GATA-5 Is Involved in Leukemia Inhibitory Factor-responsive Transcription of the β-Myosin Heavy Chain Gene in Cardiac Myocytes*

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Leukemia inhibitory factor is a member of a family of structurally related cytokines sharing the receptor component gp130. Activation of gp130 by leukemia inhibitory factor is sufficient to induce myocardial cell hypertrophy accompanied by specific changes in the pattern of gene expression. However, the molecular mechanisms that link gp130 activation to these changes have not been clarified. The present study investigated the transcriptional pathways by which leukemia inhibitory factor activates β-myosin heavy chain expression during myocardial cell hypertrophy. Mutation of the GATA motif in the β-myosin heavy chain promoter totally abolished leukemia inhibitory factor-responsive transcription without changing basal transcriptional activity. In contrast, endothelin-1 responsiveness was unaffected by the GATA mutation. Among members of the cardiac GATA transcription factor subfamily (GATA-4, -5, and -6), GATA-5 was the sole and potent transactivator for the β-myosin heavy chain promoter. This transactivation was dependent on sequence-specific binding of GATA-5 to the β-myosin heavy chain GATA element. Cardiac nuclear factors that bind to the β-myosin heavy chain GATA element were induced by leukemia inhibitory factor stimulation. Last, leukemia inhibitory factor stimulation markedly increased transcripts of cardiac GATA-5, the expression of which is normally restricted to the early embryo. Thus, GATA-5 may be involved in gp130 signaling in cardiac myocytes.

Cardiac muscle cells exit the proliferative cell cycle soon after birth, with little or no capacity for subsequent cell division. Hence, the adult myocardium responds to hemodynamic stimuli through an adaptive hypertrophic response that is characteristic of the hypertrophic program (17, 18). The receptors of this cytokine family are multimeric and share the class-specific transmembrane signal-transducing component gp130 (19–23). Signaling is triggered through the homodimerization of gp130 (24) or the heterodimerization of gp130 with a related transmembrane signal transducer, the LIF receptor subunit β (25, 26). Overexpression of both interleukin-6 and its receptor results in constitutive tyrosine phosphorylation of gp130 (i.e. activation) in the myocardium and left ventricular hypertrophy in vivo (27). Thus, the activation of cardiac myocyte hypertrophy through gp130-dependent signaling pathways is not confined to the in vitro hypertrophy assay but is also observed in vivo. Activation of gp130 by LIF or cardiotrophin-1 is also associated with specific changes in cardiac gene expression (18). The molecular mechanisms that link gp130 activation to these changes have not been clarified.

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LIF-responsive Transcription

Members of the interleukin-6-LIF cytokine family have been shown to activate the Janus kinase/signal transducer and activator of transcription (STAT) pathway and phosphorylate STAT3 (23, 28–30). It is also clear that this family of cytokines can activate Ras and mitogen-activated protein kinase cascades (23, 28, 31). An activated Ras gene, targeted to myocardi

dium in transgenic mice, elicits ventricular enlargement, atrial natriuretic factor expression, myofibrillar disarray, and impaired relaxation in diastole (32). Conversely, microinjection of dominant-negative Ras protein was reported to block α1-adren
ergic induction of both morphological changes in myofibrillar structure and expression of atrial natriuretic factor (33), demonstrating a requirement of Ras-dependent pathways for G-protein-coupled signaling in myocardial cell hypertrophy. The relative contributions of the Ras/mitogen-activated protein kinase and Janus kinase/STAT pathways to gp130-induced cardiac hypertrophy are presently unclear, however, because selective pharmacological inhibition of mitogen-activated protein kinase activation does not block hypertrophy (34). Recently, we and others have shown that zinc finger GATA transcription factors are required for transcriptional activation of the genes for angiotensin II type 1a receptor and β-MHC during pressure overload hypertrophy in vivo (35, 36). However, pressure overload is a complex stimulus consisting of multiple factors. A specific stimulus linked to GATA factors has not been clarified. In addition, although so far six members of the GATA transcription factor family have been cloned, it is unclear which member of this family plays the most important role. Thus, the present study analyzed cis-acting elements and trans-acting factors required for LIF-responsive β-MHC transcription during myocardial cell hypertrophy.

