The regulation of transcription of the gene for the cytosolic form of phosphoenolpyruvate carboxykinase (GTP) (PEPCK-C) (4.1.1.32) during diabetes is a complex process that involves a number of regulatory elements in the PEPCK-C gene promoter. The accessory factor 2 (AF2)-binding region that is contained within the glucocorticoid regulatory unit of the PEPCK-C gene promoter (AF2) binds in vivo in the action of both insulin and glucocorticoids on PEPCK-C gene transcription. To determine the role of AF2 in these processes, we have generated a mouse model bearing a transgene that contains the PEPCK-C gene promoter with a mutation in the AF2-binding region. This promoter is linked to the structural gene for human growth hormone that is biologically inactive (AF2–2000/hGx). In the absence of the AF2 regulatory element, the transcription of the transgene in the liver is not induced by diabetes but is inhibited by the administration of insulin. There is also a marked reduction in the response of the AF2–2000/hGx gene in the kidney to the administration of glucocorticoids. The AF2–2000/hGx gene in the liver responds normally to a high carbohydrate diet with a marked decrease in gene transcription. This suggests that insulin is not exerting its usual negative effect on the PEPCK-C gene promoter through the AF2 site. In contrast, the response of this transgene to a high fat/carbohydrate-free diet is severely blunted. Our results support a role for the AF2 site in the PEPCK-C gene promoter in the effect of glucocorticoids, but not insulin, on PEPCK-C gene transcription in the liver.

The cytosolic form of phosphoenolpyruvate carboxykinase (PEPCK-C) (GTP) (4.1.1.32) is a critical enzyme in hepatic gluconeogenesis and glyceroneogenesis (1) and is induced during diabetes, where it is involved in the increased rate of hepatic glucose output and triglyceride synthesis characteristic of this disease. Transcription of the gene for PEPCK-C is induced by cAMP (2–4) and glucocorticoids (5) and inhibited by insulin (6–8) and high concentrations of glucose (4, 9). There are three major factors involved in the induction of PEPCK-C during diabetes. First, the concentration of insulin in the blood is dramatically reduced resulting in the removal of the dominant negative regulator of PEPCK-C gene transcription. Second, there is an increase in the levels of glucagon and a subsequent elevation in the concentration of hepatic cAMP; this stimulates PEPCK-C gene transcription (10). Third, glucocorticoids are required to ensure the elevated expression of the PEPCK-C gene and the resultant physiological alterations, including increased gluconeogenesis (11) (12).

The mechanism(s) responsible for the induction of PEPCK-C gene transcription during diabetes has been studied in some detail (13). The effect of cAMP is exerted through two regions in the PEPCK-C gene promoter; the cAMP-response element, which maps from −90 to −83, and an up-stream regulatory element termed P3(1), which spans bases −250 to −234 in the promoter (14). The regulation of PEPCK-C gene expression by glucocorticoids is accomplished through a glucocorticoid regulatory unit (GRU) in the promoter located between −451 and −353 (15). The GRU includes a linear array of three accessory factor-binding sites, AF1 (−451 to −439), AF2 (−416 to −407), and AF3 (−326 to −321), and two glucocorticoid receptor-binding sites, GR1 and GR2 (−386 to −353) (15). The GRU can bind a variety of transcription factors. The AF1 site binds the chicken ovalbumin upstream promoter transcription factor and hepatic nuclear factor 4 (HNF-4). Both the CCAAT enhancer-binding protein (C/EBP) family members C/EBPα and C/EBPβ and HNF-3 bind to the AF2 site, but it has been shown that HNF-3 mediates PEPCK-C gene transcription (16). The AF2-binding site also contains an overlapping glucocorticoid response element (GRE) and an insulin regulatory sequence (IRS), which regulate the glucocorticoid and insulin responses. A third accessory site has been characterized by Scott et al. (15) and shown to bind chicken ovalbumin upstream promoter transcription factor and up-stream stimulatory factor. A single mutation in any one of these three AF elements results in a 60% decrease of the glucocorticoid response in hepatocytes, whereas a mutation in any two AF elements completely eliminates the glucocorticoid response of the PEPCK-C gene promoter mediated by the GRU (15). As suggested by the overlapping GRE and IRS within the AF2 element, the AF1, AF2, and AF3 are also required for other hormone responses. Both the AF1 (17) and AF3 contain GRE and retinoic acid response elements (15) and are necessary for the response of the PEPCK-C gene promoter to retinoic acid. The AF2 element
contains an IRS (18) that has been proposed to mediate part of the negative response of the gene to insulin (19).

