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Targeting of the YY1 Transcription Factor to the Nucleolus and the Nuclear Matrix In Situ: The C-Terminus Is a Principal Determinant for Nuclear Trafficking

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Abstract The multifunctional transcription factor YY1 is associated with the nuclear matrix. In osteoblasts, the interaction of several nuclear matrix-associated transcription factors with the bone specific osteocalcin gene contributes to tissue-specific and steroid hormone-mediated transcription. A canonical nuclear matrix targeting signal (NMTS) is present in all members of the AML/CBFβ transcription factor family, but not in other transcription factors. Therefore, we defined sequences that direct YY1 (414 amino acids) to the nuclear matrix. A series of epitope tagged deletion constructs were expressed in HeLa S3 and in human Saos-2 osteosarcoma cells. Subcellular distribution was determined in whole cells and nuclear matrices in situ by immunofluorescence. We demonstrated that amino acids 257–341 in the C-terminal domain of YY1 are necessary for nuclear matrix association. We also observed that sequences within the N-terminal domain of YY1 permit weak nuclear matrix binding. Our data further suggest that the Gal4 epitope tag contains sequences that affect subcellular localization, but not targeting to the nuclear matrix. The targeted association of YY1 with the nuclear matrix provides an additional level of functional regulation for this transcription factor that can exhibit positive and negative control. J. Cell. Biochem. 68:500–510, 1998.

Key words: transcription factor; nuclear matrix; YY1; amino acids; functional regulation

Recent studies have demonstrated that targeting of transcription factors to the nuclear matrix is functionally linked to transcriptional control. The nuclear matrix is a dynamic and intricate structure involved in multiple gene regulatory functions, as well as DNA replication [Fey et al., 1984, 1986, 1991; Berezney, 1991; van Driel et al., 1991; Pienta et al., 1991; Stein et al., 1994; Berezney and Coffey, 1975]. The nuclear matrix consists of a peripheral lamina-core complex and an internal filamentous ribonucleoprotein network that supports the activities of a broad spectrum of functional subnuclear compartments including the nucleolus [Scheer and Weisenberger, 1994], SC-35 RNA processing domains [Spector et al., 1991; Fu and Maniatis, 1990], as well as PML [Weis et al., 1994; Dyck et al., 1994] and coiled bodies [Andrade et al., 1991; Raska et al., 1990].

The association of transcription factors with the nuclear matrix provides a mechanism for integration of transcriptional regulatory cues within the context of nuclear architecture (reviewed in [Stein et al., 1995]). These functional interrelationships are reflected by the selective and/or temporal subnuclear partitioning of transcription factors during cell growth and differentiation. Our identification of two nuclear matrix-associated DNA binding proteins, NMP-1 [Guo et al., 1995, 1997a; Bidwell et al., 1993; Dworetzky et al., 1992] and NMP-2 [Merriman et al., 1995; Bidwell et al., 1994], initially indicated selective partitioning of regulatory fac-

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tors between the nuclear matrix and nonmatrix nuclear fractions in osseous and nonosseous cells. Specifically, NMP-1, identified as YY1, has been shown to attenuate the vitamin D responsiveness of the bone-specific osteocalcin gene [Guo et al., 1995, 1997a] and may antagonize ATF/CREB activation of a cell cycle-regulated histone H4 gene [Guo et al., 1997b]. Another example of nuclear matrix-mediated transcriptional control involves the AML/CBFα class of factors that are key regulators of the hematopoietic [Meyers and Hiebert, 1995] and osteogenic lineages [Komori et al., 1997; Ducy et al., 1997; Banerjee et al., 1997]. AML-3/CBFα1, for example, is relatively bone specific and is requisite for development of the mature osteoblast phenotype and contributes to the osteocalcin gene transcriptional activity in osteoblasts. The identification of a specific 31-amino acid nuclear matrix targeting signal in the AML factors provides a molecular basis for directing regulatory factors to transcriptionally active domains in the nuclear matrix. Although the nuclear matrix targeting signal (NMTS) can autonomously direct heterologous proteins to the nuclear matrix (NM), it is only present in the AML/CBFα class of runt-homology domain (rhd) proteins. This finding further supports the concept that interactions with the nuclear matrix contribute to promoter selective control of transcription. In contrast to AML factors, which are tissue-restricted and predominantly associated with the nuclear matrix, YY1 is a ubiquitous factor that partitions between the nuclear matrix and nonmatrix compartments. Therefore, it is necessary to identify the sequences in YY1 that contribute to association with the nuclear matrix.

YY1 regulates the expression of many cellular and viral genes by either trans-activation, trans-repression or initiation of transcription [Shrivastava and Calame, 1994; Hahn, 1992]. The diverse functions of YY1 may be attributed to the interaction of YY1 with numerous cellular factors (reviewed in [Shrivastava and Calame, 1994; Hahn, 1992]). Several functional regions of YY1 have been identified through deletion analysis. The N-terminal portion of the protein contains two acidic regions that are important for the transactivation activity of YY1 [Bushmeyer et al., 1995; Austen et al., 1997]. A sequence between the Gly/Ala-rich region (aa 154–198) and the DNA binding domain (aa 298–414) appears to enhance transactivation activity. The DNA binding domain is composed of four zinc fingers and all four are essential for DNA binding activity [Bushmeyer et al., 1995]. The second and third zinc finger are required for nuclear localization [Austen et al., 1997]. Interestingly, sequences that overlap the zinc-fingers (aa 333–397) are important for repressor activity [Bushmeyer et al., 1995]. The DNA binding and Gly/Ala rich domains of YY1 mediate protein-protein interactions with TBP, TFIIB, $TAF_{II}55$, and CBP. The two acidic transactivation domains are not required for these interactions [Austen et al., 1997].

