In Vivo Functional Characterization of the Aldolase B Gene Enhancer*

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A 400-bp intronic enhancer fragment in conjunction with the proximal promoter of the aldolase B gene provided correct tissue-specific expression in transgenic mice together with hormonal regulation in the liver. We investigated in vivo and in cultured cells the contribution of the intrinsic regulatory sequences and their interaction with the promoter elements in controlling aldolase B gene expression. Transgene activity was completely abolished by disruption of the two hepatocyte nuclear factor 1 (HNF1) binding sites in the enhancer, whereas mutation of one HNF1 site had no effect in the liver but strongly decreased activity in the kidney. Our data show that the HNF1 binding site(s) in the enhancer were key regulators of aldolase B transgene expression both in the liver and kidney. Deletion of the CCAAT/enhancer-binding protein site in the promoter completely abolished the enhancer function in HepG2 cells. These results suggest that expression of the aldolase B gene in the liver requires cooperative interactions between CCAAT/enhancer-binding protein and HNF1. Deletion of the HNF4 binding site in the enhancer suppressed expression in both liver and kidney in half of the transgenic lines, suggesting that this element might play a role in chromatin opening at the insertion site. We firmly establish that the endogenous aldolase B gene’s first response to glucagon or cyclic AMP exposure was a transient increase in the expression in the liver, followed by a secondary decline in the transcription, as previously reported. This response was reproduced by all transgenes studied, indicating that neither HNF1 nor HNF4 binding sites in the enhancer were involved in this biphasic cyclic AMP response.

The aldolase B enzyme catalyzes the reversible cleavage of fructose 1-phosphate into dihydroxyacetone phosphate and glyceraldehyde and is involved in two opposite metabolic pathways, glycolysis and gluconeogenesis. The expression of the aldolase B gene is subject to tissue-specific, hormonal, and metabolic regulation. The adult liver expresses the aldolase B gene exclusively, whereas the kidney and enterocytes co-express both aldolase A and B genes (1). In the liver, transcription of the aldolase B gene is induced by a carbohydrate-rich diet and inhibited by fasting and glucagon, whereas, in the kidney, it is almost unresponsive to dietary and hormonal regulation (2). However, even in the liver, the expression of the aldolase B gene is never completely abolished. In addition, it is restimulated after prolonged starvation. This behavior may reflect the dual role of aldolase B in hepatocytes, where it is required for the opposite glycolytic and gluconeogenic pathways.

Finally, hereditary fructose intolerance is a recessive genetic disease caused by aldolase B deficiency. Repeated ingestion of noxious sugars by homozygotes leads to hepatic and renal injury (3), with metabolic disturbances (including low concentrations of blood glucose) that may prove fatal (4).

The aldolase B gene proximal promoter (−194 to +14) is sufficient to direct cell type-specific expression in cultured hepatoma cells, but in mice a distal intronic enhancer fragment (+1916 to +2324) is required for transgene expression (5). We have previously studied both these regions extensively (6–9) and have identified the regulatory elements and cognate binding factors (see Figs. 1 and 3). Their respective contributions to promoter activity and enhancer function were investigated by transient chloramphenicol acetyltransferase (CAT) assays in HepG2 hepatoma cells. The main findings were as follows. The promoter activity was up-regulated by hepatocyte nuclear factor 1 (HNF1) and CAAT/enhancer binding protein (C/EBP), but repressed by hepatocyte nuclear factor 3 (HNF3) (7). The enhancer function was abolished by mutation or deletion of either of the two HNF1 binding sites and reduced by deletion of the HNF4 binding site (9).

Dietary regulation of a transgene directed by 1600 bp of the 5′-flanking region of the aldolase B gene and the first intron appeared paradoxical; it was down-regulated by a high carbohydrate diet and stimulated by prolonged fasting (5). In order to explain these results, we hypothesized that the endogenous gene possessed distinct glucose-dependent and fasting-dependent responsive elements, only the latter being present in the transgenic construct.

The objective of the current study was to assess, in a chromosomal in vivo context, the contribution of the different DNA elements of the intrinsic enhancer to the tissue-specific expression and metabolic regulation of the transgenes.

