NF-κB mediated transcriptional activation is enhanced by the architectural factor HMGIC

Fiamma Mantovani, Sonia Covaceuszach, Alessandra Rustighi, Riccardo Sgarra, Carol Heath¹, Graham H. Goodwin¹ and Guidalberto Manfioletti*∗

Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università di Trieste, via Giorgieri, 1 34127 Trieste, Italy and ¹The Institute of Cancer Research, Haddow Laboratories, Sutton, UK

Received November 28, 1997; Revised and Accepted February 2, 1998

ABSTRACT

High mobility group I proteins (HMGI, HMGY and HMGC) are a family of low molecular mass non-histone nuclear proteins which constitute an important component of the active chromatin structure. Two members of this family, HMGI and HMGY, have been demonstrated to contribute to the transcriptional regulation of several promoters by interacting with the DNA and with different transcription factors. On the contrary, very little is known about the third member, HMGC, which plays an important role during embryonic growth and in the process of cell transformation, its gene being rearranged in a large number of mesenchimal tumors. In this paper we show for the first time that HMGC is also able to function as architectural factor, enhancing the activity of a transcription factor, NF-κB, through the PRDII element of the β-interferon enhancer. Moreover we show that this enhancement is absolutely dependent on the binding of HMGC to its target sequence. The demonstration that HMGC is able to modulate transcription is thus an important initial step in the identification of genes regulated by this factor.

INTRODUCTION

The high mobility group proteins HMGC, HMGI and HMGY belong to a family of non-histone nuclear proteins collectively termed as HMGI proteins. These architectural factors are able to modulate DNA conformation and are involved in fundamental processes such as transcription and recombination (1). Although encoded by a different gene (2), HMGC shares some structural homologies with HMGI and HMGY, which are produced by differential splicing from a single gene, and therefore also referred as HMGI(Y) (3). All three proteins in fact have three short basic domains termed AT-hooks which recognise AT-rich DNA sequences via the minor groove, and highly acidic C-terminal tails (1).

HMGI expression levels are very low or undetectable in adult tissues, but after oncogenic transformation their expression is up-regulated reaching levels comparable to those observed during early embryo development (4,5). The existence of a correlation between the expression levels of HMGI proteins and the degree of malignant transformation has suggested their use as tools for tumor diagnosis (6,7).

Direct evidence that HMGC participates in the oncogenic process was first provided when the expression of antisense HMGC RNA was shown to prevent retrovirally induced neoplastic transformation of rat thyroid cells (8). Moreover, the human HMGC gene, mapped to a ‘multiple aberration region’ (MAR) on chromosome 12, has been reported to be a target for translocations in eight different benign solid tumor types (9–11) and to be amplified and rearranged in a number of malignant sarcomas (12). Because of the extremely high incidence of these tumors, this rearrangement can be considered one of the most frequent aberrations in human tumors (13).

It has been shown that disruption of the Hmgi-c gene in mice results in growth retardation and pygmy phenotype characterized by a marked inhibition of adipose tissue development (4). Since HMGI(Y) expression remains unaltered in these mice, this implies that HMGI(Y) cannot compensate for the lack of HMGC, suggesting that their functions are not redundant and therefore implying a specific role for each of the HMGI proteins. It could therefore be possible that HMGC regulates a different set of genes than HMGI(Y).

Although increasing evidence is accumulating on the biological relevance of the HMGC protein in proliferation and development, very little is known about its biochemical properties, such as its DNA-binding specificity and potentiality to modulate transcription. Its homology to HMGI(Y) suggests that it could have a similar function as architectural transcription factor, but despite the increasing number of gene regulatory elements where HMGI(Y) have been shown to cooperate with other proteins to modulate transcription (positively or negatively) (14–20), there are still no data showing that HMGC is able to act in a similar way.

