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Yin Yang 1 represses α-myosin heavy chain gene expression in pathologic cardiac hypertrophy

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Abstract

In the work presented here, we elucidate a mechanism for the repression of α-myosin heavy chain (MyHC) during pathological cardiac hypertrophy. We demonstrate that the transcription factor Yin Yang 1 (YY1) significantly decreases endogenous α-MyHC mRNA and protein expression in neonatal rat ventricular myocytes. Furthermore, mutation of the YY1 binding sites in the proximal rat α-MyHC promoter increases promoter activity and alleviates YY1-mediated repression of the promoter. Despite the presence of 5 sites that bind YY1, only one site, located at −94 bp of the rat α-MyHC promoter, is both necessary and sufficient for pathological repression of the promoter by phorbol esters, revealing a unique mechanism for the repression of α-MyHC expression during cardiac hypertrophy.

Keywords: Yin Yang 1; Cardiac hypertrophy; α-Myosin heavy chain; Transcription; Repression; Phorbol esters; Protein kinase C; Spontaneously hypertensive and heart failure rats

The heart expresses two isoforms of myosin heavy chain (MyHC), α and β, and their relative expression levels change during development and disease. α-MyHC has higher rates of ATP hydrolysis, and hearts expressing mainly α-MyHC have faster contractile velocities than hearts expressing mainly β-MyHC [1,2]. Therefore, the down-regulation of α-MyHC gene expression in disease states is thought to contribute to the decreased contractile function of failing hearts [3]. For this reason, understanding how α-MyHC gene expression is repressed in disease states is critical to understanding the mechanism(s) of pathological signaling.

Yin Yang 1 (YY1) is a ubiquitously expressed transcription factor that has a diverse range of regulatory functions including transcriptional activation, repression, and initiation (for reviews [4,5]). How YY1 functions appears to depend on cell type as well as promoter context. In myogenic cells and in muscle-specific promoters, however, YY1 appears to function mainly as a repressor of transcription [6–9], although there is evidence that YY1 interacts at the B-type natriuretic peptide (BNP) promoter with GATA4 to activate transcription [10].

The signaling pathways involved in cardiac hypertrophy are numerous, and protein kinase C (PKC) has been shown to play an important role in the development of cardiac hypertrophy/failure (for review see [11]). The phorbol ester, phorbol 12-myristate 13-acetate (PMA), has long been known as an activator of PKC signaling, and treatment of cardiac myocytes with PMA leads to altered calcium handling and pathological hypertrophy [12,13].

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We have recently shown that YY1 represses the expression of the human α-MyHC promoter activity [14,15]. In the work presented here, we show that the YY1 binds to and represses the rat α-MyHC promoter. More specifically, over-expression of wildtype YY1 represses the rat α-MyHC promoter activity, while over-expression of a mutant YY1(Δ170–200) protein that lacks its central repression domain does not. Furthermore, by infecting neonatal cardiac myocytes with an adenovirus construct expressing YY1, we show that YY1 in fact represses endogenous α-MyHC expression in these cells. We also show that YY1 expression is increased in the hearts of diseased spontaneously hypertensive and heart failure (SHHF) rats. More importantly, we show that YY1 is likely important for α-MyHC-mediated repression during cardiac hypertrophy since YY1 is essential for PMA-mediated repression of the rat α-MyHC promoter. Further investigation revealed that only one of the six putative YY1 sites, located −94 bp upstream of the transcription start site of the rat α-MyHC promoter, is both necessary and sufficient for the PMA response, establishing a functional role for YY1 in the diseased heart.

Materials and methods

Nuclear extract preparation. Nuclear extracts were prepared using the Nuclear Extract Kit as per the manufacturer's instructions (Pierce). Protease Inhibitor Cocktail (Sigma) and Phosphatase Inhibitor Cocktails I & II (Sigma) were used in place of individual inhibitors. Ventricles were taken from neonatal rats (1–3 days old) and aged male adults, washed twice with ice-cold PBS, frozen with liquid nitrogen, and stored at −80 °C until used for nuclear extract preparation.

Western blots. Proteins from nuclear extracts were resolved by SDS–PAGE and then transferred to a PDVF membrane (Amersham Biosciences). Blots were incubated with an anti-YY1 antibody (Santa Cruz Biotechnology, sc-7341) and then detected using an anti-mouse alkaline phosphatase-conjugated secondary antibody (Sigma). The YY1 signal was normalized to total Coomassie-stained protein as described previously [14].

