Point Mutations within AT-Hook Domains of the HMGI Homologue HMGIYL1 Affect Binding to Gene Promoter but Not to Four-Way Junction DNA†

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ABSTRACT: High-mobility group I/Y (HMGI/Y) proteins are chromosomal proteins involved in gene and chromatin regulation. Elevated levels of HMGI/Y proteins were reported in diverse malignant tumors, and rearrangements of their genes are casually involved in the development of benign tumors. In humans, the chromosomal locus Xp22 has been often found to be affected in diverse benign mesenchymal tumors. Recent studies revealed that this region contains a retropseudogene *HMGIYL1* which potentially can be activated in a way of "exonization" upon aberrations involving this region. The coding sequence of the *HMGIY-L1* is highly homologous to the *HMGI(Y)* gene. On the protein level, both HMGIYL1 and HMGI differ at few amino acid residues, including their putative DNA-binding domains (DBDs). Here we have approached the question of whether the HMGIYL1 product would be able to adopt a role of HMGI in the context of binding to gene promoters and chromatin. Comparative binding studies, employing protein footprinting technique, revealed that HMGIYL1 has lost the ability to bind to the promoter of the interferon β gene, but retained its high affinity for the four-way junction DNA. Our results stress the importance of particular residues within the DBDs for DNA binding and demonstrate that tight binding of HMGI/Y proteins to the four-way junction DNA can be achieved in alternative ways. The binding of HMGIYL1 to four-way junction DNA suggests that activation of the *HMGIYL1* gene would yield a protein sharing some binding properties with HMG1-box proteins and histone H1. Thus, the HMGIYL1 could interplay together with these components in chromatin regulation.

The high-mobility group I/Y protein family (HMGI/Y)[1] consists of diverse proteins carrying multiple copies of the AT-hook (*1*) DNA binding domain (DBD) (for reviews, see refs *2*−*4*). DBD preferentially binds within the narrowed minor groove of the AT-rich DNAs (*1*, *5*) and modulates the conformation of the DNA (*6*−*10*). Three major proteins HMGI, HMGY, and HMGI-C, each containing three DBDs, have been described for mammalian cells. HMGI and HMGY are isoforms derived from alternatively spliced transcripts encoded by the *HMGI(Y)* gene (*11*) located at chromosomal locus 6p21. The HMGI-C protein is coded by another gene (*12*) at locus 12q14-15. The proteins are involved in assembly of promoter−enhancer complexes of diverse genes (for a review, see ref *4*). In this respect, the best characterized promoters and enhancers are those of the genes encoding interferon β (IFNβ) (*13*−*15*) and the α-subunit of the interleukin 2 receptor (*16*). Because HMGI/Y proteins bind to four-way junction DNA (*17*, *18*) and nucleosomes (*19*) and compete for binding with histone H1 (*18*, *20*, *21*), it has been proposed that they play a role in organization of specific types of chromatin and chromatin opening (*20*).

Chromosomal aberrations affecting the *HMGI-C* and *HMGIY* genes have been found in a variety of benign solid tumors, such as uterine leiomyomas, endometrial polyps, lipomas, and pulmonary chondroid hamartomas (*22*−*27*). Moreover, an overexpression of the HMGI(Y) proteins is characteristic for malignant tumors (*28*−*33*), suggesting a relation between high titers of the proteins and neoplastic phenotype. The aberrations in the chromosomal region Xp22 are frequently observed in a number of benign tumors (*34*−*42*). Recently, a *HMGIYL1* retropseudogene that is homologous to *HMGI(Y)* has been mapped to this region (*43*), and a possible activation of the gene by "exonization", upon chromosomal rearrangement, has been suggested (*44*). The coding region of *HMGIYL1* is more than 94% identical with that of the *HMGI(Y)* gene. The deduced primary structure of the putative *HMGIYL1* gene product revealed that few residues within DBD 1 and 2 are exchanged (Figure 1A).

Recently, we have studied organization of DNA−HMGI/Y complexes with different DNAs, including promoter sequences of the IFNβ gene and the four-way junction DNA (*45*, *46*). In this work, we have approached the question of how structural changes in two DBDs of the HMGIYL1 affect

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[1] Abbreviations: HMG, high-mobility group; HMGI/Y family, diverse proteins containing multiple AT-hooks; HMGI(Y), HMGI and HMGY proteins that are products of a single gene; HMGIYL1, product of the *HMGIYL1* gene; IFNβ, interferon β gene; DBD, DNA binding domain containing an AT-hook; CK2, casein kinase 2.

