Biochemical and Biophysical Research Communications 378 (2009) 62–67

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Transcription factor Yin Yang 1 represses fetal troponin I gene expression in neonatal myocardial cells

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ARTICLE INFO

Article history:
Received 28 October 2008
Available online 12 November 2008

Keywords:
Gene regulation
Troponin I
Yin Yang 1
Heart development
Myocardium

ABSTRACT

Yin Yang 1 (YY1) is a transcription factor that can activate or repress expression of a variety of genes and is involved in several developmental processes. While some transcription factors are known to modulate skeletal myogenesis, the regulation of fetal troponin I (ssTnI) expression by YY1 in cardiac development has not been studied. The present study shows that the fetal troponin I gene expression in neonatal myocardium was reduced by overexpression of YY1, while cardiac troponin I (cTnI) did not show any significant decrease. And a dose–response inhibition by YY1 was observed in fetal troponin I promoter induced transcriptional activities. Mutation of YY1-binding site can abolish the inhibitory effect and YY1 silencing in neonatal myocardium resulted in an increase of ssTnI protein expression. Our results indicate that YY1 is a novel regulator of fetal TnI transcription in the heart.

Troponin, a contractile protein of the thin filament of striated muscle, consists of three subunits: troponin C (TnC), troponin T (TnT), and troponin I (TnI). TnI is the inhibitory subunit that can bind to actin-tropomyosin and regulate muscle contraction [1]. Two major isoforms of TnI are expressed in the heart under the control of a developmentally regulated program: ssTnI is expressed first and predominates throughout embryonic and fetal development, and it is therefore referred to as the fetal isoform of TnI in the heart; the expression of cTnI, i.e., adult isoform of TnI, is upregulated after birth and predominates throughout adulthood in most mammals including humans [2–4]. TnI gene family has been used as a model to study the regulation of gene expression during differentiation and maturation of cardiac muscle tissues [5]. However, the mechanisms of the fetal-to-adult isoform switch and ssTnI gene inactivation during development are not well understood.

Transcriptional activation and repression of a given gene are critical for its proper regulation. Yin Yang 1 (YY1) is a 65 kDa member of the GLI-kruppel family of zinc finger transcription factor. The expression of YY1 is ubiquitous and the protein is highly conserved among human, mouse and *Xenopus* [6]. YY1 can activate or repress transcription of a variety of genes and is involved in several developmental processes. YY1 modulates skeletal myogenesis through transcriptional silencing and repress α-myosin heavy chain gene expression in pathologic cardiac hypertrophy [7,8]. However, biological function of YY1 on fetal troponin I gene development in the heart has not been studied. We have previously cloned a 1.8 kb upstream part of mouse ssTnI gene (TnIs1800P) and confirmed its promoter activities [9]. In the present study, we identified a YY1 protein-binding site on the promoter of mouse ssTnI gene. A dose–response inhibition by YY1 in TnIs1800P-induced transcriptional activities was found and mutation of the YY1 site abolished the inhibitory effect in myocardial cells. ssTnI expression was significantly reduced by overexpression of YY1 in cultured neonatal myocardium and ssTnI expression was significantly increased when YY1 expression was inhibited by siRNA in the cells. These data indicate that YY1 plays a role on myofibril protein gene regulation and can repress ssTnI gene expression in cardiac myocytes during heart development.

Materials and methods

Cell culture. C2C12 cell line (ATCC) was maintained in DMEM (ATCC) containing 15% FBS (growth medium) and cell differentiation was induced by changing the media to DMEM containing 2% horse serum (differentiation medium). Cardiac myocytes were isolated from neonatal mice (C57BL/6) using a Neonatal Cardiomyocyte Isolation Kit (Cellutron, NJ).

Vector construction and preparation. The upstream parts of mouse ssTnI gene containing 1800 bp (TnIs1800P), 1500 bp (TnIs1500P), and 200 bp (TnIs200P) were cloned into pGL3-Basic vector (Promega, WI) as previously reported [9]. Mutation in the YY1-binding site (TnIs1800P-Mut) was performed by site-directed PCR mutagenesis. Mouse cDNA was synthesized from neonatal heart RNA using a

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doi:10.1016/j.bbrc.2008.10.174

Thermoscript RT-PCR System (Invitrogen, IL). YY1 coding region was amplified by Platinum Pfx Taq (Invitrogen, IL). Specific primers were designed as the follows: YY1-forward: 5′-AAGCTTCTCAGCCATG GCCTCGGG-3′; YY1-reverse: 5′-TCACTGGTTG TTTTTGGCTTTAGC-3′. Obtained amplicons were inserted into pcDNA3.1 (+) expression vectors. Expressed protein was confirmed by Western blotting analysis. All clones were sequenced to ensure the sequence accuracy.

