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21 June 1993; accepted 29 October 1993

Inhibition of Transcriptional Regulator Yin-Yang–1 by Association with c-Myc

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Yin-Yang–1 (YY1) regulates the transcription of many genes, including the oncogenes c-*fos* and c-*myc*. Depending on the context, YY1 acts as a transcriptional repressor, a transcriptional activator, or a transcriptional initiator. The yeast two-hybrid system was used to screen a human complementary DNA (cDNA) library for proteins that associate with YY1, and a c-*myc* cDNA was isolated. Affinity chromatography confirmed that YY1 associates with c-Myc but not with Max. In cotransfections, c-Myc inhibits both the repressor and the activator functions of YY1, which suggests that one way c-Myc acts is by modulating the activity of YY1.

Zinc finger protein Yin-Yang–1 (CF1, δ, NF-E1, or UCRBP) is a 65-kD DNA binding protein, belonging to the GLI-Krüppel family, which is widely expressed and highly conserved between humans and mice (*1*). Depending on the context, YY1 can function as an activator, a repressor, or an initiator of transcription (*1*). YY1 represses the adeno-associated virus (AAV) P5 promoter (*1*), the immunoglobulin (Ig) κ 3′ enhancer (*1*), the c-*fos* promoter (*2*), the human papilloma virus–18 promoter (*3*), the skeletal α-actin promoter (*4*), the long terminal repeat of Moloney murine leukemia virus (*1*), and on the basis of sequence similarity, the N-*ras* promoter (*3, 5*). YY1 repression of the P5 promoter is relieved by adenovirus E1A protein (*1*). In some of these genes YY1 DNA binding sites overlap with serum response elements or 12-O-tetradecanoyl phorbol-13-acetate (TPA) response elements and repression is relieved when activators such as serum response factor or AP-1 displace YY1 (*2–4, 6*). A fusion protein of YY1 and the GAL4 DNA binding domain represses transcription of a thymidine kinase promoter with GAL4 binding sites (*1*). However, YY1 is an activator for the c-*myc* promoter, the IgH intronic enhancer, and the promoters of ribosomal proteins L30 and L32 (*7*). Finally, YY1 acts as a transcriptional initiator at nucleotide position +1 in the AAV P5 promoter (*8*).

A simple model to explain the functional versatility of YY1 is that interactions

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with different cellular proteins alter its activity. To investigate this possibility, we used the yeast two-hybrid system to identify cDNAs that encoded proteins that associate with YY1 (*9*). This assay depends on the ability of the interacting proteins to bring together the GAL4 DNA binding (GAL4DB) and activation (GAL4AD) domains, which results in the expression of a *lacZ* reporter driven by a GAL1 promoter. LacZ expression, detected by the formation of blue colonies after staining with X-gal, indicates protein association.

We constructed a vector, GAL4DB-YY1, that expressed a fusion protein with the DNA binding domain of the first 147 amino acids of GAL4 [GAL4(1–147)] (*10*) and YY1. This plasmid did not activate *lacZ* transcription upon transformation into yeast strain GGY1:171 (*11*), which contains a *lacZ* gene whose expression is dependent on a GAL1 promoter (Fig. 1A). Subsequently, GAL4DB-YY1 was cotransformed into GGY1:171 with pools of plasmids from a library expressing the GAL4 activation domain fused to cDNAs from the human monocytic line HL-60 (*12*). We screened 300,000 colonies (*9*), and over 50 potential positives were identified. Subsequent rescreening produced several strong positives. Upon sequence analysis, one of these, pGADMyc, was found to encode the COOH-terminal 190 amino acids of c-Myc protein (*13*) fused to the GAL4AD (Fig. 1B).