EXPERIMENTAL PROCEDURES

Measurement of Protein Synthesis Rate—Primary ventricular cardiac myocytes were prepared from hearts of 1–2-day-old Sprague Dawley rats as described previously (37). Twenty-four hours after plating, the cells were washed twice with serum-free media and then incubated with 5 μCi/mI 3H-phenylalanine (120 Ci/mmol) and unlabeled phenylalanine (0.36 mmol/liter) in serum-free medium for 48 h in the presence of 2.5 × 10−8 M LIF (AMRAD, Melbourne, Australia), 10−7 M ET-1 (Peptide Institute, Osaka, Japan), or saline as a control. The cells were then washed twice with ice-cold phosphate-buffered saline and then resuspended in 0.15 M acid was added at 4 °C for 60 min to precipitate protein. The precipitate (Peptide Institute, Osaka, Japan), or saline as a control. The cells were then washed twice with ice-cold-phosphate-buffered saline and lyzed as described (36, 37, 40, 43, 47). Luciferase activities were determined in duplicate samples from each plate using a Monolight LB 9501 luminometer (EG & G, Berthold) (36, 37, 40, 43, 47). Chromophenol acetylactyltransferase (CAT) activities were determined in the same cell lysates as that used for the luciferase assay (36, 37, 40, 43, 47).

Electrophoretic Mobility Shift Assays (EMSAs)—Nuclear extracts were prepared from cultures of primary newborn rat cardiac myocytes as described (48, 49). GATA-4/5 protein was prepared using in vitro transcription and translation systems (Promega, Madison, WI) according to the manufacturer’s recommendation. Double-stranded oligonucleotides were designed that contained GATA motifs from the β-MHC or α1-actin 5′-flanking sequences (36, 37). The sequence strand of these oligonucleotides were as follows: β GATA, 5′-CTGGGATGTA-TAAGGAGATTTTGGTCTTACCTTTGGAACCA-3′; mut β GATA, 5′-CTG- GTGGAGTGATGATTTTTGCTTCACTTTGCTTACGGACCA-3′; EF-1, (GATA element in the TnC promoter; Ref. 48), 5′-CGCGGATCCCA-GGCTTGAGATTACGAGGATCCGGG-3′; and nonspecific oligonucleotide, 5′-GGGGATCCGGGATCCGGG-3′. Oligonucleotides were synthesized by Greiner Inc. (Tokyo, Japan) and purified by SDS-polyacrylamide gel electrophoresis.

EMSAs were carried out at 4 °C for 20 min in 15-μl reaction mixtures containing 10 μg of nuclear extract, 0.25 ng (>20,000 cpm) of radiola
depleted double-stranded oligonucleotide, 500 ng of poly(dI-dC), 5 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.5 mM dithiothreitol, 37 mM KCl, and 4% Ficoll 400. For cold competition experiments, a 100 mM excess of unlabeled competitor oligonucleotide was included in the binding reaction mixture. Protein-DNA complexes were separated by electrophoresis on 4% nondenaturing polyacrylamide gels in 0.25 × Tris-borate EDTA (1 × Tris-borate EDTA is 100 mM Tris, 100 mM boric acid, and 2 mM EDTA) at 4 °C.

Reverse Transcription-Polymerase Chain Reaction (PCR)—To detect GATA-5 transcripts in cardiac myocytes, a reverse transcriptase-polymerase chain reaction was carried out as described previously (51). For this particular experiment, we used ventricular myocytes isolated from 1–2-day-old DDY mice, because the rat GATA-5 sequence has not been published. One litter (8–12 pups) yielded >105 cells. Total RNA was isolated as described (36, 37) from these cells and subjected to reverse transcription (8 μg of total RNA/sample) with a first-strand cDNA synthesis kit (Amersham Pharmacia Biotech) according to the manufacturer’s recommendation.