In this study, we examined the in situ subnuclear localization of YY1 using a panel of YY1 deletion constructs. These studies identified domains in YY1 with low and high affinity for association with the nuclear matrix. We showed that the carboxy terminus of the YY1 protein contains a strong NMTS. Our studies also indicate that subnuclear localization, but not trafficking to the nuclear matrix, appears to be influenced by heterologous functional domains.

MATERIALS AND METHODS

Cell Culture

HeLa S3 cells were grown in Dulbecco's Minimal Essential Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Saos-2 cells were grown in McCoy's 5A Medium supplemented with 15% FBS.

Transient Transfections

HeLa cells were plated on 0.5% gelatin coated coverslips (Fisherbrand, #12-545-101, 22cir-1; Fisher, Pittsburgh, PA) in 6 well tissue culture trays at a density of 0.08×10^6 cells/well. Cells were grown approximately 24 h after plating on coverslips in the appropriate growth medium. Cell density was approximately 60-80% at the time of transfection. HeLa cells were transiently transfected using Superfect as described by the manufacturer (Qiagen, Santa Cruz, CA). Saos cells were transfected by DEAE-dextran. DNA was mixed with serum-free medium containing 0.2 mg/ml DEAE-dextran and 0.5 mg/ml chloroquine. This mixture was added to 6 well plates and incubated 1.5–2.0 h at 37°C. Cells were subjected to a 15% glycerol shock for 2 min, washed with phosphate-buffered saline (PBS), and refed complete media. Cells were processed for immunofluorescence 24 h following transfection as described below. Epitope-tagged full-length YY1 DNA constructs or epitope-tagged YY1 deletion constructs used in these transfections are described below.

Plasmid Constructions

Plasmids Gal4/YY1(1-414), Gal4/YY1(1-200), Gal4/YY1(1-256), Gal4/YY1(1-341), Gal4/YY1(201-414) were previously described [Bushmeyer et al., 1995]. Plasmid HA/YY1(1-414) was constructed by fusing the YY1 coding sequence [Shi et al., 1991] in frame with an HA epitope tag cloned into pcDNA1/Amp.

Cell Extraction and Fixation

All coverslips were rinsed 2 times with PBS. For whole cells, cells were fixed 10 min in 3.7% formaldehyde in PBS and then permeabilized 10 min in 0.1% Triton-X 100 in PBS. Unless otherwise stated, the in situ nuclear matrix intermediate filament (NMIF) preparation used was essentially that of Fey et al. [1984] as modified by Zeng et al. [1997] for transfected cells (designated in this paper as NMIF 1). Transfected cells overexpress the exogenous protein to greater levels than the endogenous protein is normally expressed and require an increased duration for cytoskeletal (CSK) extraction [Zeng et al., 1997]. For preparation of in situ nuclear matrix intermediate filaments (NMIF 1), plates were placed on ice, and cells were extracted 2 times for 15 min each with ice cold CSK buffer (10 mM Pipes, pH 6.8, 300 mM sucrose, 100 mM NaCl, 3 mM $MgCl_2$, 1 mM EGTA, 0.5% Triton) with 2 mM vanadyl ribonucleoside complex (VRC). Following CSK extraction, DNA was digested by the addition of room temperature digestion buffer (DB, 10 mM Pipes, pH 6.8, 300 mM sucrose, 50 mM NaCl, 3 mM $MgCl_2$, 1 mM EGTA, 0.5% Triton X-100) containing 50 U/ml RNase-free DNase I (Boerhringer Mannheim Biochemical, Indianapolis, IN) and 2 mM VRC. After 30 min of incubation, the DNase was removed and replaced with a fresh aliquot for an additional 30 min of incubation. Following removal of the DNase solution, chromatin and cytoskeleton proteins were removed by a 10-min incubation in ice cold digestion buffer supplemented with 0.25 M ammonium sulfate. This solution was removed and replaced with a fresh aliquot for an additional 5 min of incubation. This solution was removed and the cells fixed in ice cold digestion buffer containing 3.7% formaldehyde. After fixation, all cells were rinsed 2 times in PBS and 2 times in PBS containing 0.5% bovine serum albumin and 0.05% sodium azide (PBSA) prior to the addition of antibodies. Alternative NMIF preparations are described below. For NMIF 2, cells were extracted one time for 10 min with CSK buffer (without VRC). Following CSK extraction, cells were extracted for 10 min in 0.25 M ammonium sulfate in CSK buffer (without VRC). Nuclease digestion included 100 µg/ml DNase I (Sigma, St. Louis, MO) and 100 µg/ml RNase A (Sigma) for 20 min on ice. Nuclease digestion was followed by a 10-min 0.25 M ammonium sulfate extraction in CSK buffer (no VRC). Cells were fixed as described for NMIF 1. NMIF 3 was the same as NMIF 2 procedure, except that RNase A was excluded from the nuclease treatment step, to better preserve morphology.

