Tissue- and cell-specific expression of human *sn*-glycerol-3-phosphate dehydrogenase in transgenic mice

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Received February 6, 1992; Revised and Accepted April 28, 1992

ABSTRACT

Comparison of the promoter sequence for the *sn*-glycerol-3-phosphate dehydrogenase (GPDH, EC 1.1.1.8) genes in mice and humans showed that there were three promoter domains conserved in evolution (1). To study the functional organization of the GPDH promoter, we generated transgenic mice carrying the complete human gene, GPD1. The level of human and mouse GPDH activity was measured in each tissue and the amount of human-mouse GPDH heterodimer was used as a sensitive indicator of cell-specific expression of GPD1. During postnatal development and in adult tissues of the transgenic mice, human GPDH was expressed at levels that corresponded closely to the expression of the endogenous mouse gene, *Gdc-1*. Surprisingly, deletion of the evolutionarily conserved fat-specific elements (FSE) in the proximal promoter region failed to reveal any alterations in GPD1 expression that were specific for either white or brown adipose tissue.

INTRODUCTION

The adult isozyme of murine glycerol-3-phosphate dehydrogenase (GPDH, EC 1.1.1.8) is encoded by a single structural gene, *Gdc-1*, located on chromosome 15 (2). *Gdc-1* is expressed predominately in adult tissues where its level of expression varies over two orders of magnitude among different cell types (3). This wide range of expression levels is determined in part by a complex interaction of genetic, environmental, and hormonal factors. For example, in Bergmann glial cells of the adult cerebellum, high levels of *Gdc-1* expression depend on the sustained interaction of the glial cells with the adjacent Purkinje cells (4,5). In 3T3 preadipocyte cell lines, hormonal stimulation is required to initiate a differentiation program that includes activation of *Gdc-1* transcription (6,7,8). Thus, it appears that glial and adipose cells as well as many other cell types have unique mechanisms for regulating levels of GPDH expression in mice.

Our previous studies on sequence conservation and structural organization of the GPDH promoter in mice and humans showed that both promoters contain three domains (DPR2, DPR, and PPR) conserved in evolution (1). Within these conserved domains of the *Gdc-1* promoter we identified tissue-specific DNase I-hypersensitive sites in various mouse tissues. Methylation studies of the *Gdc-1* promoter also revealed that each adult tissue had

a unique distribution of nonmethylated sites except for testis and sperm, where the promoter was heavily methylated at all sites examined. These results suggested that tissue-specific trans-acting factors bind to the three conserved promoter domains and thereby determine the various levels of GPDH expression found in adult tissues. To further investigate this possibility, we generated transgenic mice carrying the human GPDH gene, GPD1, and then measured gene expression by assaying levels of GPDH enzyme activity in various mouse tissues. The initial experiments were designed to determine: 1) whether the human transgene was expressed in most adult mouse tissues, and 2) whether the developmental pattern and tissue distribution of human enzyme activity levels were similar to those observed for the normal endogenous mouse gene, *Gdc-1*.

MATERIALS AND METHODS

Transgenic mice

DNA was prepared for injection into the male pronucleus of zygotes in order to generate transgenic mice as previously described (9). The various founder transgenic mice were created using the following genetic crosses: C57BL/6J × SJL/J F2 cross for GPDH B, FSE d36A, FSE d36B, FSE d124A, and FSE d124B; C57BL/6J × LT/Sv F1 cross for GPDH A1 and GPDH A2. Positive founder animals (F0) were mated to C57BL/6J mice to produce positive F1 offspring. Each transgenic strain was then established and maintained by brother-sister mating. All mice were fed The Jackson Laboratory's standard 96W chow diet and maintained under strict light cycling conditions (lights on from 0600 to 1800 h).