The PCR primers were designed on the basis of published mouse cDNA sequences for GATA-5 (44) and GPDH (39) as follows; sense for GATA-5, TCCCACTCTCACAATCT; antisense for GATA-5, ACACGGCTTCTCGACGTA; sense for GPDH, TTGCCATCAACGACTTATCC; and antisense for GPDH, TTGCTAGATGCTGAGGATCCGG. Thermal cycling conditions for optimal amplification were performed using various amounts of reverse transcription products and various number cycles of PCR amplification as described (51). On the basis of these initial experiments, the linear portion of the amplification was determined for both genes. The following conditions were therefore chosen as standard for the PCR reactions in a volume of 50 μl: reverse transcription products from 300 ng of RNA for GATA-5 or 150 ng of RNA for GPDH, 2.5 units of TaqAmpli polymerase (Perkin Elmer), an Mr 35 flies of amplification for GATA-5 or 30 cycles for GPDH and 100 ng of each sense and antisense primers. The amplification was carried out as follows: denaturation, 45 s at 94 °C; annealing, 45 s at 54 °C; and extension, 90 s at 72 °C. The PCR products (10 μl/lane) were electrophoresed on a 1.5% agarose gel and stained with ethidium bromide.

Statistical Analysis—All data are expressed as means ± S.E. The significance of differences between mean values was evaluated by the two-tailed Student’s t test, and differences were considered significant at the p < 0.05 level.

Plasmids were purified by anion exchange chromatography (Qiagen, Hilden, Germany), quantified by measurement of A260, and examined on agarose gels stained with ethidium bromide before use.

Transfection and Luciferase/CAT Assays—Twenty-four hours after plating, cells were washed twice with serum-free media and then co-transfected with 4 μg of the luciferase construct of interest and 1 μg of pRSVCAT using LipofectAMINE (Life Technologies, Inc.) according to the manufacturer’s recommendation. After a 2-h incubation with DNA-LipofectAMINE complex, the cells were washed twice with serum-free media and further incubated for 48 h in serum-free media in the presence of 2.5 × 10−9 M LIF, 10−7 M ET-1, or saline as a control. The cells were then washed twice with ice-cold-phosphate-buffered saline and lysed as described (36, 37, 40, 43, 47). Luciferase activities were determined in duplicate samples from each plate using a Monolight LB 9501 luminometer (EG & G, Berthold) (36, 37, 40, 43, 47). Chromophenol acetylactyltransferase (CAT) activities were determined in the same cell lysate as that used for the luciferase assay (36, 37, 40, 43, 47).
A synthesis rate, respectively (Fig. 1 resulted in a 40 length; Ref. 18). Therefore, we have used bulk protein synthesis versus 6 and 3.0 stimuli, such as ET-1, cardiomyocytes were treated with 2.5 similar to that of other previously well defined hypertrophic whether LIF induces a hypertrophic response to an extent 10 protein synthesis and by specifically changing their patterns of 10 expression in cardiac myocytes by ET-1 and LIF. Cont 3.0 2 and 2.5 3 expression of b-MHC gene in cardiac myocytes by 3.8 2 basal activity was unaffected, however, by mutating the GATA motif. LIF- or ET-1-responsive elements (66% decrease 308 53, 56). As shown in Fig. 2A, basal transcriptional activity of the 3542-bp b-MHC promoter fragment conferred LIF- and ET-1-inducible expression to the luciferase reporter gene (1.9 ± 0.3- and 2.4 ± 0.1-fold, respectively). In contrast, neither LIF 3542-bp b-MHC promoter (0.9 ± 0.2- and 1.2 ± 0.3-fold, respectively). These findings suggest that the upregulated expression of b-MHC gene by LIF or ET-1 is mediated, at least in part, through a transcriptional mechanism and that the proximal 3542-bp b-MHC promoter sequences are sufficient to mediate LIF- and ET-1-responsive expression.