In this paper we start to address this point by asking whether HMGC is able to modulate transcription. To this aim we have used a simple system consisting of the well characterised PRDII element of the β-interferon promoter (14,21,22). Previous work by Thanos and co-workers has shown that the HMGC protein binds to this element together with the p50/p65 NF-κB heterodimer to activate transcription from the promoter. In this paper we demonstrate that HMGC is able to bind the PRDII element with similar affinity as HMGY and that it can enhance the activating properties of the transcription factor NF-κB. Moreover, we demonstrate that this enhancement is dependent on the binding of the protein to its target sequence.

∗To whom correspondence should be addressed. Tel: +39 40 6763675; Fax: +39 40 6763694; Email: manfiole@univ.trieste.it

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MATERIALS AND METHODS

Oligonucleotides

The following oligonucleotides were used in gel-retardation assays and for reporter constructs (only the upper strand sequence is shown): PRDII, 5′-GGGAAATTTCCGGGAAAATTCGAGCT-3′; mPRDII, 5′-GGGAGATTTCCGGGAGATTCCGAGCT-3′; IgkB, 5′-GGGACCTTCCGATGACTTTCCGAGCt-3′. For the band-shift analysis of the effect of HMGI-C on the binding of the NF-κB to the PRDII element, a shorter oligonucleotide (16mer), containing only one PRDII element, was used.

Protein expression and purification

Recombinant HMGI proteins were expressed as previously described (20) using the murine HMGI-C and HMGY cDNAs cloned in the vector pAR3038, expression being under control of the bacteriophage T7 promoter. Briefly, proteins were expressed using the BL21 (DE3) Escherichia coli strain which contains the T7 RNA polymerase under lacUV5 promoter control. Cultures were grown to OD_{600} = 0.6, induced with 0.5 mM isopropyl β-D-thiogalactopyranoside, grown overnight and harvested. HMGI proteins were selectively extracted from bacterial cells with 5% (v/v) perchloric acid and precipitated with acetone–HCl (23). The proteins were purified by reverse-phase HPLC on a BioRad RP304 column using a Waters apparatus as described elsewhere (24). The purified recombinant HMGI proteins were analyzed by mass spectrometry (Perkin-Elmer API I spectrometer) to confirm that they had the correct molecular masses. The NF-κB subunits, p50 and p65, were provided by Dr Ron Hay (University of St Andrews). These subunits were expressed as GST fusion proteins. After purification the proteins were cleaved with thrombin and further purified on a DNA–Sepharose column. GST fusion proteins. After purification the proteins were cleaved with thrombin and further purified on a DNA–Sepharose column.

Band-shift analyses

Double-stranded oligonucleotides were labeled at the 5′-end with [γ-32P]ATP and 10 U of T4 polynucleotide kinase (Epitecten Technologies) or by filling in the 5′ overhang with Klenow enzyme and [α-32P]dCTP. Unincorperated nucleotides were removed by chromatography through a Sephadex G-50 (Pharmacia) spin-column equilibrated in TEN buffer (10 mM Tris–HCl, pH 7.5, 1 mM EDTA, 100 mM NaCl). One hundred fmol of each probe (30 000 c.p.m.) were then separately incubated with increasing concentrations of HMGY or HMGI-C protein (5–200 ng, as indicated) in 20 μl reactions containing 20 mM Tris–HCl, pH 7.5, 75 mM KCl, 5 μg/μl BSA, 1 mM DTT, 13% glycerol for 20 min at room temperature. After incubation, protein-bound DNA and free DNA were separated on native 7% polyacrylamide gel run in 0.5× TBE, at 15 V/cm at 4°C. Gels were then dried and exposed to X-ray films (Hyperfilm MP, Amersham). For band-shifts of NF-κB and HMGI-C, 12.5 ng of HMGI-C and a range from 50 to 0.5 ng of NF-κB were used.

Plasmid construction

Basic TKluc reporter vector was constructed as follows. A BamHI–XbaI fragment, containing the Herpes Simplex Virus (HSV) Thymidine Kinase (TK) promoter, was obtained from pT109luc plasmid (25) and inserted into pGL2basic luciferase reporter vector (Promega), cleaved with BglII–XbaI. PRDII TKluc, mPRDII TKluc and IgκB TKluc reporter plasmids were constructed by inserting the corresponding double-stranded (ds) oligonucleotides listed above into the Smal–SacI sites of BasicTKluc, upstream of the TK promoter.

