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*J Immunol* 2000; 164:3157-3168; doi: 10.4049/jimmunol.164.6.3157

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The Role of High-Mobility Group I(Y) Proteins in Expression of IL-2 and T Cell Proliferation

S. Roy Himes, Raymond Reeves, Joanne Attema, Mark Nissen, Ying Li, and M. Frances Shannon

The high-mobility group I(Y) (HMG(IY)) family of proteins plays an important architectural role in chromatin and have been implicated in the control of inducible gene expression. We have previously shown that expression of HMG(IY) antisense RNA in Jurkat T cells inhibits the activity of the IL-2 promoter. Here we have investigated the role of HMG(IY) in controlling IL-2 promoter-reporter constructs as well as the endogenous IL-2 gene in both Jurkat T cells and human PBL. We found that the IL-2 promoter has numerous binding sites for HMG(IY), which overlap or are adjacent to the known transcription factor binding sites. HMG(IY) modulates binding to the IL-2 promoter of at least three transcription factor families, AP-1, NF-AT and NF-kB. By using a mutant HMG(IY) that cannot bind to DNA but can still interact with the transcription factors, we found that DNA binding by HMG(IY) was not essential for the promotion of transcription factor binding. However, the non-DNA binding mutant acts as a dominant negative protein in transfection assays, suggesting that the formation of functional HMG(IY)-containing complexes requires DNA binding as well as protein:protein interactions. The alteration of HMG(IY) levels affects IL-2 promoter activity not only in Jurkat T cells but also in PBL. Importantly, we also show here that expression of the endogenous IL-2 gene as well as proliferation of PBL are affected by changes in HMG(IY) levels. These results demonstrate a major role for HMG(IY) in IL-2 expression and hence T cell proliferation. The Journal of Immunology, 2000, 164: 3157–3168.
and have numerous modes of action by which they modify gene transcription (reviewed in Ref. 29). The HMGI(Y) family of proteins consists of three members, HMGI and HMGY, which are produced by alternative splicing of mRNA from the same gene locus, and HMGC, which is coded for by a related gene at a separate locus (29). These proteins have been classed as architectural transcription factors because they do not act as transactivators in their own right but modify the function of other proteins (30). HMGI(Y) proteins can interact directly with several families of transcription factors including Rel/NF-κB, bZip, Ets, homeodomain, and Pou domain proteins and lead to an alteration in their DNA binding to sites that either overlap or are adjacent to A:T-rich HMGI(Y) binding sites (29). The binding of HMGI(Y) also induces structural changes in DNA substrates (31, 32) that, in turn, often leads to alterations in the complex of transcription factors into higher order functional complexes (29). In addition, HMGI(Y) appears to play a critical role in chromatin architecture (33) and has been shown to interact specifically with isolated nucleosome core particles (34), to alter the rotational setting of DNA on the surface of nucleosomes (35), and to antagonize H1-mediated transcriptional repression (36, 37).

Here we show that HMGI(Y) can bind to numerous sites across the IL-2 proximal promoter region and modulate the binding of the major transcription factor families that are thought to control IL-2 gene transcription. Functional studies in both Jurkat T cells and primary T cells show that HMGI(Y) plays a major role in the regulation of the IL-2 gene and hence T cell proliferation.

Materials and Methods

Cell culture and transfection

The basic medium for Jurkat cell culture was RPMI 1640 medium containing 10% FCS, supplemented with l-glutamine and penicillin-streptomycin antibiotics (RPMI). The basic medium for PBL culture was as above but contained 20% FCS, 100 μM 2-ME, and 5% conditioned medium (RPMIL). Mononuclear cells were isolated from peripheral blood using lymphoprep (Nycomed Pharma AS, Oslo, Norway) and cultured for 4 days in RPMIL and PHA (5 μg/ml) (Boehringer Mannheim, Mannheim, Germany). Cells were then stimulated with 5 ng/ml of IL-12 (PharMingen, San Diego, CA) for 4 h to promote transition to G1 and panned to remove macrophages. Nonadherent PBLs were pelleted and resuspended in RPMIL at 1 × 10^7 cells in 400 μl media. PBLs were assayed for CD3, CD4, and CD8 expression using the Cyto-Stat assay kit from Coulter (Palo Alto, CA). Ninety-four percent of the cells were CD3 positive, 74% were CD4 positive, and 20% were CD8 positive. Cells were transfected by electroporation using a Bio-Rad Gene Pulser II (Richmond, CA) at 290 V with a capacitance of 975 μF. Jurkat cells were resuspended at 5 × 10^6 cells in 400 μl media and electroporated at 270 V, 975 μF capacitance. The efficiency of transfection was determined using the pCMVGFPP expression plasmid and flow cytometry (Epics XL-MCL; Coulter) and ranged from 2 to 4% green fluorescence protein (GFP) positive in PBLs and 15 to 25% GFP positive in Jurkat cells. Transfected cells were incubated in RPMIL with 10% conditioned media (PBLs) or RPMI media (Jurkats) for 24 h. Cells were then sorted for high fluorescence using a FACStarPlus cell sorter (Becton Dickinson, Mountain View, CA), and the top 1–2% for PBLs and 10–15% for Jurkats were collected and used in IL-2 ELISA and proliferation assays.