Our results demonstrate a major contribution of the HNF1 enhancer binding sites to the tissue-specific expression of the aldolase B gene. Deletion of the two HNF1 binding sites suppressed transgene expression in both liver and kidney. In contrast, mutation in one HNF1 site did not significantly affect transgene expression in the liver but resulted in a 100-fold loss of activity in the kidney. Findings suggested that the differential role of the HNF1 sites in the different organs could be

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2 The abbreviations used are: CAT, chloramphenicol acetyltransferase; HNF, hepatic nuclear factor; C/EBP, CCAAT/enhancer-binding protein; PEPCK, phosphoenolpyruvate carboxykinase; Bt,cAMP, dibutyryl cyclic AMP.
related to the abundance of C/EBP in the liver compared with the kidney. Indeed, we show that a C/EBP binding site previously identified in the proximal promoter is crucial for maximal expression in hepatocytes.

Deletion of the HNF4 site seemed to render the transgene activity more dependent on the insertion site, silencing it completely in about half of the transgenic lines. Finally, we demonstrate that transgene activation by fasting could be mimicked by glucagon and dibutylryl cAMP, but we have so far failed to delineate a cyclic AMP response element in the enhancer.

**EXPERIMENTAL PROCEDURES**

**Plasmid Constructions**—Aldolase B-CAT constructs used have been previously described (9). Fig. 1A presents a diagram of the aldolase B-CAT gene, indicating the main cis-acting elements and cognate DNA-binding proteins. The enhancer fragment (residues 1916–2329) was maintained in an intronic position; the splice sites are indicated by the boldface brackets. Fig. 1B shows the microinjected constructs indicating the locations of the block mutations or deletions performed in the aldolase B enhancer. Fig. 3 schematizes various constructs with deletions or mutations in the promoter. Here, the enhancer fragment was subcloned in the ClaI site located downstream of the CAT gene (8).

**Generation and Analysis of Transgenic Mice**—The DNA constructs were digested with restriction enzymes ClaI (cutting in 3' in the vector) and Hinf I (cutting in 5' in the plasmid linker). The fragments of interest were isolated by electrophoresis, electroeluted, and purified by using elutip-d columns (Schleicher and Schuell) and then microinjected into fertilized mouse eggs according to Gordon and Ruddle (10). The progeny was analyzed for the presence of the transgene by Southern blot.

**Cell Culture and Transient Transfection**—HepG2 cells were grown in Dulbecco’s Eagle’s medium in the presence of 10% (v/v) fetal calf serum, 1 μM l-triiodothyronine, 1 μM dexamethasone, and 10 μM insulin at 37°C in 5% (v/v) CO₂. Transfections were carried out by the calcium phosphate method (11), under experimental conditions previously described (9). In each experiment, 7.5 μg of the CAT plasmids and 2 μg of the luciferase plasmid were cotransfected. The pBSV luciferase standardization plasmid was used to monitor variations in transfection efficiency. The CAT assay (12) and luciferase assay (13) were performed as described (8).

**Hepatocytes in Primary Culture**—Hepatocytes were isolated from the livers of adult transgenic mice carrying the −232A100B400 wild-type transgene by perfusion with collagenase (14). Viable hepatocytes were separated from other cells using isodensity Percoll centrifugation (15). Hepatocytes (>95% viability) were seeded at a density of 3.5 × 10⁶ cells/cm². After cell attachment (4 h), the medium was replaced by M199 medium with Earle’s salts (Invitrogen) containing 20 mM glucose, supplemented with 1 μM l-triiodothyronine, 1 μM dexamethasone, 10 nM insulin at 37°C in 5% (v/v) CO₂ for 3 days. The culture medium was changed every 24 h. Cells were then cultured in the same medium either with or without 3.3 mg/liter glucagon and 3.3 mg/liter theophylline.

**Dietary and Hormonal Control**—Adult mice heterozygous for the transgene were used for analysis. One group of mice was fasted for 24 h prior to sacrifice; the other group was fasted for 24 h and then refed a high carbohydrate diet ad libitum for 18 h prior to sacrifice. Organs were stored at −80°C until use. To determine the effect of cAMP or glucagon, mice were either starved for 18 h or fed a high carbohydrate diet ad libitum for 18 h and then injected intraperitoneally every hour with either dibutyryl cyclic AMP (Bt-cAMP) (30 μg/kg of body weight) or glucagon (0.5 μg/kg of body weight) or theophylline (30 mg/kg of body weight) and sacrificed after 2 or 4 h of treatment, and then tissues were removed and kept at −80°C until use.