The pSVHMGY expression construct containing the full-length murine HMGY cDNA under the control of SV40 promoter has been described previously (20). pSVHMGY-C was constructed by inserting into the pGD57 expression vector (26) a 1.8 kb EcoRI–HindIII fragment containing the full-length murine HMGI-C cDNA (2). cDNAs for p50 and p65 NF-κB components, kindly provided by Dr Neil Perkins (Dundee University), were under control of the RSV promoter (27).

Cell culture and transfections

NIH-3T3 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum at 37°C in a humidified 5% CO2 incubator. PC Cl3 and FRTL-5 Cl2 cells were maintained in Coon’s modified Ham’s F12 medium supplemented with 5% fetal calf serum and six growth factors: the thyrotropic hormone (TSH), insulin, transferrin, somatostatin, hydrocortisone and the tripeptide glycyl-histidyl-lysine as described (28). Cells were plated at a density of 0.5 × 10^6 cells per 60 mm diameter culture dish and transfected by the calcium phosphate co-precipitation procedure. For NIH-3T3 transfection, precipitates containing 2 μg of reporter plasmid, 5.2 μg of expression constructs and 0.4 μg of pSV β-galactosidase expression vector (Promega) as internal control, were applied to subconfluent cells 16 h before changing medium. Total amounts of each expression plasmid were kept constant in all the experiments by adding the corresponding empty vectors. For PC Cl3 and FRTL-5 Cl2 transfection, precipitates were left on cells for 1 h, followed by glycerol shock treatment and medium replacement. Due to the lower transfection efficiency of thyroid cells, internal control was provided by adding 0.25 μg of pRL-TK Renilla luciferase expression vector (Promega), which allows a more sensitive detection of enzyme activity.

β-Galactosidase and luciferase assays

After transfection (48 h), cells were harvested and lysed with lysis buffer (Promega). For NIH-3T3 cells, firefly luciferase enzyme assays were performed using the Luciferase Assay System (Promega) in a luminometer (Lumat LB 9501, EG&G Berthold) according to the manufacturer’s instructions. Colorimetric β-galactosidase assays were performed using o-nitrophenol-β-D-galactoside (ONPG-Sigma) as substrate. Luciferase activity of the reporter vector was then normalized for transfection efficiency using β-galactosidase activity. For PC Cl3 and FRTL-5 Cl2 cells, reporter (firefly luciferase) and control (Renilla luciferase) enzyme assays were performed using the Dual Luciferase Reporter Assay System (Promega).
Figure 1. HMGI-C and HMGY bind to PRDII with the same affinity. The autoradiogram of an EMSA analysis is shown, in which binding affinity of HMGI-C and HMGY to the PRDII oligonucleotide was compared. \(^{32}\)P-labeled ds-PRDII oligonucleotide was incubated in separate reactions with corresponding amounts of the two purified recombinant proteins. Lane 1, free probe; lanes 2–6, decreasing amounts (100, 50, 25, 10 and 5 ng, respectively) of HMGI-C; lanes 7–11, decreasing amounts (100, 50, 25, 10 and 5 ng, respectively) of HMGY.

RESULTS

To investigate if HMGI-C could play a similar role as HMGY in the assembly of a complex able to activate transcription from a combinatorial promoter, the well characterized model of the human \(\beta\)-interferon enhancer was chosen. Previous work has shown that HMGY plays a structural role by facilitating the binding of different transcription factors to their consensus sequences and this contributes to the viral inducibility of the entire enhancer (14). Part of the enhancer consists of the PRDII element, which binds NF-\(\kappa\)B in the major groove and HMGY in the minor groove of the central AT-rich region (14). Binding of the HMGY protein has been shown to potentiate the transcriptional activation by the NF-\(\kappa\)B protein and we have sought to investigate whether the HMGI-C protein will function in a similar manner.