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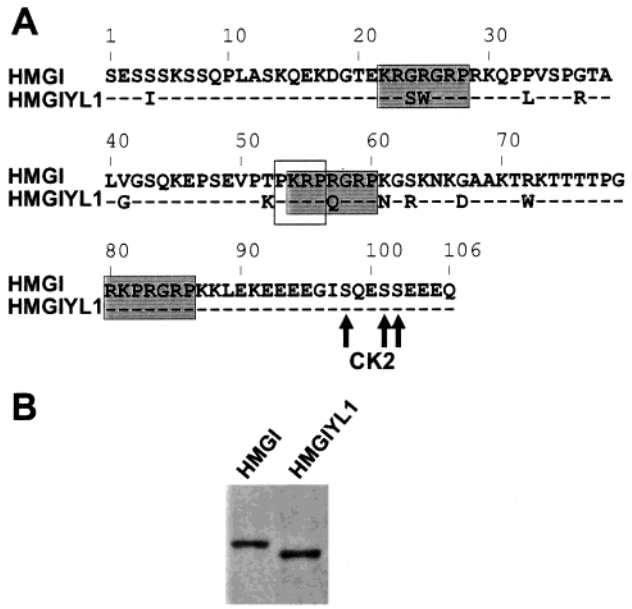

FIGURE 1: (A) Comparison of the primary structures of the HMGI and HMGIYL1 proteins. The DNA binding domains (DBDs) and the tetrapeptide PKRP are boxed. The phosphorylation sites of the CK2 kinase are marked with arrows. (B) SDS−PAGE of the purified proteins.

binding properties and the organization of the protein complexes with IFNβ promoter and four-way junction DNA. We show that the mutations within HMGIYL1 impair binding of the protein to the promoter DNA, but do not substantially weaken its interaction with four-way junction DNA.

EXPERIMENTAL PROCEDURES

Construction of Expression Vectors. The coding regions of the human HMGI and HMGIYL1 proteins were cloned in pGEM-T. For expression, the pET3a constructs were transferred into *Escherichia coli* BL21(DE3) as described previously (*47*).

Synthesis of Proteins in Bacteria. Transformed *E. coli* BL21(DE3) cells were grown at 37 °C in 5 mL of LB medium in the presence of 300 μg of ampicillin on a rotary shaker with 240 revolutions/min. A 12-hour-old culture was used to inoculate 200 mL of fresh LB medium with 20 mg of ampicillin. The culture was grown to an optical density of 0.4 measured at 600 nm. The expression of the HMG protein was then induced by adding isopropyl thiogalactoside to a concentration of 1 mM. The induced culture was incubated for an additional 2 h under the same conditions. The cells were harvested in a centrifuge at 4 °C and 5000*g*. Cell pellets were frozen at −20 °C. Proteins were extracted from bacteria with 5% $HClO_4$ and purified in a two-step procedure combining cation-exchange chromatography and reverse-phase HPLC as described previously (*47*). The homogeneity of the products was checked by SDS−PAGE.

The identity of the products was confirmed by MALDI mass spectroscopy. The HMGI was quantified on Coomassie Blue-stained polyacrylamide gels using HMGI-C as a standard. The concentration of HMGIYL1 was determined spectrophotometrically using an absorption coefficient for two tryptophans of 11 000 M^{-1} cm^{-1}.

Protein Phosphorylation by Casein Kinase 2. Fifty micrograms of purified protein was phosphorylated at 37 °C with 500 units of recombinant human CK2 (New England Biolabs Inc.) for 5 min in the presence of 200 μM ATP containing 100−150 μCi of [γ-^{32}P]ATP in 50 μL of CK2 buffer [20 mM Tris-HCl, 50 mM KCl, and 10 mM $MgCl_2$ (pH 7.5)]. Phosphorylation of cHMGI was extended for 2.5 h. The reaction products were separated by reversed-phase HPLC.

DNA and Oligonucleotides. The synthetic linear poly(dA-dT)·poly(dA-dT) DNA was obtained from Pharmacia Biotech. The approximate average length of this DNA was 5000 bp. The 34 bp fragment of the promoter of the IFNβ gene containing the PRDIII-1, PRDII, and NRDI elements was prepared from synthetic oligonucleotides (*45*, *46*). Four-way junction DNA was prepared according to the method of Bianchi (*48*). For DNA footprinting and mobility shift experiments, the oligonucleotides were ^{32}P-end-labeled with T4 polynucleotide kinase.