Construction of FSE promoter deletions

Restriction endonuclease *HindIII* was used to cut the human promoter at position –266. A nested set of DNA deletions extending from the *HindIII* site toward the first exon was generated by a time course of digestion with nuclease Bal31 (New England Biolabs). The ends of the deleted molecules were filled-in using the Klenow fragment of DNA polymerase I (Pharmacia). Then, the molecules were ligated into blunt-ended M13mp18 generated by digestion with *SphI* followed by treatment with Klenow fragment. Resulting clones were sequenced by the dideoxy chain termination method (10). Clones with desired deletions were excised from the M13 vector using the *HindIII* site immediately adjacent to the *SphI* site and then cloned back as a cassette into the full-length human GPD1 gene. This method

of deletion regenerated the original *Hind*III site and also resulted in the insertion of three additional nucleotides, ATG, derived from the *Sph*I cloning site as shown in Fig. 1.

Preparation of tissue extracts

Tissues were collected and stored at -20°C in 2.5 μl Buffer A (50mM Tris-HCl, pH 7.5; 1mM EDTA) per mg tissue weight. Tissues were thawed on ice at the time of assay and homogenized using a motor driven Teflon pestle. Homogenates were centrifuged at 15,000 rpm in a refrigerated Eppendorf 5415 centrifuge for 5 min. The resulting supernatant was transferred to another tube and used in all subsequent analyses.

Assay of GPDH activity

Spectrophotometric assay of GPDH activity was routinely performed immediately following tissue homogenization as previously described (11). Many samples required further dilution with Buffer A in order to obtain a linear reaction for the course of the assay. Protein concentration was determined as previously described (12).

Cellulose acetate plate electrophoresis

The active form of GPDH normally exists as a dimer. Homodimeric and heterodimeric forms of human and mouse GPDH were separated by electrophoresis as previously described for *Gdc-1* (2). Modifications of the procedure involved decreasing the run time from 2 h to 1 h and using a 1% noble agar (Difco Laboratories) overlay. Stain of enzyme activity was allowed to develop until optimum definition of all three species was achieved. Length of staining time varied significantly depending upon the transgenic strain and the tissue type. Color development was stopped by submerging the plates in 2% formaldehyde for 10 min followed by a 10 min rinse in water. The intensity of the stain in each band was measured on a Helena Quik Scan densitometric scanner (Helena Labs, Beaumont, Texas), using a program designed to measure stain intensity on cellulose acetate plates. Tissue homogenates for up to eight animals were scanned and the results averaged to obtain the percentages of the various dimeric species. Standard deviations for densitometric values were less than 10% unless otherwise noted. The relative amounts of human (h) and mouse (m) GPDH activity in the various tissues were derived by dividing the value for the heterodimeric band (human-mouse) in half and then adding that value to the relative amount of each homodimeric band (either human-human or mouse-mouse). Assuming random dimerization of the human and mouse polypeptide chains and identical cell-specificity for expression of the human and mouse genes, the predicted heterodimeric enzyme levels for each tissue were calculated by the following formula: fraction of total enzyme activity that is human $\times$ fraction of total enzyme activity that is mouse $\times 2$. In other words, the predicted fraction of total GPDH activity in a heterodimeric band equals 2mh.

RESULTS

Expression of the human GPDH gene (GPD1) in transgenic mice

Cloned fragments of the human GPDH gene (1) were reassembled in the plasmid pUC19 as 13.5 kb of continuous genomic DNA (Fig. 1). Digestion of the recombinant plasmid with *Sca*I released a DNA fragment containing approximately 3.5 kb of 5' flanking sequence, 7.6 kb of the structural gene,

