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IFI 16 Gene Encodes a Nuclear Protein Whose Expression Is Induced by Interferons in Human Myeloid Leukaemia Cell Lines

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Abstract We have characterized the induction of mRNA and protein products of the human *IFI 16* gene in response to IFN-γ, IFN-α, and IFN-β_2 (IL-6). We demonstrate that the IFI 16 gene product is a novel nucleoprotein expressed in association with the differentiation of myeloid precursor cell lines. In Northern blots, IFI 16 mRNA was increased ~25-fold above barely detectable levels in unstimulated promyelocytic HL-60 cells, in response to IFN-γ. Other myeloid cell lines, U937 and K562, also demonstrated a marked IFN-γ-inducibility of IFI 16 mRNA. However, all three cell lines were far less responsive to IFN-α, and there was no response to IL-6. By comparison, a panel of T and B cell lines demonstrated high constitutive expression of IFI 16 mRNA that was not regulated by these cytokines. Culture of HL-60 cells in medium containing dimethylsulfoxide, retinoic acid, and 1,25 dihydroxyvitamin D_3, agents that stimulate the differentiation of HL-60 along myeloid pathways, also caused the induction of IFI 16 mRNA. To characterize the protein product of *IFI 16,* a monoclonal antibody was raised against a recombinant bacterial protein comprising the amino terminal 159 amino acids of IFI 16 fused to glutathione S-transferase. The antibody, designated 1G7, was used in Western blotting to demonstrate the strong induction of a cluster of proteins of 85–95 kDa in the nuclear extracts of IFN-γ-treated HL-60. The nuclear localization of IFI 16 antigen was confirmed by immunohistochemical staining of HL-60 cells treated with IFN-γ, dimethylsulfoxide, and retinoic acid. IFI 16 was also detected in the nuclei of monocytes, neutrophils, and lymphocytes in normal peripheral blood. Database comparisons of the IFI 16 amino acid sequence revealed 51% identity with the recently cloned myeloid cell nuclear differentiation antigen (MNDA), and extensive similarity to protein products of the Gene 200 cluster of IFN-inducible genes, *Ifi 202* and *Ifi 204.* The amino terminal domain of IFI 16 encodes a putative nuclear localization signal, ^{124}PGAQKRKK, which is strongly conserved in MNDA and 204. Nuclear IFI 16 was able to bind double-stranded DNA in vitro and exhibited a similar elution profile from DNA-cellulose as previously observed for MNDA and 204. Therefore, IFI 16 and MNDA are members of a novel family of human DNA-binding proteins whose expression is associated with myeloid cell differentiation induced by cytokines and chemical agents.

Key words: *IFI 16* gene, interferons, HL-60 cells, IFN-γ-inducibility, nuclear protein

The molecular mechanisms involved in the maturation of the different hemopoietic lineages remain unresolved. On the premise that the expression of lineage- and stage-specific transcription factors might control orderly hemopoiesis, attempts have been made over recent years to correlate complex changes in morphology, enzymology, cell surface phenotype, and function with the patterns of expression of nuclear proteins at various stages of maturation [Chou et al., 1984; Goldberger et al., 1984; Murao et al., 1985]. A global examination of the nuclear proteins of B cells undergoing differentiation revealed that very few nonhistone proteins show appreciable variation in their level of expression [Rabilloud et al., 1991]. Nevertheless, similar approaches have successfully identified nuclear proteins that were expressed only at specific stages of myeloid development, and several of

Abbreviations used: D_3, 1,25 dihydroxyvitamin D_3; DMSO, dimethylsulfoxide; EDTA, ethylenediaminetetraacetic acid; GST, glutathione S-transferase; IFN, interferon; kb, kilobasepair; mAb, monoclonal antibody; MNDA, myeloid cell nuclear differentiation antigen; PBS, phosphate-buffered saline; PMSF, phenoxymethylsulfonylfluoride; RA, retinoic acid; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis.

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these molecules have been at least partially characterized [Choubey and Lengyel, 1992; Goldberger et al., 1986; Hromas et al., 1991].

It is evident that some stages of cellular differentiation are regulated by cytokines elaborated from bone marrow stromal cells or from other hemopoietic cells [Ogawa, 1992]. One such group of cytokines, the interferons (IFNs), are a diverse group of molecules with antiviral and immunomodulatory functions in addition to their influences on cell maturation [Pestka et al., 1987]. The phenotypic effects associated with IFN action are generally achieved through their modulation of gene transcription [Chebath et al., 1987; Grattage et al., 1992]. Recently, the IFN-α-inducible gene encoding the Myeloid cell Nuclear Differentiation Antigen (MNDA) has been identified. MNDA, a 55 kDa nuclear protein, is present only in late myeloid precursors of bone marrow, in mature myeloid cells of peripheral blood, and in myeloid cell lines such as HL-60 and U937 [Briggs et al., 1992; Burrus et al., 1992; Cousar and Briggs, 1990; Hudson et al., 1988]. The observation that MNDA expression is restricted to specific developmental stages of a single lineage of hemopoietic cells [Goldberger et al., 1986; Briggs et al., 1992; Burrus et al., 1992; Gaczynski et al., 1990], its ability to bind DNA or folded chromatin [Gaczynski et al., 1990; Duhl et al., 1989] and its IFN-inducibility suggests that it may act as a transcriptional activator or repressor in cells of the myeloid lineage. We have been interested in defining novel IFN-inducible proteins because of the involvement of IFN-γ in the step-wise maturation of myeloid precursors and cytotoxic T lymphocytes [Gromo et al., 1987]. Recently, we described a novel cDNA clone, designated IFI 16, which detected a single 2.7 kilobasepair (kb) mRNA expressed only in hemopoietic cells. IFI 16 mRNA was absent or barely detectable in various myeloid cell lines such as HL-60, K562, and U937; however mRNA expression was strongly induced in these cell lines following exposure to IFN-γ [Trapani et al., 1992]. In the present study, we have further characterized the pattern of induction of IFI 16 mRNA with IFN-γ, IFN-α and IL-6 and defined the protein product of the *IFI 16* gene, using a novel monoclonal antibody (mAb). We report that IFI 16 and MNDA are structurally and evolutionarily related, and also share extensive amino acid sequence identity and domain organization with proteins encoded on the mouse Gene 200 complex. We conclude that IFI 16 and MNDA are related nuclear proteins and may represent a family of novel IFN-inducible hemopoietic factors.