Further controls confirmed the specificity of the apparent association between YY1 and c-Myc in the two-hybrid system (Fig. 1A). The pGADMyc did not cause LacZ expression when transformed into GGY1:171 alone, and there was no LacZ expression upon cotransformation of pGADMyc with a plasmid encoding GAL4DB or a heterologous protein (human immunodeficiency virus integrase) fused to the GAL4DB (GAL4DBHI). This suggests that YY1 interacts specifically with c-Myc. A vector was also constructed that expressed YY1 as a fusion protein with the LEXA DNA binding domain, LDBYY1 (*14*). LDBYY1 and pGADMyc were cotransformed into CTY10-5d (*15*). The pGADMyc hybrid activated LacZ expression only in the presence of LDBYY1 and not in the presence of LEXADB alone. Thus, we conclude that in the two-hybrid system, YY1 associates in vivo with the COOH-terminal portion of c-Myc. We also tested the ability of the GAL4DB-YY1(1–343) and GAL4DB-YY1-(1–201) hybrids to interact with pGADMyc. Only GAL4DB-YY1(1–343) gave blue-colored yeast colonies when cotransfected with pGADMyc (Fig. 1A), although immunoblotting with a YY1 polyclonal antiserum showed both proteins were expressed in similar amounts. These results delimit a portion of YY1 essential for interaction with c-Myc to 142 amino acids. Amino acids 201 to 343 do not contain the NH_2-terminal acidic region of YY1 or the histidine-rich region of YY1 and also lack two and a half COOH-terminal zinc fingers (*1*).

We used a glutathione-S-transferase

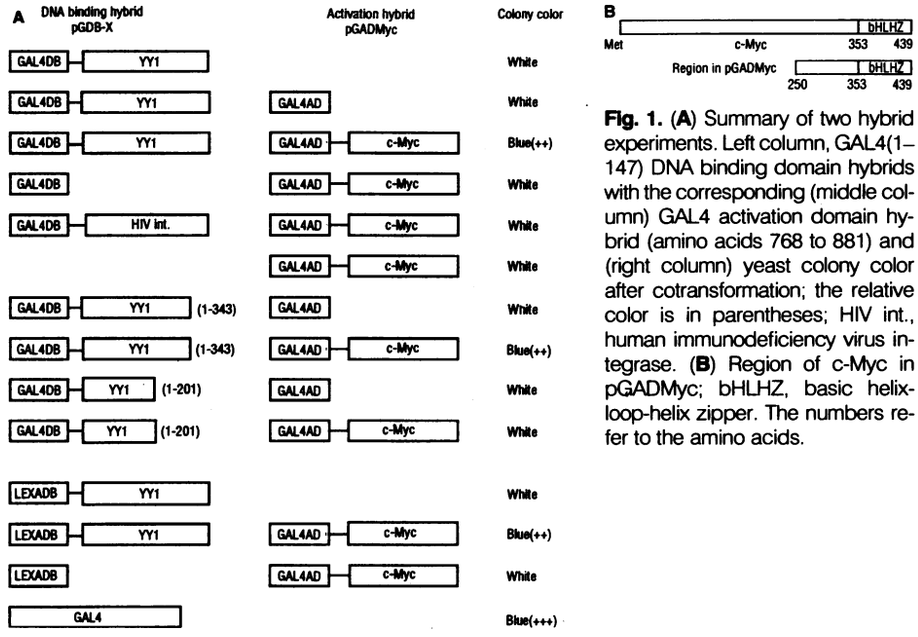

Fig. 1. (**A**) Summary of two hybrid experiments. Left column, GAL4(1–147) DNA binding domain hybrids with the corresponding (middle column) GAL4 activation domain hybrid (amino acids 768 to 881) and (right column) yeast colony color after cotransformation; the relative color is in parentheses; HIV int., human immunodeficiency virus integrase. (**B**) Region of c-Myc in pGADMyc; bHLHZ, basic helix-loop-helix zipper. The numbers refer to the amino acids.

