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ISOLATION OF A cDNA FOR THE CATALYTIC SUBUNIT OF RAT LIVER GLUCOSE-6-PHOSPHATASE: REGULATION OF GENE EXPRESSION IN FAO HEPATOMA CELLS BY INSULIN, DEXAMETHASONE AND cAMP[1]

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cDNA clones coding for the catalytic subunit of rat liver glucose-6-phosphatase (EC 3.1.3.9) were isolated from a rat liver cDNA library in lambda gt_{11} phage. The sequence of the cDNA and the amino acid sequence derived from it were greater than 90% identical to the corresponding sequences for the mouse and human forms of liver glucose-6-phosphatase. Northern blot analysis of RNA from FAO hepatoma cells revealed that dexamethasone induced the glucose-6-phosphatase mRNA while insulin suppressed its expression. When both hormones were added together insulin completely suppressed the effect of glucocorticoid. cAMP addition alone decreased the abundance of glucose-6-phosphatase mRNA. The results demonstrate multihormonal regulation of gene expression of hepatic glucose-6-phosphatase and support a dominant role for insulin.

Glucose-6-phosphatase (Glu-6-Pase), a key enzyme in the homeostatic regulation of blood glucose concentration, catalyses the terminal step in gluconeogenesis (1). Liver Glu-6-Pase activity is increased in starved, diabetic and glucocorticoid-treated adrenalectomized rats (2-5). Refeeding of starved rats and insulin administration to diabetic rats decreased Glu-6-Pase activity (2-5). However, it has not been possible to ascertain whether these changes in Glu-6-Pase activity were due to modulation of gene expression or to define which hormones were responsible for the changes because this protein had been recalcitrant to cloning. Recently, the catalytic subunit of mouse and human liver Glu-6-Pase has been cloned (6,7). It was the object of this study to isolate the rat liver Glu-6-Pase cDNA and use it to analyze the effect of insulin, dexamethasone, and cAMP on mRNA abundance by Northern blot analysis in

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FAO cells, a rat hepatoma cell line which can produce glucose from 3-carbon precursors (8). The derived amino acid sequence of the rat liver Glu-6-Pase clone was also determined and compared to those of the mouse and human liver enzyme.

EXPERIMENTAL PROCEDURES

Materials - Restriction endonucleases and polynucleotide kinase were from New England Biolabs (Beverly, MA). M-MLV-reverse transcriptase and RPMI media were from Gibco - BRL (Bethesda, MD). [γ-^{32}P]ATP was from New England Nuclear - Dupont (Wilmington, DE). Hybond N^+ nylon filters were from Amersham, Inc. (Chicago, IL). Automated sequencing kit was from ABI. Sodium cacodylate and 8-(4-chlorophenylthio)-adenosine 3':5'-cyclic monophosphate (CPT-cAMP) were from Sigma (St. Louis, MO). All other reagents were of the highest quality and obtained from Fisher Scientific (Pittsburgh, PA).

Molecular Cloning and Extention by PCR - Two oligonucleotides (45mers) based on the mouse liver Glu-6-Pase cDNA sequence were synthesized to screen a λgt11 cDNA library from Clontech (Irvine, CA): 1) AGGAAGGAATGAACATTCTCCATGA-CTTTGGGATCCAGTCGACTC in the forward direction begining just after the initiating methionine, and 2) ACTGCCTAGCCCGGATCCTGGGACAGACACACAAGAAGTCTTTG ending just before the translation stop codon. Oligonucleotides were end-labelled with $^{32}PO_4$ using T_4 polynucleotide kinase. Unincorporated counts were removed by centrifuging through spin columns. N^+-Hybond nylon filters were hybridized in the presence of labeled oligonucleotides overnight in 50% formamide, 1X SSPE, and 0.05 mg/ml salmon sperm DNA. Filters were washed 3 times in 0.1X SSC, 5 mM Na pyrophosphate, 1% SDS for 20 mins at 42°C. Sequential PCR was carried out using three nested reverse downstream primers (CCAGCCCCAACCCCAAGAGGG, GAAGAGGCTGGCAAAGGGTGT, and CAGGAGGTCCACCCCTAGCCC) which were synthesized based on the partial rat liver Glu-6-Pase and two nested forward primers (CGGGGCCAACCGGGCTTGGAC and TCACTGCACGGGCTCTGCTGG) based on the sequence of the mouse cDNA at the 5' terminus. The template for PCR was reverse-transcribed (M-MLV-RT) liver RNA prepared from 48 hr fasted rats. PCR products were analysed by Southern blot using end-labelled probes made from oligonucleotides internal to the primers used for PCR. PCR products which gave signal by Southern blot analysis were run out on agarose gels, excised, and extracted with glass milk (Qiaex, Qiagen Inc.). The purified DNA was sequenced directly on an ABI model 373A autosequencer primed with the same oligonucleotides used for PCR.