Transient transfection and luciferase assays. C2C12 myoblasts were transfected with the testing luciferase reporter constructs and reference plasmid pRL-TK. Cells transfected for 6 h were subjected to either growth medium (for myoblasts) or differentiation medium (for myotubes). The myoblasts were incubated for 48 h in growth medium and the myotubes were incubated for 96 h prior to harvesting. pGL3-Basic was used as a negative control and pGL3-Control was used as a positive control. Cardiac myocytes were first isolated in 12-well plates in NS medium for 36 h before transfection and cells were collected for analysis on day 6 after transfection.

Luciferase assays were performed using a Dual-Glo Luciferase Assay System (Promega, WI). Values were normalized to *Renilla* luciferase activities. Each experimental condition was measured in triplicate and multiple transfections were performed for each reporter construct.

RNA interference. Small interfering RNA (siRNA) specifically targeting mouse YY1 (sc-36864) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The siRNAs were introduced into the cells in the presence of Lipofectamine 2000 following the manufacture's protocols. The cells were collected 5 days post-transfection and subjected to analysis.

Electrophoretic mobility-shift assays (EMSA). Nuclear proteins were extracted from neonatal mouse hearts using NE-PER Nuclear and Cytoplasmic Extraction Reagent (Pierce, IL). Synthetic complementary oligonucleotides for determining potential YY1 bindings on ssTnI gene promoter (5′-GTTTCTAAAAATATGGCAGGTGTTC-3′) were biotinylated using the Biotin 3′-End DNA Labeling Kit (Pierce, IL). Nucleotide probe and protein binding were detected with HRP-conjugated streptavidin (LightShift Chemiluminescent EMSA kit, Pierce, IL). The competition assays were also performed following the same protocols described previously in which 100-fold excess of unlabeled double-stranded competitor oligonucleotides were added to the binding reactions. For supershift assay, 2 μg of anti-YY1 (sc-7341, Santa Cruz, CA) were used before adding the probes.

ChIP assays. Chromatin immunoprecipitation (ChIP) assays were performed by using an EZ-CHIP assay kit (Millipore, MA). Briefly, cells from mouse hearts were treated with formaldehyde and lysed with lysis buffer. DNA was sheared by sonication to yield an average fragment size of 500–2000 bp and cross-linked proteins were immunoprecipitated by incubation with anti-YY1 antibody, and the normal IgGs and no antibody treatments were used as negative control and input, respectively. The following primers were used for YY1 site A: ssTnI-ChIP-forward (5′-CACCCATATCAGAACATCACC-3′) and ssTnI-ChIP-reverse, (5′-TCAAATCCCAGAAACCACAT-3′). Another different site (Site B) on ssTnI promoter was selected as a negative control for ChIP assays. The following primers were used for site B: ssTnI-ChIP-forward (5′-CCTTAAAGGCCATCTGAAGATGTGG-3′) and ssTnI-ChIP-reverse, (5′-TGGCCAGTAAAAATAGAACAGCCGC-3′).

Western blotting. Proteins were separated on 4–12% SDS gels before being transferred onto nitrocellulose membranes. Membranes were incubated with an anti-TnI monoclonal antibody (clone cTnI-3), which recognizes both mouse cTnI and ssTnI, and a mouse anti-YY1 antibody, followed by incubation with HRP-conjugated anti-mouse IgG. Proteins were visualized by Enhanced Chemiluminescence kit (Pierce, IL).

Statistics. Data were expressed as means ± SD. Statistical analysis was carried out using ANOVA and Student's *t*-test to determine statistical significance. The criteria for significance were $P < 0.05$.

