Synergistic Gene Interactions Control the Induction of the Mitochondrial Uncoupling Protein (Ucp1) Gene in White Fat Tissue*

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Among a selected group of mouse strains susceptible to dietary obesity, those with an enhanced capacity for Ucp1 and brown adipocyte induction in white fat preferentially lost body weight following adrenergic stimulation. Based on the generality of this mechanism for reducing obesity, a genetic analysis was initiated to identify genes that control brown adipocyte induction in white fat depots in mice. Quantitative trait locus (QTL) analysis was performed using the variations of retroperitoneal fat Ucp1 mRNA expression in progeny of genetic crosses between the A/J and C57BL/6J parental strains and selected AXB recombinant inbred strains. Three A/J-derived loci on chromosomes 2, 3, and 8 and one C57BL/6J locus on chromosome 19 were linked to Ucp1 induction in retroperitoneal fat. Although A/J-derived alleles seemed to contribute to elevated Ucp1 expression, the C57BL/6J allele on chromosome 19 increased Ucp1 mRNA to levels higher than parental values. Thus, novel patterns of C57BL/6J and A/J recombinant genotypes among the four mapped loci resulted in a transgressive variation of Ucp1 phenotypes. Although the extent of the interchromosomal interactions have not been fully explored, strong synergistic interactions occur between a C57BL/6J allele on chromosome 19 and an A/J allele on chromosome 8. In addition to selective synergistic interactions between loci, variations in recessive and dominant effects also contribute to the final levels of Ucp1 expression.

Brown adipocytes are localized in the newborn animal as defined tissue masses in several regions of the body (for a review of variation among mammalian species see Nedergaard (1)). The function of the tissue at birth is to generate heat to protect the animal from the cold. As the animal matures, alternative strategies for protection against cold become available and the discrete character of the tissue is gradually lost, except in small rodents where brown fat remains well defined. In large mammals the small number of brown adipocytes that can be found in normal individuals are generally dispersed throughout the white fat masses (2). As a consequence, the age-dependent loss of brown adipocyte character has made it questionable whether brown adipocyte thermogenesis can contribute significantly to energy balance and body weight regulation as proposed originally by Rothwell and Stock (3).

For several years it has been repeatedly demonstrated by different groups that adrenergic stimulation of rodents and dogs either by cold exposure or β-adrenergic agonists induces brown adipocytes in white fat depots as evidenced by characteristic histology of the brown adipocyte and/or the analysis of mitochondrial uncoupling protein gene expression (4–8). However, the high variability and low level of induction and dispersion of the brown adipocytes among white adipocytes precluded an experimental analysis that would lead to an understanding of mechanisms controlling this induction. Recently, an experimental strategy has become possible, which can overcome this problem, that is based on the finding that induction of brown adipocytes in white fat depots is genetically variable among inbred strains of mice (9, 10). The C57BL/6J strain has a very low capacity for induction, whereas, in A/J mice, brown adipocytes may constitute over 50% of the adipocytes in traditional fat depots. Accordingly, a quantitative trait locus analysis can lead to the identification of genes that determine brown adipocyte induction in adult animals.

The first genetic analysis on induction of the brown adipocyte phenotype in white fat depots of A/J and C57BL/6J inbred strains was based on expression in the AXB; BXA recombinant inbred (RI)1 strains (10). This analysis established: 1) that variation in Ucp1 mRNA levels correlated positively with the number of brown adipocytes present in fat tissue as quantified by image analysis of tissue sections stained with anti-UCP1 antibody, therefore indicating that Ucp1 expression was an appropriate subphenotype for analyzing brown fat induction; 2) that the level of induction and the degree of difference in expression among strains of mice varied among regional fat depots; 3) that selected AXB and BXA RI strains had levels of brown fat induction and Ucp1 mRNA that greatly exceeded the phenotype of the parental A/J strain, a phenotype known as transgressive variation; and 4) that enhanced mRNA levels reduced adiposity in strains susceptible to dietary obesity following treatment with β3-adrenergic receptor agonists (10).