Tissue Culture - FAO cells, derived from a rat hepatoma cell line (8), were maintained on RPMI media containing 10% bovine fetal serum. For hormone treatments cells were maintained in RPMI media without bovine fetal serum containing 0.1% Albumin.

Preparation of mRNA and Northern Blot Analysis - mRNA was prepared from plated FAO cells and rat liver by extraction with STAT-60, following the protocol of the manufacturer (Tel-Test "B", Inc., Friendswood, TX). 20 µg of total RNA was electrophoresed per lane in a 0.8% agarose, 2.2M formaldehyde gel using a buffer containing 50 mM Boric Acid, 5 mM Na Borate, 1 mM Na Citrate, pH 8.0. RNA was denatured with 20 mM methyl mercury hydroxide for 10 min at room temperature before loading. RNA was transferred to a nylon membrane by capillary action, overnight. Northern blot lanes were scanned for UV absorption and varied less than 10%. Rat liver Glu-6-Pase cDNA fragment was isolated by digestion of pBS/G6Pase/7.1 and electrophoresis on 1% agarose gel. The fragment was extracted and labelled by the random primer method using Multi-Prime Kit by Boehringer-Mannheim. Nylon membrane was hybridized with labelled probe overnight in a buffer containing, 7% PEG, 0.5 M NaCl, 100 µg/ml Salmon Sperm DNA, 50% formamide, and 7% SDS at 42°C and then washed three times for 20 min each with 0.1XSSC and 0.1% SDS at 55°C (9). Autoradiographs were scanned on a BioRad Imaging Densitometer Model GS-670 and analyzed with the BioRad Molecular Analyst Software package.

Glu-6-Pase Activity Determinations - Gluc-6-Pase activity was determined by measuring production of P_i by the method of Ames (10) with a modification by

Bickerstaff and Burchell (11). Briefly, sonicated cells were incubated in 20 mM G-6-P, 50 mM Tris-Cacodylate, pH 6.5 in a final volume of 100 µl for 10 min at 30°C. The reaction was stopped with the addition of 900 µl of working solution (1M H_2SO_4, 5% Am Molybdate, 1% SDS, 1% Ascorbate) and incubated at 45°C for 20 min. O.D. was read at 820 nm. Activities are expressed as nanomoles formed per minute per mg total protein.

Protein Determinations - Protein concentration was determined by the BCA protein assay reagent from Pierce Chemical Co. using the enhanced protocol and bovine serum albumin as a standard.

RESULTS

Isolation of Rat Liver Glu-6-Pase cDNA - Two cDNA clones were isolated from a λgt_{11} cDNA library, λG6P-7.1 and λG6P-8.1, containing 1.1 and 1.4 Kb of sequence, respectively. Clone λG6P-7.1 spanned from bp 250 to bp 1375 of the mouse Glu-6-Pase sequence. Clone λG6P-8.1 included an additional 300 bp homologous to the mouse sequence on the 3' end. The 200 bp on the 5' end of clone λG6P-8.1 were not homologous to the corresponding mouse sequence.