HMGI-C binds to the PRDII element \textit{in vitro} with the same affinity as HMGY

As a first step, band-shift analyses were performed to test the ability of HMGI-C to interact with the PRDII element. A ds-PRDII oligonucleotide, which contains two copies of the PRDII element, was incubated with different concentrations of HMGY and HMGI-C recombinant proteins and subjected to EMSA. As shown in Figure 1, incubation with either HMGY or HMGI-C produces a protein–DNA complex, whose mobility is higher for HMGY than for HMGI-C (in accordance with the different molecular weights of the proteins), and whose intensity increases with protein concentration. Interestingly, no significant differences of intensity can be noted when comparing identical concentrations of HMGY and HMGI-C, suggesting a very similar binding affinity of the two proteins for the PRDII sequence. It should be noted that dimer band-shifts are not observed; this could be because binding of one HMGI molecule to one AT element precludes the binding of a second molecule or, more likely, because of the stronger binding of one HMGI molecule utilising two AT-hooks to occupy both AT elements simultaneously, as suggested for DNA sequences with multiple AT tracts (22,29).

Figure 2. HMGY and HMGI-C bind to the wild-type PRDII site but not to the mutated mPRDII or Ig\(\kappa\)B sites. (A) Comparison of PRDII, mPRDII and Ig\(\kappa\)B sequences. The bases which are different in mPRDII and Ig\(\kappa\)B with respect to PRDII are indicated in bold character, the central region recognized by HMGI proteins is boxed. (B) Binding of HMGY to PRDII, mPRDII and Ig\(\kappa\)B oligonucleotides. EMSA analyses were performed incubating decreasing amounts of recombinant HMGY protein with either PRDII, mPRDII or Ig\(\kappa\)B labelled oligonucleotide probes. Lanes 1, 6 and 11, free PRDII, mPRDII and Ig\(\kappa\)B probes; lanes 2–5, 7–10 and 12–15, PRDII, mPRDII and Ig\(\kappa\)B incubated with 200, 100, 50 and 25 ng of HMGY, respectively. (C) Binding of HMGI-C to PRDII, mPRDII and Ig\(\kappa\)B oligonucleotides. EMSAs were performed incubating decreasing amounts of recombinant HMGI-C protein with the same probes as in (B). Lanes 1, 6 and 11, free PRDII, mPRDII and Ig\(\kappa\)B probes; lanes 2–5, 7–10 and 12–15, PRDII, mPRDII and Ig\(\kappa\)B incubated with 200, 100, 50 and 25 ng of HMGI-C, respectively.

Disruption of the AT-rich core of PRDII abolishes the binding of both HMGY and HMGI-C

In order to test the sequence-specificity of HMGI-C binding to the PRDII element, two different oligonucleotides, mPRDII and Ig\(\kappa\)B, were synthesized where the AT-rich central core of PRDII is disrupted. As shown in Figure 2A, mPRDII differs from PRDII...
by a single base pair substitution (A–G), while Igκ oligonucleotide bears the NF-κB consensus found in the immunoglobulin light chain gene enhancer. Both sequences have been shown to have lower HMGY binding affinity when compared to PRDII, while neither mPRDII nor Igκ oligonucleotides produced a specific band-shift, although at high protein concentration, some weak and unstable complexes are generated in the case of mPRDII, indicating that although at high protein concentration, some weak and unstable complexes are generated in the case of mPRDII, indicating that a labile interaction can occur under these conditions.

When the same experiment was performed with HMGY-C (Fig. 2C), a similar behaviour was observed, since this protein also fails to bind both mPRDII and IgκB probes at the concentrations which produce a strong shift on the wild-type sequence. Again, a weak binding to the mPRDII sequence can be observed only at high protein concentrations.

HMGY-C enhances the ability of NF-κB to bind to the PRDII element in vitro

To investigate the possibility that HMGY-C, like HMGY(Y), is able to enhance the binding of NF-κB to the PRDII element, band-shift experiments were carried out using similar conditions to those described by Thanos and Maniatis (14). The PRDII oligonucleotide was incubated with decreasing amounts of HMGY protein, while neither mPRDII nor IgκB probes confirmed this finding, since only the wild-type PRDII oligonucleotide gives a specific band-shift, while neither mPRDII nor IgκB show detectable retarded bands, although at high protein concentration, some weak and unstable complexes are generated in the case of mPRDII, indicating that a labile interaction can occur under these conditions.