Figure 1. Structure of the various human GPDH gene constructs used to make transgenic mice. The upper restriction map shows the entire human gene subcloned into the plasmid vector pUC19. The 5' end of the subclone begins at a *Kpn*I (K) site located within *Hind*III (H) fragment A. The subclone extends downstream to include three additional *Hind*III fragments labeled B, C, and D (1). The full length transgene construct used to generate the GPDH A1, GPDH A2, and GPDH B strains is the *Sca*I fragment shown below the subclone. Locations of the proximal promoter region (PPR) and the two distal promoter regions (DPR and DPR2) are indicated by open rectangles. Below the map of the transgene, the nucleotide sequence of the PPR in the wild type (WT) human gene is shown from -266 to -130 and the locations of the fat-specific elements (FSE1 and FSE2) are indicated by arrows. The corresponding sequences in the human deletion mutant transgenes, FSE d36 and FSE d124, along with the normal mouse gene (23) are aligned below. The three additional bases (ATG) shown in bold in the deletion mutants were introduced during construction of the FSE d36 and FSE d124 transgenes.

and 1.2 kb of 3' flanking sequence containing both human and plasmid DNA (see below). The 5' *Sca*I site at position -3535 in the GPD1 promoter region is located 109 bp upstream of the polyadenylation signal of another conserved gene named *DISK2* in the mouse (1, 13). The 3' *Sca*I site, located within the pUC19 vector itself, removed all but 900 bp of the vector from the 3' end of the DNA fragment. The entire 12.3 kb fragment, the longest used in this study to generate transgenic mice, is referred to as the full length GPDH construct because it contained all of the promoter domains previously identified in the 5' flanking region of GPD1.

Two founder transgenic mice carrying the full length GPDH construct in the germ line were used to establish three strains of mice designated GPDH A1, GPDH A2, and GPDH B. GPDH B animals had approximately 4 copies of the transgene per haploid genome and appeared healthy either as heterozygotes or homozygotes. In contrast, as the number of transgene positive offspring from the original GPDH A founder animal increased, it became obvious by both Southern blot analysis and by the appearance of the transgenic animals themselves that there were two types of transgenic offspring. One type of positive offspring, which gave rise to the GPDH A1 strain, had approximately 6 copies of the transgene per haploid genome and was characterized by small, poorly breeding heterozygous animals that were

Figure 2. Cellulose acetate plate showing the three dimeric forms of GPDH in GPDH A1 transgenic mice. Mice from the GPDH A1 strain that were heterozygous for the full length human transgene expressed mouse and human GPDH in all tissues assayed. Tissue extracts at equal protein concentration were spotted at the origin on a cellulose acetate plate and then electrophoresed. After staining for GPDH activity, multiple bands representing human-human (HH), human-mouse (HM), and mouse-mouse (MM) dimeric forms of the enzyme were observed in the various tissues. Tissues represented on the plate shown are cerebral cortex (CX), brown fat (BF), cerebellum (CB), white fat (WF), heart (HT), kidney (KI), and liver (LV).

microphthalmic. The second type of positive offspring produced by the original GPDH A founder was used to establish the GPDH A2 strain. These animals had approximately 14 copies of the transgene per haploid genome and the heterozygotes appeared phenotypically normal. However, homozygous GPDH A2 animals were never obtained because the strain carried a recessive lethal mutation presumably related to insertion of the transgene.

Tissues of heterozygous transgenic mice from the GPDH A1, GPDH A2, and GPDH B strains were homogenized and the level of total (mouse and human) GPDH activity determined spectrophotometrically. Then, human and mouse GPDH in the homogenates were separated by cellulose acetate electrophoresis as shown in Fig. 2 for the GPDH A1 strain. After staining for GPDH activity, three bands were usually seen on the plates. The band moving most slowly towards the anode represents the normal adult mouse enzyme which exists as a homodimer (MM) and is encoded by the *Gdc-1* locus (2). The next slowest migrating band represents a heterodimer (HM) containing one molecule each of the human and mouse enzyme. The band migrating fastest towards the anode represents the homodimer (HH) of the human enzyme encoded by the *GPD1* transgene. In Fig. 2 it is easy to see that the human enzyme was expressed in every GPDH A1 mouse tissue assayed on the plate. In addition, since equal amounts of protein were run in each lane, it is also possible to observe that the relative level of human enzyme activity was different in each tissue and roughly corresponded to the tissue distribution of the endogenous mouse enzyme activity.