Reporter assay, ELISA, and proliferation assay

pRcCMV and pDNA3.1/Zeo were obtained from Invitrogen (San Diego, CA), and pRcCMVGMP, pIL-2uc, and pHIVuc have been previously described (14). Standard site-specific mutagenesis procedures, the details of which will be reported elsewhere (Li et al., unpublished observations), were used to create a non-DNA binding mutant form of the HMGI protein designated HMGI(mII,mIII) starting with the wild-type human HMGI cDNA, clone 7C (38). Briefly, HMGI(mII,mIII) had four proline to alanine substitutions introduced at amino acid residues 57, 61, 83, and 87 located in its second and third DNA-binding domains, the primary regions of the protein that interact with the minor groove of A:T-rich substrates (39). As a consequence, the recombinant HMGI(mII,mIII) protein lacks the ability to specifically bind with high affinity to A:T-rich DNA sequences in vitro but retains its ability for specific protein-protein interactions with other transcription factors (Li et al., unpublished observations). For expression in transfected mammalian cells, the mutant HMGI(mII,mIII) cDNA was subcloned into the pcDNA3.1/Zeo plasmid vector (Invitrogen) as described above. The HMGY-cDNA was also subcloned into pcDNA3.1 to generate pcDNAHMGY for expression of the protein in cells. Jurkat cells used in the reporter assay were cotransfected with 5 μg of pIL-2uc or pHIVuc together with pRcCMVGMP, pcDNAHMGI(mII,mIII), or pcDNAHMGY, in combination with the amount of parent plasmid needed to normalize DNA at 10 μg. At 24 h posttransfection, cells were pelleted and resuspended in phenol red free RPMIL media at 1 × 10^6 cells/ml. Then, 100 μl of cells/well were plated in 96-well culture plates and stimulated with 50 ng/ml PMA and 1 μM Ca^2+ ionophore (P/II) (A23187, Boehringer Mannheim) or with the above and a 1/10,000 dilution of ascites fluid containing activating Ab to the CD28 receptor (α-CD28) (Bristol-Myers Squibb, New York, NY) for 9 h. Cells were then harvested and assayed for luciferase activity using a 96-well plate luminometer (TopCount; Packard, Meriden, CT) as previously described (14). PBLs were cotransfected with 5 μg of pIL-2uc and either 10 μg of pRcCMVGMP, pcDNAHMGI (mII,mIII), or pcDNAHMGY and the appropriate parent plasmid. At 24 h posttransfection, cells were stimulated with PHA (5 μg/ml) for 16 h. Cells were then harvested and cell extracts assayed for luciferase activity as previously described (40).

ELISA and proliferation assays were performed with PBL or Jurkat cells transfected with 10 μg of the appropriate expression plasmid together with 5 μg of pCMVGFPP for cell sorting. Then, 1 × 10^6 sorted cells in 100 μl were plated in 96-well tissue culture plates and stimulated with either PHA for 24 h (PBL) or P/II+α-CD28 for 8 h (Jurkat), and supernatants were assayed for IL-2 using a Quantikine ELISA kit (R&D Systems, Minneapolis, MN). Proliferation was assayed in similarly treated cells using the chemiluminescent bromodeoxyuridine incorporation kit from Boehringer Mannheim.

Production and purification of recombinant proteins

Recombinant hexahistidine-tagged full-length Fos and Jun proteins, as well as truncated Fos16-212 and Jun122-334 proteins, were prepared and purified by affinity chromatography using a Ni-NTA-agarose column (Qiagen, Chatsworth, CA) as described (41). Fos and Jun proteins were corenaturated in vitro into the heterodimer transcription factor activity AP-1 by step-wise dialysis from 6 M urea with the final buffer containing 25 mM sodium phosphate, pH 7.6, 5% glycerol, and 5 mM DTT (42). Full-length recombinant human HMGI protein (i.e., the unspliced member of the HMGI(Y) protein family; Ref. 29) was produced by alternative splicing of mRNA from the same gene as described (48). The purity of each recombinant preparation was assessed by SDS-PAGE (43). The purity of each recombinant was determined spectrophotometrically employing either a Bio-Rad protein assay kit or using the extinction coefficient ε_280 = 74,000 L/mol·cm for wild-type HMGI protein (48).

Preparation of nuclear extracts

Nuclear extracts were prepared from Jurkat T cells stimulated with PMA (20 ng/ml), Ca^2+ ionophore (1 μM), and CD28 Ab (1:10,000) for 1 or 6 h. Nuclear extract preparation was as previously described (14).

In vitro DNase I footprinting

A cloned 410 bp XhoI/HindIII restriction fragment encompassing nucleotides (nt) −360 to +50 of the human IL-2 gene proximal promoter (49) was cloned into fragment A in vitro protein footprinting. Promoter subfragments were isolated by either selective restriction enzyme digestion or PCR amplification techniques (47). A fragment from −180 to −60 was used for some of the footprinting experiments. Standard gel electrophoretic procedures (47) were used to isolate all DNA fragments followed by purification on a QiaGen column as described by the manufacturer. Restriction enzyme fragments were 5′-end radiolabeled with T4 polynucleotide kinase and γ-[32P]ATP. The end labeled fragments were cleaved by incorporating, during the final few cycles of the amplification reaction, either one or the other of the two PCR primers that had been 5′ radiolabeled with T4 polynucleotide kinase and γ-[32P]ATP.

Footprinting of both the HMGI and AP-1 recombinant proteins on promoter DNA fragments radiolabeled on one 5′ end, employing the nuclease DNase I, followed published protocols (35, 44). For each protein and DNA substrate, optimal conditions for footprinting were empirically determined. Single-stranded DNA cleavage products were then separated by electrophoresis on a 6% sequencing gel with Maxam-Gilbert “G-lane” chemical
Oligonucleotides for gel shift analysis had the following sequences: IL-2 NF-IL-2A, 5'-GATCCTGTAGGAAACAGGGGCTT-3'; GM170, 5'-GATCCTGTAGGAAACAGGGGCTT-3'. The lower case letters represent the mutations described except that 5% glycerol was used instead of Ficoll and 0.5 mM PMSF was included. Competitor double-stranded oligonucleotides or Abs containing 0.5 μg purified BSA (New England Biolabs, Beverly, MA) (50). Protein:DNA complexes was resolved on a nondenaturing 5% polyacrylamide gel containing 0.5× TBE buffer (0.5 mM Tris, 42 mM boric acid, 1 mM EDTA, pH 8.3). Binding of recombinant proteins to the CD28RR was conducted in 10 mM Tris, pH 7.5, 10 mM MgCl2, 5 mM EDTA, pH 7.5, 10 mM DTT, 0.2% Nonidet P-40, 1% glycerol, 0.4% sucrose, 0.5 mg/ml BSA, and 100 ng polidy(dC):dG) in 20 μl reactions. A total of 0.2 ng of radiolabeled probe was generally used with the amounts of recombinant proteins indicated for individual experiments. Reactions were separated on 5% 0.5× TBE polyacrylamide gels. EMSAs for nuclear extract were conducted as previously described except that 5% glycerol was used instead of Ficoll and 0.5 mM PMSF was included. Competitor double-stranded oligonucleotides or Abs was added to the binding reactions at the concentrations indicated in individual experiments and incubated for 10 min before the addition of the radiolabeled probe. All gels were dried and exposed to x-ray film. Digital images were produced on a Fujiﬁlm LAS1000 Plus CCD camera (Tokyo, Japan) and Image Gauge software (Fujifilm).

