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Multiple Interactions of the Transcription Factor YY1 With Human Histone H4 Gene Regulatory Elements

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Abstract Multiple regulatory elements and intricate protein–DNA interactions mediate the transcription of the human histone H4 genes in a cell growth-dependent manner. Upon analysis of the regulatory elements of the FO108 histone H4 gene, we identified several potential YY1 binding sites. In this study, we have analyzed the ability of the transcription factor YY1 to interact at these sites in vitro by using electrophoretic mobility shift assays in combination with oligonucleotide competition and antibody immunoreactivity. We show that YY1 specifically binds transcriptional regulatory elements at −340 nt (site III), −100 nt (site I) and at least two domains within the coding region of the histone H4 gene. To test if these elements were functionally responsive to YY1, we performed transient expression experiments in *Drosophila* S-2 cells transfected with heterologous reporter gene constructs driven by histone H4 gene segments fused to the thymidine kinase promoter. Co-expression of YY1 stimulated promoter activity of these constructs relative to the reporter construct lacking histone H4 gene fragments. Our results suggest that YY1 contributes to transcriptional regulation of the histone H4 gene through interactions at multiple regulatory elements. J. Cell. Biochem. 72:507–516, 1999.

Key words: YY1; histone; promoter; transcription

The five classes of replication-dependent histone genes—H1, H2A, H2B, H3, and H4—encode abundant and highly conserved basic proteins required for nucleosomal packaging and proper transcriptional regulation of chromatin [reviewed in Osley, 1991; Stein et al., 1992]. Expression of histone genes is tightly linked to DNA synthesis and peaks in S phase [Plumb et al., 1983; Heintz et al., 1983; Graves and Marzluff, 1984; Baumbach et al., 1987]. Transcriptional expression is also dependent on the growth status of the cell. Quiescent or postproliferative differentiated cells have a basal level of histone gene transcription significantly lower than that of replicating cells [reviewed in Stein et al., 1992]. Consequently, histone gene promoter activity is responsive to cellular cues related to cell cycle progression and proliferation [reviewed in Stein et al., 1992]. Transcriptional control of histone genes involves cell-signaling pathways converging through multiple transcription factors that interact with composite regulatory elements in the promoter and coding sequences of the gene. The expression of the human histone H4 gene FO108 is controlled by an array of transcription factors, such as ATF-1, CREB, IRF-2, and CDP/cut, which are components of gene regulatory signaling pathways that control cell growth and cell cycle progression [Wright et al., 1995; Guo et al., 1997b; Vaughan et al., 1995; van Wijnen et al., 1996]. Recently, our laboratory showed that the nuclear matrix protein NMP-1, which interacts with the histone H4 distal regulatory element site IV, is identical to YY1 [Guo et al., 1995]. Hence, YY1 may represent a critical regulatory factor involved in modulating transcription of the histone H4 gene.

YY1 (UCRBP, δ, or NF-E1) is a multifunctional zinc finger transcription factor that is ubiquitously expressed in mammalian cells [Shi et al., 1991; Flanagan et al., 1992; Hariharan et al., 1991; Park and Atchison, 1991]. YY1 is capable of modulating transcription by multiple mechanisms [for review, see Shrivastava and Calame, 1994; Hahn, 1992], including its ability to interact with a vast array of regulatory factors, such as p300 [Lee et al., 1995],

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TAFII55 [Chiang and Roeder, 1995], TFIIB, TATA box-binding protein [Usheva and Shenk, 1994], CBP [Austen et al., 1997], SP1 [Seto et al., 1993; Lee et al., 1993], E1A, c-Myc [Shrivastava and Calame, 1994], ATF/CREB [Zhou et al., 1995], B23 [Inouye and Seto, 1994], YAF2 [Kalenik et al., 1997], RPD3 [Yang et al., 1996], cyclophilin A, and FKBP 12 [Yang et al., 1995]. In vivo, YY1 has been found to have positive, negative, and neutral influences on transcription [for review, see Shrivastava and Calame, 1994; Hahn, 1992]. In addition, YY1 is capable of initiating transcription with TFIIB and RNA polymerase II [Usheva and Shenk, 1994] and has been shown to influence transcription by its ability to bend DNA and thus regulate contact between elements [Natesan and Gilman, 1993]. Therefore, elucidation and characterization of YY1-responsive genes such as the histone H4 gene are essential to our understanding of this versatile transcription factor.

In this study, we provide evidence that YY1 is involved in the regulation of histone H4 gene expression. First, YY1 interacts with multiple gene regulatory elements in vitro. Furthermore, expression of YY1 in *Drosophila* cells influences promoter activity through the histone H4 regulatory elements suggesting that these elements are functional YY1 sites in vivo. We discuss potential regulatory mechanisms and models by which YY1 is intimately involved in histone gene regulation.

MATERIALS AND METHODS

Cell Culture

HeLa S3 cells were grown and maintained in suspension at 3–6 $\times$ 10^5 cells per ml at 37°C in Joklik-modified minimum essential medium (Gibco-BRL, Gaithersburg, MD) supplemented with 5% calf serum and 2% horse serum. *Drosophila melanogaster* Schneider's S-2 cells were grown at room temperature in Schneider's insect medium (Sigma Chemical Co., St. Louis, MO) supplemented with 10% heat-inactivated fetal calf serum (FCS).