In this paper we have used backcross and intercross analyses of the parental strains together with selected RI lines to begin to identify the chromosomal regions that encode the genes affecting the phenotypic variation of the induction of retroperitoneal (RP) brown fat in mice. For this purpose the determination of Ucp1 mRNA levels is a comparatively simple molecular phenotype that can be accurately quantified, as opposed to complex morphological or physiological phenotypes like counting the number of brown adipocytes. We also describe efforts to identify interactions occurring between specific QTLs in progeny from segregating crosses that can generate transgressive variation. The results are consistent with the hypothesis that transgressive variation arises, in part, in mice with recombinant genotypes due to synergistic interactions between A/J and C57BL/6J alleles that are located on chromosomes 8 and 19.

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The abbreviations used are: RI, recombinant inbred; RP, retroperitoneal; QTL, quantitative trait locus; RT-PCR, reverse transcription-polymerase chain reaction; LOD, logarithm of the odds favoring linkage.
**EXPERIMENTAL PROCEDURES**

**Animals and Tissue**

Male mice, 2 and 3 months old, were used in this study. A/J, C57BL/6J, (C57BL/6J × A/J)F1 hybrid mice and the AXB and BXA recombinant inbred strains (RI) were purchased from the production colonies of The Jackson Laboratory (Bar Harbor, ME). The (C57BL/6J × A/J)F1 × A/J backcross and the intercross between the AXB10 and AXB8 strains were set up in the research facilities at both the Jackson Laboratory and the Pennington Biomedical Research Center. Two or three mice per plate were placed in the cold (5 °C) for 1 week with a 12-h light/dark cycle and fed ad libitum a diet of 6.5% fat, 53% carbohydrate, and 18.6% protein. Mice were then sacrificed by cervical dislocation, and the fat pads were removed for RNA analysis, whereas the liver, spleen, and a protein. Mice were then sacrificed by cervical dislocation, and the fat pads were removed for RNA analysis, whereas the liver, spleen, and a protein. Mice were then sacrificed by cervical dislocation, and the fat pads were removed for RNA analysis, whereas the liver, spleen, and a protein. Mice were then sacrificed by cervical dislocation, and the fat pads were removed for RNA analysis, whereas the liver, spleen, and a protein. Mice were then sacrificed by cervical dislocation, and the fat pads were removed for RNA analysis, whereas the liver, spleen, and a protein.

**Quantitation of RNA**

Northern Blot Analysis—Northern blots were prepared as described by Derman et al. (12), and RNA expression was quantified according to procedures described by Guerra et al. (10). 10 μg of total RNA from each RP fat sample along with 2.7 or 1.35 μg of total brown fat RNA to serve as a standard was loaded onto each gel. Hybridization probes using [32P]dCTP were prepared using previously described methods (13). 18 S RNA was used as an internal standard to evaluate RNA loading and be almost undetectable in some animals and in others with levels as high as 3000 units; an amount corresponding to 40% of Ucp1 mRNA in cold-induced interscapular brown fat. The overall mean of RP Ucp1 in 2-month old mice (402 ± 22 S.E.) was not significantly different from 3-month old mice (364 ± 24 S.E.). The frequency distribution of the phenotypes in the backcross was without defined modes and similar to the continual range of values observed for the AXB and BXA RI strains (see Table II) (10). To determine the location of genes that contribute significantly to RP Ucp1 expression in backcross progeny, a genome-wide scan was carried out with 80 microsatellite markers at approximately 20-centimorgan spacing. The genotyping autoradiogram of a Northern blot shows the wide variation in signal intensities of RP Ucp1 mRNA in the backcross progeny (Fig. 1). RP Ucp1 induction varied up to 3000-fold, being almost undetectable in some animals and in others with levels as high as 3000 units; an amount corresponding to 40% of Ucp1 mRNA in cold-induced interscapular brown fat. The overall mean of RP Ucp1 in 2-month old mice (402 ± 22 S.E.) was not significantly different from 3-month old mice (364 ± 24 S.E.). The frequency distribution of the phenotypes in the backcross was without defined modes and similar to the continual range of values observed for the AXB and BXA RI strains (see Table II) (10). To determine the location of genes that contribute significantly to RP Ucp1 expression in backcross progeny, a genome-wide scan was carried out with 80 microsatellite markers at approximately 20-centimorgan spacing. The genotyping autoradiogram of a Northern blot shows the wide variation in signal intensities of RP Ucp1 mRNA in the backcross progeny (Fig. 1). RP Ucp1 induction varied up to 3000-fold, being almost undetectable in some animals and in others with levels as high as 3000 units; an amount corresponding to 40% of Ucp1 mRNA in cold-induced interscapular brown fat. The overall mean of RP Ucp1 in 2-month old mice (402 ± 22 S.E.) was not significantly different from 3-month old mice (364 ± 24 S.E.).

