Regulation of Cardiomyocyte Glut4 Expression by ZAC1*

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The transcription factor ZAC1 is expressed in a variety of tissues including the developing heart, but its physiological role is unclear. We examined the role of ZAC1 in regulating expression of the insulin-responsive glucose transporter GLUT4 and whether ZAC1 expression is altered in cardiomyocyte hypertrophy. We demonstrated expression of Zac1 mRNA and protein in rat cardiomyocytes by PCR and Western blotting, respectively. Using a combination of chromatin immunoprecipitation and luciferase assays, we showed that ZAC1 regulates Glut4 expression via a specific binding site in the Glut4 promoter. Overexpression of ZAC1 increased Glut4 mRNA and protein expression and resulted in increased glucose uptake in cardiomyocytes as determined by a fluorescent analog uptake assay. Induction of hypertrophy by phenylephrine or isoproterenol resulted in increased Zac1 expression. We identified a novel putative promoter in the Zac1 gene and demonstrated increased binding of MEF2 to this promoter in response to hypertrophic stimulation. MEF2 regulated transactivation of the Zac1 promoter and Zac1 protein expression. This work identifies ZAC1 as a novel and previously unknown regulator of cardiomyocyte Glut4 expression and glucose uptake. Our results also implicate MEF2 as a regulator of Zac1 expression in response to induction of hypertrophy.

The heart is a metabolic omnivore, using various substrates as fuel. Fatty acids and glucose are the primary substrates of choice, but reliance on one or the other changes depending on age. During development, the heart primarily uses glucose but shifts largely to fatty acids soon after birth (1). Fuel choice can also be altered in disease, e.g. increased reliance on fatty acids in diabetes or on glucose in pathological cardiac hypertrophy (1). The transcriptional mechanisms underlying fuel selection, either in physiological or pathological hypertrophy, are incompletely understood.

To accommodate this metabolic flexibility, cardiac fuel uptake and anabolism are tightly regulated by transcriptional, translational, and post-translational mechanisms. Only recently has a detailed understanding of transcriptional metabolic control begun to emerge. Studies into the roles of transcription factors such as the peroxisome proliferator-activated receptors (PPARs)2 and nuclear respiratory factors or co-regulators such as PGC-1α (PPARγ co-activator 1α) have improved our understanding of how fuel is selected or metabolized in the heart, as well as how disease may arise when the activity of these factors is altered (1–2). New classes of drugs have been developed that specifically regulate some of these transcription factors, e.g. thiazolidinediones, which regulate PPARγ and have shown efficacy in treating diabetes by acting as insulin sensitizers (3). Altering fuel selection in the heart may improve patient outcomes, e.g. increasing cardiac reliance on glucose following infarction may prove beneficial (1). To seriously consider metabolic therapies as viable treatments, it is critical that the mechanisms underlying fuel selection and energy metabolism are fully elucidated.

The transcription factor, transcriptional co-activator, and repressor ZAC1 regulates cell cycling and apoptosis of transformed cells and is highly expressed in the developing heart, but its physiological role in cardiomyocytes is unknown (4–5). The ZAC1 genomic locus in humans is associated with the development of transient neonatal diabetes mellitus and with type II diabetes in African Americans (6–7). ZAC1 also regulates the expression of PACAP1-R, a potent insulin secretagogue (8). These findings indicate that ZAC1 plays an important role in insulin signaling and/or glucose metabolism.

We report here that ZAC1 independently regulates cardiomyocyte gene expression of the insulin-responsive glucose transporter Glut4. Zac1 expression increases during cardiomyocyte hypertrophy, via a mechanism that appears to involve transactivation of a novel putative promoter containing a functional MEF2-binding site. Together, these findings support a role for ZAC1 as a potential regulator of glucose metabolism downstream of MEF2 signaling and may contribute to our understanding of how glucose transport is regulated during physiological forms of hypertrophy.