Plasmid Construction

PBLCAT4, the parent vector for all constructs, contains the chloramphenicol acetyltransferase (CAT) gene under the control of the HSV-thymidine kinase (TK) promoter [Jonat et al., 1990]. All inserts were cloned into a Klenow-filled *Hin*dIII site upstream of the thymidine kinase promoter using blunt end ligation. The following inserts were derived from the FO108 human histone H4 gene [Kroeger et al., 1987]: −10/+210 bp (*Bbs*I/*Xmn*I), −10/+75 bp (*Bbs*I/*Blp*I) and +75/+210 bp (*Blp*I/*Xmn*I). The −418/−210 insert was derived from the *Hin*dIII/*Sma*I fragment of an FO108-related clone pFO 002. The nucleotide sequence for the histone gene was reported by Pauli et al. [1987] (GenBank accession number M16707). Figure 1 is a schematic diagram showing the relative locations of these fragments.

In Vivo Transient Transfection, Luciferase, and CAT Assays

Drosophila Schneider's S-2 cells were plated at a density of 4 $\times$ 10^5 cells/well in six-well plates (Corning) or 3.2 $\times$ 10^6 cells per 100-mm plate. Cells were transfected by calcium phosphate coprecipitation as described [Chen and Okayama, 1988; Di Nocera and Dawid, 1983]. The *Drosophila* cells were harvested 48–72 h after transfection and cell extracts were prepared and assayed for CAT [Gorman et al., 1982] and luciferase activities [Ausubel et al., 1989]. Luciferase activity was assayed using reagents obtained from Promega (Madison, WI) and measured in a Monolight 2010 luminometer (Analytical Luminescence Laboratory, Ann Arbor, MI). CAT activities were normalized for transfection efficiency using the luciferase data.

Isolation of Histidine-Tagged YY1

Recombinant His-tagged YY1 protein was obtained by overexpression in the *Escherichia coli* K-12-derived m15[pREP4] strain transformed with a His-YY1 plasmid (kindly provided by Dr. Thomas Shenk, Princeton, NJ) [Shi et al., 1991]. YY1 expression was induced by treatment of bacterial cultures with 1 mM isopropyl-β-D-

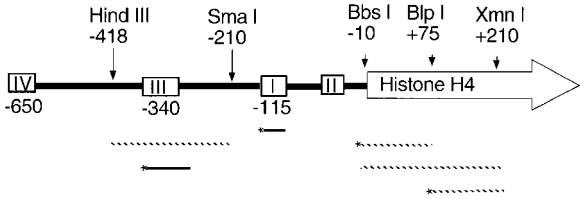

Fig. 1. Schematic of probes and gene fragments used for studying YY1 interactions on the H4 histone gene FO108. Dashed lines, DNA fragments cloned into the blunted Hind III site of PBLCAT4. Solid lines, oligonucleotide probes used in these studies. *, the DNA fragment or oligonucleotide was used as an EMSA probe.

TABLE I. Sequences of Oligonucleotides Used in This Study[a]

Full-length site III:	5′ CGCTGGCGGCCTCCTGCCAGTCTCTGGCCTCCATTTGCTCTTCCTG 3′
Proximal site III:	5′ TCTCTGGCCTCCATTTGCTCTTCCTGAG 3′
Prox. site III mutant:	5′ TCTCTGGCCTagcTTTGCTCTTCCTGAG 3′
Distal site III:	5′ GCGCTGGCGGCCTCCTGCCAGTCTCTGGCC 3′
Dist. site III mutant:	5′ GCGCTGGCGGCCTCCTtCaccTCTCTGGCC 3′
YY1 wild type:	5′ CGCTCCGCGGCCATCTTGGCGGCTGGT 3′
YY1 mutant:	5′ CGCTCCGCGattATCTTGGCGGCTGGT 3′
Distal site I:	5′ CGGAAAAGAAATGACGAAATGTCGAGA 3′

[a]Sequences of oligonucleotides used either as electrophoretic mobility shift assay (EMSA) probes or as unlabeled EMSA competitors are shown.

thiogalactopyranoside for 4 h. His-YY1 was purified from bacterial lysates by nickel chelate column chromatography [Shi et al., 1991].