The present study focuses on use of transgenic animals carrying a mutated AF2 element (in the PEPCK-C gene promoter −2000 to +73) linked to the human growth hormone structural gene (hGx), which lacks a receptor-binding site and thus is biologically inactive. We have analyzed the transcriptional response of the transgene to diabetes, insulin replacement, administration of glucocorticoids, and alterations in dietary protein and carbohydrate. The results suggest that the AF2 element of the GRU is necessary for induction of PEPCK-C mRNA by glucocorticoids during diabetes and that the effect of insulin on this site is modest at best.

EXPERIMENTAL PROCEDURES

Materials—Theophylline, Bt,cAMP, streptozotocin, and dexamethasone were purchased from Sigma. Strip-EZ DNA probe synthesis was purchased from Ambion (Austin, TX). QuickPrep total RNA extraction kit was purchased from Amersham Pharmacia Biotech. Gene Screen Plus and [α-32P]dTTP (3000 Ci/mmol) were purchased from PerkinElmer Life Sciences. Restriction enzymes and protease K were purchased from Roche Molecular Biochemicals. The synthetic diets (20) used in these studies were purchased from Nutritional Biochemical Corp. (Cleveland, OH). Human recombinant insulin was obtained from Calbiochem.

Molecular Probes—The PEPCK-C probe used was a 1.1-kilobase PotI fragment from the 3′-end of the PEPCK-C cDNA, as described previously (20). RNA was normalized with a 752-ng SacI fragment from the mouse 18 S rRNA cDNA as described by Oberbaumer (21). The 700-base pair Smal cDNA hGx fragment (22) was used to screen the hGx transgenic mouse eggs. Viable embryos were re-implanted into the oviducts of Super 57 BL6 × SJL females. On day 19 of gestation, the mice were delivered, and their livers and kidneys were removed for mRNA and analysis by Northern blotting. A, hepatic expression of WT-490/hGx, AF2−490/hGx, WT-2000/hGx, and AF2−2000/hGx is presented as the relative ratios of hGx mRNA normalized to 18 S rRNA. B, renal expression of WT-490/hGx, AF2−490/hGx, WT-2000/hGx, and AF2−2000/hGx is presented as the ratio of hGx mRNA to 18 S rRNA. The values are expressed as the mean ± the S.E. for 5 to 7 mice (*p < 0.05 relative to WT controls).

Generation of Transgenic Mice—Recombinant DNA used for microinjection was separated from plasmid sequences by digestion with XbaI for WT-2000/hGx and AF2−2000/hGx or XbaI-BglII for WT-490/hGx and AF2−490/hGx, followed by electrophoresis. DNA was electroeluted from the agarose gel and then used for microinjection into fertilized mouse eggs. The procedure for the generation of transgenic mice has been described previously (26). Briefly, fertilized eggs were flushed from the oviducts of super-ovulated C57BL/6 × SJL mice. 2 μl of DNA solution (2 ng/μl) was injected into the male pronuclei of the fertilized mouse eggs. Viable embryos were re-implanted into the oviducts of pseudo-pregnant mice as described previously (26). Transgenic mice were identified by genomic DNA analysis of tail samples taken at 4 weeks of age. Briefly, small segments of mouse tail were placed in a lysis buffer containing 50 mM KCl, 10 mM Tris-HCL, pH 8.3, 2.5 mM MgCl2, 0.01% gelatin, 0.45% Nonidet P-40, 0.45% Tween 20, and 24 μg of proteinase K overnight at 55 °C. 1 μl of this solution, containing the lysed tail cells, was used in a polymerase chain reaction to amplify the hGx gene. The primers used were hGH1, 5′-TTGCAAGCTAGTTGAGCT-3′; and hGH2, 5′-TTTTGGCCGTTGGGCGAAGT3′.