Results

Transcriptional activities of mouse ssTnI promoter in C2C12 and myocardial cells

Transcriptional activities of mouse ssTnI upstream promoter were determined with transient transfection assays in un-differentiated and differentiated C2C12 cells and in mouse myocardial cells. The cloned mouse ssTnI upstream promoter (TnIs1800P) and other deletion constructs (TnIs1500P and TnIs200P) were connected to the luciferase gene, as illustrated in Fig. 1A. The expression of the report gene driven by the tested constructs encoding ssTnI promoter was extremely low in un-differentiated C2C12 myoblasts. However, the expressions were dramatically enhanced in differentiated C2C12 myotubes that were converted from myoblasts after incubation in the differentiation medium as described

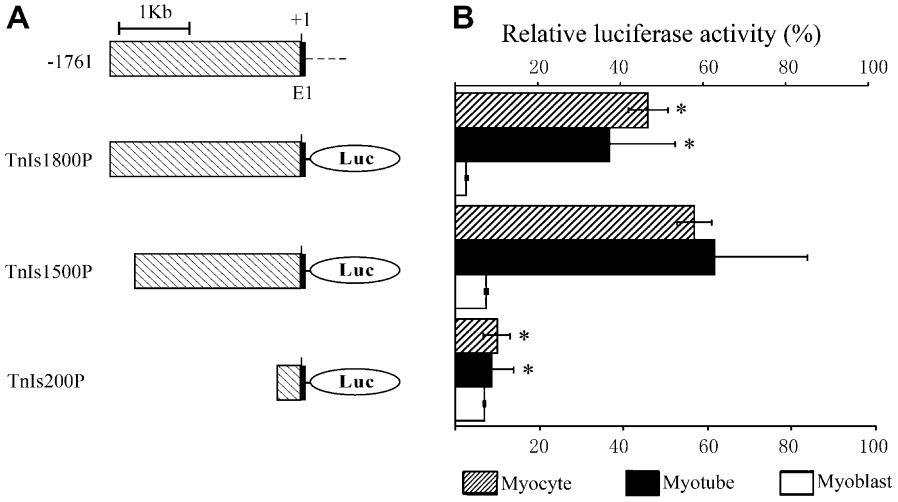

Fig. 1. Transcriptional activities of mouse ssTnI upstream promoters in C2C12 and myocardial cells. (A) Variable 5′ flanking regions of the mouse ssTnI upstream promoter were ligated with luciferase reporter gene (TnIs1800P, TnIs1500P, and TnIs200P) and subjected to transfection. (B) Luciferase activity analysis from cells after transient transfections with constructs. Luciferase expressions in C2C12 myoblasts, C2C12 myotubes and neonatal mouse myocardial cells were measured, normalized, and presented as a percentage of the positive control (pGL3-control). The values are presented as means ± SD from at least two independent triplicate assays. $^{*}P < 0.05$ compared to luciferase activity of TnIs1500P.

previously. The expression pattern is very similar in myocardial cells compared to that in C2C12 myotubes (Fig. 1B). Among these constructs, the transcriptional activities were very consistent to what we found previously with TnIs1500P the highest and TnIs200P the lowest. It was noted that a significant inhibitory effect existing in the sequence between −1500 and −1767 bp on mouse ssTnI upstream part since the transcriptional activities were significantly low with TnIs1800P compared to that with TnIs1500P.

Identification of ssTnI as a novel transcriptional target of YY1

To further investigate the inhibitory regions on mouse ssTnI upstream promoter, we performed binding sites prediction using computer-aid analysis techniques to determine the potential regulatory sites on the ssTnI gene upstream promoter. The analysis software used was from Genomatrix (www.genomatrix.de) and TESS (www.cbil.upenn.edu/cgi-bin/tess). Computer analysis data indicated that a putative YY1 repressor site containing CCAT in the antisense strand (GAACACCTGCCATATTTTTAGA reversed complementary to the sense sequence from −1658 to −1637) was found on the promoter. The 5′ CCAT 3′ core sequence is essential for YY1 consensus binding [10]. We referred to this site as Site A illustrated in Fig. 2A. To further characterize whether this site is capable of interacting with YY1 transcription factor, we performed EMSAs using biotin-labeled probes containing the sequences corresponding to the YY1 site with nuclear extracts prepared from neonatal mouse myocytes. Data from EMSAs indicated that a specific protein/DNA-binding complex was observed in nuclear extracts from mouse cardiac myocytes. To confirm that this complex contains YY1, an anti-YY1 antibody was added to the reaction and confirmed to completely prevent the formation of the complex (Fig. 2B). The reaction pattern observed in our supershift assays, i.e., a completely prevention by the antibodies of the formation of the complex, is very similar to what reported previously [8,11]. Furthermore, this binding complex was abolished when 100-fold molar excess of the same unlabeled oligonucleotides were added into the reaction (Fig. 2B), suggesting that it is a specific protein/DNA binding on YY1-binding site. The specific binding of YY1 site A was further confirmed by ChIP analysis and the results indicated that amplification of the precipitated DNA with a specific primer pair corresponding to the putative YY1-binding site confirmed the YY1 specific binding on ssTnI gene upstream promoter (Fig. 2C). Whereas Site B containing the 5′ CCAT 3′ core sequence showed no amplification (Fig. 2C).

