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Transcriptional Regulation of the Human *GLUT4* Gene Promoter in Diabetic Transgenic Mice*

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We previously reported that 2400 base pairs (bp) of 5′-flanking DNA is sufficient for tissue-specific and hormonal/metabolic regulation of the human *GLUT4* gene in transgenic mice (Liu, M.-L., Olson, A. L., Moye-Rowley, W. S., Buse, J. B., Bell, G. I., and Pessin, J. E. (1992) *J. Biol. Chem.* 267, 11673–11676). To further define the DNA sequences required for *GLUT4* expression, we generated transgenic mice carrying 1975, 1639, 1154, 730, and 412 bp of the *GLUT4* 5′-flank (hG4) fused to the chloramphenicol acetyltransferase (CAT) reporter gene. The 1975-hG4-CAT, 1639-hG4-CAT, and 1154-hG4-CAT constructs were expressed in a tissue-specific manner identical to the endogenous murine *GLUT4* mRNA. Regulation of these reporter gene constructs in insulin-deficient diabetes also paralleled the endogenous gene. In contrast, 730-hG4-CAT was expressed at high levels only in skeletal muscle and at low levels in all of the other tissues examined. Additionally, expression of 412-hG4-CAT was completely unrestricted. Neither the 730-hG4-CAT nor the 412-hG4-CAT reporter genes displayed any insulin-dependent regulation. These data demonstrate that a skeletal muscle-specific DNA element is located within 730 bp of the *GLUT4* 5′-flanking DNA but that 1154 bp is necessary to direct the full extent of tissue-specific and insulin-dependent regulation of the human *GLUT4* gene in transgenic mice.

A fundamental process in cellular metabolism is the uptake and metabolism of glucose. In all mammalian cells, facilitative glucose transport is catalyzed by at least six members of a structurally related family of glucose transporter proteins, termed GLUT1–5 and GLUT7 (For reviews see Refs. 1–3). Each of these glucose transporter isoforms have unique tissue distributions and kinetic properties, which are necessary for particular physiologic functions. For example, GLUT1 is predominantly expressed at the plasma membrane in most cell types and is generally thought to account for the majority of basal state glucose uptake (4–8). In contrast, the primary role of the GLUT4[1] glucose transporter isoform is to mediate an insulin-sensitive glucose uptake in muscle and adipose tissues (4, 9–13). In these cells, the GLUT4 protein is uniquely localized to an intracellular vesicle pool and, upon insulin treatment, is rapidly translocated to the cell surface membrane accounting for the large increase in glucose transport activity (9, 14–16).

Several studies have documented the essential role of GLUT4 in the maintenance of whole body glucose homeostasis. In rodent models of insulin deficiency (fasting or streptozotocin (STZ)-induced diabetes), the expression of GLUT4 protein and mRNA is markedly reduced and accounts for the insulin resistance of glucose transport under these conditions (17–23). Similarly, the expression of GLUT4 is also decreased in cardiac and skeletal muscle, although the insulin resistance associated with these tissues may occur prior to the loss of GLUT4 protein levels (24). In any case, transgenic mice expressing the human *GLUT4* gene specifically in adipose tissue or in both adipose and muscle tissues displayed a marked increase in basal glucose disposal and insulin-sensitive glucose uptake (6–8, 25–28). Furthermore, expression of the human *GLUT4* gene in the genetically diabetic db/db strain of mice also resulted in improved basal glycemic control concomitant with an increase in insulin sensitivity (29). Together, these data indicate that physiologic manipulation of *GLUT4* expression may be a useful strategy to alleviate insulin resistance states associated with several forms of diabetes.

To this end, a detailed molecular understanding of the regulation of GLUT4 gene expression is required. Previously, we have demonstrated that 2400 bp of the *GLUT4* 5′-flanking DNA was sufficient to direct the appropriate tissue-specific and hormonal/metabolic regulation of a CAT reporter gene in transgenic mice (30, 31). In the current study, we have performed a 5′-deletion analysis of the human *GLUT4* promoter in transgenic mice. These data have defined at least one proximal region responsible for skeletal muscle-specific expression and a more distal region responsible for cardiac and adipose tissue expression. In addition, the distal region is required for decreased *GLUT4* transcription in insulin-deficient diabetes.