Probes and electrophoretic mobility shift assays (EMSAs). Probes were created by annealing sense and antisense oligonucleotides (synthesized by Integrated DNA Technologies), radio-labeling by Klenow reaction with [α-P^{32}]dCTP, and purifying with G-25 microspin columns (Amersham Biosciences). Labeled probes were incubated with nuclear extracts for 30 min at room temperature, run on 4% polyacrylamide gels, and visualized by phosphoimager as previously described [14]. Probe sequences were as follows (mutations are indicated by lowercase letters): Rα-Y1WT, CGAGATTTCTCCATCCCAAGT AAGAAG; Rα-Y1mutYY1, GAGATTTCTCCAaCCCAAGTAAG AAG; Rα-Y2WT, CAGACAGGAGGGATGGGAGGGAGGGTC; Rα-Y2mutYY1, CCAGACAGGAGGGAacGGaAGGGAGGGTC; Rα-Y3WT, GCAGCAGACCTTTCATGGGCAAACCTC; Rα-Y3 mutYY1, GCAGCAGACCTTTCtTGGGCAAACCTC; Rα-Y3mut-SRF, GCAGCAGAggTTTCATGGGCAAACCTC; Rα-Y4WT, GT CTCTCCTCTATCTGCCCATCGGCCCT; Rα-Y4mutYY1, GTCT CTCCTCTATCTGCCCAaCGGCCCT; Rα-Y4mutGATA, GTCTC TCCTCTgcCTGCCCATCGGCCCT; Rα-Y5WT, AGGTAAGGGC CATGTGGGTAGGGGAGG; Rα-Y5mutYY1, AGGTAAGGGC CAcGTGGGTAGGGGAGG; Rα-Y5mutUSF, AGGTAAGGGCC ATGaaGGTAGGGGAGG; Rα-Y6WT, GGGAGGCTGGAATGG GAGCTTGTGTGT; and Rα-Y6mutYY1, GGGAGGCTGGAATc GGAGCTTGTGTGT.

Vector construction and preparation. The rat α-MyHC promoter (−616 to +89) was cloned upstream of the firefly luciferase gene of the pGL-3 basic vector (Promega) using the *Bgl*II and *Mlu*I restriction sites. Mutations in the YY1 binding sites of the wildtype promoter were created by site-directed PCR mutagenesis using the same oligonucleotides used to make EMSA probes. All clones were sequenced to ensure that the proper mutations were created without additional spurious sequence changes.

Dr. Koji Hasegawa (Kyoto University, Japan) provided us with the rat β-MyHC(−333 to +34)-luciferase reporter vector (Rβ-WT-luc), which is activated by hypertrophic stimuli in rat hearts [16]. The CMV-YY1(WT) expression plasmid was a kind gift from Dr. Michael Atchison (University of Pennsylvania) [17], and the CMV-YY1(Δ170–200) was given to us by Dr. Edward Seto (University of South Florida) [18]. A CMV-empty vector was created by removing the YY1 gene by restriction digest followed by re-ligation of the plasmid. The YY1 expression adenovirus was kindly given to us by Dr. Aristidis Moustakas (Ludwig Institute of Cancer Research, Sweden) [19].

Plasmid DNA was prepared using alkaline-lysis kits from Qiagen. In order to minimize variation in transfection efficiency caused by DNA quality, each plasmid vector was prepared multiple times and combined. DNA was eluted in 10 mM Tris and stored at 4 °C.

Cell culture. Neonatal rat cardiac ventricular myocytes were cultured as previously described with a few modifications [20]. Briefly, cells were isolated by trypsin digestion from the ventricles of 1- to 3-day-old rats and plated at a concentration of 1.5×10^5 cells/well in 12-well tissue culture plates. Wells were pre-coated with a 0.1% gelatin solution and dried in a hood under UV lights. In addition, all media solutions were supplemented with Hepes (pH 7.5) to a final concentration of 20 mM in order to buffer the pH of the media. Transient transfections were performed with Fugene 6 (Roche) as per the manufacturer's instructions using a ratio of 3 μl Fugene 6 reagent per 1 μg DNA. A target ratio of 5 pg DNA/cell was also used. For infections, virus was added at a concentration of 10 plaque forming units (PFU)/cell.

For experiments involving the treatment of NRVMs with PMA, the culture media were removed on the day following transfection and replaced with treated media. PMA (10,000× in DMSO) was added to a final concentration of 200 μM.

RNA preparation, slot blots, and RNase protection assays. RNA was harvested from NRVM cultures using TRIzol Reagent (Life Technologies). Slot blots were prepared with 10 μg total RNA/well, using nylon membranes (ICN, Irvine), as described previously [21]. Probes for α- and β-MyHC were created using previously published sequences [22]. In order to limit variation due to sample loading, the intensity of MyHC RNA was normalized to that of GAPDH [21]. RNase protection assays were performed as described [6,23]. Briefly, 5 μg of total RNA was hybridized against probes specific to α-MyHC, ANF, and GAPDH. RNase protection experiments were performed using the RPAII Kit (Ambion).

Results

YY1 represses the rat α-MyHC promoter

In order to establish that YY1 represses the rat α-MyHC promoter, the −612 to +34bp region of the rat promoter was cloned into the pGL3 reporter vector upstream of the firefly luciferase gene (Rα-WT-luc), and this construct was co-transfected into NRVMs with various