Immunofluorescence

PBSA was used as the wash solution and for antibody dilution, unless otherwise indicated. Antisera were as follows: a rabbit polyclonal antiserum to the yeast GAL4 DNA binding region (Upstate Biotechnology, Lake Placid, NY, #06-262) was diluted 1/1,000; a rabbit polyclonal antiserum to the HA epitope was diluted 1/1,000 (Santa Cruz Biotechnology, Santa Cruz, CA, #sc805); a monoclonal antibody to B23 was diluted 1/100 (provided by P-K. Chan, Baylor College of Medicine, TX). Diluted antibody was added to coverslips in wells (or 20 µl of diluted antibody was added to coverslips, covered lightly with parafilm and incubated for 1 h at 37°C). Coverslips were rinsed 4 times for 5 min, and secondary antibody was added. Secondary antibody was goat anti-rabbit IgG conjugated to fluorescein or donkey anti-mouse IgG conjugated to Texas Red (Jackson Immunoresearch Laboratories, West Grove, PA) diluted 1/200, added to coverslips and incubated 1 h at 37°C. Following incubation coverslips were washed one time for 3 min with PBSA containing 0.1% Triton X-100 and 0.05 µg per ml of the DNA counterstain DAPI; one time in PBSA-Triton; and twice in PBS. Coverslips were mounted in Vectashield (Vector Laboratories, Burlingame, CA) as an antifade mounting media.

Microscopy

A Zeiss axiophot microscope equipped with epifluorescence filters was used. A CCD camera interfaced with a digital imaging microscope was used to record fluorescent images.

RESULTS

C-Terminal Domain of YY1 Is Required for Targeting to the Nuclear Matrix In Situ

The transcription factor YY1 has been shown to associate with the nuclear matrix both in situ and through biochemical analysis [Guo et al., 1995]. To address whether a specific domain of YY1 mediates interaction with the nuclear matrix, we assayed a series of Gal4/YY1 fusion proteins (Fig. 1). We previously showed that Gal4 (aa 1–147) alone enters the nucleus but is not associated with the nuclear matrix [Zeng et al., 1997]. Initially, we established that a chimeric protein containing the yeast Gal4 DNA binding domain fused to the full-length wild-type YY1 protein (1–414 amino acids) is targeted to the nuclear matrix in situ (Fig. 2). Saos cells were transiently transfected with the Gal4/YY1(1–414) fusion construct, and the subcellular distribution of the expressed protein was detected by in situ immunofluorescence analysis using anti-Gal4 antibody. Gal4/YY1(1–414) is distributed throughout the nucleus of whole cells (Fig. 2A,B) and nuclear matrix (NMIF) preparations (Fig. 2C). Similar results are obtained in HeLa cells (see below).

To characterize specific domains of YY1 required for NMIF association, additional Gal4/YY1 deletion constructs were examined. HeLa cells were transfected with various deletion constructs, processed for whole cell and NMIF in situ preparations, and examined for retention of the expressed proteins in the nuclear matrix. The expression pattern of Gal4/YY1(201–414) is indistinguishable from that of the full-length fusion protein; and this half of the YY1 protein is retained in the NMIF at levels similar to the wild-type protein (Fig. 3). Both the full-length