Mobility Shift Assay. Electrophoretic mobility shift assays were carried out as described previously (*47*, *49*). Briefly, purified proteins were incubated with <1 nM labeled DNA in 180 mM NaCl, 1 mM $MgCl_2$, 0.01% BSA, 8% glycerol, and 10 mM Tris-HCl (pH 7.9) at 20 °C for 10 min. The DNA and DNA−protein complexes were run on 8% polyacrylamide gels. The gels were dried and autoradiographed.

Hydroxyl Radical Protein Footprinting (50, 51). Ten picomoles of the radioactively end-labeled protein (10000−20000 cpm) was digested in the presence or absence of DNA in a total volume of 10 μL of 180 mM NaCl and 10 mM MOPS buffer (pH 7.2) at room temperature for 30 min. The chemical digestions were started by sequential addition of 1 μL each of the following freshly prepared solutions: (i) 20 mM EDTA and 10 mM $(NH_2)_2Fe(II)(SO_4)_2$, (ii) 0.2 M sodium ascorbate, and (iii) 0.375% (v/v) H_2O_2. Reactions were stopped after 30 min by addition of 3.3 μL of a 4-fold SDS sample buffer [4% SDS, 16% glycerol, 25 mM Tris-HCl (pH 6.8), 6% β-mercaptoethanol, and 0.01% bromophenol blue]. The reaction products were separated on 16.5% polyacrylamide gels using the Tricine-SDS buffer system (*52*) and analyzed as described previously (*45*, *46*). Briefly, phosphorimages of the full-width lanes were scanned, and the intensities were plotted versus the mobility (ImageQuant Software, Molecular Dynamics). The intensity plots were aligned to correct distortions between different lanes using ALIGN software. The aligned intensity plots were imported into EXCEL (Microsoft), and gel loading efficiencies and the extents of cleavage were normalized. The electrophoretic mobilities were transformed into amino acid residue positions, and mean values for each positions were calculated. Finally, each amino acid residue position was compared in a difference plot: $\Delta_{norm} = (I_{without\ DNA} - I_{with\ DNA})/I_{without\ DNA}$, where Δ_{norm} is the normalized difference, $I_{without\ DNA}$ is the mean value of the corrected phosphorimager intensity of a single residue position measured in the absence of DNA, and $I_{with\ DNA}$ is the mean value of the corrected phosphorimager intensity of the same position measured in the presence of DNA. Due to the ambiguity of the assignment and limited resolution, the peptides at the front of the gel and those of the near-full-length protein, respectively, were excluded from the analysis. Size markers were obtained by limited digestions of 10 pmol of end-labeled HMG protein by trypsin,

thermolysin, proteinase Arg-C, proteinase Glu-C, proteinase Asp-N, or chymotrypsin in a reaction volume of 10 μL. Cleavage in the presence of 10 ng of trypsin, thermolysin, or chymotrypsin was carried out in 180 mM NaCl and 20 mM Tris-HCl (pH 7.5) at 0, 20, or 20 °C, respectively. The reactions with trypsin were stopped by addition of 1 μL of 0.14 mM N^{α}-*p*-tosyl-L-lysine chloromethyl ketone. The cleavage with proteinases Glu-C and Asp-N was carried out in the presence of 50 ng of enzyme in 25 mM sodium phosphate (pH 7.8) and 180 mM NaCl at 20 °C. Digestion of the protein in the presence of 20 ng of Arg-C was performed in 90 mM Tris-HCl containing 8.5 mM $CaCl_2$, 5 mM DTT, and 0.5 mM EDTA at 20 °C. Finally, the reactions were stopped by addition of 4-fold SDS sample buffer with 20 mM EDTA.

Labeling of Proteins with Fluoresceine Isothiocyanate. The HMGI and HMGIYL1 proteins were labeled with fluoresceine thiocyanate (FITC) as described previously (*53*). Briefly, 50 μg of protein was incubated with 1 mg of 10% FITC on Celite (Sigma) in 0.5 mL of 100 mM sodium carbonate buffer (pH 9) for 4 h. The solid carrier was removed by centrifugation, and the excess of the free label was removed by gel filtration. Under the conditions that were used, less than one FITC molecule was bound to the protein, as determined spectrophotometrically.