MATERIALS AND METHODS

Cell Culture

The human cell lines, HL-60 (promyelocytic leukemia), U937 (myelomonocytic leukemia), K562 (erythroblastic leukemia), CEM (T-acute lymphocytic leukemia), and Daudi (Burkitt's lymphoma) were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco, Grand Island, NY). Human peripheral blood leukocytes from normal subjects were separated on Ficoll-Hypaque. For IFI 16 induction experiments, exponentially dividing cells were passaged into medium supplemented with IFN-γ, 100 U/ml (Amersham, Sydney, Australia); IFN-αA, 100 U/ml, or IL-6, 1 μg/ml (Dr. Mark Smyth, Biological Response Modifiers Program, National Cancer Institute, Frederick, MD); dimethylsulfoxide (DMSO), 1.25% (v/v) (Ajax Chemicals, Melbourne, Australia); retinoic acid (RA), 1 μM, (Sigma Chemical Company, St. Louis, MO) or 1,25 dihydroxyvitamin D_3 (D_3) (10 nM) (Sigma Chemical Co., St. Louis, MO), for the times specified. Reagents used for mRNA induction were prepared and stored as described [Levine et al., 1986].

Northern Blotting

Total cellular RNA was prepared using RNAzol (Biotecx Laboratories, Houston, TX) according to the manufacturer's recommendations. RNA (10 μg/sample) was electrophoresed on 1% (w/v) agarose/formaldehyde gels, transferred to nylon membranes (Nytran, Schleicher and Schuell, Dassel, Germany), and probed with α^{32}P-dCTP-labeled nick-translated probes [Sambrook et al., 1989]. The cDNA probes used were a 1.5 kb fragment of the coding region of IFI 16 cDNA [Trapani et al., 1992] and a full-length probe encoding human γ-actin [Gunning et al., 1989]. Hybridization was in buffer containing 50% (v/v) formamide, 5 × Denhardt's solution, 0.75 M NaCl, 0.075 M Na citrate, 1% (w/v) sodium dodecylsulfate (SDS), 5% (w/v) dextran sulfate, 200 μg/ml herring sperm DNA, and 20 mM sodium phosphate buffer, pH 6.8 at 42°C overnight. Filters were washed at high stringency (0.015 M NaCl, 0.0015 M Na citrate, at 65°C for 2 × 20 min), air-dried, and autoradiographed

(Kodak XAR) for 1–4 days. Quantitation of the fold induction of mRNA levels was estimated on a laser densitometer (LKB, Wallac, Finland).

mAb Production

mAbs were raised against a bacterial fusion protein containing the amino-terminal 159 amino acids of IFI 16. Oligonucleotide primers encompassing positions 1–477 of the IFI 16 cDNA [Trapani et al., 1992] were used to generate a cDNA fragment by thermal cycling. The primers encoded *Eco* RI restriction sites to facilitate subcloning of the fragment in the correct reading frame into the bacterial expression vector, pGEX-3X (Amrad, Kew, Australia). Errors during thermal cycling amplification were excluded by nucleotide sequencing. Soluble IFI 16-glutathione S-transferase (GST) fusion proteins were generated following addition of isopropyl β-D-thiogalactopyranoside to cultures of *Escherichia coli* strain DH5αF′, as described [Smith and Johnson, 1988]. Cells were harvested by centrifugation, washed, lysed by sonication, and insoluble debris pelleted and discarded. Fusion protein was affinity purified on glutathione agarose (Sigma Chemical Co., St. Louis, MO), dialysed against phosphate-buffered saline (PBS), and used to immunize BALB/c mice for mAb production. Splenocytes were fused to NS-1 myeloma cells using polyethyleneglycol, as described [Xing et al., 1989]. We isolated a mAb designated 1G7 (IgG1,κ), which reacted in solid phase ELISA assays and in Western blots with GST-IFI 16 fusion protein, but not with the GST carrier (data not shown).

Western Blotting

Cytosolic and nuclear protein lysates were prepared as described [Dignam et al., 1983; Ramsay et al., 1989]. Briefly, cells were harvested by centrifugation and washed three times in PBS. Cytosolic protein preparations were obtained by lysing cells in buffer containing 0.5% NP-40, 5 mM $MgCl_2$, 25 mM KCl, 10 mM Tris HCl, pH 8, and 2 mM phenoxymethylsulfonylfluoride (PMSF). Nuclei and cellular debris were cleared from the supernatant by centrifugation. The nuclei were then washed with the same buffer supplemented with 1% Triton X-100 to ensure complete rupture of cells, and the integrity of nuclei was assessed by trypan blue staining (>98% staining). Nuclear proteins were extracted using high salt buffer supplemented with protease inhibitors (0.5 M NaCl, 10 mM Tris HCl, pH 8, 5 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol, 2 mM PMSF, 1 mM iodoacetamide, and 1 mM aprotinin), and nuclear debris removed by ultracentrifugation at 4°C for 20 min (100K; TLA100 rotor, Beckman model TL-100). Protein lysates were electrophoresed on 10% (w/v) SDS-polyacrylamide gels (SDS-PAGE) and electrophoretically transferred onto Immobilon P membranes (Millipore, Bedford, MA). Nonspecific binding of antibodies was blocked with 3% casein in PBS. Membranes were then probed with 1G7 mAb, and bound Ig detected by rabbit anti-mouse Ig-horse radish peroxidase conjugate (DAKO, Santa Barbara, CA) and visualized using the ECL detection system (Amersham, Sydney, Australia).

In Vitro Transcription/Translation of IFI 16

The full length IFI 16 cDNA subcloned in pBluescript KS^+ (Stratagene, La Jolla, CA) was transcribed using T3 or T7 RNA polymerase (mCAP mRNA capping kit, Stratagene) to produce sense and antisense transcripts, respectively. The resultant mRNA was translated in rabbit reticulocyte lysate (Promega, Madison, WI), at 37°C for 1 h and the lysates analysed for IFI 16 protein by SDS-PAGE and Western blotting.

Immunohistochemistry

HL-60 cells and peripheral blood leukocytes were harvested, washed in PBS and resuspended in PBS supplemented with 5% fetal calf serum for cytospin slide preparation. Following air drying for 1 h, slides were fixed in 2% paraformaldehyde, 0.01 M sodium periodate, 0.075 M L-lysine, and 0.037 M sodium phosphate buffer, according to McLean and Nakane [1974]. Immunoperoxidase staining was performed as described [Stacker et al., 1985]. Endogenous peroxidase activity was quenched by incubating the slides in 0.5% H_2O_2. Following overnight immersion in buffer containing mAb 1G7 or an isotype-matched control, bound Ig was detected with a rabbit antimouse/horseradish peroxidase conjugate, and visualized by reaction with 3,3′-diaminobenzidine (Sigma Chemical Co., St. Louis, MO).