LIF-responsive b-MHC Transcription Requires an Intact GATA Element—To more precisely determine the downstream molecular events during LIF-induced cardiac hypertrophy, we examined cis-acting elements that mediate LIF-responsive b-MHC transcription. A previous study demonstrated that the proximal 333 bp of the rat b-MHC promoter are sufficient to mediate muscle-specific transcription in cultured neonatal cardiac myocytes and in sol8 cells (52). As shown in Fig. 2B, in cultured neonatal cardiac myocytes, the transfect 333/ +34 bp b-MHC promoter responded to LIF and ET-1 stimulation, increasing the expression 2.0- and 3.0-fold, respectively. These data demonstrate that important elements exist within the rat b-MHC promoter sequences –333/+34, although they do not rule out possible elements outside these sequences.

The rat b-MHC promoter sequences –333/+34 contain distal and proximal M-CAT elements, previously demonstrated to mediate muscle-specific and α1-adrenergic-stimulated transcription of the b-MHC gene (12). These also contain a GATA element, shown to mediate cardiac-specific transcription of other genes (50, 53, 55). Thus, we mutated these elements in the context of the 333-bp b-MHC promoter. Mutations were designed to abolish the binding of cardiac nuclear factors (12, 53, 56). As shown in Fig. 2A, basal transcriptional activity of the transfected 333-bp b-MHC promoter was attenuated by simultaneous mutations in both distal and proximal M-CAT elements (66% decrease versus wild type), compatible with a role for the M-CAT element in muscle-specific transcription. Basal activity was unaffected, however, by mutating the GATA motif. LIF- or ET-1-responsive b-MHC transcription is shown in Fig. 2B. In contrast to the basal activity, mutating the M-CAT elements affected neither ET-1 nor LIF responsiveness. Notably, however, LIF- but not ET-1-responsive transcription was totally abolished by mutating the GATA element (wild type, 2.0-fold, versus GATA mutant, 0.9-fold; p < 0.001). Thus, an intact GATA element is required for LIF-responsive b-MHC transcription, suggesting a role for this element in LIF-induced cardiac hypertrophy.

GATA-5 Is a Potent Activator of the b-MHC Promoter—Among members of GATA transcription factor family, GATA-4, -5, and -6 are expressed in the heart (44–46). To determine
whether expression of GATA-4, -5, and -6 can transactivate the LIF-responsive 2333/134 bp-β-MHC promoter sequences, we performed transient transfection experiments. We co-transfected a luc expression vector driven by the −333/+34-bp β-MHC promoter with a eukaryotic expression plasmid encoding either p333wtβ-MHCluc (wild type) or p333GATAβ-MHCluc (with a mutation that ablates the LIF-responsive transcription), 2.5 μg of GATA-5 expression vector, and 0.5 μg of pRSVCAT. The results are expressed as fold activation of the normalized luciferase activity (luc/CAT) relative to co-transfection with the control β-galactosidase expression vector. The data shown are from four independent experiments, each carried out in duplicate. First M, β-actin; o, −333/+34 β-MHC; second □, α-MHC.

**Fig. 3.** Sequence-specific transactivation of the β-MHC promoter by GATA-5. A, NIH3T3 cells were transfected with 2.5 μg of GATA-4, -5, and -6 expression vector, 1.5 μg of a reporter plasmid (pβ-actinluc, p333wtβ-MHCluc, or p2936e-MHCluc), and 0.5 μg of pRSVCAT. The results are expressed as fold activation of the normalized luciferase activity (luc/CAT) relative to co-transfection with the control β-galactosidase expression vector. The data shown are from two to four independent experiments, each carried out in duplicate. B, NIH3T3 cells were transfected with 1.5 μg of luc expression vector, either p333wtβ-MHCluc (wild type) or p333GATAβ-MHCluc (with a mutation that ablates the LIF-responsive transcription), 2.5 μg of GATA-5 expression vector, and 0.5 μg of pRSVCAT. The results are expressed as fold activation of the normalized luciferase activity (luc/CAT) relative to co-transfection with the control β-galactosidase expression vector. The data shown are from four independent experiments, each carried out in duplicate. First □, β-actin; o, −333/+34 β-MHC; second □, α-MHC.