MATERIALS AND METHODS

Preparation of Transgenic Mice—The cDNA constructs used to generate transgenic mice were derived from the plasmid hGLUT4(2.4)CAT containing 2400 bp of GLUT4 5′-flanking DNA (30). Five separate constructs containing the identical 3′-end (+163) but with sequential deletions from the 5′-end (−1975, −1639, −1154, −730, and −412) were prepared as described in Table I. The DNA fragments were isolated by agarose gel electrophoresis and injected into the pronucleus of fertilized mouse embryos at either the DNX, Inc. Transgenic Animal Facility (Princeton, NJ) or The University of Iowa Transgenic Animal Facility (Iowa City, IA). Transgenic animals carrying the appropriate constructs were identified by slot blot analysis of isolated tail DNA using the 4.6-kilobase *Sac*I-*Hin*DIII fragment of hGLUT4(2.4)CAT as a probe.

STZ-induced Diabetes—Insulin-deficient diabetes was induced by a single intraperitoneal injection of STZ (200 mg/kg, body weight) following an overnight fast as previously described (32). 72 h after injection, tail vein blood samples were assayed for glucose concentration using a glucometer (Lifescan, Inc.; Miltipas, CA). Animals with blood glucose levels greater than 400 mg/dl were considered diabetic. The diabetic animals were either left untreated or treated with 2 units of regular insulin per day for 2 days. The mice were killed 5 days after STZ injection, and the tissues were snap frozen in liquid nitrogen until prepared for analysis.

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[1] The abbreviations used are: GLUT4, adipose/muscle-specific glucose transporter; STZ, streptozotocin; bp, base pair; Pipes, 1,4-piperazinediethanesulfonic acid; CAT, chloramphenicol acetyltransferase.

TABLE I
Summary of constructs used to generate the human GLUT4 transgenic mice

Fragment length	Restriction enzymes	Construct name
4277	*Tth*111I/*Hin*DIII	1975-hG4-CAT
3941	*Dra*III/*Hin*DIII	1639-hG4-CAT
3456	*Bcl*I/*Hin*DIII	1154-hG4-CAT
3032	*RSR*II/*Hin*DIII	730-hG4-CAT
2712	*Bss*HII/*Hin*DIII	412-hG4-CAT

RNA Isolation and RNase Protection Assay—Total cellular RNA was isolated from snap-frozen tissues using the guanidinium isothiocyanate extraction followed by purification on a CsCl gradient (33) as previously described (34). RNA was quantified spectrophotometrically by absorbance at 260 nM and stored as an ethanol precipitate at −70 °C.

An 890-bp *Sal*I-*Eco*RI fragment of the mouse GLUT4-CAT plasmid, p469GLUT4.CAT (obtained from Dr. M. D. Lane, Johns Hopkins Medical School, Baltimore, MD) (35) was subcloned into the *Sal*I-*Eco*RI site of pIBI30 (IBI). This plasmid was linearized with *Bsu*36I and was used as a template to generate a 616-nucleotide antisense RNA probe, which is able to anneal to the 5′-untranslated region of mouse *GLUT4* mRNA up to the *Dra*I site of the mouse *GLUT4* cDNA and to CAT mRNA corresponding to the *Xba*I-*Eco*RI fragment of the CAT cDNA. Since the 5′-untranslated region of the mouse *GLUT4* mRNA is not conserved in the human *GLUT4* transcript, this probe is specific for both the endogenous mouse *GLUT4* message and transgenic CAT mRNA. The antisense RNA was labeled with [α-^{32}P]UTP using T3 polymerase in an *in vitro* transcription assay (Promega). 10 μg of total RNA was hybridized with 5×10^5 cpm of labeled probe in 30 μl of hybridization buffer (80% deionized formamide, 0.4 M NaCl, 40 mM Pipes, pH 6.4, and 1 mM EDTA). Hybridization was carried out overnight at 50 °C, and the non-hybridized RNA was digested for 30 min at room temperature in 10 mM Tris-HCl, pH 7.5, 200 mM sodium acetate, and 5 mM EDTA containing 500 units of RNase T1/ml (Life Technologies, Inc.). The protected fragments were analyzed using 6% acrylamide, 7.5 M urea gel electrophoresis and identified by autoradiography.