Germline Transmission—Six lines were generated for AF2 2000, four lines were generated for each of WT 390 and AF2 490 transgenes, and one line was generated for the WT 2000. All lines of transgenic mice demonstrated the tissue-specific pattern of expression characteristic of the endogenous PEPCK-C gene, with highest expression levels in liver and expression in the kidney ~20% that of hepatic expression. The transgenic mice were normal in body weight and showed no evidence of hepatomegaly or other disorders.

Hormonal and Dietary Treatments—Male mice heterozygous for the transgene were back-crossed to C57BL/6 × SJL WT females. Only mice heterozygous for the transgene were used for analysis. There were no gender differences noted in the level of expression of hGx mRNA, so both male and female mice heterozygous for the transgene were used.
During the concentration of blood glucose (tail vein) using glucose streptozotocin (200 mg/kg body weight). Mice were fasted overnight, and stored for analysis at later, and their livers and kidneys were isolated, frozen in liquid nitrogen, and stored at -70 °C. For dietary studies, the mice were killed 2 h later, and their livers and kidneys were isolated, frozen in liquid nitrogen, and stored for analysis at -70 °C.

Animals were made diabetic by a single intraperitoneal injection of streptozotocin (200 mg/kg body weight). Mice were fasted overnight prior to injection and fed 2 h later. Diabetes was confirmed by measuring the concentration of blood glucose (tail vein) using glucose dipsticks and an ENCORE® glucometer (VWR Scientific). Animals treated with insulin received 1 unit per 30 grams of body weight. All diabetic animals had blood glucose concentrations of more than 300 mg/dl. To determine the effect of glucocorticoids on the PEPCK-C gene promoter from livers of the same animals was measured by Northern blotting and normalized to 18 S rRNA. The values are expressed as the mean ± S.E. for 5 to 7 mice. A statistical comparison of the results is shown: *, p < 0.05 difference in the control values for hGx or PEPCK-C mRNAs as compared with the diabetic values; **, p < 0.05 difference in the diabetic values for hGx or PEPCK-C mRNAs as compared with values noted 8 h after insulin administration.

Adult animals were 2–4 months of age at the time of study; they were given free access to water and were fed standard laboratory chow, unless otherwise specified. Adult animals were killed between 9:00 and 11:00 a.m. Mice were injected intraperitoneally with 35 mg of Bt2cAMP per kg of body weight and 30 mg of theophylline per kg of body weight in 1 ml of saline or not injected (controls). The animals were killed 2 h later, and their livers and kidneys were isolated, frozen in liquid nitrogen, and stored for analysis at -70 °C.

Animals were made diabetic by a single intraperitoneal injection of streptozotocin (200 mg/kg body weight). Mice were fasted overnight prior to injection and fed 2 h later. Diabetes was confirmed by measuring the concentration of blood glucose (tail vein) using glucose dipsticks and an ENCORE® glucometer (VWR Scientific). Animals treated with insulin received 1 unit per 30 grams of body weight. All diabetic animals had blood glucose concentrations of more than 300 mg/dl. To determine the effect of glucocorticoids on the PEPCK-C gene promoter in the transgenic mice, the animals were injected with 1 μM dexamethasone (DEX). 5 h later the livers were collected, and mRNA was extracted and analyzed by Northern blotting. A, the level of hepatic hGx mRNA was normalized to 18 S rRNA for the WT-2000/hGx and AF2-2000/hGx mice. B, the level of hGx mRNA in the kidneys was normalized to renal 18 S rRNA in the WT-2000/hGx and AF2-2000/hGx mice. The values are presented relative to wild type control mice and expressed as the mean ± S.E. for 3 to 5 mice (*, p < 0.05 relative to WT controls).