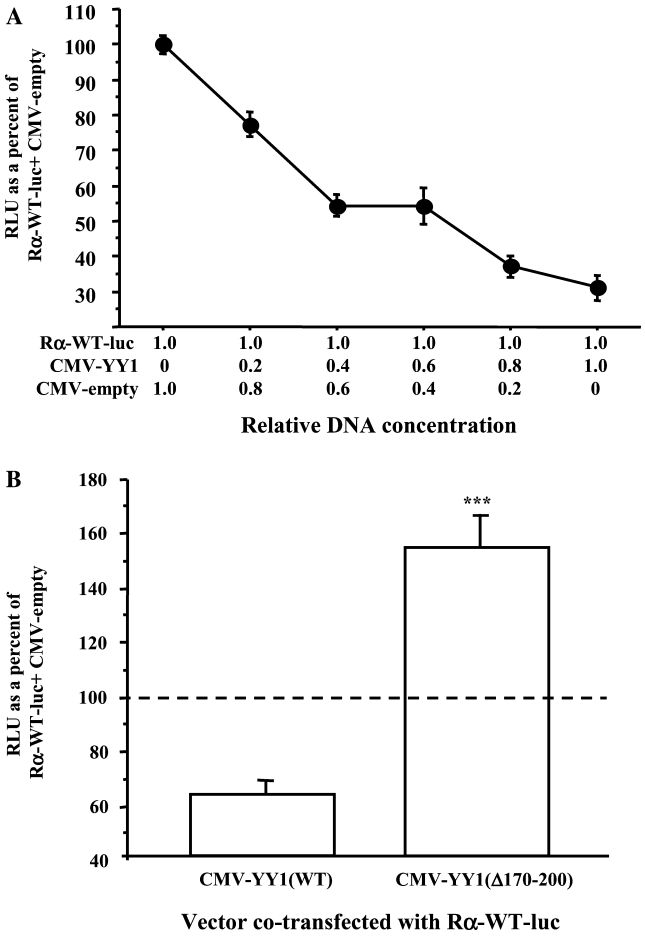

Fig. 1. Over-expression of YY1 in NRVMs represses the rat α-MyHC promoter. (A) Co-transfection of a YY1 expression vector (CMV-YYY) and a rat α-MyHC promoter reporter (Rα-WT-luc) results in a dose-dependent repression of the α-MyHC promoter. While keeping the total amount of DNA constant in each transient transfection, increasing concentrations of CMV-YY1 caused lower luciferase activities from the Rα-WT-luc reporter. (B) Co-transfection of a mutant YY1 expression vector (CMV-YYY(Δ170–200)), in which the central repression domain of YY1 had been deleted, does not repress the rat α-MyHC promoter reporter (Rα-WT-luc). An activation of the Rα-WT-luc construct is observed instead. (***$p < 0.0001$.)

combinations of a CMV-YY1(WT) expression plasmid and a CMV-empty control vector. As shown in Fig. 1A, expression of YY1 results in a dose-dependent repression of the rat α-MyHC promoter by the CMV-YY1(WT) vector. No repression is seen with a mutant YY1 expression vector, CMV-YY1(Δ170–200), in which the central repression domain of YY1 has been deleted (Fig. 1B). In fact, over-expression of the mutant YY1(Δ170–200) significantly increased the activity of the Rα-WT-luc reporter, suggesting a dominant-negative effect.

YY1 binds putative sites of the rat α-MyHC promoter

We then examined the rat α-MyHC promoter for putative YY1 binding sites. As shown in Fig. 2A, six potential YY1 sites are present within the −613 to +34 region of the rat promoter as defined by the core sequence motif of CCAT; each site is numbered (Y1, Y2, Y3, etc.) by its proximity to the transcription start site. In order to determine if YY1 binds to the predicted sites in the rat promoter, double-stranded DNA probes, whose core sequences are shown in Fig. 2B, were made for each site and used in EMSAs with nuclear extracts prepared from neonatal rat ventricles. As shown in Fig. 2C, a shifted complex containing YY1 is observed for all of the probes except the Y4 probe.

The presence of YY1 in these complexes was confirmed by incubating the nuclear extracts with an anti-YY1 antibody prior to the addition of labeled probes. This antibody recognizes the DNA binding domain of YY1 and likely interferes with the binding of YY1 to the DNA. As a result, a complex is not visible in the + antibody lane, suggesting that the addition of antibody prevented YY1 from binding to these probes.

In several cases, additional complexes are observed that do not include YY1. Three prominent complexes are formed with the Y3, Y4, and Y5 probes (Fig. 2C). The major components of these complexes have been identified previously as SRF, GATA4, and USF1, respectively [24]. We were able to confirm these identifications by antibody supershift (data not shown). Use of these antibodies supershifted only their respective complexes and did not alter the mobility of the YY1 complex, suggesting that these factors are not present in the indicated YY1–DNA complex (data not shown).

To further confirm that binding of YY1 to the rat α-MyHC promoter is specific, we designed probes which contained point mutations at each of the six sites. As shown in Fig. 2D, mutant probes do not bind YY1 nor do they display prominent shifted complexes that are not observed with wildtype probes, clearly demonstrating that YY1 binding to these probes is specific.

Mutation of YY1 binding sites increases the activity of the rat α-MyHC promoter

To study the functional significance of YY1 binding to the α-MyHC promoter region, we created a mutant reporter construct by cloning each mutation into the rat α-MyHC-luciferase vector. This mutant, Rα-Y123456-luc, was identical to Rα-WT-luc, except that all six YY1 sites were mutated. Although the Y4 probe does not appear to bind YY1 in EMSAs, the site was also mutated as a lack of in vitro binding does not preclude in vivo binding, and this site could still have a functionally significant role. As shown in Fig. 3A, disruption of YY1 binding at the rat α-MyHC promoter acted to increase promoter activity, consistent with the loss of binding of a negative regulator.