DNA-Cellulose Affinity Column

Nuclear lysate was prepared from Daudi cells as described above and diluted with nine volumes of buffer B (10 mM potassium phosphate,

pH 6.8, 1 mM $MgCl_2$, 0.5% NP-40, 1 mM dithiothreitol, and 10% glycerol). DNA-binding proteins were purified by chromatography on double-stranded calf thymus DNA covalently coupled to cellulose beads (Sigma Chemical Co., St. Louis, MO), preswollen in buffer B. The column was washed thoroughly with buffer B and bound proteins eluted with incremental increases in NaCl concentration diluted in Buffer B (three elutions at each concentration). Eluted fractions were analyzed by Western blotting.

RESULTS

Induction of IFI 16 mRNA by IFN-γ, IFN-α, and IL-6

We have previously reported that IFI 16 mRNA is strongly and rapidly induced from very low levels in myeloid cell lines such as HL-60 and U937 following their exposure to 100 U/ml IFN-γ [Trapani et al., 1992]. To determine whether related cytokines might also affect IFI 16 expression in myeloid cells or influence the constitutive mRNA levels observed in lymphoid cell lines, we examined the induction of IFI 16 mRNA expression in time-course experiments (Fig. 1). Northern blotting demonstrated a marked increase in the abundance of a single 2.7 kb mRNA transcript within 6 h of IFN-γ treatment of HL-60. The maximal mRNA signal was seen at ~12 h, with a ~25-fold increase above resting levels, as judged by densitometric scanning of the autoradiograph (Fig. 1). Interestingly, treatment of HL-60 cells with IFN-α (100 U/ml) produced a less marked mRNA accumulation, with a ~10-fold increase in signal detected. The kinetics of this response differed from the pattern observed for IFN-γ, as the induction of IFI 16 message by IFN-α was more gradual, and was greatest at 36 h. By contrast, the T cell line, CEM and the Burkitt's (B−) lymphoma cell line, Daudi demonstrated no reproducible response to either cytokine above their constitutive levels of expression. IFI 16 mRNA levels showed no response following IL-6 treatment (1 μg/ml) in any of the cell lines tested. IL-6 is also known as *IFN-β2* due to its functional similarities with IFN-β, in relation to its effects on the hemopoietic system and anti-viral activity [van Snick, 1990]. We have recently extended the Northern blot studies to include the myeloid cell lines U937, K562, the T cell lines MOLT-4 and HPB-ALL, and the B cell lines Hym-2 and RAJI. Once again the myeloid cell lines demonstrated strong induction with IFN-γ and IFN-α, while alterations in level of expression in lymphoid-derived cells were less than two-fold (up or down), and not reproducible. We therefore concluded that IFI 16 mRNA levels were strongly inducible with IFN-γ and less so with IFN-α in cell lines representing late myeloid cell precursors, but this response did not occur in lymphoid cell lines.

Definition of the IFI 16 Nuclear Antigen Using a mAb

In order to identify and characterize the protein encoded by IFI 16, we first produced a mAb, 1G7, which specifically reacts with the predicted amino terminus of this antigen (see Materials and Methods). This region of the protein was a good candidate to produce a soluble GST fusion protein, due to its high hydrophilicity and because it lacks Cys residues, therefore excluding the possibility of insolubility due to inter-chain disulfide linkages [Trapani et al., 1992]. 1G7 was used to identify IFI 16 antigen in the lysates of HL-60 and CEM cells cultured in the presence of IFN-γ (Fig. 2A). In a time course experiment, nuclear and cytoplasmic fractions were prepared and separated by SDS-PAGE, and then probed in Western blots. A strong signal was observed at 85–95 kDa in the nuclear fractions of HL-60, following exposure of the cells to IFN-γ (Fig. 2A). This signal was barely detectable in unstimulated cells but became visible 6 h after exposure to IFN-γ, reached its maximum at 12 h, and was then maintained at maximal levels. The signal was consistently resolved into three closely migrating bands at 85–95 kDa, with the central band most prominent. The reason for these multiple protein species is uncertain but may reflect different glycosylation or phosphorylation states of nuclear IFI 16. Notably, 1G7 detected only a single 90 kDa species in in vitro translation experiments, which comigrated with the central band of the triplet (Fig. 2B, lanes 1 and 3), suggesting that the heterogeneity might be dependent upon post-translational modification of IFI 16 (see Discussion). The nuclear fractions from CEM cells demonstrated strong constitutive levels of IFI 16 which were unaltered by IFN-γ treatment (Fig. 2A). By comparison, the cytoplasmic lysates from both cell lines contained very small quantities of IFI 16 antigen. Negligible accumulation of IFI 16 was noted in the cytoplasm of HL-60 cells, suggesting that virtually all of the newly synthe-