Northern blot analysis was performed as described previously (29) using 20 μg of total RNA. Gels were blotted to GeneScreen Plus and hybridized with PEPCK-C cDNA or hGx cDNA probes at 42 °C in hybridization buffer containing 50% formamide (deionized), 1% SDS, 1 M sodium chloride, 110 μg/ml denatured salmon sperm, and 10% dextran sulfate. The filters were washed at 55 °C in 2× SSC and 0.1% SDS for 20 min and 1× SSC, 0.1% SDS for 20 min. The relative radioactivity was determined using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The concentration of PEPCK-C mRNA and hGx mRNA were expressed relative to the concentration of 18 S ribosomal RNA to control for differences in loading of the RNA.

**RESULTS AND DISCUSSION**

Effect of AF2 on the Developmental Expression of the PEPCK-C Gene Transgene—Male mice heterozygous for the transgene were back-crossed to C57BL/6 × SJL non-transgenic females. On day 19 of gestation, the livers and kidneys were removed from fetal mice, and the RNA was extracted and analyzed by Northern analysis using an hGx cDNA probe. The concentration of hGxs mRNA was expressed relative to 18 S ribosomal RNA.

**FIG. 3.** The effect of a mutation in the AF2 region of the PEPCK-C gene promoter on transcription from the promoter during diabetes and after the administration of insulin. WT-2000/hGx or AF2-2000/hGx transgenic mice (8–12 weeks of age) were either untreated (controls) or given an injection of 1 μM dexamethasone (DEX). 5 h later the livers were collected, and mRNA was extracted and analyzed by Northern blotting. A, the level of hepatic hGx mRNA was normalized to 18 S rRNA for the WT-2000/hGx and AF2-2000/hGx mice. B, the level of hGx mRNA in the kidneys was normalized to renal 18 S rRNA in the WT-2000/hGx and AF2-2000/hGx mice. The values are presented relative to wild type control mice and expressed as the mean ± S.E. for 3 to 5 mice (*, p < 0.05 relative to WT controls).

**FIG. 4.** The effect of a mutation in the AF2 region of the PEPCK-C gene promoter on the glucocorticoid stimulation of transcription from the promoter. WT-2000/hGx or AF2-2000/hGx transgenic mice (8–12 weeks of age) were either untreated (controls) or given an injection of 1 μM dexamethasone (DEX). 5 h later the livers were collected, and mRNA was extracted and analyzed by Northern blotting. A, the level of hepatic hGx mRNA was normalized to 18 S rRNA for the WT-2000/hGx and AF2-2000/hGx mice. B, the level of hGx mRNA in the kidneys was normalized to renal 18 S rRNA in the WT-2000/hGx and AF2-2000/hGx mice. The values are presented relative to wild type control mice and expressed as the mean ± S.E. for 3 to 5 mice (*, p < 0.05 relative to WT controls).
Chimeric genes containing the WT-2000, AF2–2000, and WT-490 promoters expressed very little hGx mRNA in the liver and kidney before birth. The AF2–490/hGx gene, however, had an unexpected high level of expression before birth in both the kidney and liver compared with WT (**, p < 0.05). This result demonstrates the importance of the AF2 element in the PEPCK-C gene promoter for the correct developmental expression of this gene before birth. The gene for PEPCK-C is not expressed in the liver before birth (30). The high levels of glucose from the maternal circulation repress expression of the gene in the fetal liver and the subsequent production of insulin by the fetal pancreas. At birth, there is a decrease in the concentration of glucose in the blood, accompanied by a decrease in secretion of insulin and an increase in hepatic cAMP (31); this initiates transcription of the gene for PEPCK-C in the liver. In the absence of the AF2 site, we noted a premature appearance of hGx mRNA in the livers of 19-day-old fetal mice. This occurred only when the AF2 mutation was in the PEPCK-C gene promoter from −490 to +73, suggesting that a region of the promoter between −490 and −2000 contains an element required to suppress the premature expression of PEPCK-C in the fetal liver. More work will be required to identify this region of the PEPCK-C gene promoter.