To test whether Rα-Y123456-luc would be repressed by YY1 over-expression, we performed co-transfection

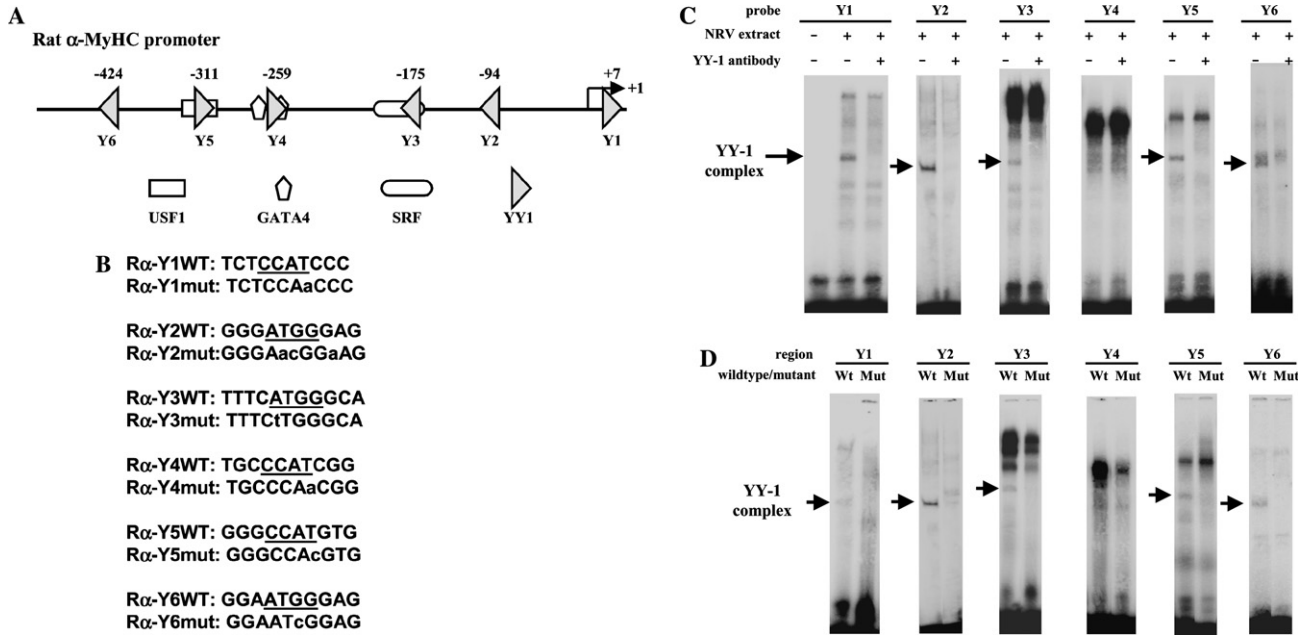

Fig. 2. Binding of YY1 to the rat α-MyHC promoter. (A) Schematic of the proximal rat α-MyHC promoter, indicating the location and numbering of each YY1 binding site (CCAT). YY1 triangles point in the 5′ to 3′ direction of the motif. In addition, the binding sites of SRF, GATA4, and USF1 are shown due to their proximity to the Y3, Y4, and Y5 sites, respectively. (B) Wildtype and mutant sequence of each YY1 site located within the proximal rat α-MyHC promoter. Mutant sequence disrupted YY1 binding in EMSAs (shown in (D)). (C) EMSA using wildtype probes for the six putative YY1 sites (Y1–Y6) in the proximal rat α-MyHC promoter. Pre-incubation of neonatal rat ventricle (NRV) nuclear extracts with anti-YY1 antibody blocked the formation of the shifted complexes, as indicated. Other complexes were identified by similar means for Y3 (SRF), Y4 (GATA-4), and Y5 (USF-1). (D) Specific mutations of each probe sequence abolished binding of YY1 as demonstrated by the absence of the shifted complex containing YY1 in lanes using mutant probes. YY1 complexes are designated with arrows.

experiments similar to those done with the wildtype Rα-WT-luc and compared the effect of YY1 over-expression on the mutant reporter. As shown in Fig. 3B, the Rα-Y123456-luc vector is not repressed to same extent as the wildtype vector. These data suggest that YY1 functions to repress the rat α-MyHC promoter through both direct and indirect means. That is, the disruption of YY1 binding sites is only able to alleviate repression that results from a direct interaction of YY1 with the promoter, but cannot block repression of the promoter that results from other downstream targets of YY1 that, in turn, decrease α-MyHC expression. These data further demonstrate that YY1 serves as a potent repressor of the rat α-MyHC expression in cardiac cells.

YY1 mutations disrupt pathological repression of the rat α-MyHC promoter

Given the strong evidence of α-MyHC repression by YY1, we then asked if YY1 plays a role in the repression of α-MyHC expression during pathological conditions. By treating NRVMs with hypertrophic agonists, it is possible to induce pathological signaling, complete with antithetical regulation of cardiac MyHC expression. The use of phorbol esters has long been known to induce such a response in NRVMs [12,25], leading us to ask whether or not PMA treatment of NRVMs would result in YY1-mediated repression of the α-MyHC promoter.