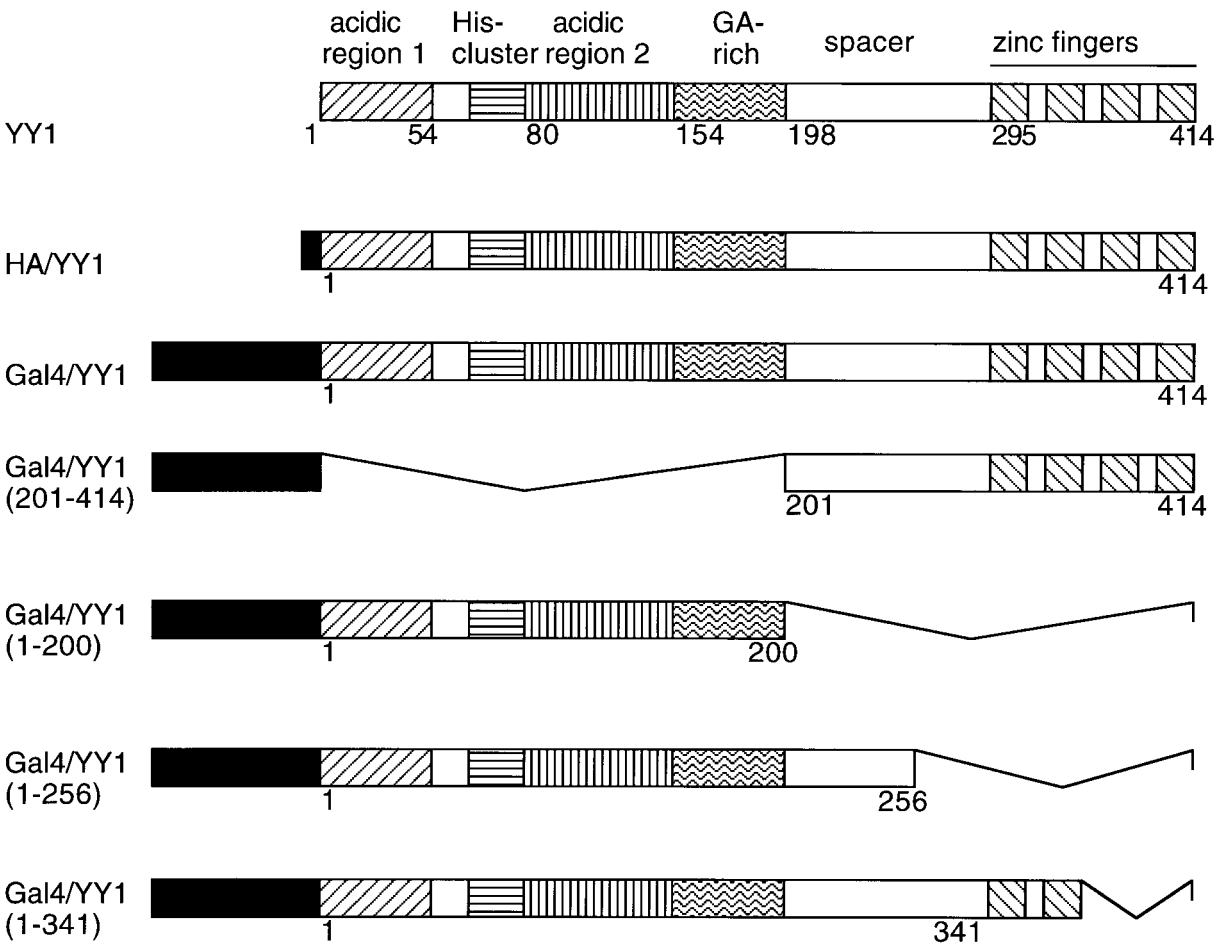

Fig. 1. Summary of Gal/YY1 and HA/YY1 constructs. The functional domains of YY1 (designated with different patterns) are shown on the upper line. The segments of YY1 in each construct used to map nuclear matrix targeting domains are shown below.

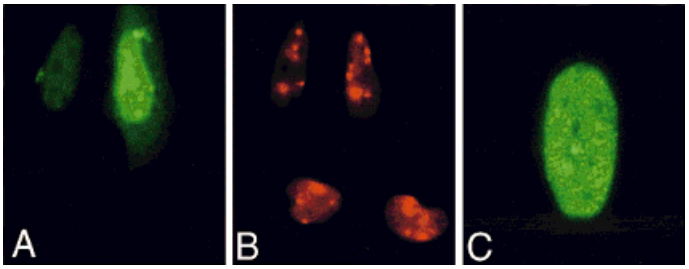

Fig. 2. Gal4/YY1 protein is targeted to the nuclear matrix when expressed in Saos cells. Saos cells were transfected with a Gal4/YY1(1–414, full length) expression construct, incubated 24 h, and whole cells and in situ nuclear matrix intermediate filament (NMIF) preparations were detected with the polyclonal anti-Gal4DNA binding domain antibody and stained with a fluorescein isothiocyanate-conjugated (FITC) second antibody (green fluorescence). Endogenous nucleolar phosphoprotein B23 was detected with the monoclonal antibody anti-B23 and visualized with a Texas Red-conjugated secondary antibody (red fluorescence). **(A)** Gal4/YY1(1–414), whole cell, 40X objective; anti-Gal4DBD; **(B)** Gal4/YY1(1–414), whole cell, 40X objective; anti-B23; **(C)** Gal4/YY1(1–414), in situ nuclear matrix preparation, 100X objective; anti-Gal4DBD. Removal of the majority of chromatin is indicated by absence of DAPI staining in the NMIF preparation (data not shown).