EXPERIMENTAL PROCEDURES

Cell Culture and Cardiomyocyte Isolation—COS7 cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% L-glutamine. Neonatal rat ventricular cardiomyocytes (NRC) were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% L-glutamine.

The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; Ad, adenovirus; eGFP, enhanced green fluorescent protein; NRC, neonatal rat cardiomyocytes; luc, luciferase; CMV, cytomegalovirus; HA, hemagglutinin; PE, phenylephrine; ISO, isoproterenol; ChIP, chromatin immunoprecipitation; RT, reverse transcription; HDAC5S/A, histone deacetylase 5 double serine-to-alanine mutant.

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2 The on-line version of this article (available at http://www.jbcd.org) contains supplemental Table S1.
were cultured from day-old pups by differential plating as described (9). Animals were treated in accordance with the guidelines of the Canadian Council on Animal Care, the Guide for the Care and Use of Laboratory Animals (49) and the University of Manitoba Animal Protocol Management and Review Committee.

Cloning of Zac1 Alternate Promoter—A 5.4-kb sequence immediately upstream of the ATG translational start codon and encompassing the first 162 nucleotides of exon VII of the mouse Zac1 gene (NCBI sequence database sequence NC_000076.5) was amplified from bacterial artificial chromosome RP1C13-82B19 (CHORI-BacPac) by high fidelity Pfx DNA polymerase (Invitrogen) (primer sequences listed in supplemental Table S1). The gel-purified PCR product was sequentially subcloned into pCR2.1-TOPO (Invitrogen) and pcDNA3.1 was added as required to normalize total DNA transfection. Luciferase activity was measured by fluorometric assay (12).

Following amplification in 293A cells, adenovirus titer was followed by packaging in 293A cells with co-transfected pJM17. mZAC1b coding region from pSG5-HA-mZac1b into pCA3, CMV promoter was generated by cloning the HA-eramerase (Invitrogen) (primer sequences listed in supplemental Table S1). The gel-purified PCR product was sequentially subcloned into pCR2.1-TOPO (Invitrogen) and pGL3 basic (Promega) to generate luciferase reporter mZac1pr-pGL3. Three point mutations (CTAAAAATA to CTAAGGATC) were generated in the mZac1pr-pGL3 putative MEF2-binding site using the QuikChange XL site-directed mutagenesis kit (Stratagene) to generate mutMEF2-mZac1pr-pGL3.

Luciferase Reporter Gene Assays—COS7 cells were seeded on six-well plates 24 h prior to transfection. Cells were transfected (Lipofectamine 2000, Invitrogen) with reporter plasmid (human GLUT4 promoter luciferase reporter hG4-luc (10); T3GN12G4-hG4-luc bearing a mutated ZAC1-binding site in hG4-luc; mZac1pr-pGL3; or mutMEF2-mZac1pr-pGL3), pCMV-lacZ for transfection normalization, and expression vectors as required (pcDNA1-mycMEF2C, pcDNA1-mycMEF2A, pcDNA1-mycMEF2D, pcDNA3.1-FLAG-HDAC5S, pSG5-HA-mZac1b (mZAC1b is a functionally identical splice variant of ZAC1 containing 11 additional amino acids (11)), and pSG4-HA-GRIP1). pcDNA3.1 was added as required to normalize total DNA transfected. Luciferase activity was measured on a T20O/20 luminometer (Turner BioSystems). β-Galactosidase activity was measured by fluorometric assay (12).

Generation of Adenovirus—An adenovirus encoding HA-tagged mZac1b under control of a constitutive cytomegalovirus (CMV) promoter was generated by cloning the HA-mZAC1b coding region from pSG5-HA-mZac1b into pCA3, followed by packaging in 293A cells with co-transfected pJM17. Following amplification in 293A cells, adenovirus titer was determined by Adeno-X kit (Clontech).