First, in order to ensure that our NRVM cultures were responding to PMA with an expected disparate regulation of the cardiac MyHC isoforms, endogenous levels of α- and β-MyHC mRNA were measured following 48 h of treatment with either PMA or the vehicle control (0.001% DMSO). As shown in a representative slot blot (Fig. 4A), PMA caused α-MyHC mRNA levels to decrease while increasing β-MyHC mRNA levels. Slot blots were repeated with cells from several NRVM preparations and the average changes in endogenous α- and β-MyHC mRNA levels are plotted to the right. GAPDH levels were also quantified and used as a loading control.

We also wanted to confirm that our transient transfection reporter system reflected a pathological response of the α- and β-MyHC promoter activities. As shown in Fig. 4B, treatment of NRVM cultures with PMA results in a decrease in the activity of the Rα-WT-luc vector while the rat β-MyHC-luciferase reporter (Rβ-WT-luc) increases, consistent with a pathological response.

Given that our cell culture system successfully induced a pathological response of our MyHC reporters, we examined the role of YY1 in the transcriptional regulation of the α-MyHC promoter under hypertrophic conditions. If YY1 acts to repress the α-MyHC

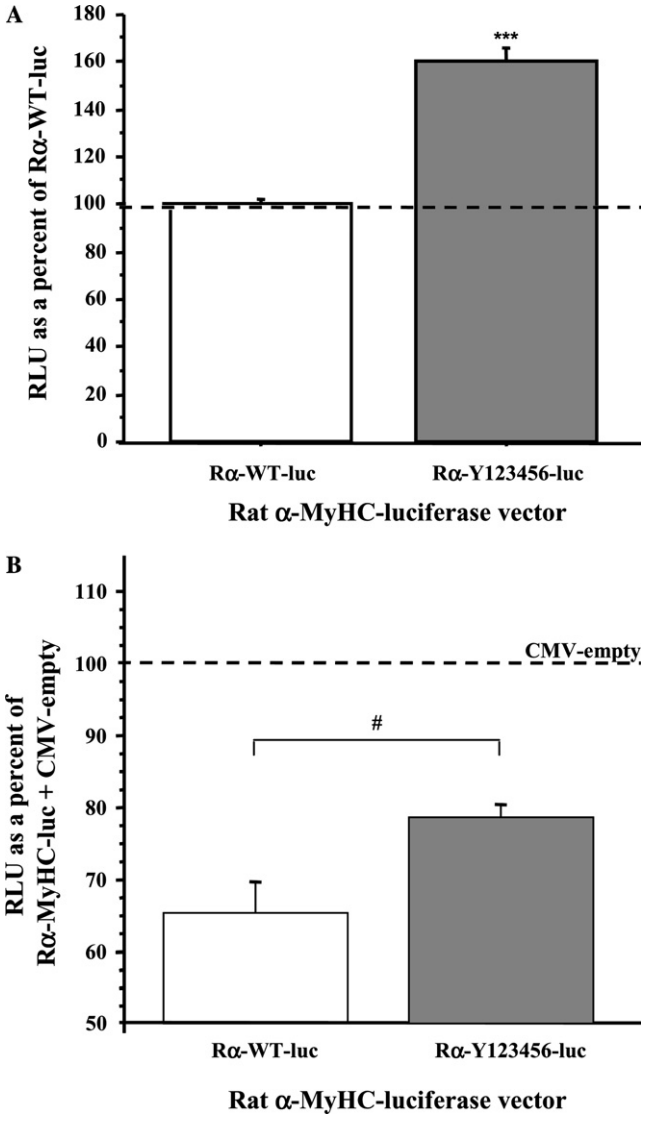

Fig. 3. Mutation of YY1 binding sites relieves YY1-mediated repression of the rat α-MyHC promoter. (A) The activity of rat α-MyHC-luciferase constructs containing each YY1 binding site mutated, Rα-Y123456-luc, is compared to the activity of the wildtype vector, Rα-WT-luc. As expected, disruption of YY1 binding to the α-MyHC promoter prohibits the ability of YY1 to repress the promoter and leads to increased luciferase activity. (B) Co-transfections of the CMV-YY1 vector with the Rα-WT-luc or Rα-Y123456-luc construct. Disruption of all YY1 binding sites in the rat α-MyHC promoter alleviates YY1-mediated repression, though complete de-repression is not observed. ($^{\#}p < 0.05$ and $^{***}p < 0.0001$.)

promoter during hypertrophy, we would expect that mutating all of the YY1 binding sites would block the response of the promoter to hypertrophic stimuli. By transfecting the Rα-WT-luc and the mutant Rα-Y123456-luc vectors into NRVMs prior to treatment with PMA, we found that the α-MyHC promoter with mutant YY1 sites is not repressed by treatment with PMA (Fig. 4C). These data clearly indicate that PMA stimulation of NRVMs results in a YY1-mediated repression of α-MyHC expression.