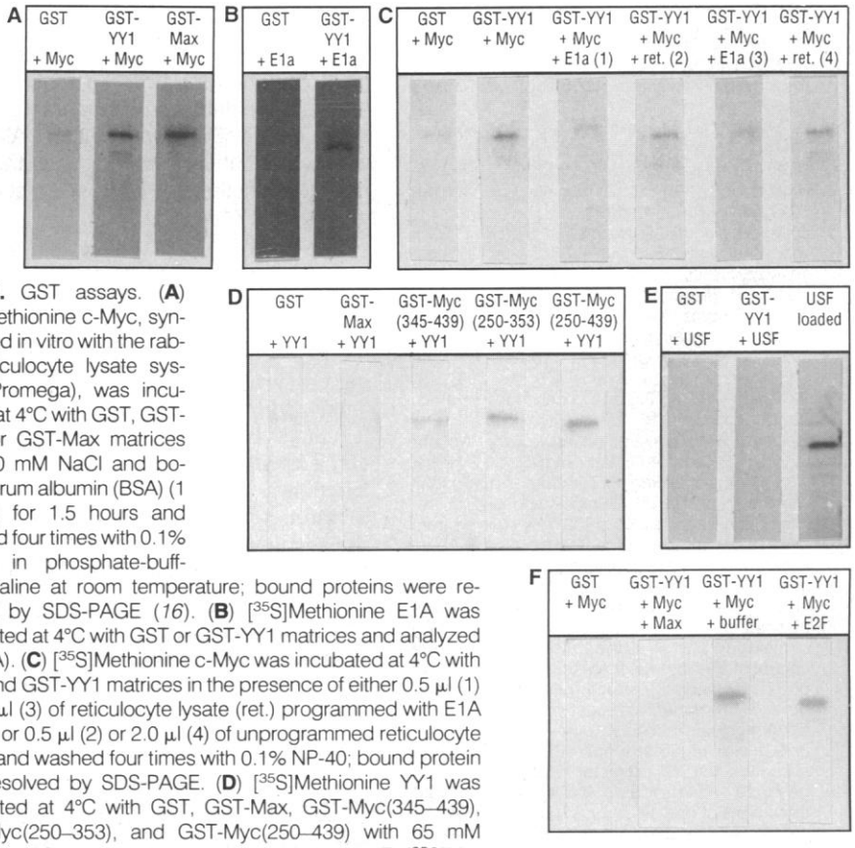

Fig. 2. GST assays. (**A**) [^{35}S]Methionine c-Myc, synthesized in vitro with the rabbit reticulocyte lysate system (Promega), was incubated at 4°C with GST, GST-YY1, or GST-Max matrices with 50 mM NaCl and bovine serum albumin (BSA) (1 mg/ml) for 1.5 hours and washed four times with 0.1% NP-40 in phosphate-buffered saline at room temperature; bound proteins were resolved by SDS-PAGE (*16*). (**B**) [^{35}S]Methionine E1A was incubated at 4°C with GST or GST-YY1 matrices and analyzed as in (A). (**C**) [^{35}S]Methionine c-Myc was incubated at 4°C with GST and GST-YY1 matrices in the presence of either 0.5 μl (1) or 2.0 μl (3) of reticulocyte lysate (ret.) programmed with E1A mRNA or 0.5 μl (2) or 2.0 μl (4) of unprogrammed reticulocyte lysate and washed four times with 0.1% NP-40; bound protein was resolved by SDS-PAGE. (**D**) [^{35}S]Methionine YY1 was incubated at 4°C with GST, GST-Max, GST-Myc(345–439), GST-Myc(250–353), and GST-Myc(250–439) with 65 mM NaCl and BSA (2 mg/ml) and analyzed as in (A). (**E**) [^{35}S]Methionine USF was incubated at 4°C with GST and GST-YY1 matrices and analyzed as in (A). (**F**) [^{35}S]Methionine c-Myc was incubated with GST, with GST-YY1 with 2 μg of bacterially expressed Max that was purified as GST protein and cleaved by thrombin, with GST-YY1 with cleavage buffer and thrombin, or with GST-YY1 with 2 μg of bacterially expressed E2F that was also purified as GST protein and then cleaved by thrombin (*3*); bound protein was resolved by SDS-PAGE and analyzed as in (A).

(GST) affinity matrix assay (*16*) to determine if YY1 and c-Myc can associate in vitro. A GST-YY1 fusion protein was bound to glutathione-agarose to make a YY1 affinity matrix; control matrices with GST alone and GST-Max were prepared in parallel. A [^{35}S]methionine-labeled c-Myc protein prepared by in vitro transcription-translation was incubated with these matrices, and the specifically bound protein was eluted and resolved by SDS–polyacrylamide gel electrophoresis (PAGE). c-Myc bound equally well to the GST-Max and GST-YY1 matrices but bound poorly to the GST matrix (Fig. 2A). This confirms that c-Myc and YY1 associate and further shows that the association is not unique to a truncated form of c-Myc, because full-length c-Myc protein was used in this experiment. Our results also show that the association between YY1 and c-Myc can occur in the absence of DNA or other transcription proteins.