Treatment of Cardiomyocytes—Isolated NRC were grown for 24–48 h and then infected with adenoviruses encoding CMV-β-galactosidase (Ad-β-gal) or CMV-FLAG-HDAC5S/A (Ad-HDAC5S/A) as described previously (9) or CMV-myc-MEF2C (Ad-MEF2C) using a multiplicity of infection of 100. In other experiments, cardiomyocytes were infected with adenoviruses encoding CMV-eGFP (Ad-eGFP) or CMV-HA-mZac1b (Ad-mZac1b). Total RNA or protein was isolated following a 48-h incubation in serum-free medium after infection. Alternatively, cardiomyocytes were treated with phenylephrine (PE) or isoproterenol (ISO) at indicated doses for 48 or 72 h.

Western Blot Analysis of Proteins—Protein lysates were isolated as described (9) and assayed using a Coomassie stain-based kit (Pierce) and then resolved on 12% SDS-polyacrylamide gels and wet-transferred onto polyvinylidene difluoride (Bio-Rad). Blots were immuno-labeled with anti-ZAC1 (Santa Cruz Biotechnology) or anti-GLUT4 (Abcam) primary antibodies and goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (Jackson ImmunoResearch Laboratories), detected using Western blotting luminol reagent (Santa Cruz Biotechnology) and visualized on a Fluor-S-Max Multimagmer (Bio-Rad). Anti-α-tubulin antibodies (Developmental Studies Hybridoma Bank, University of Iowa) were used for gel loading controls.

Analysis of RNA—Total RNA was isolated using a GenElute mammalian total RNA miniprep kit (Sigma). 1 µg total RNA was reverse transcribed using the Superscript III 1st Strand Synthesis System for RT-PCR (Invitrogen). Using appropriate primers (supplemental Table S1), optimal PCR cycle number (22 or 28 for glyceraldehyde-3-phosphate dehydrogenase and Zac1, respectively) was determined by standard curves to ensure that bands were obtained within the linear range of amplification. PCR products were resolved on 2% agarose gels and quantified by GelDoc (Bio-Rad). Alternatively, mRNA abundance was determined by quantitative RT-PCR using the 2−ΔΔCT method on an iQ5 real-time PCR system (Bio-Rad).

Chromatin Immunoprecipitation (ChiP)—ChiP was performed on isolated NRC as described (9). Immunoprecipitation was carried out using anti-MEF2C (Cell Signaling Technology), anti-ZAC1 (M-300; Santa Cruz Biotechnology) or nonspecific IgG antibodies (negative control), with pull down by protein A-agarose beads. PCR was carried out using appropriate primers (supplemental Table S1). NRC genomic DNA was used as a positive control (input).

Fluorescent Imaging—NRC were plated on coverslips 24 h prior to treatment. Cells were fixed in 4% formalin and permeabilized with 0.1% Triton X-100. After blocking (2% milk powder in phosphate-buffered saline plus 0.1% Tween 20), cells were incubated with anti-α-actinin (Sigma) or anti-GLUT4 antibodies (Abcam), followed by Alexa Fluor 488-conjugated goat-anti-mouse or Alexa Fluor 594-conjugated goat-anti-rabbit secondary antibody (Molecular Probes). Cells were co-labeled with 500 µM 4′,6-diamidino-2-phenylindole and mounted with Prolong Gold mounting medium (Molecular Probes) prior to imaging (Zeiss Axiophot 2).

Glucose Uptake—Isolated NRC were plated on gelatin-coated coverslips. Cells were allowed to attach for 24 h and then starved for 24 h. Cells were infected with Ad-β-gal or Ad-mZac1b for 24 h. Cells were washed twice with Krebs buffer without glucose and then incubated with 0.3 mM fluorescent glucose analog 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)aminol]-2-deoxy-d-glucose (2-NBDG; Molecular Probes) for 0, 15, or 30 min. Cells were then washed in Krebs buffer for 10 min and imaged for glucose analog accumulation.