Nuclear Extracts and Electrophoretic Mobility Shift

Nuclear extracts from exponentially growing HeLa S3 cells were prepared as described previously [van Wijnen et al., 1992]. Protein concentrations were determined using the Coomassie Plus Protein Assay Reagent (Pierce Chemicals, Rockford, IL). Electrophoretic mobility shift assay (EMSA) and probe preparation were performed essentially as described [van Wijnen et al., 1996]. Crude nuclear extract or bacterially expressed His-tagged YY1 fusion protein (1 µg) was incubated for 15 min at room temperature with 10 fmole of ^{32}P-end-labeled double stranded probes in a total reaction volume of 20 µl with 10 mM Hepes (pH 7.5), 50 mM KCl, 10% glycerol (v/v), 1 mM dithiothreitol (DTT), 0.2 mM EDTA, and 2 µg poly (dI-dC)·(dI-dC) and 1 µg poly (dA-dT)·(dA-dT). Competition experiments were performed by incubating the above reactions with 2.5 pmole unlabeled oligonucleotides. Antibody block-shift experiments were performed in the absence of DTT by adding 1 µl of antibody to the protein and incubating for 1 h on ice before the addition of DNA probe. Both the anti-YY1 and the anti-AP2 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The binding reactions were electrophoretically separated in 0.5 × Tris-borate/EDTA, 40:1 bis acrylamide 4% nondenaturing acrylamide gel at 200 V. Gels were dried and autoradiographed. Table I lists the EMSA oligonucleotide probes used. The *Bbs*I/*Blp*I (−10 to +75 nt) and *Blp*I/*Xmn*I (+75 to +210 nt) fragments were used as EMSA probes for analysis of the coding region (Fig. 1 for relative locations of the probes on the histone H4 gene).

TABLE II. Potential YY1 Binding Sequences in the Histone H4 Gene[a]

Site IV (−630 nt):	TGACGTC CAT GAGA
Site III (−340 nt):	TGGCCTC CAT TTGC
Site I (−120 nt):	ATTTCGT CAT TTCT
H4 gene (+30):	CAGCGGT CAT GTCC
H4 gene (+130):	AGCCTGC CAT TCGG
H4 gene (+180):	CTGGCCT CAT TTAC
YY1 consensus:	VKY CAT NWB

[a]Histone regulatory sequences for sites IV, III, I, and histone H4 coding region, shown aligned with the YY1 consensus sequences [Hyde-DeRuyscher et al., 1995], where V = A or C or G, K = G or T; Y = C or T, N = A or C or G or T, W = A or T, and B = C or G or T.

RESULTS

H4UA1 Is Identical to Transcription Factor YY1

Histone H4 gene transcription is modulated in a cell growth and cell cycle-dependent manner. The regulatory element site III (nt −418 and −215) has been shown to influence histone transcription and may facilitate regulation during cell growth [Kroeger et al., 1987; Zahradka et al., 1993; Larson et al., 1989]. Our laboratory has previously identified H4UA1 as a protein–DNA complex that interacts within this region [van der Houven van Oordt et al., 1992]. The sequences important for H4UA1 binding (5′-GGCCTCCATTTGC-3′) are also homologous to a consensus YY1 binding site (Table II). To test if H4UA1 is YY1, we performed EMSA analysis together with oligonucleotide competition and specific antibody recognition experiments. The results in Figure 2A show that the H4UA1 interaction with the site III EMSA probe (−368 to −323 nt) is specifically competed by an excess of oligonucleotide encoding the wild-type H4UA1 binding site (lane 2) or the YY1 (lane 4) consensus element, but not with oligonucleotide containing mutated H4UA1 or YY1 sites (lanes 3 and 5). The remaining binding activity