Figure 3. HMGY-C enhances the binding of NF-κB to the PRDII element. The autoradiogram of an EMSA analysis is shown where a range of concentrations of NF-κB were incubated with the PRDII oligonucleotide in the absence or in the presence of 12.5 ng of HMGY-C. Lane 1, free probe; lanes 2–9, decreasing amounts (50, 16, 1.3, 1.0, 0.8, 0.7, 0.6 and 0.5 ng, respectively) of NF-κB without HMGY-C. Lanes 10–16, decreasing amounts of NF-κB, as in lanes 3–9, with 12.5 ng of HMGY-C.

Both HMGY and HMGY-C are able to enhance NF-κB mediated transcriptional activation through the PRDII element

Having demonstrated that HMGY-C, like HMGY, is capable of sequence-specific binding to the IFN-β PRDII element and enhances the binding of NF-κB in vitro, it was of interest to verify if it could also have a similar function in cooperating in vivo with NF-κB in transcriptional activation through this enhancer element. A reporter construct, bearing an artificial enhancer containing two copies of PRDII, was then generated by subcloning the PRDII oligonucleotide, previously employed in band-shift assays, upstream of the HSV TK promoter driving the expression of the firefly luciferase gene. This reporter, named PRDII TKluc, was then assayed for transactivation by NF-κB in the differentiated rat epithelial cell line PC13. This cell line was chosen because of its total lack of endogenous HMGY expression (30); it was thus possible to establish the transactivation levels achieved solely by NF-κB. Cotransfection of a fixed amount of reporter vector with increasing amounts of an equimolar mixture of p50 and p65 expression vectors led to a linear increase (up to 14-fold) of luciferase activity (data not shown). The PRDII TKluc reporter was then cotransfected with a fixed amount of p50/p65 in order to achieve a transactivation level of ∼5-fold, and the experiment was performed in the presence of either the HMGY or HMGY-C expression vectors, or the empty SV40 expression vector. Cotransfection of HMGY resulted in a further increase of luciferase activity, up to 11–12-fold over the basal level (Fig. 4), confirming the results obtained by other groups, with different constructs and cellular systems (22,31). Interestingly, very similar levels of enhancement were observed also for the HMGY-C protein (Fig. 4). These results demonstrate that, at least in this model system, HMGY-C is able to act as a factor enhancing the activity of a transcription factor, NF-κB.

Neither of the two HMGY proteins is capable of direct transactivation, since transfection of either HMGY or HMGY-C with PRDII TKluc in the absence of p50/p65 expression vectors did not produce any detectable effect on luciferase activity (Fig. 4). The control reporter vector Basic TKluc, lacking PRDII element, was not affected by cotransfection with the expression vectors in all combinations, demonstrating that the effects are specific for the PRDII element (not shown).

NIH-3T3 cells, which express endogenous HMGY proteins (32, and our observations), were also used in the assay, cotransfecting PRDII TKluc reporter with NF-κB and HMGY-C plasmids. Figure 5 shows that in this different system overexpression of these two proteins is able to enhance transactivation to similar levels.

Mutations that decrease the binding affinity of HMGY and HMGY-C to the PRDII sequence in vitro abolish HMGY/ HMGY-C-dependent transcription enhancement in vivo