YY1 inhibits the transcriptional activities of ssTnI promoters and downregulates ssTnI expression in myocardial cells

Since an inhibitory YY1-binding site was discovered on mouse ssTnI upstream promoter, we carried out the experiments to further investigate the role of YY1 on the activity of ssTnI upstream promoter in C2C12 cells co-transfected with ssTnI promoter and YY1 expression vector (pcDNA3-YY1). The data indicated that YY1 significantly inhibited the transcriptional activities of ssTnI upstream promoter (TnIs1800P) in C2C12 myotubes and YY1-mediated inhibition on ssTnI promoter was dose dependent (Fig. 3A). The inhibition on ssTnI promoter activity by YY1 was specific in cultured cardiac myocytes, because the mutation of YY1-binding site abolished completely the inhibitory effect of YY1 (Fig. 3B). Furthermore, the inhibitory effect of YY1 on ssTnI expression was clarified in neonatal myocardium overexpressing pcDNA3-YY1. ssTnI expression level was dramatically decreased in myocardial cells transfected with pcDNA3-YY1 vectors compared to that in pcDNA3

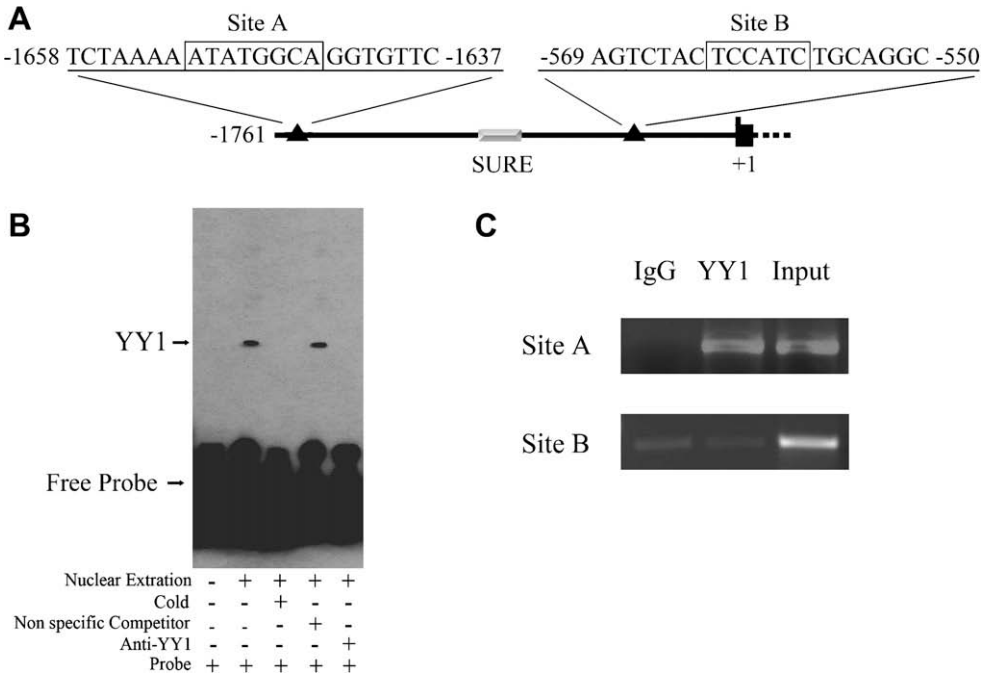

Fig. 2. YY1 binds to the mouse ssTnI upstream region in myocardial cells. (A) Schematic illustration of mouse ssTnI 5′ flanking region containing the putative YY1-binding site (Sites A and B, triangle). Position and sequence of the sites are shown above. (B) EMSA was performed to determine the interactions between the putative YY1-binding site and nuclear proteins extracted from mouse myocardial cells. The probe was biotin-labeled and incubated with neonatal mouse ventricular myocytes nuclear extract. Lane 1 (left), biotin-labeled YY1 probes alone; lane 2, YY1 probes with nuclear extracts; lane 3, addition of 100-fold excess unlabeled YY1 oligonucleotides in reaction mixtures with probes and nuclear extracts; lane 4, addition of 100-fold non-specific oligonucleotides in reactions with the biotin-labeled probes and nuclear extracts; lane 5, anti-YY1 antibody was added to the reaction with probes and nuclear extracts. (C) A representative image of ChIP assays for the putative YY1-binding site (Site A). The DNA fragments precipitated by anti-YY1 and negative control IgGs were amplified. Total input DNA is indicated (Input). A different site (Site B) of the mouse ssTnI promoter was used as a negative control for ChIP assays.