Microinjection and Microscopy. The lumen of the syncytial gonads of *Caenorhabditis elegans* hermaphrodite worms was injected with 10−20 pL of ~100 μM FITC-labeled protein. For DNA staining, 100 μM Hoechst 33342 was co-injected. The efficiency of the injection and the distribution of the fluorophore were monitored under a Zeiss Axioplan 2 microscope equipped with Nomarski differential interference contrast and epifluorescence optics. Green fluorescent images were collected with an excitation wavelength of 450−490 nm and an emission filter long pass of 520 nm (blue fluorescent images were collected with an excitation wavelength of 365 nm and an emission filter long pass of 397 nm) with a Spot RT CCD (Diagnostic Instruments) camera.

RESULTS

The HMGIYL1 Homologue Translocates into the Nucleus and Binds to Chromosomes. Sequence comparison of the HMGIYL1 product with the HMGI protein reveals that two arginyl residues located centrally within the two AT-hook domains of the HMGI are replaced in HMGIYL1 with other residues (Figure 1A). Such interruption of the positively charged stretches could affect the cellular behavior of the HMGIYL1. Basic sequences often constitute nuclear targeting signals, and therefore, it would be possible that these changes in the primary structure can impair translocation of HMGIYL1 into nuclei and/or inhibit binding to chromosomes. To test this possibility, the HMGIYL1 product and the HMGI protein were labeled with FITC and microinjected in syncytial gonads of *C. elegans* (Figure 2). We found a rapid accumulation of both labeled proteins in the nuclei (Figure 2B,C). Furthermore, in the meiotic region (M) of the gonads, binding of the labeled HMGIYL1 to the chromosomes was observed (Figure 2D,E). These results suggest that HMGIYL1 possesses the ability to move across the nuclear membrane and bind to chromosomes; thus, if expressed, it could exert a cellular function as it does with the HMGI protein.

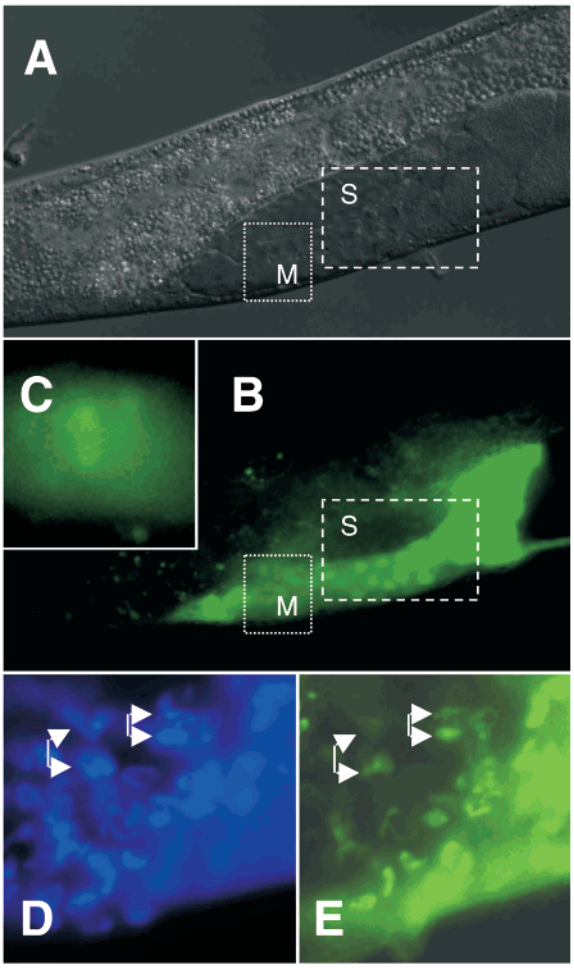

FIGURE 2: Direct fluorescence microscopy for detecting nuclear translocation and binding of HMGIYL1 to chromosomes. The syncytial gonads of the *C. elegans* were injected with FITC-labeled HMGI (B) and HMGIYL1 (C and E). Nuclear accumulation and binding to chromosomes were observed in the syncytial (S) and meiotic (M) regions of the gonads, respectively. (D) Fluorescence of the Hoechst 33352 co-injected with HMGIYL1 (E). Individual pairs of spots marked with arrows represent groups of 12 chromosomes in the anaphase.