To quantitatively analyze the data obtained from all three transgenic mouse strains carrying the full length construct, the cellulose acetate plates described above were scanned in a densitometer to measure the relative amounts of each of the three dimeric protein bands present in each tissue. This information was used to calculate how much of the total GPDH specific activity (which was measured spectrophotometrically) represented the expression of the human transgene. The results are shown in Fig. 3 where the relative amount of mouse GPDH activity present in each tissue was plotted against the relative amount of human GPDH activity present in the same tissue. Considering

Figure 3. Comparison of mouse and human GPDH activity levels in tissues from transgenic mice. For each of the two enzymes, the level of activity in the tissue with the highest specific activity was arbitrarily set at 100. Then, the relative amount of mouse GPDH activity present in each tissue was plotted against the relative amount of human GPDH activity present in the same tissue. The line represents the best fit equation for the points in the graph. Spearman's rank correlation coefficient was used as a statistical test for measuring the probability of correlation between the two variables. The graph shows the results obtained from three GPDH mouse strains carrying the full length transgene. The tissues represented are cerebellum (C), epididymal fat (E), brown fat (F), kidney (K), liver (L), spleen (S), and cerebral cortex (T).

that these results were obtained from transgenic mice with different genetic backgrounds and variable copy numbers of a human gene, the correlation between the relative levels of expression of the two enzymes in the tissues assayed was remarkably high ($r=0.681$). For example, in all three strains the spleen had very low human and mouse GPDH activity while the brown fat was exceptionally high for both enzymes as predicted.

However, there were also some instances where expression of human GPDH was significantly higher than expected relative to the mouse enzyme. In the GPDH A1, GPDH A2, and GPDH B strains the levels of human GPDH activity in the cerebral cortex were approximately 15-, 50-, and 50-fold higher, respectively, than the level of endogenous mouse GPDH activity. For each strain this was by far the highest ratio of human to mouse activity seen in any tissue. Another interesting anomaly in human enzyme expression was found in the GPDH A2 and GPDH B strains. In the kidneys of these two strains human GPDH activity was 12- and 19-fold higher, respectively, than the activity of the endogenous mouse enzyme. This elevated level of human GPDH activity in the kidney was approximately 1.5-fold higher than the level in brown fat (Fig. 3). Interestingly, in all tissues it appeared that the level of human enzyme activity had little if any effect on the normal expression of *Gdc-1* and the presence of very high levels of human GPDH activity in certain tissues had no obvious deleterious effects on the health of the animals.

Developmental regulation of human GPDH gene expression in transgenic mice

The data presented above were obtained by assaying human and mouse GPDH activity in adult transgenic mice. However, the expression of *Gdc-1* in specific tissues of normal mice can vary over 2 orders of magnitude during development and differentiation (3,11). In order to determine whether *GPD1* undergoes similar changes in expression during mammalian development, we measured the levels of human and mouse GPDH activity in three tissues during postnatal development of GPDH B transgenic mice.

Figure 4. Regulation of human GPDH transgene expression during postnatal development in mice. Specific activities of human (●) and mouse (○) GPDH were measured in epididymal fat, cerebellum, and kidney at various time points from birth until weaning at 19 to 24 days of age. The results shown were obtained using the GPDH B strain of mice.

Figure 5. Cellulose acetate plates showing the three dimeric forms of GPDH present in the FSE deletion strains. The three cellulose acetate plates shown contain extracts obtained from kidney, brown fat, and epididymal fat, respectively. Two mice from each of the four FSE deletion strains were analyzed on each plate as described in the legend of Fig. 2. For each plate, all samples were applied at equal protein concentration. However, for each tissue, the protein concentration was different. Multiple bands representing human-human (HH), human-mouse (HM), and mouse-mouse (MM) dimeric forms of the enzyme were observed in all three tissues.