The Role of AF2 in the Regulation of PEPCK-hGx Gene Transcription during Diabetes—To dissect the role of AF2 in the response of the PEPCK-C gene to diabetes and to the subsequent administration of insulin, the animals were made diabetic by injection of streptozotocin (the blood glucose concentration was in excess of 300 mg/dl in these mice). In all animals, the endogenous gene for PEPCK-C responded to diabetes as expected, i.e., an increase in hGx mRNA; insulin reduced its expression by half when administered for 8 h. The difference in the time course of response to insulin may be attributable to differences in half-life between PEPCK-C and hGx mRNAs. Transcription of the AF2–2000/hGx transgene in the liver was not induced by diabetes but was inhibited 50% by insulin treatment (Fig. 3A). (**, p < 0.05). This finding strongly suggests that AF2 is necessary for the induction of PEPCK-C by diabetes but that it is not involved in the response of the PEPCK-C gene promoter to insulin.

The AF2 regulatory element in the PEPCK-C gene promoter contains a glucocorticoid-binding site, as well as an insulin regulatory element. Because glucocorticoids are required for the increase in PEPCK-C gene transcription that occurs during diabetes (12), we next determined the response of the AF2–2000/hGx transgene to glucocorticoids. Dexamethasone (1 μM) was administered to both WT-2000/hGx and AF2–2000/hGx transgenic mice; the animals were killed 5 h later. The administration of dexamethasone had no effect on the concentration of mRNA for hGx in the livers of WT-2000/hGx and AF2–2000/hGx transgenic mice (Fig. 4A). The lack of response of the transgenes to dexamethasone was expected, because it is well established that glucocorticoids cause an increase in the secretion of insulin (11); this insulin secretion would, in turn, prevent the induction of transcription from the PEPCK-C gene promoter (12). For this reason, we determined the response to glucocorticoids of both transgenes in the kidney. There was a 3-fold induction of hGx mRNA in the kidneys of AF2–2000/hGx transgenic mice, accompanied by a 5.5-fold induction of hGx mRNA in the kidneys of WT-2000/hGx mice (Fig. 4B) (**, p < 0.05). Thus the loss of one accessory factor 2-binding site resulted in a 60% reduction in the glucocorticoid response of the transgene. These data are consistent with the in vitro studies of Scott et al. (15, 32) showing a similar reduction in PEPCK-C gene transcription when one accessory factor 2 element of the GRU was deleted.

We have shown that the AF2-binding site in the PEPCK-C gene promoter is critical for the regulation of PEPCK-C mRNA by glucocorticoids. The AF2-binding site has an overlapping GRE and a putative IRS that can potentially bind C/EBPβ and HNF-4; both can then act as accessory factors for the glucocorticoid response. In animals with a deletion in the gene for C/EBPβ, the glucocorticoid response is totally blocked.2 C/EBPβ binds to the PEPCK-C gene promoter through the cAMP-response element-1 and P3(I) regions, both of which are downstream from the AF2-binding site and GRU. Yamada et al. (33) have determined that C/EBPβ is an accessory factor for the glucocorticoid response by acting via the cAMP-response element region in the PEPCK-C gene promoter. This suggests a complex mechanism for the regulation of PEPCK-C by glucocorticoids. Recently Leahy et al. (34) have stressed the role of CREB-binding protein in coordinating the regulation of the PEPCK-C promoter. CREB-binding protein can bind both steroids and C/EBPβ, which interact with the promoter. It is likely that the AF2 region interacts with the GR or with a protein that binds the GR. Here we note that the lack of an AF2 site results in a 60% reduction in the increase in hGx mRNA when stimulated with glucocorticoids. Thus, the absence of a binding site for AF2 may disrupt the ability to form the needed complex with CREB-binding protein and C/EBPβ.