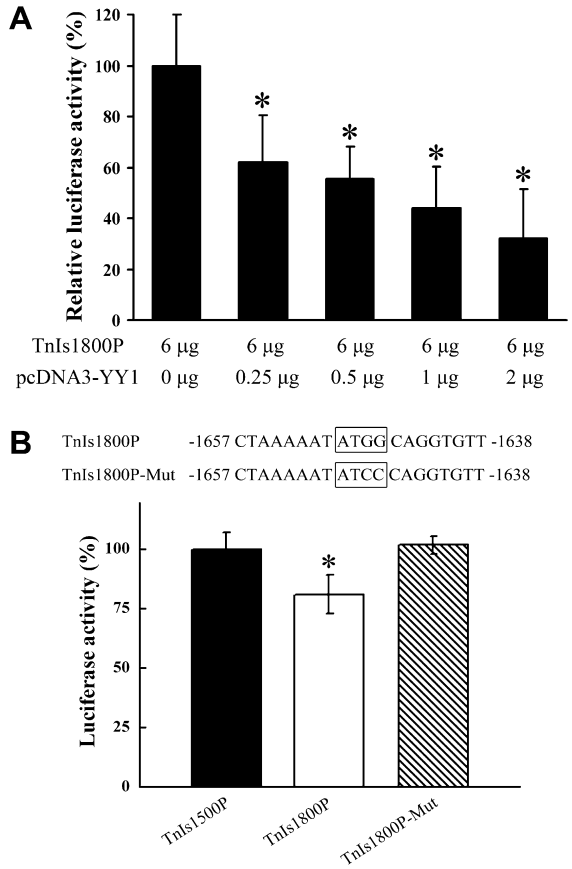

Fig. 3. YY1 inhibits ssTnI promoter activities in C2C12 and mouse neonatal myocardial cells. (A) pGL3-basic vector containing mouse ssTnI upstream promoter (TnIs1800P) was co-transfected with YY1 expression (pcDNA3-YY1) vector in differentiated C2C12 myotube cells. Co-transfection experiments were carried out using various amounts of pcDNA3-YY1 with reporter gene driven by 1800-bp ssTnI promoter. The values are expressed as means ± SD from three independent assays. The relative luciferase activity obtained from the cells transfected with plasmid TnIs1800P was the highest and is used as 100%. $^{*}P < 0.05$. (B) Transfection of WT mouse ssTnI promoter induced luciferase reporters (TnIs1500P and TnIs1800P) and an YY1-binding site mutated vector (TnIs1800P-Mut), in which a central repression domain of YY1-binding site had been changed from ATGG to ATCC, in mouse neonatal myocardial cells. $^{*}P < 0.05$.

transfection control cells, whereas the expression level of another TnI isoform, cardiac TnI (cTnI) did not change in the same cells (Fig. 4A and B). YY1-mediated repression of ssTnI gene expression in myocardial cells was also evidenced by YY1 knockdown experiments. The experimental data indicated that the inhibition of YY1 expression in neonatal myocardium caused by siRNA could increase ssTnI expression in neonatal myocardium, whereas the expression levels of cTnI or β-actin in the same cells did not change (Fig. 4C and D), suggesting that YY1 would be involved in the regulation of ssTnI gene expression in myocardial cells.

Discussion

A major level for the control of gene expression is that of transcription and this is achieved through the interaction of specific DNA-binding transcription factors with DNA sequences located in the promoters of target genes. During T lymphocyte development, a silencer is the critical controlling element that downregulates CD4 transcription at several developmental stages [12]. A 30-bp purine-rich negative regulatory element has been discovered in the first intronic region of rat cardiac β-MHC gene to be essential for negative gene regulation [13]. YY1 is involved in developmental process as a repressor of transcription in differentiated H9C2 cells [14]. Very recently, it is reported that YY1 inhibits skeletal myogenesis through transcriptional silencing of myofibrillar genes [15].