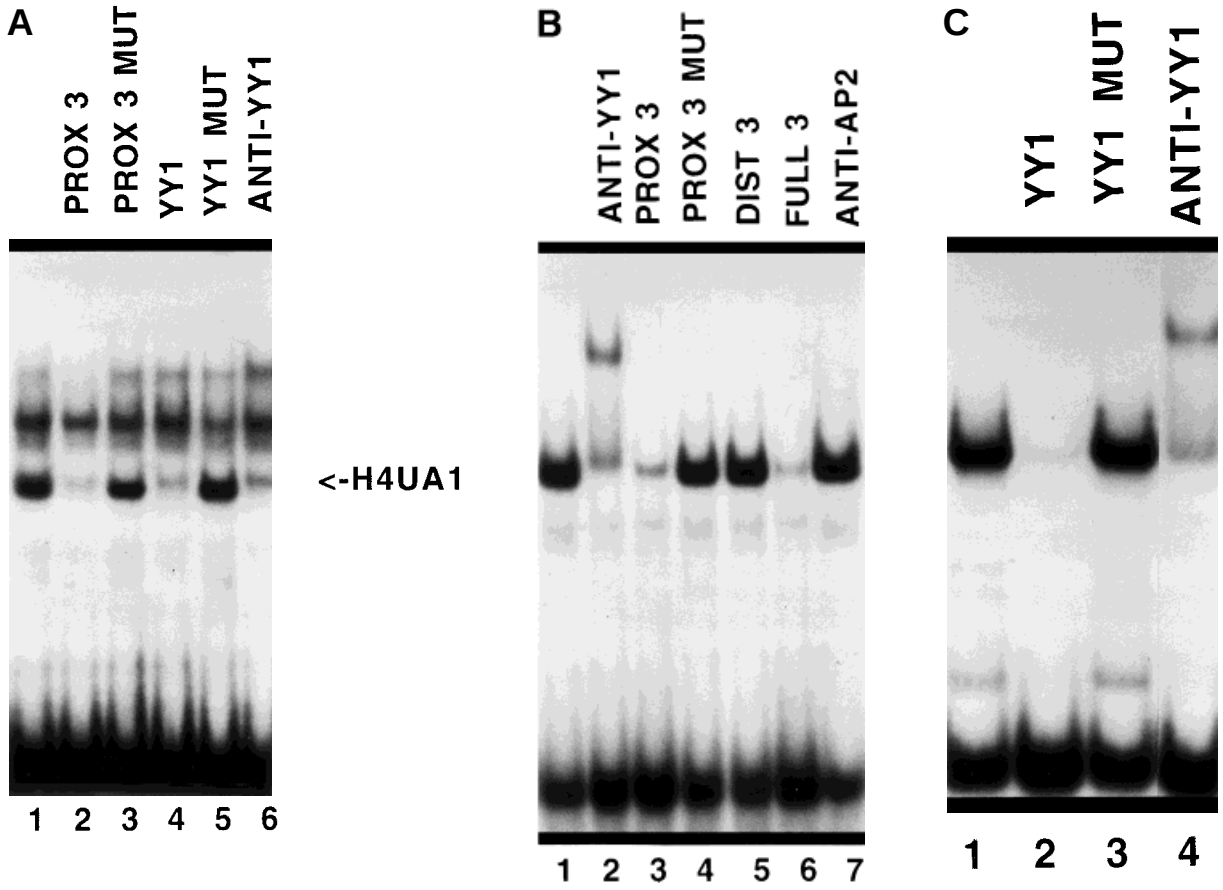

Fig. 2. The H4 site III binding protein H4UA1 is YY1. **A:** EMSA was performed using HeLa nuclear extract (3 µg/reaction) and the full site III oligonucleotide probe (**lanes 1–6**) to determine whether H4UA1 is YY1. The H4UA1 complex is competed by an excess of either unlabeled proximal site III (lane 2) or YY1 binding site (lane 4) oligonucleotide, but not by an equivalent amount (2.5 pmole, 250-fold molar excess) of either the proximal site III mutant (lane 3) or YY1 mutant binding site (lane 5) oligonucleotide. Incubation with 1 µl of YY1 antibody results in a block shift of the H4UA1 complex (lane 6). **B:** EMSA was performed using HeLa nuclear extract (3 µg/reaction) and the YY1 oligonucleotide probe (**lanes 1–7**). YY1 interaction on the YY1 probe is competed by H4UA1 binding site oligonucleotides [proximal site III oligonucleotide (lane 3) and full site III oligonucleotide (lane 6)], but not by an equivalent amount (2.5 pmole) of the oligonucleotides lacking H4UA1 binding site [distal site III (lane 5) and proximal site III mutant (lane 4)]. Incubation with YY1 antibody (lane 2) results in a block shift of the YY1 complex, whereas incubation with an AP 2 antibody (lane 7) did not. **C:** EMSA was performed to determine whether purified recombinant fusion protein His-YY1 interacts specifically with full site III probe (**lane 1–4**). His-YY1 complex is competed by an excess of unlabeled YY1 binding site oligonucleotide (lane 2), but not by an equivalent amount of unlabeled YY1 mutant binding site (lane 3). Incubation with the YY1 antibody (lane 4) results in a block shift of the His-YY1 complex.

represents a comigrating nonspecific band [van der Houven van Oordt et al., 1992]. Furthermore, the H4UA-1 complex was recognized by an anti-YY1 antibody (lane 6). Thus, these results suggest that the site III binding factor H4UA1 is YY1.

To confirm that YY1 interacts specifically with the H4UA1 binding site, we tested the ability of site III oligonucleotides to compete for YY1 interactions on a YY1 binding site EMSA probe. The results in Figure 2B show that oligonucleotides spanning the H4UA1 binding site (lanes 3 and 6) compete for the YY1 interaction on the YY1 probe, whereas oligonucleotides lacking the H4UA1 binding site (lanes 4 and 5) do not. The YY1 complex is recognized by an antibody against YY1 (lane 2), whereas a control antibody against AP-2 does not recognize the YY1 complex (lane 7). Further evidence that YY1 interacts specifically at site III is presented in Figure 2C, which shows that recombinant His-YY1 fusion protein interacts on the site III oligonucleotide probe. His-YY1 fusion protein binding to site III is competed by the consensus YY1 oligonucleotide (lane 2), but not by the corresponding mutant YY1 oligonucleotide (lane

3). Consistent with these results, the His-YY1 complex is also recognized by the YY1 antibody (lane 4). Thus, the data presented in Figure 2A–C establish that the previously defined site III binding protein H4UA1 is the transcriptional regulator YY1.