Statistical Analysis—Statistical significance of results was determined by one-way analysis of variance with Student Newman-Keuls post hoc test, or by Student’s t test as appropriate, with p < 0.05 considered significant. Results represent means ± S.E.

RESULTS

Cardiac Zac1 Expression—High Zac1 expression has been reported in the developing heart and somites, although expression specifically in cardiomyocytes has not been described
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FIGURE 1. Transactivation of the GLUT4 promoter by ZAC1. A, Western blot of isolated neonatal rat cardiomyocyte protein lysates (in triplicate), using anti-ZAC1 antibody or α-tubulin loading control (molecular mass indicated in kilodaltons). B, PCR amplification of the Zac1 coding region from total NRC cDNA, generating a single 90-bp predicted amplicon (100 bp standard indicated at left); numbers at top indicate amplification cycles. C, upper panel, partial sequence of the human GLUT4 promoter, with the putative ZAC1 binding site underlined. Numbers denote position relative to the transcription start site. Lower panel, sequence alignment of the rat, mouse, and human putative ZAC1-binding site (underlined). Asterisks denote identical nucleotides across all three species; boldface font indicates critical G4 nucleotides. D, ChIP of NRC Zac1—Misexpression of the human ZAC1 locus is associated with transient neonatal diabetes mellitus and with type II diabetes in African Americans (6–7, 15). We therefore hypothesized that ZAC1 may regulate genes involved in glucose metabolism such as the insulin-responsive glucose transporter Glut4. We noted a putative ZAC1-binding site (G4N12G4) from −107 to −88 bp relative to the transcription start site in the human GLUT4 gene promoter. This sequence is similar to reported ZAC1-binding sites containing dual G4 repeats separated by even-length spacers (16) and is highly conserved in the rat and mouse Glut4 gene promoters (Fig. 1C). We employed primers spanning the putative ZAC1-binding site of the rat Glut4 promoter to perform ChIP, confirming the presence of endogenous Zac1 bound to this region in cardiomyocytes (Fig. 1D).

ZAC1 transactivation of the GLUT4 promoter was examined by luciferase assay. ZAC1 strongly activated the GLUT4 promoter, and this activation was attenuated by mutation of the G4N12G4 motif (Fig. 1E). We confirmed activation of this promoter by MEF2C, as reported by others (Fig. 1F) (10). Both Zac1 and MEF2C bind to and are co-activated by the transcriptional co-activator GRIP1 (11, 17); therefore, we tested the effect of GRIP1 on activation of the GLUT4 promoter. GRIP1 alone had a negligible effect but augmented reporter activity induced by MEF2C. The effectiveness of ZAC1 alone in transactivating the reporter was similar to MEF2C and GRIP1 combined. Reporter activity was highest in the presence of all three factors and was significantly greater than GRIP1 + MEF2C or GRIP1 + ZAC1, demonstrating an additive effect of these transcriptional regulators (Fig. 1F).

Regulation of Glut4 Expression by Zac1—Because Zac1 was capable of regulating the human GLUT4 promoter, we assessed the effect of Zac1 on Glut4 expression in intact rat cardiomyocytes. Glut4 mRNA was strongly up-regulated by Zac1 (Fig. 2A). Protein expression was moderately but significantly up-regulated by Zac1 (Fig. 2, B and C), a result confirmed by immunofluorescence (Fig. 2D). This increase in Zac1-medi-

cDNA, generating a single 90-bp predicted amplicon (100 bp standard indicated at left); numbers at top indicate amplification cycles. C, upper panel, partial sequence of the human GLUT4 promoter, with the putative ZAC1 binding site underlined. Numbers denote position relative to the transcription start site. Lower panel, sequence alignment of the rat, mouse, and human putative ZAC1-binding site (underlined). Asterisks denote identical nucleotides across all three species; boldface font indicates critical G4 nucleotides. D, ChIP of NRC Zac1.
Zac1 genes. However, we did identify a putative MEF2-binding site between 2400 and −1400 bp relative to the first coding exon in each species (exons VII, VI, and VIII in mouse, rat, and human respectively; Fig. 4, A–B). A CCAAT box was found within 40 bases of the start of each of these exons, and numerous putative binding sites for other transcription factors are also found in this region. A database search revealed a large number of mRNAs and expressed sequence tags initiating in this region (Table 1), suggesting the existence of a proximal alternative transcription start site.