Unlike the hemoglobin gene cluster that is located within the same locus, TnI isoforms are encoded by different genes. The human ssTnI gene has been cloned and characterized [16]. It spans 12.5 kb and is divided into nine exons. In contrast to many muscle-specific genes, the ssTnI promoter does not contain consensus CCAAT or TATA elements. Deletion analysis of the TnI promoter region revealed a 157-bp enhancer conferred slow-muscle-preferential activity [17]. The rat ssTnI gene characterized by Banerjee-Basu and Buonanno [18] was reported *cis*-acting sequences conferring tissue- and development-specific transcription in skeletal muscle cells. However, the properties of mouse ssTnI gene promoter in the heart are not well studied. We have cloned and identified a 1.8 kb upstream region of the mouse ssTnI gene [9]. Sequence comparison of this mouse ssTnI promoter with those of rat and human indicates that the ssTnI gene is highly conserved. In the present studies, we have confirmed that the ssTnI upstream promoter, TnIs1800P, has a high transcriptional activity in differentiated C2C12 cells and in cultured neonatal myocardial cells. The fact that the transcriptional activities driven by both TnIs1800P and TnIs1500P are much higher than that of TnIs200P indicates that a 128-bp ssTnI upstream regulatory element (SURE), which is found highly conserved in mouse TnIs1800P, may be one of many elements residing between −200 and −1500 bp. The YY1 regulatory domain is, for the first time, revealed on mouse ssTnI upstream promoter. The transcription factor YY1 inhibits the transcriptional activities of mouse ssTnI upstream promoter and represses the ssTnI gene expression in neonatal myocardial cells. At present, it is still not clear the mechanism underlying YY1-mediated ssTnI gene transcriptional repression. More studies will be needed to elucidate the interactions between YY1 and/or other nuclear factors in myocardial cells.

The troponin I isoform switch during heart development was discovered two decades ago. However, the mechanisms of fetal isoform of TnI inactivation soon after birth are not clear yet. We first hypothesized that the up-regulation of cardiac TnI (cTnI) soon after birth might be a signal molecule to regulate the ssTnI gene expression. However, that is not the case since the ssTnI gene in heart was still turned off during post-natal development even in cTnI gene knockout mice that die 2–3 weeks after birth as the ssTnI could not maintain expression in the heart to compensate for the lack of cTnI [19]. Since thyroid hormone has been reported to play a critical role in contractile protein gene regulation [20–21], we further investigated the role of thyroid hormone in regulation of ssTnI expression in the cTnI knockout mouse model. Our data indicate that thyroid hormone can promote the inactivation of ssTnI gene expression in the heart and result in an early death of the homozygous cTnI knockout mice [22].

The similarities in regulation of TnI in lower and higher mammals imply a tight genetic control of the expression of TnI gene in the heart. Experimental cardiac hypertrophy and heart failure in the rat are associated with the re-expression of many genes normally observed during fetal cardiac development [23]. However, there is no evidence that ssTnI is re-expressed in experimental rat hearts with cardiac hypertrophy or in human hearts in end-stage heart failure. So the signals that mediate the down-regulation of ssTnI gene in maturing heart are potentially quite interesting to understand the differences between pathologic isoform shifts and maturational shifts. In this study, an YY1 biding site has been identified for the first time on mouse ssTnI promoter and its inhibitory effect on ssTnI gene expression has been revealed. However, more studies are needed to further elucidate the inhibitory mechanism and the potential co-factors in myocardial cells for this inhibitory action. The regulation of TnI isoform expression in the heart is an important area of investiga-