Western blot analysis

For Western blot, Jurkat cells were transfected, sorted, and stimulated as above except 1 × 10^6 cells were recovered from the GFP-positive population. A similar analysis using PBLs was not feasible due to the low transfection efficiency of these cells and the large numbers of cells required for the assay. Nuclei were isolated from cells and HMG I(Y) proteins were extracted with 5% perchloric acid and precipitated with TCA (44). Protein:DNA complexes was resolved on a nondenaturing 5% polyacrylamide gel containing 0.5× TBE buffer (0.5 mM Tris, 42 mM boric acid, 1 mM EDTA, pH 8.3). Band intensities were quantitatively analyzed using a PhosphorImager machine and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Results

HMG(I)Y binds to multiple elements within the IL-2 promoter

We have previously shown that transcription from a transfected IL-2 promoter reporter construct was inhibited by coexpression of antisense RNA for HMG(I)Y proteins (14). A comparison of the sensitivity of the promoters for a number of T cell-expressed genes (IL-2, IL-3, GM-CSF, and the HIV long terminal repeat (LTR)) to HMG(I)Y depletion found that IL-2 was the most significantly affected by changes in HMG(I)Y levels (data not shown). The IL-2 proximal promoter contains ~65% A:T base pairs and has many potential binding sites for HMG(I)Y with A:T sequences present within or adjacent to several known transcription factor binding sites. To identify the binding sites for HMG(I)Y on the IL-2 promoter, DNase I footprinting was performed on a fragment of the IL-2 promoter from nt −360 to +50 (Fig. 1). DNase I footprinting was conducted on both DNA strands by labeling each end of the DNA fragment independently. Fig. 1A represents footprints obtained with the DNA labeled at −360, and in Fig. 1B the DNA was labeled at +50. As shown in Fig. 1, A and B, footprints for HMG(I)Y were observed at a number of locations across the proximal promoter (indicated by vertical bars and filled arrowheads).

Fig. 1C shows the location of these HMG(I)Y binding sites in relation to the sequence and the known transcription factor binding sites on the promoter. Because different binding sites within the promoter have different affinities for HMG(I)Y (data not shown), only those sites that are consistently seen in independent replicate experiments are indicated. The HMG(I)Y footprints are within or adjacent to the 45 NF-AT/TATA-2 site (11), the NF-IL-2A region, the NF-IL-2B region, the CD28 response region (CD28RR), and the −285 NF-AT site in ARRE-2. All of these elements have been shown to be important in IL-2 promoter activity (reviewed in Refs. 3 and 5). Interestingly, at the concentrations used in this assay, HMG(I)Y was also observed to specifically footprint at the −30 TATA-1 sequence (Fig. 1), a region of the IL-2 promoter that is known to be protected in vivo by proteins in both unstimulated and stimulated T cells (21). As a control for these experiments, a Fos/Jun (AP-1) heterodimer was also footprinted to the promoter DNA, both alone and in combination with the HMG(I)Y protein (Fig. 1, A and B). AP-1 was used at two different concentrations, which were empirically determined, in Fig. 1, A and B. As expected, at the high concentration of input protein used in Fig. 1A, the Fos/Jun complex altered the footprinting pattern across the entire promoter but specific footprints were seen across known AP-1 sites in the promoter region (indicated by vertical dashed lines), including the low-affinity NF-IL-2A (ARRE-1) site located between −80 and −90. These specific footprints were confirmed in Fig. 1B at lower concentrations of AP-1. However, when both HMG(I)Y and Fos/Jun were combined together in the reaction mixture, binding of Fos/Jun to the NF-IL-2A site and the CD28RR was considerably reduced, and this is most clearly seen at the higher concentrations of protein used in Fig. 1A (see below).

HMG(I)Y modulates AP-1 and NF-AT binding to the IL-2 promoter

HMG(I)Y has previously been shown to promote or inhibit transcription factor binding to their regulatory elements overlapping or adjacent to the HMG(I)Y binding sites (reviewed in Ref. 29). We have previously shown that HMG(I)Y can modulate the binding of c-Rel to the CD28RR on the IL-2 promoter. We wished to determine whether HMG(I)Y affected the binding if other transcription factors to the IL-2 promoter. Several of the regions on the IL-2 promoter to which HMG(I)Y binds have been described as NF-AT and AP-1 binding sites (reviewed in Refs. 3 and 4). We chose to examine the effect of HMG(I)Y on NF-AT and AP-1 binding to two of these sites because of their distinct properties. The NF-IL-2A (ARRE-1) region has previously been reported to cooperatively bind NF-AT and AP-1 to form a higher order NF-AT complex on the NF-IL-2A region (11). The second site we examined, the CD28RR, has also been reported to bind NF-ATp (11, 46) and AP-1, but the binding sites are in a distinct configuration and cooperative binding is not observed (46).

At low protein concentrations, recombinant truncated Fos/Jun alone does not stably bind to the low-affinity AP-1 site in the NF-IL-2A site in EMSA (Fig. 2A, lane 1). However, the addition of recombinant HMG(I)Y resulted in the appearance of an AP-1 band (Fig. 2A, lanes 2–5). The intensity of the AP-1 band initially increased (up to 2 ng HMG(I)Y) and then decreased with the addition of increasing amounts of HMG(I)Y (Fig. 2A). These results demonstrating that high concentrations of HMG(I)Y inhibit Fos/Jun binding are consistent with the previously described footprinting results obtained with varying concentrations of these proteins (Fig. 1A). Recombinant NF-ATp protein was added to determine the effect of HMG(I)Y proteins on formation of the NF-AT/AP-1 complex. Under the binding conditions used here, NF-ATp did not bind alone to the probe and the formation of the higher-order
NF-AT complex did not occur (Fig. 2A, lane 6). Addition of HMGI(Y) protein resulted in a dose-dependent increase in formation of the NF-AT complex as well as the previously observed increase in AP-1 binding (Fig. 2A, lanes 7–10). At higher levels of HMGI(Y), the intensity of the NF-AT complex did not significantly decline as was seen for AP-1 alone (Fig. 2A, lanes 7–10). The amount of AP-1 and NF-ATp used in these experiments was sufficient for strong binding to the consensus sites contained in an