RESULTS

Tissue-specific Expression of the Human GLUT4 Gene in Transgenic Mice—To identify the DNA sequences responsible for tissue-specific expression of the human *GLUT4* gene, we have generated multiple lines of transgenic mice carrying CAT reporter gene constructs containing various deletions in the 5′-flanking DNA. Since expression of reporter genes in transgenic animals can be effected by the location of genomic integration and copy number, we analyzed a minimum of two independent founder lines per construct. RNase protection analysis of mice carrying 412 bp of *GLUT4* 5′-flanking DNA fused to CAT (412-hG4-CAT) demonstrated a lack of tissue specificity (Fig. 1*A*). The level of CAT mRNA was relatively low and present in all the tissues examined, including the brain and liver (Fig. 1*B*). Although mice carrying 730 bp of *GLUT4* 5′-flanking DNA (730-hG4-CAT) was expressed in a proportionally high level in skeletal muscle, it was also expressed in brain. The relative low level of this reporter gene mRNA in cardiac and adipose tissue indicates that the expression of this construct was generally unrestricted. However, the high level of expression in skeletal muscle suggests the presence of a skeletal muscle-specific *cis*-DNA element located downstream of −730.

In contrast to 412-hG4-CAT and 730-hG4-CAT, 1154 bp of the *GLUT4* 5′-flanking DNA (1154-hG4-CAT) displayed a pattern of CAT expression that paralleled the expression of the endogenous murine *GLUT4* mRNA (Fig. 1, *A* and *B*). The transgenic CAT and murine *GLUT4* mRNAs were expressed at highest levels in brown adipose tissue and skeletal muscle with slightly lower levels in cardiac muscle followed by white adipose tissue. Importantly, there was no measurable expression of the 1154-hG4-CAT transgenic mRNA in liver or brain, tissues which normally do not express *GLUT4*. This pattern of expression was virtually identical in the lines of transgenic

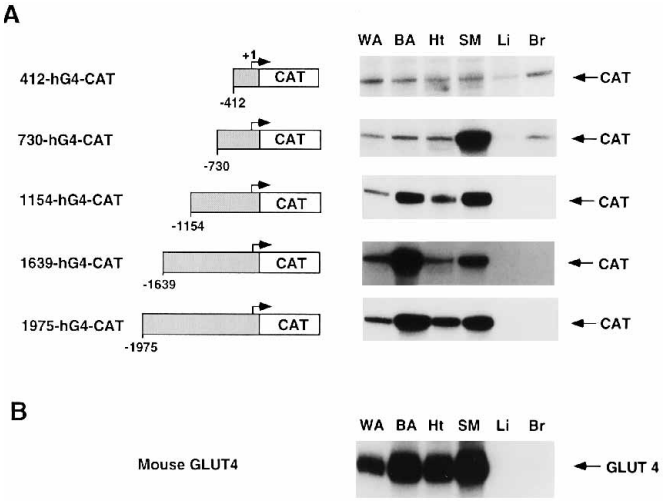

FIG. 1. **Tissue-specific expression of CAT mRNA in transgenic mice carrying different human *GLUT4* 5′-flanking DNA.** 10 μg of total RNA isolated from white adipose tissue (*WA*), brown adipose tissue (*BA*), heart (*Ht*), hindquarter skeletal muscle (*SM*), liver (*Li*), and brain (*Br*) was analyzed for CAT mRNA (*panel A*) and endogenous mouse *GLUT4* mRNA (*panel B*) using RNase protection assay as described under "Experimental Procedures." The names and schematic representation of constructs used to generate transgenic mice are shown in *panel A*. The *stippled box* represents the human *GLUT4* 5′-flank, and major transcription start site of the human *GLUT4* gene is indicated by the *arrow*. Nucleotide sequence for each construct is indicated relative to the transcription start site as +1.

animals carrying the larger 1639 (1639-hG4-CAT) and 1975 bp (1975-hG4-CAT) of *GLUT4* 5′-flanking DNA (Fig. 1*A*). From these data, we conclude that at least 1154 bp of 5′-flanking DNA is required for the appropriate tissue-specific expression of the *GLUT4* gene.