Transcription Factor YY1 Interacts at Site I

Site IV (−600 nt) and the distal region of site I (−110 nt) of the FO108 histone H4 gene bind common factors, such as ATF [Guo et al., 1997b]. The nuclear matrix associated protein NMP-1 interacts specifically at site IV, and has been identified as YY1 [Guo et al., 1995]. YY1 binds in an overlapping arrangement with ATF/CREB (see Fig. 6). We performed EMSA experiments to test whether YY1 interacts with site I in a similar overlapping arrangement. Figure 3 demonstrates that YY1 binds to an oligonucleotide probe (−125 to −101 nt) spanning distal site I. The site I-YY1 complex is specifically recognized by the YY1 antibody (lane 1) and is competed by YY1 binding site oligonucleotides (lanes 3 and 5). Oligonucleotides lacking the YY1 binding site have no effect on the YY1 complex (lanes 4, 6, and 7). The slower migrating ATF/CREB complexes interacting on the distal site I probe are competed only by the ATF/CREB consensus oligonucleotide (lane 7); this oligonucleotide does not compete for the YY1 complex. Furthermore, purified His-YY1 fusion protein interacts with the distal site I probe in EMSA experiments (data not shown). These results show that YY1 interacts specifically with histone H4 distal site I.

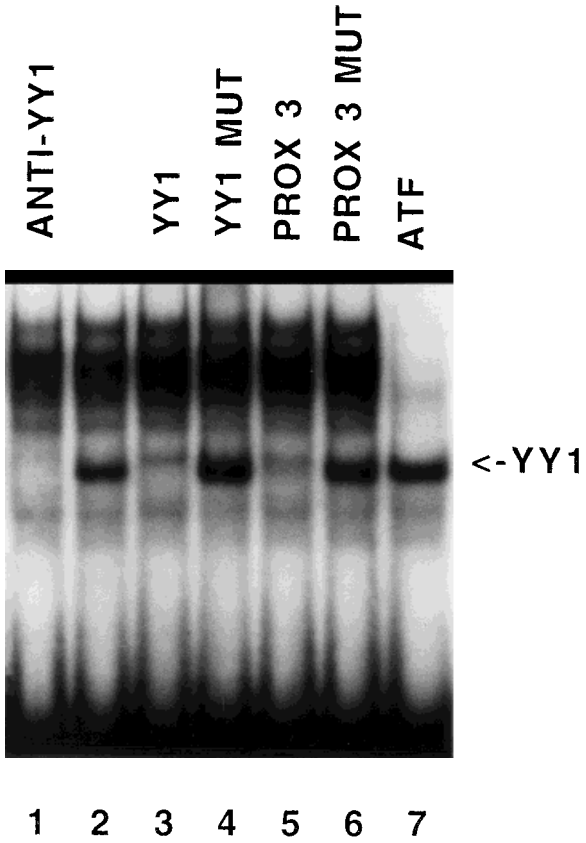

Fig. 3. YY1 can interact with distal site I. EMSA was performed using HeLa nuclear extract to determine whether YY1 interacts with distal site I probe (**lanes 1–7**) . A fast-mobility complex (marked) is specifically competed by an excess of oligonucleotides that interact with YY1 [YY1 binding site (lane 3) and proximal site III (lane 5)], but not an equivalent amount (2.5 pmole) of oligonucleotides lacking YY1 binding sites [YY1 mutant (lane 4), proximal site III mutant (lane 6) and ATF consensus (lane 7)]. Incubation with the YY1 antibody results in a block shift of the complex (lane 1).

YY1 Interacts Within the Coding Region of the Histone H4 Gene

We recently showed that the coding sequences between nt −10 and +200 of the histone H4 gene contribute to transcriptional control [Last et al., 1998]. Analysis of these coding sequences (nt −10 to +210) identified potential YY1 binding sites at +30, +130 and +180 (Table II). EMSA experiments were performed to determine whether YY1 can interact with these putative YY1 elements. Figure 4A demonstrates that an EMSA probe spanning nt −10 to +75 of the coding region forms a complex with either His-YY1 fusion protein (lane 1) or with YY1 from HeLa cell nuclear extracts (lane 6). Both the His-YY1/coding region complex (lane 2) and the YY1/coding region complex from HeLa extract (lane 5) are recognized by an antibody against YY1. Lanes 3 and 4 show that the YY1 complex in HeLa nuclear extracts is competed by a consensus YY1 oligonucleotide, but not by a mutant YY1 binding site oligonucleotide.

Figure 4B shows that the +75 to +210 coding region probe binds YY1. Purified His-YY1 interacts specifically with the nt +75 to +210 region (lane 1) and a YY1 complex was also detected in HeLa nuclear extract (lane 6). Both the His-YY1 complex (lane 2) and the YY1 complex from HeLa extract (lane 5) are recognized by an antibody against YY1. Lanes 3 and 4 show that the YY1 complex in HeLa nuclear extracts is competed by wild-type, but not by the mutant YY1 oligonucleotides. The results presented in Figure 4A,B demonstrate that YY1 interacts with at least two regulatory sites within the histone H4 coding region in vitro.