**FIGURE 1.** A and B, DNase I footprinting of HMGI(Y) and AP-1 on IL-2 promoter DNA. The DNA fragment was labeled at either −360 (A) or +50 (B). The proteins added are indicated at the top of the gels with a Maxam-Gilbert “G” chemical cleavage of the naked DNA serving as a sequence reference marker. A + H represents the simultaneous addition of HMGI(Y) and AP-1. Lanes 1–5 and 6–10 are two different loadings of the same samples on the sequencing gel that have been electrophoresed for different lengths of time. The nucleotide numbers relative to the major transcriptional start site are indicated next to the G lanes. The recognition sequences for known protein transcription factors and vertical lines next to the nucleotide numbers indicate functional elements. A vertical thick solid line and a filled arrowhead indicate the footprinted areas protected by HMGI(Y), and a vertical broken line indicates those protected by AP-1. C, Sequence of the IL-2 proximal promoter showing HMGI(Y) footprints relative to known transcription factor binding sites. The HMGI(Y) footprints that are consistently observed are marked by lower case letters in the sequence and filled dots underneath. The sequence is numbered relative to the transcription start site of +1. Horizontal lines above the sequence indicate transcription factor binding sites and control elements.
oligonucleotide from the GM-CSF enhancer (GM170) that can bind NF-ATp or AP-1 alone and also form a higher-order complex (Fig. 2A, lanes 11–15). This oligonucleotide showed no binding of HMGI(Y), and neither the binding of AP-1 or NF-ATp alone nor the formation of the NF-AT higher-order complex were affected by addition of HMGI(Y) at any concentration (Fig. 2A, lanes 11–15).

Recombinant full-length c-Fos/c-Jun (AP-1) bound to the CD28RR in a dose-dependent manner, without the addition of HMGI(Y), and neither the binding of AP-1 nor NF-ATp alone nor the formation of the NF-AT higher-order complex were affected by addition of HMGI(Y) at any concentration (Fig. 2A, lanes 11–15).

FIGURE 2. A, HMGI(Y) promotes AP-1 and NF-ATp binding to the NF-IL-2A region of the IL-2 promoter. EMSAs were performed using radiolabeled oligonucleotides spanning the NF-IL-2A region of IL-2 (lanes 1–10) or the GM170 region of the GM-CSF enhancer (lanes 11–15) and recombinant truncated NF-ATp (0.4 ng), truncated c-Fos/c-Jun (tAP-1) (2 ng), and HMGI(Y) (1, 2, 4, and 8 ng) proteins. The results were visualized using a Molecular Dynamics PhosphorImager. The proteins and probes used in the individual lanes are indicated above the lanes, and the positions of the free DNA and the individual protein:DNA complexes are indicated. B, HMGI(Y) promotes AP-1 but inhibits NF-ATp binding to the IL-2 CD28RR. EMSAs were performed as above using full-length recombinant c-Fos and c-Jun (AP-1) at 12 ng (lanes 1, 4, and 7), 6 ng (lanes 2, 5, and 8), or 3 ng (lanes 3, 6, and 9) and the CD28RR radiolabeled oligonucleotide. In lanes 10–13, 0.1 ng NF-ATp was added with 3 ng AP-1. HMGI protein was added to the binding reactions at 2 ng (lanes 4–6), 20 ng (lanes 7–9), or 5 (lane 11), 10 (lane 12), or 20 ng (lane 13). EMSAs were processed, and the diagram was labeled as in A.

These results show that HMGI(Y) can modulate the binding of transcription factors that are required for IL-2 promoter function to at least two major control regions of the IL-2 proximal promoter. However, it appears that the ratio of HMGI(Y) to AP-1 or NF-ATp
FIGURE 3. Binding of AP-1 to the CD28RR from Jurkat T cell extracts requires HMGI(Y). A, EMSAs were performed with nuclear extracts prepared from Jurkat T cells either unstimulated (lanes 1, 4, and 7) or activated with P/I/CD28 for 1 h (lanes 2, 5, and 8) or 6 h (lanes 3, 6, and 9) using radiolabeled CD28RR (lanes 1–3), RE mutant (lanes 4–6), or AP mutant (lanes 7–9) oligonucleotides. The inducible protein DNA complexes are indicated by an arrow. B, Competition experiments using the RE mutant as the radiolabeled probe and extracts from cells activated for 6 h. The CD28RR wild-type sequence (lanes 2–4), AP mutant (lanes 5–7), RE mutant (lanes 8–10), or a TRE (AP-1 consensus binding site) (lanes 11–13) were used as competitors at 1, 10, and 20 ng per reaction as indicated. C, The effect of addition of HMGI(Y) Ab on complexes binding to the RE mutant was confirmed by competition experiments where the RE mutant, the wild-type CD28RR, and a consensus AP-1 site (TRE) were able to compete for complex formation but the AP mutant was not (Fig. 3B). Thus, these complexes contain AP-1-like proteins. To determine whether the formation of the AP-1-like complex was dependent on HMGI(Y), an anti-HMGI(Y) Ab was added to the binding reactions. The addition of the HMGI(Y) Ab to the binding reactions reduced AP-1 binding to the RE mutant (Fig. 3C, lanes 1–3), as it did the c-Rel complexes binding to the AP mutant (Fig. 3C, lanes 5–7). A control Ab (C) did not affect complex formation (Fig. 3C, lanes 4, 8, and 11). We have previously shown that HMGI(Y) Ab removes the inducible complexes containing c-Rel from the CD28RR but does not affect NF-kB complexes binding to a distinct NF-kB site (14). AP-1 binding to a TRE was not significantly reduced by the addition of the HMGI(Y) Ab (Fig. 3C, lanes 9 and 10).

To confirm that HMGI(Y) could promote AP-1 binding to the RE mutant, recombinant HMGI was titrated into binding reactions with a fixed amount of AP-1 using either the wild-type CD28RR or the RE mutant. As described above, AP-1 binding to the CD28RR was increased only at high concentrations of HMGI (Fig. 3D, lanes 7 and 8). In contrast, a dose-dependent increase in AP-1 binding was observed with increasing levels of HMGI starting at 1 ng on the RE mutant (Fig. 3D, lanes 9–16). We also observed that