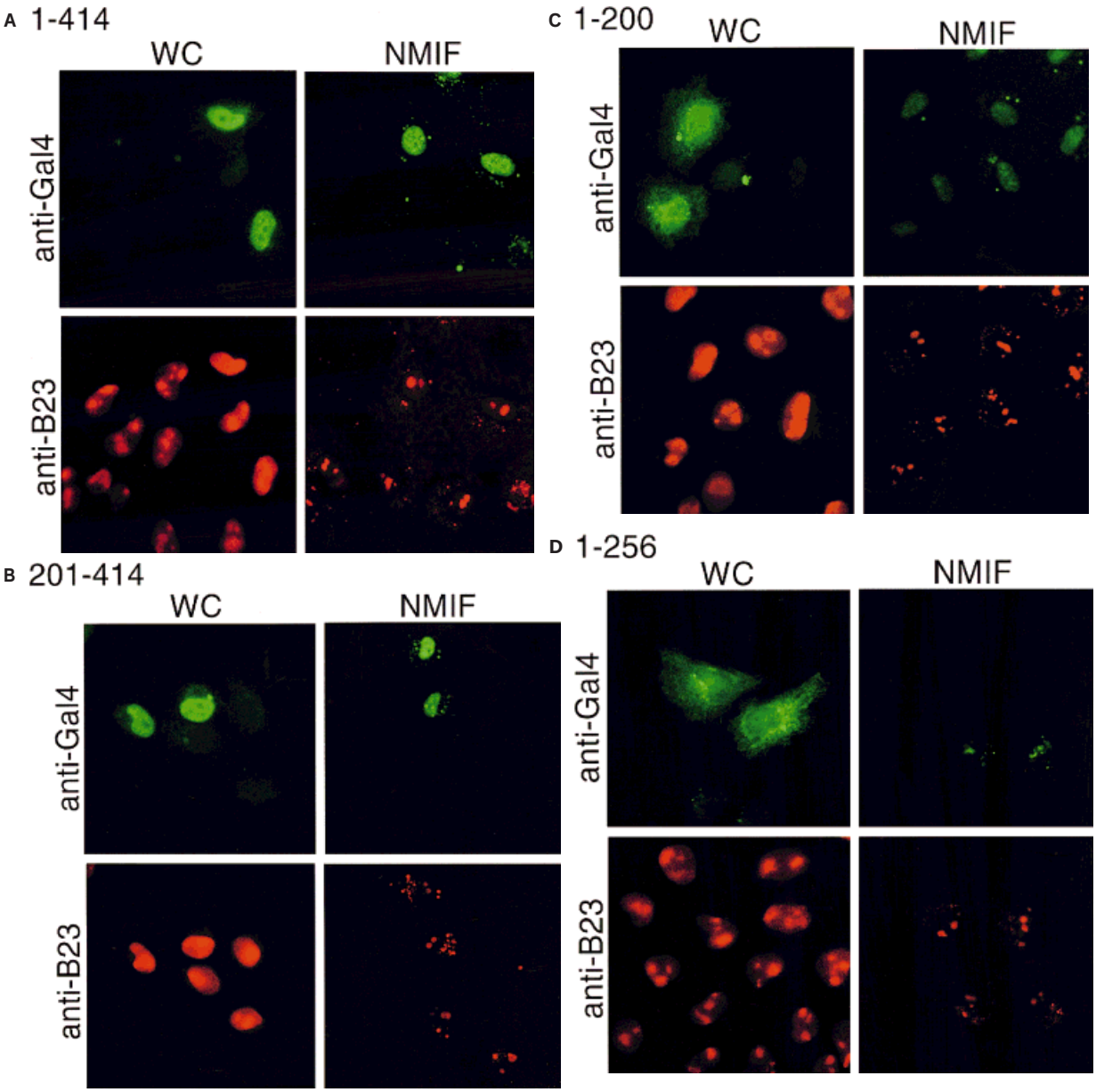

Figure 3.

Gal4/YY1 and the Gal4/YY1(201–414) fusion proteins are retained in the NMIF at levels similar to whole cell preparations (Fig. 3). However the retention of Gal4/YY1(1–200) and Gal4/YY1(1–256) fusion proteins in the nuclear matrix is significantly reduced when compared to the whole cells. Taken together, these results indicate that the C-terminal domain of the YY1 protein is necessary for efficient targeting to the nuclear matrix.

Although the N-terminal region is not required for association of YY1 with the nuclear matrix, variation was observed in the retention of Gal4/YY1(1–200) and Gal4/YY1(1–256) fusion proteins in the NMIF. These differences were evaluated by counting similar numbers of plated cells for each experiment. For the full-length construct, Gal4/YY1(1–414), we observed that most of the transfected cells retained the fusion protein in the NMIF preparation (Table I). However, for constructs lacking the C-terminal domain of YY1, the fusion proteins were retained in the NMIF in only a small percentage of cells (Table I). From these findings in both Saos and HeLa cells (Table I), we conclude that sequences between amino acids 200 and 414 are required for nuclear matrix targeting of the YY1 protein. Because the Gal4/YY1(1–200 and 1–256) fusion proteins were retained at low percentages in the NMIF preparations, sequences that reside in the N-terminal half of the YY1 protein may mediate low-affinity nuclear matrix association.

We further delineated the sequences between amino acids 200 and 414 by examining two additional C-terminal deletion constructs. These fusion proteins Gal4/YY1(1–341) (Fig. 4) and Gal4/YY1(1–370) (data not shown) were retained in the NMIF at levels quantitatively similar to those of the wild type. This result indicates that the domain between amino acids 256 and 341 is necessary for nuclear matrix association. However, the whole cell preparations of cells transfected with Gal4/YY1(1–341) show a different pattern of subcellular localization than observed for the full-length Gal4/YY1(1–414) fusion protein. The Gal4/YY1(1–341) fusion protein is observed throughout the cytoplasm, as well as the nucleus, of both Saos and HeLa transfected cells (Fig. 4 and data not shown). This unusual distribution of YY1 was also observed with the fusion protein Gal4/YY1(1–370) where only the 44 C-terminal amino acids are deleted. These data suggest that deletion of C-terminal sequences (370-414 amino acids) causes an increased accumulation of Gal4/YY1 fusion protein in the cytoplasm. These results are in agreement with those of Austen et al. [1997], which indicate that the C-terminus of YY1 contains the nuclear localization signal. Taken together, these findings suggest that there is a hierarchy of signals for protein localization within the cell.

Fig. 3. The C-terminal sequences of YY1 are sufficient for association with the nuclear matrix. HeLa cells were transfected with Gal4/YY1(1–414) and Gal4/YY1 deletion constructs and incubated 24 h. Whole cell and in situ nuclear matrix preparations were stained with anti-Gal4/DBD antibody, detected with FITC-conjugated second antibody and anti-B23 antibody, detected with Texas Red-conjugated second antibody. For each of the constructs, Gal4/YY1(1-414), **A;** Gal4/YY1(201–414), **B;** Gal4/YY1(1-200), **C;** and Gal4/YY1(1–256), **D;** immunofluorescence signals are shown for whole cell (WC) and nuclear matrix intermediate filament (NMIF) preparations. Single channel signals are shown for detection of Gal4/YY1 proteins (green) or the nucleolar protein B23 (red) are shown. All panels were imaged using an objective with 40X magnification.