**Fig. 2.** Mutation analysis of the basal transcriptional activities and LIF-responsive transcription of rat β-MHC promoter sequences in cardiac myocytes. Four μg of p333wtβ-MHCluc (wild type), p333M-CATβ-MHCluc (mutation of both M-CAT elements) or p333GATAβ-MHCluc (mutation of GATA element) and 1 μg of pRSVCAT were co-transfected into primary cardiac myocytes of neonatal rats subsequently stimulated with saline, ET-1, or LIF for 48 h. A, basal activities represent the relative luciferase activities (luc/CAT) in saline-stimulated states and were expressed by setting those of p333wtβ-MHCluc at 100% in each experiment. B, fold activation was expressed as the β-MHC promoter activities (luc/CAT) in ET-1- or LIF-stimulated myocytes relative to those in saline-stimulated cells. In both A and B, data are presented as the mean ± S.E. of at least three independent experiments. □, wild type; □, mutant M-CAT; □, mutant GATA.
GATA-5

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**Fig. 4.** Analysis of interactions between the \( \beta \)-MHC GATA site and \textit{in vitro}-translated-GATA-5. EMSA studies in which unprogrammed lysate (lane 1) or \textit{in vitro}-translated GATA-5 (lanes 2–6) were probed with a radiolabeled oligonucleotide containing the \( \beta \)-MHC GATA site. Unlabeled competitor DNAs were present at a 100-fold molar excess as indicated: wild-type \( \beta \)-MHC GATA (\( \beta \) GATA) in lane 3; CEF-1 (a previously established GATA-5 binding site in the cardiac TnT promoter) in lane 4; \( \beta \)-MHC GATA with a mutation that ablates the LIF-responsive transcription (mut \( \beta \) GATA) in lane 5; and nonspecific oligonucleotide (NS; see “Experimental Procedures”) in lane 6. The arrow indicates the complex corresponding to the GATA-specific interaction between the \( \beta \)-MHC GATA site and GATA-5.

with a previous report (53), a 2936-bp \( \alpha \)-MHC promoter was not transactivated by GATA-4, -5, and -6 (<3-fold). We showed that GATA-4, -5, and -6, to a comparable degree (>10-fold), transactivated the smooth muscle myosin heavy chain promoter, which contains two GATA motifs. Thus, among members of the cardiac GATA transcription factor subfamily (GATA-4, -5, and -6), GATA-5 is the sole potent activator of the \( \beta \)-MHC promoter. In addition, this marked activation occurs in the \( \beta \)-MHC promoter but not in the \( \alpha \)-MHC promoter, compatible with LIF responsiveness in cardiac myocytes.

Next we addressed whether GATA-5 stimulation of the \( \beta \)-MHC promoter activity occurred in a sequence-specific manner. Point mutations were introduced into the GATA site of the \(-333/+34\)-bp \( \beta \)-MHC promoter to ablate LIF responsiveness (Fig. 2B) as above. The resulting promoter construct (\(-333\)GATA-34-MHCICluc) was co-transfected with an expression plasmid, pcDNA-GATA-5, and subsequently assayed for the relative luciferase activity. As seen in Fig. 3B, transactivation of the \( \beta \)-MHC promoter was reduced by the GATA site mutation to levels only slightly greater than those exhibited by the \( \beta \)-actin promoter. These findings demonstrate that the transactivation of the \( \beta \)-MHC promoter by GATA-5 is dependent on an intact GATA sequence.

