A glycerol-3-phosphate dehydrogenase null mutant in BALB/cHeA mice

Michal Prochazka, Ulrike C. Kozak, and Leslie P. Kozak
From The Jackson Laboratory, Bar Harbor, Maine 04609

A mutation in the BALB/cHeA subline of mice has resulted in loss of the glycerol-3-phosphate dehydrogenase (glycerol-P dehydrogenase) activity in adult tissues. Analysis of F2 offspring segregating for enzyme activity levels and a PvuII restriction fragment length polymorphism in the Gdc-1 gene on Chromosome 15 indicates that the mutation has occurred in or around the structural gene. Southern blot analysis failed to detect any major change due to chromosomal insertion, deletion, or rearrangement of the mutant gene. In vitro measurements of transcription in isolated nuclei failed to detect any difference in transcription between the BALB/cHeA and BALB/cBy strains of mice. However, Northern blot analysis with poly(A+) RNA isolated from brown fat shows that the mutant has about a 50-fold reduction in hybridizable transcripts of altered size. The data suggest that the mutation affected the processing of nuclear transcripts of glycerol-P dehydrogenase. The morphological, physiological, and reproductive characteristics of the mutant mice appear normal. This suggests that alternate pathways of energy metabolism and lipid synthesis exist which obviate the function of glycerol-P dehydrogenase.

The cytoplasmic NAD+-linked glycerol-3-phosphate dehydrogenase (EC 1.1.1.8), a dimeric enzyme with a molecular weight of 74,000, catalyzes the reversible reduction and oxidation of dihydroxyacetone phosphate and glycerol phosphate (1). Because of its pivotal position in linking energy metabolism and lipid synthesis, two primary functions have been attributed to the enzyme. In some tissues, such as muscle, with a high capacity for energy metabolism, the NAD+-linked glycerol-P dehydrogenase, together with the mitochondrial flavin-linked glycerol-P dehydrogenase, forms a cycle to transport cytosolic reducing equivalents into the mitochondria (2, 3). In other tissues, such as oligodendroglia and adipocytes, glycerol-P dehydrogenase would supply the glycerol phosphate precursor for phospholipid and triglyceride synthesis (4, 5). These varied metabolic functions for the glycerol-P dehydrogenase enzyme have resulted in extremely varied patterns of tissue expression (6). Several different hormones have been implicated in regulating gene expression (7–9). High levels of this enzyme and its mRNA are characteristic of certain differentiated cell types, especially adipocytes and glial cells (9–11). This interest in the cell-specific expression of the glycerol-P dehydrogenase gene has stimulated recent work on the cloning of the gene and on investigating mechanisms controlling its expression (12–15).

Recently, it was reported that glycerol-P dehydrogenase activity, measured by staining a gel with a histochemical method, could not be detected in liver extracts of a BALB/c subline of mice (17). Surprisingly, the phenotype of these mice appears normal. The purpose of this study was to assess whether a mutation in the structural gene, Gdc-1 on Chromosome 15, had occurred and to evaluate the effects of the mutation on mRNA expression.

MATERIALS AND METHODS

Mice—The colony of homozygous mutant BALB/cHeA mice at The Jackson Laboratory was originated from breeding pairs obtained from Dr. J. Hilgers, The Netherlands Cancer Institute, Amsterdam, The Netherlands. BALB/cByJ and DBA/2J mice were obtained from the Animal Resources Colonies at The Jackson Laboratory.

Reagents—Restriction enzymes, phenol, guanidinium HCl, and CsCl were purchased from Bethesda Research Laboratories. A cDNA clone, pL10.9, of 900 bp containing the coding sequence of glycerol-P dehydrogenase was kindly provided by Drs. Deborah Dobson and Bruce Spiegelman (16).

DNA and RNA Preparation—Genomic DNA was prepared from fresh spleens by a phenol extraction technique (18). Total RNA from liver, brown adipose tissue, and testis was prepared by a modification (19) of the guanidinium HCl/Sarkosyl extraction method (20). Poly(A)+ mRNA was purified as previously described (12).

Enzyme Analyses—The specific activity of glycerol-P dehydrogenase and the heat stability of the enzyme was determined as described previously (21).

In Vitro Nuclear Transcription—Liver and cerebellar nuclei were isolated as described by Schibler et al. (22). Nuclear run-on transcription, extraction of the labeled RNA, and hybridization to blots containing subcloned genomic fragments of the Gdc-1 gene were carried out according to Groudine et al. (23). Hybridizations were carried out for 4 days with 2 x 105 cpm/ml of probe. In addition, blots were digested with RNase after hybridization with 32P-labeled probe. The human a-tubulin clone used as a control was kindly provided by Dr. Winston Salser (24).