**Real Time Quantitative RT-PCR**—RT-PCR was performed using MapManager QTb8 (15). Data generated from these experiments were analyzed using multiple molecular markers. The genotyping autoradiogram of a Northern blot shows the wide variation in signal intensities of RP Ucp1 mRNA in the backcross progeny (Fig. 1). RP Ucp1 induction varied up to 3000-fold, being almost undetectable in some animals and in others with levels as high as 3000 units; an amount corresponding to 40% of Ucp1 mRNA in cold-induced interscapular brown fat. The overall mean of RP Ucp1 in 2-month old mice (402 ± 22 S.E.) was not significantly different from 3-month old mice (364 ± 24 S.E.). The frequency distribution of the phenotypes in the backcross was without defined modes and similar to the continual range of values observed for the AXB and BXA RI strains (see Table II) (10). To determine the location of genes that contribute significantly to RP Ucp1 expression in backcross progeny, a genome-wide scan was carried out with 80 microsatellite markers at approximately 20-centimorgan spacing. The genotyping autoradiogram of a Northern blot shows the wide variation in signal intensities of RP Ucp1 mRNA in the backcross progeny (Fig. 1). RP Ucp1 induction varied up to 3000-fold, being almost undetectable in some animals and in others with levels as high as 3000 units; an amount corresponding to 40% of Ucp1 mRNA in cold-induced interscapular brown fat. The overall mean of RP Ucp1 in 2-month old mice (402 ± 22 S.E.) was not significantly different from 3-month old mice (364 ± 24 S.E.). The frequency distribution of the phenotypes in the backcross was without defined modes and similar to the continual range of values observed for the AXB and BXA RI strains (see Table II) (10). To determine the location of genes that contribute significantly to RP Ucp1 expression in backcross progeny, a genome-wide scan was carried out with 80 microsatellite markers at approximately 20-centimorgan spacing. The genotyping autoradiogram of a Northern blot shows the wide variation in signal intensities of RP Ucp1 mRNA in the backcross progeny (Fig. 1). RP Ucp1 induction varied up to 3000-fold, being almost undetectable in some animals and in others with levels as high as 3000 units; an amount corresponding to 40% of Ucp1 mRNA in cold-induced interscapular brown fat. The overall mean of RP Ucp1 in 2-month old mice (402 ± 22 S.E.) was not significantly different from 3-month old mice (364 ± 24 S.E.). The frequency distribution of the phenotypes in the backcross was without defined modes and similar to the continual range of values observed for the AXB and BXA RI strains (see Table II) (10). To determine the location of genes that contribute significantly to RP Ucp1 expression in backcross progeny, a genome-wide scan was carried out with 80 microsatellite markers at approximately 20-centimorgan spacing. The genotyping autoradiogram of a Northern blot shows the wide variation in signal intensities of RP Ucp1 mRNA in the backcross progeny (Fig. 1). RP Ucp1 induction varied up to 3000-fold, being almost undetectable in some animals and in others with levels as high as 3000 units; an amount corresponding to 40% of Ucp1 mRNA in cold-induced interscapular brown fat. The overall mean of RP Ucp1 in 2-month old mice (402 ± 22 S.E.) was not significantly different from 3-month old mice (364 ± 24 S.E.).