To test this possibility, we cloned a 5.4-kb span of mouse genomic DNA 5′ to the ATG translation start site (+162) in exon VII of the mouse Zac1 gene, which included the exon VII 5′ boundary at +1, the CCAAT box at −42, and a putative MEF2 site at −2372/−2380. Transient transfection of COS7 cells with a luciferase reporter bearing this putative promoter region resulted in a dose-dependent increase in reporter gene expression (Fig. 4C). In contrast, the empty reporter vector showed no change in luciferase activity, suggesting that the cloned region behaves as a promoter.

Mice, rats, and humans possess a putative MEF2-binding site within this region (Fig. 4D). We therefore confirmed that MEF2C is able to bind to a site within this promoter region in rat cardiomyocytes using ChIP (Fig. 4E). Induction of cardiomyocyte hypertrophy by PE or ISO treatment resulted in an increase in MEF2C binding to this site (Fig. 4F).

To examine the mechanism of hypertrophic Zac1 induction, RT–PCR was repeated using primers specific for exon I in the Zac1 5′ untranslated region (Fig. 4G). No induction of this region was observed in hypertrophic cardiomyocytes, in contrast to the significant amplification observed using primers located in the protein coding region, suggesting that induced transcripts excluded upstream sequences and supporting the idea that Zac1 is expressed from the novel putative promoter.

**MEF2 Regulates Zac1 Expression**—Luciferase assays demonstrated that MEF2C (Fig. 5A), MEF2A, and MEF2D (data not shown) strongly transactivated the putative Zac1 promoter. Transactivation was attenuated by a double serine-to-alanine mutant of histone deacetylase 5 (HDAC5S/A), which showed no change in luciferase activity, supporting the idea that Zac1 is expressed from the novel putative promoter.

**Identification of a MEF2-binding Site in a Putative Novel Zac1 Promoter**—The MEF2 transcription factors are key regulators of hypertrophic gene expression in striated muscle (22–23); thus, MEF2 may regulate Zac1 expression. In silico analysis revealed no MEF2 binding sites within 3000 bp of the canonical transcription start sites upstream of the rat, mouse, or human

Zac1 genes. However, we did identify a putative MEF2-binding site between 2400 and −1400 bp relative to the first coding exon in each species (exons VII, VI, and VIII in mouse, rat, and human respectively; Fig. 4, A–B). A CCAAT box was found within 40 bases of the start of each of these exons, and numerous putative binding sites for other transcription factors are also found in this region. A database search revealed a large number of mRNAs and expressed sequence tags initiating in this region (Table 1), suggesting the existence of a proximal alternative transcription start site.

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DISCUSSION

The transcriptional networks regulating cardiac energy metabolism are gradually being elucidated. In this study, we report that ZAC1 directly regulates expression of the insulin-responsive glucose transporter Glut4 via a highly conserved G4N12G4 motif upstream of the transcription start site. ZAC1 overexpression increases expression of Glut4, resulting in increased glucose uptake by cardiomyocytes. These results are particularly striking in light of the association of ZAC1 defects with diabetes and the recent report that ZAC1 regulates expression of PPARγ, the therapeutic target of the thiazolidinedione class of insulin sensitizers for diabetes treatment (6–7, 25). We also demonstrate that ZAC1 expression is regulated by MEF2, which may explain our observation that Zac1 expression is increased during cardiomyocyte hypertrophy.