Regulated Expression of Transgenic mRNA in Diabetic Animals—To determine the *cis*-DNA sequences involved in the regulation of the human *GLUT4* gene in an insulin-deficient state, we examined the expression of the CAT reporter genes in STZ-induced diabetes. Previous studies have demonstrated that *GLUT4* mRNA is down-regulated in STZ-diabetic rats and mice due to a decrease in transcription rate (31, 36). This is an insulin-specific response since the loss of *GLUT4* mRNA is fully reversible following insulin therapy. As expected, mice made insulin-deficient by STZ treatment had a marked reduction in the steady-state levels of the endogenous *GLUT4* mRNA in white adipose tissue (Fig. 2*B*, *lane D*) compared to control animals (Fig. 2*B*, *lane C*). Treatment of the diabetic animals with insulin resulted in a restoration of the *GLUT4* mRNA levels somewhat above the control value (Fig. 2*B*, *lane I*). In contrast to the endogenous *GLUT4* mRNA, the two smallest constructs (412-hG4-CAT and 730-hG4-CAT) did not decrease in the diabetic state and, in fact, appeared to increase (Fig. 2*A*). Furthermore, insulin treatment did not increase expression of the CAT reporter genes but did reduce the small increase observed in the untreated diabetic animals. However, the transgenic mice carrying the the three larger constructs (1154-hG4-CAT, 1639-hG4-CAT, and 1975-hG4-CAT) all displayed a pattern of reporter gene regulation, which paralleled that of the endogenous *GLUT4* gene. In each case, CAT mRNA was reduced in the diabetic state and was subsequently returned to supranormal levels following insulin treatment.

The appropriate pattern of insulin regulation of the 1154-hG4-CAT, 1639-hG4-CAT, and 1975-hG4-CAT reporter genes was reiterated in brown adipose tissue (Fig. 3*A*), heart (Fig. 4*A*), and skeletal muscle (Fig. 5*A*), all of which mirrored that of the endogenous murine GLUT4 mRNA (Figs. 3*B*, 4*B*, and 5*B*). As observed in white adipose tissue, both the 412-hG4-CAT and

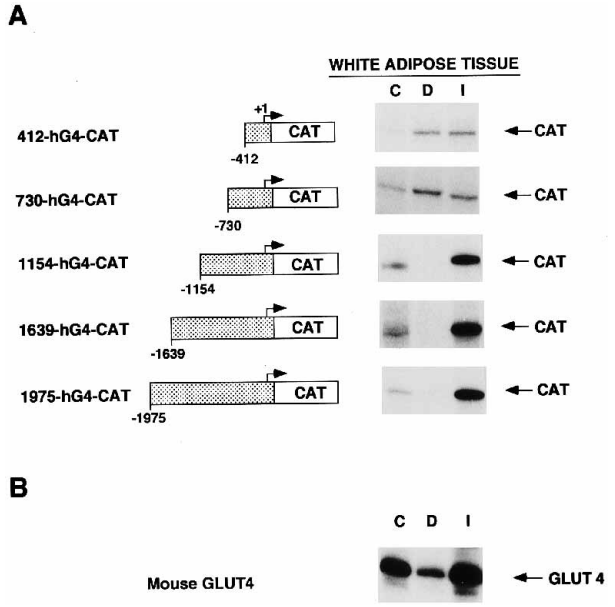

FIG. 2. **Regulation of CAT mRNA expression in white adipose tissue from STZ-diabetic transgenic mice.** Total cellular RNA was isolated from gonadal white adipose tissue from control (*C*), untreated diabetic (*D*), or insulin-treated diabetic (*I*) female mice. RNA was analyzed for CAT mRNA (*panel A*) and endogenous mouse *GLUT4* mRNA (*panel B*) using RNase protection assay as described under "Experimental Procedures." The names and schematic representation of constructs used to generate transgenic mice are shown in *panel A*. The *stippled box* represents the human *GLUT4* 5′-flank, and major transcription start site of the human *GLUT4* gene is indicated by the *arrow*. Nucleotide sequence for each construct is indicated relative to the transcription start site as +1.