The function of YY1 is altered in the presence of adenovirus E1A protein; E1A relieves YY1 repression (*1*) and synergizes with YY1 activation (*7*). Therefore, the ability of GST-YY1 to associate with E1A was tested. E1A binds to YY1 in the GST assay (Fig. 2B). In a competition experiment, unlabeled E1A competed with c-Myc for binding to GST-YY1 (Fig. 2C), which demonstrates that E1A and c-Myc cannot associate simultaneously with YY1. Others have also demonstrated a direct interaction between YY1 and E1A (*17*). It has previously been noted that E1A and c-Myc have structural similarity (*18*); thus, structurally similar regions of the two proteins may associate with YY1. Retinoblastoma protein is the only other non–helix-loop-helix zipper (HLHZ) protein known to associate with E1A as well as with c-Myc (*19*).

Max and Myn heterodimerize with c-Myc by way of the HLHZ domains (*20*). YY1 is a different kind of c-Myc partner that does not contain HLHZ domains. A GST fusion protein containing only the c-Myc basic HLHZ region, amino acids 345 to 439, does not associate with YY1 (Fig. 2D). A fusion protein containing amino acids 250 to 353 binds YY1 but less effectively than a fusion protein containing amino acids 250 to 439 (Fig. 2D). Thus, both amino acids 250 to 353 and the basic HLHZ region appear to be required for interaction with YY1. The c-Myc amino acids 250 to 353 contain a nuclear localization signal, nonspecific DNA binding activity, and a casein kinase II phosphorylation site (*13, 21*). There is evidence that the region of amino acids 250 to 353 is functionally important. (i) Amino acids 290 to 318 are conserved among species and among N-Myc, L-Myc, and c-Myc (*13*). (ii) Removal of amino acids 265 to 353 decreases (but does not abolish) the ability of c-Myc to cooperate with Ras in transforming rat embryo fibroblasts (*22*). (iii) This region is required for transformation of Rat1 cells (*23*).

The ability of YY1 to interact with two other HLHZ proteins, Max and upstream stimulatory factor (USF) (*24*), was also tested, but the association of YY1 with Max or USF was not detected (Fig. 2, D and E). This is consistent with the idea that YY1 does not associate with the HLHZ region alone and emphasizes the specificity of the YY1–c-Myc association. To probe whether YY1, c-Myc, and Max could form a ternary complex, we added unlabeled c-Myc and ^{35}S-labeled YY1 to a GST-Max column; no retention of YY1 was observed. Furthermore, addition of bacterially expressed Max abolished the binding of c-Myc to GST-YY1 (Fig. 2F). Thus, there is no evidence for ternary complex formation, which is also consistent with the finding that the basic HLHZ region of c-Myc is important for association with YY1 (Fig. 2D).

We investigated whether c-Myc affects the activator and repressor functions of YY1. The ability of c-Myc to affect YY1-GAL4 repression of a thymidine kinase promoter with upstream GAL4 binding sites (G_5tk) (*1*) in NIH 3T3 cells was tested. YY1-GAL4 repressed the G_5tk promoter approximately 55% (Fig. 3A). This repression was completely inhibited in a dose-dependent manner by cotransfection of a c-Myc expression vector, which demonstrates that c-Myc interferes with the ability of YY1 to repress transcription. The ability of c-Myc to affect YY1-dependent activation of a c-*myc* promoter was also tested with P3X plasmacytoma cells, with which we have observed the strongest activation by YY1 (*7*). Upon transfection, YY1 activated a c-*myc* promoter (pSNLUC) eightfold (Fig. 3B, bars 1 and 3). This activation increased only twofold when a c-Myc expression vector was included in the cotransfection (Fig. 3B, bar 4), although c-Myc expression had little effect on pSNLUC alone (bar 2), which demonstrates that c-Myc inhibits the activating ability of YY1.