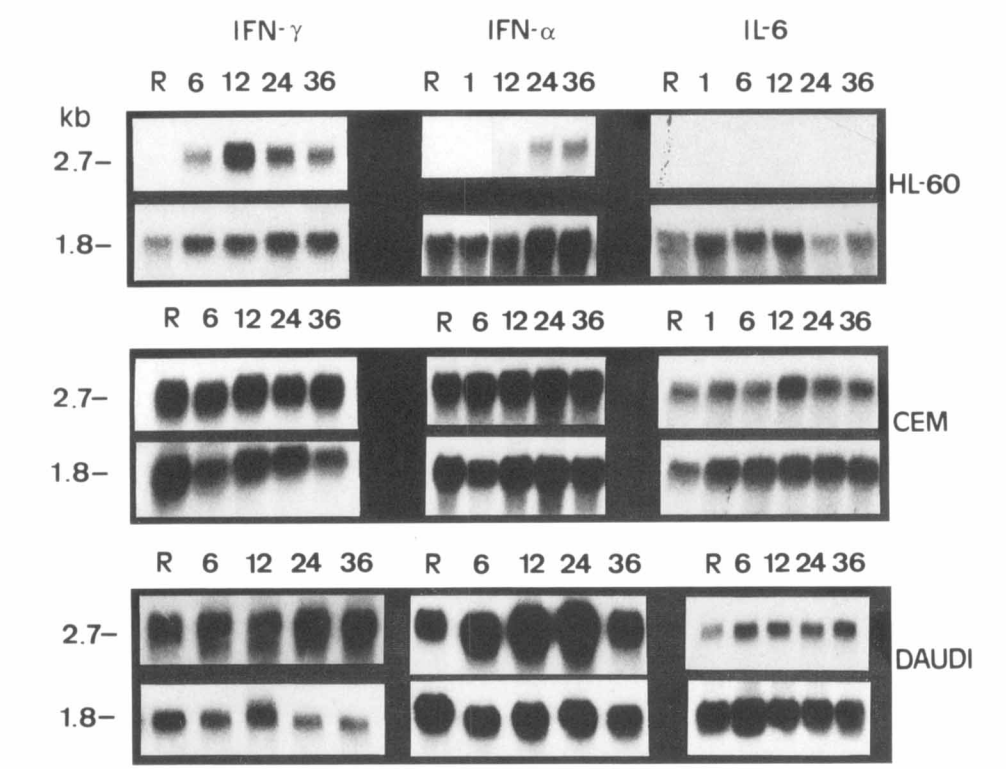

Fig. 1. Northern blot analysis of RNA (10 μg) purified from the cell lines HL-60, CEM, and Daudi and probed with IFI 16 cDNA insert. Cells were cultured for the number of hours shown at the top of each gel lane in the presence of the lymphokines shown (see Materials and Methods). "R" indicates untreated cells. In each case, to demonstrate that approximately equal amounts of RNA were loaded in each lane, the filter was stripped and reprobed with cDNA detecting γ-actin (~1.8 kb; lower panel of each set).

sized IFI 16 was rapidly transported to the nucleus.

Immunohistochemical Staining of IFN-γ-Stimulated HL-60

To confirm the nuclear localization of IFI 16 protein, immunoperoxidase staining using mAb 1G7 was performed on HL-60 cells treated with IFN-γ. More than 90% of the cells stimulated for 72 h demonstrated intense nuclear staining, while no cytoplasmic staining was noted (Fig. 3d). By comparison, few (<15%) unstimulated cells were stained with 1G7, and their level of staining was markedly less intense (Fig. 3a). Isotype-matched control mAbs demonstrated no staining in any of the preparations examined (data not shown). The staining observed in both resting and stimulated nuclei was non-uniform (punctate or patchy) and extended throughout the nucleoplasm.

Association of IFI 16 Expression With Chemically Induced Differentiation of HL-60

HL-60, derived from a patient with promyelocytic leukaemia, has served as an in vitro model for chemically induced myeloid differentiation [Breitman et al., 1980; Huberman and Callahan, 1979; Rovera et al., 1979]. Chemical agents such as DMSO, RA, and 5-azacytidine induce differentiation to a mature granulocytic phenotype, while D_3 and phorbol esters result in cells with macrophage/monocyte phenotype. The effect of some of these chemical agents on IFI 16 mRNA and protein expression was therefore examined. Treatment of HL-60 with RA, DMSO, or D_3 for 72 h resulted in each case in the induction of IFI 16 mRNA above resting levels (Fig. 4), which was concomitant with phenotypic changes associated with differentiation [Goldberger et al., 1984; data not shown]. Interestingly, the phorbol ester PMA, which like D_3 induces macrophage-like differentiation, had no effect on IFI 16 mRNA levels (data not shown). The degree of mRNA induction by RA, DMSO, and D_3 was not as great as observed for IFN-γ treatment (Fig. 1). However, the increase in IFI 16 mRNA was associated with the appearance of nuclear IFI 16 antigen, as detected by immunoperoxidase staining. Exposure to DMSO or RA resulted in staining of ~50% of the nuclei,

