of transcription factors (Pine et al., 1990). The recent observation that the IRF-1 is an immediate early response gene associated with prolactin induction of the Nb2T lymphoma cell proliferation has led to the proposal that the IRF-1 and IRF-2 are involved in regulating the expression of a network of other early response genes (Li-Yuan et al., 1990). The sequence similarity between the MNDA and the IRF-2 is limited to the presence of highly basic regions in the NH_2-terminal half of the protein adjacent to a region rich in serines and threonines (Miyamoto et al., 1988) and the determination of the biological significance of these regions in the MNDA will require a structure/function mutagenesis analysis. Although the specificity of the DNA binding by the IRF-1 and IRF-2 has been elucidated, their roles in the regulation of β-interferon gene transcription is being debated (Pine et al., 1990). However, interleukin 6 and leukemia inhibitory factor, physiological inducers of myeloid cell differentiation, induce a regulatory cascade in murine M1 myeloid cells involving the immediate early induction of IRF-1 which controls at least in part the later expression of β-interferon gene transcription (Abdollahi et al., 1991). These results indicate that the role of IRF-1 in β-interferon expression could depend on the circumstances surrounding the induction of interferon expression or on the type of cell examined.

The similarity in primary structure between the MNDA and the interferon-inducible mouse gene 204 (Choubey et al., 1989) (Fig. 7) extends over two separate regions. The mouse 204 gene is a member of a cluster of genes which share a duplicated 200 amino acid sequence (Choubey et al., 1989). The MNDA contains only a single copy of the conserved 200 amino acid sequence, and, in addition, a high level of sequence similarity to the 204 gene NH_2-terminal sequence. The NH_2-terminus of the 204 gene sequence is different from other members of the mouse gene cluster. The question of the protein product of the mouse 204 gene being expressed in a cell-specific fashion or being located in the nucleus has not been addressed (Choubey et al., 1989). In contrast, the sequence between MNDA amino

acid residue 68 and 178 shows no similarity to the 204 gene product. Because this region of the MNDA contains the sequence similar to the IRF-2, the putative DNA binding region, it is possible that the MNDA and the mouse 204 gene protein product perform different functions.

Experimental evidence has been obtained indicating that the MNDA is a DNA binding protein (Duhl et al., 1989; Gaczynski et al., 1990). The original observation that the number of S(T)PXX motifs in gene regulatory proteins was elevated over the number in other proteins was accompanied by the proposal that a non-specific DNA binding activity provided by the motif was needed to facilitate site-specific binding by stabilizing protein contacts with the DNA (Suzuki, 1989). Recently, an S(T)PXX motif near the basic DNA binding domain of the c-jun was reported to be a critical phosphorylation target responsible for inhibiting DNA binding (Boyle et al., 1991). Three of the five S(T)PXX motifs in the MNDA are located in or near the highly basic NH_2-terminal region. Phosphorylation of the serine in the S-P-X-K/R motif in histone proteins was also found to influence DNA binding (Hill et al., 1991). Although the functional significance of this motif or the variation of the motif found in histone proteins is not completely understood, the experimental evidence available at this time, suggests that it is important to the mechanism of action of many DNA binding proteins.

Previously obtained immunocytochemical and biochemical results (Goldberger et al., 1984, 1986; Hudson et al., 1988) showing a strict nuclear localization (nuclear localization signal encoded between nucleotides 1391 and 1403 in Fig. 5) and the constitutive level of MNDA expression in all late stages of granulocyte/monocyte cell differentiation suggest that MNDA does not relocate from cytoplasm to nucleus in response to an activation signal, such as takes place with factor E in response to α-interferon (Imam et al., 1990). In the latter case, the activation of E (ISGF3) by α-interferon leads to rapid translocation from the cytoplasm to the nucleus, where E binds the ISRE representing an initiating event in stimulating transcription of responsive genes (Imam et al., 1990). By contrast, the properties of the MNDA are consistent with those of a cell-specific repressor. The investigation of the regulation associated with the induction of interferon genes and the interferon-inducible gene expression has established a role for repressors (Keller and Maniatis, 1991). It is possible that the MNDA functions as a pre- and postinduction repressor associated with granulocyte/monocyte cell-specific response to interferon and/or viral infection.

Four factors have been identified that interact with the ISRE (Imam et al., 1990). Each shows a somewhat different temporal pattern of induced activity following exposure of cells to interferon. These responses vary with classes of interferon (Imam et al., 1990). The factors exhibit different affinities and specificity for ISRE binding. The factors and the presence of additional transcription factors, repressors, and extinguishers have been proposed to account for tissue-specific variations in response to the interferons (Imam et al., 1990). Our previous findings and the present results suggest that the MNDA represents a lineage-specific nuclear protein involved in the granulocyte/monocyte cell-specific response to interferon.

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