**RNA Isolation and Northern Blot**—The mRNA extraction from liver, kidney, and cultured hepatocytes was performed using the RNAzol B reagent (Bioprobe Systems) according to the manufacturer’s instructions. 1 μg of total RNA was electrophoresed through a 1.5% (v/v) denaturing formaldehyde agarose gel and transferred to Hybond N+ (Amershams Biosciences). The CDNA probes were labeled by random priming using a labeling kit, according to the manufacturer’s instructions. The CAT CDNA probe was a Clal-EcoRI fragment of the PECAT vector (5). The murine aldolase B probe was a 379-bp fragment of cDNA, and the phosphoenolpyruvate kinase (PEPCK) probe was a 1305-bp fragment of cDNA (a gift of Dr. B. Antoine (16)).

![Diagram of the aldolase B-CAT transgenes.](http://www.jbc.org/)

**RESULTS**

**In Vivo Role of the Different Enhancer Elements**—Full and tissue-specific expression of the aldolase B transgenes results from a cooperation between a proximal promoter and a distal intronic enhancer (5). The enhancer activity is provided by a 400-bp sequence (residues 1916–2329) containing binding sites for known liver-enriched nuclear factors HNF1 (residues 2212–2246 and 2275–2304), HNF4 (residues 2146–2184), and unknown factor (residues 2195 to +2220). We analyzed the contribution of each of the cis-acting elements to the expression of the aldolase B gene in vivo. For this purpose, chimeric genes that contained the wild-type aldolase B promoter (−232A100) linked to either wild-type or mutant aldolase B enhancer in its normal intronic position were ligated to the CAT gene (Fig. 1). Fig. 2 shows the results of the expression of the CAT transgenes directed by aldolase B gene regulatory sequences in the liver and kidney. Expression of the aldolase B-CAT transgenes was strictly restricted to the liver, kidney, and small intestine (not shown); no ectopic expression was detected in other tissues tested (e.g. brain and lung) (not shown). In agreement with prior data (5), the level of transgene expression was totally independent of the copy number and strongly dependent on the integration site. Among the six lines harboring the wild-type aldolase B-CAT transgene, one was very weakly expressed. The HNF1-mut transgene carries a 5-bp block mutation of the 5' HNF1 binding site (enhancer element 5). In five lines with this construct, transgene expression was conserved in the liver and kidney. HNF1-mut transgene expression was totally independent of the copy number and strongly dependent on the integration site. Among the six lines harboring the wild-type aldolase B-CAT transgene, one was very weakly expressed. The HNF1-mut transgene carries a 5-bp block mutation of the 5' HNF1 binding site (enhancer element 5). In five lines with this construct, transgene expression was conserved in the liver (although weak in two of the lines) and was very low in the kidney (a decrease on average of 100-fold compared with the wild-type construct). Among 16 lines harboring a transgene with deletion of enhancer element 7 (i.e. the 3' HNF1 binding site), only one expressed CAT activity in the liver and, very weakly, in the kidney (Fig. 2). None of the nine lines harboring the transgene with deletion of both HNF1 binding sites (construct Δ5–7) expressed CAT activity either in the liver or in the kidney (data not shown). Twelve lines harboring a Δ2 transgene with a deletion of the HNF4 binding site (enhancer element 2) were generated. In five lines, transgene expression was undetectable; in one it was weak, and in the other six it was strong. Finally, expression of a transgene with deletion of enhancer element 4, which binds a liver-enriched but uncharac-
characterized factor (9), was much higher than that of the wild-type transgene and had a high CAT activity in both liver and kidney in four lines and a lower activity in one line (Fig. 2).

Hypothetical Role of C/EBP in the Differential Effect of HNF1 Site Mutations in the Liver and Kidney.—The differential effect of the 5′ HNF1 binding site deletion from the enhancer on transcriptional activity in different cells and tissues could reflect tissue-specific differences in transcription factor content. We focused on transcription factors previously demonstrated to be active on aldolase B gene expression. The C/EBP factor was a good candidate, since its concentration is high in the liver, low in HepG2 cells, and almost zero in the kidney (17). In addition, C/EBPs like HNF1 are potent transactivators of the aldolase B promoter (7). If our hypothesis is correct, overexpression of C/EBP in HepG2 cells should restore transcriptional activity of constructs with mutation or deletion of one of the enhancer HNF1 binding sites. Indeed, when HepG2 cells were transiently cotransfected with the wild-type aldolase B-CAT plasmid and a C/EBP expression vector, CAT activity was stimulated only 5-fold. In contrast, under the same conditions, the HNF1-mut and was stimulated only 5-fold. In contrast, under the same condition, the HNF1-mut and 