The rat liver Glu-6-Pase sequence of clone λG6P-7.1 was extended 30 bp 5' of the initiating codon by PCR. PCR was done between bp 251 of the mouse sequence using 3 nested downstream reverse primers based on the rat sequence and 2 nested upstream forward primers based on the 5' mouse sequence. The template for PCR was reversed transcribed liver RNA from 48 hr fasted rats. This reaction generated a full coding length rat sequence starting 30 base pairs upstream of the initiating methionine codon. The nucleotide sequence and derived amino acid sequence are shown in Figure 1. Figure 1 shows the 3' untranslated region of clone pG6P-7.1, which diverges (38% identity)from the mouse sequence (6) at base pair 1240 (rat) but the last 85 base pairs (1308-1393) of the rat sequence are 78% identical to a region of the mouse sequence located 475 base pairs downstream. The 3' untranslated region of the other rat liver clone (pG6P-8.1) has an overall 75% identity to the mouse sequence and ends 528 base pairs from the end of the mouse sequence. These differences indicate that there are at least two different forms of the rat Glu-6-Pase mRNA.

Figure 2 is an alignment of the human, mouse, and rat liver Glu-6-Pase derived amino acid sequences. The six putative transmembrane spanning domains and the glycosylation sites, $N_{96}SS$, $N_{203}AS$, and $N_{276}SS$, are all conserved across the three species (6,7). Conservation of sequence is very high with the human and mouse sequences being 92 and 95% identical to the rat sequence, respectively. Extensive biochemical and genetic studies were performed to establish the mouse and human clones as those for Glu-6-Pase (6,7), and the high degree of identity of the rat cDNA reported here and its derived amino acid sequence with the mouse and human sequences establish this clone as that for the rat liver Glu-6-Pase.

Effect of Hormones and cAMP on Rat Liver Glu-6-Pase mRNA Abundance and Enzymic Activity in FAO Hepatoma Cells - The abundance of rat liver Glu-6-Pase message (approximately 2.4 Kb) was determined by Northern Blot analysis in order

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cagcttcctgaggtaccaaggaggaaggATGGAGGAAAGAATGAACGTGCTCCACGACTT     60
                             M  E  E  R  M  N  V  L  H  D  F      11

TGGGATCCAGTCGACTCGCTACCTCCAAGTGAATTACGAAGACTCCCAGGACTGGTTTGT    120
 G  I  Q  S  T  R  Y  L  Q  V  N  Y  E  D  S  Q  D  W  F  V     31

CTTGGTGTCTGTGATCGCTGACCTCAGGAACGCCTTCTATGTCCTCTTTCCCATCTGGTT    180
 L  V  S  V  I  A  D  L  R  N  A  F  Y  V  L  F  P  I  W  F     51

CCACATTCAAGAGACTGTGGGCATCAATCTCCTCTGGGTGGCAGTGGTTGGAGACTGGTT    240
 H  I  Q  E  T  V  G  I  N  L  L  W  V  A  V  V  G  D  W  F     71

CAACCTCGTCTTTAAGTGGATTCTTTTTGGACAGCGCCCGTATTGGTGGGTCCTGGACAC    300
 N  L  V  F  K  W  I  L  F  G  Q  R  P  Y  W  W  V  L  D  T     91

TGACTATTACAGCAACAGCTCCGTGCCTCTGATAAAACAGTTCCCCGTCACCTGTGAGAC    360
 D  Y  Y  S  N  S  S  V  P  L  I  K  Q  F  P  V  T  C  E  T    111

TGGACCAGGGAGTCCCTCGGTCCATGCCATGGGCACAGCAGGTGTATACTACGTTATGGT    420
 G  P  G  S  P  S  V  H  A  M  G  T  A  G  V  Y  Y  V  M  V    131

TACTTCTACTCTCGCTATCTTTCGTGGAAAGAAAAAGTCAACGTATGGATTCCGGTGCTT    480
 T  S  T  L  A  I  F  R  G  K  K  K  S  T  Y  G  F  R  C  L    151

GAATGTCGTCTTGTGGTTGGGATACTGGGCTGTGCAGCTGAACGTCTGTCTGTCCCGGAT    540
 N  V  V  L  W  L  G  Y  W  A  V  Q  L  N  V  C  L  S  R  I    171