Zac1 Is a Novel Regulator of Glut4 Expression—Our results agree with reports that Glut4 expression is regulated by MEF2 (26). We also found that ZAC1 additively regulates GLUT4 transactivation with MEF2 and the steroid receptor co-activator GRIP1, likely via a binding site in the GLUT4 promoter in close proximity to the previously identified MEF2 binding site at ∼470 bp (26). Because both MEF2C and ZAC1 are co-activated by GRIP1, our data suggest that these three factors work together as part of a common transcriptional complex (Fig. 6) (11, 17). We previously showed that MEF2 regulates expression of the transcriptional co-activator PGC-1α, while others have shown that PGC-1α represses expression of Glut4 in vivo but augments expression of Glut4 in vitro (9, 27–28). This further underscores the complexity of transcriptional pathway interactions that regulate net Glut4 expression.

MEF2 Regulates Zac1 Expression—Although MEF2 is insufficient to drive cardiomyocyte hypertrophy unless expressed at very high levels, it nonetheless plays a central role in this process, including regulating hypertrophic gene expression and promoting sarcomere elongation (29–31). MEF2 transactivation activity is increased in cardiac hypertrophy or exercised skeletal muscle, while class II histone deacetylases such as HDAC5, which repress MEF2 activity, are considered antihypertrophic (22–23, 32–34). In the present study, MEF2 transactivated the putative Zac1 promoter and increased ZAC1 protein expression, suggesting a mechanism for our observation that Zac1 expression increases in hypertrophy. Although HDAC5S/A blocked MEF2-mediated transactivation of the Zac1 promoter, inhibition of ZAC1 protein expression did not reach statistical significance; thus, a definitive role for HDAC5 in regulation of ZAC1 expression cannot yet be demonstrated.
However, our data is consistent with a previous report that the HDAC inhibitor trichostatin A relieves silencing of LOT1/Zac1 expression (35).

**Glut4 Expression in Hypertrophy**—Our data demonstrate that MEF2 regulates Zac1 expression and that ZAC1 regulates Glut4 expression. It is thus intriguing to speculate that ZAC1 may provide an accessory regulatory mechanism for Glut4 expression in hypertrophy downstream of or in parallel to MEF2 (Fig. 6). However, we did not observe an increase in Glut4 expression following PE or ISO treatment of cardiomyocytes (data not shown) despite induction of ZAC1 expression. Although this observation might argue against the involvement of ZAC1 in hypertrophy, it must be noted that our results parallel observations made for MEF2. MEF2 is a potent inducer of Glut4 expression and is also induced by PE (22, 26, 36). Yet, in pathological cardiac hypertrophy, MEF2...
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### TABLE 1

| GenBank™ accession no. | Species | Initiation site* |
|------------------------|---------|------------------|
| AF244741               | Mouse   | +4               |
| AA919394               | Mouse   | +5               |
| X95504                 | Mouse   | +75              |
| BX336611               | Human   | −75              |
| BP344615               | Human   | +20              |
| BP344689               | Human   | +20              |
| DA717483               | Human   | +31              |
| DA013221               | Human   | +45              |
| CN312868               | Human   | +66              |
| DR006097               | Human   | +68              |
| CN312866               | Human   | +109             |
| CN312867               | Human   | +129             |
| AW06609                | Human   | +175             |
| BC074814               | Human   | +276             |

* Numbers are relative to the 5’ boundary of exon VII in mice (ATG at +162) or exon VIII in humans (ATG at +325).

activity increases while GLUT4 expression decreases (22, 37). In contrast, GLUT4 expression increases in physiological hypertrophy (exercise), which is also associated with increased MEF2 activity (18–19). In agreement with these reports, we demonstrated increased Zac1 and Glut4 mRNA expression in hypertrophic neonatal cardiomyocytes compared with nonhypertrophic adult cells (Fig. 3G). The PE/ISO cardiomyocyte hypertrophy model employed in this study thus likely fails to recapitulate physiological hypertrophy. Further study is required to confirm the role of ZAC1 in mediating Glut4 expression during physiological hypertrophy in vivo, e.g. in response to exercise.