The first tissue assayed for enzyme activity was the epididymal fat pad where cytoplasmic lipid droplets begin to form in fibroblast-like cells at 3 days postpartum in normal mice. Between 3 and 14 days of age, enlarging lipid droplets appear in a majority of the cells and GPDH specific activity increases up to 100-fold (6). Fig. 4 shows that the developmental patterns of human and mouse GPDH expression during epididymal fat pad development were nearly identical. In addition, expression of both enzymes was very similar to that seen for mouse GPDH alone in normal inbred strains of mice (6).

The next tissue assayed was the cerebellum. During postnatal development, expression of GPDH increases dramatically in the cerebellum of normal mice (14). Immunohistochemical studies have shown that the rise in enzyme activity in this tissue reflects primarily an increase in enzyme concentration in the Bergmann glial cells (4). As shown in Fig. 4, our results in transgenic mice indicated that human and mouse GPDH had nearly identical developmental profiles in the cerebella of GPDH B mice. These profiles were similar to those seen for *Gdc-1* in normal inbred strains of mice (14).

The third tissue we examined during development was the kidney, where the human transgene was expressed very efficiently in adult GPDH B mice. Although developmental regulation of *Gdc-1* has not previously been reported in kidney, immunocytochemistry has shown that the proximal tubule cells of the adult kidney have a high concentration of GPDH in normal mice (3). Fig. 4 shows that there is a significant rise in both human and mouse GPDH activity in kidneys of transgenic mice between birth and 21 days of age. As was seen in epididymal fat and cerebellum, the developmental profiles of both enzymes were very similar to the profile observed in normal C57BL/6J

Figure 6. Graphs of actual versus expected levels of heterodimeric (human-mouse) GPDH in tissues of transgenic mice. The actual level of heterodimer activity in each tissue was measured as a percent of total GPDH activity using densitometric scans of cellulose acetate plates. The expected level was the level calculated assuming 1) random intracellular association of the human and mouse GPDH monomers, and 2) a high correlation between the cell-specific expression of the human and mouse genes in each tissue. The expected heterodimeric enzyme level was calculated using the following formula: fraction of total GPDH activity that is human $\times$ fraction of total GPDH that is mouse $\times 2$.

mice (data not shown). We concluded that, in all three mouse tissues examined, the regulation of the GPDH B human transgenes during postnatal development was similar to that seen for the endogenous mouse gene, *Gdc-1*.

Deletion of fat-specific elements in the GPD1 promoter

A region of the GPD1 promoter that appeared likely to contain tissue-specific regulatory elements was the proximal promoter region (PPR) located immediately upstream from the transcription initiation site. Within this 300 bp region of the mouse and human GPDH promoters there are sets of homologies previously named fat-specific elements (FSE) 1 and 2 (15, 16). The three FSEs

originally identified in the *Gdc-1* promoter were also present in GPD1 (1). In order to determine whether these elements were required for efficient expression of GPD1 in adipocytes or other cells in transgenic mice, we sequentially deleted these elements from the full length human promoter and then measured the specific activities of human and mouse GPDH in transgenic mouse tissues.

The first construct, FSE d36, deleted 36 bp extending from -258 to -223 as shown in Fig. 1. This deletion contained the upstream FSE1 (-257 to -246) which is identical in mouse and man (AGCACTGTCAGC). Also included in this deletion is the FSE2 which extends from -239 to -226 in the human promoter

(GCCCAGGAGAAAAA). The second construct, FSE d124, deleted an additional 88 bp (124 bp total) and extended from -258 to -135. As shown in Fig. 1, this region contained the second human FSE1 (CCTAGTCTCTGCC), which is inverted relative to the upstream FSE1. Two strains of transgenic mice were established for each of the two deletion constructs. Precise transgene copy numbers were not determined for the four strains. However, Southern blot analysis of the animals in the F1 generation indicated that the approximate copy number was 10 and 30 copies per haploid genome in the two FSE d36 strains A and B, respectively, and was 2 to 5 copies per haploid genome in both of the two FSE d124 strains A and B. All mice that were heterozygous for these transgenes appeared normal.