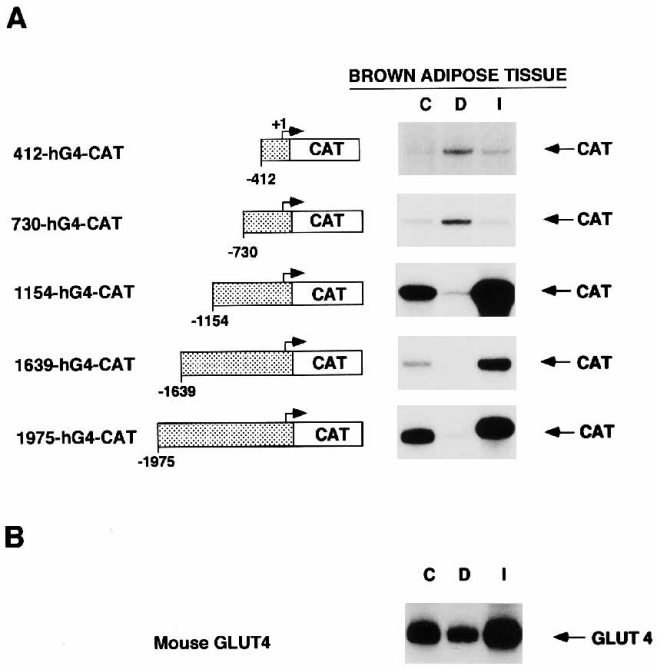

FIG. 3. **Regulation of CAT mRNA expression in brown adipose tissue from STZ-diabetic transgenic mice.** Total cellular RNA was isolated from brown adipose tissue from control (*C*), untreated diabetic (*D*), or insulin-treated diabetic (*I*) female mice. RNA was analyzed for CAT mRNA (*panel A*) and endogenous mouse *GLUT4* mRNA (*panel B*) using RNase protection assay as described under "Experimental Procedures." The names and schematic representation of constructs used to generate transgenic mice are shown in *panel A*. The *stippled box* represents the human *GLUT4* 5′-flank, and major transcription start site of the human *GLUT4* gene is indicated by the arrow. Nucleotide sequence for each construct is indicated relative to the transcription start site as +1.

730-hG4-CAT reporters did not display any significant down-regulation in brown adipose, cardiac, or skeletal muscle in the STZ-induced diabetic state. The inability of 730-hG4-CAT to undergo hormonal/metabolic regulation was surprising in that this reporter construct contained the necessary *cis*-DNA elements required for high levels of skeletal muscle expression.

DISCUSSION

Insulin-stimulated glucose disposal primarily results from a translocation of an intracellular pool of GLUT4 protein to the cell surface membrane in muscle and adipose cells (9, 14, 15). The insulin-stimulated increase in cell surface GLUT4 protein is required for the maintenance of normal glucose homeostasis, particularly in the post-prandial state (37). We and others (17–23, 38) have previously demonstrated that in some rodent models of peripheral tissue insulin resistance there is a loss of both GLUT4 protein and mRNA expression. More recently, expression of the GLUT4 protein in both normal and genetically diabetic mice has demonstrated an important role for GLUT4 in regulating insulin sensitivity (25–29, 39). These data strongly suggest that increased expression of the *GLUT4* gene would have a major beneficial effect to enhance glucose disposal and to maintain more efficient control over fluctuations in circulating glucose concentrations. Based upon this potentially important intervention, we have begun to dissect the *GLUT4* promoter in normal and pathophysiologic states *in vivo*.

Previously, we had determined that 2400 bp of 5′-flanking DNA of the human *GLUT4* promoter was sufficient for tissue-specific and normal physiologic regulation in insulin-deficient diabetes and fasted states (30, 31). The deletion analysis presented in this manuscript has demonstrated that 412 bp of 5′-flanking DNA supports a basal level of transcription but which is neither tissue restricted nor regulated by insulin deficiency. Thus, 412 bp of the *GLUT4* 5′-flank was operationally identified as the functional human *GLUT4* minimal promoter. In contrast, 730 bp of the *GLUT4* 5′-flanking DNA was sufficient to direct relatively high levels of expression in skeletal muscle but not cardiac or adipose tissue. In addition, expression of this reporter gene also failed to display the normal pattern of down-regulation in STZ-induced diabetes. These data were consistent with a skeletal muscle-specific enhancer element located between −412 and −730 of the human *GLUT4* promoter. This region is highly conserved between the mouse, rat, and human genes and contains a consensus binding sequence for the muscle-specific transcription factor MEF2. Recent promoter analysis in the differentiating C2C12 muscle cell line has demonstrated that the rat MEF2 sequence was essential for the myotube-specific expression of the rat *GLUT4* gene (40). Furthermore, this element was shown to directly bind MEF2 in cell extracts from differentiated C2C12 myotubes but not undifferentiated C2C12 myoblasts. Although this element was necessary for C2C12 myotube-specific expression, these reporter constructs were not regulated, similar to our current results in the 730-hG4-CAT-carrying transgenic mice. Currently, we are in the process of generating transgenic mice carrying reporter genes mutated in the MEF2 consensus site of the human GLUT4 promoter to directly determine its role in skeletal muscle expression *in vivo*.