HMGI(Y) modulates nuclear AP-1 binding to the CD28RR

The CD28RR consists of an AP-1 binding site as well as a c-Rel binding site (CD28RE). We have previously shown that the c-Rel-containing complexes from activated T cell nuclear extracts require HMGI(Y) to bind to the CD28RE (14). To determine whether nuclear AP-1 was also dependent on HMGI(Y), we examined binding of AP-1 from nuclear extracts of P/I/CD28-activated Jurkat T cells. Because the Rel and AP-1 complexes migrate at the same position on EMSA gels using the intact CD28RR (17), it was necessary to generate EMSA probes with mutations in either the CD28RE (RE mutant) or the AP-1 site (AP mutant). Binding of nuclear extracts to these probes showed that an inducible complex could bind to the RE mutant in extracts made both 1 and 6 h following stimulation with P/I/CD28 (Fig. 3A, lanes 4–6). These complexes migrated at the same position as the complexes binding to the wild-type probe or the Rel-containing complexes binding to the AP mutant (Fig. 3A, lanes 1–3 and lanes 7–9). The identity of the complexes binding to the RE mutant was confirmed by competition experiments where the RE mutant, the wild-type CD28RR, and a consensus AP-1 site (TRE) were able to compete for complex formation but the AP mutant was not (Fig. 3B). Thus, these complexes contain AP-1-like proteins. To determine whether the formation of the AP-1-like complex was dependent on HMGI(Y), an anti-HMGI(Y) Ab was added to the binding reactions. The addition of the HMGI(Y) Ab to the binding reactions reduced AP-1 binding to the RE mutant (Fig. 3C, lanes 1–3), as it did the c-Rel complexes binding to the AP mutant (Fig. 3C, lanes 5–7). A control Ab (C) did not affect complex formation (Fig. 3C, lanes 4, 8, and 11). We have previously shown that HMGI(Y) Ab removes the inducible complexes containing c-Rel from the CD28RR but does not affect NF-kB complexes binding to a distinct NF-kB site (14). AP-1 binding to a TRE was not significantly reduced by the addition of the HMGI(Y) Ab (Fig. 3C, lanes 9 and 10).

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HMGI(Y) no longer bound to the RE mutant (Fig. 3D), probably because the RE mutant affects the A:T stretch within the CD28RE to which HMGI(Y) most likely binds. These results 1) show that AP-1 binding in nuclear extracts is influenced by HMGI(Y) and 2) imply that the ability of HMGI(Y) to promote AP-1 binding is not dependent on DNA binding by HMGI(Y).

**HMGI(Y) binding to DNA is not required for promotion of transcription factor binding to the CD28RR**

The results above imply that the promotion of transcription factor binding may not always require DNA:HMGI(Y) interactions. To test this further, site-specific proline to alanine mutations were introduced into the second and third DNA binding domains of HMGI (two substitutions in each binding domain) to create a form of the protein (designated HMGI(mII,mIII)) that could not specifically bind to A:T-DNA under the assay conditions employed. We tested the ability of HMGI(mII,mIII) to bind to the IL-2 promoter both in footprinting and in gel shift assays. At concentrations of protein where recombinant HMGI or HMGY bound specifically to the CD28RR, HMGI(mII,mIII) protein did not bind (Fig. 4A). The same result was obtained in footprinting assays across the IL-2 promoter from −180 to −60 (Fig. 4B). Four footprints for HMGI

**FIGURE 4.**  
A and B, HMGI(mII,mIII) does not bind specifically to the IL-2 promoter. A, EMSAs were performed using the IL-2CD28RR oligonucleotide as a probe and 1, 10, and 20 ng of HMGI (lanes 1–3), HMGY (lanes 4–6), and HMGI(mII,mIII) (lanes 7–9). The HMGI/Y bands and the free DNA are indicated. B, DNase I footprinting assay using the −180 to −60 region of the IL-2 promoter. HMGI (lanes 2–5) or HMGI(mII,mIII) (lanes 6–9) at 10, 20, 50, or 100 ng were used in the footprinting reactions. Solid lines indicate the HMGI footprints, and the names of the regions are shown. C and D, HMGI(mII,mIII) can promote but not inhibit transcription factor binding to the IL-2 CD28RR. Increasing amounts (0.5, 1, 2.5, 5, 10, and 20 ng) of HMGI (lanes 4–9) or HMGI(mII,mIII) (lanes 10–15) were added to binding reactions containing the IL-2CD28RR probe and 3 ng AP-1 and 0.1 ng NF-ATp. Reactions containing NF-ATp alone (lane 1), AP-1 alone (lane 2), or AP-1 and NF-ATp together (lane 3) were also analyzed. The positions of the transcription factor:DNA complexes are indicated. D, Similar binding reactions as described in C were conducted using bacterial extract containing the Rel homology domain of c-Rel and increasing amounts (1, 5, 10, and 20 ng) of HMGI (lanes 2–5) or HMGI(mII,mIII) (lanes 6–9). Lane 1 contains c-Rel alone.
FIGURE 5. Inhibition of HMGI(Y) production reduces IL-2 promoter activity in Jurkat T cells and PBLs. A, Jurkat T cells were transfected with increasing amounts of pRcCMVIGMH and the pIL-2luc or pHIVluc reporter plasmids. Transfected cells were treated with P/I (□) or P/I+α-CD28 (■) before luciferase assays were performed. Luciferase activity is expressed as counts per second (CPS) readings from the Packard TopCount luminometer. B, Western blot showing the decrease in HMGI(Y) levels in cells expressing antisense HMGI. Cells were transfected with either pRcCMV (lanes 1 and 3) or pRcCMVIGMH antisense expression plasmid (lanes 2 and 4). Protein was extracted from nuclei of either unstimulated (lanes 1 and 2) or P/I-stimulated (lanes 3 and 4) cells, resolved on SDS polyacrylamide gels, and HMGI(Y) was detected by Western blot. Recombinant HMGI protein (lane 5) was also loaded as a reference. Numbers below represent the densitometry quantitation of HMGI(Y) protein bands. C, PBLs, pretreated to generate blasts, were transfected with the pRcCMV or pRcCMVIGMH plasmids, and the pIL-2luc reporter plasmid and luciferase activity was measured either before (□) or following PHA treatment (■). Columns represent the means of five replicate assays, and the bars show the SEMs.

were observed across this region of the promoter (Fig. 4B, lanes 2–5). In contrast, HMGI(mII,mIII) did not form specific footprints across any of these regions (Fig. 4B, lanes 6–9).

We also tested whether HMGI(mII,mIII) could interact with transcription factors by generating an affinity matrix for both HMGI and HMGI(mII,mIII). The results of binding experiments using these affinity matrices in binding experiments demonstrated that both the wild-type HMGI and mutant HMGI(mII,mIII) proteins could bind specifically to the AP-1 and NF-AT proteins but could not interact with themselves or with each other (data not shown).