Affinity-Dependent Retention of YY1 in the Nuclear Matrix

Our studies show limited but detectable retention of Gal4/YY1(1–200 and 1–256) in the nuclear matrix by in situ immunofluorescence (Table I). However, identical constructs were used by Bushmeyer et al. [1998], and the fusion proteins were not detected in the biochemical NMIF preparation. Different methods are routinely used for biochemical and in situ NMIF analyses. These variant methodologies differ in utilization of cell type, temperature of isolation, detergents, salts, and nucleases [reviewed by Fey et al., 1991]. The goal of in situ studies is to use a nuclear matrix preparation that best preserves the morphological integrity of the nuclear matrix, while extracting proteins that are not matrix associated. Therefore, we specifically compared several different methods for preparation of nuclear matrices to determine the consequences on morphological integrity and retention of YY1.

HeLa cells were transfected with Gal4/YY1 fusion constructs and then processed for in situ examination of whole cells or nuclear matrices prepared by one of three methods (described in the Methods section). The number of cells retaining the Gal4/YY1 fusion protein were counted. Table II compares the percentage of Gal4 immunopositive cells retaining the Gal4/YY1 fusion proteins in the nuclear matrix by the in situ procedure [Zeng et al., 1997; NMIF 1], a biochemical procedure [Bushmeyer et al.,

TABLE I. The C-Terminal Domain of YY1 Contains Sequences Necessary for Association With the Nuclear Matrix in Both Saos and HeLa Cells*

Construct transfected	HeLa cells (%)	Saos cells (%)
Gal4/YY1(1–414)	100	80
Gal4/YY1(201–414)	100	125
Gal4/YY1(1–200)	0	3
Gal4/YY1(1–256)	4	33
Gal4/YY1(1–341)	100	100

*Equivalent numbers of cells were examined for each construct within each experiment. For Saos cells, 300–500 cells were counted for each construct. For HeLa cells, 1,500–2,000 cells were counted for each construct. The number of Gal+ cells among the counted cells was recorded. The transfection efficiencies were determined to be consistent for each construct based on in situ β-galactosidase assays. Note percentage of transfected cells that retain the expressed Gal4/YY1 protein in the NMIF, determined using the following formula: the percentage of cells retaining fusion protein in the NMIF = number of Gal+ cells in the NMIF/number of Gal+ cells in the whole cells.

1998; NMIF 2], and the biochemical procedure omitting the RNase to better preserve nuclear morphology (NMIF 3). A high percentage of protein encoded by the full-length construct was retained in the NMIF by all these procedures (84-100%). A high percentage of protein encoded by the construct containing the C-terminal domain was also retained in the NMIF 1 and NMIF 3 preparations and was somewhat reduced in the NMIF 2 preparation. In agreement with Bushmeyer et al. [1998], the N-terminal fusion protein is clearly not retained in the NMIF biochemical procedure, but a low percentage of Gal4-positive cells are observed in the in situ NMIF preparation. In this experiment, the quantitation is based on a lower total number of transfected cells than in the experiment described in Table I (500 vs 2,000), which contributes to the variability of retention that we observed with the N-terminal domain. The structure of the nucleus in the biochemical procedures (NMIF 2 and NMIF 3) was not well preserved compared to the in situ procedure (NMIF 1) where nuclear morphology is preserved (Fig. 5). In all methods, the DNA was completely removed as detected by the observance of DAPI staining (data not shown). Our results also suggest that, at least with overexpressed YY1 proteins, there may be different affinities of interactions between YY1 domains and the nuclear matrix. The C-terminal domain of the YY1 protein appears to have a high affinity for the nuclear matrix. By contrast, the N-terminus may contain sequences that interact with lower affinity with the nuclear matrix. Taken together, these findings confirm that the C-terminus of YY1 is important for targeting to the nuclear matrix in situ.

Intranuclear Trafficking of YY1 to the Nucleolus

Endogenous YY1 protein is known to be associated with the nucleoli of whole cells and the nuclear matrix in situ [Guo et al., 1995]. None of the Gal4 epitope tagged full-length or deletion proteins that we examined in this study is detected in the nucleolus of whole cell or the NMIF preparations. We therefore compared HA/YY1 and Gal4/YY1 epitope tagged fusion proteins in situ. Cells were transfected with Gal4/YY1 or HA/YY1 fusion constructs, processed for immunofluorescence and immuno-

Fig. 4. Deletion of C-terminal sequences leads to an increased accumulation of Gal4/YY1 fusion protein in the cytoplasm in addition to nuclear localization. Saos cells were transfected with Gal4/YY1(1–414) and Gal4/YY1(1–341) and incubated 24 h. Whole cell and in situ nuclear matrix intermediate filament preparations were stained with anti-Gal4DBD antibody and Gal4/YY1 fusion proteins were detected with FITC-conjugated second antibody. **(A)** whole cells, Gal4/YY1(1–414); **(B)** whole cells, Gal4/YY1(1-341); **(C)** NMIF, Gal4/YY1(1–414); **(D)** NMIF, Gal4/YY1(1–341). Fields were imaged using a 40X objective.