In contrast to 412-hG4-CAT and 730-hG4-CAT, the larger constructs 1154-hG4-CAT, 1639-hG4-CAT, and 1975-hG4-CAT showed no evidence of CAT mRNA expression in brain or liver, tissues which do not express significant amounts of *GLUT4* mRNA. These data suggest that upstream sequences located between −730 and −1154 act to repress *GLUT4* gene expression in some tissues while enhancing specific gene expression

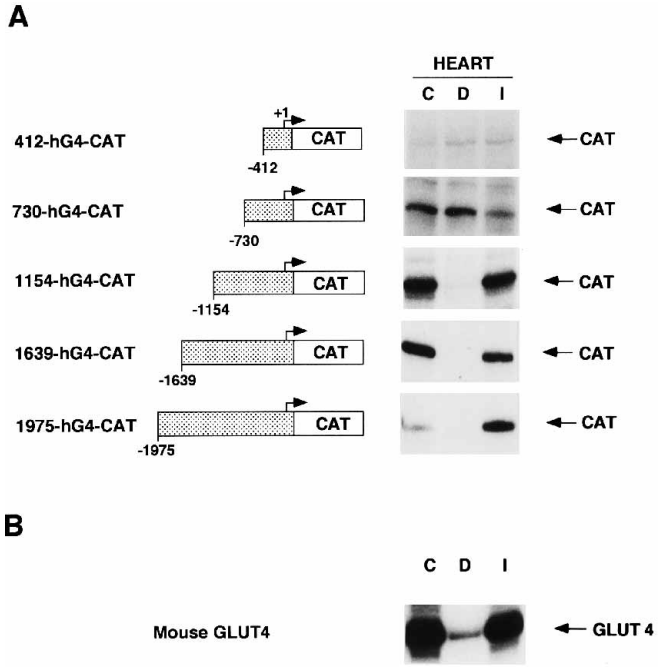

FIG. 4. **Regulation of CAT mRNA expression in cardiac muscle from STZ-diabetic transgenic mice.** Total cellular RNA was isolated from heart tissue from control (*C*), untreated diabetic (*D*), or insulin-treated diabetic (*I*) female mice. RNA was analyzed for CAT mRNA (*panel A*) and endogenous mouse *GLUT4* mRNA (*panel B*) using RNase protection assay as described under "Experimental Procedures." The names and schematic representation of constructs used to generate transgenic mice are shown in *panel A*. The *stippled box* represents the human *GLUT4* 5′-flank, and major transcription start site of the human *GLUT4* gene is indicated by the *arrow*. Nucleotide sequence for each construct is indicated relative to the transcription start site as +1.

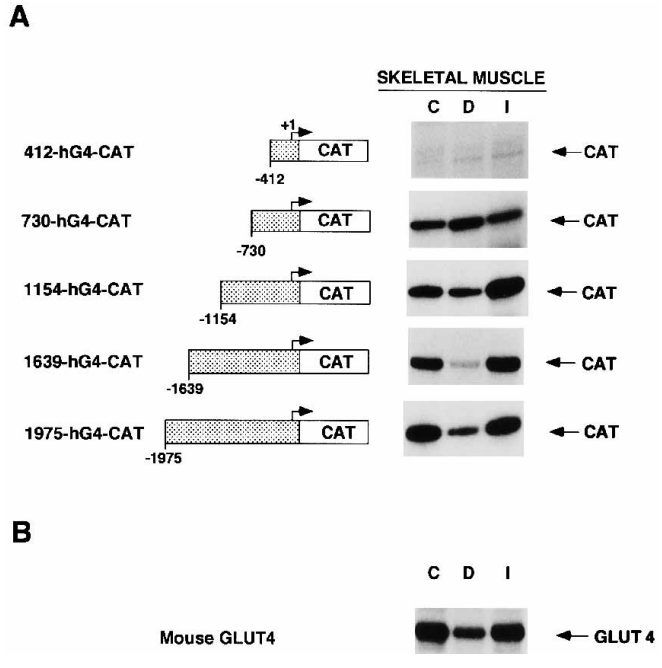

FIG. 5. **Regulation of CAT mRNA expression in skeletal muscle from STZ-diabetic transgenic mice.** Total cellular RNA was isolated from skeletal muscle from control (*C*), untreated diabetic (*D*), or insulin-treated diabetic (*I*) female mice. RNA was analyzed for CAT mRNA (*panel A*) and endogenous mouse *GLUT4* mRNA (*panel B*) using RNase protection assay as described under "Experimental Procedures." The names and schematic representation of constructs used to generate transgenic mice are shown in *panel A*. The *stippled box* represents the human *GLUT4* 5′-flank, and major transcription start site of the human *GLUT4* gene is indicated by the *arrow*. Nucleotide sequence for each construct is indicated relative to the transcription start site as +1.