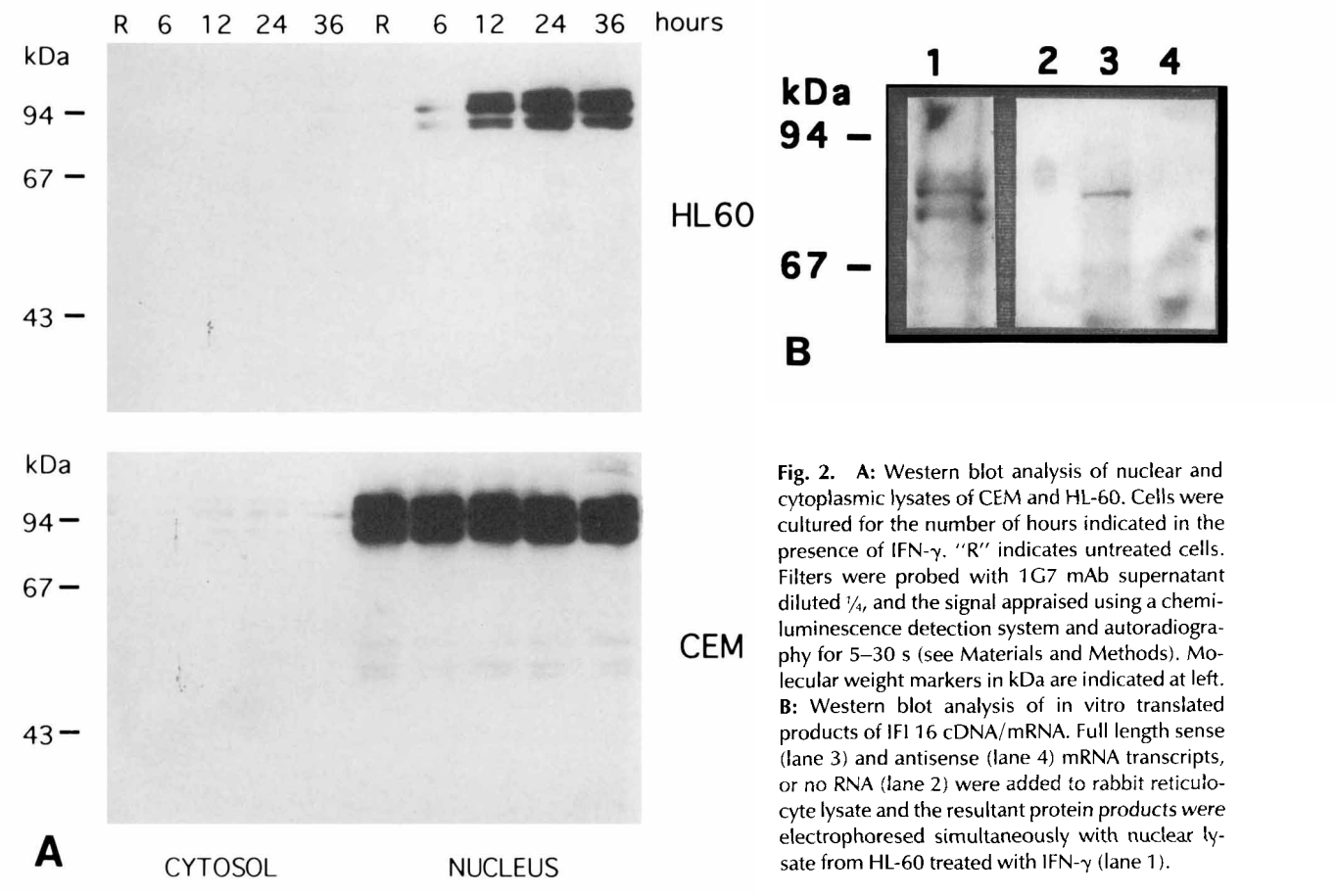

Fig. 2. **A:** Western blot analysis of nuclear and cytoplasmic lysates of CEM and HL-60. Cells were cultured for the number of hours indicated in the presence of IFN-γ. "R" indicates untreated cells. Filters were probed with 1G7 mAb supernatant diluted ¼, and the signal appraised using a chemiluminescence detection system and autoradiography for 5–30 s (see Materials and Methods). Molecular weight markers in kDa are indicated at left. **B:** Western blot analysis of in vitro translated products of IFI 16 cDNA/mRNA. Full length sense (lane 3) and antisense (lane 4) mRNA transcripts, or no RNA (lane 2) were added to rabbit reticulocyte lysate and the resultant protein products were electrophoresed simultaneously with nuclear lysate from HL-60 treated with IFN-γ (lane 1).

although the intensity of staining was less than observed in cells treated simultaneously with IFN-γ (compare Fig. 3b,c with d).

IFI 16 Antigen Expression in Peripheral Blood Leukocytes

To investigate IFI 16 protein expression in cells of peripheral blood, cytospin preparations of peripheral leukocytes from healthy individuals were examined by immunoperoxidase staining using 1G7 (Fig. 3f). Strong staining was observed in the nuclei of all peripheral lymphocytes and monocytes, whereas the polymorphonuclear cells consistently exhibited lower, but easily discernible 1G7 staining in their nuclei. No cytoplasmic staining was noted, and staining with an isotype control antibody was also negative (Fig. 3e).

IFI 16 Binds to Double-Stranded DNA In Vitro

The demonstration that IFI 16 is a nuclear antigen whose expression is associated with the differentiation of cell lines of the myeloid lineage prompted us to consider whether IFI 16 might bind DNA or chromatin. Accordingly, nuclear lysate prepared from Daudi cells was incubated with DNA-cellulose, and bound proteins sequentially eluted with increasing concentrations of NaCl. Western blotting of the eluted fractions indicated that the IFI 16 protein specifically and completely eluted from DNA-cellulose at 300 mM NaCl (Fig. 5). Furthermore, the GST-IFI 16 fusion protein showed similar binding characteristics to DNA-cellulose as native IFI 16, while GST did not bind to DNA (data not shown). These results are consistent with the findings for MNDA and 204, both of which bind DNA [Choubey and Lengyel, 1992; Duhl et al., 1989]. Furthermore, both the native IFI 16 and the GST-IFI 16 proteins eluted from heparin-agarose at 0.25–0.3 M KCl (data not shown), reminiscent of previously described DNA-binding proteins [Bell et al., 1989; Heberlain et al., 1985]. The similarity of the elution profiles of whole native IFI 16 and GST-IFI 16 fusion protein, which contains only the amino terminal 159