We then investigated whether HMGI(mII,mIII) could alter transcription factor binding when added to binding assays using the CD28RR together with AP-1 and NF-ATp. The addition of HMGI(mII,mIII) lead to a dose-dependent increase in AP-1 binding but did not inhibit NF-ATp binding as did wild-type HMGI (Fig. 4C, lanes 10–15). We observed that HMGI(mII,mIII) generated a more consistent dose dependence compared with the wild-type HMGI (Fig. 4C, compare lanes 4–9 and 10–15). c-Rel binding to the CD28RR was also increased by HMGI(mII,mIII) (Fig. 4D, lanes 1 and 6–9). In addition, inhibition of transcription factor binding sometimes seen at high concentrations of HMGI was never observed with HMGI(mII,mIII). In summary, these results show that HMGI binding to DNA is involved in the inhibition of binding but is not required for the promotion of transcription factor binding.

Alteration of HMGI(Y) expression results in the modulation of IL-2 promoter activity in both Jurkat and primary T cells

To determine whether HMGI(Y) had a functional role in IL-2 gene transcription, HMGI(Y) levels were altered in the cell by antisense or overexpression studies. The pRcCMVIGMH plasmid expressing antisense HMGI was cotransfected into Jurkat T cells with pIL2luc or pHIVluc. The HIV LTR responds to P/I and CD28 stimulation through NF-κB sites that closely match consensus sites and contain no potential binding sites for HMGI(Y) (our unpublished observations).

As previously reported (14), the expression of antisense HMGI RNA resulted in a 72% decrease in reporter activity in P/I-treated Jurkat cells and a 75% inhibition in P/I+α-CD28-treated cells at the maximum dose of antisense expression plasmid (10 μg; Fig. 5A). The pHIVluc reporter transfections showed only a small reduction in promoter activity at the maximum dose of antisense plasmid (Fig. 5A). To confirm that the antisense HMGI RNA was reducing the level of HMGI(Y) protein in the cells, a Western blot, using an anti-HMGI polyclonal Ab, was performed on nuclear extracts from transfected cells either unstimulated or P/I-stimulated following transfection. Densitometry scanning of the blot showed that there was a significant reduction in the levels of HMGI(Y) protein in the antisense-expressing cells (Fig. 5B). This experiment also showed that the amount of HMGI(Y) in the nuclei of Jurkat T cells was increased almost 3-fold by P/I activation (Fig. 5B).

HMGI(Y) expression in transformed cell lines is generally high (29), hence the Jurkat T cell leukemia may display an aberrant role for HMGI(Y) proteins in IL-2 promoter activity. In contrast, HMGI(Y) levels are quite low or not detectable in most normal cell types. Therefore, the dependence of the IL-2 promoter on HMGI(Y) proteins for activation of transcription was also examined in normal PBLs. PBLs isolated from blood were cultured for 4 days as described to generate blasts, transfected by electroporation, rested for 24 h, and restimulated with PHA. The IL-2 promoter transfected into PBLs had activity in cells that were not stimulated posttransfection most likely because of the primary stimulation with PHA before transfection to generate T cell blasts. However, these cells showed a 2- to 3-fold increase in promoter activity when stimulated with PHA posttransfection (Fig. 5C). Transfection with pRcCMVIGMH resulted in an 84% inhibition of the reporter activity in unstimulated PBLs and an 81% inhibition in PHA-stimulated cells (Fig. 5C). These results show that the activity of a transfected IL-2 promoter is dependent on HMGI(Y) in either a T cell line or in primary T cells.
Expression of HMGY in Jurkat T cells increases IL-2 promoter activity. Jurkat cells were transfected with either the pIL-2luc or the pHIVluc reporter plasmids together with the indicated amounts of the pcDNAHMGY expression plasmid or the control pcDNA3.1 plasmid. Cells were stimulated with P/I (+) or P/I+α-CD28 (−) for 8 h before harvesting for luciferase assays. pHIVluc transfected cells were only stimulated with P/I+α-CD28. Luciferase activity is expressed as counts per second (CPS) as measured by a Packard TopCount scintillation counter. Expression of HMGY(mII,mIII) in Jurkat cells inhibits IL-2 promoter activity. The pIL-2luc or pHIVluc reporter plasmids were transfected into Jurkat T cells together with the indicated amounts of the pcDNAHMGY(mII,mIII) expression plasmid or the control pcDNA plasmid. Cells were activated and analyzed, and results are presented as described in A.

We also tested whether increased levels of HMGY(Y) protein affected IL-2 promoter activity. Transfection of the pcDNAHMGY expression plasmid into Jurkat T cells together with the pIL-2luc or the pHIVluc increased luciferase activity ~2-fold for IL-2 but had no effect on HIV LTR activity (Fig. 6A). Because the mutant HMGY(mII,mIII) protein also promoted transcription factor binding, its effect in transfection assays was also tested. Cotransfection of an expression plasmid for the HMGY(mII,mIII) protein resulted in significant inhibition of the IL-2 promoter (60% in P/I and 73% in P/I+α-CD28-treated cells; Fig. 6B) at the highest amounts of transfected plasmid. The pHIVluc reporter plasmid showed no loss of reporter activity with expression of the HMGY(mII,mIII) protein (Fig. 6B). Thus, this non-DNA binding mutant appears to act as a dominant negative protein for IL-2 gene transcription.

Taken together, these results show that modulating HMGY(Y) levels or function in either Jurkat T cells or PBLs affects IL-2 promoter activity without a general effect on transcription as measured by HIV LTR function.

**IL-2 production and primary T cell proliferation are dependent on HMGY(Y)**

It is possible that the transfected IL-2 promoter may be more highly dependent on HMGY(Y) than its endogenous counterpart because of the likely differences in chromatin configuration on chromosomal and plasmid DNA. To monitor the effect of HMGY(Y) on expression from the endogenous IL-2 gene, the amount of IL-2 protein secreted into the supernatant of cells transfected with pRcCMVIGMH and pcDNAHMGY(mII,mIII) was assayed by ELISA. To enrich for transfected cells, 5 μg of pCMV-GFP was cotransfected with the HMGY plasmids above, and the cells were subsequently sorted for high level GFP expression by FACS. The sorted cells were then stimulated with either PHA for PBLs or P/I+α-CD28 for Jurkat cells to induce IL-2 expression. In PBLs and Jurkats, significant inhibition of IL-2 production was detected in cells transfected with pRcCMVIGMH (78% and 88%, respectively) or pcDNAHMGY(mII,mIII) (47% and 55%, respectively) (Table I). These results imply that the role of HMGY(Y) may be extended to endogenous IL-2 gene transcription in both Jurkat T cells and primary T cells.