It is unknown how c-Myc inhibits YY1 activity. One simple model is that association with c-Myc blocks the ability of YY1 to interact with other activators or the basal transcription machinery. Alternatively, association with c-Myc may inhibit the ability of YY1 to bind DNA. A third possibility is that c-Myc sequesters YY1.

The c-Myc protein is a potent regulator of cell growth and differentiation (*13, 25*); elevated c-Myc levels are associated with tumorigenesis or apoptosis (*13, 25*). Only

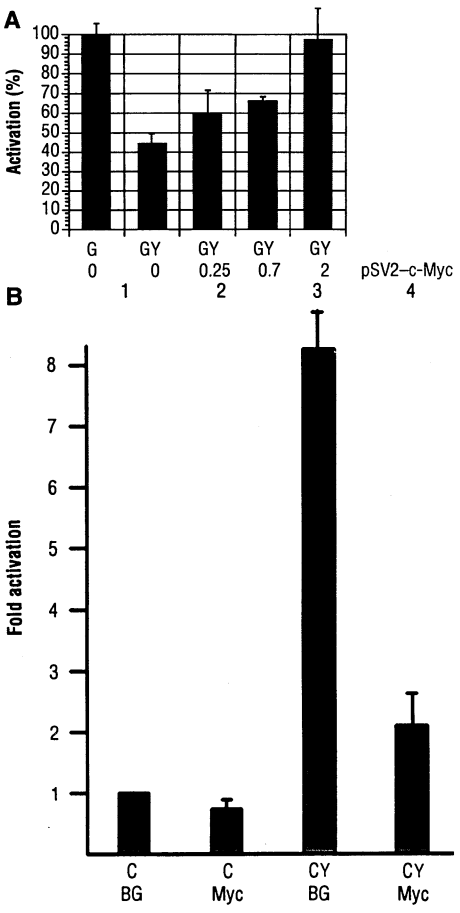

Fig. 3. Cotransfection assays for YY1 function. (**A**) Inhibition of the transcriptional repressor activity of YY1 by c-Myc. NIH 3T3 cells were cotransfected with 5 μg of a luciferase reporter dependent on the thymidine kinase promoter (nucleotides −200 to +56) with five GAL4 binding sites 5′ of the promoter. Two expression vectors were included as indicated: (i) 2 μg of pSV2–GAL4(1–147) (G) or 2 μg of pSV2–GAL4-YY1 (GY) and (ii) varying amounts of pSV2–β-gal (BG) or pSV2–c-Myc (Myc), with the sum of the two choices constant (2 μg). Total DNA was kept constant at 20 μg, and transfections were done with the $Ca_3(PO_4)_2$ method (*1*). Results represent the averages of three independent transfections and have been corrected for the effect of Myc on the reporter by simultaneously doing the experiment with GAL4 and YY1-GAL4 for each concentration of Myc; bars show 1 SD. (**B**) Inhibition of the transcriptional activator ability of YY1 by c-Myc. Plasmacytoma P3X cells were cotransfected with 10 μg of a luciferase reporter containing 758 base pairs of the murine c-Myc promoter. Two expression vectors were included as indicated: (i) 0.3 μg of cytomegalovirus (CMV) (C) or 0.3 μg of CMV-YY1 (CY) and (ii) 0.9 μg of pSV2–β-gal (BG) or 0.9 μg of pSV2–c-Myc (Myc). Twenty micrograms of pUC19 DNA was added as a carrier in each transfection, and transfections were done by electroporation at 240 V, 960 μF, and 200 ohms. Results show the average of three independent transfections, and the bars indicate 1 SD.

a few proteins have been shown to associate with c-Myc, and the exact mechanisms of c-Myc action are poorly understood. Inhibition of YY1 activity may be one mechanism by which c-Myc acts. YY1 regulates transcription of many genes, including the oncogenes c-*fos* and c-*myc*, and also acts as a transcription initiator (*8*). The amounts of c-Myc are highly regulated and differ markedly between dividing and nondividing cells. YY1 may function only when the amounts of c-Myc drop below some threshold level. Lack of YY1 activity would activate some genes and repress others. Concentrations of c-Myc compatible with YY1 activity may vary with cell type or with the YY1 target gene or with both. It is also interesting that c-Myc has recently been shown to inhibit the activity of another transcriptional initiator, TFII-I (*26*). In addition to affecting YY1 function, there is increasing evidence that c-Myc–Max heterodimers regulate gene transcription directly (*27*).