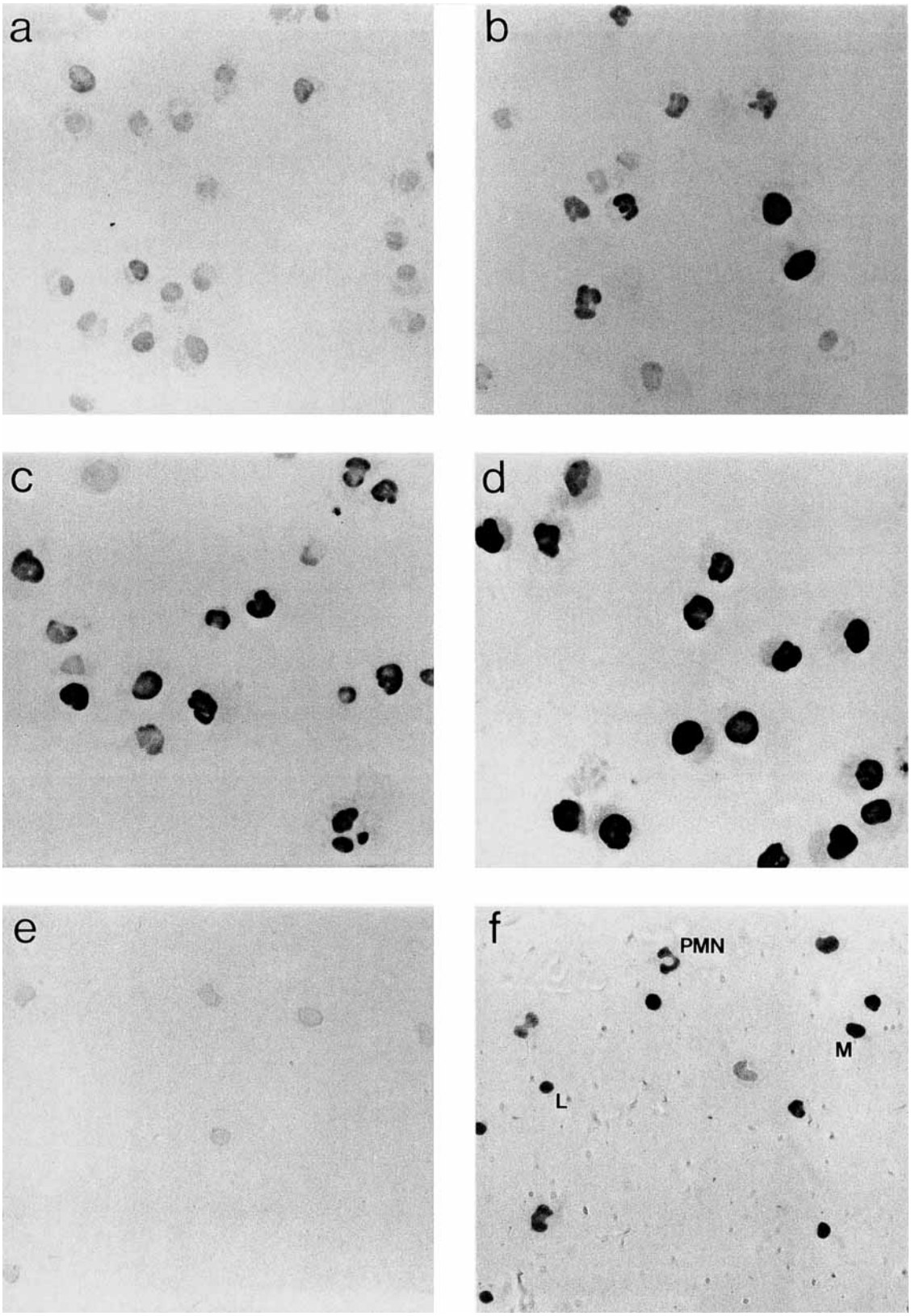

Fig. 3. Immunoperoxidase staining of HL-60 cells and normal human peripheral blood leukocytes with mAb 1G7. **a:** unstimulated HL-60; **b:** HL-60 cells exposed to RA for 72 h; **c:** HL-60 cells treated with DMSO for 72 h; **d:** HL-60 cells exposed to IFN-γ (100 U/ml) for 72 h; **e:** peripheral blood leukocytes stained with isotope-matched antibody; **f:** peripheral blood leukocytes stained with 1G7. L, lymphocyte; M, monocyte; PMN, polymorphonuclear leukocyte. To clearly demonstrate nuclear staining, the cells were not counterstained. The cytoplasm and cell membrane are therefore seen faintly. Magnification, ×100.

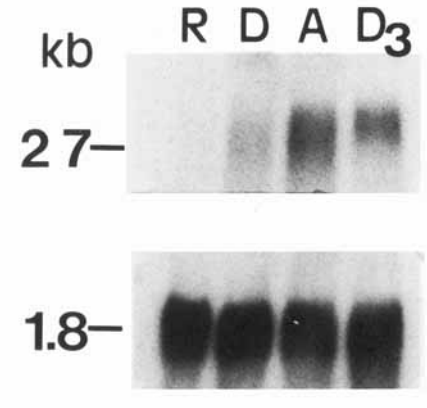

Fig. 4. Northern blot analysis of RNA (10 μg/lane) purified from unstimulated HL-60 cells (R) or cells exposed to DMSO (D), RA (A), or D_3. An identical filter was probed with cDNA detecting γ-actin to normalize the amounts of RNA probed (lower panel).

amino acids of IFI 16 suggests that this domain may be crucial in determining DNA binding activity.

DISCUSSION

This report describes the characterization of a novel nuclear protein, IFI 16, the product of the IFN-inducible *IFI 16* gene located on human chromosome 1. The inducibility of IFI 16 mRNA and its protein products is restricted to myeloid cell lines, is stronger in response to IFN-γ than to IFN-α, and is also seen in response to RA, DMSO, and D_3, agents that cause differentiation along myeloid pathways in vitro. By comparison, MNDA is induced most strongly by IFN-α and 202/204 by IFN-β [Goldberger et al., 1986; Briggs et al., 1992; Choubey et al., 1989]. We have generated a mAb that reacts specifically with the amino terminus of the IFI 16 protein and identified 85–95 kDa protein species that are inducible in HL-60 cells treated with IFNs or undergoing differentiation in response to chemical agents associated with in vitro differentiation of this cell line. IFI 16 is therefore a novel IFN-inducible nuclear protein capable of binding to double-stranded DNA in vitro, and whose expression is associated with the differentiation of myeloid cell precursors.

Although the structural similarities between IFI 16 and proteins encoded by the mouse Gene 200 cluster [Choubey and Lengyel, 1992, 1993; Choubey et al., 1989; Engel et al., 1985; Opdenakker et al., 1989; Samanta et al., 1986] have been noted previously, these observations have been extended by the availability of the human MNDA cDNA sequence [Briggs et al., 1992]. The clear conservation of a domain structure among all of these proteins, their expression in the nuclei of hemopoietic cells, and inducibility by IFNs provides strong grounds for their classifi-

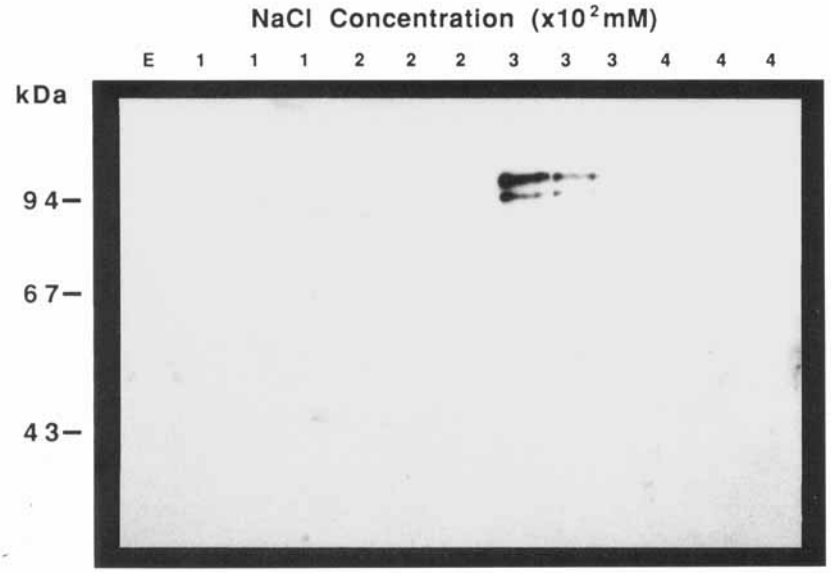

Fig. 5. Binding of IFI 16 and its elution from DNA-cellulose. Nuclear lysate from Daudi cells was equilibrated in Buffer B containing no NaCl and passed through a DNA-cellulose column. Bound proteins were sequentially eluted with Buffer B containing the NaCl concentration indicated (see Materials and Methods). E represents the column effluent. Column fractions were analyzed by Western blotting with 1G7 mAb. Molecular weight markers are shown at left. The data shown are representative of three experiments performed.