Identification of a Novel Zac1 Promoter—Intriguingly, MEF2-mediated regulation appears to occur via a novel noncanonical promoter element. The genomic region immediately upstream of the first coding exon (exon VII) of the mouse Zac1 gene exhibits several features, suggesting that it contains a novel promoter. First, a putative CCAAT box is conserved at −162 (Table 1). Second, multiple mRNAs and expressed sequence tags initiate in this region, lacking the 5′ UTR found in transcripts originating from the canonical Zac1 promoter (Table 1). Third, this region contains a large number of putative transcription factor-binding sites, including sites for myogenic transcription factors such as MyoD and myogenin (E-boxes) or nuclear factor of activated T cells. It is noteworthy that generation of an mRNA transcript from this site would produce an identical open reading frame to Zac1, depending on cell context. Zac1 expression is likely to be regulated by other as yet unidentified signaling pathways. These pathways may link upstream pro-hypertrophic signals with downstream increases in glucose metabolism in situations such as physiological hypertrophy. AMPK, AMP-regulated protein kinase; GEF, Glut4 enhancer factor.
increase in reporter activity is observed, indicating basal promoter activity. ChIP demonstrates MEF2 binding to this region, complementing luciferase assays demonstrating responsiveness of this putative promoter to MEF2 and HDAC5S/A. Most notably, MEF2 binding increases in response to PE or ISO treatment of cardiomyocytes, suggesting the intriguing possibility that this promoter responds to hypertrophic signaling. We also noted that up-regulation of Zac1 mRNA was detected when primers corresponding to the coding region (but not exon I) were used, further supporting the idea that cardiac hypertrophic expression of Zac1 is not mediated by the canonical Zac1 gene promoter. However, to date, we have been unsuccessful in isolating mRNAs originating from this promoter by 5′ rapid amplification of cDNA ends; thus, we cannot discount the possibility that this region may represent a transcriptional enhancer rather than a promoter.

Physiological Significance—Induction of Zac1 expression during cardiomyocyte growth is particularly striking in light of a recent report (39) that ZAC1 acts via an enhancer element to regulate expression of insulin-like growth factor II, a regulator of cardiomyocyte hypertrophy (40 – 43). An intriguing model is that activation of MEF2 and/or inhibition of HDACs downstream of hypertrophic growth cues results in increased expression of Zac1, in turn up-regulating insulin-like growth factor II-mediated hypertrophic signaling pathways that reinforce or augment the effect of MEF2 independently.

It was recently reported that MEF2 and Glut4 enhancer factor cooperate to regulate Glut4 expression (44). The cellular fuel sensor AMP-activated protein kinase directly activates Glut4 enhancer factor and indirectly activates MEF2 via phosphorylation of p38, which in turn phosphorylates and activates MEF2 (44 – 46). In this way, expression of Glut4 is up-regulated when fuel supply is low, permitting increased glucose uptake and generation of ATP. Our results indicate that ZAC1 interacts with this mechanism to impact Glut4 expression.

It is unknown which MEF2-independent pathways may be involved in up-regulation of Zac1 and which other ZAC1 gene targets may exist. Gain-of-function or loss-of-function studies may provide insight into other genes directly regulated by ZAC1. Although both Zac1-null mice and transgenic mice over-expressing the human ZAC1 locus exist, specific effects on cardiac gene expression or glucose metabolism have not been described (39, 47). Should ZAC1 prove to be an important regulator of glucose uptake, striated muscle-specific overexpression of ZAC1 to increase expression of Glut4 may prove useful in treating diabetes because increased Glut4 expression is beneficial in this disease (48). ZAC1 may therefore represent an intriguing novel target for therapeutic manipulation in the future.

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