RNase Protection—The assay was performed with a riboprobe made from a 0.8-kb HindIII fragment containing exon 2 and parts of introns 1 and 2 of the glycerol-P dehydrogenase gene. Conditions for preparations of the 32P-labeled riboprobe and for the RNase mapping were essentially as described by Promega. RNA samples were hybridized with 1 x 106 cpm of probe. Both sense and antisense probes were synthesized for use in the RNase mapping and for probing Northern blots to confirm the orientation and specificity of the probes.

RESULTS

Enzyme Expression—The specific activity of glycerol-P dehydrogenase was measured in several tissues of neonatal
and adult mice (Table I). The values observed in the control BALB/cBy mice were typical of those previously observed for wild-type mice (21, 25). In contrast, the enzyme-specific activity in tissues from the BALB/cHeA mice was severely depressed. In some tissues, such as liver, no enzyme activity could be detected; however, other tissues such as cerebellum and white fat exhibited a very low level of activity in both neonatal and adult tissues. We attribute this low activity to expression of the Gdc-2 gene on the basis of the extreme heat sensitivity of this enzyme. The Gdc-2 gene encodes an iso-
zymic form of glycerol-P dehydrogenase which is expressed predominantly in fetal tissues (21). This pattern of expression was also consistent with previous observations which showed that Gdc-2 is expressed in neonatal white fat and brain, but not in fetal liver (11, 21).

**Genetic Analysis**—The genetic basis of the mutation causing the reduction in glycerol-P dehydrogenase activity was investigated in an intercross mating between normal DBA/2J and mutant BALB/cHeA mice. The DBA/2J mice had normal levels of glycerol-P dehydrogenase and a 3.2-kb PvuII fragment when hybridized with a cDNA probe derived from the 3'-untranslated region of the mRNA (Fig. 1). The BALB/cHeA mice had no adult isozyme expression and a PvuII fragment of 3.0 kb, the allele normally found in the BALB/c DNA. In F1 hybrids between BALB/cHeA and DBA/2J mice both DNA fragments were present and the hybrids expressed an intermediate level of enzyme activity in liver. In F2 offspring produced by an intercross of the F1 mice only three phenotype classes were found: the original, two parental phenotypes, and the F1 phenotype with no recombinant class (Table II). Accordingly, the mutation causing absence of enzyme activity occurred at a site in or near the Gdc-1 structural gene on Chromosome 15. The allele for this phenotype has been designated Gdc-1* (17).

**Analysis of Gene Structure**—We have attempted to determine whether the mutation to Gdc-1* involves a major change in the structure of the gene as might be caused by insertion, deletion, or rearrangement. This determination was made by Southern blots where genomic DNA was cut by several restriction endonucleases and then hybridized with DNA probes derived from the 5' and 3' end of the gene (Fig. 2). The comparison of DNA fragment sizes detected from the mutant and wild-type animals suggested that no differences were present within the limits of detection of the method. Accordingly, changes in the structure of the gene resulting from insertion or deletion of 100 or more base pairs are probably not the cause of the altered expression.

**mRNA Expression**—The initial analysis of mRNA expression by Northern blot analysis and by RNase mapping failed to detect any hybridization signal in total RNA from liver (data not shown). These hybridizations were performed with a riboprobe made from a 0.6-kb PsI/HindIII fragment con-

![FIG. 1. A Southern blot showing the PvuII restriction fragment length polymorphism detected with a 700-bp cDNA probe (probe B shown in Fig. 2).](image)

**TABLE I**

| Tissue       | Age | BALB/cBy | BALB/cHeA | P value |
|--------------|-----|----------|-----------|---------|
| Cerebral     | 4 days | 0.027 ± 0.003 | 0.012 ± 0.002 | 0.001 |
| cortex*      | 6-8 weeks | 0.095 ± 0.004 | 0.007 ± 0.002 | 0.001 |
| Liver        | 6-8 weeks | 0.200 ± 0.035 | Undetected | 0.001 |
| White fat    | 6-8 weeks | 0.275 ± 0.024 | 0.066 ± 0.005 | 0.001 |
| Cerebellum   | 4 days | 0.019 | 0.010 |
|              | 6-8 weeks | 0.440 | 0.009 |

*All activity measurements were performed on individual mice except for the cerebellum where measurements were made on a pool of tissue from five mice. Pairwise comparisons for statistical significance were done by the Student's t test.