Fig. 5. Nuclear matrix association of Gal4/YY1 deletion proteins and nuclear morphology is affected by the NMIF preparation method. In situ immunofluorescence analysis of the Gal4/YY1(1–414) fusion protein expressed in HeLa cells. Immunostaining with anti-Gal4DBD antibody is observed in both whole cell (WC) and NMIF preparations. **(A)** NMIF method 1, in situ method; **(B)** NMIF method 2, biochemical method; **(C)** NMIF method 3, biochemical method without RNase.

Fig. 6. HA tagged YY1 protein is selectively targeted to the nucleolus. Gal4/YY1 and HA/YY1 constructs were transiently transfected in Saos and HeLa cells and analyzed in whole cells (not shown) and NMIF preparations. Preparations were visualized with an FITC-conjugated secondary antibody recognizing the anti-Gal4DBD polyclonal antibody, which detects Gal4/YY1 fusion proteins (green) **(A,B)** or the anti-HA polyclonal antibody, which detects the HA/YY1 fusion protein (green) **(C,D)**. The nucleolus was visualized using a Texas Red-conjugated secondary antibody detecting the anti-B23 antibody (red) **(B,D)**. DAPI fluorescent signal (blue) was observed for whole cell but not for NMIF preparations (data not shown). Wild-type HA/YY1(1–414) fusion protein, but not Gal4/YY1(1–414) fusion protein, is predominantly localized to the nucleolus. Endogenous nucleolar phosphoprotein B23 is predominantly localized to the nucleolus as detected by anti B23 antibody. HA/YY1 and B23 colocalize (yellow) as detected by both anti-HA (green) and anti-B23 (red), whereas Gal4/YY1 does not show colocalization (red nucleolus).

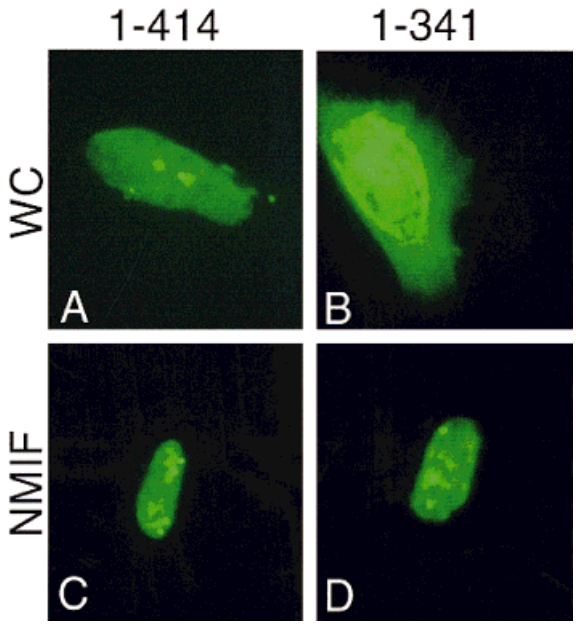

Figure 4.

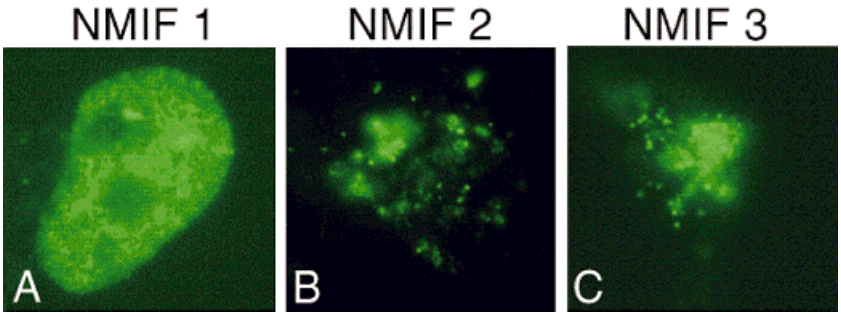

Figure 5.

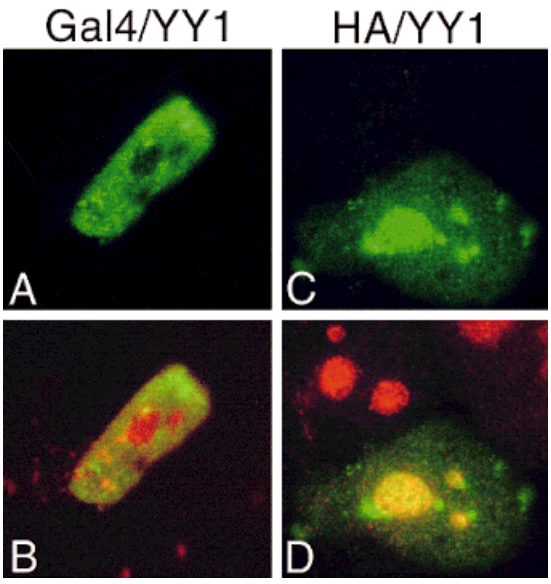

Figure 6.