*If IL-2 production in PBLs is dependent on HMGY(Y), then PBL proliferation may also be affected by changes in HMGY(Y) levels in the cells. To test the effect of inhibition of HMGY(Y) on cell growth, the IL-2-dependent PBLs and the IL-2-independent Jurkat cell line were transfected with either the parent plasmids or the expression plasmids pRcCMVIGMH and pcDNAHMGY(mII,mIII) together with the pCMV-GFP plasmid to allow sorting of transfected cells by FACS. Proliferation of PBLs was inhibited by pRcCMVIGMH (62%) or pcDNAHMGY(mII,mIII) (29%), whereas the effect of these plasmids on growth of the Jurkat cell line was not significant (Fig. 7, A and B). To test whether increased HMGY(Y) levels also altered proliferation, PBLs were transfected with the pcDNAHMGY plasmid and pCMV-GFP to allow for sorting of transfected cells. Overexpression of HMGY(Y) in PBLs increased proliferation of these cells by ~2-fold (Fig. 7C).

These results show that IL-2 production in both PBLs and Jurkats is dependent on the correct level of HMGY(Y). This dependence can also be seen at the level of proliferation in PBLs that are IL-2 dependent but not in Jurkats that are IL-2 independent.

**Table I. Decrease in IL-2 production with inhibition of HMGY(Y)**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Antisense Expression</th>
<th>Dominant-Negative Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>− (RcCMV)</td>
<td>+ (RcCMVIGMH)</td>
</tr>
<tr>
<td>PBL</td>
<td>119 ± 33a</td>
<td>26 ± 7.7</td>
</tr>
<tr>
<td>Jurkat</td>
<td>48 ± 4.4</td>
<td>5.6 ± 1.5b</td>
</tr>
</tbody>
</table>

a Values represent the mean ± SEM of four replicate assays in pg/ml of IL-2 protein.

b This value is below the reliable detection limit of the assay.
We have shown here that HMGI(Y), a protein involved in regulating transcription factors on the proximal promoter of the IL-2 gene during transcription, participates in the inducible expression of the human IL-2R beta-chain genes is an essential component of the formation of the autocrine loop that drives T cell proliferation and clonal expansion following an immune stimulus. This requirement for proliferation appears to stem from the involvement of HMGI(Y) in controlling transcription from both the IL-2 and IL-2R beta-chain genes in response to mitogenic stimuli. It has previously been shown that HMGI(Y) participates in the inducible expression of the human IL-2R gene by facilitating the assembly of multiprotein, enhanceosome-like complexes on both an upstream enhancer element that has binding sites for HMGI(Y), Elf-1 (an Ets family protein), Stat5, and a GATA family protein (52) and on the proximal promoter element that contains binding sites for HMGI(Y), Elf-1, and NF-kB proteins (53). Likewise, the present results obtained from both in vitro footprinting, EMSA, and transfection experiments strongly suggest that HMGI(Y) facilitates the formation of a functional multiprotein complex consisting of a number of different transcription factors on the proximal promoter of the IL-2 gene during transcriptional activation in vivo.

Here, and elsewhere (14), we have demonstrated that HMGI(Y) modulates the binding to the IL-2 promoter of NF-AT, AP-1, and c-Rel; three transcription factor families that play important roles in IL-2 promoter activity. This has been shown both for recombinant proteins and proteins present in nuclear extracts. In each case, the transcription factor binding site constitutes a nonconsensus element that differs markedly in sequence from the high-affinity consensus sites. This is generally, but not always, because of the presence of the A:T sequences required for HMGI(Y) binding; consequently, these sites are weakly recognized by their cognate transcription factors. Therefore, HMGI(Y) serves to lower the threshold at which transcription factors can bind to and activate these weak sites. The presence of these nonconsensus sites in the IL-2 promoter appears to be important for the T cell-restricted expression of the promoter. It has been shown that mutation of some of these sites to consensus high-affinity sites weakens the induction dependence or T cell specificity of the promoter (54–56).

There is no evidence that there are T cell-restricted members of the transcription factor families that may have selectivity for the IL-2 promoter sites. Instead, it is likely that the weak interactions together with the need for proteins such as HMGI(Y) and cooperative binding of many of the protein complexes is a requirement to generate these characteristics. Thus, HMGI(Y) may play an indirect role in the T cell specificity and induction dependence of the IL-2 promoter.

The role of HMGI(Y) in the assembly of a functional enhanceosome has been best studied for the IFN-beta promoter (57). It has been shown that HMGI(Y) promotes the coordinate binding of members of the NF-kB, activating transcription factor, and IFN-gamma regulatory factor families of transcription factors to the IFN-beta promoter leading to the assembly of a functional complex known as an enhanceosome (57). This enhanceosome is then thought to recruit coactivator complexes such as CREB binding protein to lead to chromatin reorganization and transcription activation (58). It has recently been shown that the recruitment of transcription factors to the IFN-beta promoter requires the binding of HMGI(Y) to DNA and that HMGI(Y):transcription factor interaction is not crucial for this recruitment (59). The IFN-beta promoter DNA contains an intrinsic bend that is straightened by HMGI(Y) binding, and this is important to allow transcription factor binding (27). However, HMGI(Y):transcription factor interactions are required for the completion of the enhanceosome assembly process on the IFN-beta gene (59).

We have found here that binding of either c-Rel or c-Fos/c-Jun to the CD28RR of the IL-2 promoter does not require HMGI(Y) binding to DNA. We have shown this by using either a mutant of the CD28RR that can no longer bind HMGI(Y) or a mutant HMGI protein that does not bind DNA specifically. However, the non-DNA binding mutant of HMGI can still interact with the NF-ATp and AP-1 transcription factors and may promote transcription factor binding by protein:protein interactions. The intrinsic structure of the IL-2 promoter region is not known but it is possible that the promoter DNA does not require structural alteration to allow transcription factor binding. However, the HMGI(mILmIII) non-DNA binding mutant acted as a dominant negative protein in transfection assays. This result implies that correct DNA binding as well as protein recruitment is important for the assembly of a functional complex. When expressed at sufficiently high levels, the dominant negative mutant may compete with wild-type protein in the cells to form nonfunctional complexes. Thus, it is likely that for both the IL-2 and IFN-beta promoters, HMGI(Y) needs to both bind to DNA and interact with transcription factors to generate a functional complex, although the order of events may differ.