**RESULTS**

**QTL Analysis with Backcross Progeny**

We previously reported that the induction of retroperitoneal (RP) fat Ucp1 mRNA in A/J mice (668 ± 140 S.E.) by cold exposure resulted in a 30- to 40-fold increase in expression as compared with C57BL/6J (B6; 16 ± S.E.) mice (10). We also provided preliminary evidence on expression in RI strains that suggested the trait was genetic. We have continued with the analysis of the genetic basis of Ucp1 expression in 789 two (n = 380)- and three (n = 409)-month old male (B6 × A/J)F1 × A/J backcross progeny exposed to the cold for 1 week. A representative autoradiogram of a Northern blot shows the wide variation in signal intensities of RP Ucp1 mRNA in the backcross progeny (Fig. 1). RP Ucp1 induction varied up to 3000-fold, being almost undetectable in some animals and in others with levels as high as 3000 units; an amount corresponding to 40% of Ucp1 mRNA in cold-induced interscapular brown fat. The overall mean of RP Ucp1 in 2-month old mice (402 ± 22 S.E.) was not significantly different from 3-month old mice (364 ± 24 S.E.).

**Microsatellite Analysis**

DNA isolated from tails, liver, or spleen of mice was genotyped using microsatellite markers purchased from Research Genetics, Inc. (Huntsville, AB). PCR products were separated using horizontal polyacrylamide gel electrophoresis as described previously (10) or by 4% NuSieve-agarose gel electrophoresis. A genome-wide scan was performed on the RNAs of mice with the 40 lowest and 37 highest levels of RP Ucp1 mRNA from the first 580 of 789 backcross progeny. Markers were spaced at 15- to 20-centimorgan intervals throughout the 20 mouse chromosomes. Chromosomal regions, identified by markers that show significant associations with Ucp1 mRNA levels, were analyzed across the region in detail by typing all 789 progeny with available polymorphic markers. Data generated from these experiments were analyzed using both Microsoft Excel and Statview 4.5 (Abacus Concepts, Berkeley CA). Linkage analysis and permutation analysis (14) were performed using MapManager QTb8 (15).

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19 QTL is particularly interesting, because, in contrast to the other 3 QTLs where the A/J allele is associated with high Ucp1 mRNA levels, the B6 allele is associated with "hyper-elevated" RP Ucp1 mRNA. This pattern of allelic expression may account for the transgressive variation for the RP Ucp1 mRNA phenotype in selected RI strains compared with the A/J parental strain (10), as well as in the backcross progeny. Fig. 3 represents a linkage analysis using only the mice that are recombinant within each chromosomal region. This figure displays more precisely the peak location of each QTL.
The presence of homozygosity for the A/J allele at the
Ucp1 structural gene located at 37.7 centimorgans.
And in the AXB8 strain. There are no exceptions to the combination for the 10 RI strains with the highest expression is also evident in the RI lines. A B6 allele at D19Mit86 and an A/J allele for a gene on chromosome 8 located near Ucp1 (37.7 centimorgans) and D8Mit128 (40.5 centimorgans) occurs for the 10 strains with the highest levels of expression. This allelic pattern is not interrupted until BXA11, which has 387 units of expression. From the lower end of the rank-ordered RI strains, this combination of an A/J allele on chromosome 8 and a B6 allele on chromosome 19 does not occur again until the expression is 186 units of Ucp1 mRNA in the BXA8 strain. There are no exceptions to the combination for the 10 RI strains with the highest expression or the 10 strains with the lowest level of expression. However, the wide range in Ucp1 expression evident among the 10 strains with highest expression underscores the point that quantitative expression is also associated with the action of genes on other chromosomes. Accordingly, the chromosome 8-A/J and chromosome 19-B6 combination is necessary, but not sufficient, for transgressive variation. It implies that the allelic patterns for the QTLs located on other chromosomes are also very important for expression.