Y2 site is both necessary and sufficient for PMA-mediated repression of the rat α-MyHC promoter

In order to identify the YY1 sites responsible for the PMA-mediated repression of the Rα-WT-luc reporter, α-MyHC-luc constructs were prepared which contained only single YY1 binding sites mutated. As shown in Fig. 5A, only the Rα-Y2-luc reporter had a significantly different response to PMA treatment than the wildtype Rα-WT-luc vector. In addition, a mutant construct, in which all of the YY1 sites were mutated except for the Y2 site, behaves like the wildtype reporter (Fig. 5B), suggesting that the Y2 site is both necessary and sufficient for mediating the repression of the rat α-MyHC promoter in response to PMA treatment of NRVMs.

YY1 represses endogenous α-MyHC expression

Although the promoter-reporter data clearly demonstrate that YY1 is a potent repressor of the rat α-MyHC promoter and its activity represses the Rα-WT-luc reporter during pathological signaling, it was important to examine the effect of YY1 on the expression of endogenous α-MyHC in NRVMs. Therefore, NRVMs were infected with an adenovirus encoding wildtype YY1 (AdYY1). Infection of NRVMs with the AdYY1 virus increases YY1 protein levels and significantly represses endogenous α-MyHC mRNA levels when compared to infections with viral controls expressing only GFP (Fig. 6). This means that the transfection data reflect expression of the endogenous α-MyHC gene.

YY1 expression is elevated in aged SHHF rats

We then asked whether YY1 levels are increased in an animal model of pathologic cardiac hypertrophy. Spontaneously hypertensive heart failure (SHHF) rats are a well-characterized rodent model of cardiac hypertrophy and heart failure, presenting with increased heart to body weight ratios and decreased α-MyHC levels [26,27]. In order to establish that the animals used in these experiments were in a pathological state, we first determined that α-MyHC mRNA expression was repressed and ANF (a marker of cardiac pathology) was induced in SHHF animals when compared to age-matched Sprague–Dawley (SD) controls (Fig. 7). These changes in gene expression also correlated with an increase in the heart to body weight ratio of the SHHF rats ($4.57 + 0.35$ g/kg) when compared to the SD controls ($3.02 + 0.17$ g/kg). The pathological state of these SHHF animals was accompanied by a significant increase in YY1 expression, consistent with the idea that YY1 plays a role in the reduction of α-MyHC expression during pathological cardiac hypertrophy.