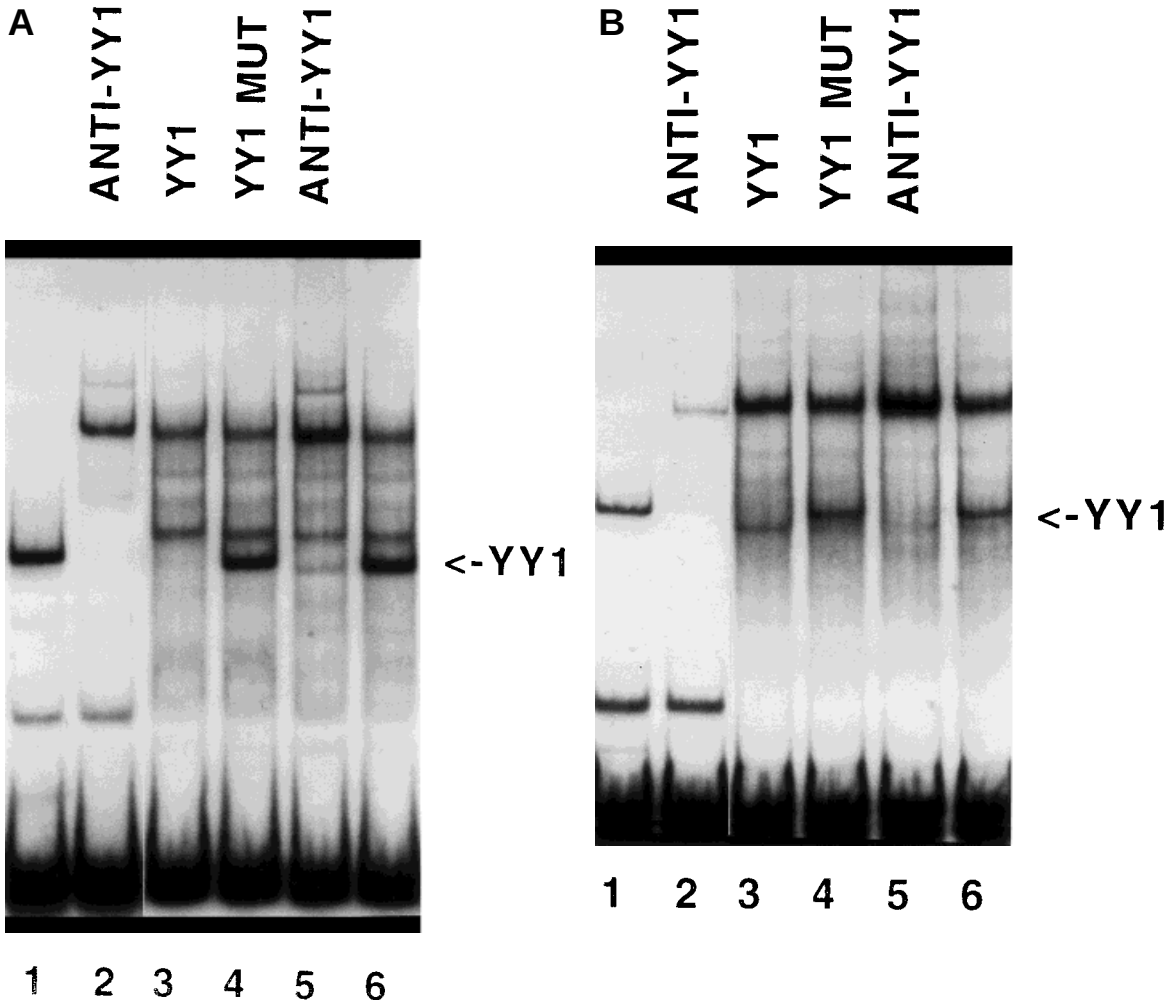

Fig. 4. YY1 can interact with H4 histone coding region. EMSA results showing that both the −10 to +75 H4 coding region probe **(A)** and the +75 to +210 H4 coding region probe **(B)** interact with purified His-YY1 (**lanes 1, 2**) and with YY1 in HeLa nuclear extract (**lanes 3–6**). The YY1 complex (marked) was competed by an excess of the YY1 binding site oligonucleotide (lanes 3), but not by an excess of the YY1 mutant oligonucleotide (lane 4). Incubation with the YY1 antibody results in a block shift of either the His-YY1 complex (lane 2) or the YY1 complex formed with HeLa nuclear extract (**lane 5**).

Histone H4 Gene FO108 Is Responsive to YY1 Expression

To study the effects of YY1 in vivo on histone H4 gene transcription, several segments of the histone gene were cloned upstream of a thymidine kinase promoter and chloramphenicol acetyltransferase reporter gene. These H4 gene segments span one or two of the YY1 binding sites characterized in this study and are located in previously defined regulatory domains (nt −10 to +200 of the coding region [Last et al., 1998] and site III [Kroeger et al., 1987]). *Drosophila* S-2 cells were chosen for transfection experiments because these cells are devoid of endogenous YY1 [Shi et al., 1991]. The reporter construct pBLCAT4, which lacks histone gene sequences, was unresponsive to YY1 expression. In contrast, reporter gene constructs containing the histone gene segments nt −10 to +210, nt −10 to +75, nt +75 to +210 or nt −418 to −210 are responsive up to twofold (Fig. 5A). Figure 5B shows that promoter activity from the −10 to +210 nt pBLCAT4 or the −418 to −215 nt (site III) pBLCAT4 constructs cotransfected with increasing amounts of YY1 expression vector is dose dependent. These re-