CTACCTTGCGGCTCACTTTCCCCATCAGGTGGTGGCTGGAGTCTTGTCAGGCATTGCTGT    600
 Y  L  A  A  H  F  P  H  Q  V  V  A  G  V  L  S  G  I  A  V    191

GGCTGAAACTTTCAGCCACATCCGGGGCATCTACAATGCCAGCCTCCAGAGGTACTGTCT    660
 A  E  T  F  S  H  I  R  G  I  Y  N  A  S  L  Q  R  Y  C  L    211

CATCACCTTCTTCCTGTTTGGTTTCGCACTTGGATTTTACCTGCTGCTAAAGGGGCTAGG    720
 I  T  F  F  L  F  G  F  A  L  G  F  Y  L  L  L  K  G  L  G    231

GGTGGACCTCCTGTGGACTTTGGAGAAGGCCAAGAGATGGTGTGAGCGCCCCGAGTGGGT    780
 V  D  L  L  W  T  L  E  K  A  K  R  W  C  E  R  P  E  W  V    251

CCACCTTGACACTACACCCTTTGCCAGCCTCTTCAAAAACCTGGGGACCCTCTTGGGGTT    840
 H  L  D  T  T  P  F  A  S  L  F  K  N  L  G  T  L  L  G  L    271

GGGGCTGGCCCTCAACTCCAGCATGTACCGCAAGAGCTGCAAAGGAGAACTGCGCAAGTC    900
 G  L  A  L  N  S  S  M  Y  R  K  S  C  K  G  E  L  R  K  S    291

GCTCCCATTCCGTTTGGCCTGCATCGTGGCCTCCTTGGGCCTGCTGCATCTCTTTGACTC    960
 L  P  F  R  L  A  C  I  V  A  S  L  G  L  L  H  L  F  D  S    311

GCTGAAGCCCCCGTCCCAGATTGAGTCGATCTTCTACATCTTGTCTTTCTGCAAGAGTGC   1020
 L  K  P  P  S  Q  I  E  S  I  F  Y  I  L  S  F  C  K  S  A    331

GACCGTCCCCTTTGCATCTGTCAGTCTTATCCCCTACTGCCTAGCCCGGCTCCTGGGACA   1080
 T  V  P  F  A  S  V  S  L  I  P  Y  C  L  A  R  L  L  G  Q    351

GACACACAAGAAGTCTTTGtaaagcgttaagtctctgacacaagtcagcggccgtgcaaa   1140
 T  H  K  K  S  L  *                                           357

ggactaggagcaacccaagccttgtgcaacccagtgtggggccagagtagttcacagcca   1200
ccctggtagccctgtctttctttgccatcgtaaccacaaaggtctgcgtgccataggact   1260
catcactgcttccctgaccacgctgctggaccctgcattgctgaaccccatgctgctgga   1320
ccctgtggtgtacggtgggcaattgtttgctggtgcttttgtagggttaagataaactct   1380
gagatcttgggcc                                                  1393
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Figure 1. **Sequence of rat liver glucose-6-phosphatase.** Noncoding bases are lowercase and coding bases are uppercase. The derived amino acid is aligned with first base of each codon.

to ascertain whether the expression of the gene in FAO cells was under hormonal control. FAO cells were chosen as an experimental model system since these cells, in contrast to many hepatoma cell lines, can produce glucose from three carbon precursors (Argaud, et al., unpublished results, and Ref. 12), which implies that they express Glu-6-Pase. FAO cells were grown to confluence and incubated with insulin [1 μM], dexamethasone [1 μM] and the cAMP analog CPT-cAMP [100 μM] for 24 hrs, individually or in combination. Cells were incubated in

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           1                                                   50
  Hg6pase  MEEGMNVLHD FGIQSThYLQ VNYQDSQDWF ILVSVIADLR NAFYVLFPIW
  Mg6pase  MEEGMNILHD FGIQSTRYLQ VNYQDSQDWF ILVSVIADLR NAFYVLFPIW
  Rg6pase  MEErMNVLHD FGIQSTRYLQ VNYeDSQDWF VLVSVIADLR NAFYVLFPIW
Consensus  MEEGMNVLHD FGIQSTRYLQ VNYQDSQDWF ILVSVIADLR NAFYVLFPIW