**TABLE II**

| RFLP | Number of mice | Enzyme activity* |
|------|----------------|------------------|
| D (3.2 kb) | 11 | 18.9 ± 3.6 |
| C (3.0 kb) | 9 | 0.3 ± 0.3 |
| CD (3.0 plus 3.2 kb) | 25 | 7.9 ± 2.2 |

*Enzyme activities are expressed as units/g wet weight of liver. The values in the table are statistically indistinguishable from the enzyme activities for the parental DBA/2J, BALB/cHeA and F1 hybrid mice.

**FIG. 2.** Southern blot analysis of enzyme activity levels and restriction fragment length polymorphism (RFLP) in F2 offspring from a (DBA/2J × BALB/cHeA)F1 cross.
oncile the observed decrease in glycerol-P dehydrogenase mRNA with an apparent normal level of transcription, the Northern blot experiments were repeated with poly(A) RNA purified by oligo(dT)-cellulose chromatography from total cellular RNA isolated from the brown fat of cold-adapted mutant BALB/cHeA mice and normal BALB/cBy mice (Fig. 4A). Cold adaptation provides a level of glycerol-P dehydrogenase mRNA in brown fat which is approximately 20-fold higher than the level present in liver (6). As previously observed (12), glycerol-P dehydrogenase mRNA in BALB/cBy mice was present as a single molecular species which migrated at the position of 23 S ribosomal RNA of Escherichia coli. The hybridization signal of the glycerol-P dehydrogenase mRNA from BALB/cHeA mice differed both qualitatively and quantitatively from the wild-type BALB/cBy mice. The apparent size of the major RNA species from the wild-type mouse was approximately 3.4 kb, whereas the diffuse major band in the mutant mRNA preparation was approximately 3.2 kb. Also present in the mutant RNA were diffuse higher molecular weight RNA species with a pattern indicative of improperly processed nuclear RNA molecules (Fig. 4A). From the signal intensity of the band, we estimated that as much as 100 times less hybridizable RNA was present in the preparation from the mutant mice (Fig. 4B). The amount of α-tubulin RNA in the brown fat of the mutant and normal mice was virtually identical (Fig. 4C).

Since the hybridization signal on the Northern blot suggested that the glycerol-P dehydrogenase RNA in the mutant mouse does not have the same size as the normal BALB/cBy RNA (Fig. 4A), we performed an RNase protection assay to determine whether any normal sized fragments could be detected. Two fragments corresponding to the expected size (estimated from the migration of MspI cut pBR322 fragments end-labeled with Klenow) were the predominant protected fragments in normal BALB/cBy RNA (lanes 1, 2, and 3 of Fig. 5). The riboprobe used in this experiment was an 0.8-kb HindIII fragment, containing part of intron 1, exon 2, and part of intron 2 (see Fig. 2). We expected to detect a single protected fragment of 177 bp; however, two protected fragments were always observed irrespective of the source of RNA. These same fragments were also protected in the mutant BALB/cHeA RNA (lanes 4 and 5 of Fig. 5) with greatly reduced signal intensity. The results suggested that glycerol-P dehydrogenase transcripts containing a sequence corresponding to exon 2 were present in poly(A) RNA preparations from the mutant BALB/cHeA mice.

**Metabolic Consequences of the Mutations**—No obvious disease phenotype is associated with the mutation. Litter sizes and body weight were normal. However, the weights of some tissues were outside the normal range (17). Since brown fat has the highest known level of glycerol-P dehydrogenase expression, corresponding to 0.7% of total protein or mRNA (16), we expected that the enzyme served an essential role in thermogenesis. When mutant BALB/cHeA mice were housed at 5 °C, no adverse affect on the ability of the mice to thermoregulate could be detected.

**DISCUSSION**

A mutation in the Gdc-1 gene has occurred in BALB/cHeA. According to Hilgers et al. (17), the mutation in this BALB/c subline probably occurred since 1950. Mice homozygous for the mutant allele do not produce active enzyme nor is im-
Glycerol-3-phosphate Dehydrogenase

In vitro transcription. Isolated nuclei from 20 cerebella each of BALB/cBy and BALB/cHeA mice were incubated with [32P]UTP as described by Groudine et al. (23). 32P-Labeled RNA was isolated and used to probe a Southern blot containing an α-tubulin cDNA and the coding region of a glycerol-P dehydrogenase cDNA. Lanes 1, 3, and 5 contain 1 µg of α-tubulin cDNA in p-GEM2 vector cut with PstI to release a cDNA insert of 1.1 kb. Lanes 2, 4, and 6 contain 1 µg of a glycerol-P dehydrogenase cDNA clone, pL10.9, cut with EcoRI to release a 900-bp cDNA insert. Lanes 1 and 2 show the ethidium bromide-stained gel. Lanes 3 and 4 show an autoradiograph of hybridization to BALB/cHeA transcripts, and lanes 5 and 6 show the results of hybridization to the BALB/cBy transcripts. Films were exposed for 9 days with intensifying screens. Nonspecific hybridization to vector sequences was not observed.