**GATA-5 Strongly Binds to the \( \beta \)-MHC GATA Element—**To determine whether the GATA motif in the \( \beta \)-MHC promoter can interact with GATA-5, EMSAs were performed. \textit{In vitro}-translated GATA-5 was probed with a radiolabeled oligonucleotide containing the \( \beta \)-MHC GATA site (Fig. 4, lanes 2–6) in the presence or absence of competitor DNAs. Competition EMSAs revealed that a retarded band represented specific binding (Fig. 4, lane 2), as evidenced by the fact that it was competed by a 100-fold molar excess of unlabeled \( \beta \)-MHC GATA oligonucleotide (Fig. 4, lane 3). The retarded band represents an interaction of the probe with GATA-5, because it was absent in unprogrammed lysate (Fig. 4, lane 1). The retarded band was also competed by an unlabeled CEF-1 oligonucleotide (Fig. 4, lane 4), which contains the GATA motif in the cTnC promoter (48) previously demonstrated to be a binding site of GATA-5. In contrast to the wild-type \( \beta \)-MHC GATA site, the gel shift could not be competed by an excess of an oligonucleotide containing the \( \beta \)-MHC GATA site into which point mutations (Fig. 2B) that ablate LIF responsiveness had been introduced (Fig. 4, lane 5) or by the same amount of a nonspecific oligonucleotide (Fig. 4, lane 6), confirming the sequence-specific nature of the interaction. These findings demonstrate that GATA-5 can bind the \( \beta \)-MHC GATA site in a sequence-specific manner.

Although cTnC promoter has been shown to be efficiently transactivated by both GATA-4 and -5 (44, 50), the present study demonstrated that the \( \beta \)-MHC promoter was activated by GATA-5 but not by GATA-4. We have investigated whether this difference in the transactivation intensity is attributable to the ability of cTnC and \( \beta \)-MHC GATA sites to bind GATA-5 relative to GATA-4. \textit{In vitro}-translated GATA-4 or GATA-5 was probed with a radiolabeled \( \beta \)-MHC GATA oligonucleotide (Fig. 5A, lanes 3–6). Although a retarded band showing the interaction of the \( \beta \)-MHC GATA site with GATA-5 was strong in its intensity (Fig 5A, lane 5), a band showing the interaction with GATA-4 was very weak (Fig. 5A, lane 3). \textit{In vitro}-translated GATA-4 or GATA-5 derived from the same lysates with those used for the \( \beta \)-MHC GATA site was also probed with CEF-1 oligonucleotide containing the GATA motif in the cTnC promoter (Fig. 5B, lanes 3–6). In contrast to the \( \beta \)-MHC GATA site, the intensity of the band showing the interaction of the CEF-1 with GATA-5 (Fig. 5B, lane 5) was similar to that showing the interaction with GATA-4 (Fig. 5B, lane 3). Thus, the ability of GATA elements to bind GATA-5 relative to GATA-4 differs between \( \beta \)-MHC and cTnC promoters.

**LIF Induces the Expression of GATA-5 in Neonatal Cardiac Myocytes—**To determine whether LIF modulates the \( \beta \)-MHC GATA binding activity in cardiac myocytes, EMSAs were performed with nuclear extracts from saline- and LIF-stimulated neonatal cardiac myocytes. Nuclear extracts were probed with a radiolabeled \( \beta \)-MHC GATA oligonucleotide in the presence or absence of competitor oligonucleotides (Fig. 6, lanes 1–5). Competition EMSAs revealed that one retarded band (Fig. 6, lanes 1 and 2, arrow) represented GATA sequence-specific binding, as evidenced by the fact that it was competed by an unlabeled \( \beta \)-MHC GATA oligonucleotide (Fig. 6, lane 3) or by an oligonucleotide containing a previously demonstrated GATA site in the cTnC promoter (CEF-1; Fig. 6, lane 4), but not by an excess of the \( \beta \)-MHC GATA site containing point mutations that ablate LIF responsiveness (Fig. 6, lane 5) or by a nonspecific oligonucleotide (data not shown). Notably, the activity of the specific band was increased in nuclear extracts from LIF-stimulated myocytes (Fig. 6, lane 2) compared with those from saline-stimulated cells (Fig. 6, lane 1). This experiment was repeated three times using three independent preparations of cells and found to be reproducible. Thus, LIF up-regulated the \( \beta \)-MHC GATA binding activity in nuclear extracts from cardiac myocytes.