An Intercross between AXB8 and AXB10—To further assess the allelic interactions between chromosomes 19 and 8, we analyzed the progeny from an intercross between the AXB10 and AXB8 RI strains. These strains have the lowest (13 units) and highest (1719 units) expression of Ucp1, respectively, and are variant for microsatellite markers within the QTLs on chromosomes 2, 8, and 19 and for the Ucp1 structural gene located at 37.7 centimorgans. Both strains have a B6 allele on chromosome 3. The interpretation of the effects of other genes on the chromosome 8 and 19 interactions is therefore a little less complex. There were three reasons for making this cross: one was to provide some insight into dominant, recessive, or codominant effects of this interaction; a second was to clarify the location of the gene on chromosome 8 that interacts with chromosome 19; and a third was to confirm the QTL with an independent cross albeit with the same alleles. Regarding the problem of chromosomal location, the backcross data showed the most significant linkage for the control of Ucp1 expression with D8Mit128 and less linkage to Ucp1 or D8Mit45 (Figs. 2 and 3). On the other hand, the strain distribution patterns in RI lines suggest that the association of Ucp1 mRNA levels with chromosome 8 is stronger in a region more distal to D8Mit128, nearer to the Ucp1 structural gene and D8Mit45 (Table II). The interaction plots of the intercross progeny show that the allelic-specific interactions clearly exist between the B6 allele at D19Mit86 and an A/J allele on chromosome 8 that is stronger for D8Mit45 than D8Mit128 (Fig. 5). In addition, the effects are codominant for the alleles on chromosome 19, but recessive for the A/J allele on chromosome 8 in determining Ucp1 mRNA levels (Fig. 5). The presence of homozygosity for the B6 allele at D19Mit86 and homozygosity for the A/J allele at the Ucp1-D8Mit45 region nearly triples the level of Ucp1 mRNA compared with heterozygosity or homozygosity for the B6 allele at Ucp1-D8Mit45. To assess whether these effects shown in the interaction plots are in some way dependent on contributions
Comparison of combinations of Iba genotypes on RP Ucp1 mRNA induction in 789 backcross mice

Mean Ucp1 mRNA levels and associated genotypes are listed for all combinations of Iba loci in 789 backcross mice. Positive loci are defined as genotypes contributing to elevated RP Ucp1 expression. An “A” genotype indicates a positive locus for chromosomes 2, 3, and 8, whereas an “F” (heterozygous) indicates a positive locus for chromosome 19. No overlap between the means of any subgroup of “positive” alleles was observed. Significance levels were compared vertically between each set of allelic combination of animals. The letter “N” denotes nonsignificance (p > 0.05), whereas boxes marked 1, 2, 3, and 4 indicate significance values of p < 0.05, p < 0.01, p < 0.001, and p < 0.0001, respectively.

| Set | # mice | 1  | 2  | 3  | 4  | Ucp1 mRNA |
|-----|--------|----|----|----|----|-----------|
| 1   | 50     | A  | A  | A  | A  | 878       |
| 2   | 45     | A  | A  | A  | A  | 778       |
| 3   | 51     | A  | F  | A  | F  | 668       |
| 4   | 42     | F  | A  | A  | F  | 516       |
| 5   | 47     | A  | F  | A  | F  | 470       |
| 6   | 48     | F  | A  | A  | A  | 392       |
| 7   | 50     | A  | F  | A  | A  | 352       |
| 8   | 55     | F  | A  | A  | F  | 325       |
| 9   | 37     | A  | A  | A  | F  | 320       |
| 10  | 55     | A  | F  | F  | F  | 320       |
| 11  | 58     | F  | F  | A  | F  | 316       |
| 12  | 46     | F  | F  | A  | A  | 298       |
| 13  | 52     | A  | F  | F  | A  | 222       |
| 14  | 49     | F  | F  | F  | F  | 187       |
| 15  | 57     | F  | A  | F  | A  | 150       |
| 16  | 47     | F  | F  | F  | F  | 118       |

Table I

Fig. 4. Synergy effect of Iba QTLs on retroperitoneal Ucp1 mRNA induction. This is a graphic representation demonstrating the complex epistatic and additive interactions between the four Iba loci. The solid line represents the experimental RP Ucp1 mRNA induction levels in relationship to the number of positive contributing loci. The dashed line indicates the theoretical value if interactions between Iba loci were only additive.

from chromosome 2, the genotypes for D2Mit66 were determined. It was found that the same 1:2:1 distribution of chromosome 2 alleles were associated with each subset of mice sorted in Fig. 5. This indicates that the effects of allelic interactions between chromosomes 8 and 19 occur even when variation at chromosome 2 is constant. Accordingly, the synergistic interaction between chromosomes 8 and 19 appears to be a major determinant of the quantitative phenotype.