Various tissues from FSE d36 strains A and B were assayed for human and mouse GPDH activity as described above. The FSE d36 mice had a tissue distribution of human enzyme expression that correlated well with the relative distribution of endogenous mouse enzyme ($r=0.900$). In fact, the expression of the FSE d36 gene was remarkably similar to that seen with the full length GPDH construct in Fig 3 (data not shown). Similar results were obtained in FSE d124 strains A and B. Surprisingly, even with the larger deletion in the human promoter, there was still a significant correlation between the relative levels of human and mouse enzyme in epididymal fat pads and most other tissues ($r=0.875$) (data not shown).

The most interesting FSE deletion results are summarized in Fig. 5 where we show that all four of the FSE d36 and FSE d124 strains expressed moderate levels of the human enzyme in brown fat, epididymal fat, and kidney. We concluded that the presence of the fat specific elements (FSE1 and FSE2), which are conserved in evolution between mouse and man, is not an absolute prerequisite for expression of the human GPDH gene in adipose tissue or any other tissue thus far examined in transgenic mice.

Cell-specific expression of GPDH in transgenic mice

The results presented above were obtained by assaying tissue homogenates for total (human and mouse) GPDH activity. The relative contributions of human and mouse GPDH to total enzyme activity were determined by densitometric scanning of electrophoretic cellulose acetate plates stained for GPDH activity. Since isozymes of mouse GPDH do not spontaneously form heterodimers in vitro (2) and since the human and mouse enzymes do not form heterodimers in vitro unless homogenates are repeatedly frozen and thawed in 1M NaCl (17), we reasoned that the relative amount of the mouse-human GPDH heterodimers in the various transgenic tissues was a good indicator of whether the human and mouse enzymes were expressed in the same cells within a given tissue. By assuming random dimerization of the mouse and human GPDH polypeptide chains and identical cell-specificity for expression of the human and mouse genes, we calculated the expected level of heterodimer enzyme activity in each tissue.

The results of comparing measured heterodimer levels to the predicted levels are shown in Fig. 6 for six tissues. All seven of the transgenic strains described above were included for each tissue. The three full length promoter strains (GPDH A1, GPDH A2, and GPDH B) showed a close correlation between actual and expected heterodimer levels. The only significant deviation was seen in the cerebellum of the GPDH A1 strain (Fig. 6E). Another significant result is seen in Fig. 6A where all four of the FSE deletion strains (FSE d36A, FSE d36B, FSE d124A, and FSE d124B) showed exceptionally high correlation between

actual and predicted heterodimer levels in the epididymal fat pad. Interestingly, the correlation was not as good in the brown fat pads of the four FSE strains. The two FSE d124 strains also showed a similar deviation from predicted levels in kidney (Fig. 6C) and liver (Fig. 6D). However, the poorest correlations were seen in the central nervous system where the cell-specific expression of human GPDH appeared to be very sensitive to alterations of promoter sequence. All of the mice that contained transgenes with FSE deletions showed decreased correlation between actual and expected heterodimer levels in both the cerebellum (Fig. 6E) and the cerebral cortex (Fig. 6F). The data suggest that FSE promoter elements have a role in directing cell-specific expression of the human transgene in many mouse tissues.

DISCUSSION

These experiments used transgenic mice to delineate the functional promoter of the human GPDH gene, GPD1. Comparison of the GPD1 promoter to that of the corresponding mouse gene, *Gdc-1*, was reported previously (1). This comparison showed that there were two conserved upstream or distal promoter regions named DPR and DPR2. There was also a third highly conserved proximal promoter region named PPR and located immediately 5' to the first exon of both GPD1 and *Gdc-1*. The PPR contained a TATA-like box sequence and the fat-specific elements, FSE1 and FSE2. The full length GPD1 transgene used in the present study was a 12.3 kb fragment that included the complete structural gene and all of the conserved 5' flanking promoter sequences located between the first exon of GPD1 and the 3' end of the next gene upstream. This transgene was expressed in all mouse tissues examined and the levels of expression corresponded closely to that of the endogenous mouse gene. The most likely explanation for these results was that mouse trans-acting transcription factors were interacting with the conserved human promoter sequences in a way that was very similar to the way they normally interacted with the mouse gene. The recognition of human DNA sequences by these mouse regulatory factors was sufficiently accurate to produce an appropriate tissue distribution of transgene expression.