munoreactive protein detected by Western blot. Since the level of hybridizable RNA in the mutant animal is approximately 2 orders of magnitude lower than in wild-type mice, the lack of protein is undoubtedly due to the lack of glycerol-P dehydrogenase mRNA. The mechanism resulting in the low level of mature cytoplasmic glycerol-P dehydrogenase mRNA is not clear. The fact that we have been unable to detect differences in the level of run-on transcripts in nuclei suggests that mechanisms controlling transcription of the gene are not impaired in the BALB/cHeA mutant. Subsequently, in looking for evidence of defects in RNA processing, we detected glycerol-P dehydrogenase transcripts with altered size at very low levels. The RNA transcripts are larger than the minor forms which can occasionally be seen on Northern blots of wild-type RNA (Fig. 4B, lane 2, and Ref. 16). One interpretation of this result is that glycerol-P dehydrogenase transcripts are abnormally processed in the mutant.

No abnormal sized DNA fragments from mutant DNA could be detected by Southern blot analysis suggesting that a chromosome rearrangement, deletion, or insertion is not the basis of the mutation. If a single base substitution was the cause of the phenotype, then more detailed DNA mapping or sequencing is required to identify the mutation. Since there is some confusion concerning the origin of various BALB/c sublines, finding a single base substitution would require additional expression analysis to prove the cause and effect relationship between a base change and the phenotype.

Fig. 3. Northern blot analysis of poly(A+) RNA isolated from the brown fat of cold-adapted BALB/cBy and BALB/cHeA mice. A, Lanes 1–4 contain 1, 0.2, 0.1, and 0.02 µg of poly(A+) RNA isolated from the brown fat of cold-adapted BALB/cBy mice. Lane 5 contains 20 µg of poly(A+) RNA isolated from the brown fat of BALB/cHeA mice. The blot in panel A was hybridized with a glycerol-P dehydrogenase probe containing exon 2 of the gene. B, a Northern blot containing 1 µg and 10 µg of poly(A+) RNA from BALB/cBy brown fat (lanes 1 and 2) and 1 µg and 10 µg of poly(A+) RNA from BALB/cHeA brown fat (lanes 3 and 4). The blot was hybridized to the same glycerol-P dehydrogenase probe as used in A. C, the blot in B was erased, although not completely for the lane containing 10 µg of BALB/cBy RNA, and hybridized to an α-tubulin probe. This blot indicates that equal amounts of intact RNA are on the blot of B.
The Gdc-I gene accounts for greater than 90% of the glycerol-P dehydrogenase enzyme activity in all adult tissues and is the only source of enzyme in liver and possibly kidney. It was therefore surprising that the absence of expression in tissues like liver and kidney has so little effect on the normal phenotype of the mouse. In Drosophila melanogaster, a large family of glycerol-P dehydrogenase mutants with varying levels of enzyme has been analyzed for viability and flight ability (26). Effects on these characteristics began to be seen when the level of enzyme dropped to around 5% of the wild-type. In one study, flies which were glycerol-P dehydrogenase-null and which had lost the ability to fly had reacquired this ability after another 20 breeding generations even though they were still null for glycerol-P dehydrogenase (27). It was argued that adaptive changes enabled the animals to develop a buffering system to protect the metabolism from loss of glycerol-P dehydrogenase function. Mechanisms that could substitute for loss of Gdc-1 expression in mice include the following.

1. The Gdc-2 gene which encodes an isozyme of glycerol-P dehydrogenase may be sufficiently active to provide the minimal amount of enzyme essential for survival.

2. The role of glycerol-P dehydrogenase in the synthesis of triglycerides may be assumed completely by the glycerol-P shuttle. Since the BALB/cHeA mutant mouse is the only source of enzyme in liver and possibly kidney, it seems that this shuttle is not essential. It may be that the high level of mitochondrial glycerol-P dehydrogenase is primarily involved in oxidation of the glycerol phosphate released by lipase action during utilization of the triglyceride pool (for reviews of brown fat metabolism, see Refs. 33 and 34). Why such high levels of cytoplasmic glycerol-P-dehydrogenase are found in brown fat and glial cells of the brain when other pathways can substitute remains enigmatic.

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