The analysis of the synergistic interactions using the recombinant inbred lines described above indicated that the location of the Iba3 QTL was not in total agreement with the interval map generated with the backcross data. Accordingly, the QTL linkage analysis of chromosome 8 was determined for AXB10 and AXB8 intercross progeny that were respectively homozygous AA, heterozygous AB, and homozygous BB at chromosome 19 (Fig. 6). The most significant effects occur when D19Mit86 is homozygous BB and with a recessive model for inheritance. The results show that the QTL is spread over a broad region that includes both D8Mit128 and D8Mit45. Surprisingly, the single QTL on chromosome 8 resolved into two QTLs that were centromeric and telomeric of the backcross QTL (Fig. 2). The data suggest that the QTL peak at D8Mit128 is a “phantom QTL” generated when more than one QTL lies on the same chromosome (14, 21, 22). A consequence of this change in the position of the QTL is that significant effects on Ucp1 mRNA expression are located nearer D8Mit45 and the Ucp1 structural gene than D8Mit128, an association that is also evident in the strain distribution patterns of the RI lines (see Table II).

The AXB10-AXB8 intercross also provided useful linkage information with respect to the location of the QTL on chromosome 19. Inspection of the linkage relationships for markers on chromosome 19 shows that D19Mit106 and D19Mit86 have two recombinants in BXA4 and AXB10 (Table II). The order of markers and their centimorgan positions were determined from the (B6 × A/J)F1 × A/J backcross as shown in Fig. 2. The location of the QTL from the backcross progeny was slightly more significant for D19Mit106 than D19Mit86. However, because the AXB10-AXB8 intercross is not variant for D19Mit106 and phenotypic effects are still present in the intercross progeny, the QTL on chromosome 19 must lie distal to D19Mit106.

Discussion

QTL analysis of (B6 × A/J)F1 × A/J backcross indicated that four highly significant (Iba) loci on chromosomes 2, 3, 8, and 19 control differences in Ucp1 induction. We have evaluated whether genes, known to be involved in the regulation of Ucp1 expression and the differentiation of the brown adipocyte, map to these locations and are, accordingly, candidate genes. The number of physiological systems and, therefore, genes that could be involved in brown adipocyte differentiation could be very large, because the hypothalamus and the sympathetic nervous system are also involved. However, the fact that the same induction phenotype occurs whether the adipocytes are stimulated by cold exposure and, therefore, mediated by the hypothalamus or by intraperitoneal injection of a β3-adrenergic agonist and are hypothalamus-independent, leads us to think that the phenotype is restricted to variant gene expres-
sion in the adipocytes of the retroperitoneal fat depot (10). Therefore, any gene functioning from the stimulation of adrenergic receptors on the surface of the adipocyte, through intracellular signal transduction pathways, to transcription complexes that control gene expression in the nucleus and the mitochondria could be involved. Many of these that have been shown to serve a function in \textit{Ucp1} expression have been mapped to the Mouse Genome Database; however, only the \textit{Ucp1} structural gene itself maps reasonably close to the QTL on chromosome 8. This suggests that major regulatory genes of brown adipocyte differentiation, which also include genes involved in mitochondrial biogenesis, remain to be discovered.