Effect of the AF2 Regulatory Element on the Response of the PEPCK-C Gene Promoter to Alterations in the Carbohydrate Content of the Diet—Feeding mice a diet high in carbohydrate (or a high fat/carbohydrate-free diet) markedly decreases (or increases) the transcription of the gene for PEPCK-C in the liver (20). This effect is presumably because of the secretion of insulin by the pancreas that in turn represses hepatic PEPCK-C gene transcription. To determine whether the AF2 regulatory element in the PEPCK-C gene promoter is involved in the response of the gene to dietary carbohydrate, WT-2000/
hGX and AF2–2000/hGX transgenic mice were fed a high carbohydrate diet for 1 week. This diet did not significantly alter the blood glucose concentration of the animals. Control mice fed a normal lab chow diet had a blood glucose level of 124 mg/dl ± 6.4, whereas mice fed a high carbohydrate diet had a blood glucose level of 114 mg/dl ± 12.9. The high carbohydrate diet dramatically decreased the level of hGX mRNA in the livers of both the WT-2000/hGX and AF2–2000/hGX transgenic mice. This demonstrates that the AF2 site in the PEPCK-C gene promoter is not required for the transcriptional response of the promoter to dietary carbohydrate and presumably to insulin (Fig. 5). Mice that were fed the high carbohydrate diet for 1 week were then injected with Bt,cAMP and theophylline to stimulate transcription from the PEPCK-C gene promoter. There was no difference in the response to Bt,cAMP between mice that were fed the high carbohydrate diet. Alternatively, the AF2 site is adjacent to a PPAR-binding domain that maps at 451 to 439 of the liver (37). Therefore, in the absence of the AF2 site, PPARα might be less effective in inducing PEPCK-C gene transcription. Clearly, further work is needed to establish the exact effect of a high fat/carbohydrate-free diet on the control of PEPCK-C gene transcription.

Acknowledgments—We are indebted to Dr. Ifeanyi J. Arinze for critically reading the manuscript and to Jianqi Yang for help in preparing the figures.