High concentrations of HMGI appear to inhibit the binding of certain transcription factors at specific sites, e.g., AP-1 to the NF-IL-2A site and NF-ATp to the CD28RR. This inhibition was dependent on DNA binding by HMGI and would appear to be a direct competitive effect. It has previously been shown that HMGI(Y) can inhibit NF-AT binding to the IL-4 promoter (60, 61). In the case of IL-4, this inhibition of DNA binding translates into the ability of increased HMGI(Y) to inhibit IL-4 promoter function (61). In contrast, our results strongly suggest that HMGI(Y) is an activator of the IL-2 promoter. The data that support this conclusion are 1) antisense HMGI RNA expression strongly inhibits IL-2 promoter activity in both Jurkat T cells and PBLs, 2) increased expression of HMGI, either in response to P/I stimulation from both the IL-2 and IL-2R beta-chain genes is an essential component of the formation of the autocrine loop that drives T cell proliferation and clonal expansion following an immune stimulus. We have shown here that HMGI(Y), a protein involved in regulating transcription factors on the proximal promoter of the IL-2 gene during transcription, participates in the inducible expression of the human IL-2R beta-chain genes is an essential component of the formation of the autocrine loop that drives T cell proliferation and clonal expansion following an immune stimulus. This requirement for proliferation appears to stem from the involvement of HMGI(Y) in controlling transcription from both the IL-2 and IL-2R beta-chain genes in response to mitogenic stimuli. It has previously been shown that HMGI(Y) participates in the inducible expression of the human IL-2R gene by facilitating the assembly of multiprotein, enhanceosome-like complexes on both an upstream enhancer element that has binding sites for HMGI(Y), Elf-1 (an Ets family protein), Stat5, and a GATA family protein (52) and on the proximal promoter element that contains binding sites for HMGI(Y), Elf-1, and NF-kB proteins (53). Likewise, the present results obtained from both in vitro footprinting, EMSA, and transfection experiments strongly suggest that HMGI(Y) facilitates the formation of a functional multiprotein complex consisting of a number of different transcription factors on the proximal promoter of the IL-2 gene during transcriptional activation in vivo.

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activation or by cotransfection of an expression plasmid, correlates with increased IL-2 promoter activity, and 3) production of IL-2 from the endogenous IL-2 gene is inhibited by antisense HMGI RNA in both Jurkat T cells and PBLS. These experiments not only show that a promoter in the context of a reporter plasmid, but also the endogenous gene, in both a T cell line and normal T cells is dependent on HMGI(Y) for activity. The ability of HMGI(Y) to promote transcription factor binding may be an important aspect of the positive function of HMGI(Y) on the IL-2 promoter. Whether inhibition of transcription factor binding is an artifact of high concentrations of HMGI or plays a role in the removal of inhibitors or down-regulation of the promoter following activation remains to be determined. One possible explanation for the results observed here is that at high concentrations of HMGI(Y) following T cell activation NF-ATp is displaced by HMGI(Y), which then promotes the binding of c-Rel. The appearance of NF-ATp in the nucleus (4) at early times following activation is likely to proceed the increased levels of c-Rel that requires new protein synthesis (12). A time-dependent change in the proteins that bind to the CD28RR may play a role in correct activation.

We have previously shown that the GM-CSF promoter (14) and the IL-3 promoter (our unpublished observations) are dependent on HMGI(Y) for activity. All of these cytokines are expressed following a primary T cell activation, before the development of effector T cell function (62, 63). In contrast, IL-4 requires at least three cell divisions before expression is detected (64, 65) and is the hallmark of the Th2 effector phenotype (62, 63). Signaling from the IL-4 receptor has also been shown to lead to phosphorylation of HMGI(Y) and a consequent reduction in DNA binding (66). Because IL-4 activation of T cells leads to increased IL-4 synthesis, it is intriguing to speculate that the phosphorylation of HMGI(Y) and its removal from the DNA may be one mechanism by which this increased IL-4 production is achieved (61). The same signaling pathway may consequently decrease IL-2 production. It may be of interest to examine the role of HMGI(Y) in expression of other Th1- or Th2-specific cytokines and to test the consequences of different levels of HMGI(Y) on the expression of these cytokines and the development of the T cell subtypes. Alteration of the level of HMGI(Y) in PBLS leads to an effect on proliferation of these cells. It would appear that the expression of antisense HMGI RNA does not have a general effect on the cell cycle machinery because antisense HMGI RNA expression had little effect on the proliferation of Jurkat T cells. This change in proliferation in PBLS is likely to be the result of changes in IL-2 expression in these cells, although we have not definitively proven this link. Attempts to rescue the proliferative defect in HMGI antisenes RNA-expressing cells lead to only a small reversal of this inhibition. This may be due to the fact that the expression of the IL-2Rα-chain gene is also controlled by HMGI(Y) as discussed above. The IL-2Rα-chain protein is required for high-affinity IL-2 receptor complex formation (reviewed in Ref. 67). Indeed, the PBLS expressing antisense HMGI RNA had a reduced level of IL-2Rα-chain on the cell surface as measured by FACS analysis (data not shown).

We have found here that HMGI(Y) levels increase in Jurkat T cells in response to P/I treatment. PMA treatment has previously been shown to increase HMGI(Y) levels in other cell types (68, 69), as have other signals such as exposure to growth factors such as serum (70, 71) and epidermal growth factor (72) or proinflammatory agent such as endotoxin and IL-1β (73). The increase observed in HMGI(Y) levels may directly translate into increased promoter activity mediated by HMGI(Y) interactions with the other inducible transcription factors such as c-Rel or AP-1.

The results presented here show that the architectural transcription factor HMGI(Y) is critical for the correct regulation of IL-2. The finding that HMGI(Y) not only affects the function of a trans- fected IL-2 promoter but also the endogenous IL-2 gene and T cell proliferation implies that the level of HMGI(Y) in cells is a critical determinant of IL-2 gene activation and that modulating such levels either by physiological or pharmacological means may provide a means of modulating the immune response.

Acknowledgments

We thank Dr. D. Tremethick for supplying us with recombinant Fos and Jun proteins and also for many helpful discussions during the course of these experiments. We also thank Dr. Peter Cockey for recombinant NF-ATp and truncated fos and Jun proteins and Dr. Bunks Wattenberg for the pCMVGFp plasmid.

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