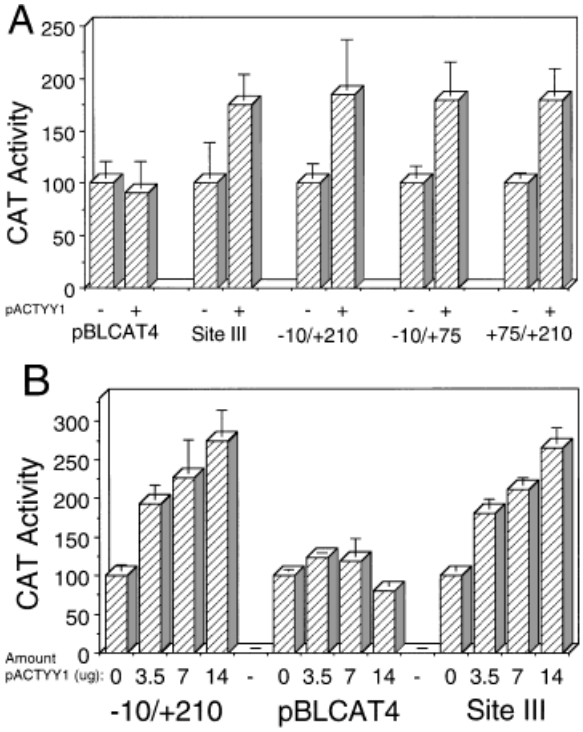

Fig. 5. Functional evidence showing that YY1 overexpression up-regulates transcription through the histone H4 regulatory elements. **A:** Graphic representation of the relative CAT activity of test constructs in *Drosophila* Schneider's S-2 cells co-transfected in the presence or absence of YY1. The heterologous reporter CAT constructs (pBLCAT4, −10/+75, +75/+210, −10/+210 and site III) were individually (1.5 μg) co-transfected with either 3 μg of the expression plasmid pACT YY1 (+) or pACT vector (lacking YY1) (−) in 6-well plates. **B:** The effect of YY1 expression on −10/+210 and on site III reporter constructs is dose dependent. Increasing amounts (0, 3.5, 7, 14 μg) of pACT YY1 expression plasmid was cotransfected with 7 μg of CAT reporter plasmids (−10/+210, site III or PBLCAT4) into *Drosophila* Schneider's S-2 cells grown on 100-mm plates. The total amount of DNA transfected was 22 μg. The differences in the amount of pACT YY1 vectors were corrected with a complementary amount of pACT. Transfected cells were harvested after 48–72 h and analyzed for CAT and luciferase activity. CAT activities were normalized for transfection efficiency using luciferase results. Error bars designate standard deviation. Results are expressed as percentage of the reporter vector CAT activity (100%) without any pACTYY1 cotransfected.

sults suggest that YY1 can stimulate transcription up to 2.7-fold through histone H4 regulatory elements in vivo.

DISCUSSION

The cell growth and cell cycle regulation of histone H4 gene expression is mediated by multiple regulatory elements located throughout the gene. Although significant progress has been made in understanding molecular mechanisms involved in the control of histone transcription, the identities of some of the factors and pathways involved still need to be elucidated. The purpose of this study was to determine if the ubiquitous transcription factor YY1 interacts with functional histone H4 gene regulatory elements.

Our data suggest that the versatile transcription factor YY1 has a functional role in regulating human histone H4 gene transcription. Transient expression experiments in eukaryotic cells lacking YY1 demonstrate that YY1 functionally interacts at Site III in the histone H4 promoter and within the coding region. Protein–DNA interaction experiments show that both endogenous YY1 in HeLa extracts and affinity-purified recombinant YY1 interact specifically with multiple histone gene regulatory elements, including site I, site III, and coding region elements. Hence, several lines of evidence support the intimate involvement of YY1 in histone gene regulation.

YY1 may regulate histone gene expression by mediating protein–protein interactions with other gene regulatory factors or by altering the responsiveness of other factors, perhaps by mutually exclusive binding. For example, YY1 may compete with other H4 gene transcription factors (e.g., ATF1/CREB) [Wright et al., 1995; Guo et al., 1997b] for binding to the H4 gene promoter. The regulatory elements site IV (nt −643 to −636) and site I (nt −117 to −110) both contain functional ATF/CREB motifs that overlap with YY1 binding sites (Fig. 6). Modulation of histone H4 gene transcription may depend on critical threshold levels in the binding activities of YY1 and the opposing factors. There are many examples of YY1 influencing gene expression by competing with DNA binding factors involved in cell signaling pathways, e.g., hormone receptors [Guo et al., 1997a; reviewed in Shrivastava and Calame, 1994]. Interestingly, the multifunctional YY1 participates in protein–protein interactions with Sp1 [Seto et al., 1993; Lee et al., 1993]. Sp1 has been shown to regulate histone H4 gene expression [Guo et al., 1997b; Birnbaum et al., 1995]. Therefore, YY1 might also modulate histone transcription by altering ATF/CREB or Sp1 activity.