REFERENCES
1. Hanson, R. W., and Patel, Y. M. (1994) Adv. Enzymol. Relat. Areas Mol. Biol. 65, 263–281.
2. Lamers, W. H., Hanson, R. W., and Meisner, H. M. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 5317–5321.
3. Liu, J., Park, E. A., Gurney, A. L., Roessler, W. J., and Hanson, R. W. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6482–6486.
4. Sasaki, K., Crispe, T. P., Koch, S. R., Andronis, L. L., Peterson, D. D., Beale, E. G., and Granner, D. K. (1984) J. Biol. Chem. 259, 15242–15251.
5. Inoue, M., Stromstedt, P. E., Quinn, P. G., Carlstedt-Duke, J., Gustafsson, J., and Granner, D. K. (1990) Mol. Cell. Biol. 10, 4712–4719.
6. Granner, D. K., Andronis, T., Sasaki, K., and Beale, E. (1987) Nature 325, 549–551.
7. Forest, C. D., O'Brien, R. M., Lucas, P. C., Magnuson, M. A., and Granner, D. K. (1990) Mol. Endocrinol. 4, 1302–1310.
8. Scott, D. K., O'Doherty, R. M., Stafford, J. M., Newgard, C. B., and Granner, D. K. (1998) J. Biol. Chem. 273, 12445–12451.
9. Short, J. M., Wynshaw-Boris, A., Short, H. P., and Hanson, R. W. (1986) J. Biol. Chem. 261, 9721–9726.
10. Hanson, R. W., and Reshef, L. (1997) Annu. Rev. Biochem. 66, 581–611.
11. Gunn, J. M., Hanson, R. W., Meyshaus, O., Reshef, L., and Ballard, F. J. (1975) Biochem. J. 150, 195–203.
12. Friedman, J. E., Yun, J. S., Patel, Y. M., McGrane, M. M., and Hanson, R. W. (1995) J. Biol. Chem. 268, 12952–12957.
13. Granner, D. K., and O'Brien, R. M. (1992) Diabetes Care 15, 369–385.
14. Liu, J., Distefano, C., and Zaret, K. S. (1991) Mol. Cell. Biol. 11, 773–784.
15. Scott, D. K., Mitchell, J. A., and Granner, D. K. (1996) J. Biol. Chem. 271, 31909–31914.
16. Wang, J., Stromstedt, P., O'Brien, R., and Granner, D. (1996) Mol. Endocrinol. 10, 794–800.
17. Hall, R. K., Sladec, F. M., and Granner, D. K. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 412–416.
18. O'Brien, R. M., Lucas, P. C., Forest, C. D., Magnuson, M. A., and Granner, D. K. (1990) Sci. 249, 533–537.
19. O'Brien, R. M., Noisou, E. L., Szuwanicki, A., Yamashita, T., Lucas, P. C., Wang, J. C., Powell, D. R., and Granner, D. K. (1995) Mol. Cell. Biol. 15, 1147–1158.
20. McGrane, M. M., deVente, J., Yun, J., Bloom, J., Park, E. A., Wynshaw-Boris, A., Wagner, T., Rottman, F. M., and Hanson, R. W. (1988). J. Biol. Chem. 263, 11443–11451.
21. Oberbaumer, I. (1990) Nucleic Acids 20, 671–677.
22. Spremanti, I., Romani, L., Masiani, P., Modesti, A., Fattori, E., Lazzaro, D., Sellitto, C., Scarpa, S., Bellavita, D., Lattanzio, G., Bistoni, F., Frati, L., Cortese, F., Gutino, A., Cliberti, G., Costanzini, F., and Poli, V. (1995) EMBO J. 14, 1932–1941.
23. Kunkel, T. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 488–492.
24. Liu, J., Roessler, W. J., and Hanson, R. W. (1990) Biotechniques 9, 738–741.
25. Iida, R. L., Behringer, R. R., Theisen, M., Huggenvik, J. I., McKnight, G. S., and Brinster, R. L. (1989) Mol. Cell. Biol. 9, 5154–5162.
26. Patel, Y. M., Yun, J. S., Liu, J., McGrane, M. M., and Hanson, R. W. (1994) J. Biol. Chem. 269, 5619–5627.
27. McGrane, M. M., deVente, J., Yun, J., Bloom, J., Park, E. A., Wynshaw-Boris, A., Wagner, T., Rottman, F. M., and Hanson, R. W. (1988). J. Biol. Chem. 263, 11443–11451.
28. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
29. Eisenberger, C. L., Nechustan, H., Cohen, H., Shanti, M., and Reshef, L. (1993) Mol. Cell. Biol. 13, 1036–1043.
30. Ballard, F. J., and Hanson, R. W. (1987) Biochem. J. 240, 866–871.
31. Girard, J. R., Cuenet, G. S., Marlsie, E. B., Kervran, A., Rieutort, M., and Assan, R. (1973) J. Clin. Invest. 52, 3190–3200.
32. Scott, D. K., Stromstedt, P. E., Wang, J. C., and Granner, D. K. (1998) Mol. Endocrinol. 12, 482–491.
33. Yamada, K., Duong, D. T., Scott, D. K., Wang, J. C., and Granner, D. K. (1999) J. Biol. Chem. 274, 5580–5585.
34. Leahy, P., Crawford, D. R., Grosman, G., Chaulbry, F., Gronostajski, R., and Granner, D. K. (1999) J. Biol. Chem. 274, 8813–8822.
35. Hasty, A. H., Shimano, H., Yahagi, T., Shimokura, K., Naka, M., Sato, S., Yoshikawa, T., Osuga, T., Okazaki, H., Tamura, Y., Iizuka, Y., Shinohara, F., Ohashi, K., Harada, K., Gotoda, T., Nagai, R., Ishibashi, S., and Yamada, N. (2000) J. Biol. Chem. 275, 31069–31077.
36. Tontonoz, P., Hu, E., Devine, J., Beale, E. G., and Spiegelman, B. M. (1995) Mol. Cell. Biol. 15, 351–357.
37. Keller, H., Dreyer, C., Medin, J., Mahfoudi, A., Ozato, K., and Wahl, W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2160–2164.

K. Chakravarty, P. Ferre, F. Foufelle, and R. W. Hanson, unpublished data.