The availability of the AXB and BXA RI lines have been a source of much insight and power in the analysis of this genetic

### Table II

| Strain | Chromosome 8 | Chromosome 19 |
|--------|--------------|--------------|
|        | 8M128 | 8M13 | 8M14 | 8M15 | 8M242 | 8M31 | 8M34 | 37.7 cM | 40.5 cM | 47 cM | 18.5 cM | 20 cM | 20.3 cM | 21.4 cM |
| A/J    | A | A | A | A | A | 668 | A | A | A | A | A | A | A | A |
| C57BL/6J | B | B | B | B | B | 16 | B | B | B | B | B | B | B | B |
| (B6 X A/J)F1 | AB | AB | AB | AB | AB | 129 | AB | AB | AB | AB | AB | AB | AB | AB |
| AXB 8  | A | A | A | A | A | 1719 | B | B | B | B | B | B | B | B |
| AXB 4  | A | A | A | A | A | 1700 | B | B | B | B | B | B | B | B |
| AXB 15 | A | A | A | A | A | 1490 | B | B | B | B | B | B | B | B |
| BX A14 | A | A | A | A | A | 1439 | B | B | B | B | B | B | B | B |
| AXB 14 | A | A | A | A | A | 1247 | B | B | B | B | B | B | B | B |
| AXB 19 | A | A | A | A | A | 1181 | B | B | B | B | B | B | B | B |
| AXB 20 | A | A | A | A | A | 896 | B | B | B | B | B | B | B | B |
| AXB 18 | A | A | A | A | A | 630 | B | B | B | B | B | B | B | B |
| BX A 25 | A | A | A | A | A | 576 | B | B | B | B | B | B | B | B |
| AXB 24 | A | A | A | A | A | 562 | B | B | B | B | B | B | B | B |
| BX A 11 | B | A | A | A | A | 387 | A | A | A | A | A | A | A | A |
| BX A 4 | A | A | A | A | A | 359 | A | A | A | A | A | A | A | A |
| BX A 12 | A | A | A | A | A | 295 | B | B | B | B | B | B | B | B |
| AXB 6  | B | B | B | B | B | 226 | A | A | A | A | A | A | A | A |
| BX A 17 | B | A | A | A | A | 200 | B | B | B | B | B | B | B | B |
| BX A 8  | B | A | A | A | A | 186 | B | B | B | B | B | B | B | B |
| AXB 5  | B | B | B | B | B | 153 | B | B | B | B | B | B | B | B |
| AXB 12 | B | B | B | B | B | 122 | A | A | A | A | A | A | A | A |
| BX A 7 | B | B | B | B | B | 86 | A | A | A | A | A | A | A | A |
| AXB 10 | B | B | B | B | B | 77 | A | A | A | A | A | A | A | A |
| AXB 24 | B | B | B | B | B | 55 | A | A | A | A | A | A | A | A |
| AXB 2  | B | B | B | B | B | 51 | A | A | A | A | A | A | A | A |
| AXB 13 | B | B | B | B | B | 51 | A | A | A | A | A | A | A | A |
| AXB 1  | B | B | B | B | B | 40 | B | B | B | B | B | B | B | B |
| AXB 10 | B | B | B | B | B | 37 | B | B | B | B | B | B | B | B |
| AXB 14 | A | A | A | A | A | 13 | B | A | A | A | A | A | A | A |

**Fig. 5.** Effect of interactions between loci near \textit{Ucp1} (\textit{D8Mit45}; 40.5 centimorgans), \textit{D8Mit128} (31 centimorgans), and \textit{D19Mit86} on retroperitoneal fat \textit{Ucp1} mRNA induction in 286 intercross progeny from AXB10 and AXB8. Line interaction plots demonstrate a strong epistatic interaction between loci on chromosomes 8 and 19. This interaction appears to be stronger for \textit{D8Mit45}, a locus nearer to \textit{Ucp1} than for \textit{D8Mit128}, the major QTL defined in the (\textit{A/J} X \textit{C57BL/6J})F1 \times \textit{A/J} backcross. Interaction plot data was generated using Statview v.4.5.