To investigate whether the up-regulated \( \beta \)-MHC GATA binding activity represents increased GATA-5 transcripts in cardiac

\[ ^{2} \text{H. Wada, K. Hasegawa, T. Kakita, S. Kaburagi, T. Morimoto, and S. Sasayama, unpublished data.} \]
myocytes, we examined GATA-5 mRNA levels in saline- and LIF-stimulated cells. Previous studies demonstrated that the GATA-5 expression in the heart is restricted to the early embryonic stage and not detectable in the late embryo or in the adult by Northern blots. Using highly sensitive reverse transcriptase-PCR, a faint band indicating GATA-5 was detectable in saline-stimulated neonatal rat cardiac myocytes (Fig. 7). Notably, the band intensity markedly increased in LIF-stimulated cardiac myocytes. We confirmed by sequencing that this band represents a specific PCR product derived from GATA-5 cDNA. In contrast, the intensity of the band indicating GAPDH was almost comparable between saline- and LIF-stimulated cardiac myocytes. We repeated these experiments with three independent preparations of cardiac myocytes. With the use of a previously described semiquantitative reverse transcriptase-PCR (51), the cumulative results indicated that GATA-5 mRNA relative to GAPDH mRNA was 6.2 ± 0.5-fold higher in the LIF-stimulated cardiac myocytes than in the saline-stimulated cells.

**DISCUSSION**

Cardiac myocyte hypertrophy is a central feature of all types of cardiac muscle disease and is an interesting example of the response of a terminally differentiated cell type to growth stimulation. Current insights into the mechanisms controlling cardiomyocyte hypertrophy have been obtained primarily from a cell culture model, in which growth factors signaling through G-protein-coupled receptors induce hypertrophy (9–16). Growing evidence suggests that gp130 activation is also coupled to myocardial cell hypertrophy (17, 18, 27). Using neonatal cardiac myocytes in culture, the present study demonstrates that mutation of the GATA motif in the β-MHC promoter totally abolished LIF-responsive transcription. Among members of the cardiac GATA transcription factor subfamily, GATA-5 alone was able to potently transactivate the β-MHC promoter. This transactivation was dependent on sequence-specific binding of GATA-5 to the β-MHC GATA element. Last, LIF stimulation markedly increased levels of GATA-5 transcripts in cardiac myocytes. These findings demonstrate that GATA-5 is important in the LIF-mediated up-regulation of β-MHC expression in cardiac myocytes and may represent the mechanism underlying the cardiac hypertrophy induced by the gp130 ligand family.

**Role of M-CAT and GATA Elements in β-MHC Transcription**—Once the hypertrophy signal is transduced from the membrane to the nucleus, a fundamental reprogramming occurs within cardiac myocytes that results in the reexpression of genes encoding fetal protein isoforms. Genes such as skeletal α-actin, β-MHC, and atrial natriuretic factor become highly expressed within ventricular myocytes (4–8). Studies focused on elucidating the transcriptional regulation of these genes have identified a group of cis-acting regulatory elements that might mediate the nuclear response to hypertrophic stimuli.
Analysis of the β-MHC promoter has demonstrated that the M-CAT element, a binding site of the transcription enhancer factor-1 family, may play a role in both basal and hypertrophic-responsive transcription. For example, this element mediates both α1-adrenergic and β-protein kinase C-stimulated β-MHC transcription (11, 12). M-CAT elements have also been implicated in α1-adrenergic-stimulated expression of the skeletal α-actin and β-type natriuretic peptide promoters (13, 57). In addition, the M-CAT motif is present in the promoters of several striated muscle-specific genes, where it functions to positively regulate basal transcription (57–63). Our observation that simultaneous disruption of both M-CAT elements in the setting of a 333-bp β-MHC promoter decreases transcriptional activity is consistent with previous reports demonstrating that M-CAT elements play an important role in basal β-MHC transcription. Unexpectedly, however, simultaneous mutations in both M-CAT elements, adequate to abrogate binding of nuclear proteins and to destroy enhancer function, had no effect on the ET-1 or LIF responsiveness of the 333-bp rat β-MHC promoter. Although this finding does not rule out the possibility that M-CAT elements contribute to ET-1 or LIF responsiveness, it demonstrates that other elements within these sequences suffice to mediate this transcriptional response.