           51                                                  100
  Hg6pase  FHLQEaVGIk LLWVAVIGDW LNLVFKWILF GQRPYWWVLD TDYYSNtSVP
  Mg6pase  FHLkETVGIN LLWVAVVGDW FNLVFKWILF GQRPYWWVLD TDYYSNSSVP
  Rg6pase  FHiQETVGIN LLWVAVVGDW FNLVFKWILF GQRPYWWVLD TDYYSNSSVP
Consensus  FHLQETVGIN LLWVAVVGDW FNLVFKWILF GQRPYWWVLD TDYYSNSSVP

           101                                                 150
  Hg6pase  LIKQFPVTCE TGPGSPSGHA MGTAGVYYVM VTSTLsIFqG KiKPTYrFRC
  Mg6pase  iIKQFPVTCE TGPGSPSGHA MGaAGVYYVM VTSTLAIFRG KKKPTYGFRC
  Rg6pase  LIKQFPVTCE TGPGSPSvHA MGTAGVYYVM VTSTLAIFRG KKKsTYGFRC
Consensus  LIKQFPVTCE TGPGSPSGHA MGTAGVYYVM VTSTLAIFRG KKKPTYGFRC

           151                                                 200
  Hg6pase  LNVILWLGFW AVQLNVCLSR IYLAAHFPHQ VVAGVLSGIA VtETFSHIhs
  Mg6pase  LNVILWLGFW AVQLNVCLSR IYLAAHFPHQ VVAGVLSGIA VAETFSHIRG
  Rg6pase  LNVVLWLGYW AVQLNVCLSR IYLAAHFPHQ VVAGVLSGIA VAETFSHIRG
Consensus  LNVILWLGFW AVQLNVCLSR IYLAAHFPHQ VVAGVLSGIA VAETFSHIRG

           201                                                 250
  Hg6pase  IYNASLkKYf LITFFLFsFA iGFYLLLKGL GVDLLWTLEK AqRWCEqPEW
  Mg6pase  IYNASLrKYC LITiFLFGFA LGFYLLLKGL GVDLLWTLEK AKRWCERPEW
  Rg6pase  IYNASLqrYC LITFFLFGFA LGFYLLLKGL GVDLLWTLEK AKRWCERPEW
Consensus  IYNASL-KYC LITFFLFGFA LGFYLLLKGL GVDLLWTLEK AKRWCERPEW

           251                                                 300
  Hg6pase  VHiDTTPFAS LLKNLGTLFG LGLALNSSMY ReSCKGkLSK wLPFRLssIV
  Mg6pase  VHLDTTPFAS LFKNLGTLLG LGLALNSSMY RKSCKGELSK SFPFRFACIV
  Rg6pase  VHLDTTPFAS LFKNLGTLLG LGLALNSSMY RKSCKGELrK SLPFRLACIV
Consensus  VHLDTTPFAS LFKNLGTLLG LGLALNSSMY RKSCKGELSK SLPFRLACIV

           301                                                 350
  Hg6pase  ASLVLLHvFD SLKPPSQVEL VFYVLSFCKS AvVPLASVSv IPYCLAqVLG
  Mg6pase  ASLVLLHLFD SLKPPSQVEL IFYILSFCKS ATVPFASVSL IPYCLARILG
  Rg6pase  ASLgLLHLFD SLKPPSQIEs IFYILSFCKS ATVPFASVSL IPYCLARILG
Consensus  ASLVLLHLFD SLKPPSQVEL IFYILSFCKS ATVPFASVSL IPYCLAR-LG