To determine whether binding to the AT-rich core was required for HMGY proteins to facilitate transactivation of the PRDII by NF-κB, two reporter constructs were generated in which the oligonucleotides mPRDII and IgκB, which are not bound by HMGY proteins as demonstrated above, were inserted upstream of the TK promoter. The constructs mPRDII TKluc and IgκB TKluc, as well as the wild-type reporter PRDII TK luc, were then cotransfected with p50/p65 expression vectors alone or in combination with either HMGY or HMGY-C. Figure 6 shows the
Figure 4. HMGY and HMGI-C show similar abilities in enhancing NF-κB mediated activation of PRDII Tkluc. PC Cl3 cells were transiently transfected with 2 µg of PRDII Tkluc reporter vector alone or in various combinations with 200 ng of an equimolar mixture of p50/p65 expression vectors and 5 µg of either pSVHMGY or pSVHMGI-C expression plasmids, as indicated in the lower part of the figure. Total amounts of each expression plasmid were kept constant in all the experiments by adding the corresponding empty vectors. pRL-TK Renilla luciferase expression vector (0.25 µg) was included as internal standard. Transcriptional activation is indicated as percentage of the luciferase activity measured for reporter alone; columns and bars represent the mean and standard deviation, respectively, of at least five independent experiments.

Figure 5. Enhancement of transcriptional activity by HMGY and HMGI-C occurs also in NIH-3T3 cells. NIH-3T3 cells were transiently transfected with 2 µg of PRDII Tkluc reporter vector alone or in combination with 200 ng of p50/p65 mixture and 5 µg of either pSVHMGY or pSVHMGI-C expression vectors, as indicated in the scheme. Total amounts of each expression plasmid were kept constant in all the experiments by adding the corresponding empty vectors. Samples (400 ng) of pSV β-galactosidase expression vector were included as internal standard. Three separate experiments were performed and means and standard deviations calculated. Transcriptional activity is indicated as percentage of that obtained for reporter alone.

DISCUSSION

Eukaryotic cells are able to respond to a large number of different extracellular signals and environmental stresses, leading to the activation of specific sets of genes. The understanding of this process is complicated by the fact that many transcription factors like homeodomain-containing proteins and members of the Rel family, once activated can bind specifically to regulatory sequences upstream of a large number of different genes (33), but only a subset of such genes are activated in response to a given signal. One mechanism through which this specificity is achieved is the interaction with accessory factors that can bind nearby, altering the DNA conformation and consequently facilitating or inhibiting the interaction of a transcription factor with DNA. HMGI proteins are a class of non-histone chromosomal proteins that have been shown to fulfill this role, acting as ancillary or architectural factors. By doing so they can increase the affinity (14–18) or can compete for the binding (19,20) of the transcription factor to the DNA. Probably the best characterized example is the virus-inducible enhancer of the human interferon-β (IFN-β) gene (14,21,22) where four positive regulatory domains (PRDI, PRDII, PRDIII and PRDIV) are present. HMGI(Y) are required for the transcriptional activities of both NF-κB and ATF-2/c-Jun, binding respectively to the PRDII and PRDIV elements of the promoter. For this reason we chose the PRDII element to

results of parallel experiments in PC Cl3 cells. Expression of HMGY and HMGI-C was not able to cause a transcriptional enhancement above the levels achieved by NF-κB when the mPRDII TKluc and the IgκB TKluc reporters were used, in contrast to the enhancement observed with the PRDII TKluc reporter. These results were confirmed using another rat thyroid cell line, FRTL-5 Cl2, that also lacks HMGI proteins (30), and again HMGI-dependent enhancement was seen only with reporters that had the AT-core sequence (not shown).
investigate whether another member of the HMGI family, HMGI-C, is able to function as an architectural factor facilitating the transactivating ability of NF-κB.

HMGI(Y) proteins contain a DNA-binding motif, the AT-hook, which is repeated three times in the protein sequence. It has been shown that this motif is able to specifically bind to the minor groove of AT-rich DNA sequences (1). HMGI-C also contains three AT-hooks suggesting that this protein binds to similar sequences to those bound by HMGI(Y), but since the proteins differ in amino acid sequence outside the AT-hooks and the acidic C-terminals, it is possible that HMGI-C functions differently to HMGI(Y) in the regulation of transcription. There are in fact many examples of nuclear factors sharing the same type of DNA binding domain which, however, display quite different transacting capabilities.