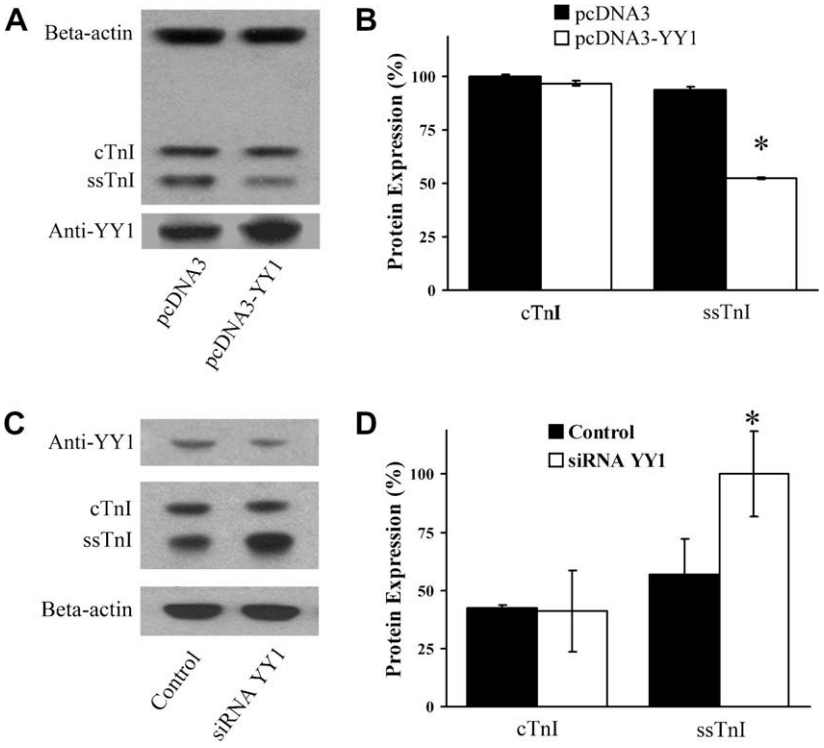

Fig. 4. YY1 causes an increase of ssTnI expression in neonatal myocardial cells. (A) Representative Western blots indicate that ssTnI protein level is significantly reduced in pcDNA3-YY1 transfected neonatal myocardial cells, whereas no significant changes for cTnI or β-actin in the same cells. (B) A summary of the Western blotting data of troponin I concentration in pcDNA3-YY1 transfected cells and pcDNA3 transfected control cells from three independent assays. Data are expressed as means ± SD, $^{*}P < 0.05$. (C) Western blot assays indicate that the ssTnI protein level is increased significantly in cultured neonatal myocardial cells in which YY1 expression is knockdown by siRNA. Upper panel, decreased YY1 expression level in neonatal myocardial cells transfected with siRNA against YY1; middle panel, increased ssTnI protein, but not cTnI, in neonatal myocardial cells with less YY1 expression; lower panel, β-actin level was determined as an internal control. (D) A summary of Western blotting data indicates that when YY1 expression is prohibited by siRNA, ssTnI expression level increases significantly whereas no change in cTnI expression in neonatal myocardial cells. Data are expressed as means ± SD from at least two separated experiments. $^{*}P < 0.05$.

tion as congenital and early cardiovascular disorders are one of the most common causes of neonatal death and diseases. The understanding of the TnI isoform switching and TnI expression regulation will shed light on important aspects of cardiac functional development.

Acknowledgments

The authors thank Jianfeng Du and Chi Zhang at Florida Atlantic University for their technical assistance in mouse ssTnI promoter cloning. This work was supported by grant from NIH (S6-GM0 73621) and American Heart Association Florida/Puerto Rico Affiliate and from the Center of Excellence for Biomedical and Marine Biotechnology at Florida Atlantic University.