           351
  Hg6pase  QpHKKSL*
  Mg6pase  QTHKKSL*
  Rg6pase  QTHKKSL*
Consensus  QTHKKSL-
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Figure 2. **Alignment of derived rat, mouse and human amino acid sequences.** Sequences are denoted: Hg6pase, human liver Glu-6-Pase; Mg6pase, mouse liver Glu-6-Pase; Rg6pase, rat liver Glu-6-Pase. Lower case symbols represent non-conservative differences in amino acid sequence.

the absence or presence of glucose (25 mM). In cells incubated in the presence of glucose, dexamethasone increased the Glu-6-Pase mRNA abundance 10-fold over the basal level and insulin decreased it to non-detectable levels. In cells incubated with both dexamethasone and insulin, insulin completely suppressed the increase seen with dexamethasone alone. Addition of CPT-cAMP decreased mRNA abundance only slightly (25%) versus control but did not suppress the stimulatory effect of dexamethasone. However, CPT-cAMP opposed the action of insulin to suppress Glu-6-Pase mRNA. When FAO cells were incubated in the absence of glucose, there was a large response to dexamethasone and insulin completely blocked the glucocorticoid effect, but the mRNA abundance was very

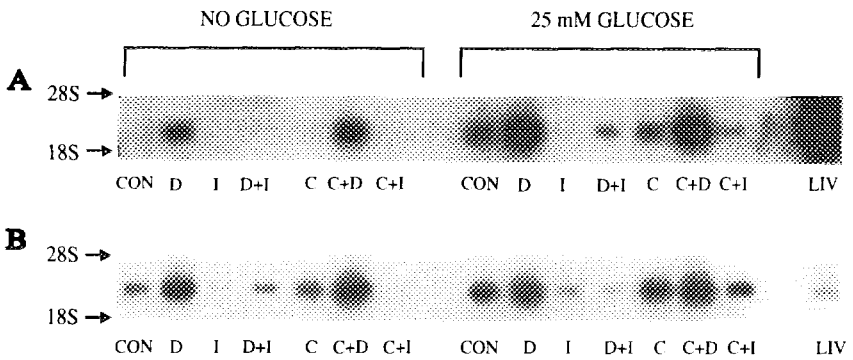

Figure 3. **Northern Blot analysis.** Autoradiograph images of FAO cell Glu-6-Pase mRNA (A), and PEPCK mRNA (B). FAO cells were incubated with hormones for 24 hr. Conditions are Control (CON), 1μM dexamethasone (D), 1μM insulin (I), 1μM dexamethasone plus 1μM insulin (D+I), 100μM CPT-cAMP (C), 100μM CPT-cAMP plus 1μM dexamethasone (C+D), 100μM CPT-cAMP plus 1μM insulin (C+I), and rat liver (LIV). Arrows designate 28S and 18S ribosomal RNA bands, Glu-6-Pase mRNA and PEPCK mRNA sizes were approximately 2.4 and 2.6 Kb, respectively. Images shown are representitive data of 3 experiments, all of which gave identical results.

low in the basal state compared to that in cells incubated in glucose containing media.

Glu-6-Pase activity increased from 15±2 nanomoles/mg protein/min to 29±3 nanomoles/mg protein/min, approximately 2-fold, in extracts of cells incubated in the presence of dexamethasone, and this increase in activity was not seen when cells were incubated with both dexamethasone and insulin. No differences in activity were detected in extracts from cells incubated with CPT-cAMP alone (data not shown).

Changes in phosphoenolpyruvate carboxykinase (PEPCK) mRNA abundance were also determined from the same set of hormonal conditions. PEPCK, like Glu-6-Pase is a key enzyme in the gluconeogenic pathway and changes in its gene expression under various hormonal conditions have been characterized in hepatoma cells (13). Figure 3 shows that mRNA abundance was increased by 8-fold by addition of dexamethasone, suppressed by 95% by insulin alone, and that insulin suppressed the stimulatory effect of dexamethasone completely. CPT-cAMP induced (1.3-fold) PEPCK mRNA abundance, and the induction was suppressed by insulin. These observations agree well with published reports on the hormonal regulation of PEPCK (13) and indicate that both genes for these gluconeogenic enzymes are regulated reciprocally by dexamethasone and insulin, but that their response to cAMP is different.