In this report we show that HMGI-C is able to bind to the PRDII element with the same affinity as HMGY. The integrity of the AT-rich core present in the PRDII element is necessary for the binding of both HMGY and HMGI-C since a single base pair substitution is sufficient to drastically decrease their affinity for the DNA. We also demonstrate that HMGI-C is able to enhance the binding of NF-κB to this element. Furthermore like HMGY, HMGI-C enhances the NF-κB mediated transcriptional activation of the PRDII element in different cell lines. Since it was not able to affect the basal activity of the PRDII TKluc reporter in the absence of NF-κB, we can conclude that all the HMGI proteins do not possess transactivation capabilities on their own, but rather act by influencing the activities of classical transcription factors. It is apparent that the HMGI proteins need to bind to DNA in order to exert their effect since HMGY and HMGI-C are not able to enhance NF-κB mediated transactivation when transfected with the reporter vectors mPRDII TKluc and IgκB TKluc which, although able to bind NF-κB, do not bind HMGY and HMGI-C strongly. It has been shown that HMGI(Y) are able to interact with NF-κB in the absence of DNA and our data would suggest that such protein–protein interactions might stabilize the ternary complex. This mechanism is thus different to that described for other nuclear proteins like the viral tax protein that can facilitate the binding of transcription factors without binding to the DNA (34).

Thanos and Maniatis originally demonstrated that the HMGI(Y) protein was required for the virally-induced and NF-κB-dependent promoter activity of the PRDII element, by using an antisense HMGI(Y) construct in transient transfection experiments (14). Transfection of the antisense plasmid into HeLa and Jurkat T cells inhibited the PRDII element, demonstrating the requirement for HMGI(Y), but furthermore may suggest that HMGI-C cannot substitute for HMGI(Y). However T cells do not express HMGI-C (35) and HeLa express very low levels, as judged by northern analysis of HeLa cell RNA (unpublished data). Also, in experiments with antisense HMGI-C we found that the expression of HMGI(Y) as well as HMGI-C is inhibited (8) and so it is possible that an HMGI(Y) antisense would inhibit the expression of HMGI-C as well. Thus, the antisense experiments of Thanos and Maniatis do not exclude the possibility that HMGI-C can bind to and activate the PRDII element in the similar manner as HMGI(Y).

HMGI(Y) act as accessory factors on a number of other inducible promoter elements, interacting with the transcription factors ATF-2 (36), IRF-1 (21), Elf-1 (16) and Oct-2A (17), but it is not known whether HMGI-C interacts with any of these proteins. Whilst it is possible that the three HMGI proteins interact with some common partners, such as NF-κB, it is likely that the functional specificity of these proteins resides in their interactions with different subsets of transcription factors. Thus,
since an altered HMGI-C protein is involved in the abnormal growth and development of fat cells resulting in lipomas (9–11), and that HMGI-C knock-out is responsible in the pygmy mouse for a disproportionate reduction in body fat (14) which is not compensated by HMGI(Y), it is feasible that HMGI-C might specifically interact with transcription factors involved in adipocyte growth and development.

Promoter structure may also play a role in determining whether HMGI(Y) or HMGI-C is recruited. In fact, regulatory elements which contain multiple AT sites interact with HMGI proteins with higher affinity, this cooperative binding is possible since two or three AT-hooks of the same molecule are simultaneously employed in the interaction with DNA (22,29). In this context the different lengths of the spacer regions between the AT-hooks of the HMGI proteins might be relevant (2); AT elements that are different lengths of the spacer regions between the AT-hooks of HMGI(Y) or HMGI-C is recruited. In fact, regulatory elements specifically interact with transcription factors involved in adipocyte growth and development.

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ACKNOWLEDGEMENTS

This study was supported by Associazione Italiana per la Ricerca sul Cancro (AIRC), Milano, Italy and Università degli Studi di Trieste, Italy. F.M. was a recipient of a fellowship from Fondazione Italiana per la Ricerca sul Cancro (FIRC). G.H.G. is supported by the Cancer Research Campaign. We thank V.Giancotti for continuous encouragement and advice during this work.

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