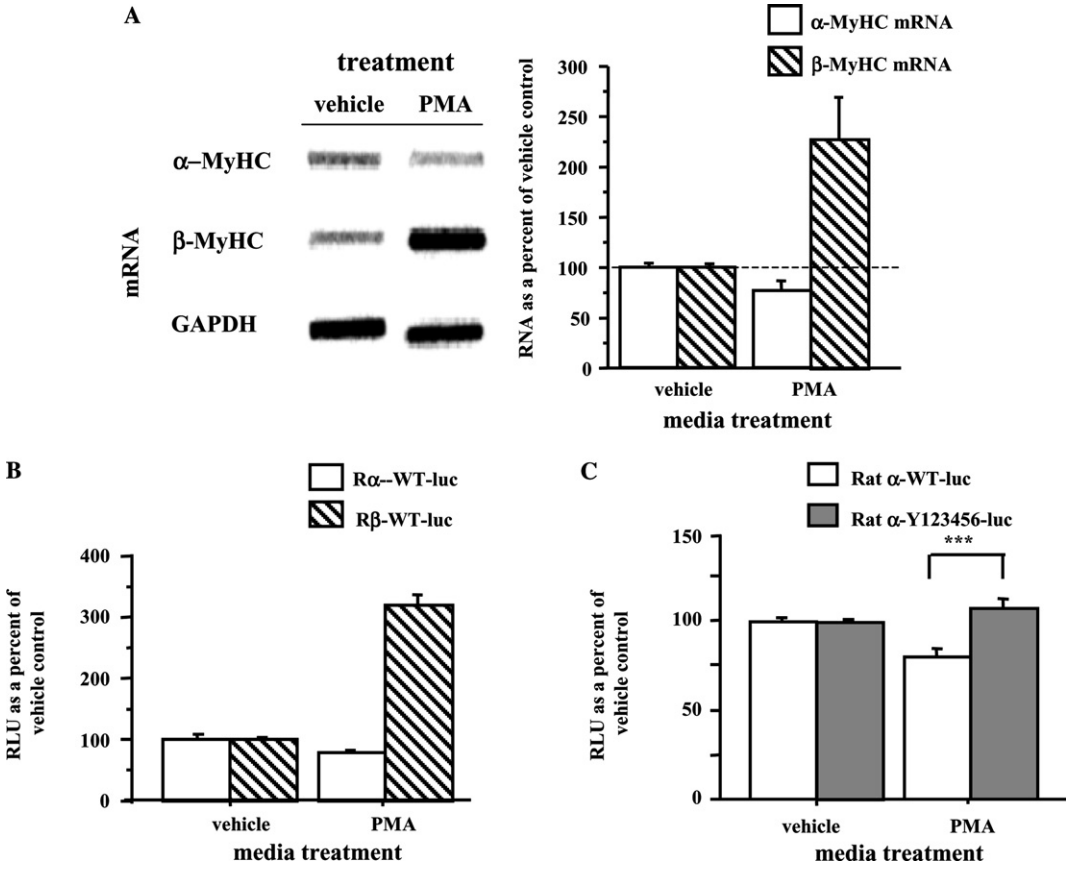

Fig. 4. YY1 is responsible for the pathological response of cardiac MyHC isoforms in NRVMs. (A) Slot blot of total RNA extracted from NRVMs treated with PMA or the vehicle control (0.001% DMSO). The expected pathological response is observed in these cells in that PMA causes antithetical changes in α- and β-MyHC mRNA levels. To the right, a histogram shows the relative levels of α- and β-MyHC mRNA. Data were pooled from multiple slot blots, using RNA harvested form several NRVM preparations. RNA levels were normalized internally to GAPDH. (B) PMA also causes disparate transcriptional responses of the α- and β-MyHC promoters. Transient transfection of NRVMs with Rα-WT-luc and Rβ-WT-luc reporters shows that PMA treatment decreases the luciferase activity of the Rα-WT-luc vector and increases the luciferase activity of the Rβ-WT-luc vector. (C) A comparison of the response of the Rα-WT-luc vector and the mutant Rα-Y123456-luc vector shows that disruption of YY1 binding sites abolishes the PMA-mediated repression of the rat α-MyHC promoter. Activity of the Rα-Y123456-luc vector is not repressed by PMA treatment and remains at untreated levels.

Discussion

Pathological cardiac hypertrophy is characterized by changes in gene expression patterns, which ultimately lead to deleterious alterations of the heart muscle. One change that appears to be universal, independent of the etiology of the disease, is the disparate regulation of the MyHC isoforms such that α-MyHC levels are repressed while β-MyHC levels are increased [28]. Moreover, it has been hypothesized that the decrease in the α-MyHC:β-MyHC ratio plays an important role in the contractile dysfunction observed in pathologic conditions [29]. For this reason, understanding the molecular regulation of the MyHC isoforms is central to uncovering the mechanism(s) of hypertrophic expression patterns and may possibly lead to advances in the treatment of heart disease.

In the work presented here, we show that YY1 decreases the activity of a rat α-MyHC-luciferase reporter vector (Fig. 1C). More importantly, we show that over-expression of wildtype YY1 in rat myocytes represses endogenous expression of α-MyHC (Fig. 6B). Sequence analysis of the proximal rat α-MyHC promoter revealed six putative YY1 binding sites. In vitro DNA binding assays showed that YY1 from nuclear extracts prepared from neonatal rat hearts is capable of binding five of the six predicted YY1 sites within the rat α-MyHC promoter. Disruption of all six of these sites together increased promoter activity and partially alleviated YY1-mediated repression of the promoter. Complete alleviation of repression was not observed, however, suggesting that YY1's role in repressing the rat promoter is both direct and indirect. YY1 is known to interact with HDACs and HATs whose chromatin-remodeling activities are capable of regulating transcription in a more global manner. As a result, the over-expression of YY1 will also likely have a general effect on transcription at a cellular level. In addition, the