DISCUSSION

Until the recent cloning of mouse and human liver Glu-6-Pases, this had been the only key regulatory enzyme in the glycolytic/gluconeogenic pathway that had not been cloned or sequenced and for which there was little or no definitive

structure/function information. The nucleotide sequences of the cDNA for the catalytic subunit of mouse, human and rat liver Glu-6-Pase and their corresponding derived amino acid sequences have not, however, provided any immediate insight into structure/function relationships of this protein, since comparison of the nucleotide and deduced amino acid sequences with that in the databases revealed no identity to any sequence reported to date (6,7, and this report). The catalytic subunit of mammalian Glu-6-Pase catalyzes its reaction via a phosphohistidine enzyme intermediate (14,15). Pilkis, et al. (16), postulated that Glu-6-Pase was a member of the Fru-2,6-P_2ase/phosphoglycerate mutase/acid phosphatase family of enzymes. This family catalyzes phosphohydrolase reactions which are also mediated by phosphohistidine intermediates (17). All enzymes in this family have a characteristic RHG motif, surrounding the phospho-acceptor active site histidine. Glu-6-Pase sequences, however, do not contain this motif, nor is there any conservation of sequence with the Fru-2,6-P_2ase/mutase/acid phosphatase family, which strongly suggests that Glu-6-Pase is not a member of this family. This is not surprising since Glu-6-Pase is an integral endoplasmic reticulum membrane protein consisting of several transmembrane spanning domains and would not be expected to have the α/β structure of the soluble cytosolic enzymes of this family (17). The rat, human, and mouse Glu-6-Pase contain eight conserved histidines, one of which is presumably the phospho-acceptor histidine which mediates Glu-6-P hydrolysis. The availability of a full length cDNA for Glu-6-Pase will permit identification of the active site histidine and other substrate/product binding site residues by site directed mutagenesis, using a eukaryotic expression system. Such work is in progress.

Comparison of Glu-6-Pase mRNA abundance with and without glucose (25 mM) indicates that the level of expression of mRNA is much lower without glucose. One might expect that cells incubated in the absence of glucose would be in a gluconeogenic mode and therefore have elevated Glu-6-Pase. However these cells are probably not producing glucose since they were incubated without three carbon precursors. Gluconeogenic precursors and/or glucose may be necessary to trigger expression of Glu-6-Pase and PEPCK in these substrate starved cells. Further studies on the effect of three carbon precursors and glucose on Glu-6-Pase gene expression are in progress.

The slight decrease in Glu-6-Pase mRNA abundance seen with CPT-cAMP is in contrast to the positive effect of this second messenger on PEPCK (see Figure 3 and Ref.18) and Fru-1,6-P_2ase (19) gene expression. Further work is needed to determine whether this negative effect of CPT-cAMP on Glu-6-Pase mRNA abundance is also observed in primary hepatocytes and in vivo. Although CPT-cAMP had little effect on Glu-6-Pase gene expression when added alone, it inhibited the insulin suppression of mRNA. These opposing actions of insulin and cAMP are similar to those on PEPCK and Fru-1,6-P_2ase gene expression (5). The hormonal

effects of insulin and dexamethasone on the gene expression of Glu-6-Pase reported here in FAO cells are consistent with changes in activity levels reported in rat in vivo studies. For example, dexamethasone increased mRNA abundance in FAO cells and glucocorticoid administration to adrenalectomized rats increased liver Glu-6-Pase activity (3). Insulin decreased mRNA abundance in FAO cells and insulin treatment of streptozotocin-diabetic rats decreased Glu-6-Pase activity (4). The results presented indicate that the previously reported activity changes, in vivo, are due to changes in mRNA brought about by hormone-induced changes in gene expression. This report demonstrates that regulation of the Glu-6-Pase gene resembles that of other gluconeogenic enzyme genes. The finding that insulin is able to completely suppress the stimulatory effect of dexamethasone in FAO cells indicates that insulin plays a dominant negative role in the regulation of Glu-6-Pase gene expression and is likely to be the primary mediator of this regulation in vivo.

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