FIG. 2. Level of expression of aldolase B CAT transgenes in the liver and kidney of transgenic mice. CAT activity was assayed as described in liver (black) and kidney (gray) homogenates from chow-fed animals. Each value represents the mean of independent assays on different mice (at least three). The CAT activity in the kidney of −232A100B400HNF1mut transgenic mice is shown on a large scale in the inset.

Deletion of the HNF4 binding site from the promoter did not change this stimulation. Deletion or mutation of the PAB element suppressing binding of either HNF1 or HNF3 or both HNF1 and HNF3 seemed rather to increase the stimulatory effect of the enhancer. Finally, as expected, all mutants devoid of the promoter C/EBP binding site were weakly sensitive to the enhancer action. When the PAB element was replaced by a high affinity HNF1, the enhancer lost practically all stimulatory activity, most likely because the C/EBP/HNF1 interaction between promoter and enhancer is replaced by the same type of interaction inside the promoter.

Dietary Regulation of the Transgenes—Northern blot analysis of transgene expression as a function of diet was performed on at least two different mouse lines for each transgene. Feeding mice a high carbohydrate diet stimulates aldolase B gene transcription in liver, whereas starvation decreases its expression (Ref. 2 and Fig. 4). Prior transgenic analysis indicated that a transgene with 1600 bp of the 5′-flanking sequence and the whole first intron responds to diet in an opposite manner (5). Northern blots in Fig. 4 demonstrate that exactly the same expression pattern was reproduced using the “wild-type” construct with only 232 bp of the promoter and the 400-bp intronic enhancer. HNF1 mut, Δ2, and Δ4 transgenes were also induced in mice fasted for 24 h and inhibited in mice refed a high carbohydrate diet. Therefore, the investigated enhancer elements do not seem to be implicated in the long term dietary regulation of the aldolase B gene in the liver.

The dietary response of the aldolase B transgenes resembles that of neoglucogenic genes such as the PEPCK gene. Since such genes are known to be responsive to glucagon and cAMP, we tested the effects of the hormone and of cAMP analogues on the expression of the −232A100B400wild-CAT transgene. Fig. 5A shows that Bt2cAMP induced parallel accumulation of PEPCK and CAT transgenic mRNA 2 and 4 h after injection into carbohydrate-fed mice. In fasted mice, these messengers were already abundant before Bt2cAMP treatment; consequently, the Bt2cAMP effect was weak or nil (Fig. 5B).

As expected, the effect of Bt2cAMP in stimulating the transgene was mimicked by glucagon (Fig. 5C). The response to glucagon was conserved for the Δ2 and HNF1-mut transgenes, characterized by a deleted HNF4 binding site and a mutated HNF1 binding site, respectively (not shown). Although the endogenous aldolase B gene has been described rather as a typical “glycolytic gene,” up-regulated by carbohydrates and down-regulated by fasting and glucagon (2), the results observed with the aldolase B-CAT transgenes prompted us to reevaluate more precisely the response of the endogenous gene to glucagon. Fig. 5C shows that, in fact, endogenous aldolase B mRNA accumulated 2 and 4 h after glucagon administration to carbohydrate-fed animals, in parallel with accumulation of the PEPCK mRNA.

In primary hepatocytes isolated from the liver of mice bearing the −232A100B400wild-CAT transgene and cultured for 3 days in the presence of 25 mM glucose and 20 nM insulin, the addition of glucagon led first to aldolase B mRNA accumulation, with a peak at 3 h and thereafter a decline. Aldolase B and PEPCK mRNA inductions were parallel. In contrast, the re-
TABLE I
Effect of CIEBP-α overexpression on activity of aldolase B CAT vectors containing an impaired HNF1 binding site in the enhancer