To identify the presence of tissue specific regulatory elements within the GPDH promoter, we decided to examine the proximal promoter region (PPR) which is immediately 5' of the site of transcription initiation. The human and mouse PPRs contain sets of homologies known as fat-specific elements (FSE) 1 and 2. The FSEs were originally found by comparing the 5'-flanking sequences in the three mouse genes *Gdc-1*, *Adn*, and *Ap2* (15, 16). These genes have similar patterns of differentiation-dependent expression in adipocytes (6, 7, 18), and previous work by others suggested that at least some of the FSEs may be involved in regulating this expression (19, 20, 21). Thus, we predicted that deletion of the FSEs in the human transgene would perturb or prevent expression of human GPDH in adipose tissue. We were also interested in determining whether the pattern of transgene expression would be altered in other tissues as well. Surprisingly, even after deletion of three FSEs and almost half the PPR sequence, the relative tissue distribution of human GPDH appeared remarkably similar to that seen with the full length construct. Brown adipose tissue continued to have the highest level of transgene expression. In other tissues, including the epididymal fat pad, expression of human GPDH still showed a relatively high correlation with the level of the endogenous mouse

enzyme activity. Thus, the presence of the FSEs was not a prerequisite for transgene expression in any mouse tissue examined including fat. These results parallel those found with a tissue-specific gene, *Ap2*, which is only expressed in differentiated adipocytes. In transgenic mice, an upstream adipose-specific enhancer was the major determinant of tissue specificity. The *Ap2* proximal promoter elements, which included an AP-1 binding site (FSE 2) and a C/EBP binding site, were not sufficient or necessary for adipose-tissue-specific expression (24).

Since deletion of the FSEs did not alter GPD1 transgene expression in a tissue-specific manner, we considered the possibility that cell-specific expression could still be altered in certain tissues even though the total activity appeared relatively normal. To test this possibility, we assumed that there was random association between human and mouse GPDH monomers to form heterodimers if the monomers were produced within the same cell. Since human and mouse GPDH dimers do not reassociate in vitro to form heterodimers under the homogenization conditions we used, the heterodimer levels present in transgenic mouse tissues were a measure of how well the cell-specific expression of human GPDH correlated with the expression of the mouse enzyme. Using this indirect method to examine expression of the full length human transgene revealed that the predicted heterodimer level was usually close to the level that was actually observed. This was also the case for the FSE deletions except in the central nervous system. In the cerebellum and frontal cortex, the four FSE deletion strains had heterodimer levels significantly below the level expected. These results are consistent with the possibility that the human FSE promoter elements modulate levels of GPDH expression in a cell-specific fashion within the central nervous system. This is particularly interesting given the fact that genetic background and specific mutations can alter levels of mouse GPDH expression in a cell-specific fashion within the cerebellum (22). Perhaps the FSE sequences are a component of the genetic mechanisms involved in regulating expression of *Gdc-1* in the central nervous system. It will be interesting to confirm these results using either immunocytochemistry for GPDH or a reporter gene such as lacZ.

ACKNOWLEDGMENTS

We would like to thank Pamela S. Ganschow who was a summer student at The Jackson Laboratory and worked on the preliminary studies of human transgene expression during postnatal development. We also thank Dr. Leslie P. Kozak for helpful suggestions during the early phases of this research. This work was supported by Public Health Service grant DK-34384 to Edward H. Birkenmeier from the National Institutes of Health.

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