cation as a family of structurally and functionally related proteins (Table I). Although some differences exist in subcellular localisation, all are essentially nuclear proteins. To date, IFI 16 and mouse protein 204 have only been detected in the nucleus, whereas MNDA and 202 exhibit a broader intracellular distribution. When induced, the 202 protein localizes firstly to the outer surface of a membranous fraction in the cytoplasm, and later to the nucleus [Choubey and Lengyel, 1993]. By contrast, the nuclear localisation of MNDA is cell-cycle dependent, leaving the nucleus in anaphase, becoming totally cytoplasmic by metaphase, and returning to the nucleus during telophase [Goldberger et al., 1986]. This cyclic redistribution is reminiscent of another nuclear protein, lamin, whose subcellular localization is entirely dependent upon its phosphorylation state [Gerace and Blobel, 1980]. MNDA, 202, and 204 are all capable of being phosphorylated, and due to its large number of conserved kinase recognition sequences, it is predicted that IFI 16 will also prove to be a nucleophosphoprotein. The tissue distribution has not been completely characterized for all four proteins, but all are associated with expression and/or induction in myeloid cell lines such as HL-60. The expression of MNDA is restricted to the promyelocytic and later stages of myeloid differentiation, whereas IFI 16 is also expressed in lymphoid cells, but is inducible specifically in cells of the myeloid lineage. By comparison, the mouse proteins 202 and 204 have been detected in IFN-stimulated lymphoid, myeloid, and fibroblastoid cell lines [Choubey et al., 1989; Choubey and Lengyel, 1993; Engel et al., 1985; Opdenakker et al., 1989; Samanta et al., 1986].

The IFI 16 protein was localized by two methods to the nuclei of unstimulated lymphoid and stimulated myeloid cell lines. In Western blotting experiments, three protein species ranging from 85–95 kDa were detected specifically in the nuclear lysates; however the nature of the three species is yet to be resolved. The narrow range of molecular weights is reminiscent of alternative phosphorylation states. Importantly, it is known that proteins bearing a structural resemblance to IFI 16, such as 204 also undergo phosphorylation, a key feature of many proteins translocated to the nucleus [Choubey and Lengyel, 1992]. Alternatively, the apparent molecular weight of the largest species on SDS-PAGE is only marginally greater than the unmodified protein and may be consistent with minor glycosylation. The three bands could also represent allelic forms, the products of alternative mRNA splicing, or may be related to proteolysis. While Southern blotting suggests that the 5′ end of IFI 16 is present as a single copy gene in humans [Trapani et al., 1992], multiple related mouse genes exist [Choubey et al., 1989]. The possibility that three proteins that contain the 1G7 epitope arise from multiple human genes or from alternatively spliced mRNA originating from *IFI* 16 still requires formal exclusion. The subnuclear localization of IFI 16 also remains unknown. The punctate, nonuniform staining

TABLE I. Characteristics of a Family of IFN-Inducible Proteins*

	IFI 16	MNDA	202	204
Species	H	H	M	M
Molecular weight[a]	85–95	55	52	72
Subcellular location	N	N, C	N, C	N
Gene mapping	chr 1	?	chr 1	chr 1
Inducing agents	IFN-γ, -α, DMSO, RA, D_3	IFN-α, PMA, D_3	IFN-β	IFN-β
Cellular distribution	Lymphoid/myeloid[b]	Myeloid	Lymphoid/myeloid fibroblast	Myeloid fibroblast
Substrate for phosphorylation	?	Yes	Yes	Yes
200 amino acid repeat motifs	2	1	2	2

*H, human; M, mouse; N, nuclear; C, cytoplasmic; chr, chromosome. The information in this table was compiled from the following references: Briggs et al., 1992; Burrus et al., 1992; Choubey et al., 1989, 1992, 1993; Cousar et al., 1990; Duhl et al., 1989; Engel et al., 1985; Goldberger et al., 1984, 1986; Opdenakker et al., 1989; Trapani et al., 1992.

[a]kDa.

[b]The following nonhemopoietic cell lines also express IFI 16 mRNA: Hela (cervical carcinoma), Panc 89 and HPAF (pancreatic adenocarcinoma), Colo 319 (ovarian carcinoma), Colo 368 (melanoma), CAKI (kidney carcinoma). The following cadaver tissues and cell lines tested negative for IFI 16 mRNA expression: liver, testis, and adrenal (cadaver RNA), Colo 205 and LIM 1215 (colon carcinoma), MCF-7 and T47D (breast carcinoma), and OAW-42 (ovarian carcinoma).

of HL-60 observed by immunoperoxidase may be consistent with nucleolar localization, as has been observed with the related protein, 204 [Choubey and Lengyel, 1992]. Experiments involving co-localisation with nucleolar markers and actinomycin D treatment should provide further information on the intranuclear localization of IFI 16 protein.

We have previously reported that IFI 16 and the protein products of the mouse 202 and 204 genes are similarly organized, and share a common domain structure [Trapani et al., 1992]. An evolutionary association between these genes was proposed, supported by the observations that both the human and mouse genes are IFN-inducible and located on syntenic regions of chromosome 1 [Kingsmore et al., 1989a,b]. Moreover, sequence alignment of the recently reported MNDA cDNA with 202 and 204 revealed that MNDA is a close structural relative of these proteins [Briggs et al., 1992; Burrus et al., 1992]. It is also clear that MNDA and IFI 16 are themselves closely related (Fig. 6A). The two proteins share 51% amino acid identity, largely accounted for by a common 200 amino acid domain which is reiterated in IFI 16, but is present as a single copy in MNDA. This strongly suggests that *MNDA* and *IFI 16* may have arisen from a common precursor gene, by gene duplication and mutation to introduce a "premature" termi-