<table>
<thead>
<tr>
<th>Constructs</th>
<th>Without CIEBP-α</th>
<th>With CIEBP-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>232A100B400wild</td>
<td>70 ± 25</td>
<td>15,000 (4)</td>
</tr>
<tr>
<td>232A100B400mut</td>
<td>2003 ± 312</td>
<td>10,794 (4)</td>
</tr>
<tr>
<td>232A100B400Δ2</td>
<td>54 ± 9</td>
<td>10,268 (4)</td>
</tr>
<tr>
<td>140A100</td>
<td>64 ± 11</td>
<td>10,355 (4)</td>
</tr>
<tr>
<td>140A100B400mut</td>
<td>50 ± 19</td>
<td>1500 (4)</td>
</tr>
</tbody>
</table>

FIG. 3. Analysis of the element in the promoter required for enhancer function. At the top is an illustration of the wild-type construct with the location of the regulatory elements and transcription factors known to bind to these sites. 7.5 μg of the indicated constructs mutated in the promoter linked or not linked to the enhancer fragment were transfected into HepG2 cells with 2.5 μg of the reference plasmid RSV luciferase. CAT activity was normalized with respect to the transfection efficiency by measuring the luciferase activity. Results give the ratio of the CAT activity of the construct with and without the enhancer fragment.

spontaneous expression of the aldolase B-CAT transgene was delayed, with a peak at 24 h (Fig. 6).

DISCUSSION
Tissue-specific transcription of the aldolase B gene is governed by a short 200-bp promoter stimulated by a 400-bp intronic enhancer (5, 9). In previous studies using transient transfection assays, both the promoter and the enhancer were shown to be cell-specific (9, 18). The nuclear factors mainly involved in liver-specific gene transcription include HNF1 (19), HNF3 (20), HNF4 (21), HNF6 (22), C/EBP (23), and albinin D binding protein (24). Both the aldolase B gene promoter and the enhancer fragments contain HNF1 and HNF4 binding sites; in addition, a binding site for C/EBP is present in the promoter. Therefore, the aldolase B gene was an attractive model for identifying, in vivo, the promoter and enhancer elements necessary for tissue-specific, dietary-regulated gene expression.

In the present study, we have shown that mutation of the 5′ HNF1 binding site in the enhancer, preventing binding of HNF1, reduced expression of the transgene (−232A100B400HNF1mut) in the kidney but did not alter its transcription in the liver. A similar role for HNF1 in the differential control of PEPPCK gene transcription in the liver and kidney has been reported (25). In contrast, the aldolase B gene was normally transcribed in the liver (26) and in the kidney of HNF1 knock-out mice. However, these results are not inconsistent with our own data, since the two isoforms HNF1α and HNF1β, binding to the same element, are coexpressed in the liver and the kidney, HNF1β being increased by a compensatory mechanism in HNF1α−/− (26).

Deletion of the second HNF1 site in the enhancer (element 7) led to an inactive transgene in 11 of the 12 lines tested. In the only line expressing the −232 A100B400Δ7 transgene, expression was weak but easily detectable in the liver and almost nil in the kidney, which confirms that here again abrogation of the HNF1 binding site has a more severe effect in the kidney than in the liver. Since the mutation in the first HNF1 site changed 5 bp whereas deletion of the second site excised 47 bp from the transgene, we cannot deduce from our results that the two HNF1 binding sites in the enhancer play different roles. Indeed, it could be that the 47-bp sequence binds factors other than HNF1 or destroys a binding site 5′ to the deletion, although we did not detect such a site either by in vivo footprinting (9) or by gel shift analysis (not shown).

3 M. Pontoglio and M. Yaniv, personal communication.
A transgene deleted from the HNF4 site was silent in both livers and kidneys in half of the mouse lines. It is possible that the HNF4 mutation affects the recruitment of chromatin-remodeling factors (27, 28). Accordingly, the –232A100B400wild-CAT transgene could remain silent when inserted into a closed chromatin region and would be active when integrated into a permissive, open chromatin region. Although both HNF1 (29, 30) and HNF4 binding sites (31, 32) have been reported to play a role in the response of some genes to cAMP, the corresponding elements present in the enhancer do not seem to be involved in the dietary and hormonal regulation of the aldolase B gene. Indeed, the response of our mutated transgenes to fasting and cAMP was conserved.