The YY1-Myc association gives a partial indication of the complex equilibria that appear to exist among transcriptional regulators. For example, c-Myc transcription is negatively autoregulated by c-Myc (*28*), c-*myc* transcription is activated by YY1 (*7*), and, as shown here, c-Myc blocks this activation, explaining in part the autoregulation mechanism. Thus, c-*myc* transcription is probably sensitive to changes in relative and absolute amounts of c-Myc and YY1.

Another regulatory loop may involve putative cellular E1A-like proteins. E1A and c-Myc compete for association with YY1. E1A relieves YY1 repression (*1*) and synergizes with YY1 activation (*7*), whereas c-Myc inhibits YY1 repression and activation. Cellular E1A-like proteins could have a strong effect on YY1 activity by simultaneously displacing the inhibitor (c-Myc) and directly activating YY1.

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29. We thank A. Berk, S. Chellapan, R. Eisenman, S. Fields, P. Gregor, S. Hanes, R. Perry, R. Roeder, and R. Schwartz for various constructs and R. Sternglanz for strain CTY10-5d. We thank R. Dalla-Favera, D. Shore, and M. Carlson for reading the manuscript and members of our laboratories for helpful discussions. Supported by U.S. Public Health Service grants CA 38571 and GM29361.

8 July 1993; accepted 21 October 1993

Attachment of *Helicobacter pylori* to Human Gastric Epithelium Mediated by Blood Group Antigens

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Helicobacter pylori **is associated with development of gastritis, gastric ulcers, and adenocarcinomas in humans. The Lewis^b (Le^b) blood group antigen mediates *H. pylori* attachment to human gastric mucosa. Soluble glycoproteins presenting the Le^b antigen or antibodies to the Le^b antigen inhibited bacterial binding. Gastric tissue lacking Le^b expression did not bind *H. pylori*. Bacteria did not bind to Le^b antigen substituted with a terminal GalNAcα1-3 residue (blood group A determinant), suggesting that the availability of *H. pylori* receptors might be reduced in individuals of blood group A and B phenotypes, as compared with blood group O individuals.**

Helicobacter pylori, a prevalent human-specific pathogen, is a causative agent in chronic active gastritis (*1*), gastric and duodenal ulcers (*2*), and gastric adenocarcinoma (*3*), one of the most common forms of cancer in humans. This genetically diverse bacterial species (*4*) has been estimated to infect the gastric mucosa of >60% of adults over the age of 60 in industrialized countries (*5*). In developing countries, most individuals are infected during childhood (*6*).

Attachment is a prerequisite for microbial colonization of epithelial surfaces and is mediated by molecules on the bacterial surface, adhesins (*7*), that recognize proteins or glycoconjugates on the surface of the eukaryotic cell (*8*). The specificity of this interaction and the limited distribution of receptors often results in a restricted range of hosts and tissues utilized for colonization. This phenomenon is known as tropism. Bacteria unable to adhere to epithelium tend to be rapidly removed by shedding of surface cells and mucus layer.

Helicobacter pylori expresses sialic acid–specific hemagglutinins, the gene for one of which has been cloned (*9*). In addition to sialylated glycoconjugates, *H. pylori* binds to sulfatide (SO_3-Galβ1-1Cer) (*10*). However, *H. pylori* adhesion to HeLa cells appears to be independent of sialic acid (*11*). Cell-specific attachment of *H. pylori* to human gastric surface mucous cells is inhibited by human colostrum secretory immunoglobulin A (sc-IgA) (*12*, *13*), a glycoprotein carrying a highly variable set of N- and O-linked oligosaccharides (*14*). The less glycosylated serum IgA (S-IgA) (*15*) is devoid of such inhibitory properties. The adherence-inhibiting activity of sc-IgA is

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