YY1 may facilitate a process that remodels local chromatin of the histone H4 gene. Sites I and IV both reside near DNase I hypersensitive sites that are devoid of normal nucleosomes

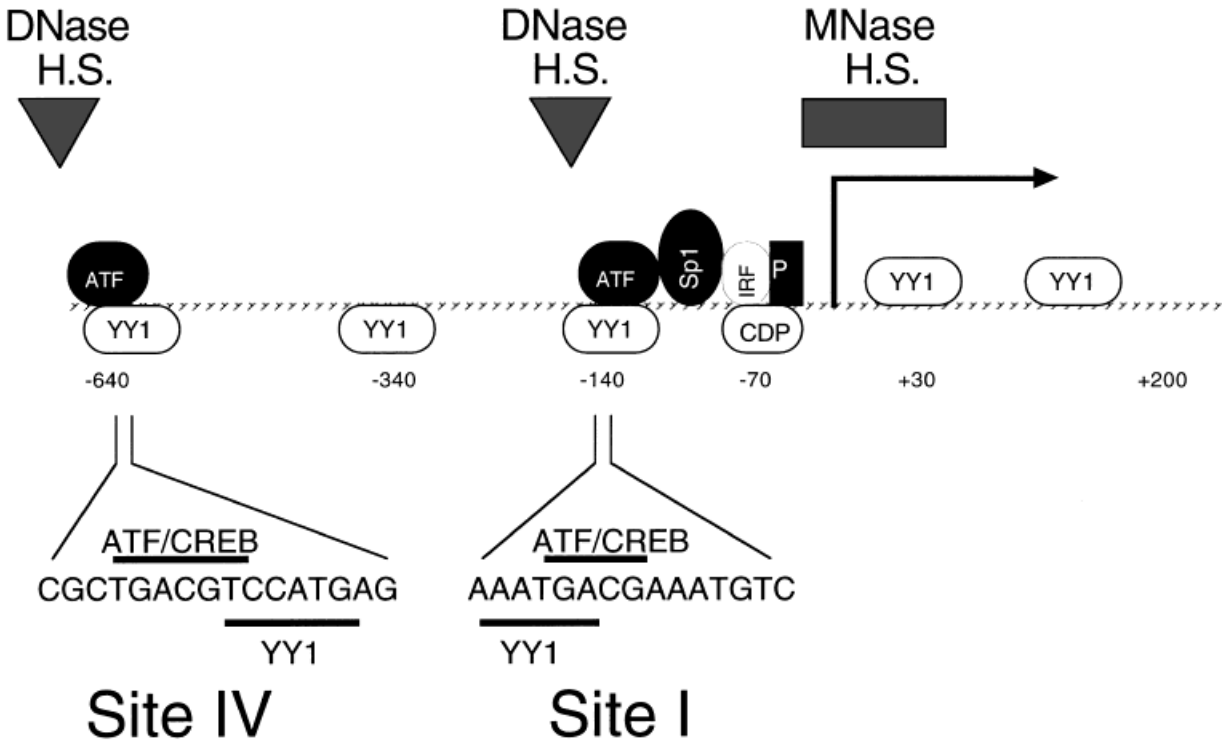

Fig. 6. Schematic model of multiple transcription factor interactions of the FO108 histone H4 gene. Large arrowheads, DNase I hypersensitive sites, marked DNase H.S. A micrococcal nuclease-sensitive site is marked by a large box, labeled MNase H.S. The various identified transcription factors are indicated and relative locations for IRF-2 (IRF), CDP/*cut* (CDP), Sp1, ATF/CREB (ATF), and HiNF-P (P) on the promoter are shown. Overlapping YY1 and ATF/CREB binding motifs for sites I and IV are also shown.

[Chrysogelos et al., 1989]. Site III and coding region elements are within segments of the gene that change in accessibility to nucleases during the cell cycle [Chrysogelos et al., 1989]. Thus, chromatin structure near YY1 binding sites is modified in a cell cycle stage-specific manner. We have previously observed that both YY1 DNA binding activity and overall protein levels remain constant during the cell cycle [Last et al., 1998; data not shown]. If YY1 contributes to cell cycle control of the histone H4 gene it may do so through protein–protein interactions with other gene regulatory factors, or by altering the responsiveness to these other factors. YY1 is known to interact with co-activators and repressors that alter the acetylation status of histones [Austen et al., 1997; Lee et al., 1995; Yang et al., 1996; reviewed in Pazin and Kadonaga, 1997]. Therefore, YY1 may have a critical role in recruiting factors that modify the chromatin structure of histone H4 gene regulatory elements in conjunction with other sequence specific factors and histone-modifying enzymes.

Regulating nuclear matrix attachment is another transcriptional mechanism by which YY1 might be involved in modifying histone H4 gene architecture. Because YY1 is an established nuclear matrix protein [Guo et al., 1995; McNeil et al., 1998], YY1 may support association of the H4 gene with the nuclear matrix in a transient open chromatin state. This association might enhance promoter accessibility and allow other transcription factors to interact with the H4 promoter to facilitate transcription. Although the details of the mechanism by which YY1 influences histone H4 transcription need to be established, the data presented in this study suggest that one mechanism involves YY1 interacting at multiple regulatory elements of the gene.

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