Changes within the Two AT-Hooks of the HMGIYL1 Protein Impair Binding to IFNβ Promoter but Not to Four-Way Junction DNA. Earlier work has shown that the HMGI/Y proteins bind with high affinity to intrinsically prebent DNAs carrying spaced AT-tracts like that of the IFNβ promoter (*8*). Moreover, HMGI/Y proteins bind tightly to synthetic four-way junction DNA (*17*, *18*) which is often used as a model for DNA structure in chromatin at the sites where it enters and exits the nucleosome (*54*). The HMGI and HMGIYL1 proteins (Figure 1B) were assayed for binding to a 34 bp fragment of IFNβ promoter and four-way junction DNA by means of the mobility shift assay (Figure 3). In agreement with previous studies (*55*), under the conditions that were used, the HMGI protein interacted with the IFNβ promoter with high affinity (in a nanomolar range), yielding a stable complex (black arrow in Figure 3A). In contrast, even at a HMGIYL1 protein concentration of 1 μM, only a smear of shifted DNA was observed, suggesting weak and unstable interaction between the protein and DNA. Mobility shift studies using four-way junction DNA revealed that HMGIYL1 possesses similar affinity for this DNA as the HMGI protein (Figure 3B). When the changes in the

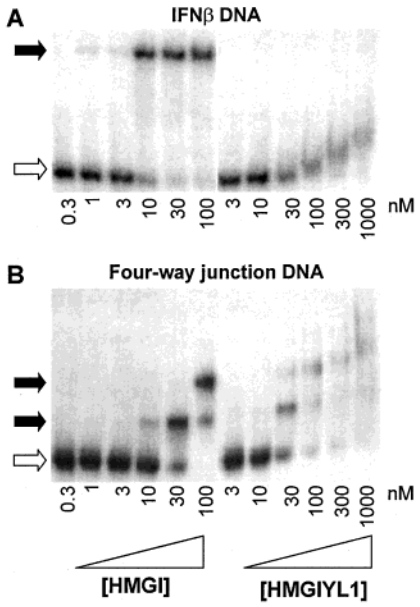

FIGURE 3: Binding of HMGI and HMGIYL1 to IFNβ promoter fragment (A) and four-way junction DNA (B). ^{32}P-labeled IFNβ DNA (<1 nM) or four-way junction DNA was incubated with increasing concentrations of HMGI or HMGIYL1 and electrophoresed on 8% polyacrylamide gels. The gels were dried and autoradiographed.

primary structure of the HMGIYL1 are considered, which include alterations in the first and the second DBDs, the loss of the affinity for the IFNβ promoter was expected. On the other hand, however, retaining the high-affinity binding to four-way junction DNA by HMGIYL1 suggested that regions outside the DBDs can be involved in HMGIYL1 binding to this DNA. To gain insights into the nature of the complexes of the proteins with both DNAs, protein footprinting studies were performed.

Protein Phosphorylation and Protein Footprinting. Protein footprinting is a novel method that allows mapping of contacts between proteins of interest and other macromolecules as well as studying conformational changes. With this technique, a protein of interest is labeled either at the N-terminal end or at the C-terminal end, incubated in the presence of an interacting molecule, and digested with hydroxyl radicals. The digestion products are analyzed by means of SDS electrophoresis. Comparison of the electrophoretic patterns of the end-labeled protein after digestion in the presence or absence of an interacting molecule reveals regions involved in protein binding. This technique was recently applied for characterization of the contacts between diverse HMGI/Y proteins with DNA of different conformations, like IFNβ promoter and four-way junction DNA (*45*, *46*).

The HMGI/Y proteins can be selectively end-labeled within their C-terminal region using casein kinase 2 (*46*). The C-terminal regions of HMGI and HMGIYL1 are identical. Both proteins possess three serine residues, Ser-98, Ser-101, and Ser-102 (Figure 1A), that can be easily in vitro phosphorylated by CK2. The phosphorylation of the C-terminal serine residues by CK2 reflects the native state of the HMGI/Y proteins (*56*−*58*).

One of the most important steps in protein footprinting analysis is the elicitation of molecular size markers that enable assignment of residue positions to the hydroxyl radical