Sequences ~333/+34 of the rat β-MHC promoter also contain a GATA element (36). GATA elements have been shown to be important for cardiac-specific transcription in many cardiac genes, including α-MHC, β-type natriuretic peptide, myosin light chain 1/2 and cTnC (50, 53–55). We show here that mutation of the GATA element in the 333-bp β-MHC promoter totally abolished LIF-responsive transcription without changing basal transcriptional activity. Thus, this GATA element plays a critical role in LIF-responsive β-MHC transcription. In contrast, ET-1 responsiveness was unaffected by the GATA mutation. These findings suggest that LIF and ET-1 activate β-MHC gene transcription through distinct cis-acting elements. Previous work has shown that G-protein and gp130 pathways elicit morphologically distinct forms of myocardial cell hypertrophy (18). Thus, it appears that these two stimuli induce distinct hypertrophic processes through different pathways. The elucidation of the differences in these signaling pathways and the pathophysiological significance of these two forms of hypertrophy would be of particular interest.

GATA Factors Mediate gp130 Signaling in Cardiac Myocytes—To date, six related zinc finger-containing proteins have been described, which recognize and bind the GATA motif (44, 45, 64). The proteins fall into two subgroups: those containing GATA-1, -2, and -3, and those with GATA-4, -5, and -6. The subgroups are defined by both sequence homology and expression pattern, with GATA-1, -2, and -3 predominating in blood and ectodermal derivatives and GATA-4, -5, and -6 in heart and endodermal derivatives. Interestingly, the genes encoding GATA-4 and -6 are expressed in the heart throughout embryonic and postnatal development, whereas the murine GATA-5 gene is normally expressed in a temporally and spatially restricted pattern within the embryonic heart (44, 45). These findings raise the possibility that GATA-4, -5, and -6 play differential roles during LIF-induced hypertrophy. The present study demonstrated that neither GATA-4 nor -6 significantly activated the LIF-responsive 333-bp β-MHC promoter. In contrast, GATA-5 markedly stimulated this promoter. This activation required an intact GATA element, suggesting a direct effect. Consistent with this model, GATA-5 bound the β-MHC GATA element in a sequence-specific manner. Importantly, LIF stimulation increased β-MHC GATA binding activity in cardiac nuclear extracts. Although GATA-5-specific antiserum for supershift experiments is not available at present, a complex formed with the β-MHC GATA element is clearly GATA sequence specific. In addition, LIF stimulation increased expression of GATA-5 in neonatal cardiac myocytes. Taken together, these findings demonstrate that GATA factors are involved in LIF-responsive β-MHC transcription and that GATA-5 is the factor that is primarily involved.

The signal transduction pathways by which members of the gp130 ligand family activate target genes have been well studied in several cell types (28–31). Typically, the phosphorylated STAT proteins dimerize, translocate into the nucleus, and bind to the promoter of target genes. The DNA binding targets of STATs include the interferon-γ activation site-like elements (TTC/ANNG/TTA) and the interferon-γ-stimulated response elements (AGTTCCNNTTTCN/CT) (31). Our data demonstrate that GATA-5 markedly activated the β-MHC promoter through specific binding to the GATA element and that β-MHC GATA binding activity in cardiac myocytes is induced by LIF stimulation. At present, the molecular events that may link the Janus kinase/STAT pathway to this augmentation are unknown. LIF activates STATs within 15 min after LIF stimulation, whereas the induction of the β-MHC expression occurs much later (48 h after LIF stimulation). Therefore, it is unlikely that STATs directly associate with GATA factors in the activation of the β-MHC promoter. We demonstrate here that LIF stimulation increased GATA-5 transcripts in neonatal cardiac myocytes. Thus, one model is that the Janus kinase/STAT pathway is linked to the regulation of GATA-5 gene expression. Another model is that LIF-induced intracellular signaling cascades activate GATA factors or GATA co-activators post-translationally by phosphorylation or other mechanisms. In any event, because gp130 activation is one component of the hemodynamic overload stimulus (65), additional studies to delineate the precise mechanisms by which gp130 activation induces β-MHC transcription are likely to provide significant insight into the pathways that mediate hemodynamic overload-induced hypertrophy in vivo.

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