in cardiac and adipose tissues. Furthermore, the elements confirming the appropriate pattern of metabolic regulation for the endogenous *GLUT4* gene appear to reside within 1154 bp of the human *GLUT4* promoter. However, it is important to recognize that these data have defined boundaries of these tissue-specific and regulatory elements. It remains to be determined whether the sequences responsible for physiologic regulation coincide with the cardiac and adipose tissue-specific enhancers.

In summary, we have performed a 5′-deletion analysis of the human *GLUT4* promoter in transgenic mice to define the DNA sequences responsible for both tissue-specific and hormonal/metabolic regulation of *GLUT4* expression *in vivo*. The data presented in this manuscript have defined −1154 as the 5′-boundary of the human *GLUT4* promoter that contains the necessary elements for tissue-specific expression and down-regulation by insulin deficiency. Furthermore, a skeletal muscle-specific element was located within −730 bp of the transcription initiation site; however, these DNA sequences were not sufficient to confer regulation in STZ-induced diabetes. Thus, the basal *GLUT4* promoter (−412) was not a tissue-specific promoter, and other upstream regulatory elements function to suppress the basal promoter in irrelevant tissue while enhancing promoter activity in appropriate tissues. Currently, we are in the process of performing additional functional deletion and biochemical analysis of these regions to further elucidate the specific sequences and transcription factors involved in this complex regulation of the human *GLUT4* gene.

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REFERENCES

1. Bell, G. I., Burant, C. F., Takeda, J., and Gould, G. W. (1993) *J. Biol. Chem.* **268,** 19161–19164
2. Mueckler, M. (1994) *Eur. J. Biochem.* **219,** 713–725
3. Pessin, J. E., and Bell, G. I. (1992) *Annu. Rev. Physiol.* **54,** 911–930
4. Marette, A., Richardson, J. M., Ramlal, T., Balon, T. W., Vranic, M., Pessin, J. E., and Klip, A. (1992) *Am. J. Physiol.* **263,** C443–C452
5. Zorzano, A., Wilkinson, W., Kotliar, N., Thoidis, G., Wadzinkski, B. E., Ruoho, A. E., and Pilch, P. F. (1989) *J. Biol. Chem.* **264,** 12358–12363
6. Marshall, B. A., Ren, J. M., Johnson, D. W., Gibbs, E. M., Lillquist, J. S., Soeller, W. C., Holloszy, J. O., and Mueckler, M. (1993) *J. Biol. Chem.* **268,** 18442–18445
7. Gulve, E. A., Ren, J. M., Marshall, B. A., Gao, J., Hansen, P. A., Holloszy, J. O., and Mueckler, M. (1994) *J. Biol. Chem.* **269,** 18366–18370
8. Ren, J. M., Marshall, B. A., Gulve, E. A., Gao, J., Johnson, D. W., Holloszy, J. O., and Mueckler, M. (1993) *J. Biol. Chem.* **268,** 16113–16115
9. Holman, G. D., Kozka, I. J., Clark, A. E., Flower, C. J., Saltis, J., Habberfield, A. D., Simpson, I. A., and Cushman, S. W. (1990) *J. Biol. Chem.* **265,** 18172–18179
10. Tordjman, K. M., Leingang, K. A., James, D. E., and Mueckler, M. M. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 7761–7765
11. Henriksen, E. J., Bourey, R. E., Rodnick, K. J., Koranyi, L., Permutt, A., and Holloszy, J. O. (1990) *Am. J. Physiol.* **259,** E593–E598
12. James, D. E., Strube, M., and Mueckler, M. (1989) *Nature* **338,** 83–87
13. Lawrence, J. C., Jr., Piper, R. C., Robinson, L. J., and James, D. E. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 3493–3497
14. Cushman, S. W., and Wardzala, L. J. (1980) *J. Biol. Chem.* **255,** 4758–4762
15. Suzuki, K., and Kono, T. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 2542–2545
16. Karnieli, E., Zarnowski, M. J., Hissin, P. J., Simpson, I. A., Salans, L. B., and Cushman, S. W. (1981) *J. Biol. Chem.* **256,** 4772–4777
17. Berger, J., Biswas, C., Vicario, P. P., Strout, H. V., Saperstein, R., and Pilch, P. F. (1989) *Nature* **340,** 70–72
18. Garvey, W. T., Huecksteadt, T. P., and Birnbaum, M. J. (1989) *Science* **245,** 60–63
19. Garvey, W. T., Maianu, L., Huecksteadt, T. P., Birnbaum, M. J., Molina, J. M., and Ciaraldi, T. P. (1991) *J. Clin. Invest.* **87,** 1072–1081
20. Sivitz, W. I., DeSautel, S. L., Kayano, T., Bell, G. I., and Pessin, J. E. (1989) *Nature* **340,** 72–74
21. Sivitz, W. I., DeSautel, S. L., Kayano, T., Bell, G. I., and Pessin, J. E. (1990) *Mol. Endocrinol.* **4,** 583–588
22. Charron, M. J., and Kahn, B. B. (1990) *J. Biol. Chem.* **265,** 7994–8000
23. Bourey, R. E., Koranyi, L., James, D. E., Mueckler, M., and Permutt, M. A. (1990) *J. Clin. Invest.* **86,** 542–547
24. Richardson, J. M., Balon, T. W., Treadway, J. L., and Pessin, J. E. (1991) *J. Biol. Chem.* **266,** 12690–12694