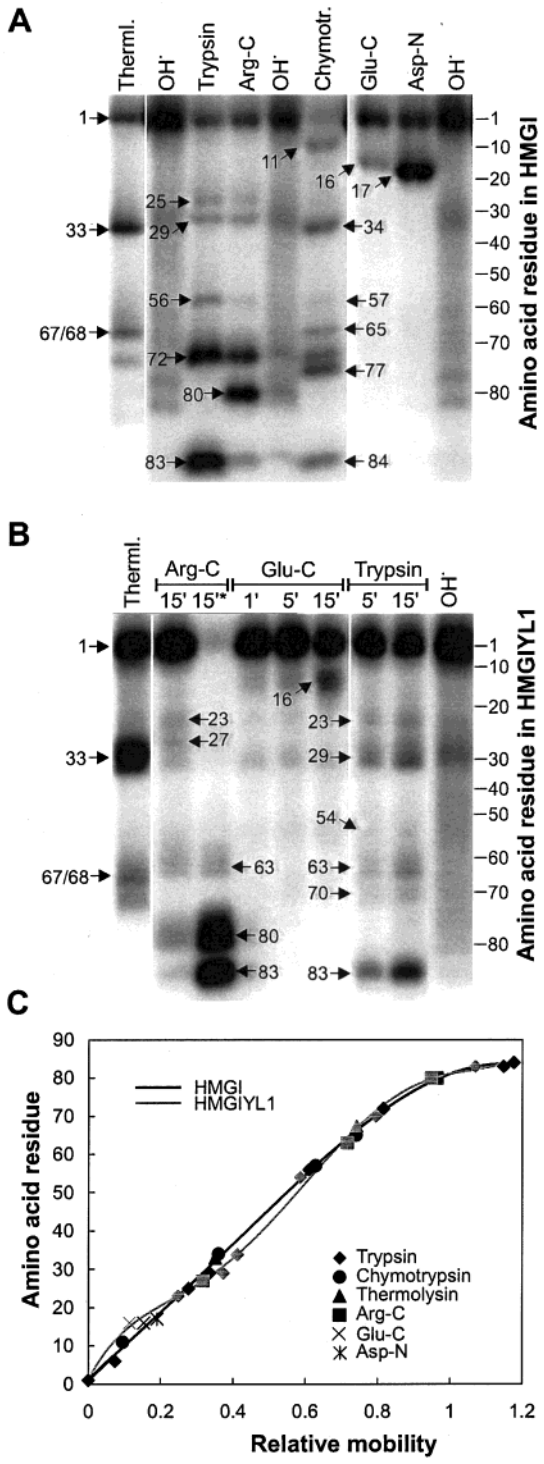

FIGURE 4: Size markers and assignment of the bands for protein footprinting of HMGI (A and C) and HMGIYL1 (B and C). Size markers were generated by site specific cleavage of ^{32}P-end-labeled HMGI with thermolysin (Therml.), trypsin, chymotrypsin (Chymotr.), and endoproteinases Glu-C, Asp-N, and Arg-C. OH$^{\bullet}$ lanes show peptide patterns of the protein digested with the hydroxyl radicals. (C) Plot of the size of peptide markers vs relative mobility. The relative mobility of noncleaved HMG protein was defined as 0 and the most rapidly migrating distinct band of hydroxyl radical cleavage as 1. Black and gray symbols correspond to peptide markers of HMGI and HMGIYL1, respectively.

patterns. For this purpose, the end-labeled HMGI and HMGIYL1 were digested with different proteinases. The digestion patterns obtained for both proteins are shown in panels A and B of Figure 4. The relative mobilities of the