References

[1] L.S. Tobacman, Thin filament-mediated regulation of cardiac contraction, Annu. Rev. Physiol. 58 (1996) 447–481.
[2] M.A. Sabry, G.K. Dhoot, Identification and pattern of expression of a developmental isoform of troponin I in chicken and rat cardiac muscle, J. Muscle Res. Cell Motil. 10 (1989) 85–91.
[3] S. Ausoni, C. De Nardi, P. Moretti, L. Gorza, S. Schiaffino, Developmental expression of rat cardiac troponin I mRNA, Development 112 (1991) 1041–1051.
[4] S. Schiaffino, L. Gorza, S. Ausoni, Troponin isoform switching in the developing heart and its functional consequences, Trends Cardiovasc. Med. 3 (1993) 12–17.
[5] L. Zhu, G.E. Lyons, O. Juhasz, J.E. Joya, E.C. Hardeman, R. Wade, Developmental regulation of troponin I isoform genes in striated muscles of transgenic mice, Dev. Biol. 169 (1995) 487–503.
[6] Y. Shi, J.S. Lee, K.M. Galvin, Everything you have ever wanted to know about Yin Yang 1, Biochim. Biophys. Acta 1332 (1997) F49–F66.
[7] W.R. MacLellan, T.C. Lee, R.J. Schwartz, M.D. Schneider, Transforming growth factor-beta response elements of the skeletal alpha-actin gene. Combinatorial action of serum response factor, YY1, and the SV40 enhancer-binding protein, TEF-1, J. Biol. Chem. 269 (1994) 16754–16760.
[8] P.D. Mariner, S.W. Luckey, C.S. Long, C.C. Sucharov, L.A. Leinwand, Yin Yang 1 represses α-myosin heavy chain gene expression in pathologic cardiac hypertrophy, Biochem. Biophys. Res. Commun. 326 (2005) 79–86.
[9] J. Du, C. Nan, J.J. Huang, C. Zhang, J. Liu, P. Jia, M. Abers, X.P. Huang, Functional characterization of mouse fetal TnI gene promoters in myocardial cells, J. Biomed. Sci. (Epub: May 2008).
[10] R.P. Hyde-DeRuyscher, E. Jennings, T. Shenk, DNA binding sites for the transcriptional activator/repressor YY1, Nucleic Acids Res. 23 (1995) 4457–4465.
[11] C.C. Sucharov, P. Mariner, C. Long, M. Bristow, L. Leinwand, Yin Yang 1 is increased in human heart failure and represses the activity of the human α-myosin heavy chain promoter, J. Biol. Chem. 15 (2003) 31233–31239.
[12] S. Sawada, J.D. Scarborough, N. Killeen, D.R. Littman, A lineage-specific transcriptional silencer regulates CD4 gene expression during T lymphocyte development, Cell 77 (1994) 917–929.
[13] M. Gupta, R. Zak, T.A. Libermann, M.P. Gupta, Tissue-restricted expression of the cardiac alpha-myosin heavy chain gene is controlled by a downstream repressor element containing a palindrome of two Ets-binding sites, Mol. Cell. Biol. 18 (1998) 7243–7258.
[14] C.C. Sucharov, S. Langer, M. Bristow, L. Leinwand, Shuttling of HDAC5 in H9C2 cells regulates YY1 function through CaMKIV/PKD and PP2A, Am. J. Physiol. Cell Physiol. 291 (2006) C1029–C1037.
[15] H. Wang, E. Hertlein, N. Bakkar, H. Sun, S. Acharyya, J. Wang, M. Carathers, R. Davuluri, D.C. Guttridge, NF-κB regulation of YY1 inhibits skeletal myogenesis through transcriptional silencing of myofibrillar genes, Mol. Cell. Biol. 27 (2007) 4374–4387.
[16] S.J. Corin, O. Juhasz, L. Zhu, P. Conley, L. Kedes, R. Wade, Structure and expression of the human slow twitch skeletal muscle troponin I gene, J. Biol. Chem. 269 (1994) 10651–10659.
[17] S.J. Corin, L.K. Levitt, J.V. O'Mahoney, J.E. Joya, E. Hardeman, R. Wade, Delineation of a slow-twitch-myofiber-specific transcriptional element by using in vivo somatic gene transfer, Proc. Natl. Acad. Sci. USA 92 (1995) 6185–6189.
[18] S. Banerjee-Basu, A. Buonanno, *cis*-Acting sequences of the rat troponin I slow gene confer tissue- and development-specific transcription in cultured muscle

cells as well as fiber type specificity in transgenic mice, Mol. Cell. Biol. 13 (1993) 7019–7028.
[19] X.P. Huang, Y. Pi, K.J. Lee, A.S. Henkel, R.G. Gregg, P.A. Powers, J.W. Walker, Cardiac troponin I gene knockout. A mouse model of myocardial troponin I deficiency, Circ. Res. 84 (1999) 1–8.
[20] V. Averyhart-Fullard, L.D. Fraker, A.M. Murphy, R.J. Solaro, Differential regulation of slow-skeletal and cardiac troponin I mRNA during development and by thyroid hormone in rat heart, J. Mol. Cell. Cardiol. 26 (1994) 609–616.
[21] K. Ojamaa, J.D. Klemperer, S.S. MacGilvray, I. Klein, A. Samarel, Thyroid hormone and hemodynamic regulation of α-myosin heavy chain promoter in the heart, Endocrinology 137 (1996) 802–808.
[22] X.P. Huang, K.J. Lee, B. Riedel, C. Zhang, L.F. Lemanski, J.W. Walker, Thyroid hormone regulates slow skeletal troponin I gene inactivation in cardiac troponin I null mouse hearts, J. Mol. Cell. Cardiol. 32 (2000) 2221–2228.
[23] K.R. Boheler, K. Schwartz, Gene expression in cardiac hypertrophy, Trends Cardiovasc. Med. 2 (1992) 172–178.