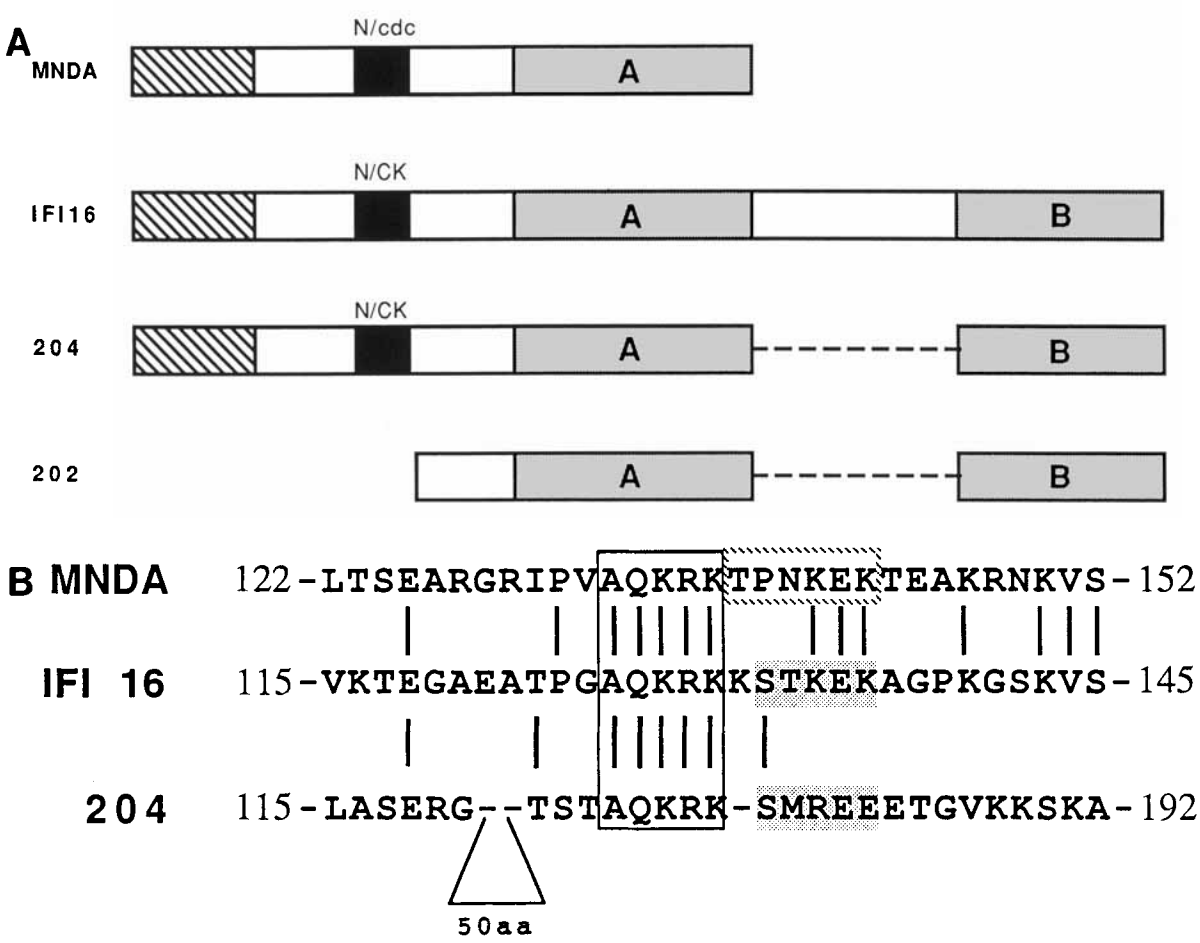

Fig. 6. **A:** Alignment of putative domains of IFI 16, MNDA, 204, and 202. A and B represent the 200 amino acid repeat domains, while the highly conserved basic amino-terminal region is hatched. Consensus sequences for nuclear localisation (N) and potential sites for phosphorylation [cdc-2 or casein kinase II (CKII)] are darkly shaded and labeled. Regions of low amino acid similarity are unshaded. **B:** A highly conserved consensus sequence for nuclear localization in IFI 16, MNDA, and 204. The strongly conserved basic core residues are boxed. Possible sites for phosphorylation by casein kinase II or cdc2 are shaded and stipple-boxed, respectively. Numerals indicate the amino acid sequence positions as published in Trapani et al. [1992] (IFI 16), Briggs et al. [1992] (MNDA), and Choubey et al. [1989] (204). Vertical lines indicate amino acid identity, and a 50 amino acid deletion in the 204 sequence was introduced to optimize the alignment.

nation codon in MNDA. By comparison, both mouse proteins, 202 and 204, are predicted to encode two reiterated domains (Fig. 6A).

The present study has demonstrated that IFI 16 is localized to the nucleus of human leukocytes and hemopoietic cell lines. MNDA is also known to be nuclear, having originally being defined by antisera raised against neutrophil nuclei [Goldberger et al., 1986]. The close similarities between the two proteins caused us to search for possible consensus sequences for nuclear localization. One such region close to the carboxyl terminal of MNDA, ^{401}KKNKE, has already been proposed as being of potential significance [Briggs et al., 1992]. The Lys-rich domain of IFI 16 contains two sequences which are similar to nuclear localization signals [Silver, 1991], ^{92}PALSRKRKK and ^{124}PGAQKRKK, which resemble the nuclear localization signals for SV40 large T antigen [Kalderon et al., 1984] and nucleoplasmin [Robbins et al., 1991], respectively. The sequence ^{92}PALSRKRKK is not conserved in 204 or MNDA, but ^{124}PG*AQKRKK* in IFI 16 is similar to ^{172}ST*AQKRK*, which occurs (only once) in the heptamer repeat region of 204 and to ^{131}PV*AQKRK* in MNDA (Fig. 6B). Importantly, these sequences in MNDA and 204 are located in regions that are otherwise poorly conserved in IFI 16. Furthermore, the regulation of nuclear import has recently been shown to involve phosphorylation of nuclear proteins by casein kinase II [Rihs et al., 1991] and cdc 2 kinase [Jans et al., 1991], and target consensus sequences for these kinases are present in the vicinity of the putative NLS of IFI 16, MNDA, and 204 (Fig. 6B).

The obvious similarities in structure, inducibility of expression, and subcellular localization described for IFI 16, MNDA, 202, and 204 may reflect a general function for these molecules. All four proteins contain at least one copy of a conserved 200 amino acid domain, in addition to domains that are peculiar to that individual family member, e.g., the C and C′ domains of IFI 16, which intervene the 200 amino acid motifs [Trapani et al., 1992]. We speculate that the reiterated 200 amino acid domain may confer a function common to all members of the family, while individually expressed domains may afford other tissue- or organelle-specific functions. It is evident that at least some of these proteins are capable of interacting with DNA or chromatin. This property would be consistent with a role in the regulation of hemopoietic differentiation, through activation of unknown target genes. In order to address this question, studies of IFI 16 expression in normal bone marrow and defined populations of myeloid precursors, and its response to cytokines such as GM-CSF and IL-3 are currently underway. Unlike MNDA, whose expression is restricted to cells of the myeloid lineage, IFI 16 is additionally expressed constitutively in the nucleus of lymphoid cells. It remains to be determined whether the association between IFI 16 expression and hemopoietic differentiation is a causal one.

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