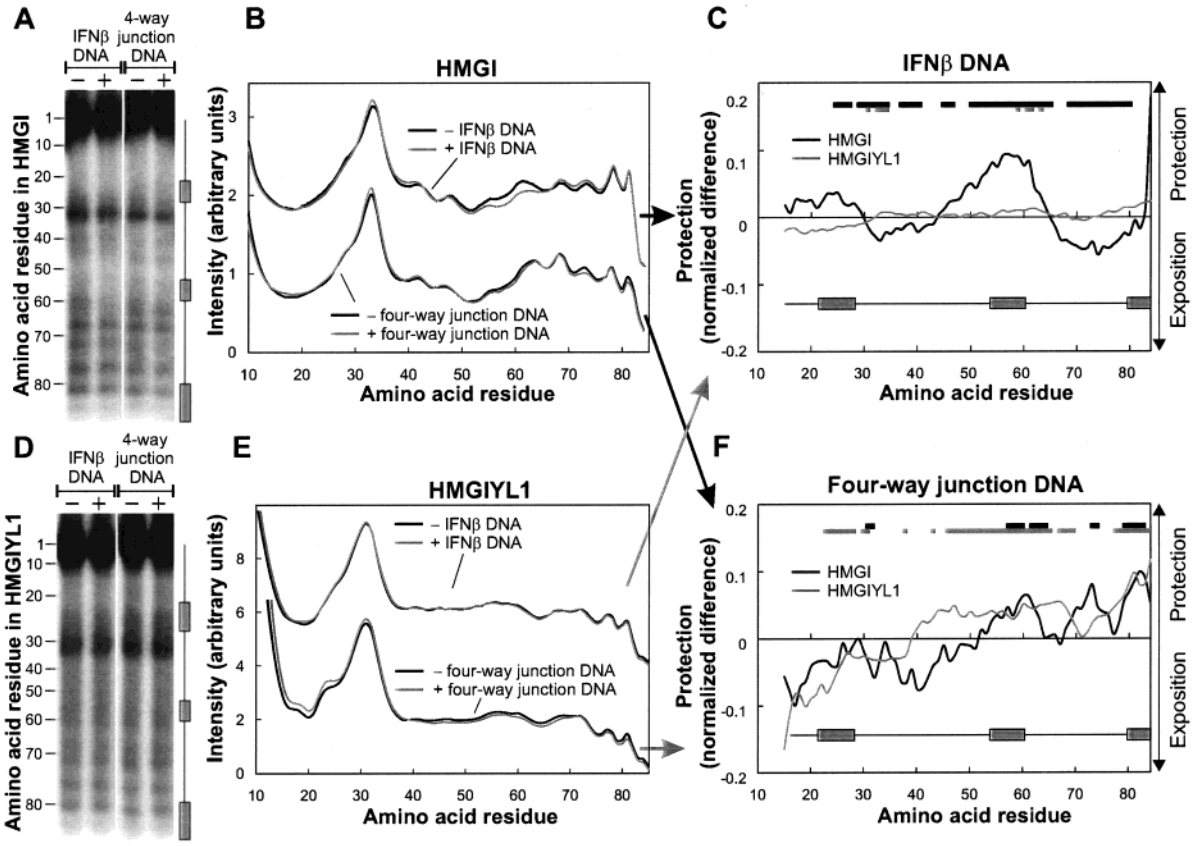

FIGURE 5: Hydroxyl radical protein footprinting of IFNβ fragment (A−E) and four-way junction DNA (4-way junction DNA) (A, B, and D−F) on the end-labeled proteins. Representative electrophoretic patterns of hydroxyl radical digestions of the HMGI (A) and HMGIYL1 (D) proteins in the absence (−) or presence (+) of IFNβ fragment (5/1 DNA/protein ratio) or four-way junction DNA (5/1 DNA/protein ratio). Plots of the corrected PhosphorImager intensities of the hydroxyl radical-digested HMGI (B) and HMGIYL1 (E). (C and F) Difference plots. Plots of the corrected intensities and the difference plots show averaged data from 6 to 12 independent experiments. Schematic primary structures of the proteins with DBD (boxes) are shown in the lower parts of the panels. Bold lines above the plots in panels C and F indicate regions where the observed protection or exposition of HMGI (black) or HMGIYL1 (gray) was statistically significant according to a Student's *t* test at a confidence level of 0.95.

end-labeled peptides were transformed by nonlinear regression into amino acid residues within the protein (Figure 4C), which then allowed the alignment of hydroxyl radical cleavage products as shown in panels A and B of Figure 4.

Point Mutations within the First and the Second DBDs Impair Binding of the HMGIYL1 to IFNβ Promoter but Not to Four-Way Junction DNA. Protein footprinting of the IFNβ promoter on the HMGI protein revealed that a region comprising the central DBD of the protein is strongly protected from proteolysis (Figure 5A−C). The protection of a region containing the first and third AT-hooks was much weaker. Footprinting of the IFNβ promoter on the HMGIYL1 protein did not show any significant protected region of the protein (Figure 5C−E). This result is consistent with the mobility shift experiments, showing weak and unspecific binding of HMGIYL1 to the IFNβ fragment (Figure 3A).

Protein footprinting of the four-way junction DNA on the HMGI protein showed that three regions of the protein are protected (Figure 5A,B,F). The major one corresponds to the protein region comprising the central DBD. In the C-terminal portion of the protein, regions between residues 68 and 76 as well as a region beyond residue 78 are protected (Figure 5F). The first region contains three positively charged residues, which are flanked by apolar residues. Interestingly, this region of the protein has the highest concentration of positive charges outside the DBDs. The second protected region corresponds to the N-terminal portion of the third AT-hook.

Binding of the four-way junction DNA to the HMGIYL1 resulted in the protection of the broad region between residues 40 and 70, as well as the C-terminally located portion of the protein beginning with residue 72 (Figure 5F). The first protected region contains the central AT-hook and the stretch of mainly apolar residues located N-terminal to them. The protection at residues above position 80 reflects binding of the third DBD.