TABLE II. Nuclear Matrix Association of Gal4/YY1 Deletion Proteins Is Affected by the NMIF Preparation Method*

Construct transfected	NMIF 1 (%)	NMIF 2 (%)	NMIF 3 (%)
Gal4/YY1(1–414)	100	96	84
Gal4/YY1(1–200)	20	0	0
Gal4/YY1(201–414)	100	60	94

*HeLa cells were transiently transfected with Gal4/YY1 constructs; 24 h later, the cells were processed for whole cells, and one of three nuclear matrix preparations before immunostaining with anti-Gal4DBD antibody and detection with FITC-conjugated second antibody. Equivalent numbers of cells were examined for each construct within each experiment (500 total cells were examined for each preparation). The percentage of transfected cells that retain the expressed Gal4/YY1 protein in the NMIF were determined as described in Table I.

stained with anti-Gal or anti-HA, and anti-B23, which detects the nucleolar phosphoprotein B23. Gal4/YY1(1–414) fusion protein is not associated with nucleoli and does not colocalize with B23 in NMIF preparations (Fig. 6A,B). HA/YY1 (1–414) fusion protein is detected throughout the nucleus and prominently in the nucleoli. Colocalization with the nucleolar phosphoprotein B23 occurs in NMIF preparations (Fig. 6C,D). Our findings suggest that the nucleolar detection of HA/YY1 fusion protein more closely reflects the endogenous localization than Gal4/YY1 fusion protein. These results were consistent in both HeLa and Saos cells. Although the Gal4/YY1 fusion proteins interact with the nuclear matrix in situ, signals for subcellular trafficking to sites within the nuclear matrix may be affected by the Gal4 epitope tag.

DISCUSSION

In this study, we established that the C-terminal domain of YY1 is necessary for high-affinity interactions with the nuclear matrix. The N-terminal domain of YY1 only supports low-affinity association with the nuclear matrix. We note that the stringency of nuclear matrix protein isolation influences the experimental ability to detect low-affinity interactions of YY1 with the nuclear matrix in situ. Similar to YY1, there are several examples of transcription factors with more than one protein domain for nuclear matrix interaction. The human androgen receptor (hAR) and the human glucocorticoid receptor (hGR) were both shown to interact with the nuclear matrix through their C-terminal domains and the hGR also requires the DNA binding domain for matrix association. The hAR also retains a weak interaction with the nuclear matrix upon deletion of the C-terminal domain, which was shown to be required for strong nuclear matrix association. This weak interaction is variable and is dependent on the method of matrix preparation [van Steensel et al., 1995].

The association of YY1 with the nuclear matrix occurs in both nucleolar and non-nucleolar domains. The nucleolus is an integral part of the NMIF scaffold and represents the subnuclear location of protein/protein interaction between YY1 and the nucleolar phosphoprotein B23. This interaction relieves YY1 mediated transcriptional repression [Inouye and Seto, 1994]. The B23 protein may be involved in subcellular trafficking [Borer et al., 1989], as well as ribosome assembly [Busch et al., 1984; Yung et al., 1985; Prestayko et al., 1974]. The localization of a subset of YY1 to the nucleolus with the B23 protein, is an example of the targeted distribution of transcription factors to different subnuclear domains, which support distinct gene regulatory functions.

Subcellular targeting to specific sites within the nuclear matrix may be affected by the addition of chimeric sequences to the YY1 protein. We observed that fusion of the Gal4 DNA binding domain (aa 1–147) results in an apparent lack of Gal4/YY1 in the nucleolus. However, fusion of a short HA epitope tag (11 amino acids), results in association with the nucleolus, as is observed for the endogenous YY1 protein. This result suggests that there could be a signal present in the Gal4 DNA binding domain (aa 1–147) that inhibits trafficking to the nucleolus. This observation is novel and may only affect proteins that are normally directed to the nucleolus. Zeng et al. [1997] observed no difference in the targeting of Gal4/AML1B and HA/AML1B. AML1B is not located in the nucleolus. Further analyses will be required to better understand the hierarchy of signals that control selective trafficking to the nuclear matrix and the nucleolus.

The presence of both high- and low-affinity nuclear matrix targeting signals in distinct segments of YY1 suggest that YY1 interfaces with different components of nuclear architecture to facilitate its multiple transcriptional functions. The YY1 C-terminal domain that contains the high-affinity nuclear matrix targeting signal

has been shown to be important for repressor activity [Bushmeyer et al., 1995]. This C-terminal domain of YY1 interacts with proteins that relieve repressor activity including c-Myc [Shrivastava et al., 1993] and the adenovirus E1A product [Shi et al., 1991], both of which are also associated with the nuclear matrix [Carvalho et al., 1995; Eisenman et al., 1985]. Transcriptional activity of YY1 is mediated by protein–protein interactions with several transcriptional co-factors, including histone acetylase CBP/p300 [Austen et al., 1997; Lee et al., 1995] and the histone deacetylase Rpd3p [Yang et al., 1996]. Histone acetylase and deacetylase activities have been shown to be associated with the nuclear matrix [Davie, 1997]. The association of YY1 with these chromatin modifying factors at the nuclear matrix may mediate nucleosomal remodelling within specific nuclear domains to modulate transcription.

In summary, the results of this study are consistent with the concept that the subcellular localization of YY1 and/or its interactions with other nuclear matrix-associated regulatory factors may be determinants of YY1 mediated transcriptional control within the context of nuclear architecture.

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