**Fig. 6.** This figure represents the linkage analysis of a QTL on chromosome 8 using 286 intercross progeny from AXB10 and AXB8. The QTL analysis was performed for individual groups of mice having the AA, AB, and BB genotypes for a QTL on chromosome 19 near \textit{D19Mit86}. Results demonstrate a very strong recessive gene affect near a locus at 40 centimorgans on chromosome 8 for RP fat \textit{Ucp1} mRNA induction. This affect is only observed in animals with a BB allele on chromosome 19. Data for these plots was generated using MapManager QTb8 (15).
system. Intercross data from the RI lines suggests that the QTL on chromosome 8 is composed of two loci due to the tendency of multiple loci on a chromosome to form a phantom peak on QTL analysis (14, 21, 22). However, these loci do not account for all of the genetic determinants involved in the variance associated with Ucp1 induction. Based on the data in Table I and evidence suggesting that the B6-derived allele on chromosome 19 exerts codominant affects on chromosome 8 (Fig. 5), we estimate that the highest level of Ucp1 mRNA in mice with the AABA genotype at chromosomes 2, 3, 8, and 19, respectively, would be 1021 units. Because the highest level of mRNA present in RI lines is 1730 units for AXB8, which have the AAB genotype at the 4 Iba loci, these four chromosomal regions account for approximately 60% of maximal levels of expression. Whether the remaining 40% of the genetic variance comes from many minor gene effects or one or two major loci that are recessive for the B6 allele, and therefore not revealed in the backcross to the A/J parent, remains to be determined. Additional intercrosses among the RI lines can identify new major loci, if they exist.

Ucp1 mRNA expression in the RI lines suggests that not all QTLs can independently increase Ucp1 expression. The A/J allele at chromosomes 2, 3, and 8 conferred higher levels of Ucp1 expression. In contrast, the B6 allele at the Iba4 locus on chromosome 19 was associated with elevated Ucp1 expression. Because B6 mice have very low levels of expression, it suggests that the B6 allele at Iba4 cannot by itself increase Ucp1 mRNA levels. This is evident from intercross data in Fig. 5, which shows that increases in Ucp1 mRNA depend on an interaction with a gene on chromosome 8 that carries the A/J allele. In addition, AXB1 and BXA1 strains with a B6 allele at Iba4 have the lowest levels of expression when the allele at Iba3 is derived from B6 (Table II). Thus Iba4 has no main gene effect by itself, but only through its interactions with Iba3. Of course Iba4 could also be interacting with other chromosomes to determine other phenotypes. Although it is possible that Iba4 on chromosome 19 is interacting with the Ucp1 structural gene on chromosome 8, it is difficult to reconcile a recessive rather than a codominant phenotype for a gene at which transcription is occurring, as it is in this case, i.e. production of Ucp1 mRNA. It is easier to imagine a post-transcriptional mechanism akin to dominant-negative interactions of protein complexes involved in the initiation of transcription to explain recessivity.

The ability to induce brown adipocytes and Ucp1 expression in white fat is not unique to rodents models. Many animals, including humans appear to retain some capacity to induce brown adipose tissue. As newborns, humans have an abundance of brown fat, which is mostly lost by early adulthood (23). However, brown adipocytes have been observed to be interspersed with peri-renal white fat in human adults (24), and adults with pheochromocytoma have an abundance of brown fat (25, 26). These observations suggest that the capacity for brown adipocyte formation in human adults is not permanently lost, but may very well lie dormant and can be reactivated with the proper stimuli. It has been shown that human adipocytes in culture can be stimulated to express Ucp1 following exposure to adrenergic agonists or thiazolidinediones (27, 28). Using the mouse models described in this manuscript to gain further insight about the genetics controlling Ucp1 expression in white fat and its relationship to adiposity may ultimately provide new mechanisms for the development of therapeutics to control obesity in humans.

The conclusions from this study underscore the genetic complexity controlling metabolic variation among closely related strains of mice. Ucp1, the core of one of the systems for the regulation of obesity in rodents, (29–32) is differentially expressed in white fat tissue of B6 and A/J mice, strains previously identified as models of genetic obesity and diabetes (33). A minimum of four to five genes are involved in Ucp1 expression alone, and additional variant genes will be involved in the regulation of mitochondrial biogenesis and other processes relevant to the induction of brown adipocytes in white fat depots. Hence, the number of variant genes involved in metabolic systems associated with the obese phenotype could be much larger than currently estimated.

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