DISCUSSION

The major finding emerging from this work is the demonstration that the product of transcription of the *HMGIYL1* retropseudogene retained the HMGI(Y) protein properties for translocating across the nuclear membrane, binding to chromosomes, and tight interaction with a particular type of DNA like the four-way junction DNA. Thus, probably despite mutations within two of its DBDs, the HMGIYL1 product still possesses binding activity which could affect organization of particular regions of chromatin. The HMGI/Y proteins share some DNA binding properties with linker histone H1. This includes recognition of binding to four-way junction DNA (*17*, *18*) and interaction with isolated nucleosomes (*19*), and a preference for AT-rich DNA. The HMGI/Y proteins bind to four-way junction DNA

with higher affinity than histone H1 and therefore compete efficiently for binding to this DNA (*18*, *59*).

The HMGIYL1 product has lost its affinity and specificity for the intrinsically prebent promoter of the IFNβ gene, and thus, it appears that this protein would not be able to exert a role in organization of higher-order promoter−enhancer complexes like those of the IFNβ gene. Moreover, the results of the mobility shift and footprinting analyses explain why HMGIYL1 has lost its unbending activity against the IFNβ promoter (our unpublished results) and stress the importance of intramolecular cooperativity of AT-hooks in HMGI/Y proteins (*17*, *60*, *61*). A single AT-hook binds with only moderate affinity (micromolar range) (*17*, *62*) to DNAs and confers this binding specificity to chimeric proteins consisting of additional domains (*63*).

Previous analyses employing fragments of HMGI proteins (*61*, *64*) and protein footprinting analysis (Figure 5) demonstrated that the second DBD of the protein plays the central role in binding to DNA. Mutation of an arginine residue to a glutamine residue within the central core tripeptide RGR of the DBD2 is probably responsible for the loss of DNA binding within this region of the protein. These data emphasize the importance of the N-terminal arginine residue in the organization of the binding region. The structure of the complex of the N-terminally located DBD1 of the HMGI-(Y) with DNA has still not been determined. Our results suggest, however, that it is similar to DNA complexes of DBD2 and DBD3 (*62*) because mutation of the corresponding arginine residue within the DBD1 impairs DNA contacts.

The HMGIYL1 protein consists of four additional bulky hydrophobic residues, two tryptophans, one leucine, and one isoleucine, that are not present in the HMGI protein. This is a doubling of the number of this type of residue, which may affect the organization of the HMGIYL1−DNA complex in an indirect way via hydrophobic interaction(s) within the protein. Comparison of the footprinting patterns obtained for HMGI and HMGIYL1 on the four-way junction suggest that each of the analyzed proteins interacts in a specific way with the DNA (Figure 5F). Differences observed in the protein regions comprising residues 40−50 and 65−75 cannot be explained solely by the existence of a few mutated residues (Figure 1A). Therefore, we suggest that intramolecular interactions in the HMGIYL1 protein are responsible for differences in the DNA contacts in comparison to those exerted by HMGI.

The tight binding of the HMGIYL1 product to four-way junction DNA raises the question as to which structural elements are primarily responsible for this protein property. The HMG proteins representing diverse HMG families like those of the HMG1 and HMG14/17 family carry the peptide PKRP. Interestingly, proteins belonging to these groups exhibit high affinity for binding to four-way junction DNA. In the HMG1 proteins, this peptide is primarily contributed to the minor groove contacts with DNA ligands (*49*); therefore, it appears that this peptide located N-terminally adjacent to the central RGR tripeptide of the DBD2 is also involved in organization of the HMGIYL1−four-way junction complex. Thus, in this complex, a cooperation in binding between the PKRP region and the third DBD could explain tight binding of the protein mutant to this DNA.

Proteins carrying AT-hooks have been reported in diverse organisms ranging from bacteria to humans. They occur not only in canonical HMGI/Y proteins found in mammals, but the majority of the AT-hooks was found in proteins possessing other DNA binding domains (*65*). In fact, however, in many of the identified AT-hooks, the N-terminal arginine residue in the RGR core peptide is lacking. Therefore, they are probably not able to exert their minor groove binding activity. For example, the *Drosophila* D1 protein contains 10 AT-hook-like regions, but only seven of them have the intact RGR tripeptide (*66*).

This study carried on the "natural mutant" of the HMGI protein is the first mutational analysis in which a protein was mutated within its AT-hooks. Future studies in which designed protein mutants will be elicited and used in binding studies are necessary for understanding in detail the important role of particular residues with the HMGI/Y proteins for binding to ligands of diverse conformations.

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