25. Liu, M.-L., Gibbs, E. M., McCoid, S. C., Milici, A. J., Stukenbrok, H. A., McPherson, R. K., Treadway, J. L., and Pessin, J. E. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 11346–11350
26. Shepherd, P. R., Gnudi, L., Tozzo, E., Yang, H., Leach, F., and Kahn, B. B. (1993) *J. Biol. Chem.* **268,** 22243–22246
27. Ren, J. M., Marshall, B. A., Mueckler, M. M., McCaleb, M., Amatruda, J. M., and Shulman, G. I. (1995) *J. Clin. Invest.* **95,** 429–432
28. Hansen, P. A., Gulve, E. A., Marshall, B. A., Gao, J., Pessin, J. E., Holloszy, J. O., and Mueckler, M. (1995) *J. Biol. Chem.* **270,** 1679–1684
29. Gibbs, E. M., Stock, J. L., McCoid, S. C., Stukenbrok, H. A., Pessin, J. E., Stevenson, R. W., Milici, A. J., and McNeish, J. D. (1995) *J. Clin. Invest.* **95,** 1512–1518
30. Liu, M.-L., Olson, A. L., Moye-Rowley, W. S., Buse, J. B., Bell, G. I., and Pessin, J. E. (1992) *J. Biol. Chem.* **267,** 11673–11676
31. Olson, A. L., Liu, M.-L., Moye-Rowley, W. S., Buse, J. B., Bell, G. I., and Pessin, J. E. (1993) *J. Biol. Chem.* **268,** 9839–9846
32. LeMarchand-Brustel, Y., Olichon-Berthe, C., Gremeaux, T., Tant, J. F., Rochet, N., and Van Obberghen, E. (1990) *Endocrinology* **127,** 2687–2695
33. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299
34. Olson, A. L., Perlman, S., and Robillard, J. E. (1990) *Pediatr. Res.* **28,** 183–185
35. Kaestner, K. H., Christy, R. J., and Lane, M. D. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 251–255
36. Gerrits, P. M., Olson, A. L., and Pessin, J. E. (1993) *J. Biol. Chem.* **268,** 640–644
37. Koranyi, L. I., Bourey, R. E., Vuorinen-Markkola, H., Koivisto, V. A., Mueckler, M., Permutt, M. A., and Yki-Jarvinen, H. (1991) *Diabetologia* **34,** 763–765
38. Camps, M., Castello, A., Munoz, P., Monfar, M., Testar, X., Palacin, M., and Zorzano, A. (1992) *Biochem. J.* **282,** 765–772
39. Treadway, J. L., Hargrove, D. M., Nardone, N. A., McPherson, R. K., Russo, J. F., Milici, A. J., Stukenbrok, H. A., Gibbs, E. M., Stevenson, R. W., and Pessin, J. E. (1994) *J. Biol. Chem.* **269,** 29956–29961
40. Liu, M. L., Olson, A. L., Edgington, N. P., Moye-Rowley, W. S., and Pessin, J. E. (1994) *J. Biol. Chem.* **269,** 28514–28521