To explain the differential effect of enhancer HNF1 binding site mutations according to the cells and tissues, we hypothesized that HNF1 is involved in a cooperative interaction of the enhancer with another DNA element able to bind a factor differentially abundant in the liver, hepatoma cells, and kidneys. C/EBPα, present and abundant in adult hepatocytes, at low concentration in hepatoma cells, and essentially absent from kidney (33), is a good candidate. Mouse models also suggest a major role for C/EBPα in liver-specific expression of aldolase B. The liver of albino lethal mice shows reduced aldolase B and C/EBP levels (34), whereas other transcription factors are normally expressed. In contrast, aldolase B is normally expressed in the kidney of these animals. Aldolase B gene expression is also depressed in the liver of C/EBP α/– mice (35). A C/EBP binding site (−170 to −140) has been characterized in the aldolase B promoter (6), and both C/EBP and HNF1 have been shown to be potent transactivators of the promoter (7). To confirm that the different levels of C/EBP in different tissues could account for the tissue-specific cis effects of the enhancer HNF1 binding sites, we studied by transient transfection in HepG2 cells the inducibility by C/EBP of aldolase B-CAT constructs carrying either the wild-type enhancer or enhancers devoid of one of the two HNF1 binding sites. The constructs with mutations of the HNF1 binding site(s) in the enhancer were highly responsive to overexpression of C/EBP (100-fold induction) compared with the 5-fold induction of the construct with an intact enhancer. Furthermore, deletion of the C/EBP site in the promoter impaired the enhancer function, whereas mutations or deletions of the other known sites (binding HNF1, HNF3, or HNF4), alone or in combination, did not affect the enhancer function. These findings suggest that liver-specific expression of the aldolase B gene requires cooperation between the C/EBP site in the promoter and at least one HNF1 site in the enhancer. In contrast, expression in the kidney (lacking C/EBP) requires the presence of the two enhancer HNF1 binding sites. Cooperation between HNF1 and C/EBP in the control of gene expression in the liver has already been reported for the PEPCK gene (25), the phenylalanine hydroxylase gene (36), the apolipoprotein B gene (37), and the albumin gene (38).

Our data indicate that neither HNF1 nor HNF4 binding sites mediate the aldolase B gene regulation by fasting and cAMP. However, C/EBP was a possible alternative candidate, since its involvement in the response to cAMP has been established for several genes, in particular the PEPCK gene (25), the phenylalanine hydroxylase gene (36), the apolipoprotein B gene (37), and the albumin gene (38).

Fig. 5. Effects of cAMP and glucagon on the level of expression of the −232A100B400wild-CAT transgene and the endogenous aldolase B gene in the liver. Mice were fed a 75% carbohydrate diet for 18 h (A and C) or fasted for 24 h (B) prior to intraperitoneal injection of either Bt2cAMP or glucagon as indicated under “Experimental Procedures.” Total liver RNAs were prepared at the indicated time after the first injection, and Northern blots were analyzed as described in the legend to Fig. 4. PEPCK served as a positive control of the hormonal status.

Fig. 6. Effect of glucagon on aldolase B gene expression in primary cultured hepatocytes. Hepatocytes isolated from −232A100B400wild-CAT transgenic mice were cultured for 3 days without glucagon in the medium described under “Experimental Procedures” and then incubated 3–24 h in the presence or absence of glucagon and theophylline. Total RNAs were extracted and analyzed for the level of transgenic CAT mRNA, endogenous aldolase B, and PEPCK mRNAs. The Northern blots are representative of two culture experiments with two different lines of mice.
glucagon, maximal at the 3rd hour, is followed by a decline that reached undetectable levels at the 24th hour. Accordingly, the transcription rate of the aldolase B gene measured by run-on assay showed first a transient increase in transcription 10 min after glucagon injection, followed by transcriptional inhibition (41).

Hormonal regulation of the aldolase B gene reflects its dual role in both glycolysis and gluconeogenesis. Glucagon and cAMP are involved in hypoglycemic and stress conditions in which a rapid increase in glucose production is essential. This requires, in particular, a transient increase in aldolase B gene transcription, leading to an increase in mRNA and enzyme abundance. However, this gene must also be stimulated to respond to a carbohydrate-rich diet. As previously discussed, the regulatory domains responsible for the response to cAMP (and fasting) are retained in the –232A100B400CAT transgene, while the region required for the positive response to carbohydrates is absent from this construct.

In conclusion, we report in this paper the complex, tissue-specific functional interplay between the promoter and intronic enhancer of the aldolase B gene, which can be considered to be both a glycolytic and gluconeogenic gene.

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