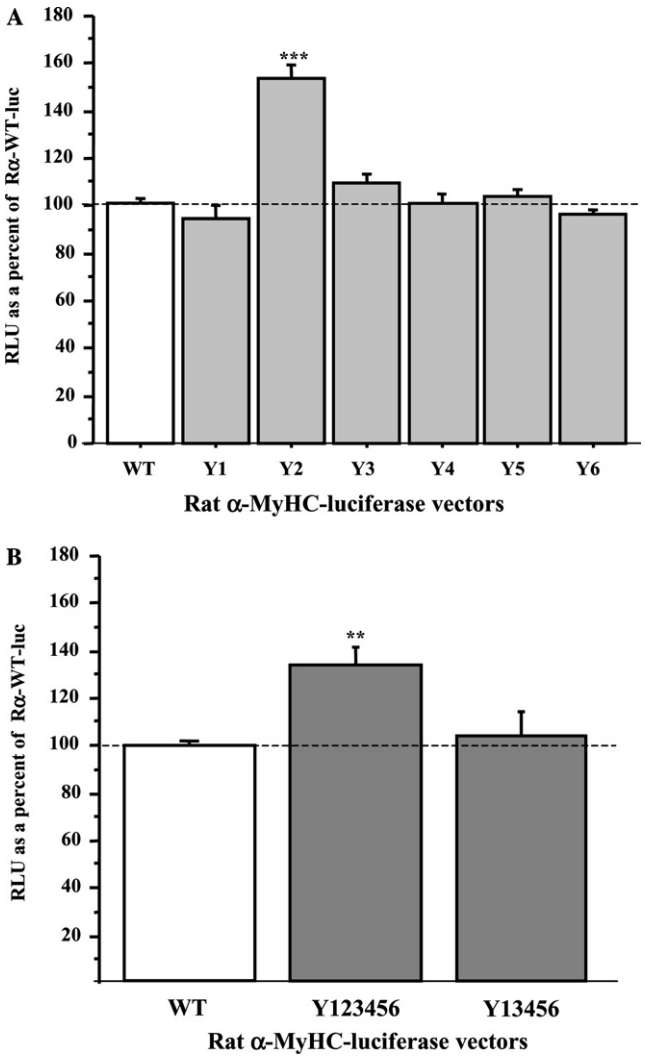

Fig. 5. The Y2 site is necessary and sufficient for PMA-mediated repression of the rat α-MyHC promoter. (A) The response of luciferase constructs carrying individual YY1 binding site mutations to PMA treatment is compared. Only the construct containing the Y2 site mutated, Rα-Y2-luc, responded differently than the wildtype vector, Rα-WT-luc, demonstrating that the Y2 site is sufficient for the PMA-mediated repression of the promoter. (B) A mutant vector in which all YY1 sites had been disrupted, except the Y2 site does not respond to PMA treatment differently than the wildtype vector, indicating that this site is also necessary for PMA-mediated repression of the promoter. (**$p < 0.001$, ***$p < 0.0001$.)

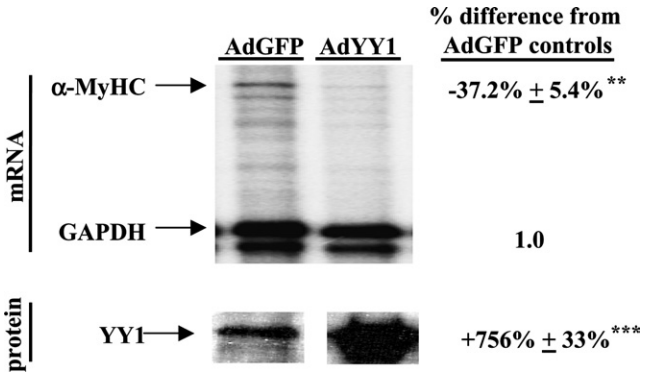

Fig. 6. YY1 over-expression represses endogenous expression of α-MyHC in NRVM. RNase protection assay using total RNA isolated from NRVM cultures that had been infected with either a wildtype YY1 expression virus or control virus expressing GFP. Protected fragments from α-MyHC and GAPDH are indicated. Below, a Western blot using an anti-YY1 antibody (Santa Cruz) was used to confirm that viral infection resulted in YY1 over-expression. Data pooled from separate experiments are shown as a percentage change from controls infected with the GFP virus. (**$p < 0.001$ and ***$p < 0.0001$.)

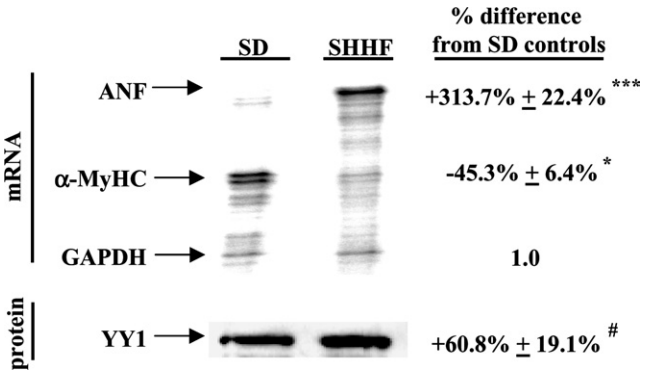

Fig. 7. YY1 expression is elevated in aged SHHF rat hearts. RNase protection assay using total RNA isolated from SD ($n = 4$) and SHHF ($n = 6$) rat hearts. Protected fragments from ANF, α-MyHC, and GAPDH are indicated. Also, a representative Western blot using an anti-YY1 antibody (Santa Cruz). Tissue samples were taken from the left ventricles of 20-month-old affected male spontaneously hypertensive heart failure rats (SHHF) and age-matched male Sprague–Dawley rats (SD). Data pooled from separate experiments are shown as a percentage change from SD controls. ($^{\#}p < 0.05$, *$p < 0.01$, and ***$p < 0.0001$.)

ability of YY1 to control the transcription of multiple endogenous genes makes it possible that the over-expression of YY1 alters the levels of other factors that, in turn, repress the rat α-MyHC promoter.

Ultimately, we wanted to define the role of YY1 in the repression of α-MyHC during cardiac hypertrophy. By culturing NRVMs in the presence of PMA, we were able to elicit a hypertrophic response that included a decrease in endogenous levels of α-MyHC and a repression of the α-MyHC promoter. Mutational analysis revealed that the Y2 site, located 94 bp upstream of the transcription start site, is both necessary and sufficient for this response. These results are extremely exciting because they provide a direct link between the repression of α-MyHC expression and PMA-induced cardiac hypertrophy. In vivo, we found that the hearts of aged SHHF rats displayed increased YY1 expression and reduced α-MyHC expression. These data provide a novel mechanism by which α-MyHC expression is repressed during pathological cardiac hypertrophy and offer valuable insights into the signaling of heart disease.

Acknowledgments

Generous contributions of reagents were as follows: Michael Atchison (University of Pennsylvania) provided us with the CMV-YY1(WT) expression plasmid, Edward Seto (University of South Florida) the CMV-YY1(Δ170–200) plasmid, Koji Hasegawa (Kyoto University, Japan) the Rβ-MyHC-luc vector, and Aristidis Moustakas (Ludwig Institute of Cancer Research, Sweden) the YY1 expression adenovirus.

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